MEDICINAL PLANTS USED BY BERBER AND ARAB PEOPLES OF MOROCCO:

Ethnopharmacology and Phytochemistry

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Botany

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

FEBRUARY 2002

Department of Botany

The University of British Columbia
Vancouver, Canada

Date March 1st, 2002

Abstract

Medicinal plants were selected on the basis of their use by Berber and Arab people to treat infectious diseases caused by viruses and microbes, as determined in interviews with 55 healers and herbalists of all regions of Morocco. Plants used traditionally to treat colds, flu, coughs, wounds, cuts, rashes, diarrhea, and fever were studied. Rare and endemic plants (205 plants of 55 families) of potential medicinal value, not investigated before, were collected from mountains (Rif, High, Middle and Anti Atlas) and eastern and western Moroccan Sahara, in the summers of 1997-2000. Many in use are toxic at high doses; consequently dosage is extremely important and may influence whether a plant is an effective medicine or a poison. Methanolic extracts of 75 Moroccan plants were evaluated against three mammalian viruses: herpes simplex, Sindbis and polio, at non-cytotoxic concentrations. Five extracts were active against all three viruses, 16 against two, and 24 against one. Thirty-two extracts showed light-enhanced, and two, light-dependent, activities. These results indicate that some of these plants are potential medicines against viral infections. Several plant species were also analyzed for antimicrobial activities. They displayed different patterns: a broad spectrum, or selective activity, against fungi or bacteria or in light only. A chemical survey of fifty plant species was performed by Electron Spin Resonance spectroscopy that detected fingerprints of phenolics that may have medicinal significance. The analysis of *Nigella sativa* revealed the presence of a high content of thymohydroquinones that have potent antimicrobial properties and may explain the activity of *Nigella*, in light and dark, against both gram-positive and gram-negative bacteria. The black seeds are used in Moroccan traditional medicine for infectious pulmonary and cough diseases as well as for other ailments. The significance of this research lies in the discovery of novel and useful biological activities from plants, and as a scientific documentation of the value of Moroccan Berber and Arab ethnomedicine. The availability of a local pharmacopoeia supported by scientific data could be beneficial to large populations who do not have access to modern drugs.

Abstract in French

Résumé

Les plantes médicinales etaient sélectionnées sur la base de leur utilisation par les Berbers et Arabs ā traiter les maladies infectieuses causées par les viruses et microbes, comme préalablement determiné dans les interviews avec 55 tradi-praticiens et herbalistes de toutes les regions du Maroc. Les plantes utilisées traditionnellement ā traiter les rhumes, inflenza, toux, blessures, rougeurs, diarrhée, et la fievre étaient étudiées. Les plantes rares et endemiques (205 plantes de 55 familles) ayant un potentiel médicinal, non etudiées auparavant, étaient collectées des montagnes (Rif, High, Middle and Anti Atlas) et du Sahara Marocain de l'est et de l'ouest du pays, durant les périodes d'été du 1997-2000. Beaucoup de ces plantes sont toxiques ā haute dose, par consequent le dosage est extrěmement important et peut influencer si une plante est un medicament éfficace ou un poison. Les extraits méthanoliques de 75 plantes marocaines étaient évalués contre trois viruses mammalians: herpes simplex, Sindbis et polio, ā des concentrations non-toxiques. Cinque extraits étaient active contre les trois viruses, 16

contre deux seulement, et 24 contre un virus. L'activité de trente deux extraits était stimulée par la lumière, tandis que l'activité de deux extraits était dépendante de la lumi ère. Ces résultats demontrent que certaines plantes étudiées sont des médicaments potentiels contre les infections virales. Plusieurs plantes étaient aussi analysées pour l'activité microbienne. Elles montrent des patterns differents: un large spectre d'activité ou une activité plus selective, contre les bacteries et les champignons, en presence de lumière. Une étude chimique de 50 plantes était faite par Electron Spin Resonance spectroscopie qui a détectée des fingerprints de phénoliques qui peuvent presenter une importance medicinale. L'analyse de *Nigella sativa* L., a révélée la presence d'une haute teneur de thymohydroquinones qui ont des propriétés antimicrobiales fortes et peut expliquer l'activité de *Nigella*, en presence de lumière et dans l'ombre, contre les bacteries gram-positive et gram-negative. *Nigella* est utilisée dans la médecine traditionnelle Marocaine pour les maladies infectieuses pulmonaires et pour la toux, ainsi que pour beaucoup d'autres affections. L' importance de cette recherche est demontrée non seulement par la découverte d'activités biologiques utiles des plantes, mais aussi comme documentation scientifique de la valeur de la médecine Marocaine ethnique Berbere et Arabe. La disponibilité d'une pharmacopoéia locale supportée par des données scientifiques pourraient être bénéfiques aux nombreuses populations qui n'ont pas l' accès aux médicaments modernes.

Abstract in Arabic

ملخص

لقد تم اختيار النباتات الطبية على اساس استعمالاتها من طرف الامازيغ والعرب بالمغرب ضد الامراض التي تسببها الفيروسات والميكروبات كما هو مبين في استطلاع للرأي مع 55 عشاب او طبيب تقليدي في كل انحاء المغرب.

تناولت الدراسة النباتات المستعملة لعلاج البرد، الزكام، السعال، الجروح، الاسهال والحمى. ونشير الى انه قد تم جمع بعض النباتات المستوطنة والمهددة بالانقراض (205 نوع تنتمي الى 55 فصيلة) من المناطق الجبلية (الريف، الاطلس الكبير والمتوسط والصغير) ومن شرق وغرب الصحراء المغربية في صيف 1997 - 2000. مجمل النباتات المستعملة تكون سامة اذا تم استعمالها بكمية كبيرة ولذا فالمقادير من الاهمية بمكان في تحديد ما اذا كان النبات دواء او سما. تم تقييم فعالية 75 عصارة النباتات ضد ثلاث فيروسات ثديية: هربس سمبلكس (herpes simplex)، سندبس (Sindbis)، بوليو (Polio) في تركيزات غير سامة.
تبين أن خمسة من العصارات كانت فعالة ضد كل الفيروسات في حين 16 فعالة ضد فيروسين و 24 فعالة ضد فيروس واحد فقط. توصلنا كذلك الى ان 32 من العصارات يدعمها الضوء واثنين من العصارات تعتمد بالضرورة على الضوء. اسفرت نتائج هذه الدراسة على ان بعض هذه النباتات يمكن استعماله ضد الأمراض

الفيروسية. تم كذلك دراسة آثار هذه النباتات على الأمراض ذات اصل مكروبي حيث تبين ان هناك مستويات مختلفة ضد الفطريات والبكتيريا. اشتملت الدراسة كذلك على مسح كميائي لـ50 نوع عن طريف ESRS مما أظهر آثار للحامض الفينولي ذو الفعالية الطبية. ولقد اظهرت دراسة الحبة السوداء (الشانوج) وجود مركب Thymohydroquinone الذي له خاصيات هامة ضد الميكروبات مما يفسر آثار الحبة السوداء في الضوء والظلام وضد البكتيريا gram+ و gram-.

تستعمل الحبة السوداء بالمغرب في معالجة امراض الرئة والسعال كما تستعمل ضد امراض آخرى.

تكمن اهمية هذه الدراسة في اكتشاف خاصيات جديدة ومهمة من النباتات. كما تعتبر وثيقة علمية للطب التقليدي للمغاربة. فوجود تطبيب تقليدي مدعم بمعطيات علمية قد يساهم في دعم فئة عريضة من السكان الذين يتعذر عليهم استعمال الادوية العصرية.

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Abbreviations

UV	=	Ultraviolet
MeOH	=	Methanol
EtOH	=	Ethanol
NaOH	=	Sodium hydroxide
EtOH-H_2O	=	Ethanol-water
ESR	=	Electron Spin Resonance Spectroscopy

Acknowledgements

I thank the International Development Research Center (Ottawa) for providing financial support for the field research in Morocco. Laboratory work was partly financed by the Natural Sciences and Engineering Research Council (Ottawa, Canada) and the Canadian Bacterial Diseases Network.

I am especially grateful to Pr. Moh Rejdali, head of the Department of Botany, Institute Agronomic and Veterinarian Sciences Hassan II (IAV), Rabat, Morocco. He provided extensive help in the ethnobotanical study, including the collection and identification of plants from different ecosystems in Morocco, as well as acquiring local information on medicinal plants, and use of facilities in his Department. The IAV also provided assistance with establishing contacts in the field, particularly in order to locate Arab and Berber healers and to interview people. They also facilitated interaction with other Moroccan researchers. The cooperation and kindness shown by the Moroccan affiliation was indispensable to the success of my research. I also would like to thank Pr. Rejdali for suggesting IDRC for financial support. Many thanks to Pr. Rejdali's technician, Mohamed Ait Lafqui, as well as other Moroccan scientists, especially Pr. Zoubida Charrouff-Chaouni, and assistants, drivers, Hussein and Ahmed and Hussein M., who helped in the field collection trips. The interactions with other local scientists, such as Pr. Othmani, Jabri, Harrouni and others were also very helpful to me.

In addition, I wish to thank Moroccan healers and others who kindly shared ethnobotanical information with me. Particularly, the family Oudghiri, all contributed in their own way: Mohamed Larbi, Azzedine, Mohamed (Meknes, Rabat, and Casablanca), and Aicha Jamal Eddine (in her memory), Aicha, and Oudghiri Ahmed, M. (in his memory). The last contributed a lot to the initial phase of this work. He was a knowledgeable healer and scholar who suggested most of the pertinent publications. Mohamed and Khadija Jamal Eddine shared their knowledge about desert plants and suggested some interesting sources of information to me.

I also thank Mohamed Sbai, Sekkat Ibrahim, Cadi Mohamed (Oujda-Figuig); Said, M. ("Souk al Joumoua", Derb Sultan, Casablanca), herbalists knowledgeable about plants and their uses. The last belongs to a family of inherited traditional practice. He provided a major part of the ethnobotanical information. Abandar, Lazaar, also known as "Lazaar" meaning the blond, is a Berber healer and druggist in Agourai, a village near Meknes, in the Middle Atlas. In most Berber villages, there is a " Thursday market " held on Thursday only, where Berbers gather to buy their needs and sale items for the week. His contribution was important because he collected plants himself from the Middle Atlas, and knew their benefits and drawbacks. He also suggested the localities of other plants that grew in the desert or other parts of Morocco. Fatna, Zhoor, Khadija, Ali and other Berber people of the Middle Atlas (Agourai and Hajeb) assisted in the collection of plants with me, and helped in their preparation (drying and crushing). I wish to acknowledge the following healers from the High Atlas, Anti Atlas and Sahara: Dhiba Abdelhadi and Ahmed, Arjdal Elhassane, Boukantar Mohamed, (Marrakech), Lahbib Mazouar, (Rissani), Bokbot M'barak (Merzougua), Mohamed Laqzadri, (Agdz), Touda Hassou, (Tamezmout), Krimo Jemaah, (Zaouiat Amzrou, Zagora), Akouchar Lahcen, (Agadir), Bennani Mohamed Assoussi, (Laayoune), as well as many others who prefer to remain anonymous. The ethnobotanical research was carried out in accordance

with the code of ethics pertaining to the protection of intellectual proprietary rights (ISE, 1998; Grenier, 1998; Posey, and Dutfield, 1997). All the information used in this thesis was obtained from Healers who signed an informed consent form. Information was not disclosed in cases where healers were not in agreement.

[Grenier, L. (1998). "Working with Indigenous Knowledge: A guide for researchers. Published by IDRC, Ottawa, Canada.

International Society of Ethnobiology (ISE). (1998). Code of Ethics.

Posey, D., and Dutfield, G. (1997). Le marche mondial de la propriete intellectuelle. Published by IDRC, Ottawa, Canada, and WWF, Geneve, Suisse.]

I would like to thank my committee members for all their help over the past years. Many thanks to my supervisor, Neil Towers, for his support, understanding and guidance, and for his enthusiasm and passion, first, for all kind of people, and second for the study of phytochemistry of medicinal plants from all over the world. He is a continual inspiration for me. Thank you also for believing in me. Jim Hudson taught me antiviral techniques and helped with the applications to IDRC. His advice during many stages of this research was very helpful. I also would like to thank him for his visit to Morocco. Many thanks to my co-supervisor, Iain Taylor, for his excellent editorial suggestions for the writing of this thesis, as well as for his help. Many thanks to Gary Bradfield for his continual encouragement, support, and excellent directions particularly in times of crisis. Thank you to Jens Pedersen for introducing me to ESR technique.

I also would like to thank the office staff of the Department of Botany for their help, as well as the staff in the Herbarium at the University of British Columbia and the

Institute in Morocco who helped with the collection and storage of plant material. Many thanks to all the people who helped me in Towers' laboratory. Thank you to all my friends for their support, particularly, Sultan, Mrs. Elizabeth Towers, Mohamed, Azzedine, Mohamed Larbi, Jamal, Lalla, Abdel Aziz, Shehrazad, Yousseff, Othman, Aicha, Zine El Abidine, Hamza, Myriam and many others.

Finally I would like to thank my family in Morocco for believing in me and for their support, understanding, patience and encouragement.

My journey towards the accomplishment of this task was a rocky one, with many detours, but in the end, very interesting and rewarding.

Dedication

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

ٱقْرَأْ بِٱسْمِ رَبِّكَ ٱلَّذِى خَلَقَ ﴿١﴾ خَلَقَ ٱلْإِنسَـٰنَ مِنْ عَلَقٍ ﴿٢﴾ ٱقْرَأْ وَرَبُّكَ
ٱلْأَكْرَمُ ﴿٣﴾ ٱلَّذِى عَلَّمَ بِٱلْقَلَمِ ﴿٤﴾ عَلَّمَ ٱلْإِنسَـٰنَ مَا لَمْ يَعْلَمْ ﴿٥﴾

To My Family

Forward

Portions of this thesis were previously written and published by the author. Their citations are as follows.

Mouhajir F., Hudson, J., Rejdali, M., and Towers, G.H.N. (2001). Multiple Antiviral Activities of Endemic Medicinal Plants used by Berber People of Morocco. *Pharmaceutical Biology* **39** (5): 364-374.

Mouhajir F., Pedersen, J. A., Rejdali, M., and Towers, G.H.N. (2001). Phenolics in Moroccan medicinal plant species as studied by Electron Spin Resonance Spectroscopy. *Pharmaceutical Biology* **39** (5): 391-398.

Mouhajir F., Pedersen, J. A., Rejdali, M., and Towers, G.H.N. (1999). Thymohydroquinones of Moroccan *Nigella sativa* seeds detected by Electron Spin Resonance. *Pharmaceutical Biology* **37**: 1-5.

Portions of this thesis were also presented at the following conferences:

Symposium "Lead Compounds from higher Plants": University of Lausanne, Switzerland, September 2001: *Multiple Antiviral Activities of Endemic Medicinal Plants used by Berber People of Morocco.*

Canadian Bacterial Diseases Network: Canmore, Alberta, May 2000: *Antimicrobial Thymohydroquinones of Moroccan Nigella sativa seeds detected by Electron Spin Resonance.*

UBC, Botany Seminar: *Medicinal Plants of Morocco: Characterization and Potential for long term Health Benefits*, February 27, 2001.

UBC, Microbiology Department, **Canadian Bacterial Diseases Network:** November 1999. *Antimicrobial Thymohydroquinones of Moroccan Nigella sativa seeds detected by Electron Spin Resonance.*

The author, under the supervision of Pr. M. Rejdali, Head of the Department of Botany, Institut Agronomique et Veterinaire Hassan II, Rabat, Morocco, collected plant material and ethnobotanical data. Extraction of all 80 Moroccan plants and their antibacterial and antifungal assays were done by the author in the laboratory and under the supervision of Pr. G.H.N. Towers, Department of Botany, University of British Columbia (UBC). Antiviral assays were performed in the laboratory of Pr. J.B. Hudson, under his supervision, Department of Pathology and Laboratory Medecine, (UBC). ESR characterization of Moroccan plants were done in the laboratory of Pr. F.G. Herring, Department of Chemistry, (UBC), with the help of Pr. J.A. Pedersen, a visiting scientist from Denmark, Aarhus University, Department of Chemistry.

F. Mouhajir G.H.N. Towers

CHAPTER I

General Introduction

Ethnomedicine

The search for medicinal plants has been an integral part of human society since the earliest recorded history. Some plants were used for food and others for medicine. In several cases, humans discovered multiple uses for the same plant. Plants used, as food can also be considered medicinal, as many fruits and vegetables contain many beneficial compounds (e.g. anti-oxidant in citrus fruits). Some plants were used to relieve pain and others (e.g. *Cannabis sativa* L., Cannabaceae, called kif or hashis in Morocco; and *Papaver somniferum* L. (opium), Papaveraceae, Table 1.1) had psychoactive properties. It is worth mentioning the present debate (Randall, 1991) about marijuana's use to relieve pain for patients suffering from cancer or AIDS. Chinese monks used green tea, an excellent source of the stimulant caffeine, to stay awake and increase mental awareness. Furthermore, some plants were avoided or used as poisons, e.g. poison hemlock (*Conium maculatum* L. (hemlock), Apiaceae) with which Socrates was poisoned. Some poisons are useful; for example, curare is used as anesthetic. Other plants were considered sacred and were worshipped like the lotus flower and also *Nigella sativa* L., Ranunculaceae, which is considered a blessed and sacred plant in North Africa and the Middle East. The sacred plants may also be considered medicinal in the context of psychosomatic diseases.

After food plants, the most beneficial ones were and are still the medicinal plants used to relieve pains, cure diseases, block the spread of epidemics, etc. Thus, the flowering plants are our main sources of food crops, timber, fibers, vegetable oils, gums, herbs, spices, flavorings, drugs, stimulants, and narcotics. They also adorn our parks, gardens, streets, and other public places as trees, shrubs, and flowers.

Medicinal plants discovered by traditional societies are proving to be an important source of potentially therapeutic drugs. Such appreciation has emerged in part because of recent discoveries made by a small but growing group of ethnobotanists who study the relationships between plants and people. Fieldwork exploring the medicinal uses of plants by indigenous peoples in remote parts of the world, coupled with the introduction of sophisticated assays to determine whether plants exert a biological effect, has facilitated the discovery of bioactive molecules made by medicinal plants. Some of these molecules show promise as possible therapies for a range of diseases, including AIDS and cancer. The drug development process is a long and arduous one, designed to ensure that therapeutics released to market are effective and safe. It can thus take many years for a substance to become commercially available as a drug. Until the 1950s, almost all-pharmaceutical research relied heavily on vascular plants as sources of medicines (Cox, 1994). Flowering plants and ferns (as opposed to microscopic organisms and fungi) have given rise to more than 120 commercially sold drugs and account for more than 25 percent of all prescriptions issued every year in North America (Cox, 1994). Many are now synthesized in the laboratory, but others are still isolated from plants. Studying indigenous uses has generated much knowledge about drugs in plants. Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against predation or infection (Cox, 1994). In the late 1970s, several groups of scientists independently set off to different corners of the world for the express purpose of finding new drugs through ethnobotanical research, e.g. Finn Sandberg, Lars Bohlin and Gunnar Samuelsson, University of Uppsala; Richard Evans Schultes of Harvard University (Cox and Balick, 1994). It is estimated that 250,000 flowering species grace the earth (Heywood, 1993). Of these, less than half of 1 percent have been studied exhaustively for their chemical composition and medicinal value. Cox and Banack (1991) found that 86 percent of the plants used by Samoan healers displayed significant biological activity in a variety of assays. Balick and Arvigo (1993) and Balick et al. (1994), discovered that crude extracts of the plants that one healer considered to be the most powerful gave rise to four times as many positive results in a preliminary laboratory test for activity against HIV than did specimens collected randomly. However, few of the compounds exhibiting activity in laboratory tests ever prove to be useful new drugs. Some will turn out to be identical to, or less potent than, existing agents are, others will prove too toxic for commercial use.

Ethnobotanists choose the societies they study according to different criteria. The society should be located in a floristically diverse area, such as a tropical rain forest. However, although floristically diverse areas do have a greater number of plant species available, one should not exclude less diverse areas from ethnobotanical research. All plants have the potential to contain pharmacologically interesting secondary metabolites. The societies should have remained in the region for many generations. Those societies presumably have had ample opportunity to explore and experiment with local vegetation. The cultures must have a tradition in which healers transmit their plant knowledge from generation to generation, usually through apprentices. Consistent application of a given species for an ailment over millennia can only be known to have occurred where there are strong oral traditions and written historical documents of sufficient age and detail. These records provide information rather analogous to that produced by large-scale clinical trials. Such repetitive, long-term use of botanical species can be expected to led to identification of both the most effective medicinal plants and those that are too toxic for use. Folk medicine of the type normally studied in ethnobotanical research identifies remedies used within a traditional context, but that is no assurance of great antiquity. Such practices are part of a dynamic living culture that has been exposed to new plants, new diseases, and new ideas about healing from other cultures. Many traditional healers are experimenters and innovators who may develop new ways to help their patients depending on their needs. Thus, a particular remedy may have been used for millennia or in fact only a few years, months, weeks, or days, yet still be traditional because of how it was discovered, applied, and understood to be efficacious within that particular cultural context.

In the islands of Samoa, most healers are female and specialize in herbal medicine. The herbalists typically make use of more than 100 species of flowering plants and ferns. The demonstrated ability of ethnobotany to generate exciting leads for drug discovery, suggests that the approach will occupy an expanding role in future drug development (Balick and Arvigo, 1993; Balick et al., 1994; Cox, 1994). However, it is not advisable to generalize too much about the efficacy and safety of traditional remedies, for example, the widespread yet clearly unsafe use of heavy metals such as lead, antimony, mercury, and arsenic in some Arabian traditional medicines.

Our understanding of the origins of medicine have progressed substantially in the last 50 years. In fact, traditional remedies are discovered by traditional healers through experience with side effects of plants in the diet, testing by smell and taste, observation of animal behavior, the "Doctrine of Signatures", and more esoteric means such as dream interpretation. Rarely do they randomly try plants by "trial-and-error experiments" (Johns, 1990).

Various materia medica, consisting of vegetable, animal and mineral products, have existed since the earliest times (Sigerist, 1951). A long time must have passed before humans really appreciated many uses of plants: drinks made from tea and coffee had desirable properties, alcoholic beverages were known since ancient times (Sigerist, 1951) and spices had both a preservative and taste-enhancing effect on food. Extracts of both plants and animals were also used as arrow-and spear-poisons (Sigerist, 1951). The effects of these poisons were noted and understood. Many traditional healers have a quite sophisticated knowledge of anatomy and physiology, so it is merely a question of the level of understanding to which one refers. It would not be at the molecular level obviously, but quite possibly at more broad physiological levels, and sometimes within a cultural context not directly comparable to modern medicine, e.g. the concepts of energy flow modulation found in Ayurvedic and Chinese Traditional Medicine systems (Johns, 1990).

Magic and religion became associated with the preparation of drugs and thus witch doctors, priests and eventually physicians practiced medicine. Because of many cases of poisoning due to excessive dosage, primitive humans evolved the kind of materia medica that excluded the more potent alkaloid and glycoside remedies. Drug assay and standardization were not available for an accurate dosage. Well-known folk medicines are known as effective purgatives and remedies for expelling worms or killing lice. Because no chemical or pharmacological investigations were available at that time, many seemingly trivial drugs became popular by trial-and-error methods (Trease, 1964; Trease and Evans 1978). Because the search for medicinal plants started very early in history, an historical study of the evolution of medicine allows us to explore findings for which past interest has been lost in the hope of rediscovering useful medicinal plants or new valuable information about them. The knowledge of medicinal plants of the past combined with the new technology of the present could bring unsuspected results. It may be a useful and inexpensive source of potential therapeutic compounds. As Holland (1994) stated, "The mass screening of plants in the search for new drugs is vastly expensive and inefficient. It would be cheaper and perhaps more productive to re-examine plant remedies described in ancient and mediaeval texts". These valuable and ancient herbals provide food for thought and problems for investigation; they are potentially of great benefit to humankind.

Origin of folk remedies

How did folk remedies originate? Initially they seem to have been introduced and persisted in a culture in ways that were related to primitive beliefs of the cause of disease, and a variety of folklore and superstitions. Over the somewhat serendipitous millennia of experimentation many have emerged with what we have described to be a valid scientific basis. This ancient knowledge became a tradition and was transmitted from one generation to the next. Traditional healers and elderly people were and continue to be treasures of information on medicinal plants. Their knowledge is valuable because they remember the effects of the plants on different peoples and diseases. Traditional ethnobotanical medicine has become a treasury of valuable information about medicinal plants, hence the need to document and exploit this knowledge before it is lost forever (Schultes, 1994). It is experimental and practical and requires documentation for scientific purposes and for future generations before it is lost (Balick and Cox, 1996; Schultes, 1994; Lewington, 1990; Stockwell, 1989).

Brief history of Mediterranean ethnomedicine:

Egypt 3000 BC to ca. 1200 BC

The knowledge of Egyptian materia medica comes from two papyri, the Edwin Smith Papyrus of ca. 1600 BC and the Ebers Papyrus of ca. 1550 BC (Bryan, 1930). Although some drugs cannot be identified, their sources are similar to present day drugs (opium from poppy, *Papaver somniferum*, aloes from *Aloe socotrina* lamk, castor oil from *Ricinus communis* L., Euphorbiaceae, and squills...). Squill is a Mediterranean bulbous herb (*Urginea indica* Roxb.) of the Hyacinthaceae family, called also sea onion. The white dried sliced bulb scales of a squill are still used as an expectorant, cardiac stimulant, and diuretic. The red squill, *Urginea maritima* (L.) Stearn, Hyacinthaceae, is used mostly as a rat poison. Animal products such as fat (milks and livers) and minerals such as copper carbonate also were used. Egyptian medicine was primitive in the sense that it combined magic, religious and empirical elements but they also practiced surgery and trepanning.

Assyria and Chaldea ca. 1900 to 391 BC

These medicine records were on clay tablets instead of papyri. Religion and magic had more influence on their medicine than in Egypt. Thompson (1924) published a synthesis of this medicine: The Assyrian Herbal. Drugs like opium, storax, myrrh, and liquorice were used at that time.

Greece 1250-285 BC

In its early days, Greek medicine was closely linked with religion and the sick went to temples where treatment included bathing, diet, exercise and a form of psychotherapy. Aesculepius, who was deified as the god of medicine, was actually a man who lived at Delphi about 1250 BC. Hippocrates, the father of medicine, was born in 460 BC. Socrates the philosopher and teacher lived at the time of Hippocrates. The Hippocratic Collection shows evidence of new medical ethics and a scientific spirit (Chadwick and Mann, 1950). More than 400 drugs are mentioned in these works. For example, white hellebore, *Veratrum album* L., Liliaceae (Melanthiaceae), that was used as an emetic and black hellebore, *Helleborus niger* L. (Christmas rose), Ranunculaceae, that was used as a purgative. Theophrastus (370-285 BC), also called physician of the gods, was the most celebrated botanist of antiquity and his works, which were compilations and original observations, were studied for twenty centuries. His "Historia Plantarum" or "Enquiry into Plants" contains information about the collection and preparation of vegetable drugs such as peony root, foodstuffs, perfumes, plant diseases and weather signs (e.g. water, fire, air, and earth). Hart (1916) states that "The English translation should be examined by every student of pharmacognosy". Dioscorides (AD

50-100) was born in Cilicia, served as a surgeon in the armies of Nero, and wrote "De Materia Medica". The work contains descriptions of about 600 plants (Dioscorides receives the mandrake root from Eurisis, goddess of discovery), and shows close observation of Nature (Gunther, 1934). Galen (ca. AD 130-201) was born at Pergamon in Asia Minor. He was a very successful practicing physician and surgeon, an experimentalist particularly in anatomy and physiology, and a prolific writer. Galen's theory of pathology combined the humoral ideas of Hippocrates, the Pythagorean theory of the four elements (fire=hot+dry; air=hot+moist; earth=cold+dry; water=cold+moist) and his own doctrine of the four temperaments. The Hippocratic doctrine stated that the body was composed of four "humours": blood, phlegm, black bile (melancholy), and yellow bile (choler), and that disease was produced by their maladjustment. The humors were correlated with the elements (fire, air, water and earth) and led to attractively simple but erroneous relationships. These were held so strongly until the Renaissance that many argue that they retarded understanding of both chemistry and medicine for many centuries. Galen made intelligent use of drugs such as hyoscyamus, opium, and hellebore but wrongly regarded others as specifics, e.g. pepper for fevers and scammony for jaundice. In later days the name "galenical" became a general term for a drug produced directly from plant or animal materials (Trease, 1964).

Arab Medicine

In the period of the golden age of the Muslims (AD 632-1150), the Arabs, having conquered Egypt, Syria, Iraq and Persia, extended their Empire from the borders of India to Spain. The concepts and contents relating to the materia medica and therapeutics of the Muslims in the period from 750 AD to about 1350 were qualitatively and quantitatively far ahead of those of preceding cultures. In quantity, the number of simples and compound remedies rose to about 4,000. This is a fantastic number when compared with the mere hundreds in the Greek works. The Arab rulers of the Middle East included Mansur (754-775), Harun-al-Rashid (780-809) and his son Mamun (813-833). Mamun rebuilt Baghdad and founded there a hospital and University ("House of Wisdom") where scholars were provided with a large library and encouraged to translate influential works such as those of Plato, Aristotle, Hippocrates, Galen and Dioscorides. In addition to translating Greco-Roman works, the Arabs collected knowledge from India and other sources, which they added to their own knowledge, findings and experience. They were particularly interested in alchemy. Four medical writers of the period were very influential: Ibn Hayyan, or Jabir (776), was a man of great influence at the court of Harun-al-Rashid. He was one of the greatest alchemists. Al Razi or Rhazes (865-925) was a physician and prolific writer. His comprehensive book is a medical encyclopaedia derived from the best Greek, Syrian, Arab, Persian and Indian sources. As an alchemist his interest was on practical chemistry rather than alchemy theory. To Rhazes we owe the familiar division of substances into animal, vegetable and mineral. Abu Mansur (ca. 970), a Persian, was the author of an important book on materia medica in which are described 466 vegetable drugs, 75 mineral drugs and 44 animal drugs. Ibn Sina or Avicenna (ca. 980-1036) was a Persian physician who wrote not only on medicine but on music, physics, mathematics and alchemy (Afnan, 1958). His great work on medicine, the Canon (Canon Medicinae), was "a magnificent attempt to coordinate systematically all the medical doctrines of Hippocrates and Galen with the biological concepts of

Aristotle" (Bulaq, 1877). Avicenna had a well-equipped laboratory at that time (Gruner, 1930; Holmyard, 1957). Arabic medicine reached its peak in Spain when it was in decline in the East. It was the product of many cultures including Arabs, Moors, Spaniards and Jews. Learning flourished at Cordoba, which is said to have had a library of 300,000 volumes, and at Seville, Toledo and Murcia (Gruner, 1930; Holmyard, 1957). Among the scholars were Abulcasis, who in the eleventh century wrote on surgery: Ibn Rushed or Averroes, a philosopher who wrote General Rules of Medicine; Ibn Zuhr or Avenzoar (1091-1161), a court physician; and Ibn al Khatib who wrote on infection and the plague. Of particular interest to pharmacists were Abu Zakaria Yahya ibn al-Awwam al-Ishbili of Seville who in the twelfth century wrote on agriculture (Ibn al Awwam, 1864-1867), and most important of all the pharmacognosist Ibn al Baitar (1197-1248) of Malaga (Leclerc Baitar, 1877-1883). The latter's principal work, the Jami or Corpus of Simples, describes some 1400 drugs and plants. Most of these were found in the work of Dioscorides and other classical writers, but about 300 were new and characteristic of Arab pharmacy, for example, musk, cloves, dragon's blood, galanga root, betel nut, sandal wood, rhubarb, nutmeg, tamarind, cassia bark, croton oil and nux vomica. The use of sugar was also a characteristic of Arab pharmacy. From India, cultivation of the sugar cane spread to Persia and was later introduced by the Arabs into Cyprus, Sicily and Spain. Extensive use and knowledge of chemistry and biological activity in the practice of Arabic medieval medicine accelerated the development of botany, the cultivation of plants, and other relevant areas of study. Following its capture by the Christians in 1085, Toledo became a great center for the collection and translation into Latin of Arabic manuscripts such as those by Rhazes, and Avicenna. Europeans translated Arabic alchemical work. In making these translations it was often difficult to find suitable Latin equivalents, and many Arabic words thus found their way into European languages, e.g. al-qali "=" alkali; al-kuhl "=" alcohol; qarabah "="carboy; tutiya "="tutty (zinc oxide). Even today, many of the original Arabic nouns are found in modern languages, for example, naranj = orange, kafur = camphor, kabbar = caper, misk = musk, al-kakanj = alkekenge (ground cherry), zaafaran = saffron, summaq = sumac. Berber was also affected in this way, so that the language is heavily laden with Arabic names of botanicals and minerals (Meyerhof, 1935). The latter author derived much of his information from a synonymatic text Tuhfa (of 1700) ordered according to the old Semitic alphabet (abjad), entitled "A precious gift to friends on the attributes of plants and herbs", translated by Renaud and Colin (1934). This is a glossary of 462 Moroccan simple drugs by an unknown author, which contains material from the botanical, mineralogical, and zoological kingdoms. The author seems to have been a native of Marrakech (Morocco) and so the text is rich in Berber terminology. This kind of treatise was particularly useful to apothecaries, merchants, herbalists, and physicians or healers. Al-Ghafiqi who wrote "Book of Simple Drugs", lived near Cordova. In his time, he was the greatest authority on biological activity (Al-Ghafiqi et al., 1938). Al-Idrissi (1100-1166) wrote one of the most comprehensive botanical-pharmacological works of his time, called "Universal Collection". As a botanist and a geographer, his work was carried out from his own travels. He finally settled in Sicily at the court of King Roger II. Another author worth mentioning is Ibn Biklarish who wrote Tables, a rich source of information for physicians on topics such as galenic nature and grade, synonyms (in Persian, Greek, Latin, and old Spanish), substitute drugs, preparation, therapeutic value, and drug uses (Renaud, 1927; Bedevian and Bedevian, 1936).

Not only did the Muslims systematize the pharmacological knowledge of the Greeks, Persians, and Babylonians but also in many highly specialized treatises, they made genuinely original contributions to enhance knowledge of the medieval materia medica. At the end of the eleventh century, only traces of Greek science were possessed by Latin writers and scholars (Meyerhof, 1952). Early medieval writers in Latin inherited not only a storehouse of chemical facts and many experimental results, but also a wealth of theoretical ideas. As the strongest fact-based biological science, Arabic pharmacology lasted into the nineteenth century. As a result of the accumulation of data over thousands of years' experience, this science may still lead to something of value to modern workers in pharmacology (Levey, 1973,1971, 1966). Even today the Greek-Arabic Unani tradition is practiced among more than 100 million people in the Middle East, not only in popular and folklore medicine, but it is also taught in Unani (Greek-Arabic) and Ayurvedic (traditional health care system) medical colleges in India and Pakistan. From Morocco to India, druggists in smaller towns compounded their remedies according to the ancient classical traditions of a thousand years ago (Campbell, 1904-5). A pharmacist's handbook, which greatly influenced Arabic medicine and is still very popular today, mainly outside the large cities, is that written by al-Kuhin al-Attar (meaning the druggist priest or Kohen the druggist) (Kuhin, 1940). In 1259, he wrote the treatise "Manual for the Pharmacy". Al-Kuhun, a practicing apothecary, was a contemporary of Ibn-al-Baitar and may have been in touch with him (Levey, 1971). Another work worth mentioning because of its popularity in the Near East written by a

Cairo authority, al-Antaki (1599):"Memorandum for Intelligent people", is an alphabetical list of drugs (Al-Antaki, 1997). There is much to be learned from these early drug treatises.

In the constant effort to improve the efficacy of Western medicine, researchers are increasingly turning their attention to folk medicine for new drugs. Different approaches can be taken to establish the scientific basis for the successes of folk medicine. One approach is anthropological which seeks to understand and appreciate how and why a particular medical practice became established and then to explore the chemical and biological reasons for the success of the practice. Another approach is to focus more directly on the collection, description, and testing of plants for their active ingredients (Steiner, 1986). In a broad sense this "ethnopharmacological" (looking for plants with specific effects) approach is the predominant theme in this thesis.

The increase in infectious diseases (caused by bacteria, viruses or fungi) and the increased resistance of microorganisms to antibiotics and antiviral agents, urges us to search for new and effective medicinal compounds. The major diseases addressed by Moroccan traditional medicine include digestive pathology, skin, bronchi-pulmonary, and urinary and liver disorders as well as gynecology and dental care, and many infectious diseases. The most common infectious diseases in Morocco (as listed in the American National Center for Infectious Diseases, Atlanta, GA) are 1) bacterial: diphtheria and tuberculosis, and dental plaque which is a worldwide problem, 2) viral: influenza (Orthomyxoviridae), herpes and chickenpox (Herpesviridae), polio (Picornaviridae), coughs and colds (Rhinovirus), hepatitis A, B, and C (Hepadnaviridae, Flaviviridae), and measles (Paramyxoviridae). Other common diseases in Morocco include cholera, meningitis, tetanus, and typhoid. Malaria is a problem in the south during the summer. *Schistosoma* (bilharzia) is present in some rivers and pools. Water-borne pathogens include many bacteria, worms, amoebae and viruses. Insect bites or stings, cuts and scratches that become infected, tuberculosis, and rabies are also important. Health problems that are common in the West, such as tonsillitis, candidiasis and skin infections also occur in Morocco. The increased resistance of microorganisms to many available western drugs has dramatized this picture of infectious diseases. Thus many traditional medicinal plants in Morocco are being used to treat these infections and other ailments.

Although the flora is well known (Maire, 1952-1980), only a few studies have been done on Moroccan traditional medicine and there are no reports of research on biological activity (Bellakhdar et al., 1991; Bellakhdar, 1997; Boulos, 1983; Claisse, 1990, 1985; Nauroy, 1954; Renaud et Colin, 1934; Sijelmassi 1993; Ziyyat et al., 1997). There have been some ethnobotanical studies of Moroccan plants (Balick and Cox, 1996; Campbell, 1904-5; Cox, 1994; Cox and Balick, 1994; Khallouki et al., 2000; Mouhajir et al., 2001; Schultes, 1994; Ziyyat et al., 1997). Moroccan ethnobotanical medicines have been derived from two major sources: classical Arab medicine, practiced in the Middle East from which it was spread to North Africa and other countries by Arabs, and secondly local popular Berber medicine (native). About 600 plant species are reported to be of medicinal use (Al-Antaki, 1997; Bellakhdar et al., 1991; Bellakhdar, 1997; Benjilali et al., 1997; Rejdali and Birouk, 1996; Zahwelee, 1997).

People and society at large are turning more and more to alternatives like traditional medicine for treatment of disease (cancer, HIV…) for which modern medicine has as yet no known cures. Scientists have recognized this need, as seen by the number of published papers in the subject. Instead of rejecting this knowledge, as was the case before, a new attitude has arisen which is to exploit this tradition by studying it scientifically. The discovery of remedies (Table 1.1) such as morphine (*Papaver somniferum* L., Papaveraceae), digoxin (*Digitalis purpurea* L., Scrophulariaceae), quinine, (*Cinchona calisaya* Wedd., Rubiaceae), theophylline (*Camellia sinensis* L., Theaceae) and ephedrine (*Ephedra sinica* Stapf, Ephedraceae) has provided a great impetus for the continuing isolation and characterization of plant secondary compounds. However, with well over 250,000 plant species worldwide (Heywood, 1993), a random screening of plants for compounds with biological activity is certain to be frustrating and inefficient. Cox and Balick (1994) stressed the need for some collection strategy to explore the large pool of plants for potential medicines. Many strategies were proposed. The random search, the first strategy, which has not been very successful. This involves the collection of all the plants found in the study area, independent of the ethnobotanical uses. For example, the screening of 114,000 plant extracts from 35,000 species of plants (The National Cancer Institute, USA, 1994) resulted in the discovery of three clinically active anticancer drugs. Not one of those drugs came from a plant collected on an ethnobotanical basis (Gragg et al., 1994). For example, Taxol, an anti-cancer compound isolated from the Western Yew tree (*Taxus brevifolia* Nutt., Taxaceae) was discovered in a random survey. Although this strategy is time consuming and expensive (Holland, 1994), there are in fact some economic justifications for random screening where mechanized high throughput bioassay screening methods are available. The NCI supplements random screening with ethnobotanical based screening (Gragg et al., 1994). Another strategy involves a phylogenetic survey, or phytochemical targeting, which is the collection of all species of a plant family known to be rich in bioactive compounds (Khafagi and Dewedar, 2000; Cox and Balick, 1994). The chemotaxonomic approach is the collection of plants having similar chemicals. The specific plant parts approach is the collection of a particular part of a plant, seeds for example (Khafagi and Dewedar, 2000). The ecological survey is a strategy in which plants are selected because they live in particular habitats and have certain characteristics that suggest that they will likely produce interesting chemicals. Plants that are toxic to herbivores might be useful as medicinal agents (Khafagi and Dewedar, 2000). Finally, the last strategy is the ethnobotanical approach (Cox and Balick, 1994) based upon the local flora, which has been used traditionally in each culture as a medicine. However, the introduction of western health care not only in developing but also developed countries has replaced and in some cases eradicated the traditional medicine. Three types of drug discovery have resulted from the ethnobotanical strategy (Cox, 1994). In the first type, the ethnobotanical use matches clinical efficacy of unmodified natural products (e.g. Digitalis ssp., Scrophulariaceae, Table 1.1, digoxin: heart drug). In the second type, the traditional use only suggested the clinical efficacy (e.g. vincristine: anti-cancer drug, Table 1.1.), of unmodified natural products. In the third type, the traditionally used natural product is transformed to more potent chemically modified natural or synthetic products (e.g. aspirin or acetyl salicylic acid, Table 1.1). Many workers have therefore chosen the ethnobotanical approach as a means of homing in on plants with pharmacologically active compounds of potential therapeutic interest (Balick and Cox, 1996). I also chose the ethnobotanical approach to study the medicinal plants from

Morocco, because it is the most productive and the most successful of the plant-surveying methods, as confirmed by recent findings (Cox, 1994; Cox and Balick, 1994).

Medicinal plants discovered by traditional societies are proving to be an important source of potentially therapeutic drugs. Even though the types of drug discovery that have resulted from the ethnobotanical strategy indicate that in ideal cases, the ethnobotanical use matches clinical efficacy, in other cases it only suggested the clinical efficacy. In yet other cases the traditionally used natural product is transformed to more potent chemically modified natural or synthetic products. The ethnobotanical approach seemed still the best choice available for this thesis research.

Table 1.1

Examples of medicines developed from plants based on ethnobotanical information. Medical use is also listed.

Plant source	Medicines	Medical Use
Cinchona calisaya Wedd. Rubiaceae (South America)	Quinine, an alkaloid	Malaria
Digitalis purpurea L. Scrophulariaceae (Shropshire, West England)	Digoxin, a glycoside	Dropsy: accumulation of fluid caused by the heart's failure to pump adequately
Rauvolfia serpentina (L.) Benth. Apocynaceae (India)	Reserpine, an alkaloid	Hypertension
Camellia sinensis L. Theaceae (China)	Theophylline, an alkaloid	Bronchial dilator
Papaver somniferum L. Papaveraceae (Eurasia)	Morphine, an alkaloid (opium)	Analgesic and sedative
Filipendula ulmaria (L.) Maxim. (Eurasia)	Aspirin, salicylic acid	Analgesic and anti-inflammatory Arthritis
Ephedra sinica Stapf. Ephedraceae (China)	Ephedrine, an alkaloid	Bronchial-dilator, hay fever, asthma, nasal congestion
Datura stramonium L. (North America)	Scopolamine Stramonium	Eases motion sickness. Asthma
Podophyllum peltatum L. Berberidaceae (Eastern North America)	Peltatin, a lignan	Leukemia, lymphoma and lung cancer

Scope and Objectives of Thesis

The goal of my research was to characterize the biological activity from selected plants used medicinally in Morocco (Fig.1.1, Map), and surrounding Arab countries, including the Middle East.

I had the following specific objectives:

(1) To obtain information on medicinal plants used for infectious diseases, from traditional Berber and Arab healers and knowledgeable people (1997-2000), as well as further checking this information by re-examining plant remedies described in ancient and recent Arabic texts. The documentation of the Berber oral traditional uses was the goal.

(2) To collect potentially active plants, to evaluate the biological activity of their crude extracts on representative human pathogenic Gram-positive and Gram-negative bacteria, pathogenic fungi and viruses.

(3) To characterize some active compounds by electron spin resonance (ESR) spectroscopy. ESR is ideal to detect phenolics with free ortho- or para-dihydroxy groupings as well as to detect quinones. In the method, pertinent compounds are transformed to a radical stage (semiquinones) by suitable redox reactions. The quinones are reduced and the aromatic compounds oxidized in base to semiquinone radicals and detected and identified, absolutely or partly in the crude extract by way of their unique spectra.

(4) To study the mechanisms of action of some of the active compounds.

(5) To obtain information on medicinal plants of traditional Berber and Arab healers and knowledgeable people:

a) Document Berber oral traditions regarding plant uses

b) Re-examine plant remedies described in ancient and recent Arabic texts

(6) To collect potentially active plants and evaluate the biological activity of their crude extracts against representative human pathogens.

(7) To investigate their phytochemistry by Electron Spin Resonance (ESR) spectroscopy.

This thesis has three main sections:

1-Ethnomedicine

2-Biological activity

3-Phytochemistry of selected active compounds

The science of ethnomedicine is involved with the documentation and scientific study of indigenous medical knowledge or medicines (related terms: ethnopharmacology, ethnobotany). Possibly because of its ethnic diversity, Morocco may be expected to have a wealth of traditional medicines, which were inherited from ancient Arab medicine as well as the Berber traditions. Most Berber local medicine is an oral tradition; hence we need to preserve this knowledge for future generations. All these factors make Morocco an excellent site to conduct an ethnobotanical study.

The significance of this research lies in the possibility of discovering novel and useful biologically active plants and in documenting Berber oral tradition and showing the value of ethnobotanical medicine as recorded in ancient texts that have still a wealth of information valuable in today's world.

It is hoped that not only would these medicinal plants provide interesting biological activity, but also that by documenting this ethnobotanical study, both governmental and non-governmental agencies would have a valuable resource to use for sustainable development, such as reforestation, conservation of biological diversity, or rural health care.

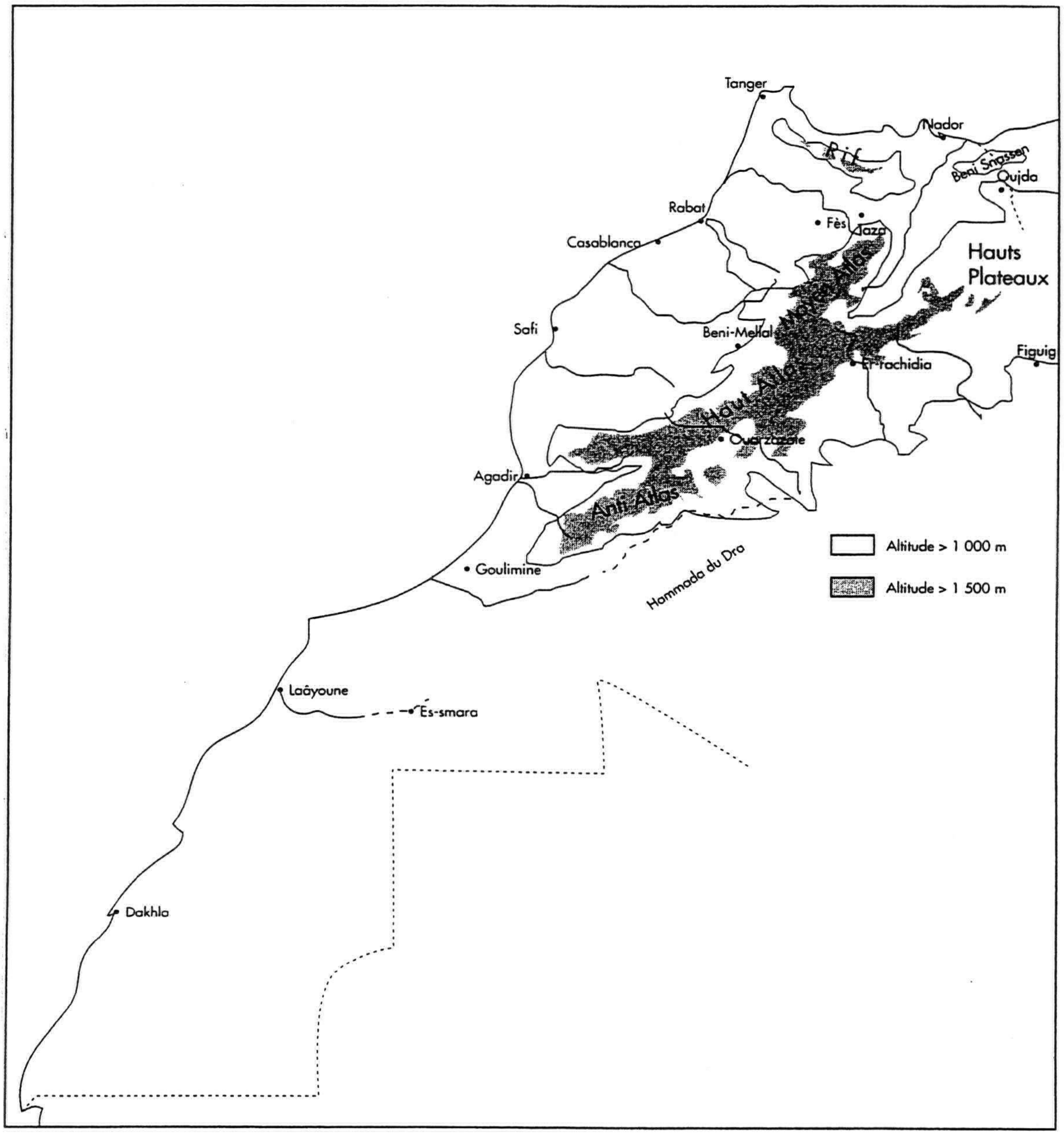

Figure 1.1. Map of Morocco

(Bellakhdar, 1997)

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CHAPTER II

FIELD RESEARCH

Introduction

Morocco is a monarchy of 710,000 sq. km, with 28,000,000 inhabitants. Its major resource industries are phosphate mining, cultivation of citrus species (e.g. oranges), and tourism. Recently oil deposits were discovered. Although there is a rich fishery in the south, it is little exploited. Morocco's economy is fast growing due to several factors. For example, foreign investments are increasing due to the fact that labor-intensive products can be made cheaper locally than in any other place close to Europe. Skilled labor and more and more people with higher education also contribute to this booming economy.

Morocco is in many ways a country apart. It is situated in the northwestern tip of Africa. Two major physical features separate it from the rest of Africa: the towering Atlas Mountains and the Sahara desert. Its climate, geography, and history are all more closely related to the Mediterranean than to the rest of Africa. The coastline (3500 km) borders the Mediterranean Sea in the North, and the Atlantic Ocean in the west. The Sahara desert occupies the southern (east and west) part with Mauritania to the South and Algeria to the East (Fig. 1.1). The climate is also as varied as the geography of the country. The coastal climate is moderate and subtropical, cooled by the Mediterranean

Sea and Atlantic Ocean. The average temperature ranges around 20°C. In the interior, the temperatures are more extreme.

These conditions of diverse biomes (desert, coast, well irrigated highlands), allow for a rich and varied flora: 4500 native vascular plant species with a high level of endemism of at least 700 endemic species, of which 600 are medicinal and about 1500 botanical introductions species (industrial, alimentary, ornamental etc.) (Bellakhdar et al., 1991; Bellakhdar, 1997; Emberger and Maire 1941; Jahandiez and Maire, 1931-1934; Maire, from 1952 to 1980: 16 volumes; Rejdali, 1996; Rejdali and Heywood, 1991).

The Atlas Mountains

The rich flora of a Morocco as well as the Berber culture are due at least inpart to the Atlas Mountains that fill most of the northwestern corner of Africa, with the exception of the Rif in northern Morocco. The Atlas is divided into 5 regions, and stretches from Morocco, throughout northern Algeria, before it reaches Tunisia. Morocco has the High Atlas, the highest peak being Jabal Toubkal (4, 165 meters). Anti Atlas is around 2, 531 meters, but still has some of the most rugged landscape. The Middle Atlas reaches 3, 350 meters.

The Atlas Mountain region includes many fertile valleys because the high peaks bring down a large part of the rainfall of this region. Several million people live in the Atlas Mountains and in smaller settlements; almost all of these count themselves as Berbers, a group living in the region stretching from Morocco's West Coast to the Siwa oases in Egypt, and from Tunisia's north tip to the oases in mid-Sahara. Historically, Maghreb was one country composed of modern Morocco, Algeria, Tunisia and Libya.

The current divisions were established after the French (Morocco [protectorate], Algeria and Tunisia) and the Italian (Libya) colonization.

Berbers are the majority of the population of North Africa. They form 80% of the population in Morocco, where their origins can be traced to the 2nd millenium BC, and Algeria, more than 60% in Tunisia and Libya, and 2% in Egypt. They have blended with other people, and stories of European slaves and war captives may explain the blond hair and red hair, as well as green and blue eyes that have become part of Berber population. The origin of the Berbers is not certain either. Some believe they came from Europe or Yemen, but it is most likely that they were the original population of North Africa (Bellakhdar, 1997).

There are around 300 local dialects among the Berbers. Berbers are Muslims, but there are other local popular practices found among Berbers. More Berbers than Arabs live in rural areas, where local popular practices are generally found.

Moroccan Berbers are divided in three groups with different languages and localization:

-The Rifains are originally from the Rif Mountain. They speak the Tarifit language.

-The Chlouhs are originally from the middle Atlas. They speak the Tamazight language.

-The Soussis are originally from the high and anti-Atlas. They speak the Tassoussit language.

The Berber linguistic tradition is oral rather than written; hence there is very little written Berber history or linguistic documentation.

The Arab communities in Morocco are originally from the southern desert areas and cities and many came from Al-Andalous or Arabian Andalouzia in southern Spain.

A major event in Moroccan history was the conquest and Islamisation of the country around 647 AD by the Arabs. The Idrissids founded the first Moroccan State in the 8^{th} century. The Arabs brought knowledge, sciences, religion, order, culture, etc. They speak Arabic, which is the country's official language, now spoken by three-quarters of the population. In addition, there are other languages, the Moroccan spoken languages and French and Spanish. Grimes (1996), in the "Ethnologue" (catalog of the World's languages), lists 11 different languages in Morocco. This ethnic diversity has created a unique and rich culture. Hispano-Moorish, Jewish, Middle Oriental and Saharan cultural backgrounds have influenced the Arab-Berber civilization.

Moroccan Ethnobotanical medicine seems to have two major sources. Classical Arab medicine was practiced in the Middle East and spread with the Arab conquest of North Africa and other countries as well. Berber popular medicine probably has local roots and may have unique aspects arising from Berber isolation before Arab conquest.

Although the flora and the vegetation of Morocco are well known now and 600 plant species are reported to be of medicinal use, only a few studies have been done on traditional medicine (Bellakhdar *et al*, 1991; Bellakhdar, 1997; Boulos, 1983; Claisse, 1985; Merzouki et al., 2000; Nauroy, 1954; Hassar, 1986).

Furthermore, Morocco is still a very traditional country, even though it has many features of modern societies and is open to outsiders. Thus, although modern medicine is available to many people, the use of medicinal plants is still popular in everyday life for illnesses such as colds, flu, wounds, rashes, fever, and other minor infections. Some of the major diseases addressed by Moroccan traditional medicine are related to digestive pathology (stomach & intestine), skin and hair-care, bronchi-pulmonary, urinary system and liver disorders, gynecologic as well as dental care. For example, *Juglans regia* (Juglandaceae), (leaves & bark & fruit husk), has been traditionally used, as well as *Olea spp.* (Oleaceae) (leaves) for dental hygiene. *Nigella sativa* and *Lawsonia inermis* (henna) are widely used in the Arab world for many ailments and in religious ceremonies and celebrations of happy events. In cosmetology, henna is very popular particularly among Berber women who use it to tattoo their hands and feet. This form of tattooing has been so successful, that it has spread to California to provide temporary tattoos as opposed to western invasive methods, which are permanent. An extract of *Mentha spp.* in combination with green tea is the national drink in summer, whereas in winter, *Artemisia absynthium* L. ("sheeba") is used instead. *Mentha spp.* (e.g., Fig. 2.2.) are also used as a flavor in many foods. Other species of mint are used for colds (e.g. *Mentha pulegium*, Fig. 2.1.). *Thymus* species are much used as a disinfecting agent for meats as well as to treat indigestion and provide dental care. *Lavandula* species (e.g., Fig. 2.3.) are used for colds, as well as in perfumes, and in the protection of clothing from bacteria.

Figure 2.1. *Mentha pulegium* L., Lamiaceae

(Boulos, 1983)

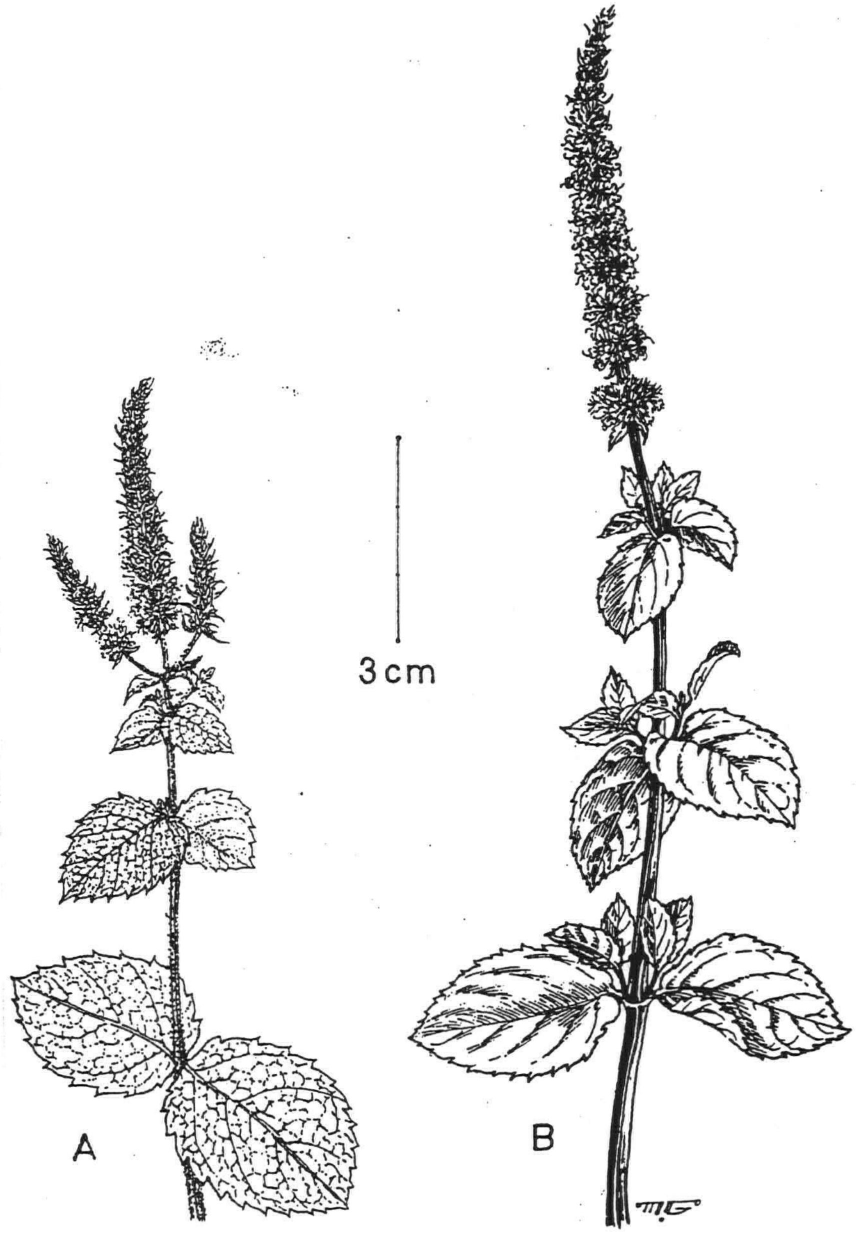


Figure 2.2. *Mentha suaveolens* Ehr. (A) & *Mentha* x *villosa* Huds. (B), Lamiaceae

(Boulos, 1983)

Figure 2.3. *Lavandula stoechas* L., Lamiaceae

(Boulos, 1983)

My research contained novel parts. First, I specifically studied endemic species from the Atlas Mountains and Sahara, in order to increase my chances of discovering some novel phytochemicals from medicinally useful but unstudied species. I also tested selected plants traditionally used to treat dental problems such as plaque or tooth decay (e.g. *Ammoides*, *Mentha*, *Juglans*, *Olea*, *Lawsonia*, *Nigella*, *Thymus* and *Lavandula*).

This was particularly interesting and original because, with some exceptions, plants are hardly used for dental hygiene in the western world. The human mouth hosts hundreds species of fungi, protozoa, viruses, and intracellular parasites and, above all, bacteria. Most oral cavity microorganisms are permanent residents, and many are found nowhere else, not even in the mouths of other mammals. These microorganisms form a very elaborate ecosystem with a temperature of 36°C, constant moisture and a steady influx of nutrients. Every square millimeter of cheek tissue, every fold beneath the gum line, every crevice in the tongue is swarming with occupants. Even a well-brushed tooth may host billions of bacteria.

For decades, dental researchers have been seeking methods to eliminate or control these microorganisms. Their focus was mainly *Streptococcus nutans*. This sphere-shaped bacterium is only found on human teeth. It causes tooth decay by degrading sugar and secreting lactic acid. Researchers have tried hard to develop an antimicrobial agent or vaccine that could destroy it. Dental plaque consists of different layers of bacterial colonies. Eight hours after brushing, a human tooth is swarming with spherical bacteria such as *Streptococcus oralis* and *Streptococcus mitis*, which bind to receptors in the film of salivary proteins and bacterial fragments. Subsequent colonizers stick to the first residents. These are the early colonizers, followed by late colonizers made up of bacteria of different species. The aerobic organisms are on the outside of this oral ecosystem, and deeper than a few cell depths they are anaerobic (Hamilton, 1998). It was interesting to test these Arab and Berber medicinal plants for their efficacy or otherwise against the oral flora (e.g. *Candida albicans*).

Methodology

Plant Selection, Collection and Interviews

The plants in this research were collected under the guidance of Prof. Moh Rejdali, a plant taxonomist and Head of the Department of Botany of the Institute Agronomic and Veterinarian Sciences (Rabat, Morocco). He provided extensive help in this ethnobotanical study, such as collection, and identification of plants, information on medicinal plants, use of the Moroccan facilities in his Department and local literature, etc.

I used methods described by Hedberg, 1993; Lipp, 1989; Waller, 1993; and Mishler, 1986, and established the field inventories using the form shown in (Fig. 2.4) (Samuelsson et al., 1991). The book published by IDRC "Working with Indigenous Knowledge: A guide for researchers" (Grenier, 1998), provided formal guidelines for study. Healers, herbalists and other knowledgeable people were interviewed. Information was verified by comparison between information from at least five unrelated traditional healers from different villages and further confirmed from written sources. Steps were taken to ensure that the people being interviewed were comfortable with the procedure. This involved dressing appropriately, explaining the purpose of the collecting

Date:____________ Collection SMP no.__________

1. **Locality**: District______________________________Village________________

Distance and direction from major town________________________________

Longitude/latitude____________________ Habitat______________________

____

2. **Type of plant:**

Tree Herb Parasite

Shrub Liana Aqueous plant

3. **For trees:** Height & bulk______________________ Bark description______________

Slash____

4. **Flower colour:**_______________________**5. Fruit description:**______________

6. **Smell:**____________________________ **7. Latex present**____________________

8. **Attacked by insects etc.:** _______________

9. **Provisional identification:**____

10. **Vernacular name:**____

11. **Name of traditional healer:**_______________________________________

12. **Preparation of remedy:**

Plant part__________________________ Fresh_________ Dried __________

Amount taken ____

Crushed_______ Powdered_______Mixed with water (amount)________________

Cold water_______Boiled_______Boiling water poured over_________________

Mixed with other vehicle (amount)_________________________________

Other preparation ____

13. **Dose and regimen:**____

____

14. **Disease or symptoms treated:** _________________________________

____

15. **Plant used together with the following plants:** (Vernacular name or SMP-number. Part of plant. Note amount of plant part taken in preparation or other details if preparation differs from description in 12 above).

____

____

____

____

17. **Additional notes:**

____

____

____

Figure 2.4. Form suggested for field inventories in ethnopharmacological research (Samuelsson et al., 1991)

<u>Note</u>: 11* Anonymous: The name will not be recorded for healer requesting confidentiality
***Gender will be noted as requested by IDRC→G: Female / Male**

trip in the language of the villager so that informed consent was given, as well as sharing life of the villagers whenever possible.

Medicinal plants were selected on the basis of their use by Berber and Arab people to treat infectious diseases caused by bacterial, viral or fungal pathogens as determined in questionnaires and interviews addressed to local healers, herbalists and other knowledgeable people. This included plants used to treat fever, colds, flu, coughs, wounds, cuts, diarrhea, dysentery, sore throats, venereal diseases, hepatitis and skin problems such as rashes, blemishes, boils and poxes as well as other infectious diseases. Only species consistently used to treat the same illness in several villages were selected, and only those parts of the plant used in the traditional treatment were collected.

Plants were collected from the main regions and ecosystems (Mountains, Sahara, and along the Atlantic and Mediterranean Sea) and at different altitudes in Morocco. The (Rif & High, Middle and Anti Atlas) mountains separate the Atlantic and Mediterranean coastal plain littorals from oriental high plateaux of Figuig (Fig. 1.1.) and large pre-Sahara and Sahara hammadas.

Two key words are important in describing the latter regions. The first one is "Reg" which means a large stretch of plateau covered with pebbles (region between Rissani & Merzouga, Fig.1.1). The second one is "Erg or Hammada" which means high dunes or vast stretch of sand in the desert (Merzouga). Morocco seems to be mainly a country of mountains. Jabal Toubkal 4, 165 m, in the High Atlas, is the highest peak in North Africa. In the North, the Rif Mountain has an elevation of 2, 465 m. In the centre of the country, the Middle Atlas has an elevation of 3, 350 m. The Anti Atlas in the south has an elevation of 2, 531 m. The climate is Mediterranean (with the exception of the southern areas that are a transition between Mediterranean and tropical desert. It is moderate and warm with four well defined seasons: a warm and dry summer, a humid and cold winter with abundant rains, with snow on mountains, and mild spring and a rainy fall. The Atlantic Ocean moderates the climate. The climate in the centre is continental: warm and dry. Mountain areas in the Rif, Middle Atlas (more than 1000mm of rain), and High Atlas, are colder with storms in summer. The pre-Sahara and Sahara, are very dry with less than 200 mm rain, and large differences of temperature between day and night (Bellakhdar, 1997). There are five bio-climate zones (Table 2.1): The Sahara and pre- Sahara or Arid zones, Semi-arid zone includes the Atlantic and Mediterranean coastal plain littorals, the Sub-humid and Humid and High Mountain sub-zones include the Rif and High, Middle and Anti Atlas Mountains. The Sahara region covers 50% of the country in the south from Figuig to the Dra valley (Zagora) and further west from Guelmim-Es Smara to Lagouira (Fig. 2.5). Species found in this area include *Acacia raddianna*, *Tamarix*, *Zizyphus lotus*, *Retama raetam* and *Peganum harmala*, as well as *Phoenix dactilifera* (Palmae). The High & Anti Atlas Mountains region includes the Arid zone of Marrakech, Agadir, and Oriental High Plateaux (Fig. 2.5). Species found in this ecosystem include *Pistacia atlantica*, *Acacia gummifera*, *Argania spinosa*, all found in the pre-steppe area. In the Steppe zone are found *Stipa tenacissima* or *Artemisia herba-alba*. The Atlantic and Mediterranean coastal plain littorals correponds to the Semi-arid zone, and includes plains of the Atlantic between Rabat and Agadir, the Mediterranean coast (Al-Hoceima, Nador, Saidia). Large forests of *Tetraclinis articulata*, *Juniperus phoenicea*, *Juniperus oxycedrus*, *Pinus halepensis*, *Olea europea* var. *Oleaster*, *Pistacia lentiscus* cover this region. The Rif and Atlas Mountains corresponds to the Sub-humid and humid zones located in the mountain zones, and these are covered with forests. Some examples of species are *Cedrus atlantica* (endemic to Morocco), *Quercus rotundifolia*, *Quercus suber*, *Quercus faginea*, *Quercus pyrenaica*, and *Abies pinsapo* ssp. *maroccana*. Other species found in High Mountains are adapted to dry cold weather such as *Cytisus balansae*, and *Astragalus boissieri*.

Collections were made over a period of 3 months, in spring (April-May: Sahara and plains) and summer (June-September: Mountains), each year of 1997-2000. Three major distinct ethnic groups were encountered: Rifains, Chlouh and Soussis.

The goals of the field research were to obtain ethnobotanical data on medicinal plants from Arab and Berber traditional healers and herbalists from all regions of Morocco and to collect potentially bioactive plants. I targeted endemic species from the Atlas and Rif Mountains and the Sahara. These specimens were previously pressed flat, dried and labeled carefully. The labels indicate species name (Latin), indigenous names (Arabic and Berber) and application, as well as any directions needed to locate the plant again. They were identified and authenticated by depositing voucher specimens in the herbaria at the University of British Columbia and the Institute of Agronomy and Veterinarian Sciences, Hassan II, Rabat, Morocco. This survey highlights the traditional phytotherapy practices by the people and traditional healers of Morocco employed in the treatment of various infectious diseases.

Table 2.1. The five bio-climate zones, sub-zones, and local species

Zones	Sub Zones	Species
1-Sahara	From Figuig to the Dra valley (Zagora) and further west from Guelmim-Es Smara to Lagouira.	*Acacia raddianna*, *Tamarix*, *Zizyphus lotus*, *Retama raetam*, *Peganum harmala*, and *Phoenix dactilifera* (Palmae).
2-Arid or pre-Sahara or pre-steppe	Marrakech, Agadir, and Oriental High Plateaux	*Pistacia atlantica*, *Acacia gummifera*, *Argania spinosa*.
3-Steppe	Zagora, north of Agadir.	*Stipa tenacissima*, *Artemisia herba-alba*.
4-Semi-Arid	The Atlantic and Mediterranean coastal plain littorals between Rabat and Agadir, Al-Hoceima, Nador, Saidia.	Large forests of *Tetraclinis articulata*, *Juniperus phoenicea*, *Juniperus oxycedrus*, *Pinus halepensis*, *Olea europea* var. *Oleaster*, *Pistacia lentiscus*.
5-Mountain	-Sub-humid: High, Anti Atlas -Humid: Rif & Middle Atlas	Forests of *Cedrus atlantica*, *Quercus rotundifolia*, *Q. suber*, *Q. faginea*, *Q. pyrenaica*, and *Abies pinsapo* ssp. *maroccana*. *Cytisus balansae*, *Astragalus boissieri*.

Ethnobotanical medicine

1-INTERVIEWS

A gender analysis showed that 60% of the healers in Morocco are women family "doctors". Information was also collected from patients or users of medicinal plants during informal conversations in the villages. All the ethnobotanical information collected in the field research was further compared and checked in ancient and recent written documents (Avicenna, 1980; Al Antaque, 1997; Bellakhdar et al., 1991; Bellakhdar, 1997; Benjilali et al., 1997; Campbell, 1904-1905; Ibn Al Baytar, 1448; Ghazanfar, 1994; Levey, 1973; Rejdali and Birouk, 1996; Renaud and Colin, 1934; Siouti, 1994; Zahwelee, 1997). Answers received were verified by comparison with answers of other healers or with already known written sources (Mishler, 1986). Ancient and modern Moroccan literature relevant to the project, both in French and Arabic were consulted to authenticate the ethnobotanical data that was gathered in the field (Al Antaque, 1997; Bellakhdar et al., 1991; Bellakhdar, 1997; Benjilali et al., 1997; Rejdali and Birouk, 1996; Zahwelee, 1997, Charrouf and Guillaume, 1999).

I interviewed 55 people (relatives, friends, herbalists and traditional healers) from all the regions of Morocco: Rif (North); High, Middle, and Anti Atlas Mountains; Sahara including western areas (Fig. 1.1 and Fig. 2.5). Most of the interviews were recorded on tapes and some were written. Before each interview, I made a brief explanation of the project and its goal (Mishler, 1986). The people interviewed were told to give only public information, and avoid revealing any secrets (note lines 1-3 on paragraph "Study Procedures" on Fig 2.6 informed consent form). The interviews lasted from one to several hours. I spent around one month, e.g. in a village near El Hajeb (Meknes area,

Fig. 1.1), with women healers (Mishler, 1986). I gathered ethnobotanical and medicinal data on these plants (Table 2.2).

Different types of traditional medical practices exist in Morocco and consequently healers of 3 types exist:

-a)Physicians called *fqih* or *taleb*, diagnose the disease, cure and prescribe traditional medicines. Bonesetters are auxiliaries of the physicians.

-b)Herbalists (*Hasabin*) sell medicinal plants and give recommendations.

-c)Women family "doctors", and midwives who act as auxiliaries of the physicians.

For each plant, information (Samuelsson et al., 1991) was gathered whenever possible about:

1-The name of the traditional healer or informant with their usual language and ethnic group.

2-The dates of collection.

3-Locality: District, distance from major town, village name, and ecosystem category or habitat, and longitude/latitude.

4-The place of origin.

5-The name of the plant: the botanical name and the vernacular name in Arabic or Berber.

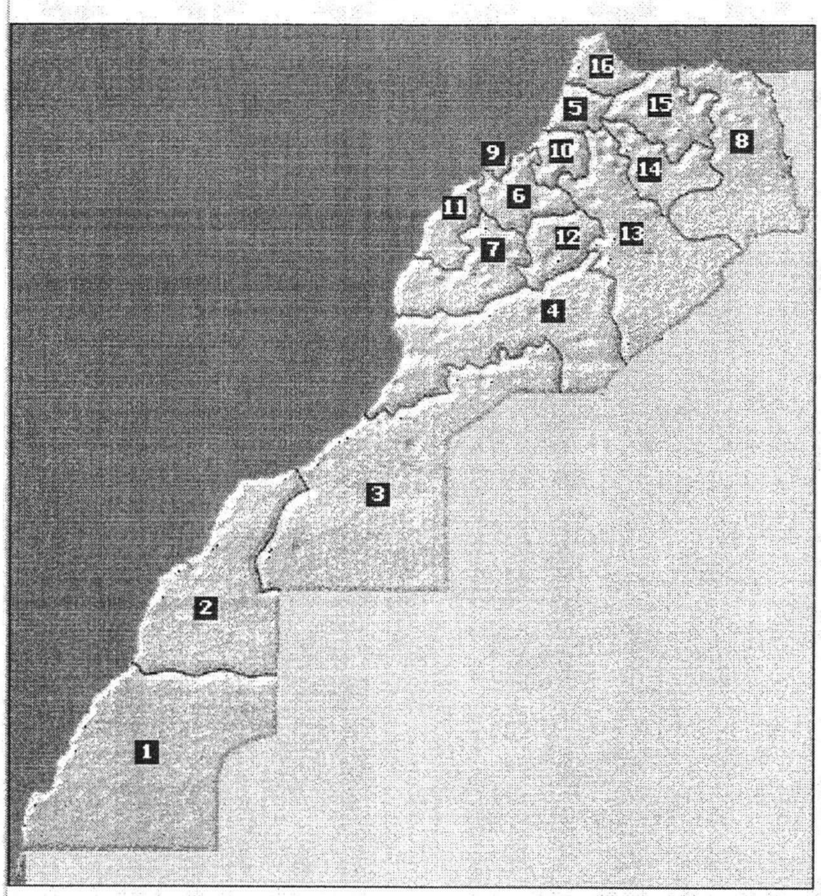
6-Type of plant: tree (bark description), herb, or shrub; flower (color); fruit (description); fragrance; presence of latex; etc.

7-Preparation of remedy:

→The part used (fresh or dried) was recommended for some plants: whole, aerial parts, leaves, flowers, fruits, seeds, stem, roots, bark, rhizome, gum, oil.

→Dose: amount taken, mixture with other vehicle or synergetic mixtures (plant used together with other plants) and administration of the drugs were also recorded.

8-The diseases and symptoms treated, toxicity, proprieties of the plant and additional notes were also gathered.



	Régions Administratives
1	Oued Eddahab-Lagouira
2	Laâyoune-Boujdour-Sakia El Hamra
3	Guelmim-Es Smara
4	Souss-Massa-Draâ
5	Gharb-Chrarda-Beni Hssen
6	Chaouia-Ourdigha
7	Marrakech-Tensift-El Haouz
8	Oriental
9	Casablanca
10	Rabat-Salé-Zemmour-Zaër
11	Doukkala-Abda
12	Tadla-Azilal
13	Meknès-Tafilalt
14	Fès-Boulmane
15	Taza-Al Hoceima-Taounate
16	Tanger-Tétouan

Figure 2.5. Collection areas

(www.mincom.gov.ma)

2-COLLECTION

Optimum Conditions for Plant Selection

It seems appropriate to consider the observed optimum growth conditions when collecting plants for medicinal purposes. The ancient Greek herbalist, *Dioscorides* summarized the importance of some factors that affected the efficacy of medicinal plants. These general remarks are still valid. The following quote described the exactness and intensity of the botanical studies:

"Before all else it is proper to use care both in the storing up and in the gathering of herbs each at its due season, for it is according to this that medicines either do their work, or become quite ineffectual. We ought to gather herbs when the weather is clear, for there is a great difference whether it be dry or rainy when the gathering is made. The place also makes a difference: whether the localities be mountainous and high, whether they lie open to the wind, whether they be cold and dry; upon this the stronger forces of drugs depend. Medicinal plants found growing on plains, in plashy and shady localities, where the wind cannot blow through, are for the most part the weaker; and especially those that are not gathered at the right season, or else are decayed through weakness. It must also not be forgotten that herbs frequently ripen earlier or later according to the characteristics of the country and the temperature of the year, and that while some of them by an innate property bear flowers and leaves in winter, others flower twice a year. Now it behooves anyone who desires to be skillful herbalist, to be present when the plants first shoot out of the earth, when they are fully-grown, and when they begin to fade. For he who is only present at the budding of the herbs cannot know it when full-grown nor can he who hath examined a full-grown herb, recognize it when it has only just appeared above the ground. Owing to changes in the shape of leaves and the size of stalks, and of the flower and fruits, and of certain other known characteristics, great mistake has been made by some who have not paid proper attention to them in this manner...We must likewise be aware that only those Medicinal Herbs, the White and Black Hellebore, *Veratrum album* and *Helleborus niger*, retain their power for many years; the others, for the most part, will only keep good for use for three years. But herbs which are full of branches...should be gathered whilst they are great with seeds; flowers ought to be gathered before they fall; fruits when they are ripe, and seeds when they begin to dry, and before they fall out. To extract the juice of herbs, take their stalks when they are newly sprouted; and so too with the leaves. But for taking juices and tears, the stems should be cut while yet in their ripeness. Roots for storing or for the extraction of juices and the peeling of barks should be collected when the herbs are beginning to lose their leaves, when those which are clean may be set to dry forthwith in dry places, but those which have earth or clay sticking to them must be washed with water. Flowers and sweet-scented things should be laid up in dry boxes of Lime-wood: but there are some herbs which do well enough if wrapped up in papers or leaves for the preservation of their seeds. For moist medicines some thicker material such as silver, or glass or horn will agree best. Yes, and earthenware, if it be not thin, is fitting enough, and so wood, particularly if it be boxwood. Vessels of brass will be suitable for eye medicines and for liquids and for all that are compounded of vinegar or of liquid pitch or of Cedria, but fats and marrows ought to be put up in vessels of tin" (*Dioscorides* as cited by Goodyer, 1934).

Plant Collection

Two preliminary field trips took place in the summers of 1997-98. Two IDRC collaborative Canadian-Moroccan botanical expeditions took place in 1999 and 2000. These two major field trips to different Moroccan ecosystems were in collaboration with Professor Rejdali. An expedition to the plains took place in April-May (1999), and a second expedition in June-September was to the High, Middle and Anti Atlas & Rif Mountains & Sahara. In the second year, 2000, a further study investigated women healer contributions. The plant collecting was more selective and focussed on rare, newly discovered endemic plants of the Atlas Mountains & Sahara. Remote regions were particularly investigated for rare species as well as to locate women healers in these areas. Plants that I had tested previously in the laboratory, and were bioactive were particularly targeted.

I collected 197 medicinal plants for the herbarium (see Table 2.2 for full list) and 80 plants for laboratory testing (Table 2.3). These plants were identified at the Department of Botany (Morocco) with the help of Professor Rejdali and further checked by using Moroccan floristic documents, herbarium specimens, Bertrand's work (Bertrand, 1991), and by consulting published encyclopedias (Maire, 1952-1980). Voucher specimens were filed in the Moroccan Institute Agronomic and Veterinarian Hassan II, (Rabat) and the University of British Columbia (Vancouver) herbaria. The species collected as well as their ethnobotanical uses are listed in Table 2.3.

A-1st Field trip Itinerary

The first IDRC year field trip from June to October (1999) focussed mainly on collecting medicinal plants from different ecosystems in the country. Herbarium specimens, seeds, and bulk plants for laboratory extractions were collected and several photographs were taken. I also gathered ethnobotanical data by interviewing healers in the field was also achieved.

The research team was G.H.N. Towers, I.E.P. Taylor, J.B. Hudson, from the University of British Columbia, Canada, and myself. In Morocco, the research team in the field was myself, Moh. Rejdali from the Institute Agronomic and Veterinarian Hassan II, IAV, Rabat, and local assistants: technicians, trained in processing collected plants and the drivers familiar with remote roads and travel in Morocco. Local scientists and their assistants, healers and their assistants as well as guides joined the team. Other details of the expedition (e.g. collection material, lodging, Land Rover, the latter was useful in mountains and desert ecosystems...) were also important in the preparation of the field trip.

The itinerary was as follows:

a) The north of the country in the Rif Mountain:

Rabat→Ouazzane→Chefchaouen→Tetouan→Moulay-Abdessalaam→Bou-Ahmed→Chefchaouen→Bab-Taza→Bab-Berred→Ketama-Issaguen→Taounate→Ain-Aicha→Tissa→Fes →Rabat. (Fig. 1.1 & Fig. 2.5, refer to numbers 16, 15, 5, and 10).

The different ecosystems encountered were forests, adjacent mountains (Rif) and plains and ecosystems near the ocean. Thick forests of *Quercus faginea* L., were found between Chefchaouen and Ketama. A few kilometers before Ketama, cedar forests, *Cedrus atlantica* a conifer endemic to Morocco, was found. Many plants were found to be rare. Some plants collected from this area still wait to be identified (Table 2.2, list of herbarium specimens).

Collecting and photographing specimens and processing the data obtained were done in the field. In total, one hundred and fifteen plants were collected some of them new (never named before) and rare species of potential medicinal value which had never been collected before by the Institute.

On different occasions, the opportunity to interact and learn about the lifestyle of the locals who directed us to healers or knowledgeable people was very much appreciated and tremendously helpful. In the north, the Berber tribes within this area were mostly the Rifains, Berbers of the Rif Mountain that spoke the Tarifit language.

b) The center of Morocco in the Middle Atlas:

Rabat→Tiflet→Maaziz→Rommani→Oulmes→Tidass→Meknes→Azrou→AinLouh →Ouawane→Ifrane→ElHajeb→Ifrane→Michelifen→Timahdit→Azrou→Ain-Kaala→Aguelmane-sidi-Ali-Agulmouss→Khenifra→Midelt→Rich→Tizint-El-Talghoumt→Imilchil→Khenifra→Amrirt→El-Hajeb→Rabat. (Fig. 1.1 & Fig. 2.5, refer to numbers 10, 12, 14, 6).

In the Middle Atlas the ecosystems were mostly abundant forests of cedar and oak (El-Hajeb→Ifrane→Michelifen→Timahdit→Azrou→AinKaala→Ain-Louh→Ouawane), mountains (Michelifen, the ski resort), and desert in the southern area (Rich). It is the most fertile region in Morocco. Many species were collected from this region. The Berber tribes of this region, the Chlouh, speak the Tamazight language. Their lifestyle, traditions and languages are completely different from the former Berber tribes of the north of Morocco, the Rifains. They are also wealthier, with large agricultural fields and flocks of sheep. Some endemic species of monkeys (apes) were seen in the forests of this region. The plant *Irinecea* was dominant in Aguelmane sidi Ali towards Midelt. Ait Ahdiddou, another Berber tribe dwelled in Imilchil, found in the national park, between snowy mountains and the two famous lakes Tisli &Tislit.

c) The south of the country in the High Atlas:

Rabat→BeniMellal→Marrakech→Oukaimden→Asni→Tizintest→Agadir→Tiznit→Bou izakarne→Rabat. (Fig. 1.1 & Fig. 2.5, refer to numbers 9, 7, 11, and 4).

c) The Sahara and the Anti Atlas:

Rabat→Taza→Oujda→Debdou→Tendrara→Bouarfa→Figuig→Boudnib→Errachidia→ Ouarzazate→Tizintichka→Marrakech→Rabat. (Fig. 1.1 & Fig. 2.5, refer to numbers 8, 13, 12, 4).

The time left was spent in completing the interview process, the identification and authentication of plant specimens as well as the processing of plant materials and discussion of data and the experience as a whole. Some time was also spent in establishing links and exchanges, with scientists and people in general who were interested in medicinal plants.

B-2nd Field trip itinerary

The second year IDRC field trip from May-October 2000 focussed mainly on gathering data from women healers. I spent time interviewing, interacting and sharing their life, as well as learning from their knowledge, and practice of healing. I chose to live for sometime with them in order to learn their healing skills and observe and share their daily lives. I liked to call myself, at this time of my training, a student healer.

The first region that I visited was Meknes→Hajeb. I met the women healers from last year as well as new ones. I spent the first month of June sharing their lives. I felt that this way they were more comfortable in disclosing some of their knowledge and practices without the pressure of the first interview. I also tried to move from the level of gathering information to really being involved in practicing their healing skills whenever the opportunity presented itself.

The second region that I visited was the entire Sahara region of Morocco. My itinerary was as follows:

2_Rabat→Casablanca→Marrakech→Ouarzazate→Zagora→M'Hamid-al-Ghazlan→Cheguagua

3_Zagora→Rissani→Merzougua

4_Tinghir→Todgha→Dades→Marrakech

5_Marrakech→Tafraoute→Tiznit→Tan Tan→Tarfaya

6_Western Sahara: Laayoune→Boujdour→Dakhla

7_Agadir→Essaouira→Asfi→El-Jadida→Casablanca→Rabat (Fig. 1.1 & Fig. 2.5, refer to numbers 1, 2 3, 4, 7, 11, 9).

Most of the time was spent collecting medicinal plants from different ecosystems and interviewing Berber & Arab Women and some Men healers. Some other activities were the visit to the three cooperatives on *Argania spinosa* of Prof. Charrouf-Chaouni (Charrouf and Guillaume, 1999), (Tidzi, Tamari & Tiznit (El-Jadida) and to the *Artemisia* distillation village at Taznakht (Ouarzazate).

RESULTS

A survey of medicinal plants, different ecosystems and information resources (healers) was accomplished during the summers of 1997-2000. Herbarium specimens and bulk plant material for pharmacological tests were collected. I selected endemic species from the Rif, High, and Middle and Anti Atlas Mountains & Sahara. The toxicity information about some plants was gathered from healers and was further evaluated in the laboratory. Seeds for some plants were also collected. Informative slides and photographs were taken in all the regions visited.

Fifty-five interviews with women and men, Arab and Berber herbalists from all regions of Morocco were conducted from 1997-2000, in order to investigate the ethnobotanical bases of local pharmacopoeia. Some healers were recorded in tapes, or written data, while explaining some local medicinal plants used for infectious diseases.

Of particular interest, were the women healers from the Middle Atlas (Hajeb & Agourai). Healers from Figuig, Oujda, Agadir, Merzouga and Marrakech were particularly helpful as were the women from Zagora. Healers were also interviewed from Western Sahara (Dakhla, Laayoune, Tantan) in the year 2000. The names of healers and persons who were especially helpful, and who were among those who provided information for this study are indicated in the acknowledgements part of the thesis.

The role of women was very important in this study because as many as 60 % of healers in Morocco, are women. They have the knowledge and experience of healing with plants. Younger women are in charge of the health of their family. The majority of them try to avoid synthetic drugs in favor of natural ones. Older women have a much deeper knowledge and experience and are in charge of community health. Most women family "doctors" incorporate medicinal plants in their everyday life in the use of specific spices in cooking or provision of medicines for colds, stomachaches or other ailments. Problems of toxicity and dosage seemed to be particularly urgent problems in need of investigation. Women healers obtain low economical returns from the practice of healing. I had valuable training in practicing as a student healer with local women.

Table 2.2 provides an alphabetical list of the botanical names, including familial, generic and specific names, together with the vernacular name, region and ecosystems in which they are located in Morocco (Mountains, Sahara, Semi-arid). The plant part used and traditional uses or treatment are also shown. Preparation and route of administration are sometimes indicated (Table 2.3). Ethnobotanical data of 97 species with medicinal use, belonging to 43 families, were gathered. Analysis of the data showed that pulmonary infections, colds, poison antidote, gastrointestinal disorders, dermatological complaints, cough, fever, headache were the majors afflictions of people within my study (Table 2.3). A total of 22 species are used for treatment of gastric-intestinal problems, such as stomachache, diarrhea and dysentery, while 19 species are used to treat dermatological problems. Sixty-eight species are used to treat cough, fever, colds, headache and pulmonary ailments, 15 for gynecological-urinary infections, and 14 for cuts and wounds (Table 2.3). Various forms of preparation are used such as infusions, decoctions, pastes and juices. Some plants are used in cosmetology.

The families most represented in Table 2.3, are the Lamiaceae with 13 species, followed by the Asteraceae with 12 species, Apiaceae with 8 species and Fabaceae with 6 species. Lamiaceae form 5.4% of the Moroccan flora and Asteraceae represent 14.3% (Bellakhdar, 1997). Plants in these families are also widely used as medicines.

The herbs specifically used by women and not men are those used for childbirth, (e.g. *Sorghum bicolor* (L.) Moench, Poaceae); herbs inducing milk production or lactation (e.g. *Trigonella foenum-graecum* L. Fabaceae), menstruation, for PMS or premenstrual syndrome (e.g. *Lavandula angustifolia* Mill. Lamiaceae); inducing menses (e.g. *Nigella sativa*, chaste tree or *Vitex agnus-castus* L., Verbenaceae) facilitating abortion (e.g. *Mentha pulegium* L., and *Iris germanica* L., Iridaceae), uterus (e.g. *Warionia saharae* Benth.& Coss. , Asteraceae) and vaginal infections (e.g. *Punica granatum* L., Punicaceae). Furthermore women use all the plants used for cosmetology and hair care exclusively. For example women use henna or *Lawsonia inermis* L., Lythraceae to dye their hair as well as tattooing their body.

Given that gender differences exist, I would modify my field research approach in a follow-up study to obtain a more comprehensive inventory of traditional medicinal practices, by focusing on ailments specific to gender. For example by singling out medicinal plants used specifically for child birth related ailments or infections, the results will include only women. Whereas by singling out medicinal plants used specifically for men related ailments, the results will include only men. For examples, the results of the gender analysis showed that men used all all herbs of the Apiaceae family, whereas women used the herbs of the Lamiaceae.

A valuable collection of rare specimens and a new set of herbarium voucher specimens are now available for research and teaching. Different plants species were found in different regions (e.g. *Cedrus atlantica*, *Thymus marocanus* and different species of *Lavandula* in the north and Center; *Aloysia triphylla* and *Olea* species in the middle Atlas; *Acacia radiana* & *Calotropis procera* in the south, *Cytisus* in Imilchil). Some plants species encountered were endemic to Morocco (e.g. *Pyrus mamorensis* (Mamora forest, Rabat-Sale), *Ammoides pusilla* (Oujda, Meknes), *Artemesia ifranensis* (Ifrane), *Hertia marocana*, *Hirschia marocana*; *Euphorbia resinefira*, *Dracaena draco*, *Argania spinosa* in the Agadir region) (Charrouf and Guillaume, 1999). Other plants were encountered in remote regions of Morocco (e.g. *Hammada scoparium* in the Sahara; *Citrullus colocyntis*, *Anabasis aphylla* and *Chenopodium album*. Extremely rare and new plants of potential medicinal value, which have never been investigated chemically, were collected. They might have other medicinal proprieties (e.g. anti cancer activity).

The information recorded in the field, for the following herbarium specimens (Table 2.2) was Family, Species, Common name, Locality, Habitat, Abundance, Phenology, Date, Collector (s). The collectors were Mouhajir, Fatima; Prof. Rejdali, Moh; his Technician Ait Lafkih, Mohamed; and other occasional assistants' Hussain Ahmed & Mohamed, and healers, etc.

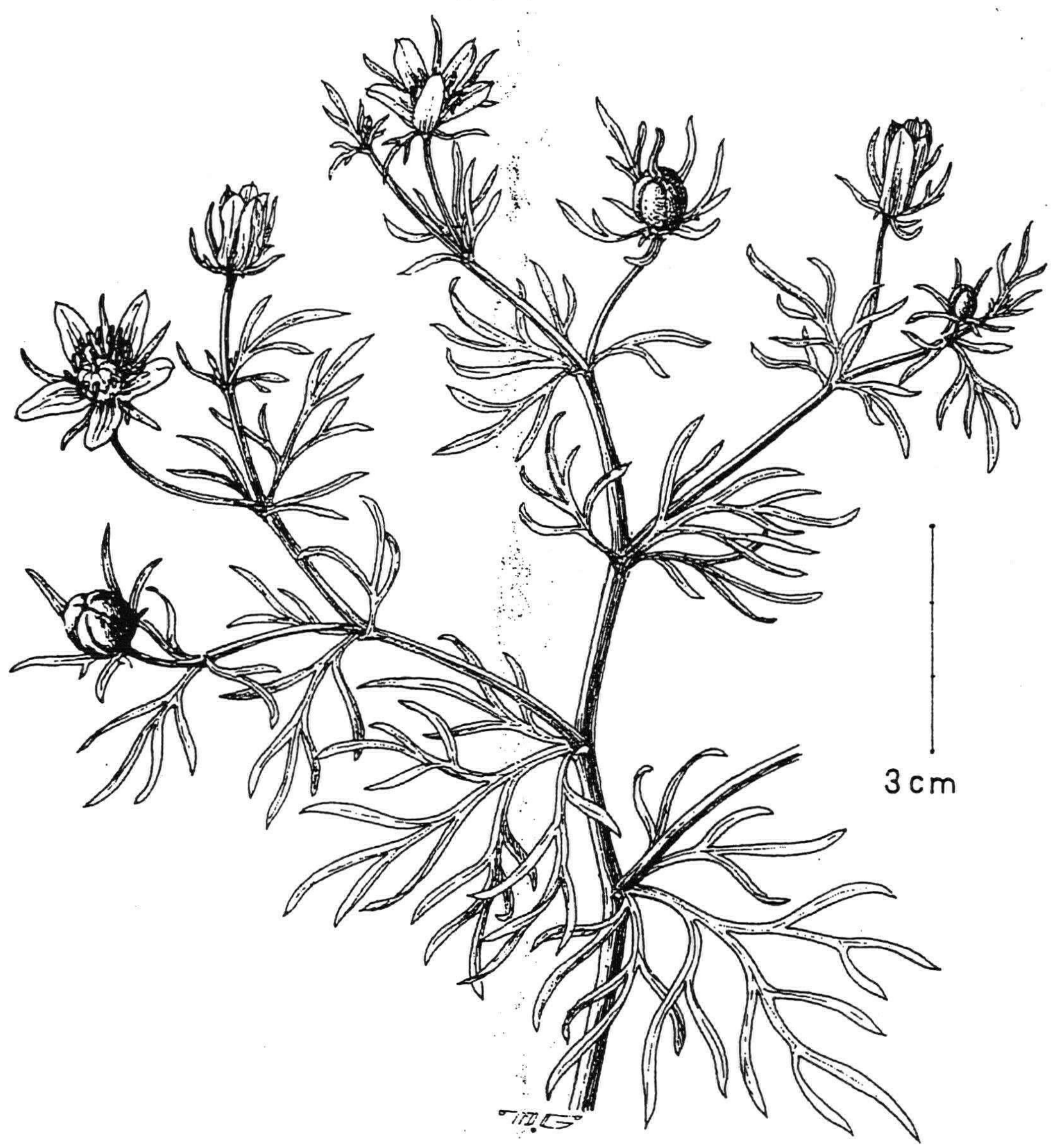


Figure 2.7. Example of steppe medicinal plant: *Peganum harmala* L.,

Zygophyllaceae

(Boulos, 1983)

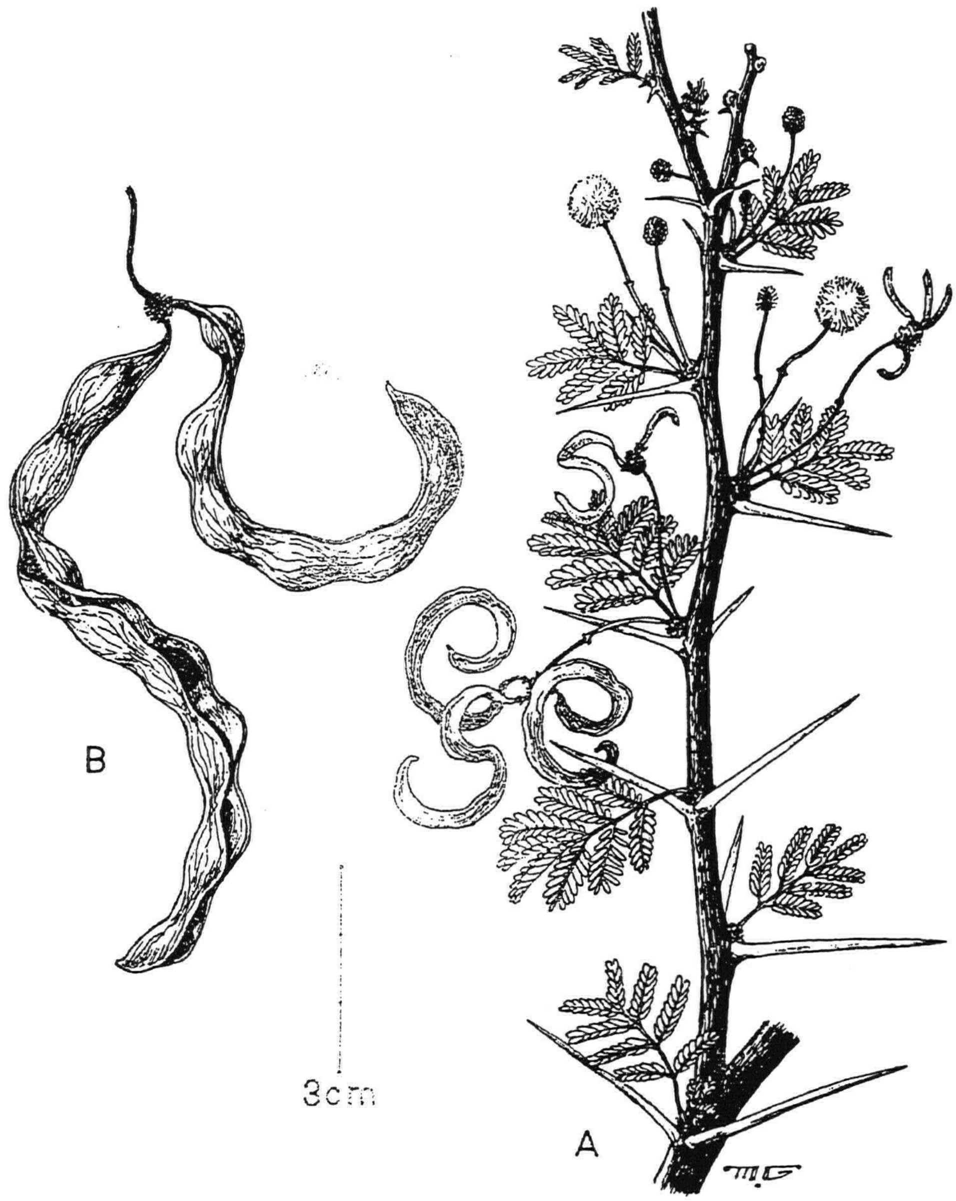


Figure 2.8. Example of steppe medicinal plant: *Acacia raddiana* L.

Fabaceae

(Boulos, 1983)

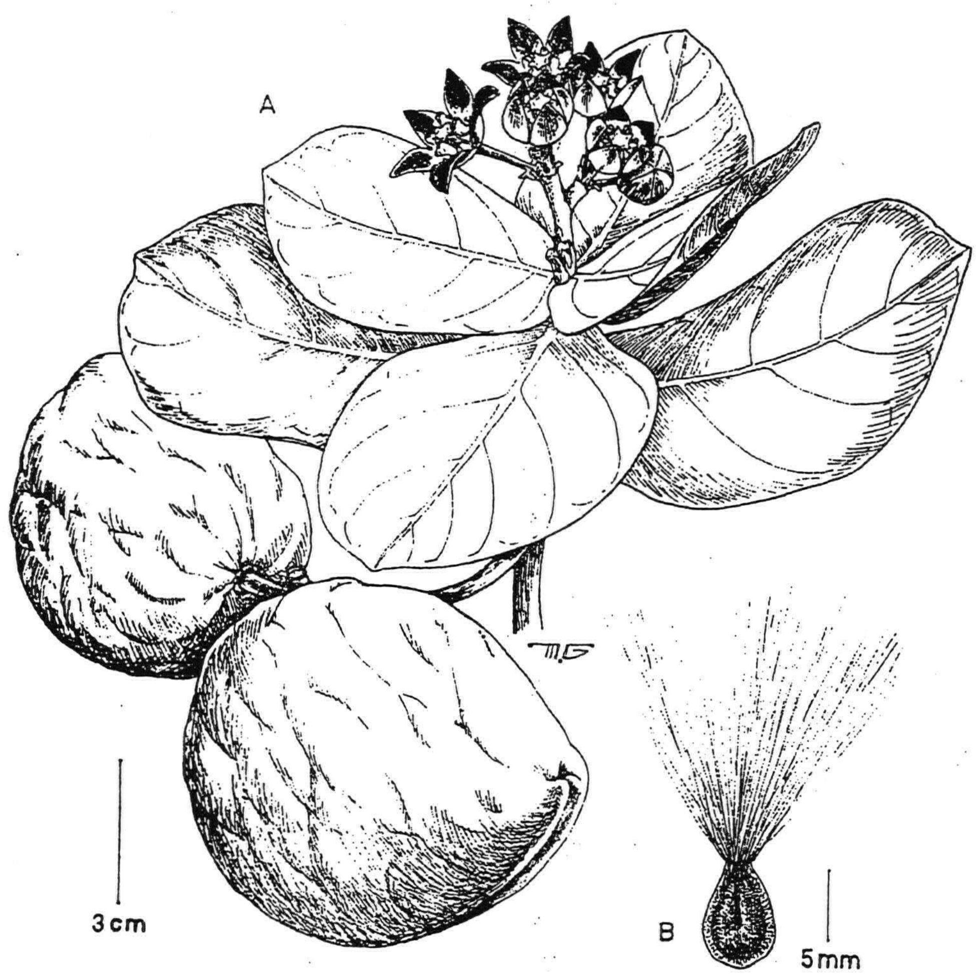


Figure 2.9. Example of steppe medicinal plant: *Calotropis procera* (Ait.) Ait.

Asclepiadaceae

(Boulos, 1983)

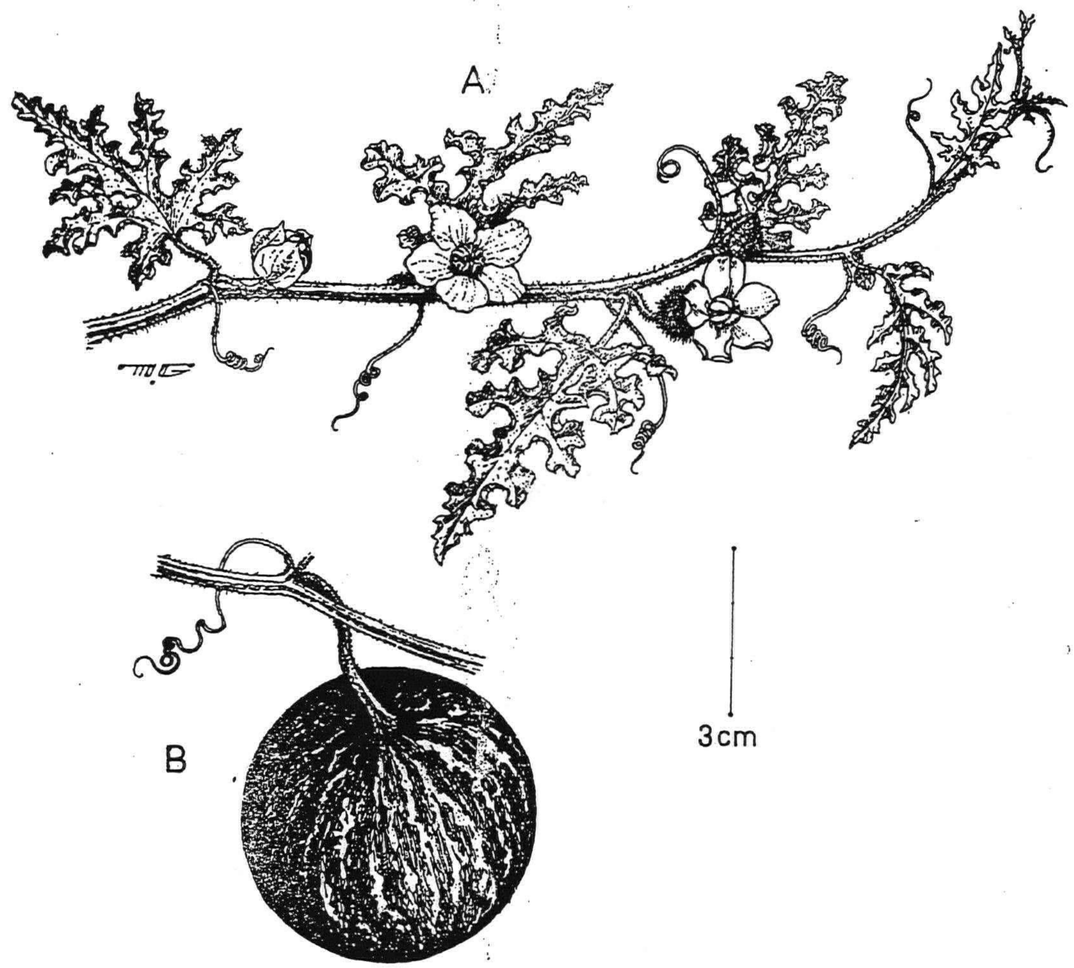


Figure 2.10. Sahara medicinal plant: *Citrullus colocynthis* (L.) Schrad.

Cucurbitaceae

(Boulos, 1983)

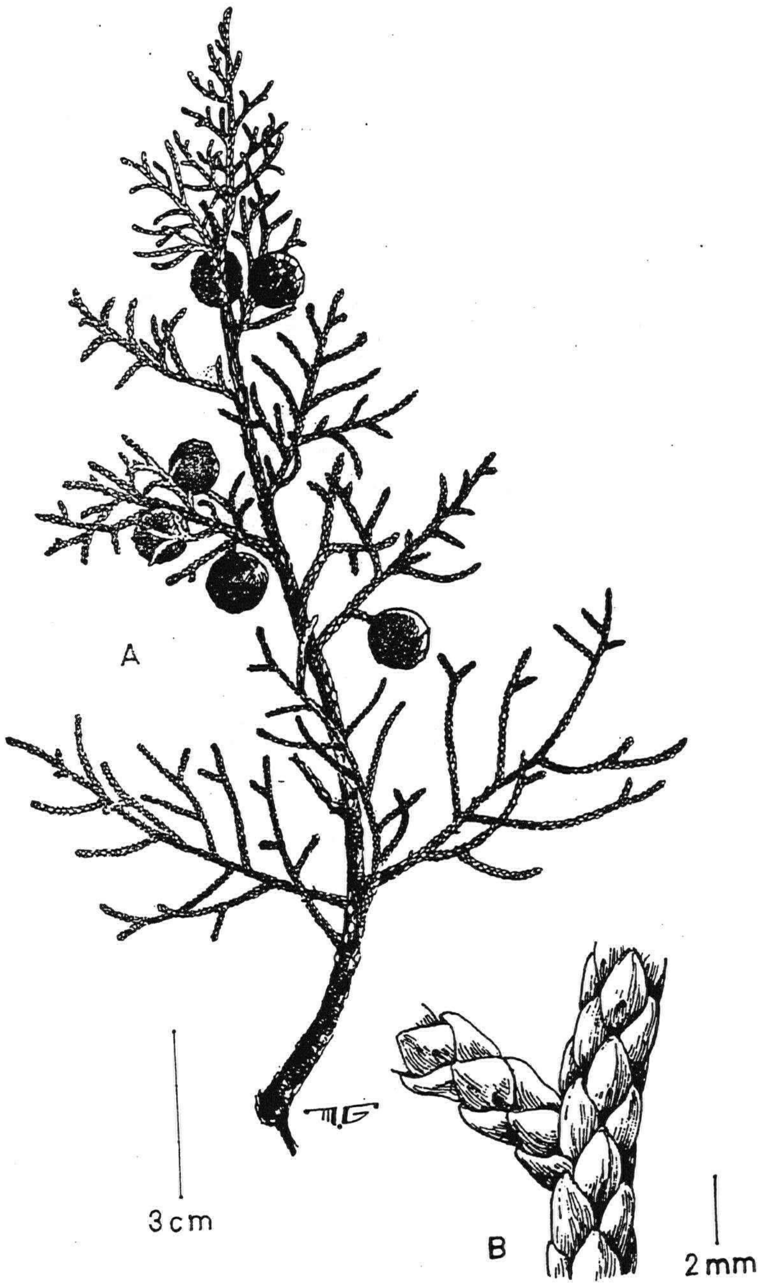


Figure 2.11. Forest medicinal plant: *Juniperus phoenicea* L.

Cupressaceae

(Boulos, 1983)

Table 2.2 List of Herbarium specimens, (Family and *Species*), their Herbarium number, date of collection, description, vernacular name, endemic, Location. Collectors are Fatima Mouhajir & Rejdali Moh, et al.

Family
Species
Amaranthaceae
Amaranthus blitoides **S. Watson.**
99-90-[UBC-V217179]-30-7-99-flower. Tetouan at 2 km to Ouad Laou, roadside at foot of mountain elev. 3000m, ca. 35° 00' N, 06° 10' W
Amaranthus deflexus **L.**
99-91-[UBC-V217180]-30-7-99-flower. Tetouan at 2 km to Ouad Laou, roadside at foot of mountain elev. 3000m, ca. 35° 00' N, 06° 10' W
Anacardiaceae
Pistacia atlantica **Desf.**
99-122-[UBC-V217195]-5-8-99-v.n. Dru, fruit. At 2 km to Maaziz. Valley elev. ca. 34° 00' N, 06° 30' W
Pistacia lentiscus **L.**
Ecotype a = 98-20-[UBC]-6-9-98-v.n. Dru. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W
Ecotype b = 99-1-[UBC-V217118]-26-7-99-Dru, fruit (Shrub)-Chaouen (forest, Rif) between Souk al Arbaa-Ouazane (Ouezzane), Souk Mas Mouda (2 Km.). Watilou Forest ca. 34°50' N, 05° 40'W
Rhus tripartita **(Ucria) DC.**
99-212-[UBC-V217263]-4-9-99-Vegetative. Figuig, jbel Grouz mountain, at 24 km to Figuig, on Bouarfa road, ravine elev. ca. 1200 m, ca. 32° 10' N, 01° 20' W
Schinus molle **L.**
98-14-[UBC]-6-9-98-v.n.Faux poivrier, fruit red, pepper-like, tree. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W
Apiaceae
Ammi visnaga **(L.) Lam.**
99-3-[UBC-V217119]-26-7-99-Bachnikha, fruit green, yellow; flower white. Chaouen (forest, Rif), between Souk al Arbaa-Ouazane (Ouezzane), Souk Mas Mouda (2 Km.). Watilou Forest , side of roads, valley ca. 34°50' N, 05° 40' W
Ammoides pusilla **(Brot.) Breistr.**
98-21-[UBC]-6-9-98-v.n. Nanukha, flower. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W
Apium graveolens **L.**
99-149-[UBC -V217220]-7-8-99-v.n. Krafess, flower, fruit. Ifrane (4.5 km to Michlifen), from Timahdite 32 Km, valley elev. ca. 33° 20' N, 05° 10' W
Bupleurum canescens **Schousb.**
99-177-[UBC-V217239]-8-8-99-Umbell flower, yellow, fruit, shrubEndemic. Khenifra, at 30 km to Midelt, 49 km to Rich (road Midelt-Rich), mountain slope elev. ca. 32° 20' N, 04° 35' W
Bupleurum spinosum **Gouan.**
99-107-[UBC-V217186]-31-7-99-Flower yellow, small, fragrance, no latex, shrub. Road Chaouen to Ketama, at 30 km of Issaguen, high altitude forest, roadside elev., ca. 35°00' N, 05° 00' W
Eryngium ilicifolium **Lam.**
99-134-[UBC -V217207]-6-8-99-Flower green, fruit, latex, shrub. Hajeb at 12 km d'Azrou. Valley, roadside elev. ca. 33° 43' N, 05° 15' W
Eryngium tricuspidatum **L.**
99-133-[UBC -V217206]-6-8-99-Flower, fruit, latex. Hajeb at 12 km d'Azrou. Valley, roadside elev. ca. 33° 43' N, 05° 15' W
Pituranthos scoparius (Coss. & Dur.) Benth. & Hook. **f.** Synon. ***Deverra scoparia*** Coss. & Dur.
99-172-[UBC-V217235]-8-8-99-Umbell flower, fragrance of parsley. Khenifra, 8 km to Midelt, entree from Zaida Tadamout, high valley elev. ca. 32° 40' N, 04° 50' W

***Thapsia garganica* L.**
99-124-[UBC-V217197]-5-8-99-Fruit, latex, shrub, toxic for bovins. Oulmes at 28 km. Valley ca. 33° 30' N, 06° 10' W
Unknown spp.
99-112-[UBC-V217190]-31-7-99-Flower yellow umbell. Road Chaouen to Ketama, at 15 km of Issaguen, cedrus forest, roadside elev., ca. 35°00' N, 05° 00' W
99-157-[UBC]-7-8-99-Green fruit, shrub. Azrou, Ain Al Kahla, 33 km near entrance, cedar forest elev. ca. 33° 00' N, 05° 00' W

Apocynaceae

***Nerium oleander* L.**
99-7-[UBC-V217122]-26-7-99-v.n. Dafflaa, flower pink, 5 petals, fragrance, shrub (toxic) Souk al Arbaa-Ouazane (5 Km from station 1) Valley, river edges ca. 34°50' N, 05° 40' W

Aquifoliaceae

***Ilex aquifolium* L.**
99-159-[UBC-V217228]-7-8-99-v.n. Houx, no flower or fruit, tree. Azrou, Ain Al Kahla, 33 km near entrance, cedar forest elev. ca. 33° 00' N, 05° 00' W
Unknown sp.
99-106-[UBC-V217106]-31-7-99-v.n. Houx, flower. Road Chefchaouen to Bab Berred, at 1 km to bab Berred, mountain forest 35° 00' N, 05° 00' W

Asteraceae

***Artemisia campestris* L.**
99-192-[UBC-V217249]-9-8-99-Flower buds, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W
***Artemisia herba-alba* Asso, synonym *Artemisia inculta* Asso**
99-213-[UBC-V217264]-4-9-99-Flower, fruit. Figuig, jbel Grouz mountain, at 24 km to Figuig, on Bouarfa road, ravine elev. ca. 1200 m, ca. 32° 10' N, 01° 20' W
***Artemisia ifranensis* J. Didier.**
99-148-[UBC -V217219]-7-8-99-v.n. Shih, flower buds, fragrance. Endemic. Ifrane (4.5 km to Michlifen), from Timahdite 32 Km, mountain forest elev. ca. 33° 20' N, 05° 10' W
***Carlina acaulis* L.**
99-53-[UBC-V217150]-28-7-99- Flower. Chaouen, 23 km from Tetouan. Valley, ca. 35° 30' N, 05° 22' W
***Carlina involucrata* Poir.**
99-135-[UBC -V217208]-6-8-99-Flower green, fruit, shrub. Hajeb Ain Allouh forest, roadside, on rocks elev. ca. 33° 00' N, 05° 00' W
***Carthamus tinctorius* L.**
99-136-[UBC -V217209]-6-8-99-Flower, fruit, shrub. Hajeb Ain Allouh forest, roadside, on rocks elev. ca. 33° 00' N, 05° 00' W
***Catananche caerulea* L.**
99-143-[UBC -V217215]-7-8-99-Flower purple aster, latex, shrub. Ifrane, Michlifen road (ski resort) at 3 Km, from Timahdite 33 Kkm, mountain forest elev. ca. 33° 20' N, 05° 10' W
***Chamaemelum mixtum* (L.) All.**
99-73-[UBC-V217167]-29-7-99-v.n. Babnouj, flower, fragrance. Tetouan at 7km to Moulay Abdessallaam, oued (river bed) ca. 35° 12' N, 06° 10' W
***Cynara cardunculus* L.**
99-153-[UBC -V217224]-7-8-99-v.n. Quq, purple aster. Ifrane (3 km to Michlifen), from Timahdite 32 Km, cedar forest elev. ca. 33° 20' N, 05° 10' W
***Dittrichia viscosa* (L.) Greuter, Synon. *Inula viscosa* (L.) Aiton.**
99-16-[UBC-V217276]-26-7-99-Flower, fruit. 8 km to Ouazzane, oued river bed elev. ca. 34° 50' N, 05° 35' W
***Echinops spinosus* L.**
99-189-[UBC-V217246]-9-8-99-Aster fruit, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W
***Hertia marocana* L.**
99-220-[UBC-V217269]-5-9-99-v.n. Allazzaz in this region only, fruit. Endemic Errachidia road, Boudnib to Errachidia, at 30 km, desert, oued river elev.ca. 32° 00' N, 02° 00' W

Hirschia marocana **Baker.**
99-179-[UBC-V217240]-8-8-99-White flower, fragrance subtle, shrub. Endemic. Khenifra, at 20 km to Rich (road Midelt-Rich), camel back elev. ca. 32° 18' N, 04° 30' W

Inula viscosa **(L.) Aiton .**
99-84-[UBC-V217174]-30-7-99-v.n. Tarahla, flower, red fruits. Road ouad (river) Laou, Zaouiat Sidi Kacem at 5 km to Ouad Laou, mountain forest elev. ca. 35° 00' N, 05° 00' W

Lactuca sativa **L.**
99-144-[UBC -V217216]-7-8-99-Flower yellow, latex. Ifrane, Michlifen road (ski resort) at 3 Km, from Timahdite 33 Kkm, mountain forest elev. ca. 33° 20' N, 05° 10' W

Launea arborescens **(Batt.) Maire.**
Ecotype a = 99-182-[UBC-V217243]-8-8-99-Yellow flower, latex, shrub. Errachidia road, (Rich to Imilchil) at 34 km d'Amellaguou, valley elev. ca. 32° 08' N, 05° 40' W
Ecotype b = 99-210-[UBC-V217261]-4-9-99-v.n. Oum Lbina, vegetative. Figuig, jbel Grouz mountain, at 24 km to Figuig, on Bouarfa road, ravine elev. ca. 1200 m, ca. 32° 10' N, 01° 20' W

Mantisalca salmantica **Cass.**
99-150-[UBC -V217221]-7-8-99-Flower aster purple. Ifrane (4.5 km to Michlifen), from Timahdite 32 Km, valley elev. ca. 33° 20' N, 05° 10' W

Ormenis scariosa **(Ball.) Lit.& Maire.**
99-175-[UBC-V217237]-8-8-99-Yellow flower (comme chamomille), lemon fragrance, shrub. Endemic to Imilchil. Khenifra, at 51 km to Rich (road Midelt-Rich), valley, eastern slopes elev. ca. 32° 40' N, 04° 50' W

Phagnalon saxatile **Cass.**
99-125-[UBC-V217198]-5-8-99-Flower, shrub, abondant on rocks. At 80 km of Khenifra, on rocks, roadside elev. ca. 33° 10' N, 05° 30' W

Pulicaria arabica **(L.) Cass.**
98-17-[UBC]-17-9-98-v.n. Hatassa, yellow flower, fragrance intense induce sneezing. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Unknown sp.
99-161-[UBC]-7-8-99-Green fluorescent flower, like cynara, spines, shrub. Timahdite 3 Km, at 225 km from Errachidia, mid mountain elev. ca. 33° 15' N, 05° 09' W

Boraginaceae

Echium plantagineum **L.**
Ecotype a = 99-87-[UBC-V217177]-30-7-99-v.n. Lisan Attir, flower. Tetouan at 5 km to Ouad Laou, roadside at foot of mountain elev. 3000m, ca. 35° 00' N, 06° 10' W
Ecotype b = 99-105-[UBC-V217185]-31-7-99-Road Bab Berred at 30 km, mountain valley ca. 35° 00' N, 05° 00' W

Brassicaceae

Zilla spinosa **Forssk.**
99-203-[UBC-V217256]-9-8-99-Silique fruit, shrub. Errachidia , at 1 km to Rich, valley elev. ca. 32° 16' N, 04° 30' W

Buxaceae

Buxus baleriaca **L.**
99-199-[UBC-V217253]-9-8-99-No flower, nor fruit, red shrub, toxic to bovin. Errachidia , road Imilchil to Rich, at 37 km of Imilchil, mountain elev. ca. 32° 00' N, 04° 00' W

Capparidaceae

Capparis spinosa **L.**
Ecotype a = 99-180-[UBC-V217241]-8-8-99-Fruit green many sizes, red when ripe open with black seeds inside, black seeds, shrub. Errachidia road, at 20 km to Rich (road Midelt-Rich), side of mountain slope elev. ca. 32° 18' N, 04° 30' W
Ecotype b = 99-214-[UBC-V217265]-4-9-99-v.n. Kebbar, fruit. Figuig, jbel Grouz mountain, at 24 km to Figuig, on Bouarfa road, eastern slope elev. ca. 1200 m, ca. 32° 10' N, 01° 20' W

Caryophyllaceae

Herniaria cinerea **DC.**
98-22-[UBC]-6-9-98-v.n. Herrast l-hjaar, flower. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Cesalpinaceae

***Ceratonia siliqua* L.**
99-49-[UBC-V2172zz]-v.n. Kharroub, nofruit or flower. Rwidaa, Chaouen Rwidaa, Chaouen, mountain-forest, ca. 35° 00' N, 05° 16' W

Chenopodiaceae

***Anabasis aphylla* L.**
99-205-[UBC-V217258]-1-9-99-Vegetative state, insecticide. Endemic. Road Taza to Oujda, at 30 km of Taza, steppe elev. ca. 34° 18' N, 03° 50' W

***Atriplex halimus* L.**
99-216-[UBC-V217266]-5-9-99-v.n. Fruit. Errachidia road at 51 km to Bouarfa, river bed elev.ca. 32° 25' N, 02° 30' W

***Chenopodium album* L.**
99-89-[UBC-V217178]-30-7-99-v.n. Mkhinza, flower and fruit. Tetouan at 2 km to Ouad Laou, roadside at foot of mountain elev. 3000m, ca. 35° 00' N, 06° 10' W

Hammada scoparium Iljin=Syn.***Haloxylum scoparium*** Pomel=***Arthrophyton scoparium*** (Pomel) Iljin.
Ecotype a = 99-185-[UBC-V217244]-8-8-99-Yellow flower, anti-poison, shrub. Errachidia road, (Rich to Imilchil), slope elev., ca. 32° 08' N, 05° 40' W
Ecotype b= 99-208-[UBC-V217260]-3-9-99-n.v. Ramt, (division des tiges, survive desert). Figuig, at 40 km of Bouarfa, Sahara elev. ca. 32° 10' N, 01° 15' W

***Noaea mucronata* Moq.**
Ecotype a = 99-169-[UBC-V217233]-7-8-99-Flower buds, spiny, shrub. Khenifra-Midelt road, 5 km from Zaida, valley elev. ca. 33° 00' N, 05° 35' W
Ecotype b = 99-206-[UBC-V217259]-3-9-99-Endemic. Jerrada (Ain BeniMathaar, at 29 km of BeniMathaar), steppe of alfaa (*Stipa tenacissima*) and Noaea, elev. ca. 34° 17' N, 02° 13' W

***Salsola longifolia* Forsk.**
99-204-[UBC-V217257]-1-9-99-Vegetative state leaves succulent. Road Taza to Oujda, at 30 km of Taza, steppe elev. ca. 34° 18' N, 03° 50' W

***Suaeda mollis* (Desf.) Del.**
99-191-[UBC-V217248]-9-8-99-Fruit, flower, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W

Unknown sp.
99-218-[UBC]-5-9-99-v.n. Hagaya, vegetative, succulent leaves. Errachidia road at 51 km to Bouarfa, river bed elev.ca. 32° 25' N, 02° 30' W

Cistaceae

***Cistus albidus* L.**
Ecotype a = 99-27-[UBC-V217132]-26-7-99- No flower or fruit, shrub, associated with *C. libanotus*. 57 km before Chaouen. Valley, ca. 35° 00' N, 05° 16' W
Ecotype b = 99-59-[UBC-V2171xx]-28-7-99- Fruit. Ain Lakchour at 2 km to Moulay Abdessallaam, mountain forest ca. 35° 20' N, 05° 55' W

***Cistus crispus* L.**
Ecotype a = 99-58-[UBC-V217155]-28-7-99- Fruit. Ain Lakchour at 2 km to Moulay Abdessallaam, mountain forest ca.35° 20' N, 05° 55' W
Ecotype b = 99-109-[UBC-V217188]-31-7-99-Fruit orange-like. Road Chaouen to Ketama, at 26 km of Issaguen, high altitude forest elev., ca. 35°00' N, 05° 00' W

Cistus sp.
99-59-[UBC-V217156]-28-7-99- Fruit. Ain Lakchour at 2 km to Moulay Abdessallaam, mountain forest ca.35° 20' N, 05° 55' W

***Cistus ladanifer* L.**
99-86-[UBC-V217176]-30-7-99- Fruit. Tetouan at 8 km to Ouad Laou, 61 km from Chefchaouen, mountain forest elev., ca. 35° 12' N, 06° 10' W

***Cistus libanotus* L. *Halimium libanotis* L.**
99-26-[UBC-V217131]-26-7-99-orange-brown fruit, leaves (lamelles), shrub. Chaouen-Road to Ouazane-Moulay Abdessalaam, Valley, ca. 35° 10' N, 05° 16' W

Cistus monspeliensis **L.**
99-111-[UBC-V217111]-31-7-99-Shrub, no fruit or flower. Fruit. Endemic. Rif, road Chaouen to Ketama, at 20 km of Issaguen, high altitude dense forest elev., ca. 35°00' N, 05° 00' W

Cistus populifolius **L.**
99-154-[UBC -V217154]-7-8-99- tree. Azrou, Ain AlKahla forest ca. 33° 27' N, 05° 14' W

Halimium libanotis **L.**
99-102-[UBC-V217183]-30-7-99-Fruit from last year. Bab Taza at 3 km from road Bab Taza, mountain elev., ca. 35° 04' N, 05° 09' W

Convolvulaceae

Cuscuta epithymum **L.**
99-196-[UBC-V217252]-9-8-99-Full bloom, flower pink, shrub (parasite on *Calicotome*). Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W

Cucurbitaceae

Citrullus colocynthis **(L.) Schrad.**
99-219-[UBC-V217268]-5-9-99-v.n. Alhadjja, Alhandal, flower. Errachidia road, Boudnib to Errachidia, at 30 km, desert, oued river elev.ca. 32° 00' N, 02° 00' W

Ecballium elaterium **(L.) Rich..**
99-39-[UBC-V217142]-27-7-99- Fruit (when touched, splash and eject a liquid useful for Jaundice). Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W

Lagenaria siceraria **(Mol.) Standl.**
98-6-[UBC]-23-8-98-v.n. Slawiya, flower white trumpet-like. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Cupressaceae

Cupressus arizonica **L.**
99-145-[UBC -V217217]-7-8-99-Fruit green & brown cone, pollen, bark papery, tree conifer. Ifrane, Michlifen road (ski resort) at 3 Km, from Timahdite 33 Km, mountain forest elev. ca. 33° 20' N, 05° 10' W

Cupressus atlantica **L.**
99-233-[UBC-V217274]-12-9-99-v.n. Imijid, fruit, cones with seeds. Endemic to high Atlas .Ighil nohasshi douar, rocks on mountain elev.ca. 31° 00' N, 07° 00' W

Juniperus oxycedrus **L.**
<u>Ecotype a</u> = 99-99-[UBC-V217182]-30-7-99-v.n. Getran, fruit, berry dry red-brown. Talamboot road, Tetouan Ouad Laou (21 km from Talamboot, 33 km from Dar Quobbaa, mountain elev. ca. 35° 00' N, 05° 00' W

<u>Ecotype b</u> = 99-128-[UBC-V217201]-5-8-99-Fruit. Khenifra road after Aguelmoiss at 35 km of Khenifra, mountain, roadside elev. ca. 33° 05' N, 05° 35' W

<u>Ecotype c</u> = 98-23-[UBC]-6-9-98-v.n. Harhar, forest. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

<u>Ecotype d</u> = 99-156-[UBC-V217226]-7-8-99-v.n. Harhar, fruit, tree. Road of Azrou, Ain Al Kahla, forest edge elev. ca. 33° 00' N, 05° 00' W

<u>Ecotype e</u> = 99-176-[UBC-V217238]-8-8-99-Berry green dry, tree. Khenifra, at 50 km to Rich (road Midelt-Rich), mountain slope elev. ca. 32° 00' N, 04° 00' W

Tetraclinis articulata **(Vahl.)Masters.**
99-54-[UBC-V217151]-28-7-99-v.n. Harhar, fruit, leaves. Chaouen, 23 km from Tetouan, forest, ca. 35° 32' N, 05° 22' W

Unknown sp.
99-96-[UBC-V217181]-30-7-99-fruit. Tetouan Ouad Laou (21 km from Talamboot, 33 km from Dar Quobaa, mountain elev. ca. 35° 00' N, 05° 00' W

Ephedraceae

Ephedra alata **Decne.**
<u>Ecotype a</u> = 99-187-[UBC-V217245]-9-8-99-v.n. Asiir, fruit, 1 seed dark enveloped of 4 Sepals orange watery, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W

<u>Ecotype b</u> = 99-222-[UBC-V217270]-6-9-99-v.n. Tamoutart, vegetative. Errachidia road, Ras al Harq, dunes elev.ca. 32° 00' N, 02° 00' W

Unknown sp.
99-163-[UBC]-7-8-99- Khenifra-Midelt road, black rock elev. ca. 32° 50' N, 05° 00' W

Ericaceae

***Arbutus unedo* L.**
99-32-[UBC-V217137]-27-7-99-v.n. Bakhanoo, no fruit, shrub. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W

***Erica arborea* L.**
Ecotype a = 99-30-[UBC-V217135]-27-7-99-Fruit, tree. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W
Ecotype b = 99-100-[UBC-V217275]-30-7-99-Last year's fruit. Talamboot road, mountain elev.ca. 35° 00' N, 05° 00' W

Euphorbiaceae

***Euphorbia falcata* L.**
98-18-[UBC]-17-9-98-v.n. Hayat Alnoufouss, flower.Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

***Euphorbia medicaginea* L.**
99-83-[UBC-V217173]-30-7-99-v.n. Flower, fruit. Tetouan, Zaouiat ouad (river) Laou,Zaouiat Sidi Kacem (village centre). Valley, road side elev. ca. 35° 00' N, 05° 00' W

***Euphorbia nicaehnsis* All.**
99-132-[UBC -V217205]-6-8-99-Flower, fruit, latex, shrub. Hajeb at 12 km d'Azrou. Valley, roadside elev. ca. 33° 43' N, 05° 15' W

***Mercurialis annua* L.**
99-36-[UBC-V217140]-27-7-99- Fruit, flower. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W

***Ricinus communis* L.**
99-y-[UBC-V21714y]-26-7-99-v.n. Kharouah, Yellow flower male, female flower red, leaves palm-like, fruit rond dark-green. Road Ouazane-Chafchaouen-Ghzzaoua-Sidi Abdessalam, valley. ca. 35° 10' N, 05° 16' W

unknown sp.
99-78-[UBC-V217170]-29-7-99-v.n. Chmitta, flower white (5 numerous), fruit (cylindrical, hairy), rare plant, latex toxic, burns fingers. Zaouiat Sidi Kacem (village centre). Valley, road side ca. 35° 00' N, 05° 00' W

Fabaceae

***Acacia gummifera* L.**
99-131-[UBC -V217204]-6-8-99-Full bloom tree. Endemic Souss. Hajeb at 14 km d'Azrou, Azrou road fountain (278 km of Errachidia), roadside, on rocks elev. ca. 33° 43' N, 05° 15' W

***Adenocarpus bacquei* L.**
99-61-[UBC-V217158]-28-7-99- Fruit. Ain Lakchour at 2 km to Moulay Abdessallaam, mountain forest ca.35° 20' N, 05° 55' W

Adenocarpus sp.
99-142-[UBC -V217214]-7-8-99-v.n. Fruit (pod), shrub. Ifrane, Michlifen road (ski resort) at 3 Km, from Timahdite 33 Kkm, mountain forest elev. ca. 33° 20' N, 05° 10' W

***Astragalus armatus* Lam.**
99-160-[UBC -V217229]-7-8-99-Fruit, gousse.lower aster purple. Timahdite 3 Km, at 225 km from Errachidia, mid mountain elev. ca. 33° 20' N, 05° 10' W

***Calicotome villosa* L.**
99-52-[UBC-V217149]-28-7-99-Fruit. Chaouen, 23 km from Tetouan. Valley, ca. 35° 30' N, 05° 22' W

***Ceratonia siliqua* L.**
99-49-[UBC-V217148]-27-7-99-v.n.Kharoub,Rwidaa,Chaouen, mountain-forest, ca. 35° 00' N, 05° 16' W

***Genista linifolia* L. Synon. *Cytisus linifolus* L. or *Teline linifolia* L.**
99-115-[UBC-V217191]-5-8-99-Flower, no fruit, shrub. Road Allal Bahraoui, Tiflet, Rabat (Maamora Forest, Quercus suber)), sandy soil elev. ca. 34° 02' N, 06° 51' W

***Genista quadriflora* L.**
99-155-[UBC-V217225]-7-8-99-Flower yellow (papillon), weak fragrance, shrub. Road of Azrou, Ain Al Kahla, high valley, forest edge elev. ca. 33° 27' N, 05° 14' W

***Ononis pseudoserotina* Batt. & Pit.**
99-158-[UBC-V217227]-7-8-99-v.n. Henne, butterfly yellow flower with red lines on large petals. **Endemic** to Middle Atlas. Azrou, Ain Al Kahla, 33 km near entrance, cedar forest elev. ca. 33° 00' N, 05° 00' W

***Ononis spinosa* L.**
99-171-[UBC-V217234]-7-8-99-Flower purple, spines, papillon, pod fruit, shrub. Khenifra, 26 km to Midelt, 8 km from Zaida, valley elev. ca. 32° 40' N, 04° 50' W

***Retama raetam* (Forsk.)Webb.**
99-164-[UBC-V217231]-7-8-99-Green plant (comme *Juncus*), fruit shaped as beans, white outside & inside black seeds, shrub. Errachidia road, at 46 km of Midelt, flat valley elev. ca. 32° 45' N, 04° 55' W

Fagaceae

***Quercus rotunfolia* Lamk. Synon. *Quercus ilex subsp bellota* L.**
Ecotype a = 99-29-[UBC-V217134]-27-7-99-v.n. Ballot, fruit, tree. Chaouen, Douar el Ballotta, mountain-forest, ca.35° 10' N, 05° 16' W
Ecotype b = 99-141-[UBC -V217213]-6-8-99-v.n. Chene vert, fruit, tree. Hajeb Ain Allouh forest, valley ca. 33° 00' N, 05° 00' W

Globulariaceae

***Globularia alypum* L.**
99-56-[UBC-V217153]-28-7-99- Last year fruit, shrub. Route Tetouan, Moulay Abdessalaam, 70 km from Larache, mountain-forest, ca.35° 20' N, 05° 55' W

Grossulariaceae

***Ribes grossularia* L.**
99-162-[UBC-V217230]-7-8-99-Leaves same as geranium, mini, spines. Khenifra-Midelt road, black rock elev. ca. 32° 50' N, 05° 00' W

Juglandaceae

***Juglans regia* L.**
Ecotype a = 99-201-[UBC-V217255]-9-8-99-v.n. Gargah, fruit, tree. Errachidia , road Imilchil to Rich, at 45 km of Imilchil, valley elev. ca. 32° 00' N, 04° 00' W
Ecotype b = 98-4-[UBC]-6-9-98-v.n. Gargah, fruit, tree. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Juncaceae

***Juncus maritimus* Lamk.**
99-22-[UBC-V217129]-26-7-99-v.n. Smaar, flower, fruit brown, shrub. Road to Ouazane- Zaouia Moulay Abdessalaam, 56 km from Chaouen. Valley ca. 35° 00' N, 05° 16' W

Lamiaceae

***Ballota hirsuta* L.**
99-120-[UBC-V217194]-5-8-99-Flower, fruit. At 5 km to Maaziz. Valley elev. ca. 34° 00' N, 06° 30' W

***Lavandula dentata* L.**
99-85-[UBC-V217175]-30-7-99-v.n. Khzama, flower, intense fragrance. Road Ouad Laou, Zaouiat Sidi Kacem at 19 km to Ouad Laou, mountain forest elev. ca. 35° 00' N, 05° 00' W

***Lavandula multifida* L.**
99-181-[UBC-V217242]-8-8-99-v.n. Khzama, flower, shrub. Errachidia road, at 20 km to Rich (road Midelt-Rich), side of mountain slope elev. ca. 32° 18' N, 04° 30' W

***Lavandula stoechas* L.**
Ecotype a = 99-31-[UBC-V217136]-27-7-99-v.n. Halhal, Hshish Al Quatt, flower. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W
Ecotype b = 99-48-[UBC-V217147]-27-7-99-v.n. Khzama, flower. Rwidaa, Chaouen, mountain-forest, ca. 35° 00' N, 05° 16' W

Lavandula sp.
99-229-[UBC-V217271]-11-9-99-v.n. Al Aoudaa, flower. Tiznit, Agadir Ajgal (*Dracena tininia*), high mountains, steep cliff, south-east elev.ca. 29° 43' N, 09° 44' W

***Marrubium vulgare* L.**
Ecotype a = 98-13-[UBC]-6-9-98-v.n. Marriouta, fruit. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Ecotype b = 99-137-[UBC -V217210]-6-8-99-Flower, fruit, shrub. Hajeb Ain Allouh forest, roadside, on rocks elev. ca. 33° 00' N, 05° 00' W

***Mentha pulegium* L.**

Ecotype a = 98-24-[UBC]-6-9-98-v.n. Flyou. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Ecotype b = 99-14-[UBC-V217165]-29-7-99-v.n. Flyou, flower, fragrance. Tetouan at 7 km to Moulay Abdessalaam, oued (river bed), ca. 35°12' N, 06° 10' W

Ecotype c = 99-71-[UBC-V217127]-26-7-99-v.n. Flyou, flower, fragrance. 8 km from Ouazane, Valley, river bed, ca. 34°50' N, 05° 35' W

Ecotype d = 99-76-[UBC-V217169]-29-7-99-v.n. Menta, flower. Tetouan at 7km to Moulay Abdessallaam, oued (river bed) ca. 35° 12' N, 06° 10' W

Ecotype e = 99-127-[UBC-V217200]-5-8-99-v.n. Flyou, flower. Khenifra road at 3 km of Aguelmass. Valley elev. ca. 33° 05' N, 05° 35' W

***Mentha rotundifolia* (L.) Hudson.**

99-76-[UBC-V217176]-30-7-99-v.n. Menta, flower (dominant species & *Inula, Morus alba*). Tetouan, 7 Km to Moulay Abdessalaam, on rocks, in forest ca. 35°12' N, 06° 10' W

***Mentha suaveolens* Ehr.**

Ecotype a = 99-15-[UBC-V217128]-26-7-99-v.n. Timijja, flower, fruit, fragrance. 8 km from Ouazane. Valley, river ca. 34°50' N, 05° 35' W

Ecotype b = 99-126-[UBC-V217199]-5-8-99-v.n. Marseta, timijja, flower. Khenifra road at 3 km of Aguelmass. Valley elev. ca. 33° 05' N, 05° 35' W

***Mentha sylvestris* L.**

99-70-[UBC-V217164]-29-7-99-v.n. Liquama Nah Nah. Tetouan, 25 Km to Moulay Abdessalaam, on rocks, in forest ca. 35°12' N, 06° 10' W

***Origanum vulgare* L.**

Ecotype a = 99-43-[UBC-V217145]-27-7-99-v.n. Zahtaar, flower, fruit. Rwidaa, Chaouen, mountain-forest, ca. 35° 00' N, 05° 16' W

Ecotype b = 99-x- [UBC-V217154]-28-7-99-69-v.n. Zahtaar, flower purple, intense fragrance. 69 km from Larache, at 25 Km to Moulay Abdessalaam. Habitat, mountain forest, ca. 35° 15' N, 06° 00' W

***Rosmarinus officinalis* L.**

Ecotype a = 99-211-[UBC-V217262]-4-9-99-v.n. Aziir, flower. Figuig, jbel Grouz mountain, at 24 km to Figuig, on Bouarfa road, ravine elev. ca. 1200 m, ca. 32° 10' N, 01° 20' W

Ecotype b = 99-101-[UBC-V217278]-30-7-99-v.n. Aziir. Talamboot road, mountain elev. ca. 35° 00' N, 05° 00' W

***Salvia officinalis* L.**

99-103-[UBC-V217184]-31-7-99-Fruit from last year. Road Ouad Laou to Chaouen, mountain forest elev., ca. 35° 00' N, 05° 00' W

***Salvia phlomoides* Asso.**

99-65-[UBC-V217161]-29-7-99-v.n. Salmia, endemic. Tetouan, mountain forest ca. 35° 12' N, 06° 10' W

***Salvia sclarea* L.**

98-3-[UBC]-6-9-98-v.n. Salmia, vegetative. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

***Satureja granatensis* (Boiss. & Reut.) R. Fernandes.**

26-7-99-[UBC-V217123]-26-7-99-v.n. Zahtaar, no flower, no fruit-Souk al Arbaa-Ouazane (5 km from station 1). Valley, ca. 34°50' N, 05° 40' W

***Stachys hirta* L.**

99-72-[UBC-V217166]-29-7-99- Flower. Tetouan at 7km to Moulay Abdessallaam, Oued (river bed) ca. 35° 12' N, 06° 10' W

Stachys sp.

99-67-[UBC-V217162]-29-7-99- Flower, fruit. Tetouan at 7km to Moulay Abdessallaam, on rocks in forest. ca. 35° 12' N, 06° 10' W

***Teucrium polium* L.**

99-200-[UBC-V217277]-9-8-99-White flower with central part yellow, fruit, shrub. Road from Rich to Imilchil, at 34 km Ammelgous, valley elev. ca. 32° 08' N, 05° 40' W

Thymus marocanus **Ball.**
99-183-[UBC-V217276]-8-8-99-v.n. Zaatar. Yellow flower, latex, shrub. Endemic. Road Rich to Imilchil at 34 km d'Amellaguou. valley elev. ca. 32° 08' N, 05° 40' W

Unknown sp.
99-114-[UBC]-31-7-99-Flower, bloom centipede (comme d'en haut). Road Chaouen to Ketama, at 15 km of Issaguen, cedrus forest, roadside elev., ca. 35°00' N, 05° 00' W

Lauraceae

Laurus nobilis **L.**
99-62-[UBC-V217159]-29-7-99- Fruit. Road Moulay Abdessallaam to Tetouan at 5 km to Moulay Abdessallaam, forest ca.35° 20' N, 05° 55' W

Liliaceae

Asparagus acutifolius **L.**
99-37-[UBC-V217141]-27-7-99- Flower. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W

Asparagus alba **L.**
99-34-[UBC-V218138]-27-7-99-v.n. Sekkum, ziou ziou, in full bloom, nice fragrance, shrub. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W

Urginea maritima **(L.) Baker.**
98-19-[UBC]-16-9-98-v.n. Feruna, flower. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Linaceae

Linum usitatissimum **L.**
99-80-[UBC-V217172]-30-7-99-v.n. Kattan. Tetouan, Zaouiat ouad (river) Laou,Zaouiat Sidi Kacem (village centre). Valley, road side elev. ca. 35° 00' N, 05° 00' W

Loranthaceae

Viscum cruciatum **Cieber.**
99-140-[UBC -V217212]-6-8-99-Fruit, small tree (parasite of aubepine), Hajeb Ain Allouh forest, valley ca. 33° 00' N, 05° 00' W

Moraceae

Ficus carica **L.**
Ecotype a = 99-173-[UBC-V217236]-8-8-99-Fruit (fig) unripe, latex, tree. Khenifra, 8 km to Midelt, entree from Zaida Tadamout, high valley elev. ca. 32° 40' N, 04° 50' W

Ficus carica **L.**
Ecotype b = 98-5-[UBC]-6-9-98-v.n. Karmouss, fruit, latex, tree. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Myoporaceae

Myoporum sp.
99-35-[UBC-V217139]-27-7-99- Red fruit. Chaouen, Douar el Ballotta. Valley-forest, ca. 35° 10' N, 05° 16' W

Myrtaceae

Eucalyptus gomphocephala **A. de C.**
Ecotype a = 98-15-[UBC]-17-9-98-v.n. Kalitus, tree, fruit. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W
Ecotype b = 99-5-[UBC-V217121]-26-7-99- Kalitus, fruit, tree. Chaouen (forest, Rif), ca. 34°50' N, 05° 40' W.

Eucalyptus spp.
99-5-[UBC-V217121]-26-7-99-v.n. Kalitus, fruit (tree)-Chaouen (forest, Rif), between Souk al Arbaa-Ouazane (Ouezzane), Souk Mas Mouda (2 Km.). Watilou Forest. ca. 34°50' N, 05° 40' W

Myrtus communis **L.**
99-46-[UBC-V217146]-27-7-99-v.n. Rihan, fruit, flower. Rwidaa, Chaouen, mountain-forest, ca. 35° 00' N, 05° 16' W

Oleaceae

***Olea europaea* L.**
Ecotype a = 99-123-[UBC-V217196]-5-8-99-v.n. Zitoun, olivier sauvage, zitoun burrii, Fruit, olives, tree. Oulmes at 34 km, mountain, collines elev. ca. 33° 30' N, 06° 10' W
Ecotype b = 98-1-[UBC]-23-8-98-v.n. Zitoun, olivier sauvage, zitoun burrii, Fruit drupe dark, olives, tree.Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

***Phillyrea latifolia* L.**
99-28-[UBC-V217133]-27-7-99- No flower, fruit, tree. Chaouen, Douar el Ballotta, mountain-forest, ca. 35° 10' N, 05° 16' W

Palmae

***Chamaerops humilis* L.**
98-12-[UBC]-6-9-98-v.n. Addoum, fruit. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Pinaceae

***Cedrus atlantica* (Endl.) Carrière.**
99-110-[UBC-V217189]-31-7-99-Fruit male. Endemic. Road Chaouen to Ketama, at 20 km of Issaguen, high altitude dense forest elev., ca. 35°00' N, 05° 00' W

Plumbaginaceae

***Limonium bonduelli* (Lestib) Sauv. & Vindt.**
99-146-[UBC -V217218]-7-8-99-Flower, small white, lemon fragrance. Ifrane, Michlifen road (ski resort) at 3 Km, from Timahdite 33 Km, mountain forest elev. ca. 33° 20' N, 05° 10' W

Poaceae

***Hyparrhenia hirta* L.**
99-11-[UBC-V217125]-26-7-99-flower. 8 km from Ouazane (la gare-airport). Valley, river, ca. 34°50' N, 05° 35' W

Polygonaceae

***Polygonum aviculare* L.**
99-40-[UBC-V217143]-27-7-99-Chaouen, Douar AlBaydaa. Valley, road ca. 35° 00' N, 05° 16' W

***Rumex acetosa* L.**
99-151-[UBC -V217222]-7-8-99-Flower red-pink, shrub. Ifrane (4.5 km to Michlifen), from Timahdite 32 Km, mountain forest, valley elev. ca. 33° 20' N, 05° 10' W

***Rumex bucephalophorus* L.**
99-152-[UBC -V217223]-7-8-99-Flower orange, shrub. Ifrane (4.5 km to Michlifen), from Timahdite 32 Km, valley elev. ca. 33° 20' N, 05° 10' W

***Rumex pulcher* L.**
99-12-[UBC-V217126]-26-7-99-v.n. Hammida, flower & fruit. 8 km from Ouazane, Valley, river, road sides ca. 34°50' N, 05° 35'W

Polypodiaceae

***Pteridium aquilinum* L.**
99-60-[UBC-V217157]-28-7-99-v.n. Fougere, vegetative- Ain Lakchour at 2 km to Moulay Abdessallaam, mountain forest ca.35° 20' N, 05° 55' W

***Adiantum capillus-veneris* L.**
99-64-[UBC-V217160]-29-7-99-v.n. Fougere- Road Moulay Abdessallaam to Tetouan at 6km to Moulay Abdessallaam, forest ca.35° 20' N, 05° 55' W

Ranunculaceae

***Clematis alba* L.**
99-23-[UBC-V217130]-26-7-99- Flower white, shrub. Road of Ouazane to Chaouen-Moulay Abdessalaam, foot of mountain, forest, oued ca. 35° 00' N, 05° 16' W

Resedaceae

***Reseda phyteuma* L.**
99-190-[UBC-V217247]-9-8-99-Fruit, flower, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W

Rhamnaceae

***Ziziphus lotus* (L.) Lam.**
98-11-[UBC]-6-9-98-v.n. Aseddra, fruit. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Rosaceae

***Crataegus monogyna* Jacq.**
Ecotype a = 99-4-[UBC -V217120]-26-7-99-Ouard, fruit rose hips. Chaouen (Forest, Rif), between Souk al Arbaa-Ouazane (Ouezzane), Souk Mas Mouda (2 Km.). Watilou Forest valley ca. 34°50' N, 05° 40' W
Ecotype b = 99-139-[UBC -V217211]-6-8-99-v.n. Aubepine, fruit pink, small tree. Hajeb Ain Allouh forest, valley ca. 33° 00' N, 05° 00' W

***Pyrus mamorensis* Trab.**
99-117-[UBC-V217193]-5-8-99-v.n. Poirier sauvage, angass, bouhwid burrii, fruit (mini pear), tree. Endemic. Road Allal Bahraoui, Tiflet, Rabat (Maamora Forest, Quercus suber)), 32 km to Rabat, or 106 km to Meknes, ca. 34° 02' N, 06° 40' W

***Rosa canina* L.**
99-129-[UBC-V217202]-5-8-99-Fruit, rose hips, tree-shrub. Khenifra road at 35 km of Khenifra, rocks elev. ca. 33° 05' N, 05° 35' W

Rubiaceae

***Rubia peregrina* L.**
99-68-[UBC -V217163]-29-7-99. No flower or fruit, shrub. Tetouan at 7km to Moulay Abdessallaam, on rocks in forest ca. 35°12' N, 06° 10' W

Rutaceae

***Ruta chalepensis* L.**
98-10-[UBC]-6-9-98-v.n. Al-fijel. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Scrophulariaceae

***Scrophularia sambucifolia* L.**
Ecotype a = 99-74-[UBC-V217168]-29-7-99-v.n. Flower. Tetouan at 7km to Moulay Abdessallaam, oued (river bed), on rocks ca. 35° 12' N, 06° 10' W
Ecotype b = 99-108-[UBC-V217187]-31-7-99-Fruit. Road Chaouen to Ketama, at 30 km of Issaguen, high altitude forest, roadside elev., ca. 35°00' N, 05° 00' W

***Verbascum sinuatum* L.**
98-16-[UBC]-17-9-98-v.n. Maslah Andaar, flower. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Solanaceae

***Solanum nigrum* L.**
98-7-[UBC]-6-9-98-v.n. Hinab Addib, fruit. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Thymeleaceae

***Daphne gnidium* L.**
Ecotype a = 98-8-[UBC]-6-9-98-v.n. Lazzaz. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W
Ecotype b = 99-55-[UBC -V217152]-28-7-99-v.n. Lazzazz, flower, fruit, shrub. Route de Tetouan, Moulay Abdessalaam, 70 km from Larache, mountain-forest, ca. 35° 20' N, 05° 55' W

***Thymelaea hirsuta* (L.) Endl.**
Ecotype a = 99-42-[UBC-V217144]-27-7-99- Flower, fragrance high in the evening. Bousbah, Bab al Khems, Chaouen. Valley, ca. 35° 00' N, 05° 16' W
Ecotype b = 99-192-[UBC-V217250]-9-8-99-v.n. Metnan, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W

***Thymelaea lythroides* Barr & Murb.**
Ecotype a = 99-116-[UBC-V217192]-5-8-99-v.n. Metnan, flower, shrub. Road Allal Bahraoui, Tiflet, Rabat (Maamora Forest, Quercus suber)), sandy soil elev. ca. 34° 02' N, 06° 51' W
Ecotype b = 99-231-[UBC-V217272]-11-9-99-v.n. Metnan. Tiznit, Agadir Ajgal (*Dracena tirinia*), high mountains, steep cliff, southeast elev.ca. 29° 43' N, 09° 44' W

Thymelaea tartonraira **(L.) All.**
99-130-[UBC -V217203]-11-9-99-Flower. Hajeb at 14 km d'Azrou, Azrou road fountain Ito (278 km of Errachidia), roadside, on rocks elev. ca. 33° 43' N, 05° 15' W

Valerianaceae

Centranthus ruber **(L.) DC.**
99-232-[UBC-V217273]-12-9-99-Flower. Ifsdadan Tizintest at 71 km to Asni, mountain, rocks elev.ca. 31° 14' N, 07° 55' W

Verbenaceae

Aloysia triphylla **Britt.**
98-2-[UBC]-6-9-98-v.n. Lwiza, flower. Province de Meknes, Cercle d'El Hajeb, Boulouzen, commune du dir, Ait Halla, valley, hills.elev. ca. 33° 30' N, 06° 10' W

Verbena officinalis **L.**
99-79-[UBC-V217171]-30-7-99-v.n. Lwiza, flower. Zaouiat ouad (river) Laou, Zaouiat Sidi Kacem (village centre). Valley, road side elev. ca. 35° 00' N, 05° 00' W

Vitex agnus-castus **L.**
99-9-[UBC-V217124]-26-7-99-v.n. Kharouah, fruit & flower purple, shrub. 8 km from Ouazane. Valley, river, ca. 34°50' N, 05° 35' W

Zygophyllaceae

Peganum harmala **L.**
Ecotype a = 99-168-[UBC-V217232]-7-8-99-v.n. Harmal, fruit (gousse with black seeds), shrub. Khenifra, 1 km from Zaida, valley elev. ca. 33° 00' N, 05° 40' W
Ecotype b = 99-195-[UBC-V217251]-9-8-99-v.n. Harmal, red fruit, polymorphism, shrub. Errachidia Imilchil, Lac Tislit-Tisli (Lake), lake side elev., ca. 32° 00' N, 05° 00' W

Zygophylum gaetulum **Emb. & Maire.**
99-217-[UBC-V217267]-5-9-99-v.n. Haguaya, vegetative, succulent leaves. Errachidia road at 51 km to Bouarfa, river bed elev.ca. 32° 25' N, 02° 30' W

It is worth noting that some specimens were not identified: *Chmitta,* Unknown, 161, 157 (Herbarium numbers) and others: these seemingly are rare specimens.

Table 2.3. List of medicinal plants (Family and Species), their common names (Vernacular name) and region, plant part and medicinal use (s)

Family and Species	Vernacular name (region)	Plant part	Treatment
Aizoaceae			
Aizoon canariense L.	Ghassoul (High Atlas)	Whole	Induce vomiting for intoxication.
Anacardiaceae			
Pistacia lentiscus L.	Dru (Agourai)	Leave / flowers	Stomachaches, ulcer.
Apiaceae			
Ammi visnaga Lam.	Bachnikha (Hajeb)	fruit/ umbel	Asthma.
Ammodaucus leucotrichus Coss. Dur.	Kemmun Sufi (Sahara)	seeds	Cough, fever, diarrhea, colic, cold.
Ammoides pusilla (Brot.) Breistr.	Nanukha (Oujda)	aerial	Cold (influenza) fevers.
Apium graveolens L.	Krafes (Meknes)	aerial	Cold, rheumatism, urinary disorders.
Carum carvi L.	Kerwiya (Sahara).	seeds	Cold, diarrhea, indigestion.
Ferula communis L.	Fasukh (Sahara).		Antidote-poison.
Petroselinum crispum (Mill.) Nyman ex A.W. Hill.	Maeadnus (Hajeb)	aerial	Cold.
Ridolfia segetum Moris	Tebsh (Sidi Kacem)	aerial	Jaundice.
Aristolochiaceae			
Aristolochia paucinervis Pomel	Bareztem (Agourai)	root	Asthma, pimples (face).
Asclepiadaceae			
Calotropis procera (Ait.) Ait.	Turza (Dra, Zagora)	leaves/ latex	Asthma, gunpowder. Skin diseases, toxic.
Asteraceae			
Chamaemelum nobile (L) All.	Chajarat myriem (Hajeb)	aerial	Gastric-intestinal troubles.
Artemisia absinthium L.	Siba (Meknes)	aerial	Cold.
Artemisia herba-alba Asso	Shih (Desert, Figuig)	aerial	Cold, indigestion, wound (abscess).Antipoison (Sahara snakes).
Cichorum intybus L.	Hendaba (Hajeb)	aerial	Cold.
Conyza canadensis (L.) Cronquist	Terehla (Rif)	leaves	Cold.
Cotula cinerea Delile	Qertufa (Atlas)	whole	Pulmonary infections.
Cynara cardunculus L.	Quq (Hajeb)	fruit	Liver aches: taken raw with radish.
Dittrichia viscosa (L.) Greuter = *Inula viscosa* (L.) Aiton	Bagraman (Sidi Kacem)	aerial	Cold.
Matricaria recutita L.	Babnuj (Hajeb)	flower	Cold mixture (used also as a dye for hair), soothing (infusion).

Pulicaria arabica (L.) Cass.	Hatassa (Hajeb)	aerial	Tobacco
Sonchus oleraceus L.	Tifaf	aerial	System cleanser (depurative)
Warionia saharae Benth. & Coss.	Afezdad (Desert Ocean)	llaiteron/ leaves	Uterus infections.
Brassicaceae			
Lepidium latifolum L.	Habb r-rshad (Sidi Kacem)	seeds	Bronchi pulmonary infection,cough. Strengthen the heart.
Capparaceae			
Capparis ssp.	Afsas (Sahara)	fruit	Stomachaches, cold.
Capparis spinosa L.	Kebbar (Fez)	fruit	Cold.
Cleome amblyocarpa Barr. & Murb.	Mkhinza (Hajeb)	aerial	Typhoid, meningitis, diphtheria, cyst, abscess.
Caryophyllaceae			
Corrigiola telephiifolia Pour.	Serghina (Agourai)	roots	Cold, lung disorders.
Dianthus sylvestris Wult.	Qrenfel	clove	Cold (powder used on skin after hot bath).
Herniaria cinerea DC.	Herrast l-hjar	aerial	Kidney stones.
Cucurbitaceae			
Cucurbita pepo L.	Bsibsiya	flower	Cold mixture.
Lagenaria siceraria (Mol.) Standl.	S- Slawiya (Hajeb)	seeds	Antibacterial (ulcer), headache, and bronchi-pulmonary infections.
Cupressaceae			
Juniperus phoenicea L.	Earear (Agourai)	stem/ leaves	Fever, incense for psychological problems.
Cyperaceae			
Scirpus holoschoenus L.	Smar (Agourai / riverbank)	aerial	Cold, internal blood cleaner.
Euphorbiaceae			
Euphorbia falcata L.	Hayath al noufouss (Hajeb)	aerial	Cold.
Ricinus communis L.	Kherwae (Sidi Kacem)	seeds	Cold.
Fabaceae			
Acacia gummifera Willd.	Talh (Zagora)	aerial	Bronchitis, antitussive.
Crotalaria saharae Coss.	L-Fula (Sahara)	root	Wound stitching.
Melilotus macrocarpus Coss. & Dur.	Azrud (Figuig)	fruit	Antiseptic, hair-care.
Retama retam (Forsk.) Webb.	Rtem (Sahara)	root	Diphtheria, epidemic (root poisonous).
Retama sphaerocarpa (L.) Boiss.	Rtem (Sahara)	root	Idem as *R. Retam*
Trigonella foenum-graecum L.	Helba (Meknes)	seeds	Ulcer (increase appetite) weight gain, cleans blood.
Fagaceae			
Quercus faginea Lam.	Eafsa (Middle Atlas)	fruit	Cold,astringent, antibacterial.

Castanea sativa Mill.	Qestal (Rif)	leaves	Cold, urinary antiseptic.
Gentianaceae			
Centaurium spicatum L.	Gussat l-hayya (Meknes)	whole	Cold, depurative.
Iridaceae			
Iris germanica L.	Eanber (Anti-Atlas)	rhizome	Cold mixture, abortive.
Juglandaceae			
Juglans regia L.	Swak (Hajeb)	leaves/ bark	Sinusitis,cold,stomachache, Skin infection, rashes (a paste from the Leaves & *Lawsonia* is used).
Juncaceae			
Juncus acutus L.	Ssmar (Hajeb)	fruit	Diuretic, fever.
Lamiaceae			
Ajuga iva L.	Shendgura (Agourai)	whole	Generalist used for many ailments.
Lavandula angustifolia Mill.	Khzama (Agourai)	aerial	Kills worms in children's intestine. Used for pains: PMS,gynecologic infections,cold, rheumatism and sinusitis. Used to get rid of germs in clothes.
Marrubium vulgare L.	Merriut (Agourai)	aerial	Cold, abscess (taken in Small quantity).
Mentha pulegium L.	Fliyu (Middle Atlas)	aerial	Bronchitis, cold.
Nepeta apulaei L.	Questal	whole	Idem as *N. spp.*
Nepeta spp.	Hashish al qatt	aerial	Venereal diseases.
Rosmarinus officinalis L.	Azir (Desert).	leaves	Bronchitis.
Salvia clandestina L.	Khiyyata (Agourai)	aerial	Burns & stitching wounds.
Salvia officinalis L	Salmiya (Agourai)	aerial	Cold, hypertension.
Stachys arvensis L.	Laktira	aerial	Cold,antitussive,antiasthma
Thymus broussonetti Boiss.	Zeitra (Agourai)	stem/ leaves	Cold, wound antiseptic (distillate).
Thymus maroccanus Ball.	Zaatar (Midelt)	leaves	Indigestion & food disinfectant. Cough, bronchitis, cold.
Thymus satureioides Cos.	Azukenni (Midelt)	aerial	Cold, colic, taken with tea. Food disinfectant.
Lauraceae			
Laurus nobilis L.	Rend (Rif)	leaves	Liver disorders.
Liliaceae			
Asphodelus microcarpus Salzm. & Viv.	Berwak	root	Abscess.
Linaceae			
Linum usitatissimum L.	Kettan (Meknes)	seeds	Cold mixture (plant used With other plants), antitussive.

Lythraceae			
Lawsonia inermis L.	Henna (Sahara)	leaves	Dye for hair & hands, feet. Skin Diseases
Myrtaceae			
Eucalyptus spp.	Kalitus, Kritus (Agourai)	leaves	Cold, cough, throat.
Oleaceae			
Fraxinus angustifolia Vahl	Lsan t-tir	seeds	Cold mixture.
Olea europaea L. var sativa	Zitun (Mrirt)	leaves	Antimicrobial.
Papaveraceae			
Papaver rhoeas L.	Beleaman (Mrirt)	petal/ capsule	Inflammation,cold, measles, Children's fever.
Pinaceae			
Pinus halepensis Mill.	Tayda (Hajeb)	bark	Astringent,diarrhea,wounds
Piperaceae			
Piper longum L.	Dar falfal (Sahara)	seeds	Colds, aphrodisiac.
Piper cubeba L.	Alkabbaba (Sahara)	cubebe	Cold.
Poaceae			
Sorghum bicolor (L.) Moench	Illan (Agourai)	seeds	Generalist: cold, used by Women after giving birth (abscess).
Cynodon dactylon (L.) Pers.	Njem (Agourai)	rhizome	Cold, diuretic.
Polygonaceae			
Rumex bucephalophorus L.	Hummida (Agourai)	aerial	Intestinal gas, cold in stomach, liver disorders.
Punicaceae			
Punica granatum L.	Qchur romman	fruit peel	Ulcer, vaginal infection.
Ranunculaceae			
Nigella sativa L.	Sanuj	seeds	Heals from all illness. (3-5 Drops of sanuj oil taken with tea or coffee)cough pulmonary diseases. Liver, heartaches, powders taken with honey. Influenza, allergy, hypertension, stomachache.
Rhamnaceae			
Rhamnus alaternus L.	Amliles	leaves/ stem	Jaundice (used boiled with lemon juice).
Rosaceae			
Rosa canina L.	Busrud (Dades)	fruit	Diarrhea.
Sorbus aria (L.) Crantz	Zaerur	flower	Anti-diarrhea.
Rubiaceae			
Rubia peregrina L.	Fuwwa	root	Jaundice.

Rutaceae			
Ruta graveolens L.	Mrijo	seeds	Children cold.
Santalaceae			
Osyris quadripartita Salz.	Bu lila		Cold.
Sapotaceae			
Argania spinoza (L.) Skeels.	Argan (Agadir)	fruit	Chickenpox scars.
Simarubaceae			
Balanites aegyptiaca (L.) Del.	Almanaa (Sidi Kacem)	fruit peel	Cold.
Solanaceae			
Solanum nigrum L.	Eneb d-dib (Meknes)	fruit	Cold, kidney problems.
Hyoscyamus albus L.	Sikran (Agourai)	seeds/ leaves	Antifungal,anti-hemorrhoid.
Thymelaeaceae			
Thymelaea microphylla Coss. & Dur.	Metnan	whole	Cold in the urinary tract.
Verbenaceae			
Aloysia triphylla Britt.	Lwiza	aerial	Children colic (as an infusion).
Zingiberaceae			
Alpinia officinarum Hance	Khdanjal (Meknes)	rhizome	Cold, back pain.
Ellettaria cardamomum White &Maton	Qaeqalla	seeds	Cold mixture, heart invigorating.
Miscellaneous			
"Indass"		root	Cold
"Ghanghass"endemic		root	Cold
(*Anacyclus pyrethrum* L.)		root	Cold
"Tahantizt"		root	Infections
"Al Ghassoul Assabssi"		root	Cold
Sea urchin (animal)		shell	Cold

Discussion

This ethnopharmacobotanical survey of the medicinal plants of Morocco was carried out in order to evaluate the reported effectiveness of the plants used for infectious diseases. Ethnopharmacological considerations of the collected data as well as the ethnomedicine and ethical issues were raised. The significance of this research lies not only in the possibility of discovering novel and useful biologically active compounds (e.g. thymohydroquinones & others), but also in documenting the apparent value of Moroccan ethnobotanical medicines. The World Health Organization (WHO) has recommended, especially in developing countries, the initiation of programs designed to use medicinal plants more effectively in the traditional health system (WHO, 1978). The resolution of the 31st WHO Assembly requested a complete inventory, evaluation of the efficacy and safety, and standardization of medicinal plants (Farnsworth, 1980). This study presented in this thesis is a response to this need.

From the ethnobotanical information gathered for the 97 plant species reported in Table 2.3, 68 species are used to treat illnesses that are potentially viral in nature, 15 species to treat illnesses that are probably caused by bacterial infection and 14 possible fungal diseases.

Most often, the whole plant is used in these treatments, particularly when it is a small, herbaceous species. The root is generally used from perennial woody species. In only 25 cases of the treatments are aerial parts used. In only two cases, the bark, and in 13 cases, seeds are used.

In most cases, herbal treatments are taken internally as an infusion or decoction. For skin diseases, an external application of a paste is used e.g. from the leaves of *Juglans regia* and *Lawsonia.*

The plant material is better used fresh if available seasonally. When not available, dried material is used. In some cases substitution of one plant is used in place of the original. Only a knowledgeable healer and written ancient sources determine the right substitute: e.g. *Lavander* is substituted with horehound or *Marrubium*, *Usnia* (Lichen) is substituted with *cardamom*, *Asarum* is substituted with caraway (Levey, 1973). The Moroccan medicinal uses of *Quercus*, *Nepeta*, *Papaver*, *Punica* and a sea urchin have not been previously documented.

In Morocco, some medicinal plants are also used as foods or condiments (spices): e.g. *Ammodaucus*, *Capparis* (Fig.2.12), *Thymus*, *Elletaria, Piper* (condiments), *and Cucurbita, Lagenaria* (food). Some plants in Morocco are considered sacred e.g. *Nigella*, *Lawsonia*, *Peganum.*

- Ethnomedicine Issues encountered in the field

a) Diagnosis, prescription, and plant preparation and administration.

I met with several healers from whom I learned that the preparation and administration of the plant material is very important. A correct diagnosis enables the healer to make an appropriate prescription. Only very experienced and skilled healers performed them well. The way healers act towards their patients is also very important. I was impressed by their devotion to their patients with little interest to the material gain from it. The ability of the healer to be a good psychologist is crucial in their relationship with the patient as well as in the healing process. Furthermore their ability to identify correctly useful plant material, collected at the right time, was noteworthy. Plants, for a defined purpose may be best used fresh rather than dry, or in vegetative or flowering state, or with or without seeds. Plant identification is also crucial to avoid a toxic plant being mistaken for a nontoxic one. Collection of a plant at the right time is important as some plants are effective, or toxic in the flowering or fruit stage and vice versa (e.g. the flower of *Artemisia absinthium* is toxic, hence the plant is only used in winter). Some healers were able to prescribe the right medicines by only listening to the description of the symptoms made by the patient, whereas other healers were very active by asking questions and used their hands or other means in reaching the right diagnosis. Some patients who had access to a modern doctor provided the diagnosis and the analysis form to the healer who made good use of it in the prescription of the medicine.

It is important to point out the fact that the only diseases of interest in my research are infectious diseases caused by viruses or bacteria or fungi. Fever was related to viral problems. Colds were related to bacteria and viruses, whereas skin infections were related to fungi and bacteria and viruses. This was a simplified approach but proved very effective and easily understood by lay or common people.

As many healers in Morocco are women family "doctors", the gender analysis, which I conducted brought an interesting dimension to the research (Browner and Ortiz de Montellano, 1986). I learned from women healers the way they prepare as well as administer medicinal plants to patients. The administration route depends on the part of the plant used and of the symptoms. Leaves are generally used in infusion for colds or fever. Several examples (Table 2.3) are in the Lamiaceae, particularly aromatic plants such as the species *Thymus maroccanus*, *T. Broussonetti*, and *T. Satureioides, as well as Mentha pulegium*, *Nepeta apulaei*, *Rosmarinus officinalis*, *Salvia clandestina*, *Salvia officinalis*, and *Stachys arvensis*. Leaves and flowers are generally used in the Asteraceae family. Some examples are *Anthemis nobilis, Artemisia absinthium*, *Artemisia herba-alba*, *Cichorum intybus*, *Conyza canadensis*, *Cotula cinerum* (used whole), *Dittrichia viscosa* (*Inula v.*), *Matricaria chamomilla* (flower), *Pulicaria arabica* (flower). Roots are used as decoction (e.g. *Crotalaria saharae*, *Retama retam*, *Retama sphaerocarpa* (Fabaceae). A paste of one or several plants is used for skin infections externally (e.g. *Lawsonia inermis* (Henna) and *Juglans regia*). Seeds of some plants are also used (e.g. *Lagenaria siceraria* (Cucurbitaceae); *Trigonella foenum-graecum*(Fabaceae). The oil of a plant is useful for coughs and colds although *Nigella sativa* oil is toxic and a very low dose should be used. Some mild plant oils as well as honey are used as a vehicle for other medicines. The oil has generally a comforting and relieving effect on the patient. In case of toxic plants, fumigation or inhalation is the preferred and safest administration route (e.g. *Peganum harmala*). Distillation is useful in extracting all the volatile compounds of a plant and is a very popular practice in the extraction of the fragrance of aromatic plants (e.g. *Lavandula*, *Thymus*).

b) Problems of dosage versus toxicity

Some observations and problems were noted during this field research. The problem of plant toxicity and the way healers solve it seems to be essential in the medicinal plant business. Many medicinal plants are toxic at high doses. The problem of dosage was closely related to the problem of toxicity in a cause effect relationship.

Figure 2.12. Medicinal plant and condiment: *Capparis spinosa* L.

Capparaceae

(Boulos, 1983)

Some healers are still ignorant of the toxicity of some plants. The dosage is most often randomly assigned. Consequently dosage was extremely important and definitely influenced the effect of the plant used. It varied with the general state, age, allergy, sensitivity, etc of a patient, the prescription being modified according to these different factors. The problem of dosage-toxicity remains an urgent concern in Moroccan ethnomedicine.

This study addressed the issue of toxicity. For example, toxicity tests were performed on 80 medicinal plants during viral laboratory assays (see the Chapter IV, Antiviral activity and Cytotoxicity assays). These tests and the interviews revealed potential health hazards associated with the use of the medicinal plants investigated. By informing local healers about the toxic effects of some plants and the benefits of others, this research study could benefit the local people or communities. These results will eventually be disseminated to the public and will be highlighted in the planned booklet.

c) Problem of language

A problem was present with the use of one language (Arab/Berber) or the other in naming plants. Some confusion arose between ethnic groups and limited qualitative exchange of information.

d) Healing practice

It seemed that healers are very skilled and successful in their job and they usually healed their patients. They were also very good in following the health progress of their patient thoroughly and for the time needed. The same healers were also very open and taught their patients about their diseases. They were also good at interpreting the symptoms felt by them by making good use of pictures of human anatomy and explaining what happens if the patient suffered in any way. This last aspect had a psychological beneficial effect on their patient.

It was important to note the observation made by a healer in order to distinguish between a competent and less competent healer, namely the importance of making sure that the patient had no problem with her / his stomach and digestive tract. This was essential, as the administration route of most plants was most often by mouth.

e) Miscellaneous

My field experience with healers was extremely interesting and rewarding. Most of them were helpful and happy to contribute and be part of my project. An effort was made to approach mainly women. In few cases, where no women were available then men were interviewed.

Whenever possible, the exchange of information was mutual. I tried to share my own knowledge about plants, diseases, toxicity, and dosage. For example, on one occasion, one healer used only steam distillates as medicines. I felt the responsibility to explain to her that in this way, she lost other compounds that might be active and not water-soluble. She was happy to learn that and wished for more information in this regard for some particular plants.

The methodological approaches of my field research were kept practical and simple. The informal and spontaneous approach of the healers was preferred and more effective in the interview, life sharing, etc. Data were recorded on tapes (around 50) then sorted and analyzed. The analysis was presented as well as a summary of results and findings.

Endangered as well as rare plants were brought to the attention of those responsible so that they can be protected and saved. A particular effort was spent encouraging local people to cultivate the endangered and rare plants as well as the most interesting medicinal plants. Rare plants were collected in the field and cultivated in IAV Botanical Garden in Morocco. Some seeds were planted in UBC Botanical Garden.

New knowledge in ethnobotanical medicine was gained in my field research experience. Particularly the practice of healing from women with whom I spent a long time (one month). I also learned how to approach healers for an interview and obtain information efficiently without being abrupt. Respect of their knowledge, lifestyle and traditions was very important. They also needed to feel that the exchange was mutual and that they also learned from me.

The problems encountered in data collection activities were minimal. Two healers refused to cooperate and I did not have further discussion with them. Others did not want to be taped so their information was written. Furthermore, some of them preferred to remain anonymous and this was respected. Most of them expected a return of the research results in a written form or by oral communication.

Other activities were accomplished besides the interaction with healers. For example, an *Artemisia* distillation unit in a village near Taznakht (Ouarzazate, see map, Fig. 1.1) was visited, where the oil extracted from *Artemisia herba-alba*, is exported to Europe for pharmaceutical purposes. Another example, was the visit to Prof. Charrouf-Chaouni Cooperatives on *Argania spinosa* (locally known as "Argan"), near Essaouira at

Tidzi, Tamari, and Tiznit. In those cooperatives, financed by the International Development Research Centre, and other agencies and operated by local women, the fruits of argan trees are used to prepare an edible and marketable oil that provides up to 25% of the dweller daily lipid diet (Charrouf and Guillaume, 1999). As side product, a cake used to feed the cattle complemented the forage furnished by the leaves and fruits of this same plant. Argan tree, of the family Sapotaceae, is endemic in southwestern Morocco where it grows over about 320 000 square miles. This slow-growing and spiny tree, that may be either shrubby or up to seven or ten meters high has played an essential ecological function in this part of Morocco. It effectively protects the soil against heavy rain or wind-induced erosion and, by shading all kind of cultivations, maintains soil fertility (Charrouf and Guillaume, 1999; Bellakhdar, 1997). Because of its ability to survive to arid climate, it has contributed to slow down the desert progression. Furthermore, Argan trees also economically support indigenous populations. Furthermore, Argan oil, rich in saponines, is exported in Europe for soap industries as well as cosmetology uses.

In conclusion, the field trip was very fruitful in the evolution of my project. I tested two main approaches of collecting ethnobotanical data. In the first one, I spent longer time with a few women healers. In the second one, I met for a shorter time, a number of healers, from different backgrounds, tribes, and languages from all the regions of Morocco. Both approaches complemented each other. The first one was relevant to my learning and development of healing skills and the other approach was relevant to the exposure and interaction with a variety of healing practices.

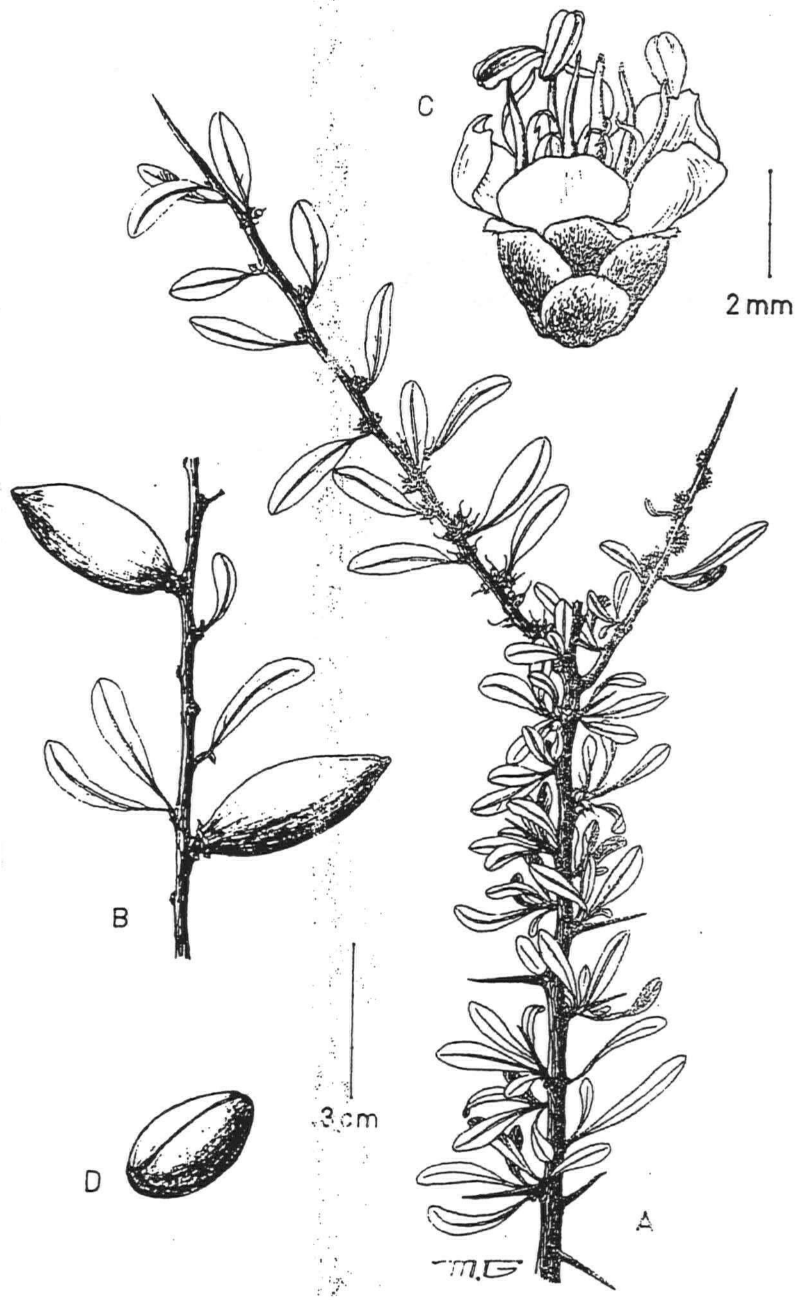


Figure 2.13. Medicinal plant endemic to Morocco: *Argania spinosa* (L.) Skeels. Sapotaceae. Marketable oil is extracted from the fruit. (Boulos, 1983)

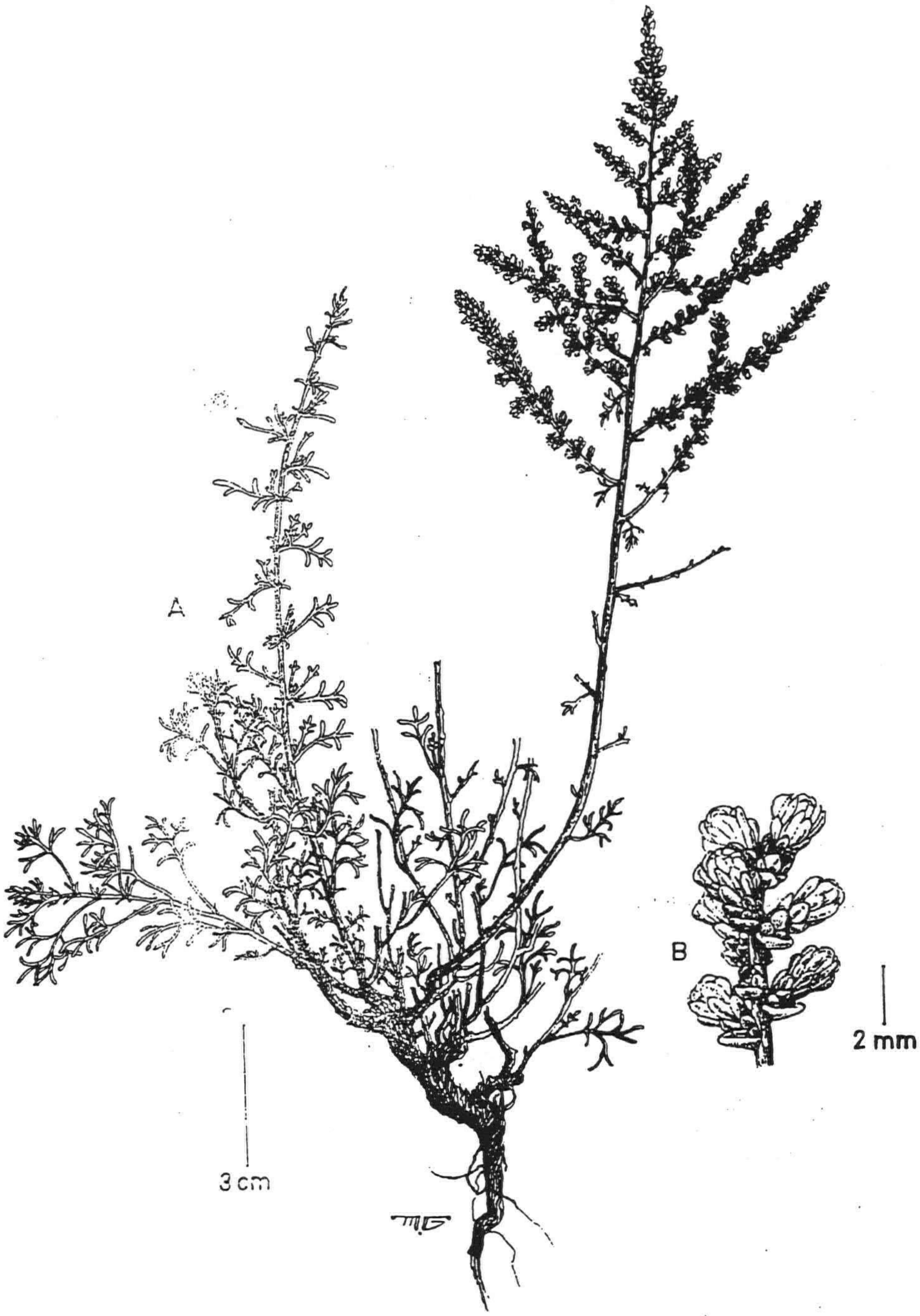


Figure 2.14. *Artemisia herba-alba* Asso

Asteraceae

(Boulos, 1983)

However some urgent issues remain. There is a need to raise the awareness of healers about the ethical problems related to their sharing of knowledge. There was a need to raise the awareness of women about the economical and social value of their healing ability. The issues of toxicity and dosage of medicinal plants remain the main concern in ethnomedicine.

It is worth mentioning the economical state of healers. Although they are usually well accepted in their society, they are generally not well paid. Usually, a visit to these healers was very cheap. In some cases if they were successful, they became popular, and people from all over the country traveled to them.

Women/men healers' educational role in society is extremely important, as "Lectures" were going on in homes, shops or market places where skillful healers were displaying their knowledge, teaching, warning and giving hope to people. The interest in my study was not confined to a few specialized persons but extended to large numbers of people. During my fieldtrips everybody seemed to have a genuine interest to learn more. It was also important to be critical and to distinguish between genuine and charlatan healers. This was achieved by comparison and consensus: only plants used consistently to treat the same illness by healers of a broad array of villages were selected (Cox and Balick, 1994; Cox, 1994). A study like mine was desperately needed as some healers tried to combine yesterday's knowledge with today's Science. This study may assist healers to update their knowledge and practice with medicinal plants. Consequently, the return of results is essential at the final stage of my study.

In Morocco as well as in some other Mediterranean countries like Italy, there are examples of plants that are considered to be a panacea active against all kinds of diseases, for example the horehound, *Marrubium vulgare* L. (Lamiaceae) is considered a universal remedy. People show great respect for these magical remedies. In Italy, a ritual is associated with the use of this plant for any kind of recovery or cure. When people are close to the horehound, they must kneel down three times, take off their cap, and recite the magical rhyme: [Hail to you my horehound friend, I came to pick you. So, if you can, take away the disease that (name of the sick person) has]. After the ritual, three leaves are picked to prepare an infusion, for the sick person to drink every morning for 7 days (Lentini, 2000).

Ethnobotanical research resulted some times in the discovery of some particular uses of plants, such as *Hyoscyamus albus* L. (Solanaceae), "Sikran", for example in my study, used as an anti-haemorrhoid; or *Salvia clandestina* L. (Lamiaceae), used to protect against the "evil-eye".

Ethnobotanists document and preserve traditional knowledge belonging to popular imagination, often an association of superstition and empirical know-how. But in fact, the contribution of ethnobotanists is important in the recovery of a rich heritage of traditional knowledge of medicinal plant uses, handed on orally and inevitably destined to get lost over the years (Schultes, 1994). For example, my study documents Berber ethnobotany. The contribution of ethnobotanists is also crucial in the singling out of new medicinal plants. For example, my study reported the interest of new medicinal plants (e.g. *Thymus maroccanus*). Through interdisciplinary expertise in chemotaxonomy, new active principles may also be singled out in species used only traditionally. Toxic aspects of some plants that induce poisoning, may also be pointed out by ethnobotanical research. Furthermore, ethnobotanists contribute in the conservation of rare and endangered plant

Figure 2.15. *Marrubium vulgare* L.

Lamiaceae

(Boulos, 1983)

species (Schultes, 1994). Consequently, ethnobotanical documentation represents a precious source of information.

This research was also multidisciplinary with aspects in ethnobotany, virology, microbiology and chemistry with a new social dimension in gender analysis, interviewing of healers, toxicity study. Similar studies, although not quite as extensive as mine, have been carried out in other parts of the world (Al-douri, 2000; Ali-Shtayeh et. al., 2000; Joshi and Joshi, 2000).

An ideal ethnobotanist was defined by Balick and Cox (1996) as a muldimensional expert: an "anthropologist, archeologist, botanist, chemist, phytochemist, psychologist, ecologist, explorer, folklorist, pharmacologist and diplomat." I may add other areas of expertise:a virologist, a microbiologist, a sociologist, a politician and ethicist, a humanist... Too many demands from one person.

- Ethical Issues

In order to protect the intellectual property rights of the Moroccans, different measures were taken. First of all, I followed the clause proposed by IDRC: **Biological Knowledge-Ethics**

""""The Centre supports the objectives of the 1992 Convention on Biological Diversity; in particular that of promoting the fair and equitable sharing of benefits arising out of the utilization of genetic resources. As this project may entail the use or dissemination of knowledge to which a person or group of persons may claim moral or legal rights, the awardee will not take steps which would prejudice such claims or diminish their value. In particular, the awardee will not profit or act in such away as to facilitate profit by third parties through the commercialization or dissemination of such knowledge without the concurrence of the Centre. Where the use or dissemination of such knowledge is envisioned, the awardee will ensure that the moral and/or legal rights of any potential claimants are respected, through consultation with them and compensation to them where available. This clause will endure beyond the termination of this agreement.""'

Beforehand, ethical considerations were taken in order to protect Moroccan intellectual proprietary rights (Elisabetsky, 1991; Grenier, 1998; ISE, 1998; Posey, 1990). Absolute adherence to Moroccan law was critical. Working with a local scientist Prof. Moh Rejdali was one example. He can represent ethical concerns of local and government communities in morocco. During my field research, Prof. Rejdali checked my plant collection removed endangered plants and agreed on the plants to be exported to Canada. For biodiversity and conservation purposes, rare plants collected were planted in the Botanical Garden of the Moroccan Institute.

UBC Behavioral Research Ethics Board approved this project. Each person approached for medicinal information signed a prior informed consent form (Fig. 2.6). Confidentiality was guaranteed to persons wishing to remain anonymous. Only public information given voluntarily by the people was recorded. Results will be made available as a booklet written in Arabic, French and English to be given to people who gave the information, to health authorities and other pertinent parties involved. All the people involved in this project were acknowledged in all the publications that resulted from this work (Thesis, publications, etc...). The booklet that will be available in the near future will be useful for the literate healers. However, during my consecutive field trips, I realized that most of the healers, particularly the women were illiterate. Thus there will be further contact to report the results orally. I believe this will make a most useful contribution to the health of local rural peoples in Morocco (Anderson and Towers, 2000). Moreover, knowledge of social, economic and political context of medicinal plant research was carefully studied in the literature and other published material as it relates to ethical aspects (Grenier, 1998; Elisabetsky, 1991; Posey, 1990). Literature from Morocco, relevant to the project, was consulted as there are many ancient and modern books and other publications in French and Arabic (Avicenna, 1980; Al Antaque, 1997; Bellakhdar et al., 1991; Bellakhdar, 1997; Benjilali et al., 1997; Campbell, 1904-1905; Ibn Al Baytar, 1448; Ghazanfar, 1994; Levey, 1973; Rejdali and Birouk, 1996; Renaud and Colin, 1934; Siouti, 1994; Zahwelee, 1997). It was very useful for this research as well as the exchange of information with local scientists working on similar projects (e.g. Prof. Zoubida Charrouf, Prof. Rejdali and many others).

Conclusion

In summary, this original study resulted in a possible validation of medicinal plant applications, usefulness to local people, and emphasized the role of women in Moroccan phytotherapy etc.

The significance of this research lies in documenting and showing the value of Berber and Arab Moroccan ethnobotanical medicines. The rich and varied ethnic background of Morocco increases our chances of obtaining something novel. Although the Arab ethnomedicine had been well studied, I believe there is still a lot to discover.

Berber ethnomedicine is definitely less studied particularly for rare endemic species, located in the Mountains or Sahara. This aspect hopefully could lead to the finding of new drugs for infectious diseases.

I hope that the results of this multidisciplinary study will have short- and long-term impacts on the health and development of the people in the region. The availability of local pharmacopoeia supported by scientific data could be beneficial to large populations who do not have access to modern drugs.

Conservation of rare & endangered medicinal plant species (e.g. *Dracaena draco* L. subsp. *Ajgal* Benabid et Cuzin (Benabid and Cuzin, 1997) has resulted through their cultivation by local people (private & public gardens, botanical garden of the Moroccan Institute). So, the availability of endangered medicinal plants will be assured and will contribute to the health of local people. Healers, local communities, particularly poor people will reap long-term health benefits from this research as well as Canadian-Moroccan research collaboration.

In addition women will benefit from this study by the communication of the scientific results, by the attempt to raise their awareness concerning their valuable role in the society as family and community doctors. They also benefit by being informed and educated about the ethical issues involved with practicing and knowledge sharing.

The exchange of scientific information for traditional knowledge seemed a fair deal at this moment because all results and information from this study were shared with the local peoples, the collaborative team and the public, and health authorities as well as the scientific communities (publications, local and national, international symposia).

Furthermore, validation of local medicinal plant uses and their potential toxicity through inappropriate usage also resulted from this study. As some medicinal plants are also used as food (fruits), or as condiments in food (spices), information was also relevant for these.

As an overall assessment, finally, the investments of time, effort, and funding for this project were valuable to emphasize the role of women in healing as well as to validate the uses of medicinal plants and at the same time draw people's attention to the toxicity of some plant remedies. Hopefully this will contribute to the appropriate use of medicinal plants. Particular attention was given to women healers and other practitioners, so that only efficacious and safe medicinal plants were used while discouraging the use of ineffective and potentially toxic ones. Raising women's awareness about the economic, social value of their practice as well as about ethical issues related to knowledge sharing was crucial. Of equal importance was the impact to modern world that these results might have if a new antiviral drug was discovered.

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CHAPTER III

Antimicrobial Activities in Selected Plant Species

Introduction

Infectious diseases are a leading cause of premature deaths, killing almost 55, 000 people every day. Drug resistance to human pathogenic bacteria has been widely reported in recent years (Piddock and Wise, 1989; Mulligen et al., 1993; Robin et al., 1998). The indiscriminate use of antibiotics in developing and developed countries is alarming. In immunocompromised AIDS and cancer patients, drug-resistant bacteria and fungal pathogens have complicated treatment strategies (Rinaldi, 1991; Diamond, 1993; David, 1994.). Interest in the discovery of new antimicrobial substances from other sources, such as plants, has increased since the emergence of multiple drug resistance in human pathogenic organisms.

In developing countries, unsanitary conditions e.g. contaminated water; overcrowding, poverty and poor nutrition may lead to many sites of infection. Although these conditions are not as prevalent in the West, microbial infections are still common. Particularly, hospital-acquired, or nosocomial infections are problematic in developing as well as in developed countries. According to the Center for Disease Control in the United States, approximately 5-15% of all hospitalized patients develop a nosocomial infection, with 20, 000 dying each year (Tortora et al., 1989). Urinary tract infections resulting from urinary catheterization, are the most common nosocomial infections. Cutaneous and surgical wounds as well as lower respiratory infections are also very common (Neu, 1993). Methicillin-resistant *Staphylococcus aureus* and multi-resistant *Pseudomonas aeruginosa*, microorganisms used in this study, are major problems in hospitals, because strains have developed which are resistant to all known antibiotic agents. *Staphylococcus aureus* induces pneumonia, bacteraemia and infects surgical wounds. *Pseudomonas aeruginosa* is responsible for urinary tract infections, bacteraemia and pneumonia (Neu, 1993). Table 3.1 lists some of the common nosocomial infections and their associated human pathogens.

Medicinal plants used traditionally provide a variety of compounds of known therapeutic value (Harborne and Baxter, 1995). The strongest candidates for developing new antimicrobial drugs are the compounds that either inhibit the growth of pathogens (bacteriostatic), or kill them outright and display the least toxicity to host cells (bactericidal). Many antibiotics (Berdy et al., 1982) and antifungals (Grayer and Harborne, 1994) have been isolated from plants. They present a variety of aliphatic and aromatic chemical structures and include carbohydrates, quinones, lactams, peptides and terpenoids (mono-, di-, sesqui-, terpenes lactones, steroidal alkaloids and steroids). Examples include chlorochimaphilin, a chlorinated naphthoquinone from *Moneses uniflora* A. Gray (Ericaceae) (Saxena et al., 1996) and 6-O-methylacrylylplenolin, a sesquiterpene lactone isolated from *Centipeda minima* (L.) A. Br.& Aschers, that are active against *Bacillus subtilis* and *Staphylococcus aureus* (Taylor, 1996).

Table 3.1. Examples of Hospital-acquired pathogens

Nosocomial Infection	Pathogen
Pneumonia, bacteraemia and surgical wounds infections.	*Staphylococcus aureus*
Candidiasis, skin infections.	*Candida albicans*
Gastrointestinal inflammations and neonatal meningitis.	*Escherichia coli* and *Klebsiella pneumoniae*
Urinary tract, burn and surgical wound infections, pneumonia	*Salmonella typhimurium* and *Pseudomonas aeruginosa*

Towers and Hudson stressed the need to test for light-mediated activities when screening plants for antimicrobial or antiviral activities (Hudson et al., 1993a, 1993b; Hudson, 1995; Hudson et al., 1999; Towers et al., 1997). Previous studies may have missed potentially important activities because of this lack of consideration (Hudson and Towers, 1999). The presence of natural photosensitizers in plants explains the light-dependent or light enhanced activity. When excited by light, some antimicrobial and antiviral chemical compounds exhibit toxicity towards living cells or organisms causing their damage or death (Hudson and Towers, 1999; Towers et al., 1997). For example, cells, organisms and biologically important molecules can be damaged or destroyed when incubated in the light in the presence of photosensitizing compounds such as acridine orange, riboflavin, phenylthiazines and furocoumarins such as 8-methoxy-psoralen (McKenna and Towers, 1981). One of the primary determinants of photoactivity in these compounds is related to the degree of unsaturation of the photosensitizer (1-2 double bond, or triple bonds). Phototoxicity or light-mediated (or photodynamic) biological activity describes this phenomenon. Photosensitizers (phototoxins) are natural compounds involved in this process. Photosensitizers may be activated by wavelengths of light ranging from near UV to red, depending on the chromophores of the photosensitizer. Ultraviolet-A (UV-A) (320-400 nm) and visible radiation, unlike the shorter wavelengths of UV-B (290-320 nm) and UV-C (200-290 nm) do not cause direct damage to cells (often DNA damage) unless compounds absorbing at these wavelengths are accumulated in them.

Natural photosensitizers include naphtho- and anthra-quinones, porphyrins, derivatives of chlorophyll, certain polyketides or polyynes (e.g. thiarubrines) present in members of the Apiaceae and Asteraceae. Thiophenes (Towers, 1981), their sulfur-derivatives, cinnamate derivatives, aromatic alkaloids or alkaloids based on tryptamine such as harmine (*Peganum harmala*, Zygophyllaceae), or phenylalanine and tyrosine such as berberine, are present in the Ranunculaceae (Hudson and Towers, 1991). Sanguinarine (Papaveraceae) or anthranilic acid (furanoquinoline, dictamnine (Rutaceae) (Downum and Towers, 1983), and furanocoumarins (8-methoxypsoralen, Rutaceae) (Apiaceae and Asteraceae), furochromones (visnagin, *Ammi visnaga*, Apiaceae, Fabaceae), are other examples of natural photosensitizers (Towers et al., 1997). All these compounds share a characteristic that gives them this ability. They have chromophores or chemical structures that are activated by light. Particularly the presence of double bonds, or triple bonds (Towers et al., 1997).

Specific glandular structures such as trichomes or laticifers sometimes store photosensitizers. For example, in rutaceous species, furocoumarins may be stored in different parts of the plant including resin canals and cuticle. The mechanism of action of phototoxic compounds is complex and varied. Quinones, polyynes and thiophenes act as photooxidants, producing radicals, which are oxidized to toxic superoxide anion or singlet oxygen (Towers et al., 1997). Furocoumarins interact with nucleic acids (RNA & DNA) and form photo-adducts or cross-linkages when irradiated with UV light resulting in DNA breaks and thus inhibiting DNA, RNA and protein synthesis.

The major diseases addressed by Moroccan traditional medicine are related to digestive pathology (stomach & intestine), skin and hair-care, bronchi-pulmonary, urinary system and liver disorders, gynecologic as well as dental diseases (Bellakhdar et al., 1991; Bellakhdar, 1997; Boulos, 1982; Maire, from 1952 to 1980).

The aim of the present investigation was to evaluate the antimicrobial activities of methanol extracts of the following Berber and endemic medicinal species: the black seeds of *Nigella sativa* L. (Ranunculaceae), the aerial parts of *Ammoides pusilla* (Brot.) Breistr. (Apiaceae), *Lavandula angustifolia* Mill., and *Mentha pulegium* L., as well as the stem and leaves of *Thymus broussonetii* Boiss. (Lamiaceae). These plants were chosen because they were used particularly against oral infections. Assays were performed in the presence and absence of light, since many antimicrobial compounds are photosensitizers (Hudson, 1995; Towers et al., 1997). Ethnobotanical information on these species was also obtained.

Materials and Methods

Plant material

Fresh seeds of *Nigella sativa* L., collected by Moroccan herbalists, were purchased from stores in Rabat, Morocco, and analyzed two months later. *Ammoides pusilla* (Brot.) Breistr. (Apiaceae), *Lavandula angustifolia* Mill., *Mentha pulegium* L., and *Thymus broussonetii* Boiss. (Lamiaceae) were collected in the field in Morocco. They were identified at the Department of Botany, IAV Hassan II (Rabat, Morocco) and further checked by using Moroccan floristic documents and herbarium specimens. Voucher specimens were filed in herbaria of the Moroccan Institute Agronomic and Veterinarian Hassan II, Rabat, and the University of British Columbia. The plant specimens were air dried in the shade; ground and the powders kept in paper bags until used. Small aliquots (200 g) were used for antimicrobial analysis.

Plants were selected on the basis of their medicinal use by Berber and Arab people as determined in questionnaires and interviews addressed to local healers and other knowledgeable people. Most species selected were used as panacea, to treat cuts, wounds, colds, coughs, diarrhea, dysentery, fever, sore throat and skin problems such as rashes, poxes, as well as other infectious diseases. Of particular interest, those plants were also used traditionally in dental infections.

Preparation of Methanol Plant Extracts and Discs for microbial Assays

Plant material was airdried and ground in a domestic grinder. A 100-200 g sample of ground material was soaked in 100-200 ml methanol (MeOH) for 24 h. The sample was then suction-filtered through Whatman No.1 filter paper and washed with 200ml of MeOH. The filtrate was evaporated to dryness under reduced pressure. The samples were freeze-dried for at least 48 h. They were dissolved in MeOH at a concentration of 1mg/ml. Paper discs were impregnated with 20-μl (20μg) of this extract and allowed to dry at room temperature.

Eight strains of bacteria and two strains of fungi were used: Gram-positive bacteria: *Bacillus subtilis, Staphylococcus aureus* methicillin sensitive and methicillin resistant, and *Streptococcus faecalis*; Gram-negative bacteria: Escherichia coli, Klebsiella pneumoniae, Mycobacterium phlei, Pseudomonas aeruginosa; Fungi: Candida albicans, Trichophyton mentagrophytes. All cultures were standard laboratory strains from The University of British Columbia microbiological collection, Department of Microbiology.

An inoculum of each bacterial strain was suspended in 5 ml of Muller-Hinton broth (commercial brand, BBL) and incubated overnight at 37°C. The cultures were diluted 1/10 with Müller-Hinton broth before use.

The preparation of fungal cultures consisted of swabbing the parent plate with a cotton swab, and then transferring the inoculum to a vial containing 5 ml of Sabouraud dextrose broth (BBL).

Antibiotic/antifungal assays

The disc-diffusion assay (Lennette, 1985) was used to measure antibiotic and antifungal activity. Sterile Müller-Hinton agar plates (for bacteria) or sterile Sabouraud dextrose agar plates (for fungi), were inoculated with 100µl of the diluted culture. Discs impregnated with 20µg of plant extract were placed on the agar and incubated for 30 min in order to facilitate diffusion. Positive controls (for bacteria) were Gentamicin (10 µg per disc), and Fungizone (25 µg per disc) (for fungi). A negative control of MeOH (20 µl per disc) was used.

In order to test for light-mediated activities, three replicates were made. One was exposed to ultraviolet (UV)-A light (5 W/m^2 320-400 nm, with a maximum emission around 350 nm, from four Sylvania F20T12-BLB lamps) for two hours at 30°C. The second replicate was treated with visible light from fluorescent lamps for two hours at 30°C, while the third replicate was kept in the dark. The plates were incubated for 18 h (48 h for *Mycobacterium phlei*) and for varying times with *Candida* before the zones of inhibition were observed and recorded. Tests were repeated three times.

Results

Ethnobotanical results

In Morocco, *Nigella sativa* L. (Ranunculaceae), also called (Sanuj) is a generalist medicinal plant used for diverse ailments. For cough and pulmonary infections (asthma), the prescription recommended by herbalists and traditional healers is 3-5 drops of Sanuj oil, taken with tea or coffee. For liver ailments or heart pain, the seed powder is taken with honey. *N. sativa* seeds are also used to treat influenza, allergy, hypertension, and stomachache.

The aerial parts of *Ammoides pusilla* (Brot.) Breistr. (Apiaceae) are used in Moroccan traditional medicine for colds and fever and dental ailments. The aerial parts of *Lavandula angustifolia* Mill. (Lamiaceae) are a panacea and used in Moroccan traditional medicine for many ailments such as gynecologic infections, intestinal worms in children, rheumatism and sinusitis, colds and fever. The plant is also popular in perfumes and "pot pourri". The aerial parts of *Mentha pulegium* L. (Lamiaceae) are used in Moroccan traditional medicine for bronchitis and colds and in mouth hygiene. The stem and leaves of *Thymus broussonetii* Boiss. (Lamiaceae) are used in Moroccan traditional medicine for colds, as a wound antiseptic, indigestion and as a food preservative.

Table 3.2. Antimicrobial activities of selected plant species

Microorganisms	Species				
	N. L/D[1]	*A.* L/D	*L.* L/D	*M.* L/D	*T.* L/D
Gram-positive bacteria					
Bacillus subtilis	+/+ [2]	+/+	_/_	_/_	+/+
Staphylococcus aureus					
*methicillin sensitive	+/+	+/+	+/+	+/+	+/+
*methicillin resistant	+/+	+/+	+/_	+/+	+/+
Streptococcus faecalis	_/_	_/_	_/_	_/_	_/_
Gram-negative bacteria					
Escherichia coli	_/_	+/+	_/_	_/_	_/_
Klebsiella pneumoniae	+/+	+/+	+/+	+/+	_/_
Mycobacterium phlei	+/+	+/+	+/+	+/+	_/_
Pseudomonas aeruginosa	_/_	_/_	+/+	_/_	_/_
Fungi					
Candida albicans	_/_	+/+	_/_	+/_	_/_
Trichophyton mentagrophytes	_/_	+/+	_/_	_/_	_/_

[1]Abbreviations: L/D, light/dark. *N., Nigella, A., Ammoides, L., Lavandula, M., Mentha, T., Thymus.*
[2]Indicated by zone of inhibition in disk assay: +, Extract active;
--, no activity observed.
Organism resistant to all extracts: *Streptococcus faecalis*.

Results of Antibiotic Testing

Table 3.2 summarizes the antibiotic data. Seed extracts of *Nigella sativa* were active against *Bacillus subtilis* and methicillin sensitive and resistant *Staphylococcus aureus* as well as *Klebsiella pneumoniae* and *Mycobacterium phlei*. Seed extracts were inactive against *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* and against the fungi, *Candida albicans* and *Trichophyton mentagrophytes.* The active compounds of this plant are not photosensitizers because the activity in dark and light were the same.

Ammoides pusilla (Fig. 3.1) had a broad spectrum of activity. The crude extract was active against *Bacillus subtilis,* methicillin sensitive and resistant *Staphylococcus aureus, Mycobacterium phlei, Candida albicans, Trichophyton mentagrophytes, Escherichia coli* and *Klebsiella pneumoniae.* However, this species was inactive against *Streptococcus faecalis* and *Pseudomonas aeruginosa.* The active compounds of this plant are not photoactive as the activity in dark and light were the same.

Lavandula angustifolia had also a broad spectrum of activity (active against five microorganisms). The crude extract was active against *Staphylococcus* aureus-methicilin sensitive and resistant, *Pseudomonas aeruginosa, Mycobacterium phlei, Klebsiella pneumoniae.* However, the plant was inactive against *Bacillus subtilis, Streptococcus faecalis, Candida albicans, Trichophyton mentagrophytes* and *Escherichia coli.* The plant contained a photosensitizer as the activity against methicillin sensitive and resistant *Staphylococcus aureus* was light activated.

A crude extract of the aerial parts of *Mentha pulegium* was active against methicillin sensitive and resistant *Staphylococcus aureus, Mycobacterium phlei, Candida* *albicans* and *Klebsiella pneumoniae.* However, the plant was inactive against *Bacillus subtilis, Streptococcus faecalis, Pseudomonas aeruginosa, Trichophyton mentagrophytes* and *Escherichia coli.* The plant contains a photosensitizer as the extract was activated by light against *Candida albicans*.

A crude extract of the stems and leaves of *Thymus broussonetii* was active against *Bacillus subtilis* and methicillin sensitive and resistant *Staphylococcus aureus,* but inactive against *Streptococcus faecalis, Pseudomonas aeruginosa, Mycobacterium phlei, Candida albicans, Trichophyton mentagrophytes, Escherichia coli* and *Klebsiella pneumoniae*.

Discussion and Conclusion

The results shown in Table 3.2 indicate that some of these plants are potential medicines against infectious diseases caused by microbes. Most of the extracts had some antibiotic activities, although the spectrum of sensitivities varied. Selective activities were shown by *Thymus broussonetii.* However, from the point- of- view of medicinal plant applications, extracts with broad-spectrum activities and minimal cytotoxic effects might be more important. Their discriminatory effect against specific microorganisms suggests the presence of different chemical compounds. These differences may also be attributed to the difference of the structure of the cell wall in gram-positive bacteria (Yao and Moellering, 1995). In addition certain activities required UVA, or were enhanced by UVA, indicating the presence of a photosensitizer. Thus light is a determining factor in the activity of photosensitizers and should be definitely taken into account in this kind of test. Furthermore, *Streptococcus faecalis* bacterium was unaffected by all the extracts tested.

No information on the nature of the bioactive phytochemicals is shown from these results. However, it is worth noting that because of the presence of so many different combinations of microbial targets for different extracts, together with indications of photosensitizers in some cases, it would appear that different types of compounds are involved. Although the bioactive compound is not known, one may speculate on the probable candidate from these results based on plant species family, microbial target, presence or otherwise of photosensitizers and other factors. Two extracts *Lavandula angustifolia* and *Mentha pulegium* were inactive in the dark, but were activated by light treatment. These light enhanced activities could be due to the presence of photodynamic compounds (Towers et al., 1997).

It is interesting to attempt to correlate the traditional applications of the plant extracts with the microbial targets. In some cases this is feasible, e.g. *Mentha pulegium* and *Ammoides pusilla* are both used to treat skin diseases and dental problems. Both of them were active against *Candida*. However, such correlations were not apparent in other cases. This may indicate that some extracts contain other kinds of bioactive phytochemicals, in addition to or instead of direct-acting antibiotics. They may also stimulate the host immune system. Neither ethnobotanical information nor biological activities were previously reported for *Ammoides pusilla*. This is a novel and interesting result. Very few chemical analyses were reported. One example, cynaroside (flavonoid), quercetin-3-o-β-D-glucuronide was reported in the leaf and stem from a species from Egypt (Saleh et al., 1983).

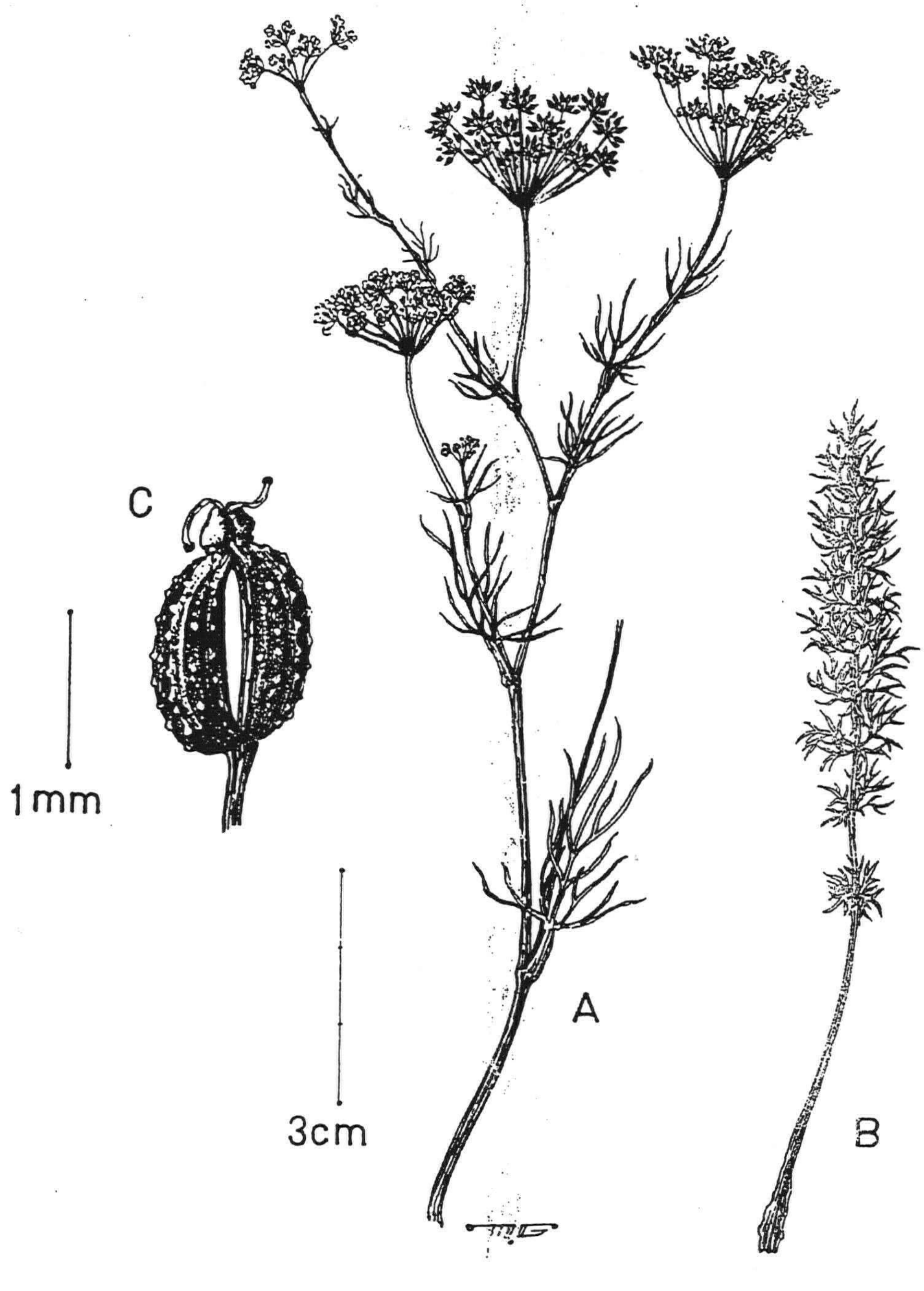

Figure 3.1. *Ammoides pusilla* (Brot.) Breistr.

Apiaceae

(Boulos, 1983)

Three extracts, *Lavandula, Nigella* and *Ammoides* had a broad spectrum of activity. These results suggest the presence of either good antibacterial potency or high concentrations of an active principle in the extract. These plants could be useful in antiseptic and disinfectant formulations as well as in chemotherapy (Olukoya et al., 1993). The results also confirm the use of these plants in traditional medicine. The selection of a plant for evaluation based on its use in ethnomedicine is an important criterion and a useful predictive factor in identifying new anti-infective agents (Balick and Cox, 1996; Cox, 1994; Cox and Balick, 1994).

The crude extract of the stems and leaves of *Thymus broussonetii* was active against Gram positive bacteria but inactive against Gram negative bacteria. This is in agreement with the general expectation that a much greater number of extracts are active against the Gram positive bacteria (McCutcheon et al., 1992). *Thymus capitatus* L. has been found to contain thymol, an essential oil and phenols which possess antibacterial activity (Benouda et al., 1988; Benjilali et al., 1986). One may predict that probably the same compounds are involved in the antimicrobial activity of *T. broussonetii*. This may provide an interpretation for the use of *T. broussonetii*, as an infusion, in the treatment of bacterial infection in Moroccan traditional medicine.

Seed extracts of *Nigella* were inactive against *Streptococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli* and against the fungi, *Candida albicans* and *Trichophyton mentagrophytes.* Seed extracts of *Nigella* were active against a number of bacteria, for example *Staphylococcus aureus*. This latter activity (20 μg/disc) reported here is higher than the one (400μg/disc) reported by Hanafy and Hatem (1991). Thymohydroquinones have been detected by electron spin resonance in *Nigella sativa* L.

They are known to have antimicrobial activity (Mouhajir et al., 1999; El-Alfy et al., 1975). The pharmacological properties of the oil support the traditional use of *Nigella* as a generalist medicinal plant. The results of this investigation show that it is active against a number of bacteria at low concentrations of a crude extract. However, the black seeds are inactive against fungi, and this might explain why the seeds are not prescribed for skin diseases caused by fungi.

The selection of plants for antimicrobial purposes by Berber and Arab peoples has clear association with activity. The traditional medicinal knowledge acquired by these people shows an understanding of species and plant parts that can be used to treat conditions associated with microbial infection. In the results shown in Table 3.2, all the plants tested are active against one or several microbes. In both the bacteria and fungi as well as viruses tested the preferred plant parts and preferred species exhibited greater antimicrobial activity than less preferred sources. This correlation indicates knowledge of medicinal plants that can be equated to an understanding of aspects of chemical ecology.

In recent years there has been an increase in incidence of antibiotic resistance in these pathogenic organisms, and the persistence of pathogens in immune-compromised individuals is of great concern (Georgopapadakou and Walsh, 1994). Some of these plant extracts may yield pure substances appropriate for the commercial pharmaceutical stream. The search for new antimicrobial substances exhibiting minimal side effects is warranted since many of the drugs currently in use result in adverse side effects and some are harmful. Antibiotics are sometimes associated with adverse effects that include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions (Idsoe et al., 1968, Kandil et al., 1994). The studied medicinal plants therefore could provide a safe and inexpensive alternative to many current synthetic drugs.

The standard diffusion disc assay (Barry, 1976; Brantner et al., 1994) used in this study for crude methanol extracts, although easy and reproducible is not quantitative. Due to the qualitative nature of the antibiotic disk assay, it is difficult to make conclusions about relative potencies. However, purified compounds may be analyzed by the minimum inhibitory concentration assay (MIC). The latter assay is quantitative and will also give information about the bactericidal or bacteriostatic state of pure compounds. However, it is not practical to isolate active compounds from a large number of plants studied.

Because the available bioassay is not quantitative, this was one major reason why tests on other plant species were not pursued any further. Furthermore, some potential draw-backs of the disc-diffusion assay for natural products relate to the diffusibility of compounds or lack of quantitative results. A technique that could be used to obtain more quantitative data is the minimum inhibitory concentration assay (MIC), but it is only useful for purified compounds.

There are a number of quite potent proteinaceous antimicrobial natural products known. Steps could be taken in the preparation of test samples to separate small molecule natural products from macromolecules, for example microfiltration e.g. at 0.4 microns.

Some of the plant species selected for this antimicrobial study are used on dental microbes. *Ammoides* and *Mentha* species showed activity against *Candida albicans*, which is also a dental pathogen. It would have been relevant to test these against oral bacteria, if the opportunity was available or time allowed it. The most common oral infectious diseases are dental caries (*Streptococcus mutans* and *S. sobrinus*) and periodontitis (streptococcal species such as *S. oralis*, *S. rattus*, *S. cricetus*, *S. salivarius*, *S. mitis*, *S. sanguis*, *S. milleri*, *S. constellatus* are aerobes) and Gram negative anaerobes such as *Prevotella oris*, *P. buccae* and *P. intermedia*). Even though antibiotics prevent and treat dental caries, they have considerable side effects such as the disturbance of oral and gut flora ecosystem. Moreover, streptococci including *Steptococcus mitis*, *S. sanguis* and *S. mutans*, the most representative human cariogenic bacteria, are resistant to antibiotics (Leclercq et al., 1988) and other antimicrobial agents like chlorhexidine (Jarwinen et al., 1993). Thus, antimicrobial phytochemicals could be effective and promising in the prevention and therapy for oral infectious diseases. Didry et al., (1998, 1994), reported the presence of the active compounds, naphthoquinones against oral bacteria, in the genus *Drosera* (Droseraceae) (insectivorous herbs). Similarly, *Juglans regia* L., a plant species from Morocco (see Chap IV) for antiviral activity, is known to contain juglone (naphthoquinone), which I speculate to be active against dental bacteria. The same speculation can be made for *Lawsonia* (lawsone, naphoquinone) as well as leaves of *Olea europea* that have been used for dental hygiene and treatment for centuries in the Arab world and particularly in Morocco.

Synergistic studies could have been interesting to do as the activity of many plants is increased when mixed with others (Salomi et al., 1991). Furthermore, in Moroccan traditional medicine, many mixtures made with up to 55 plant species are prescribed by healers.

Recent studies conducted in Towers' and Hudson's laboratories, and elsewhere, have revealed that traditional plant medicines from various parts of the world can provide a rich source of antiviral and antimicrobial activities (NAPRALERT, 1975-1998). Such types of study have often been justified in the context of phytochemical leads for pharmaceutical development. The ability to correlate ethnomedical reports with corresponding scientific studies could lead to improved selection of plants for further study in the areas of arthritis, cancer, diabetes, epilepsy, hypertension, malaria, pain and fungal and viral infections. This combination of analysing ethnomedical information and published scientific studies on plant extracts (ethnopharmacology) may reduce the number of plants that need to be screened for drug discovery attempts, resulting in a corresponding greater success rate than by random selection and mass bioscreening.

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CHAPTER IV

Antiviral Activities of Endemic Medicinal Plants Used by Berber Peoples of Morocco

Introduction

The development of an infectious viral disease in an individual involves a sequence of interactions between the virus and the host. These include entry of the virus, invasion and colonization of host tissues, evasion of host immunity mechanisms, and tissue injury or functional impairment. Many features of microorganisms determine their virulence, and many diverse mechanisms contribute to the pathogenesis of infectious diseases (Fields et al., 1996; White and Fenner, 1994).

Viruses are obligatory intracellular microorganisms that replicate within cells, using the nucleic acid and protein synthetic machineries of the host. Many viruses enter host cells by binding to physiologically important, normal cell surface molecules. Three well-known examples are (1) human immunodeficiency virus-1 (HIV-1), which binds to the CD4 molecule on human T cells; (2) Epstein-Barr virus (EBV), which binds to the type 2 complement receptor (CD21) on human B cells; and (3) rhinovirus, the agent of the common cold, which binds to intercellular adhesion molecules (ICAM-1, or CD54) expressed on a variety of cell types, including airway epithelium (Fields et al., 1996; White and Fenner, 1994).

After entering cells, viruses can cause tissue injury and disease by any of several mechanisms. Viral replication interferes with normal cellular protein synthesis and function, leading to injury to and, ultimately, death of the infected cell. This is one type of cytopathic effect of viruses, and the infection is said to be "lytic" because the infected cell is lysed. Noncytopathic viruses may cause latent infections during which they reside in host cells and produce proteins that are foreign to the host and stimulate specific immunity. As a result, infected cells are recognized and killed by viral antigen-specific cytolytic T lymphocytes (CTLs). Released viral proteins may also stimulate delayed type hypersensitivity (DTH) reactions. In these situations, cell injury is a direct consequence of physiologic immune responses to the virus. Relatively little is known about the pathogenic mechanisms in many viral infections (Fields et al., 1996; White and Fenner, 1994).

There are two principal mechanisms of natural immunity against viruses:

1. Viral infection directly stimulates the production of interferon by infected cells. This cytokine-induced "antiviral state" functions to inhibit viral replication.

2. Natural killer (NK) cells lyse a wide variety of virally infected cells.

In addition, complement activation and phagocytosis serve to eliminate viruses from extracellular sites and from the circulation (Fields et al., 1996; White and Fenner, 1994).

Viruses are not autonomous organisms and need a host to survive and replicate their genomes and propagate their progeny. Despite the vigorous and hostile host immune system towards viruses, these microorganisms are effective in infecting and successfully replicating in their host. How do viruses achieve this? Do all viruses have the same means of neutralizing the host immune defenses?

Herpesviruses (HSV, EBV), adenoviruses, poxviruses, and cytomegaloviruses, all have their own strategy in order to escape the host immune defenses. Influenza viruses and HIV have escaped by antigenic variation. Large DNA viruses, such as poxviruses, adenoviruses, and herpes viruses, encode proteins that neutralize host weapons *in vivo* (e.g. neutralizing apoptosis is a strategy used by HSV, EBV). Herpes viruses establish latent infection in immunoprivileged sites (e.g. central nervous system) and survive this way to the threat of the host defenses. Escaping CTLs as well as NK cell is the way CMVs as well as adenoviruses deal with the immune system. Capturing cytokines and chemokines is the strategy used by HSV and poxviruses in the fight against host immunity. Finally, interfering with interferon is another strategy used by poxviruses in order to survive in the host (Hill et al., 1998).

The immunomodulatory molecules encoded by viruses provide new insights for therapies, and for more understanding of the immune system as well as the advancement of research in the prevention and treatment of infectious diseases.

Viruses have left no fossil record; hence we must rely on scrutiny of existing viruses for clues to viral origins and evolution. The origin of viruses is still unknown: maybe they evolved originally from DNA or RNA already present in a cellular organelle or chromosome, or from some form of intracellular parasite such as a bacterium, or from protoviruses.

The Body's Defenses

Some of the body's defenses include mechanical barriers such as skin, membranes and mucus, chemical barriers (such as tears and the juices in the stomach), and bacterial barriers (which are the presence of productive bacteria that counter-balance the harmful ones). Another defense is the production of both white blood cells that surround and attack harmful bacteria and antibodies that kill or weaken the invaders. The body has thousands of different antibodies, each capable of weakening or destroying a certain disease. Certain antibodies can neutralize toxins from the microorganisms, and if the body doesn't produce enough of these antitoxins, a physician may inject more antitoxins. T-cells in the body protect against viruses that grow inside human cells. The body "remembers" these battles because the antibodies remain in the body for long periods of time, which produces immunity to the disease. The body also may become feverish, which increases the rate of the immune system's reactions, speeds up white blood cells, and may kill or weaken the dangerous invaders.

Viral Infections

In order to get rid of a virus, the cell, which has been invaded by the virus, must be killed, which results in damage to the cells themselves. For this reason doctors can only control the symptoms of a viral infection, but have not found cures. When viruses invade the body, the immune system releases white blood cells. These cells produce antibodies, which cover the virus's protein coat and prevent it from attaching itself to the cell. White blood cells also destroy infected cells and thus kill the viruses before they can reproduce. Unfortunately, some viruses such as measles, influenza, and HIV (AIDS) suppress the immune system.

Viruses are dangerous because they are infectious and may spread easily and cause an epidemic that can further develop into a pandemic. Mad cow disease caused by prions, as well as foot and mouth diseases are examples of damage caused by uncontrollable viruses or prions. Influenza viruses may be very serious killing large numbers of people in the course of a short time. First isolated in 1933, flu viruses have been since subdivided into types A, B, and C. Type A is the most prevalent and is associated with the most serious epidemics, being transmitted from animals to humans. Types B & C are transmitted among humans (Webster et al., 1982; Webster, 1995).

Influenza, commonly known as flu, is an acute respiratory infection caused by a variety of influenza viruses. It differs in several ways from the common cold, a respiratory infection also caused by viruses. It is usually signaled by headache, chills, and dry cough, which are followed rapidly by body aches and fever. Typically, the fever starts declining on the second or third day of the illness. It is then that upper respiratory symptom including nasal congestion and sore throat become noticeable.

Viruses that cause flu spread primarily from person to person, especially by coughing and sneezing (via airborne droplets of respiratory fluids). Flu viruses can enter the body through the mucous membranes of the eyes, nose, or mouth. After a person has been infected with the virus, symptoms usually appear within 2 to 4 days. The infection is considered contagious for another 3 to 4 days after symptoms appear. The greatest risk of infection is in highly populated areas, where people live in crowded conditions, and in schools.

Besides the rapid onset of the outbreaks and the large numbers of people affected, flu is important because of the seriousness of the complications that can develop. These complications (which can either accompany or follow the illness) generally result from bacterial infections in the lower respiratory tract. *Pneumococcal* bacteria usually cause the ensuing pneumonia, but infections with staphylococci, streptococci, and *Haemophilus influenzae* can also occur.

Human influenza viruses have been found worldwide, and studied extensively, particularly for their antigenic properties, which show evolutionary changes (Webster et al., 1982; Webster, 1995). These periodic changes result in new strains of viruses that circumvent antibodies and complicate vaccine development. Outbreaks of influenza virus usually begin abruptly and have been hard to control. This is due in part to influenza virus's high variability (recombination) rates, resulting in new strains for which a new vaccine or a new immunity has yet to be achieved (Webster et al., 1982; Webster, 1995). Their segmented RNA genomes (8 segments in influenza A, B and 7 segments in influenza C) are prone to reassortment or genetic recombination (Brink and Coleman, 1996; Linden, 1996).

The increase of global travel during the 20th century has facilitated the quick spread of many infectious diseases, from one continent to another. Consequently, an epidemic can quickly develop into a pandemic (Linden, 1996; Shu, 1996).

Pandemics are the result of antigenic shifts (genetic reassortment) (Webster et al., 1982; Webster, 1995), and are associated with severe illness and significant mortality on a global scale. Within this century, at least five pandemics and numerous epidemics (regional outbreaks involving fewer people) have occurred. Five pandemics of human influenza have been recorded, in 1890, 1900, 1918, 1957, and 1968. After the First World War, the influenza pandemic (Spanish Flu) (H1N1 subtype), killed more people (20 million people) than the War itself (7 million people). An "Asian flu" appeared in 1957, with a new subtype H2N2, replacing the former subtype H1N1. Over 1 billion people were infected in the short time of one year. (White and Fenner, 1994; Shu et al., 1996).

Viruses resisted prophylaxis longer than other microorganisms (Vanden Berghe et al., 1986). Prophylaxis refers to measures designed to prevent the occurrence of disease or its dissemination. Immunization against serious diseases such as smallpox or diphtheria is one example. Quarantine to confine communicable disease is another example. Other examples are public health measures to ensure the safety of food, milk, and water; the care of teeth to offset decay; and restrictions put on persons with such disorders as diabetes or heart disease to prevent the aggravation of these conditions.

Multiplication of a virus during an infection offers many opportunities for antiviral drugs to halt its life cycle. Such drugs attack the virus at the host cell receptor, or at the membrane when the virus enters the cell, uncoats from its capsid, duplicates its genetic material, and when it assembles for release from the infected cell (Vanden Berghe et al., 1986). Antiviral compounds can either have virucidal activity by directly attacking the virus, or by interrupting the virus life cycle. It may be difficult to find the ideal compound that acts only on the virus and is not toxic to the host cell. Lytic viruses are more easily neutralized than latent ones integrated into the host genome.

Although viruses are a serious health concern around the globe, few antivirals are available in the market. Until recently, only seven antiviral drugs were approved in the

USA (Prusoff et al, 1989). Some examples are amantadine, that prevents penetration of influenza A virus, and acyclovir or 9-((2-Hydroxyethoxy) methyl) guanine that neutralizes a range of herpesviruses, including HSV, CMV, as well as Epstein Barr virus (EBV). Acyclovir prevents DNA replication.

Many phytochemicals have displayed antiviral activity *in vitro*. Some examples are: hypericin derived from *Hypericum perforatum* L., active against HIV, HSV, and SINV (Hudson et al., 1991); quercetin, widely distributed in the plant kingdom and active against HSV (Vanden Berghe et al., 1986); perivine from *Catharanthus roseus* (L.) G. Don.), active against vaccinia polio (Farnsworth et al., 1968). There are three ways in which an antiviral activity could manifest itself in virus-cell culture systems. The active phytochemical could protect cells from virus infection, by means of an interferon-like effect (even if interferon itself is not involved). The activity could be virucidal causing direct inactivation of the virus, this pattern is common among antiviral phytochemicals (Hudson, 1995). Or the phytochemical could inhibit some stage of the virus replication cycle in the infected cells (Vlietinck and Vanden Berghe, 1991).

The foregoing problems underlie the urgent need to search for new medicinal compounds, to combat viruses more efficiently. Traditional medicinal plants have sometimes been found to be sources of valuable potential medicines (Schultes, 1994; Hudson, 1995; Hudson & Towers, 1999; Hudson et al., 1999; NAPRALERT, 1975-1998). However, with well over 275,000 plant species worldwide, a random screening of plants for compounds with biological activity is doomed to be frustrating and inefficient. Many workers have therefore chosen the ethnobotanical approach as a means of focussing on plants with pharmacologically active compounds of potential therapeutic interest (Balick & Cox, 1996). This principle has been shown to work efficiently in screening for antivirals, where a high percentage of positive results have been obtained (Vanden Berghe et al., 1986; Hudson, 1995; Towers et al., 1997; Vlietinck & Vanden Berghe, 1991,1998; Hudson & Towers, 1999; Semple et al., 1998). In Hudson's and Towers' laboratories, a large number of medicinal plants from around the world have been tested against microorganisms after ethnobotanical investigation has been done in the country of origin (Mouhajir et al., 1999; Hudson et al., 2000; Hudson & Towers, 1991, 1999; Taylor, 1996; Taylor et al., 1996; Yip et al., 1991; Yip, 1993).

Moreover, Towers and Hudson have stressed the need to test for light-mediated activities when screening plants for antimicrobial or antiviral activities (Hudson et al., 1993a, 1993b; Hudson, 1995; Towers et al., 1997). Previous studies may have missed potentially important activities because of this lack of consideration (Hudson & Towers, 1999).

The aim of the present investigation was to evaluate the antiviral activities of 75 endemic plant species used by Berber and Arab peoples of Morocco to treat diseases that could be caused by viral pathogens, for example fever, coughs, colds, influenza, herpes. These medicinal plants were tested against three pathogenic viruses: poliovirus, Sindbis virus, and herpes simplex virus. Assays were performed in the presence and absence of light, since many antivirals are photosensitizers (Hudson, 1995; Towers et al., 1997). This is the first study on antiviral activities reported on Berber and endemic Moroccan plants.

Materials and Methods

Plant Collections

The plants were collected in several regions of Morocco in the summers (Atlas & Rif Mountains and Sahara) of 1997-2000 as described in Chapter II. The plant specimens were air-dried in the shade, ground, and the powders kept in paper bags until used.

Preparation of Plant Extracts

Plant material was air-dried and ground in a domestic grinder. A 100-200 g sample of each ground plant was soaked in 100-200 ml MeOH for 24 h. The sample was then suction-filtered through Whatman No.1 filter paper and washed with another 200ml of MeOH. The filtrate was evaporated to dryness under reduced pressure. Then the samples were lyophilized for at least 48 h. Plant extracts were dissolved in MeOH, at a concentration of 100 mg/ml. Each extract was then diluted 1:50 in Modified Eagle Medium (Hudson ET al., 1994) to give a concentration of 200 μg/ml and 1 % methanol. It was then filtered through a sterile syringe filter, 0.2 μm pore diameter. This filtrate was the starting material.

Antiviral assays

Methanolic extracts were tested against poliovirus, Sindbis virus (SINV) and herpes Simplex virus (HSV). Human viruses were used in the antiviral assays. Sindbis virus (Togaviridae), is an enveloped virus, with a genome of a single-stranded RNA (11 000 nucleotides in length), an icosahedral capsid of 40 nm of diameter. Included in this family are rubella virus (German measles), yellow fever virus, dengue fever virus and many viruses causing encephalitis. The HSV1 has an envelope, a genome of double-stranded DNA (approximately 170 000 nucleotides), and an icosahedral capsid of 180 nm diameter. Besides herpes diseases, chicken pox, infectious mononucleosis and shingles are also caused by viruses included in the herpesviridae .The human poliovirus 1 (polio) (Picornaviridae), has no-envelope, a genome of single-stranded RNA (8000 nucleotides), and an icosahedral capsid of 25 nm diameter. Hepatitis A virus, viruses that cause enteritis, and rhinoviruses, which are the most common of colds, are included in the viruses of the Picornaviridae family.

Vero cells (an African green monkey kidney cell line, American Type Culture Collection) were grown in 96 well microtest trays (Falcon), 0.2 ml per well, in Dulbecco's MEM with 5% fetal bovine serum (FBS) (all cell culture reagents were obtained from GIBCO Life Sciences, Ontario) and 25 μg/ml gentamicin sulfate (Sigma). They were incubated at 37 °C in a 5% carbon dioxide and 95% air atmosphere. They were used for the assays when they reached confluent monolayers.

Starting at 200 μg/ml, two-fold serial dilutions of plant extracts were made (in duplicate), in MEM + 0.1% serum, across a row of wells in an empty 96 well microtest tray. The transfer of the diluted extracts, with the aid of a multipipettor, was made to the aspirated Vero cell monolayers of another 96 well tray, 0.1 ml per well. To test for possible immediate cytotoxic effects, these cultures were incubated at 37 °C for 60 min and examined microscopically. Then, 0.1 ml (equivalent to one hundred plaque forming units (PFU) of virus) in MEM + 0.1% serum, the standard concentration, was added to each well (Taylor et al., 1996). The tray was transferred immediately to an environmental chamber (37 °C) and exposed to a combination of visible light ($5W/m^2$, with a wavelength maximum of 580 nm, from four Westinghouse 20 W fluorescent tubes (F20T12/cw)), plus UVA (long-wave ultraviolet: $5W/m^2$, with a wavelength maximum of 350 nm, from four Sylvania F20T12-BLB lamps) for 30 min, with continuous gentle shaking of the tray. Following the light exposure, the trays were returned to the cell culture incubator. Controls consisted of cells with no virus, which should remain healthy for the duration of the test, and cells infected with untreated virus, which should show maximum characteristic virus-induced cytopathic effects (cpe). Periodically, cultures were microscopically checked for viral cpe. Poliovirus required 2 days for complete cell destruction (100% viral cpe), SINV required 3days, and HSV required 4 days. Complete inactivation of the 100 pfu of virus was indicated by the absence of cpe, whereas partial inactivation was indicated by less than 100% cpe.

Antiviral photosensitizers

Duplicate sets of trays were used to test for photosensitizers with antiviral activity. One set was exposed to the combination of fluorescent and BLB lamps, for 60 min instead of 30 min. The other set received the same treatment except that they were covered with aluminum foil throughout to assess non-light mediated activity. The foil was removed for subsequent manipulations, in dim light, and for microscopic observations.

Cytotoxicity assays

The protocol was the same as the one described above for antiviral assays, with the exception that the cells were not infected with virus. After light exposure, the trays were returned to the incubator and checked frequently under a microscope for changes in cell morphology or for visible toxic effects (lysis or any obvious cellular damage).

Results

A total of 75 plant extracts were investigated. The full botanical names, parts used and traditional uses, are summarized in Table 2.2 (Chapter II). Antiviral activities were evaluated, at non-cytotoxic concentrations, against the three human viruses: HSV, SINV, and poliovirus. These results are summarized in Table 4.1.

Forty-five extracts were active. Five were very active against all three viruses, 16 were active against two viruses, and 24 were active against only one virus. Thirty-two extracts contained photosensitizers, indicated by the presence of either light-enhanced antiviral activity or light-dependent activity.

Punica granatum extract, which was the most active, inhibited the three viruses at a minimum concentration of 1.5 μg/ml, although its activity was not light enhanced. The extracts of *Acacia gummifera*, *Juglans regia*, *Thymus maroccanus*, *Lawsonia inermis*, *Pinus halepensis*, and *Rosa canina* inhibited SINV at a minimum concentration of 1.5 μg/ml. *Thymus maroccanus* and *Rosa canina* activities were light enhanced. *Pistacia* *lentiscus* and *Thymus maroccanus* were active against HSV. *Ricinus communis* was light active against both HSV and SINV at 6 μg/ml.

Extracts most active against poliovirus were those from *Pinus halepensis* and *Punica granatum*, which inhibited the virus at a minimum concentration of 6.5 μg/ml, although these were not light, enhanced.

The activities of *Rumex bucephalophorus, Artemisia herba-alba, Quercus faginea, Pinus halepensis* and *Punica granatum* were light independent.

Finally Table 4.2 shows the correlation between traditional use(s) and the antiviral activity. There is a correlation for some plants, e.g, *Punica granatum*, *Pinus halepensis*, *Pistacia lentiscus*, *Thymus maroccanus*. For other plants, the correlation seems less obvious.

Table 4.1. Antiviral activities of Berber and Arab plant species.

	SINV[a]		HSV[a]		Polio[a]	
	Light[b]	Dark[b]	Light	Dark	Light	Dark
Family *Species*			MIC	μg/ml[c]		
Aizoaceae						
Aizoon canariense	100	200[d]	50	200	---[e]	---/tox[f]
Apiaceae						
Ammi visnaga	---	---	200	---	---	---
Ammodaucus leucotrichus	200	---	200	---	---	---
Ammoides pusilla	---	---	100	---	50	50
Apium graveolens	100	100	50	100	200	---/tox
Carum carvi	50	---	100	100	---	---
Petroselinum crispum	200	200	---	---	---	---
Ridolfia segetum	200	200	200	200	---	---
Anacardiaceae						
Pistacia lentiscus	200	200	1.5[g]	6	---	---
tox.high conc.						
Aristolochiaceae						
Aristolochia paucinervis	---	---	100	100	---	---
Asteraceae						
Chamaemelum nobile	200	200	50	---	---	---
Artemisia absinthium	100	100	100	200	200	---
Artemisia herba-alba	50	50	50	50	100	100
Cotula cinerea	100	100	100	100	---	---
Dittrichia viscosa	3.5	25	3.5	50	50	50
Inula viscosa	25	50	6	12	50	50
Matricaria recutita	25	100	50	100	200	---
Pulicaria arabica	50	100	100	100	---	---
Sonchus oleraceus	25	---	200	---	---	---
Brassicaceae						
Lepidium latifolum L.	---	---	---	---	---	---
Cactaceae						
Opuntia megacantha	50	100	25	25	---	---
Capparaceae						
Capparis spinosa	50	200	200	---	---	---
Cleome arabica	25	200	12	50	100	200
Caryophyllaceae						
Corrigiola telephiifolia	100	100	100	100	50	50
Herniaria cinerea	6.5/tox	50	12.5	---	---	---
Cucurbitaceae						
Cucurbita pepo	100/tox	100	---/tox	12	200	200/tox
Euphorbiaceae						
Euphorbia falcata	---	---	---	---	200	200
Ricinus communis	3.5	50	3.5	25	50	50
Fabaceae						
Acacia gummifera	1.5	6.5	1.5	6	---	---
Crotalaria saharae	100	100	200	200	---	---
Retama retam	200	200	100	---	200	---
Retama sphaerocarpa	100	200	50	100	---	---
Trigonella foenum-graecum	200	200	00	200	200	200

Fagaceae						
Quercus faginea	12	12	1.5	1.5	50	50/tox
Iridaceae						
Iris germanica	200	200	50	100	---	---
Juglandaceae						
Juglans regia	1.5	12.5	1.5	1.5	25	2.5
Juncaceae						
Juncus acutus	100	100	1.5	3.5	200	100
Lamiaceae						
Lavandula angustifolia	---	---	200	200	---	---
Marrubium vulgare	200	100	12	50	100	100
Mentha pulegium	---	---	6	100	---	---
Nepeta apulaei	100	100	50	50	---	---
Nepeta spp.	100	100	100	100	---	---
Rosmarinus officinalis	50	50	50	50	200	200
Salvia clandestina	6	200	3.5	50	---	---
Salvia officinalis	50	50	50	50	50	50
Stachys arvensis	200	200	200	200	---	---
Thymus broussonetii	50	50	12	100	---	---
Thymus maroccanus	1.5	3.5	1.5	3.5	---	---
Thymus satureioides	200	200	200	200	---	---
Liliaceae						
Asphodelus microcarpus	50	100	200	---	---	---
Linaceae						
Linum usitatissimum	50	50	50	---/tox	100	100/tox
Lythraceae						
Lawsonia inermis	1.5	50	200	200	200	---
Pinaceae						
Pinus halepensis	1.5	1.5	---	---	6.5	6.5
Piperaceae						
Piper longum	50	200	50	50	---	---
Piper cubeba	50	100	---	---	---	---
Polygonaceae						
Rumex bucephalophorus	6	12.5	1.5	1.5	50	100/tox
Punicaceae						
Punica granatum	6.5	6.5	12.5	12.5	6.5	6.5
Ranunculaceae						
Nigella sativa	---	---	200	200	---	---
Rhamnaceae						
Rhamnus alaternus	---	---/tox	100	100	---	---
Rosaceae						
Rosa canina	1.5	12.5	200	200	25	---
Sorbus aria	50	50	1.5	6	100	100/tox
Rubiaceae						
Rubia peregrina	100	200	50	50	---	---/tox
Rutaceae						
Ruta graveolens	50	50	200	200	100	100
Santalaceae						
Osyris quadripartita	50	100	200	200	100	---
Sapotaceae						
Argania spinoza	---	---	---	---	200	---
Solanaceae						
Solanum nigrum	200/tox	100	1.5	6	---	---

Thymelaeaceae						
Thymelaea microphylla	100	100	1.5	3.5	---	200
Verbenaceae						
Aloysia triphylla	---	---	---	---	---	---
Zingiberaceae						
Alpinia officinarum	12	12	3.5	6	25/tox	12.5/tox
Ellettaria cardamomum	100	200	200	200	---	---
Miscellaneous						
"Indass"	50	50	50	50	---	---
"Ghanghass"endemic	50	50	50	50	---	---
"Tahantizt"	---	---	50	100	---	---
"Al Ghassoul Assabssi"	50	50	100	100	---	---
Sea urchin	200	100	---	---	---	---

[a]SINV, Sindbis virus; HSV, Herpes Simplex virus; Polio, polio virus
[b] Light and Dark activities
[c] Minimum Inhibitory Concentration (MIC μg/ml)
[d] Maximum concentration 200 μg/ml (extract inactivates virus at 200 μg/ml or maximum concentration, μg/ml, required to inactivate 100 infectious virus).
[e] ---, No detectable antiviral activity
[f] Toxicity observed
[g] Minimum antiviral concentration 1.5 μg/ml

Table 4.2. Correlation between traditional use (s) and antiviral activity

Plant species	Active against	Traditional use (s)
Punica granatum	HSV & SINV & Poliovirus	Stomachache & ulcer.
Juglans regia	SINV	Colds, sinusitis, skin infection, rash & stomachache.
Thymus maroccanus	HSV & SINV	Colds wound antiseptic. Indigestion & food preservative.
Lawsonia inermis	SINV	Epidemic.
Pinus halepensis	SINV & Poliovirus	Infectious wounds, anti-cough.
Rosa canina	SINV	Fever & eye conjunctivitis.
Pistacia lentiscus	HSV	Stomachache & ulcer.
Acacia gummifera	SINV	Bronchitis & anti-cough.
Ricinus communis	HSV & SINV	Colds

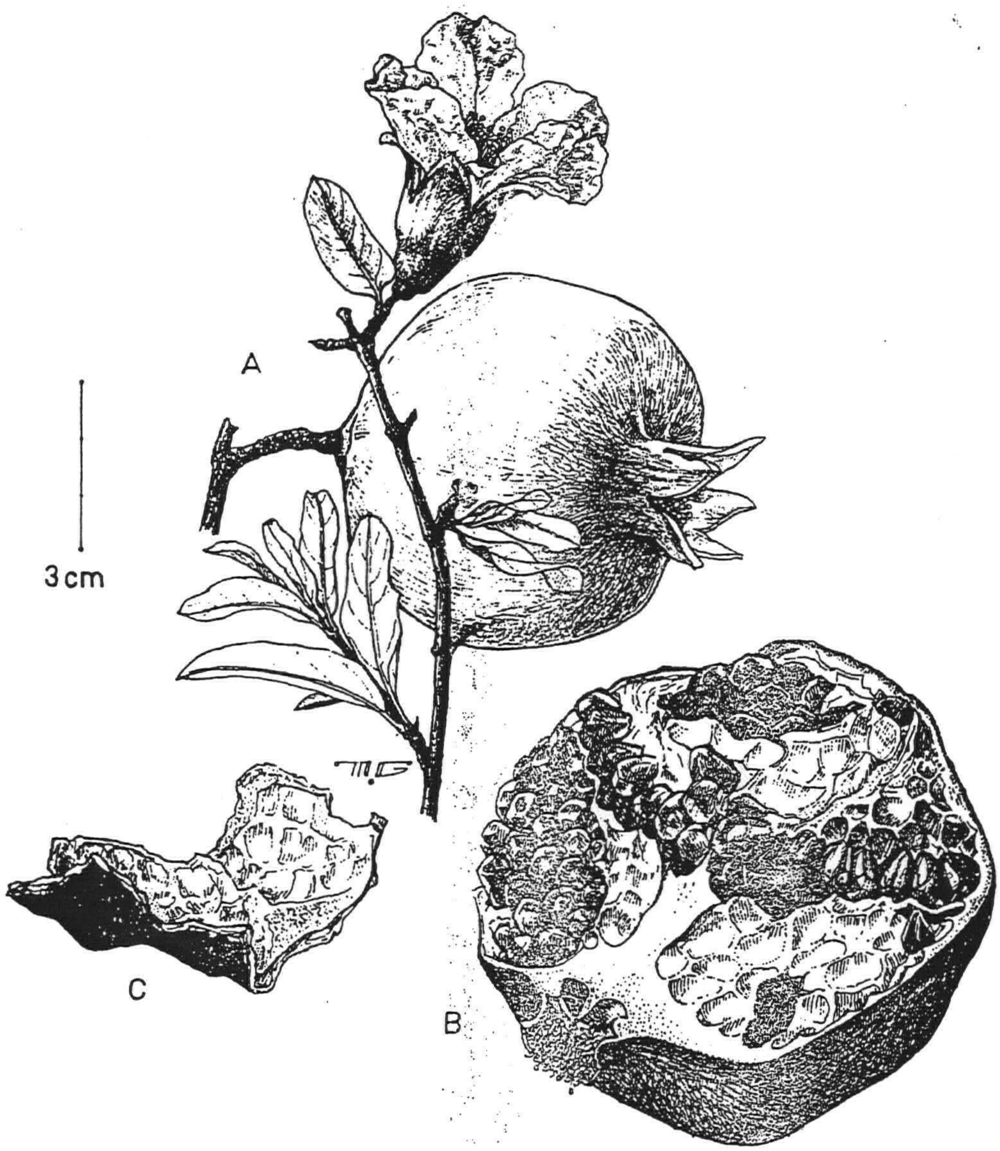

Figure 4.1. *Punica granatum* L., Punicaceae: most active against SinV, HSV and polio

(Boulos, 1983)

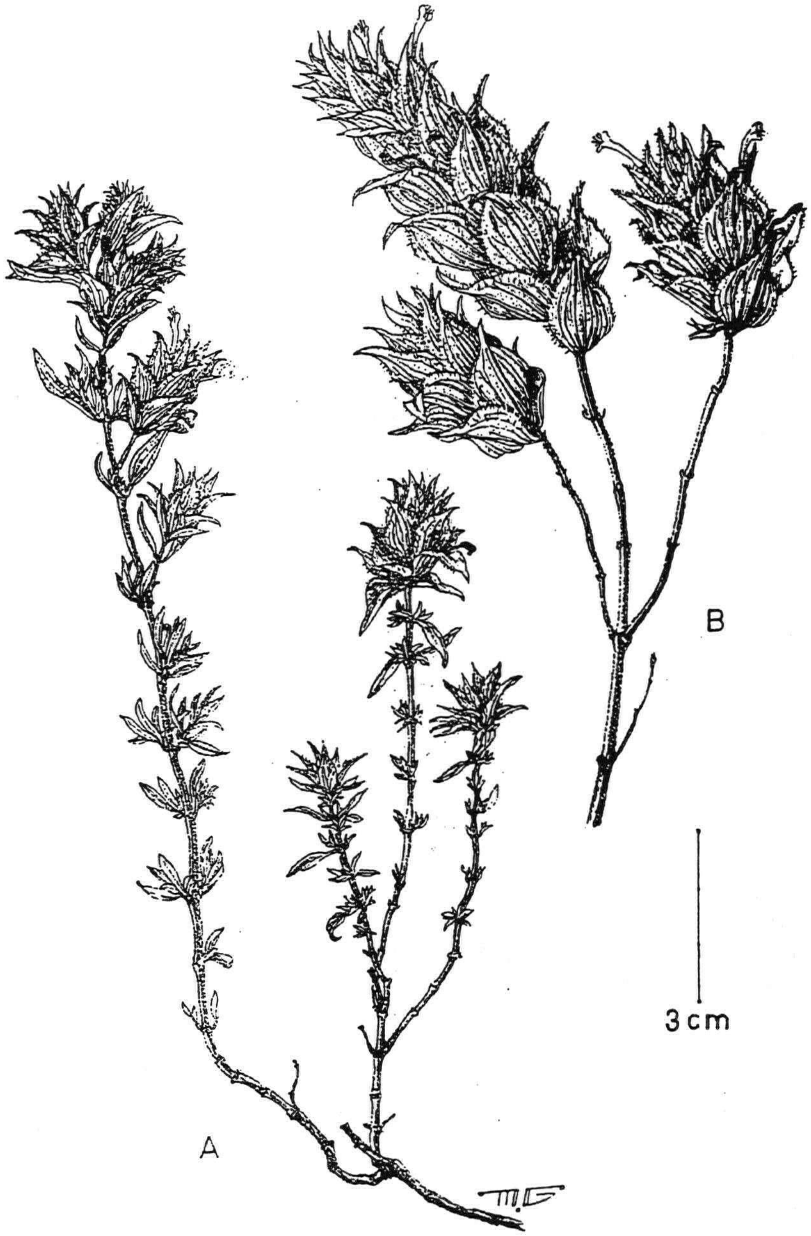

Figure 4.2. *Thymus broussonettii* Bois., Lamiaceae: active against HSV, and SinV

(Boulos, 1983)

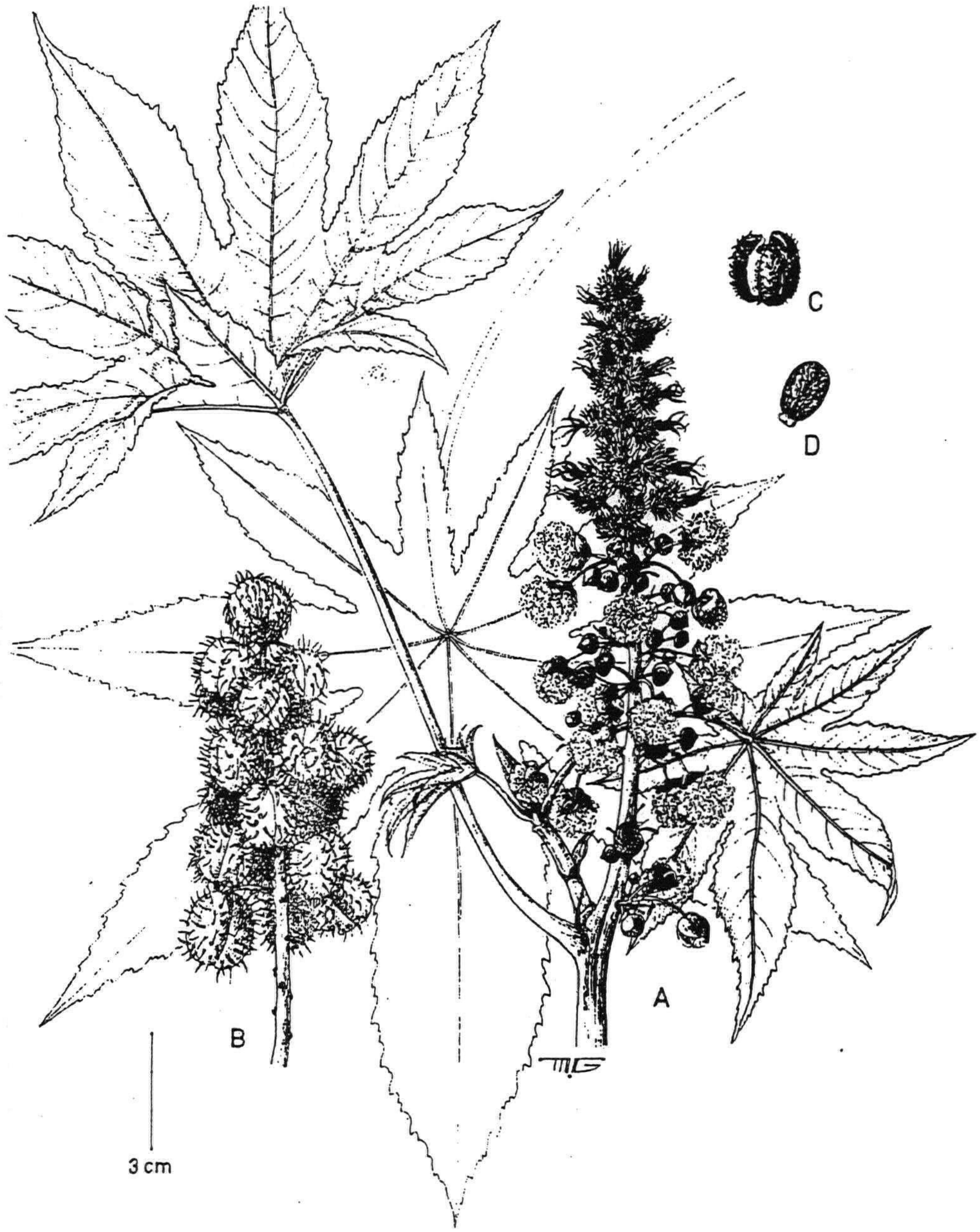

Figure 4.3. *Ricinus communis* L., Euphorbiaceae: active against both HSV and SINV.

(Boulos, 1983)

Figure 4.4. *Inula viscosa* Ait., Asteraceae: photoactive against HSV, SinV and polio.

(Boulos, 1983)

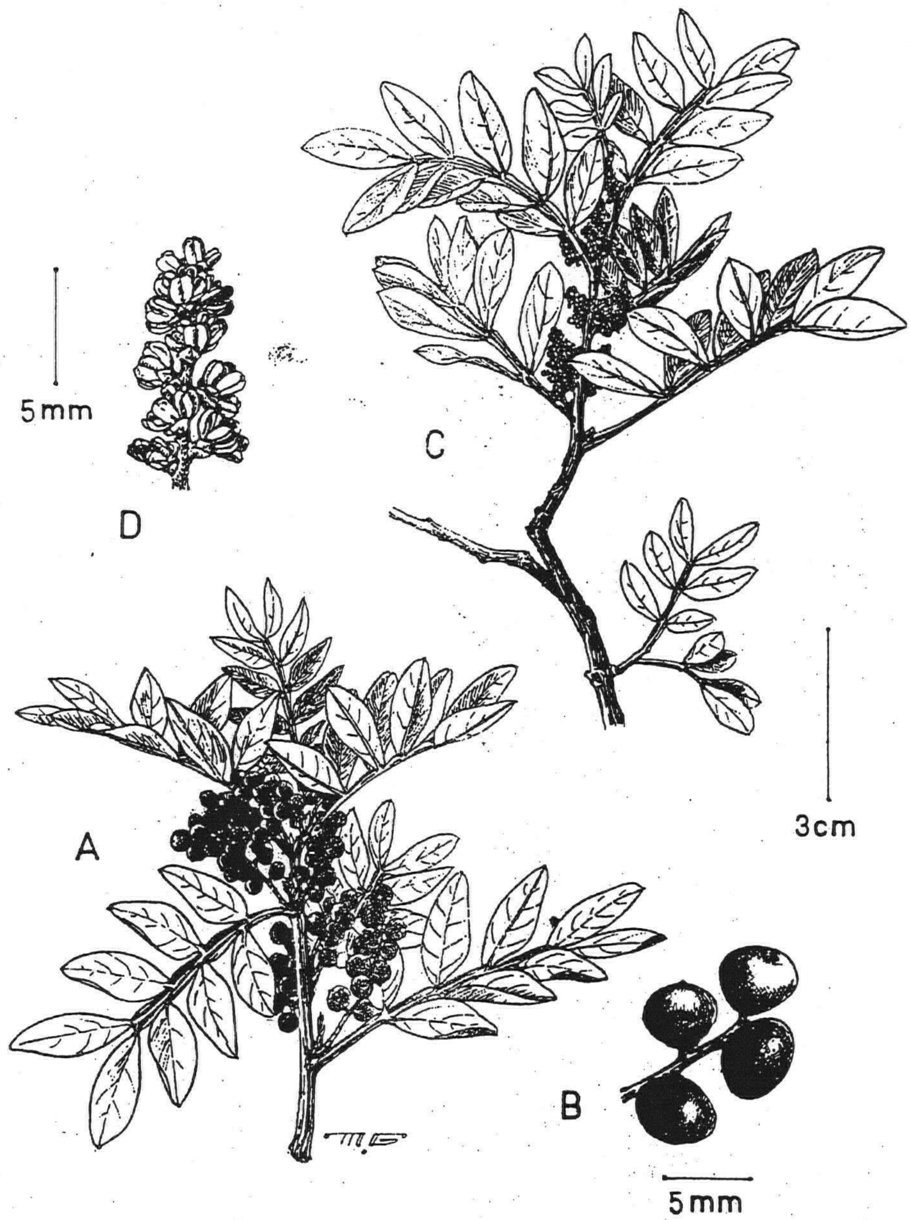

Figure 4.5. *Pistacia lentiscus* L., Anacardiaceae: correlation between traditional use and antiviral activity

(Boulos, 1983)

Discussion and Conclusion

All the antiviral tests were carried out with non-cytotoxic concentrations of the extracts, since we were interested in potent antiviral activities without deleterious effects on the host cells. Although the cytotoxicity assays were carried out in a very qualitative manner, based on visual examination, some quantitative cytotoxicity assay methods could had been used to establish the threshold concentration. Some examples are trypan blue dye exclusion or assays for leakage of lactate dehydrogenase or other intracellular enzymes characteristic of the particular cell type used, among others.

Five (*Dittrichia viscosa*, *Quercus faginea*, *Juglans regia*, *Punica granatum* and *Alpinia officinarum*) of the 75 extracts (70% Berber and 30% Arab) were found to have activity against all three viruses tested. These viruses represent a broad spectrum of potential targets (DNA/RNA, membrane/no membrane, and viral proteins) for antiviral compounds. They are also potential human pathogens, and they constitute good viral models of skin infections (HSV), gastric-intestinal infections (polio), and mosquito-borne infections (SINV). The relative potencies of these five extracts compared favorably with those determined in some previous studies, e.g., of plants from Nepal and Togo (Taylor, 1996; Anani et al., 2000). The minimal inhibitory activity presented here is 70% lower than the ones published from these authors.

The relatively high activity of *Punica granatum* against the three viruses could be due to the presence of an array of phytochemicals. The plant species has been reported to have antioxidant activity due to the high content in phenolic compounds that scavenge free radicals (Gil et al., 2000). It contains the pomegranate tannin punicalagin, (punicacortein) in the fruit rind (husk) that has antitumoral activity (Kashiwada et al., 1992). In addition, anthocyanins, phenolics, ellagic acid derivatives, and hydrolyzable tannins were detected.

The majority of the extracts were active against only one or two viruses, but there was considerable variation among them. Some were active only against viruses with a membrane, SINV and HSV. Some activities were light-enhanced/dependent; others were not affected by light. This latter observation supports the use of light exposure in testing for antiviral activities, since many of the activities would not otherwise have been so impressive. These results indicate the presence of a variety of different antiviral compounds, or combinations, rather than one or two compounds common to many extracts.

Poliovirus was the most resistant of the viruses, with only five extracts, those of *Alpinia officinarum, Rosa canina*, *Punica granatum*, *Pinus halepensis* and *Juglans regia*, showing complete inactivation of the standard virus dose. These plants were also active against one or both of the other viruses.

The traditional medicinal uses recorded for these plant species are listed in Table 2.2 of Chapter II. *Acacia gummifera,* which is used to treat colds in Morocco, was not active against poliovirus in this screening. It was however active against the membrane bound SINV or HSV, its activity increasing with exposure to light.

Only two extracts, those from *Ammodaucus leucotrichus* and *Sonchus oleraceus*, showed an absolute requirement for light (i.e., light-dependency, no detectable activity in the dark). *Ammodaucus* belongs to the Apiaceae known to contain furanocoumarins, whereas *Sonchus* belongs to the Asteraceae, known to contain many sesquiterpene photosensitizers. 15 extracts showed light-enhanced activity, with some activity in the dark. This indicates the presence of photosensitizers that also have bioactivities in the dark, or mixtures of photosensitizers and non-photosensitizers.

Few extracts were not active at the concentration of 200 μg/ml. Thus true antiviral compounds may have been present in only tiny quantities that were insufficient to inactivate infectious viruses in the standard preparations. *Aizoon canariense, Apium graveolens, Pistacia lentiscus, Herniaria cinerea, Cucurbita pepo, Quercus faginea, Linum usitatissimum, Rumex bucephalophorus, Rhamnus alaternus, Sorbus aria, Rubia peregrina, Solanum nigrum* and *Alpinia officinarum* displayed some cytotoxicity at high concentrations. Thus, caution should be taken when considering these plants as medicines, as lower concentrations have to be maintained in therapeutic use.

The antiviral properties of *Juglans regia, Thymus maroccanus, Lawsonia inermis, Pinus halepensis, Rosa canina, Acacia gummifera,* and *Ricinus communis* support their traditional use(s) in colds and fever treatments which are, usually viral symptoms (Table 4.2). The results of this investigation show that many of the medicinal plants tested are active against a number of viruses at low concentrations of a crude extract. However, for some plants, the traditional use doesn't appear to match the results obtained. This might suggest a discrepancy at the diagnostic level or at the prescription level, meaning that the plant may have other uses than treatment of infectious diseases. It may also reflect difficulty for a healer when diagnosing infectious diseases. Furthermore, in several cases the medicinal plants are also used in a holistic approach that is impossible to test with the bioassays proposed in here, which are restricted to looking for direct antiviral effects. It should also be considered that the plant might exhibit indirect activity *in vivo*, such as stimulation of certain host defenses, including the immune system.

Further research should prove useful to test some of these plants for medicinal properties against diseases other than infection, e.g., cancer. Future studies directed against other viruses, as well as the isolation and identification of specific antiviral compounds hold promise for broadening the scope of natural plant products in the control of human diseases.

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CHAPTER V

Phenolics in Moroccan medicinal plant species as studied by Electron Spin Resonance Spectroscopy.

Introduction

Moroccan medicinal plant species are widely used in traditional medicine for a number of infectious diseases of the lung, the skin, the stomach and other parts of the body (Bellakhdar et al., 1991). This use is ancient, dating back to the Greco-Roman & Arabian times. Even though people use these medicinal plants extensively in Morocco and the Middle East, there are very few reports of scientific testing and chemical analysis of active compounds (NAPRALERT, 1975-1998).

The present study was undertaken in order to see whether the medicinal uses of many Moroccan medicinal plant species were mirrored in the actual content of simple phenolics in the plants. Many phenolics, e.g. phenylethanoid glycosides often found in medicinal plants, are known to have various biological activities (Jiménez and Riguera, 1994).

A detailed study is presented for *Nigella sativa* L. (Ranunculaceae). It is also called black cumin and is widely used in Morocco and the Middle East for various ailments including colds, infections (microbial & viral), as well as asthma and pulmonary diseases (Bellakhdar, 1991). A fixed oil and alkaloids have been noted in this species (Aboul-Enein *et al.*, 1995). Compounds previously identified in seeds of this species are alkaloids, triterpenes, monoterpenes (thymol and thymoquinone), dithymoquinone, a quinoid compound (nigellone), an isoquinoline alkaloid and tannins (NAPRALERT, 1975-1998).

From previous research on *Nigella sativa*, El-Dakhakny (1963) reported the presence of thymoquinone in the seeds, whereas El-Alfy *et al.* (1975) reported thymohydroquinone from the oil of seeds. Since the two compounds exhibit different therapeutic effects, it was of interest to know whether they co-exist in the seeds or whether only one form (quinone/quinol) is present, the "wrong form" occuring as a result of the isolation procedure. More recent research on *Nigella*, using TLC assay of the methanol extract of the black seed oil, has shown the presence of thymoquinone, dithymoquinone and thymol. However, the identification of these compounds was only achieved by the use of marker compounds and recourse to Rf values obtained by TLC (Abou Basha *et al.*, 1995). Finally, Aboul-Enein *et al* (1995) have reported the presence of thymoquinone in the seed oil, by the use of HPLC.

To handle the fairly large number of species that was available (50 species from 45 genera in 27 families), I performed a screening by using Electron Spin Resonance (ESR) spectroscopy.

Electron Spin Resonance spectroscopy (ESR) also known as Electron Paramagnetic Resonance (EPR) employs microwave radiation ($\approx$ 3 cm, 9500 MHz) to induce transitions between energy levels of electrons with unpaired spins in an imposed static magnetic field (0.5 - 16, 000 G). This imposed magnetic field is modified by local magnetic fields within the molecule similar to chemical shifts in NMR. The imposed magnetic field forces the electron's magnetic moment to be either aligned (low level) or antiparallel (higher energy). The resonance interaction between the magnetic dipole of the electron and the oscillating magnetic field accompanying the microwave radiation causes the transition, with microwave energy absorption being measured. Unpaired electrons are relatively unusual, being found in free radicals and ions of transition metals and rare earths. ESR only detects free radicals, or more general systems that contain unpaired electrons. The technique is not always easy to apply because of the instability of free radicals. In case of semiquinones, the radicals are fairly stable (life times from msec to hours). They are therefore suitable targets for analytical studies by ESR.

ESR is particularly useful to detect phenolics with free ortho- or para-dihydroxy groupings as well as to detect quinones. In the method, pertinent compounds are transformed to a radical stage (semiquinones) by suitable redox reactions. The quinones are reduced and the aromatic compounds oxidized in base to semiquinone radicals and detected and identified, absolutely or partly in the crude extract by way of their unique spectra (Pedersen, 1985, 1978). The technique requires only tiny amounts of plant material, e.g. detached leaves of fingernail size, generally in the form of a crude extract. This method does not necessitate chemical isolation. However, the procedure identifies only the semiquinone nucleus. This may furnish an absolute proof of identity for some compounds (e.g. hydroquinone, thymoquinone or the naphthoquinone e.g. juglone or the anthraquinone alizarin). For other compounds the semiquinone nucleus acts as a reporter group that can help in further identification (e.g. catechol moieties in caffeic acid derivatives or in phenylethanoid glycosides), (Pedersen, 1985, 1978; Mouhajir et al., 2001,1999; Rhodes, 1996, 1994). Finally, ESR spectroscopy is a useful chemical technique for characterizing plant compounds in chemotaxonomy studies (Pedersen, 2000).

The results that were obtained from the ESR study of the 50 Moroccan plant species are reported here. The characterization of the dihydric phenolics of the black seeds of *Nigella sativa* is also presented.

Materials and Methods

Plant material

Fifty species of plants were analyzed by ESR. They were air dried in the shade, were ground and the powders kept in paper bags until used. Small aliquots (1-2 g) were used for ESR analysis.

Electron Spin Resonance analysis:

The plant material was extracted with EtOH-H_2O (4:1) and the extracts used as obtained. The semiquinone radicals were formed from ortho- or para-quinols by raising the pH and shaking the mixture in air (Pedersen, 2000). In general two experiments were run on each extract.

Reaction I: Twenty µl of the extract was mixed with 5 µl 0.1-0.2 M NaOH and the mixture was shaken in air. Ten µl of the mixture in a capillary was introduced into the magnetic resonance cavity and ESR spectra recorded immediately or after a few seconds. In the case of rapid disappearance, a spectrum was often restorable by turning the capillary upside down and letting an air bubble pass through the solution. The spectrum was seen immediately after reintroduction of the sample into the cavity. This process could be repeated several times depending on the actual concentration in the sample.

Reaction II: Twenty μl of the extract was mixed with 5 μl 1 M NaOH and the mixture was shaken in air. The increased pH made hydrolysis and hydroxylation more rapid for the pertinent compounds, and spectra of the secondary products appeared after a delay of seconds to minutes.

Results and Discussion

Electron Spin Resonance results for *Nigella*

When a crude extract of *N. sativa* is mixed with alkaline water, the addition of molecular oxygen (air), gave a strong signal of the thymosemiquinone radical ($Q^{-\bullet}$ see scheme and Figure 5.1) proving that large amounts of either thymoquinone (Q) or thymohydroquinone (QH_2) were present in the seeds. The process taking place is as follows:

$$\mathbf{QH_2 \rightarrow Q_2^{-} \rightarrow \rightarrow Q^{-\bullet} \rightarrow Q}$$

Due to the high pH the first step is dissociation of the hydroxyl protons (Q_2^-). This is followed by oxidation of the dianion to the semiquinone radical (Q^-) by molecular oxygen, the oxygen being reduced to superoxide (O_2^-) in the process. Further oxidation eventually leads to the quinone (Q).

Usually the oxidation stage of observed compounds (quinones/ quinols) is determined in the ESR procedure, e.g. naphtho- and anthraquinones are observed only after use of a reducing agent. However, certain benzoquinones, e.g. thymoquinone, are auto-reduced in the alkaline medium due to secondary processes taking place (Pedersen, 1973). In fact, the small lines indicated by arrows in Figure 5.2 derive from an intermediate semiquinone radical eventually leading to the observation of the artifact 2-hydroxy-3-methyl-6-isopropylsemiquinone, arising as a result of nucleophilic attack of OH^-. Accordingly, it was not possible from the ESR study to determine whether thymoquinone or its corresponding hydroquinone was present in the extract.

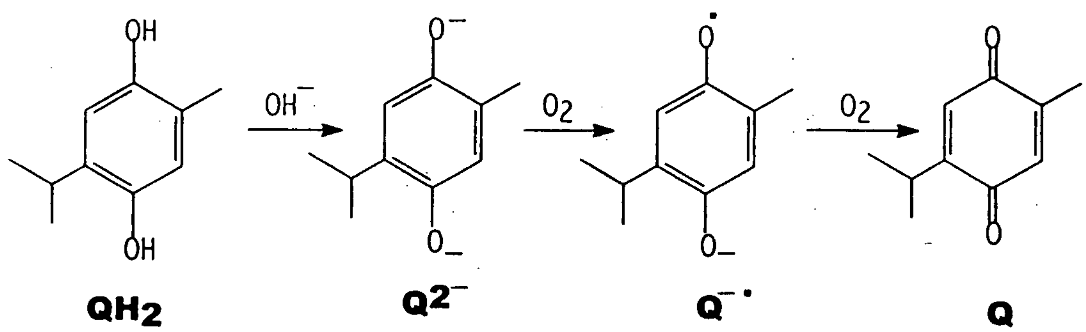


Figure 5.1. Thymohydroquinone production in ESR analysis

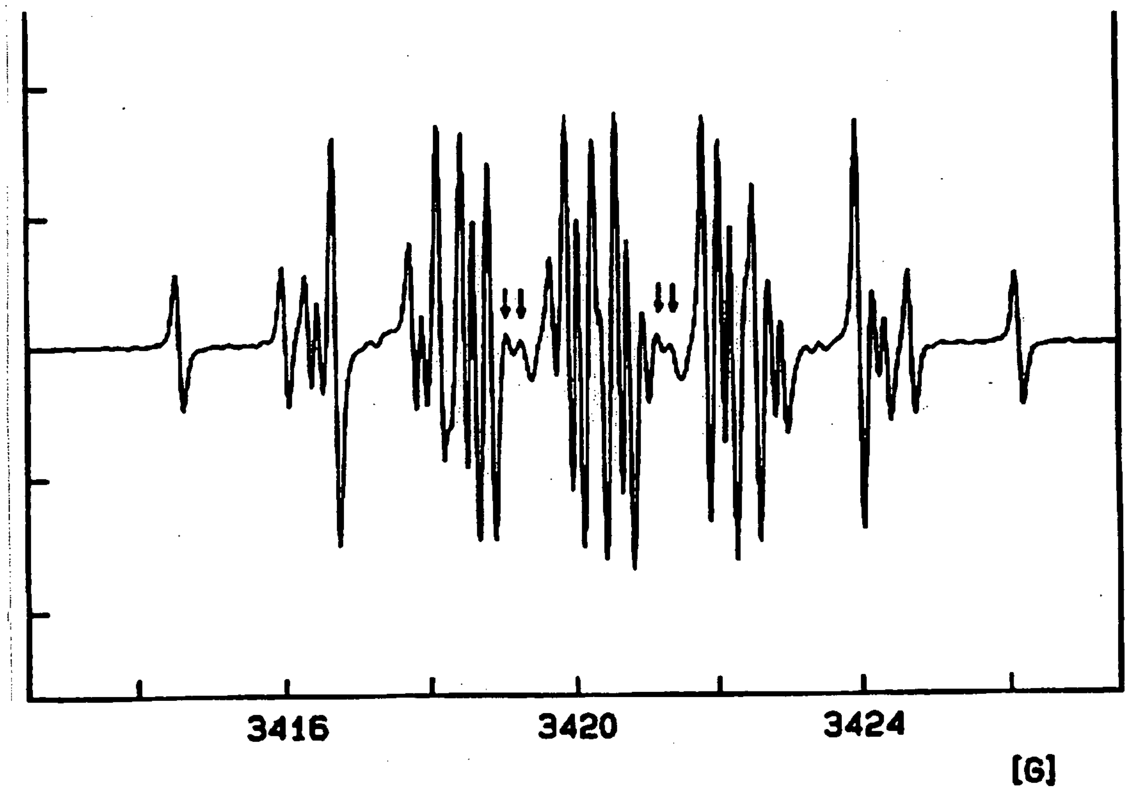


Figure 5.2. The ESR spectrum of the semiquinone radical of thymohydroquinone from *Nigella sativa* L. obtained from a crude alcoholic seed extract. The hyperfine constants observed are as follows (Gauss): $a_{Me} = 2.15$, $a_3 = 1.95$, $a_6 = 1.79$, $a_{isopropyl} = 1.43$. The isopropyl methyl groups are unobserved. The arrows indicate lines from the semiquinone radical of the artifact 3-hydroxy-thymoquinone.

No other quinones or dihydric phenolics were observed from the seeds by the ESR procedure (thymol is not detected in the procedure). Furthermore, thymohydroquinone has been observed recently by ESR in a number of Lamiaceae species (Pedersen, 1999). Finally, I observed the compound from the following Moroccan species: *Thymus maroccanus* Ball. (Lamiaceae), *Piper longum* L. (Piperaceae), *Crotalaria saharae* Coss. (Fabaceae), and *Cucurbita pepo* L. (Cucurbitaceae).

From the ESR study of the crude extract, it was observed that extraction of whole, unbroken seeds for several hours did not reveal the presence of thymohydroquinone. However, from an extract of broken seeds, a strong signal of thymosemiquinone appeared after few minutes of extraction. This clearly demonstrates that ESR is a valuable tool in the study of many smaller dihydric phenolics.

Thymoquinone has been reported to have potent therapeutic value. Particularly, Mutabagani *et al.* (1997) reported on the anti-inflammatory activity of thymoquinone in the volatile oil of *Nigella*. Thymoquinone has also been reported to be an efficient cytoprotective agent against chemically induced hepatic damage (Daba and Abdel Rahman, 1998). Anti-cancer activity has also been reported for thymoquinone isolated from *Nigella sativa* L. (Worthern *et al.*, 1998). Isolation of an antitumor principle from the black seeds, a monodesmosidic triterpene saponin, α-Hederin, was also reported (Swamy et al., 2001). Analgesic and anti-inflammatory activity as well as no effect on yeast induced pyrexia was reported for the aqueous extract of *Nigella* (Al-Ghamdi, 2001). Houghton et al. (1995) showed that both pure thymoquinone and the crude fixed oil inhibited eicosanoid generation in leukocytes and membrane lipid peroxidation. This last result may explain why whole *Nigella* is efficient in the treatment of rheumatism and related inflammatory diseases (Houghton et al., 1995).

Electron Spin Resonance results for fifty plant species

A number of prominent semiquinone spectra are frequently observed after Reaction I (see Materials and Methods) by aerial oxidation in dilute alkali, especially from specimens of families within the subclass Asteridae, and in particular those families belonging to the orders Lamiales, Callitrichales, Plantaginales and Scrophulariales. For example, the spectrum, derived from rosmarinic acid (Fig. 5.3) is clearly identified from the spectrum obtained with an authentic sample of this compound (Fig. 5.4). I shall designate both rosmarinic acid and its semiquinone radical with an **R**. Another example is a 24-line spectrum derived from 3,4-dihydroxyphenylethanoid glycosides (Fig. 5.5 and 5.6) identified since an identical spectrum is obtained from an authentic sample of acteoside. Only the 3,4-dihydroxyphenylethanoid moiety is identified (Fig. 5.6 A&B). I shall collectively designate the radical leading to the 24-line spectrum and the mother compound (s) with **A**. A third example is the spectrum of chlorogenic acid (Fig. 5.3), which I shall designate, as **C**. It should be noticed that in the routine ESR procedure neochlorogenic acid gives rise to an identical spectrum. **A**, **C**, and **R**, however, are easily distinguished in the procedure.

Other compounds of scattered occurrence are observed in Reaction I, e.g. spectra of simple hydroquinones (Fig. 5.7 B) such as thymohydroquinone (Fig. 5.7 C) (designated **T**) or naphthoquinones such as juglone (**J**) or lawsone (**L**), all absolutely identified from their ESR spectra. For details and spectra of pertinent compounds see

Pedersen (2000). The hyperfine splitting constants obtained from the observed semiquinone radical spectra are collected in Table 5.1. Specific observations are as follows:

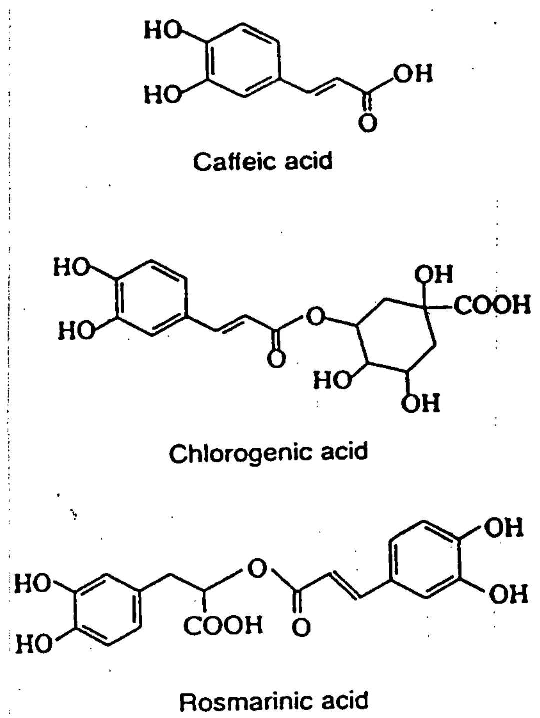


Figure 5.3. Structures of Rosmarinic acid, Chlorogenic acid, and Caffeic acid

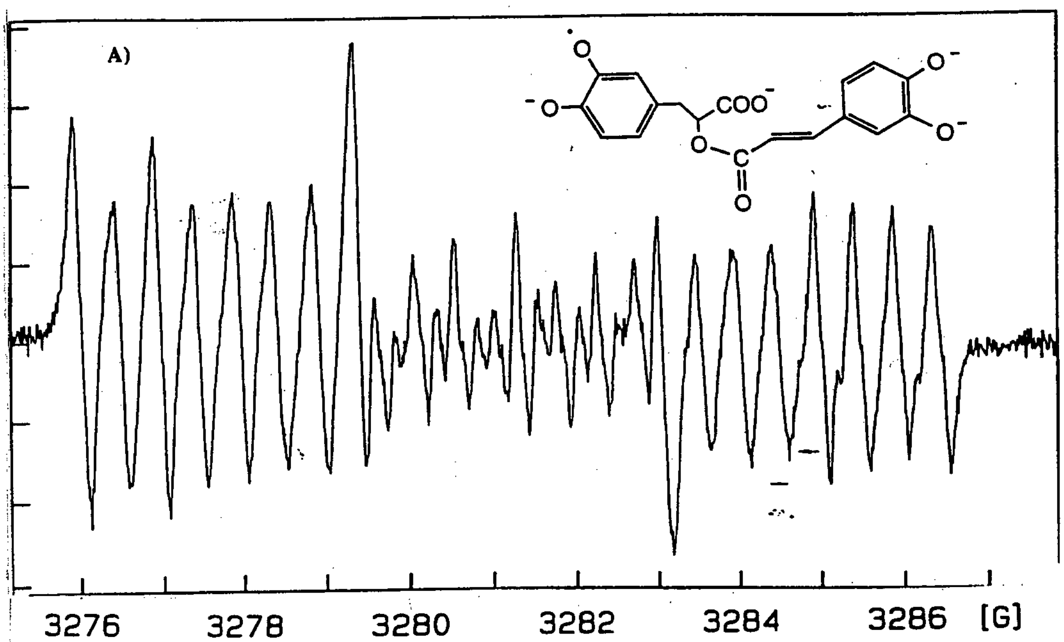
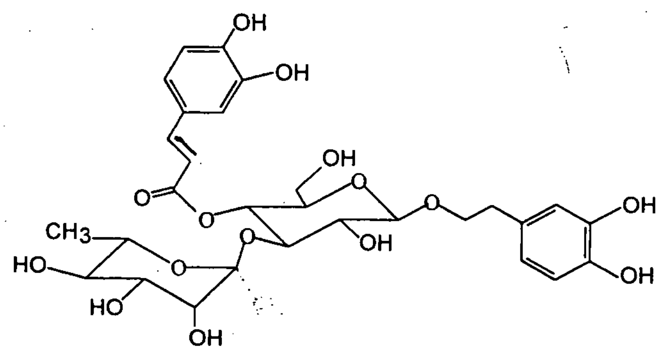


Figure 5.4. The ESR semiquinone spectrum of Rosmarinic acid



Verbascoside (Acteoside)

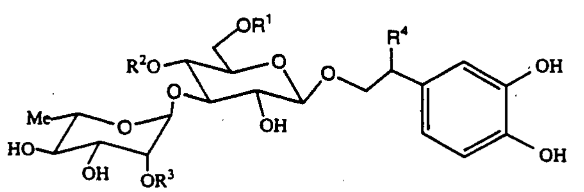


Figure 5.5. Structure of Acteoside

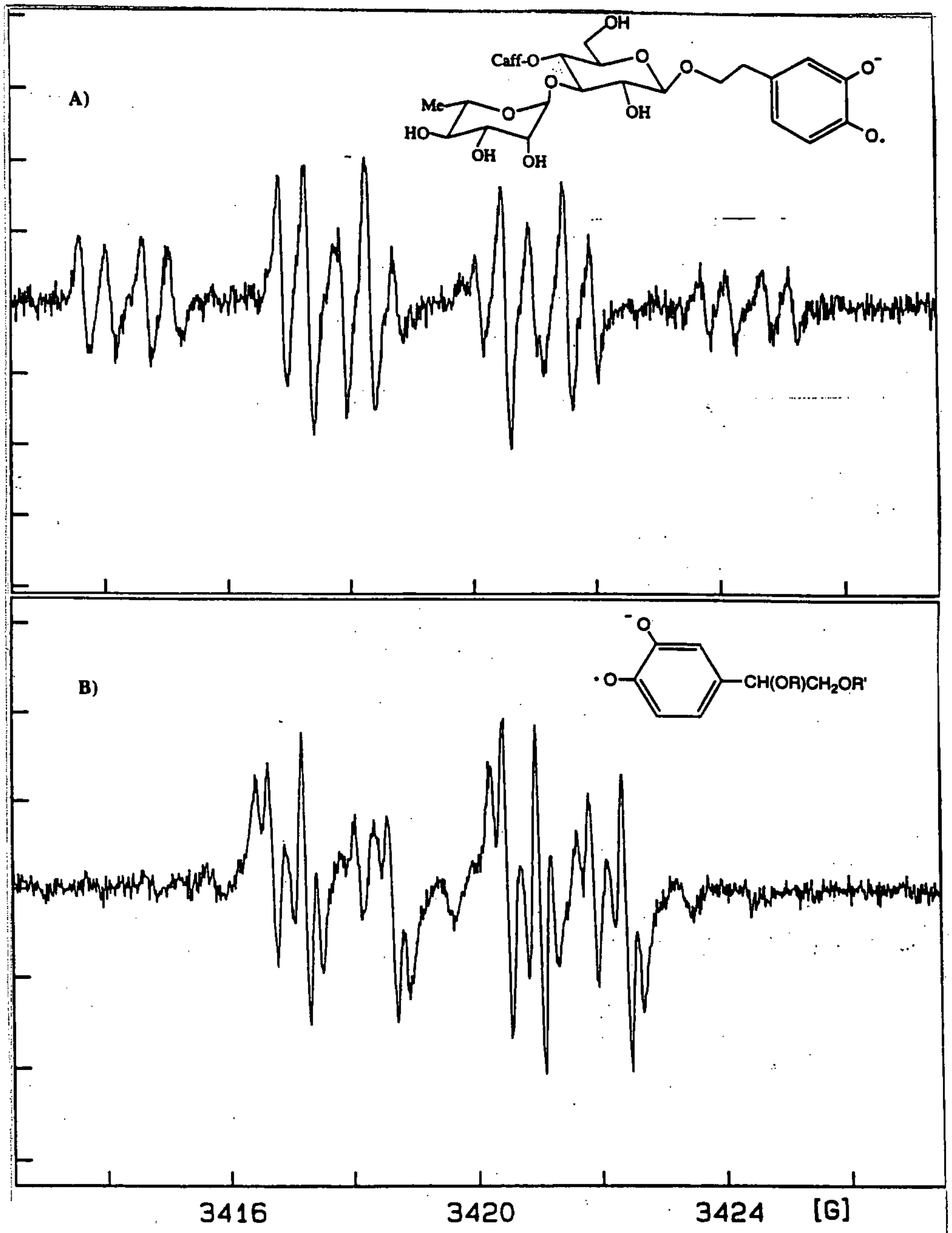


Figure 5.6. The ESR semiquinone spectrum of Acteoside

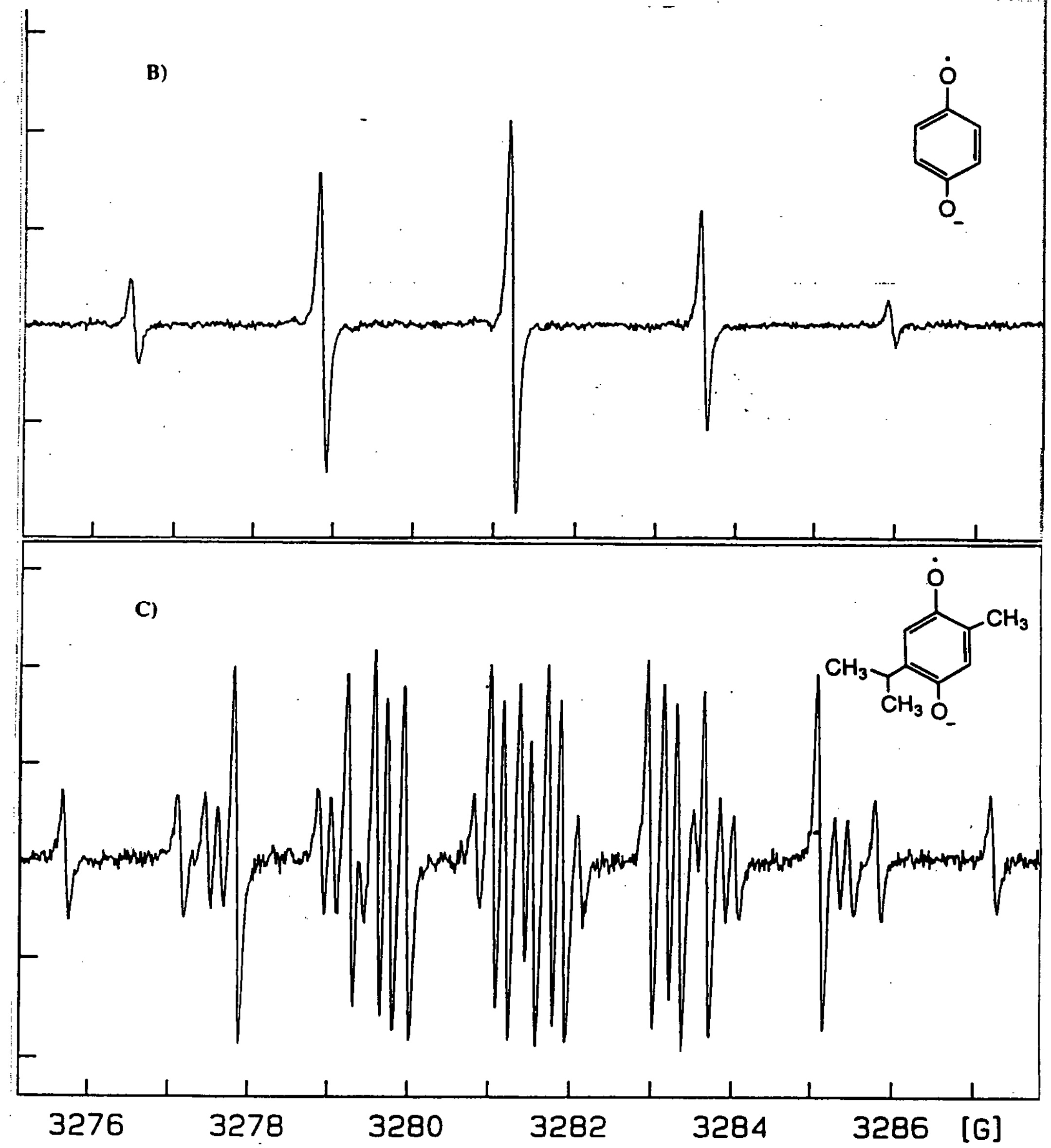


Figure 5.7. The ESR semiquinone spectrum of hydroquinone (B) and thymohydroquinone (C)

3,4-Dihydroxyphenylethanoid glycosides

Dihydroxyphenylethanoid glycosides (**A**) were identified in *Marrubium vulgare* L. (Lamiaceae), in *Aloysia triphylla* (Verbenaceae) and in *Osyris quadripartita* Salzm (Fig. 5.8). (Santalaceae) possibly recorded for the first time in this family (Table 5.2). In Lamiaceae, **A** seems confined to the subfamily Lamioideae *sensu* Erdtman (1945). It was observed recently in 111 species all confined to Lamioideae (see Pedersen, 2000) and is a strong chemotaxonomic marker for this subfamily.

Chlorogenic acid

In the present investigation this acid (**C**) (Fig. 5.3) was observed in *Artemisia herba alba* Asso. but not in *A. absinthium* L. or in two other Asteraceae. **C** is often observed in species of Asteraceae by way of the ESR technique. It is also often observed in species of Apiaceae, however, it was not detectable from any of the five Apiaceae taxa studied here.

Rosmarinic acid

I have observed rosmarinic acid (**R**) in *Mentha spicata* L. (Fig. 5.9 & 5.10), *Nepeta apulaei* Ucr., *Nepeta* sp. and in *Thymus maroccanus* Ball. and *T. saturoides* Cos., but not in four other Lamiaceae species studied. **R** was recently recorded from 110 species of Lamiaceae out of 127 studied (Pedersen, 2000) and all were confined to the subfamily Nepetoideae *sensu* Erdman (1945). Similarly to the role-played by **A** for Lamioideae the acid **R** seems a strong chemotaxonomic marker for Nepetoideae. It is also observed in many species of the related Boraginaceae. Otherwise rosmarinic acid is uncommon in the plant kingdom.

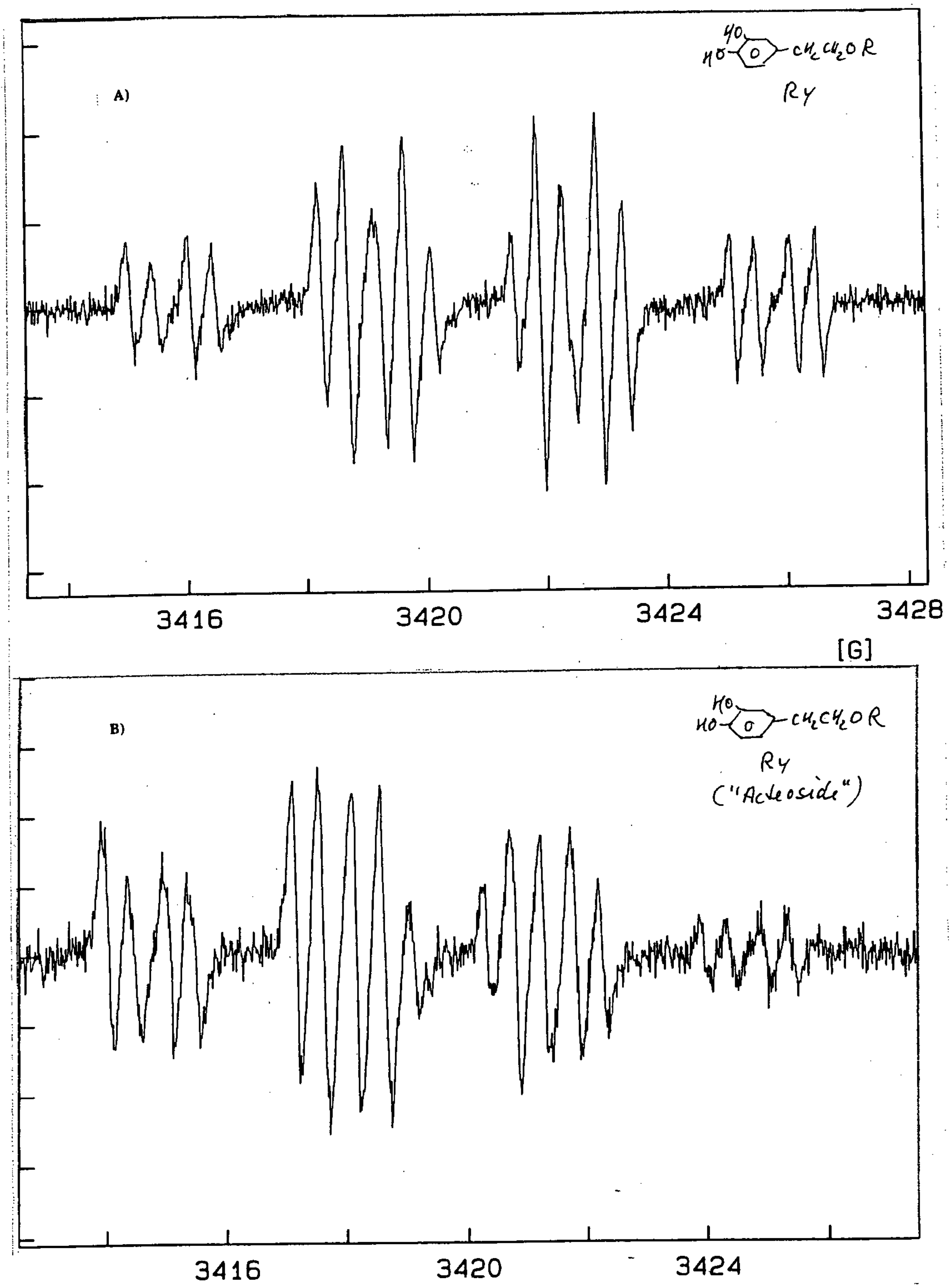


Figure 5.8. The ESR semiquinone spectrum of Acteoside of *Aloysia triphylla* (A) and *Osyris quadripartita* (B)

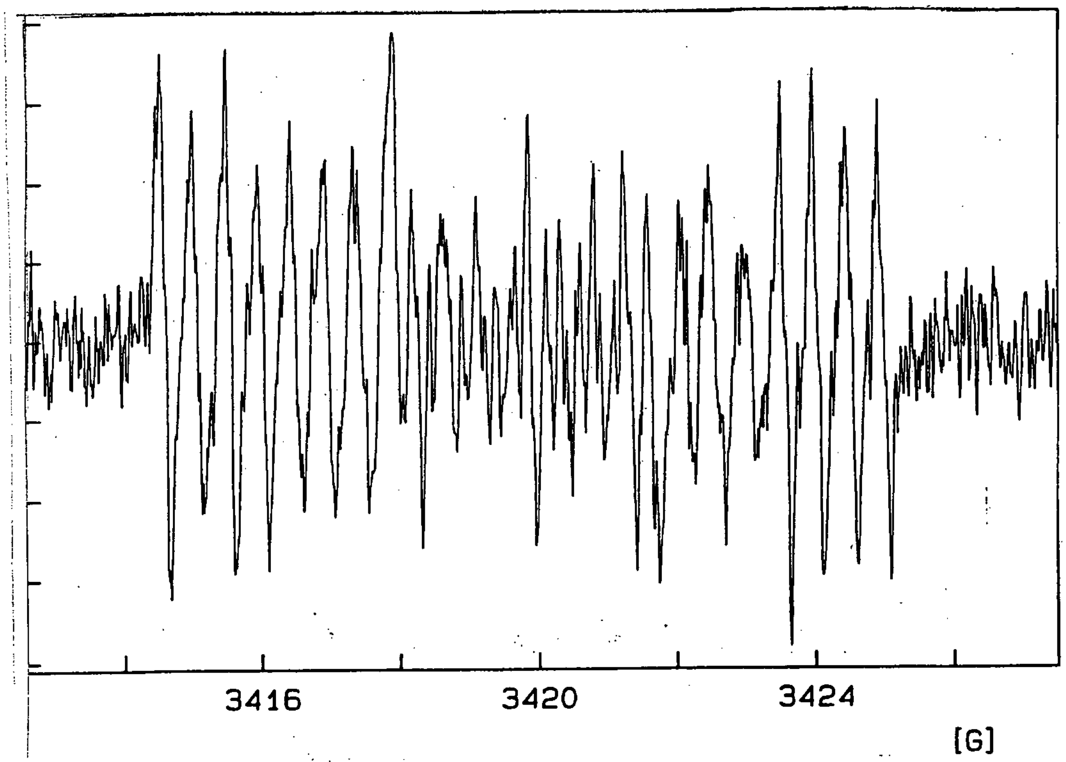


Figure 5.9. The ESR semiquinone spectrum of Rosmarinic acid of *Mentha spicata*

Figure 5.10. *Mentha spicata* L., Lamiaceae

(Boulos, 1983)

Thymohydroquinone

Thymoquinone (**T**) has previously been observed in a number of plant families, usually in the form of thymoquinol. The quinol oxidises easily to the quinone stage during isolation. I have observed thymoquinol in *Curcubita pepo* (Curcubitaceae) and in *Crotalaria saharae* (Leguminosae), in *Piper longum* (Piperaceae) but not in *P. Cubeba*. I found the compound in *Thymus maroccanus* (Lamiaceae) but not in *T. Saturoides*. Thymohydroquinone (Fig. 5.7) was recently observed in seven specimens of Lamiaceae, but it was not seen in *T. nitens* and in *T. polygoides* (Pedersen, 2000).

Naphthohydroquinones

From *Lawsonia inermis*, a strong signal for lawsone (**L**) (Fig. 5.11) was observed and as expected, a strong signal of juglone (**J**) (Fig. 5.12) was observed from the stem bark of *Juglans regia*. A strong and characteristic spectrum from *J. regia*, presumably derived from a naphthohydroquinone (**N**) of unknown structure was further observed (Fig. 5.13).

If the oxidation is performed on the ethanolic extract in stronger base (see Materials and Methods, Reaction II), I might observe degradation/hydroxylation products of compounds present in the extract. Thus, rosmarinic acid eventually leads to a product (designated **R_1**) that is selectively hydroxylated at the C-6 phenyl carbon of the dihydrocaffeic acid unit. The lack of **R_1** in some extracts clearly containing **R** is due to the fact that the hydroxylation process and subsequent transfer to the radical state (semiquinone) are delicate reactions where the optimum conditions may vary from extract to extract. Similarly, the alkaline (stronger base) oxidation of the **A**-compound leads to the selective hydroxylation at the C-6 phenyl carbon of the 3,4-dihydroxyphenylethyl moiety. The semiquinone radical of this artifact, designated **A_1**, exhibits a characteristic 12 line spectrum. Another artifact, 6-hydroxycaffeic acid (**K_1**) (Fig. 5.14) is frequently observed from extracts containing compounds possessing caffeoyl or dihydrocaffeoyl moieties. **R**, **C** and some **A** compounds fulfil this requirement.

Certain phenolics are unobserved by the ESR technique since they occur in a bound form in the extract, but the phenolic part ("aglycone") will appear when released by hydrolysis at the elevated pH of Reaction II. Thus caffeic acid (**K**) and protocatechuic acid (**P**) are often observed this way as discussed in the following:

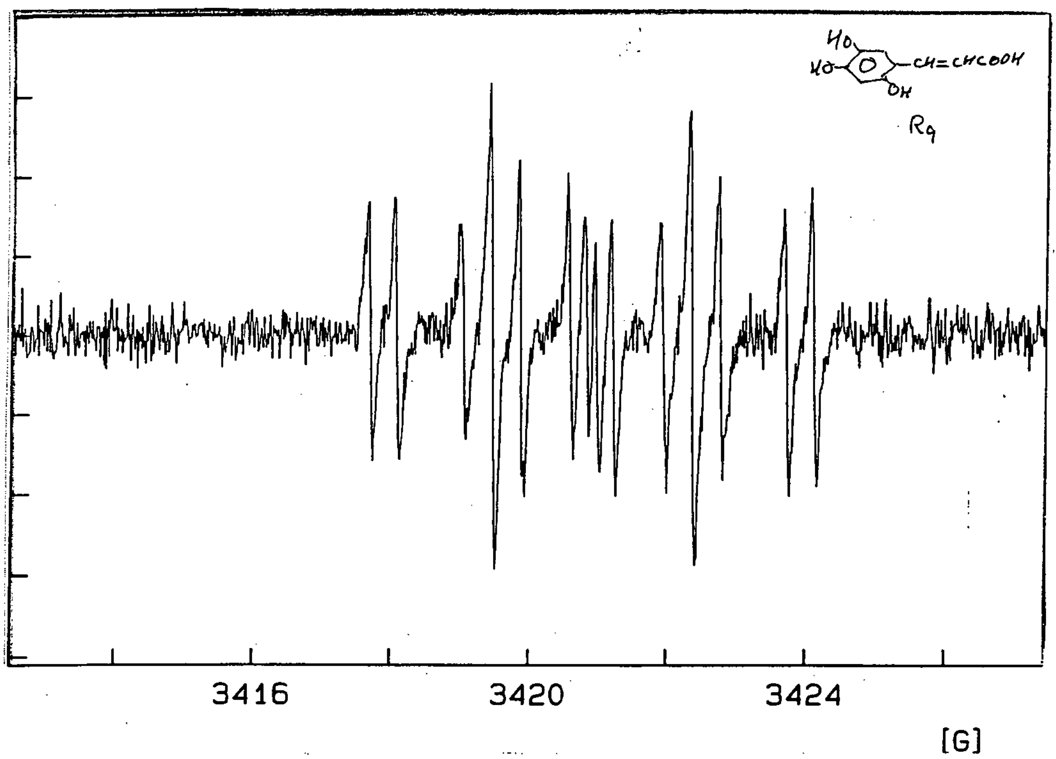


Figure 5.14. The ESR semiquinone spectrum of artifact, 6-hydroxycaffeic acid of *Aloysia triphylla*

Protocatechuic and caffeic acids

Neither of the above acids (**P** and **K**) has in the present study been observed as free acids in the extracts, e.g. they do not appear in Reaction I. Both acids are observed in Reaction II and are accordingly expected to be released from a bound form by hydrolysis in the alkaline milieu. **P** (see scheme) and **K** were observed this way in the seeds of *Ricinus communis* L. and **K** further observed in *Aloysia triphylla* (Fig. 5.15) and in *Nepeta* sp. The artifact 6-hydroxycaffeic acid (**K_1**) was observed in a number of species (Fig. 5.14).

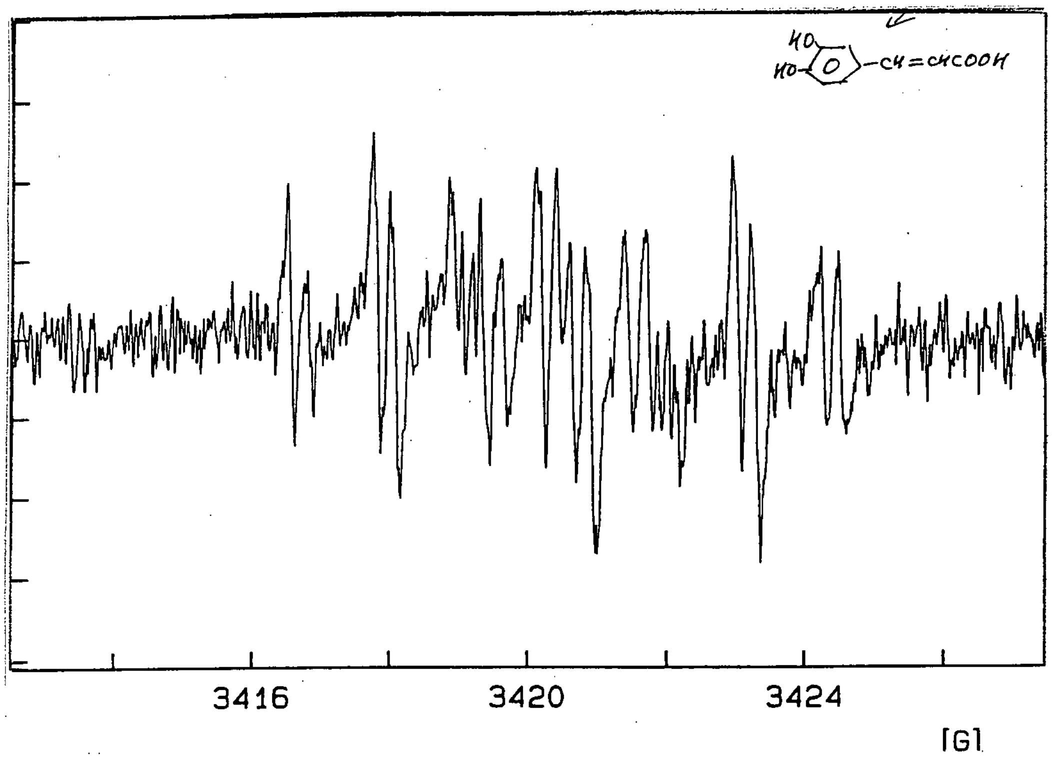
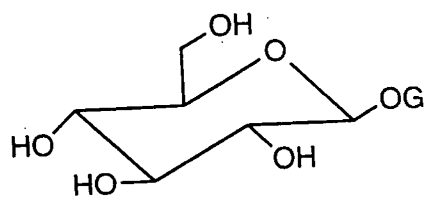
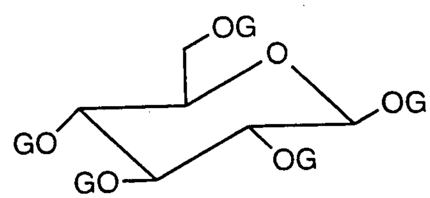


Figure 5.15. The ESR semiquinone spectrum of caffeic acid of *Aloysia triphylla*

Gallic acid esters are widely distributed in the Plant Kingdom, particularly herbaceous dicotyledons, and have been observed from more than 20 families (Haslam, 1982). Many of these esters as well as the free acid are easily observed by the ESR technique. The acid, usually liberated through hydrolysis, is unequivocally identified from its unique ESR spectrum and distinguished from its esters due to differences in g-factors and in hyperfine constants (Table 5.3). Thus, the two aromatic symmetric positioned galloyl protons, giving rise to a 1:2:1 triplet, furnish a splitting constant of 1.058 Gauss in case of the acid and a constant around 1.11 Gauss in case of the esters (Table 5.3). The galloyl moiety is unequivocally identified as well as the number of protons present at the alpha-carbon of the alcohol moiety. I shall designate gallic acid with **G** and the esters $\mathbf{E_3}$ (methyl gallate), $\mathbf{E_2}$, $\mathbf{E_1}$ and $\mathbf{E_0}$, the index assigning the number of alpha-protons present. It should be emphasised that $\mathbf{E_0}$, $\mathbf{E_1}$ and $\mathbf{E_2}$ are identified only in part. $\mathbf{E_1}$, however, yields a spectrum, which does not arise from isopropyl gallate, since isopropyl gallate furnishes a larger methylene proton constant than the one observed from $\mathbf{E_1}$. I tentatively assign $\mathbf{E_1}$ as derived from structures such as ß-D-glucogallin (cf. Figure 5.16 and Data of Table 5.3). Likewise, $\mathbf{E_2}$ (as well as $\mathbf{E_1}$) could be derived from structures such as ß-penta-galloyl-D-glucose (Figure 5.16).



ß-D-glucogallin, **E_1** type gallate



ß-penta-galloyl-D-glucose, **E_1** and E_2 type gallates

Figure 5.16

Two glucogallin structures, which could lead to simple ESR spectra of the type E_1 and E_2, cf. Table 5.3 and Figure 5.17, c and d, respectively. a) ß-D-glucogallin and E_1 type gallate (G = galloyl) and b) ß-penta-galloyl-D-glucose, E_1 and E_2 type gallates.

Gallic acid and its esters are often observed together by the ESR technique. Figure 5.17, (a) shows the spectrum observed from the crude extract of leaves of *Euphorbia falcata*. In the analysis of the complex spectrum of Fig. 5.17, (a) one has to perform a simultaneous simulation of all involved spectra in order to obtain a close match. Fig. 5.17, (a) contains spectra from three radicals. When first simulated I can subtract all but one spectrum in turn obtaining hereby the spectrum of gallic acid, Fig. 5.17, (b), and spectra of $\underline{\mathbf{E_1}}$ and $\underline{\mathbf{E_2}}$, Figs. 5.17, (c) and (d), respectively.

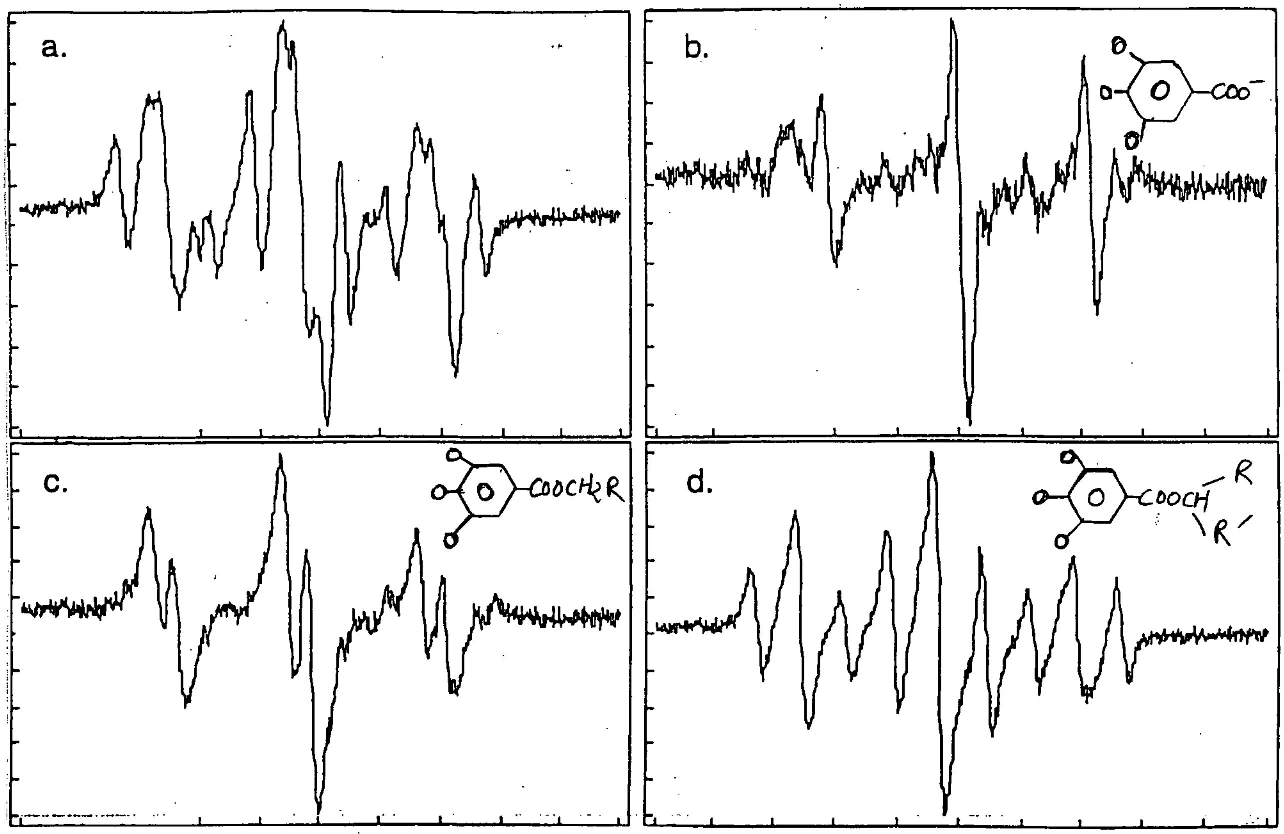


Figure 5.17

ESR spectra obtained from a crude extract of leaves of *Euphorbia falcata* L.
a) The original spectrum consisting of three spectra extracted in turn by spectral data subtraction: b) Spectrum of free gallic acid (artifact liberated by hydrolysis). c) Spectrum of E_1 type gallate. d) Spectrum of E_2 type gallate.

Finally, among the artefacts observed in Reaction II, I have selected two designated $\underline{\mathbf{X_5}}$ and $\underline{\mathbf{X_6}}$. They give rise to characteristic spectra and represent particular structures in the extracts. They are fingerprints for pertinent compounds. $\underline{\mathbf{X_6}}$ gives rise to an 8-line spectrum from three protons of the semiquinone nucleus. $\underline{\mathbf{X_5}}$ yields a 2-line spectrum. It is known (Kvist and Pedersen, 1986) that 5,6,7-trihydroxyflavones (e.g. 4'-methyl scutellarein) give rise to 2-line spectra from the single proton of the A-ring, with a splitting parameter nearly identical to the one of $\underline{\mathbf{X_5}}$. $\underline{\mathbf{X_5}}$ has been previously observed in 20 species of Gesneriaceae (Kvist and Pedersen, 1986) and in 23 species of Lamiaceae (Pedersen, 2000). From a number of extracts spectral line patterns appear as a definite proof of further phenolics being present in the plant extracts. These observations are indicated with an **X** in Table 5.2, since complexity or low intensity has precluded a safe analysis of the patterns.

Compounds of scarce occurrence

Dopamine (**D**) is a compound easily observed in Reaction I by the ESR technique. In Reaction II it is changed uniquely into 6-hydroxydopamine. I have observed 6-hydroxydopamine ($\underline{\mathbf{D_1}}$) (Fig. 5.18) from *Piper longum* in Reaction I, but have been unable to observe dopamine itself. Accordingly, $\underline{\mathbf{D_1}}$ seems to be a genuine constituent in this plant.

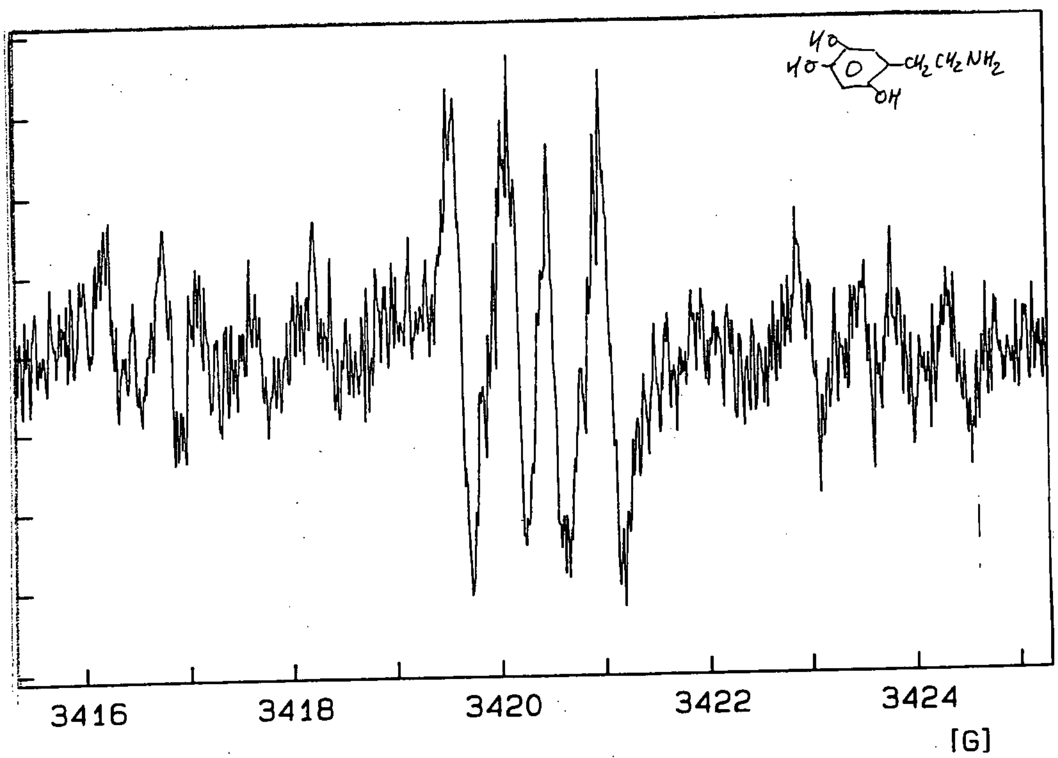


Figure 5.18. The ESR semiquinone spectrum of 6-hydroxydopamine of *Piper longum*

For the following specimens no phenolics were observed in the ESR screening, or lines observed had too low a S/N ratio to lead to confident spectral identifications: Apiaceae (*Ammi visnaga, Apium graveolens, Petroselinum sativum, Ridolfia segetum*); Asteraceae (*Artemisia absinthium, Cotula cinerum*); Brassicaceae (*Lepidium latifolium*); Caryophyllaceae (*Corrigiola telephiifolia*); Juncaceae (*Juncus acutus*); Linaceae (*Linum usitatissimum*); Leguminosae (*Acacia gummifera, Trigonella foenum-graecum*); Piperaceae (*Piper cubeba*); Punicaceae (*Punica granatum*); Rutaceae (*Haplophyllum vermiculare, Ruta graveolens*); Solanaceae (*Solanum nigrum*); Thymelaceae (*Thymelaea microphylla*); Zingiberaceae (*Ellettaria cardamomum*) and finally Aizoaceae (*Aizoon* sp.).

Table 5.1 ESR Data of Semiquinone Radicals observed from Moroccan Medicinal Plants

Radical	Structure	▶ ESR hyperfine splitting constants (Gauss)
A	Acteoside	0.45 (H), 1.00 (H), 3.60 (H), 3.10 (2H)
A_1	6-Hydroxyacteoside	0.58 (H), 0.96 (H), 3.08 (2H)
C	Chlorogenic acid	0.65 (H),1.17 (H), 1.17 (H), 2.32(H), 2.60 (H)
K	Caffeic acid	0.28 (H), 1.23 (H), 1.27 (H), 2.36 (H), 2.83 (H)
K_1	6-Hydroxycaffeic acid	0.39 (H), 1.35 (H), 1.78 (H), 2.90 (H)
R	Rosmarinic acid	0.52 (H), 1.06 (H), 2.21 (H), 3.30 (H), 3.80 (H)
R_1	6-Hydroxyrosmarinic acid*	0.21 (H), 0.54 (H), 1.02 (H), 2.40 (H), 4.10 (H)
T	Thymohydroquinone	1.43 (H), 1.79 (H), 1.95 (H), 2.16 (3H)
T_1	3-Hydroxythymohydroquinone	0.72 (H), 0.86 (3H), 4.14 (H)
G	Gallic acid	1.06 (2H)**
E_i	Gallic acid ester (Gallates)	1.11 (2H)**
P	Protocatechuic acid	0.80 (H), 1.35 (H), 3.30 (H)
D	Dopamine	0.41 (H), 1.031 (H), 2.95 (H), 3.65 (2H)
D_1	6-Hydroxydopamine	0.53 (H), 0.89 (H), 3.34 (2H)
J	Juglone	0.31 (H), 0.66 (H), 1.26 (H), 1.27 (H), 3.00 (H), 3.40(H)
L	Lawsone	0.09 (H), 0.15 (H), 0.23 (H), 1.53 (H), 1.94 (H)
N	Unknown (naphthoquinone?)	0.09 (H), 0.25 (H), 0.36 (H), 2.14 (H), 2.48 (H)
X_5	Unknown	0.20 (H)
X_6	Unknown	0.61 (H), 0.99 (H), 5.79 (H)
X	Compounds with unknown structures (safe analysis of spectral patterns not obtained)	

* The analysis of the complex spectrum of **R_1** is incomplete, viz. a = 2.40 and 4.10 might read 2.95 and 3.55.

** See Table 5.3.

▶ Hyperfine constant (hfs) is a measure of how strongly the unpaired electron interacts with nuclei in the radical. It is only nuclei with nuclear spin different from zero who are of interest, i.e. H, N, C-13 etc. C-12 or O-16 does not give rise to hyperfine interactions. The hfs-constants are measured in Gauss or in Tesla.
In case of semiquinones, hfs-constants are only observed from the protons present. The unpaired electron is distributed over the radical skeleton and it is the probability of meeting the electron at a specific proton that determine how strongly the interaction will be and hereby determining the size of the hfs-constant.
In NMR, there are spin-spin interactions between the various nuclei, whereas in ESR, there are spin-spin interactions between the unpaired electron spin and spin from the various nuclei.

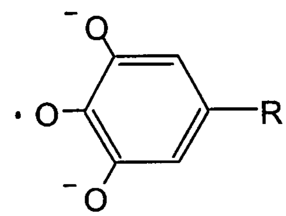
Table 5.2. Occurrence of dihydroxyphenolics in studied taxa

Family/name	Local name	Plant part	Compounds	Artifacts	Others
Aizoaceae					
Aizoon sp.	Al Ghassoul	whole	-		
Apiaceae					
Ammi visnaga (L.)Lam.	Bachnikha	flowers	-		
Apium graveolens L.	Lakrafass	leaves	-		
Carum carvi L.	Carwia	seeds	-		
Petroselinum sativum Hoffm.	Al mahadnouss	leaves	-		
Ridolfia segetum Moris	Tebsh	aerial	-		
Asteraceae					
Artemisia absinthium L.	Shiba	aerial	-		
Artemisia herba alba Asso	Al shih	aerial	**C**		
Cotula cinerea Delile	Qertufa	aerial	-		
Matricaria recutita L.	Babnouj	flowers	-		**X**
Brassicaceae					
Lepidium latifolium L.	Habb r-shad	seeds	-		
Caryophyllaceae					
Corrigiola telephiifolia Pour.	Sarghina	roots	-		
Cucurbitaceae					
Cucurbita pepo L.	Bsibsiya	flowers	**T, E_2**	**G**	
Cupressaceae					
Juniperus phoenicea L.	Earear	stem/leaves	**E_0**		
Euphorbiaceae					
Euphorbia falcata L.	Hayat al noufouss	aerial	**E_1, E_2**	**G**	
Ricinus communis L.	Kherwae	seeds	-	**K, P, K_1**	
Juglandaceae					
Juglans regia L.	Swak	stem/bark	**J, N, E_0, E_1, E_2**	**G**	**X**
Juncaceae					
Juncus acutus L.	Zariht Smaar	seeds	-		
Lamiaceae					
Lavandula angustifolia Mill.	Khzama	flowers	**X_6**		
Marrubium vulgare L.	Merriut	aerial	**A**		**X**
Mentha pulegium L.	Fliyu	aerial	**X_6**	**K_1**	
M. spicata L.	Mint	leaves	**R**	**K**	
Nepeta apulaei Ucr.	Qestal	aerial	**R, X_6**	**R_1, K_1**	
Nepeta sp.	Hashish Al Quatt	aerial	**R, X_6**	**K, K_1**	
Rosmarinus officinalis L.	Aziir	stem/leaves	**X_6**		**X**
Stachys arvensis L.	Ktira	aerial	-		
Thymus maroccanus Ball	Zahtar	aerial	**R, T**	**K_1**	
T. satureioides Cos.	Azukenni	aerial	**R, X_6**	**K, K_1**	**X**
Leguminosae					
Acacia gummifera Willd.	Talh	aerial	-		
Crotalaria saharae Coss.	L-Fula	roots	**T**		
Trigonella foenum-graecum L.	Helba	seeds	-		
Linaceae					
Linum usitatissimum L.	Kettan	seeds	-		
Lythraceae					
Lawsonia inermis L.	Henna	leaves	**L**		**X**

Pinaceae					
Pinus halepensis Mill.	Taida	bark	-		
Piperaceae					
Piper cubeba L.F.	Kabbaba	cubebe	-		
Piper longum L.	Dhar Falfal	seeds	**T, D_1**		
Polygonaceae					
Rumex bucephalophorus L.	Hummida	aerial	**E_1**	**G**	
Punicaceae					
Punica granatum L.	Romman	peel of fruit	-		
Ranunculaceae					
Nigella sativa L.	Sanuj	seeds	**T**	**T_1**	
Rosaceae					
Rosa canina L.	Ouard	flowers	**E_0, E_2**	**G**	**X**
Rutaceae					
Haplophyllum vermiculare Hand. & Maz.	Chajarah er-rih	whole	-		
Ruta graveolens L.	Mrijou	aerial	-		
Santalaceae					
Osyris quadripartita Salz.	Bu lila	roots	**A**	**A_1**	
Sapotaceae					
Argania spinosa (L.) Skeels	Argan	fruits/ oil	-		
Solanaceae					
Solanum nigrum L.	Eneb d-dib	fruits	-		
Thymelaeaceae					
Thymelaea microphylla Coos. & Dur.	Metnan	aerial	-		
Verbenaceae					
Aloysia triphylla Britt.	Lwiza	leaves	**A**	**A_1, K,K_1**	
Zingiberaceae					
Alpinia officinarum Hance	Khdanjaal	rhizomes			
Elettaria cardamomum	Qaeqalla	seeds	-		

A Acteoside, **A_1** 6-Hydroxyacteoside, **C** Chlorogenic acid, **K** Caffeic acid, **K_1** 6-Hydroxycaffeic acid, **R** Rosmarinic acid, **R_1** 6-Hydroxyrosmarinic acid, **T** Thymohydroquinone, **T_1** 3-Hydroxythymohydroquinone, **G** Gallic acid, **E_i** Gallic acid ester (Gallates), **P** Protocatechuic acid, **D** Dopamine, **D_1** 6-Hydroxydopamine, **J** Juglone, **L** Lawsone, **N**Unknown (naphthoquinone?), **X_5** Unknown, **X_6** Unknown,**X** Compounds with unknown structures

Table 5.3. ESR coupling constants/Gauss and g-factors from the semiquinone radicals of gallic acid and some of its esters.



R		a_{arom}	a_{alif}	►g	Ref.
COO^-	G	1.058	-	2.00479	a,b
$COOCH_3$	G-Me (E_3)	1.111	0.376	2.00491	b
$COOCH_2CH_3$	G-Et	1.104	0.366	2.00490	b
$COOCH_2R'$	E_2	1.113	0.369	2.00490	a
$COOCH(CH_3)_2$	G-iPr	1.104	0.220	2.00490	b
COOCH(R'R'')	E_1	1.113	0.196	2.00490	a
COOCR'(R''R''')	E_0	1.113	-	2.00490	a

[a] This work. [b] Pedersen, 1985.

►The g-factor determines where the spectrum will be found on the x-axe (B-axe). It is measured from the following resonance equation:

$h\nu = g\beta B$
h = Planck's constant
$\nu = \approx 9.5$ GHz
β = Bohr magnetron
$B = \approx 3400$ Gauss

The x-axe is a field axe in Gauss or Tesla (B-field) and the ESR spectrum is found lying symmetric around a resonance field B_0. B_0 is usually found around 3400 Gauss for instruments constructed with X-band (9.5 Hz). The y-axe is a kind of concentration axe, however it is not used as such. Quantification is very difficult to perform and often not performed. Using a standard sample in connection with advanced spectral simulations sometimes makes only relative concentration estimates. When there is more than one spectrum from different radicals, a simulation is performed involving all the radicals (spectra). A simulation is performed for one spectrum; i.e. spectrum 1 and all the remaining simulated spectra are subtracted. The result will be the experimental spectrum 1, plus what remains in case the simulation is not perfect.

Limitations of ESR technique

The present study has demonstrated that the ESR technique is a useful alternative tool to traditional Liquid Chromatography / Mass Spectroscopy (LC/MS) techniques in detecting known phenolics in plants. The technique was developed by Pedersen for use with plants in chemical taxonomy research (Pedersen, 2000). The power of the technique is demonstrated by its ability to detect specific quinols/quinones/phenolic acids or acid esters from small amounts of plant material without prior fractionation. While the ESR technique is very suitable and applicable to chemotaxonomic studies of larger groups of plants (family or genera levels), it is only complimentary to validated methods of compound identification (e.g. NMR. LC/MS) with known ESR spectra. Thus pure compounds that have not been previously analysed by ESR (e.g. **X5** and **X6**, Table 5.1 and 5.2) can be observed, but not identified without isolation and identification using other validated analytical techniques. Specifically, only quinones and compounds with an ortho or a para dihydroxy grouping, e.g. oxidizable phenolics can be immediately observed by the technique. Furthermore, compounds without oxidizable phenolics cannot be observed using this technique.

Quantification in ESR is very difficult to perform and often not attempted. Using a standard sample in connection with advanced spectral simulations sometimes makes only relative concentration estimates. Finally, quantitative concentration estimates are obtainable, especially in chemotaxonomic studies of larger groups of plants (Kvist and Pedersen, 1986; Pedersen, 2000). However, since the studies are performed at an elevated pH, original compounds might undergo transformations resulting in lowered concentration estimates, further amplified by the presence of endogenous quencher compounds. ESR quantification may be complemented by some more unambiguous techniques that could be used to identify and quantify phenolics in plants. Some examples are, iron (III) chloride chromogenic reagent followed by spectrophotometric analysis, TL (or paper) chromatography with densitometry of chromogenic reactions, or HPLC analysis using internal standards for quantitation and suitable detection e.g. LC/MS; or in situ NMR, without prior chromatographic separation, for some small molecules with very characteristic functional groups, e.g. sesquiterpene lactones.

Medicinal Significance

From the chemical survey of forty-nine Moroccan medicinal plant species comprising 45 genera of 27 families performed by (ESR) spectroscopy, I can conclude the following: Many phenolic compounds identified by ESR are common (e.g. caffeic acid, gallic acid) and rosmarinic acid proved to be a strong chemotaxonomic marker for the subfamily Nepetoideae. Chlorogenic acid was only observed in *Artemisia herba-alba* but not in *A. absinthium*. Thymoquinol was not confined to Lamiaceae but was observed in different families, and thus may not be as useful in chemotaxonomy.

I found either of these phenolic compound plastohydroquinones and 3,4-dihydroxyphenylethanoid glycosides, to be genuine, the latter being a strong chemotaxonomic marker for the Lamiaceae. The 3,4-dihydroxyphenylethanoid glycosides also occur in an unrelated family, Santalaceae. Some compounds were scarce e.g. thymohydroquinone or naphthoquinones such as juglone or lawsone, but also unique to some plants investigated. One example is 6-hydroxydopamine (**$\underline{D_1}$**) which was detected in *Piper longum*. These unique compounds identified in this study could be important for taxonomy purposes (Pedersen, 2000). They may also have medicinal significance. As I reported in a previous paper (Mouhajir et al., 1999), thymohydroquinones are potent antimicrobials. Furthermore, Saxena et al. (1994) reported the antibacterial effect of methyl gallate. Gallic acid and its esters were often observed together in the leaves of *Euphorbia falcata*. 6-Hydroxydopamine, present in *Piper longum*, has been recorded in only one other species of plants, *Musa paradisiaca* L. (Duke, 1992). *Piper longum* is used in Moroccan traditional medicine against colds and also as an aphrodisiac. One may speculate that the presence of dopamine explains its use in folk medicine. Future studies could be useful if directed towards these unique compounds detected by ESR.

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Chapter VI

Summary Discussion

The scope of this project was broad and multidisciplinary, with aspects in ethnobotany, pharmacology, virology, microbiology and phytochemistry.

Its synopsis is as follows:

1. Ethnomedicine in Morocco

With well over 4500 plant species in Morocco, of which 600 are reported to have medicinal value, a random screening of plants for compounds with biological activity would be inefficient. Cox and Balick (1994) stressed the need for a systematic collection strategy to explore the large pool of plants for potential medicines. Several approaches can be taken to establish a scientific basis for the successes of folk medicine (Balick and Cox, 1996; Cox, 1994).

Like many other workers, I chose the ethnobotanical approach as a means of homing in on plants with pharmacologically active compounds of potential therapeutic interest. This approach was useful and successful in my research, as many plants collected in the field were shown to be active in the laboratory tests against microbes and viruses.

Although I included many Arab medicinal plants, I was more interested to study endemic and Berber medicinal plants and this aspect of my research was unique and original as no studies of Berber medicinal species had been carried out before. This resulted in the evaluation and documentation of Berber and endemic traditional uses, which was, prior to this research, an oral tradition. The latter would have been probably destined to be lost if not recorded for scientific purposes and for future generations, as many healers are elderly and lack apprentices to transmit medical wisdom accumulated by many.

Re-examining plant remedies described in ancient Arabic texts was also part of this research. To investigate further the ethnobotanical uses of local pharmacopoeias, fifty-five interviews were conducted. Arab and Berber traditional healers, herbalists and knowledgeable people from a variety of different ethnic groups from the Northern mountainous region of the Rif, the Middle Atlas and the Anti Atlas and the eastern and southwestern region of the Sahara contributed information.

I selected plants traditionally used to treat infectious diseases caused by bacterial, viral and fungal pathogens, such as fever, colds, flu, coughs, wounds, cuts, diarrhea, dysentery, venereal diseases, hepatitis and skin problems such as rashes, blemishes, boils and poxes.

Some issues in medicinal plant uses were encountered like diagnosis, prescription, dosage, toxicity, preparation and administration route, as well as the social, economic, political and ethical context of medicinal plant research. It was found that a correct diagnosis enables the Healer to make an appropriate prescription. Some Healers may experience difficulty reaching the right diagnosis, and may experiment with patients.

Many plants in use are toxic at high doses; consequently dosage is extremely important and may influence whether a plant is an effective medicine or a poison. Identification of plant material is also crucial in order to avoid a toxic plant being mistaken for a nontoxic one, as well as its collection at the right time, as some plants are efficient or toxic in the flowering or fruit stage and vice versa. The right preparation (infusions, decoctions, pastes or juices) and administration route of the plant material are also very important.

2. Plant Collection

Two hundred and five herbarium vouchers of fifty-five plant families were collected and filed at the University of British Columbia herbarium, UBC, Vancouver, Canada and at the Institute of Agronomy and Veterinarian Sciences, Hassan II herbarium, Rabat, Morocco. Eighty medicinal plants were collected and dried in Morocco for biological and chemical analysis.

3. Antimicrobial activities in selected plant species

Crude extracts of selected plant species were analyzed for activities against a variety of bacteria and fungi. The presence of natural photosensitizers was investigated by varying light conditions. Activities were observed against gram-negative as well as gram-positive bacteria and against fungi. A broad spectrum of activity was also observed for some extracts. Light had an important effect on the activity of some plants, which showed light-dependent or light enhanced activity. The presence of natural photosensitizers in plants explains the light-dependent or light enhanced activity. Towers and Hudson stressed the need to test for light-mediated activities when screening plants for antimicrobial or antiviral activities (Towers et al., 1997). Previous studies may have missed potentially important activities because of this lack of consideration (Hudson and Towers, 1999).

4. Antiviral activities

I investigated the antiviral activities of methanol extracts of 75 Moroccan plants (64 genera from 35 families). They were evaluated against three mammalian viruses: herpes simplex virus, Sindbis virus and poliovirus, at non-cytotoxic concentrations. Five extracts were very active against the three viruses, at minimal inhibitory concentration of only 1.5 μg/ml. Sixteen were active against two viruses, and 24 were only active against one virus. Thirty-two extracts contained photosensitizers, indicated by the presence of either light enhanced or light-dependent activity. The toxicity of these extracts against Vero cells was also investigated. Approximately 10 plants displayed some cytotoxicity at high concentration (200μg/ml). Thus caution should be taken when considering these plants as medicines. Lower concentrations have to be maintained in therapeutic use.

These results indicate that some of these plants are potential medicines against infectious diseases caused by viruses. Their discriminatory effects against specific viruses suggest the presence of different phytochemicals. However there are severe limitations in predicting *in vivo* drug efficacy from *in vitro* results, because ingested chemicals may undergo metabolic transformation in the body.

The antiviral properties of *Juglans regia*, *Thymus maroccanus*, *Lawsonia inermis*, *Pinus halepensis*, *Rosa canina*, *Acacia gummifera*, *Ricinus communis*, support their traditional use (s). However, for others the traditional use does not appear to match the results obtained. This might suggest a discrepancy at the diagnostic level or at the prescription level, meaning that the plant may have other uses than treatment of infectious diseases. It may also mean difficulty for a healer to diagnose infectious diseases. Furthermore, in several cases the medicinal plants are also used in a holistic approach that is impossible to test with the bioassays proposed in here which are restricted to looking for direct antiviral effects. It should also be considered that the plant might exhibit indirect activity *in vivo*, such as stimulation of certain host defenses, including the immune system.

Punica granatum extract, which was the most active, inhibited the three viruses at a minimum concentration of 1.5 μg/ml, may be considered as a potential antiviral lead for drug development. *Thymus maroccanus*, which inhibited Sindbis virus and HSV, was light activated, and is endemic to Morocco, has promising properties and may also be a good source for antiviral. It is also interesting to note that *Lawsonia inermis*, which is traditionally used often for cosmetic purposes (tattooing), as well as epidemic, is very active against SinV. Extracts of *Pinus halepensis* and *Punica granatum* the most active, inhibited poliovirus the most resistant of the viruses, with at a minimum concentration of 6.5 μg/ml. The activities of *Rumex bucephalophorus, Artemisia herba-alba, Quercus* *faginea, Pinus halepensis* and *Punica granatum* were light independent, thus these extracts have no photosensitizers.

It is worth mentioning that this is the first and unique report in medical history on antiviral activities of Berber and endemic Moroccan plants and thus represents an original contribution to knowledge. For example, there was no prior record of the ethnobotanical or biological activity of *Ammoides pusilla* (Apiaceae) in the litterature. The three Berber and endemic species of *Thymus* (*T. maroccanus*, *T. broussonetii*, and *T. satureioides*), are very active against viruses and are also photoactive. It is interesting to note that *Thymus maroccanus* is the most active. This is the first report of antiviral activity of *Ammoides pusilla, Crotalaria saharae, Pulicaria arabica, and Salvia clandestina.* Another original result is the antiviral activity of a miscellaneous material, e.g. sea urchin, reported for the first time in this thesis. These results indicate that the choice of selecting Berber and endemic species for biological activities was judicious. It may also confirm that biological activity is not limited to plants but may be extended to other kind of material, e.g. mineral, animal, which has been known for centuries. The Moroccan pharmacopoeia makes good use of the vegetable, animal, and mineral kingdom (Bellakhdar (1997).

5. Phytochemistry

Thymohydroquinones in *Nigella sativa* L. were detected by ESR. ESR also detected other phenolics in fifty Moroccan medicinal plant species comprising 45 genera from 27 families. These phenolics are useful as fingerprints for pertinent compounds and markers for chemotaxonomic research. Some phenolics were found to be common (e.g.

gallic and caffeic acids); interestingly, others were less common (e.g. dopamine). These compounds could be important not only for taxonomic purposes but also may have medicinal significance as antimicrobial thymohydroquinones. 6-Hydroxydopamine, present in *Piper longum*, has been recorded in only one other species of plants. One may speculate that the presence of this chemical explains its use in folk medicine, as an aphrodisiac and against colds. Thus, the ESR technique, is an alternative, useful tool to traditional LC/MS techniques in detecting known phenolics in plants. The power of the technique is demonstrated with its ability to detect specific quinols/quinones/phenolic acids or acid esters from small amounts of plant material without prior fractionation. While ESR technique is highly applicable to chemotaxonomic studies of larger groups of plants, with known ESR spectra, it is nevertheless only complimentary to validated methods of compound identification. Thus pure compounds that have not been previously analyzed by ESR (e.g., **X5** and **X6**, in Table 5.1 and 5.2) can be observed, but not identified without isolation and identification using other validated analytical techniques. Specifically, ESR can immediately detect only quinones and compounds with an ortho or a para dihydroxy grouping, e.g., oxidizable phenolics. Furthermore, compounds without oxidizable phenolics cannot be observed.

6. Further investigations

Further investigations of each part of this study are definitely worthwhile. I would have profited if I had been able to carry out more work in the field to obtain a more complete understanding of the role of women in Moroccan ethnomedicine, and to communicate results of my studies to traditional healers. I will comply with the ethical aspects of my project by returning the results to the Moroccans. A copy of this thesis will be sent to the Institute Agronomic and Veterinarian Sciences, Rabat, Morocco, and to the International Development Research Centre (Ottawa, Canada).

A booklet written in Arabic, French and English, summarizing the final results of this study on medicinal plants is planned for the people who gave the information, and for health authorities and the Moroccan government following the guidelines outlined by Grenier, (1998), ISE, (1998), Posey, (1997), Richter and Carlson, (1998). The traditional knowledge as well as the *in vitro* antimicrobial, antiviral results, plus any known chemistry of the plants will be translated for distribution to parties involved.

Validation of local medicinal plant uses and potential toxicity from inappropriate usage were also shown from this study. Cytoxicity tests in the laboratory as well as the interviews revealed potential health hazards associated with the use of the medicinal plants investigated. The results will be disseminated to the public by means of the booklet described above.

Although the booklet will be useful for the literate healers, during my consecutive field trips, I realized that many of the healers, particularly the women, are illiterate. Thus, further contact with them will allow me to report my results orally. I believe this will make a most useful contribution to the health of local rural peoples in Morocco. The availability of local pharmacopoeia supported by scientific data could be beneficial to large populations without access to modern drugs as well as to host country collaborators including scientists, professors, field botanists, traditional healers and many others

A major portion of the time spent on this project was allocated to bioassays, particularly concerning viruses. If more time were available, studies on mode of action and possible synergistic effects also could be tested. Identification of antiviral compounds would have been a worthwhile investigation.

7. Thesis

Definition of a thesis include (a) A proposition that a person (as a candidate for scholastic honors) advances and offers to maintain by argument. (b) A dissertation embodying results of original research and especially substantiating a specific view especially: one written by a candidate for an academic degree. (c) A thorough, written presentation of an original point of view. (d) It is also the process of arriving at the truth by stating a thesis, developing a contradictory antithesis, and combining and resolving them into a coherent synthesis. Analytical philosophy is defined as a cluster of philosophical traditions holding that argumentation and clarity are vital to productive philosophical inquiry.

My thesis in this work is that the Moroccan local pharmacopoeia has some scientific basis? In other words, does the experimental bioassay system proposed here support the usage of these plants as medicines, for infectious diseases, by Moroccan people? Do the medicinal plants have biological activity against bacteria, fungi, and viruses?

The antithesis will be that the medicinal plants used for infectious diseases have no scientific value, or no biological activity in the system used in this research.

The results of the biological activities, antimicrobial and particularly antiviral activities, clearly demonstrate that use of the local pharmacopoeia is effective in approximately 70% of the cases for all the plants tested. Furthermore, some plants like *Punica granatum*, identified through ethnobotanical information, proved to be very potent, with activity of 1.5 μg/ml against SINV, herpes and polio viruses. This single result is extremely interesting because antiviral drugs are sorely needed, given the high incidence of infectious viral diseases around the globe, the prevalent resistance of microorganisms to antibiotics and antiviral agents, and the lack of potent antivirals drugs, only few available in the market. Until recently, only six antiviral drugs had been approved in the USA (Tortora et al, 1989). Furthermore, to my knowledge, among those drugs, none is active against SINV or polio viruses. The drug amantadine is active against influenza A virus, and acyclovir neutralizes a range of herpesviruses. Prostratin is active against HIV.

Although 70% of the plants tested showed impressive biological activities, 30% didn't perform as well in the system used here. Further studies could be useful if these plants were tested against an array of biological tests. For example, the National Cancer Institute (NCI) examines plants in a variety of bioassays (anticancer, HIV, etc). Tiny amounts of material can be tested rapidly against an array of up to 60 distinct human tumor cell lines (Cox and Balick, 1994). Many other assays assess the ability of an extract to influence the activity of a single enzyme (Proteomics) involved in the biochemical interactions that underlie a disease. For example, for AIDS treatment, the extract is evaluated for its ability to inhibit the activity of the enzyme reverse transcriptase in cells, needed for replication of the viral particles.

It is surprising to realize that many researchers disregard the effect of light in the evaluation of biological activity in plants. Many plants tested in this thesis are photosensitizers. In Moroccan pharmacopoeia, species containing photosensitizing furocoumarins, such as *Ammoides pusilla* (Apiaceae), *Ammi visnaga* (Apiaceae), and *Ficus* (Moraceae) as well as *Lawsonia* (Lythraceae), *Juglans regia* (Juglandaceae), species containing naphtoquinones (lawsone and juglone,), have been used for centuries, for chemotherapy, sunshine been advocated for treatment of skin diseases, e.g. vitiligo.

There is an advantage of selecting plants using the ethnobotanical approach. In this thesis, this approach proved to be productive (antiviral results), as it facilitated the discovery of the most potent, and interesting plants. NAPRALERT (1975-1998) was a good tool in providing extensive verification of the literature, and facilitated the targeting of the most rare and unstudied species from Morocco. Ethnobotanical information was also helpful in identifying the toxic plants. Such repetitive, long-term use of botanical species by Berber people can be expected to have identified both the most effective medicinal plants and those that are too toxic for use.

It is difficult to evaluate the activity of medicinal plants used as a panacea for diverse ailments, in the system proposed here. Plants used exclusively for infectious diseases are more active. It is also not possible to generalize the biological activity obtained, e.g. inactivation of the virus tested in the cell model system (Vero Cell line) to other viruses or cell lines.

The documentation of medicinal knowledge shouldn't hide the problem of extinction of flora and fauna and destruction of the environment. What use will it be if the knowledge is documented but the plant is extinct. Conservation of plant species is essential by establishing park, botanical gardens, for example, encouraging people to use plants in a sustainable way that guarantees their ongoing availability.

What is being done to protect the interests of healers and their communities? The probability that drug companies, universities and Western scientists will profit financially from information provided by healers in traditional societies raises this serious question. The healers provide significant intellectual guidance. Usually, compensation is given to those communities as royalties earned from the drug. What will be the compensation in my case, as biological activity was shown for some plants but no drug was developed? Will the return of results (booklet) to host country collaborators be a fair compensation?

Healers often practice both as generalists and specialists. Specialized information or secret can be easily traced to few people, however who owns public information? In that case how to return benefits and to whom is often a tough question. It is estimated that 95% of the world's biological diversity is in the world's poorest countries. Sharing the findings or the wealth, if any, from bioprospecting to help people in these countries, could be in the best interests for all, and may save biodiversity and possibly protect potential new drugs for saving lives.

For Berber and Arab people, living in a rural environment, the local pharmacopoeia offers many advantages. The use of plants is simple, inexpensive and appropriate for large population, with few means and no or limited access to western drugs. Then, what is the use of this study? How will it help these people? It might help by confirming the effectiveness of some plants used for infectious diseases. It will also confirm the toxicity of others. I do hope that this study will be both useful in Morocco, Canada and possibly extended to the world.

Will proteomic and genomic products replace plants in therapy? It seems not probable, particularly in the area of infectious diseases. Those high-tech approaches will probably be very promising in genetic diseases, cancer and other 'internal" disorders. However, for diseases linked with the environment, like viruses, particularly the ones with high mutation rates, they will probably not be applied.

It is interesting to realize and be part of the cycle of the transfer of plant knowledge. I started my introduction by reporting the contributions of past scientists. I opened up with the beginning of humanity, then talked about Egyptians, Greeks, Arabs, and modern world medicines. All these "researchers" had and still have the same goal, to discover new and potent medicines against diseases.

Bridging the gap between yesterday's knowledge and today's science, and hopefully the future discovery, in different time frames, generations, societies, cultures, and worlds, may provide new openings, or unconsidered avenues for drug discovery and success in fighting diseases.

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Appendix 1

A few Illustrations on the Legacy of Science and Scholarship in Al-Andalus (see Chapter I).

From "ARAMCO and Its World: Arabia And The Middle East", Edited by Ismail I. Nawwab, Peter C. Speers & Paul F. Hoye, Islam and Islamic History Section, published in 1980 by Arabian American Oil Company, Washington D.C.

Photo: A fourteenth-century manuscript of a pharmaceutical text.

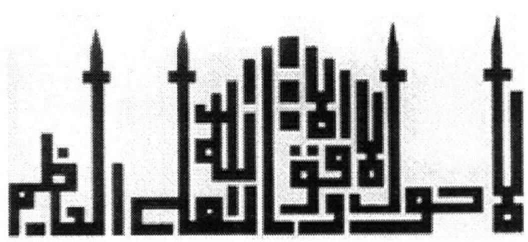

Photo: *The angular kufic script is here used to put a well known religious expression into the form of a mosque with four minarets.*

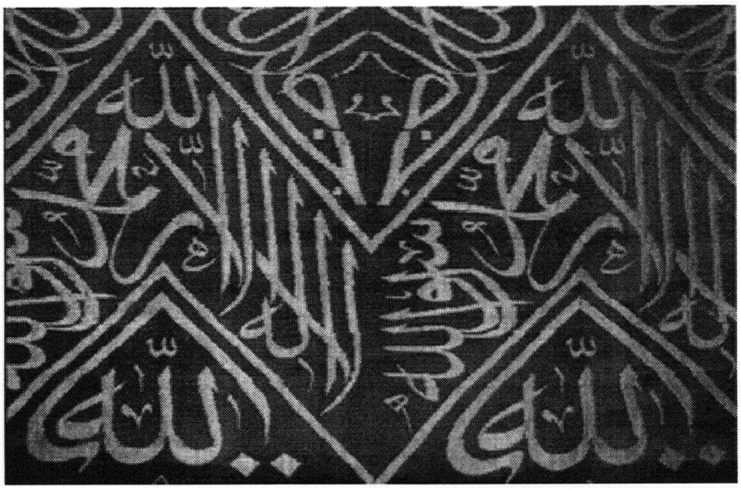

Photo: *Cursive script on a section of gold-embroidered kiswah, the black cloth covering of the Ka'bah, which is renewed each year at the time of the pilgrimage.*

IBN AL-BAITAR

Abu Muhammad Abdallah Ibn Ahmad Ibn al-Baitar Dhiya al-Din al-Malaqi was one of the greatest scientists of Muslim Spain and was the greatest botanist and pharmacist of the Middle Ages. He was born in the Spanish City of Malaqa (Malaga) towards the end of the 12th century. He learned botany from Abu al-Abbas al-Nabati, a learned botanist, with whom he started collecting plants in and around Spain. In 1219 he left Spain on a plant-collecting expedition and travelled along the northern coast of Africa as far as Asia Minor. The exact modes of his travel (whether by land or sea) are not known, but the major stations he visited include Bugia, Qastantunia (Constantinople), Tunis, Tripoli, Barqa and Adalia. After 1224 he entered the service of al-Kamil, the Egyptian Gover- nor, and was appointed chief herbalist. In 1227 al-Kamil extended his domination to Damaseus, and Ibn al-Baitar accompanied him there which provided him an opportunity to collect plants in Syria His researches on plants extended over a vast area: including Arabia and Palestine, which he either visited or managed to collect plants from stations located there. He died in Damascus in 1248.

Ibn Baitar's major contribution, *Kitab al-Jami fi al-Adwiya al- Mufrada*, is one of the greatest botanical compilations dealing with medicinal plants in Arabic. It enjoyed a high status among botanists up to the 16th century and is a systematic work that embodies earlier works, with due criticism, and adds a great part of original contribution. The encyclopedia comprises some 1,400 different items, largely medicinal plants and vegetables, of which about 200 plants were not known earlier. The book refers to the work of some 150 authors mostly Arabic, and it also quotes about 20 early Greek scientists. It was translated into Latin and published in 1758.

His second monumental treatise *Kitab al-Mlughni fi al-Adwiya al-Mufrada* is an encyclopedia of medicine. The drugs are listed in accordance with their therapeutical value. Thus, its 20 different chapters deal with the plants bearing significance to diseases of head, ear, eye, etc. On surgical issues he has frequently quoted the famous Muslim surgeon, Abul Qasim Zahrawi. Besides Arabic, Baitar has given Greek and Latin names of the plants, thus facilitating transfer of knowledge.

Ibn Baitar's contributions are characterized by observation, analysis and classification and have exerted a profound influence on Eastern as well as Western botany and medicine. Though the *Jami* was translated/published late in the western languages as mentioned above, yet many scientists had earlier studied various parts of the book and made several references to it.