Journal of Medicinal Plant Research

Volume 8 Number 3 17 January, 2014
ISSN 2009-9723

Academic Journals

ABOUT JMPR

The Journal of Medicinal Plant Research is published weekly (one volume per year) by Academic Journals.

The Journal of Medicinal Plants Research (JMPR) is an open access journal that provides rapid publication (weekly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peerreviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jmpr@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Medicinal Plant Research will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editorial Board

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JMPR to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.
Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $600 handling fee. Publication of an article in the Journal of Medicinal Plant Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Medicinal Plants Research

Table of Contents: Volume 8 Number 3 17 January, 2014

ARTICLES

Research Articles

academicJournals

Vol. 8(3), pp. 144-157, 17 January, 2014
DOI: 10.5897/JMPR09.106
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Toward a clinical research framework for collaboration among selected stakeholders in traditional herbal medical practice in seme and gem sub-locations in Nyanza province, Kenya

Job Isaac Jondiko Ogoche

Chemistry Department, Maseno University, P.O. Box 333 Maseno, Kenya. E-mail: jjondiko@yahoo.com.

Accepted 23 December, 2013

Both World Health Organization (WHO) and Kenya government have recognized the role of herbal traditional medicinal practice in primary health management due to the observation that 80% of the rural communities consult traditional healers before they go for the orthodox medical services. The traditional herbal practice remains familiar and artitional, thus least developed and hence the patients do not get the best values for their input in the services. The research strategies executed by researchers have never focussed on the plight of the patients but covered phytochemical, pharmacological and no clinical evaluations. Effectively, the data so far available remain less useful than should have been to the development of herbal traditional practices. The current project thus aimed at focussing on the establishment of a research strategy that used observational studies to establish the possibility for designing a rigorous clinical trials of herbal medical practice involving participation of three herbalists, patients, one orthodox medical doctor, a plant taxonomist and a phytochemist with the objectives of proofing and validating the practice, using principles of biomedical and bioscience in Gem and Seme sublocations in Siaya and Kisumu Districts, respectively in Nyanza province, Kenya. Through the ethnobotanical field survey, the taxonomist identified 95 plant species used by the herbalists. Through literature review it was found that 100% of the plants are used by other herbalists in East Africa to treat human and animal ailments while 30% of the species had received phytochemical and pharmacological evaluations, thus validating their therapeutic values. About 10% of these species had been used in clinical studies. The observation indicate that the herbalists have reasonable potentials for management of the diseases despite their ignorance on the literature data on these plants as well as modern medical practical procedures. The inclusion of modern diagnosis of diseases by a medical doctor and medical laboratory tests improved the rate of the healing outcome by 20% when the healing rate was compared with that observed for the treatment of patients before the intervention through this project. The results so far indicate that it is practicable to implement a rigorous clinical trial in which both herbalists, patients and researchers collaborate. Such strategies shall not only give critical data for validation of herbal traditional medicinal practice but be useful for prospection for phytochemicines based on indigenous knowledge and also be used for the improvement and management of the practice.

Key words: Ethnobotany, pharmacology, phytochemistry, herbalist, patients, malaria, diarrhoea, Got Ramogi.

INTRODUCTION

In Africa, it is estimated that 75% of the rural population rely on herbal medicine for their healthcare. Herbal remedies have the advantage of being readily available, biodegradable and the process of isolation of active

ingredients is cheaper than formulating and producing synthetic drugs (Ampofo and Johnson-Romauld, 1978). The benefit of this observation has not been realized in Africa because the research strategies used in the study of traditional medicinal practices have not been focused on the objectives which are meant to support the practitioners and the patients. The research have been on phytochemical and pharmacological evaluations without any on clinical trials and toxicological evaluations including those on standardization of the herbal remedies as well as on modern medical diagnostic practices. There have never been attempts at training the traditional practitioners and integration of medical skills in the practice including the good harvesting, manufacturing and administration of the remedies. The research paradigms neglected the integration of clinical and laboratory diagnostic techniques in the traditional practice. The ethnobotanical, ethnomedical, phytochemical and pharmacological evaluations done in Kenya have neglected the patients' feelings, attitudes, benefits and welfare (Sidiga 1995; Kokwaro, 1994; Jeruto, 2008; Gakunju, 1995; Ostrom, 2008) including the evaluation of the practitioners' practice.

Patient-centred study by Alamo (2002) was found more effective than the usual consultant-centred evaluation of effectiveness of treatment of musculoskeletal chronic pain using Chinese herbal remedies. It is also observed that there is no published information in Kenya that evaluates the reliability, efficacy, safety and validity of the traditional medical practices' performance using an approach recommended by Lewis and Elvin-Lewis (1994) and Steven and Jeffrey (2003) and participatory research approach developed for Canadian overseas projects (Found, 1995) in which all stakeholders are participants in all stages of the research.

In 1980, Clinicians working in cooperation with Chinese medicine practitioners using aqueous extracts of ten Chinese medical herbs in London treated children suffering from severe atopic eczema. In a double blind placebo controlled trial, using 37 young patients, the research proved that treatment was achieved only if all the ten plants were used (Williamson, 2001). Other challenges which must be considered in such research paradigms are ethical issues and positive attitudinal changes. The intellectual property rights, indigenous knowledge, benefit sharing, efficacy and safety must be factored into the research activities. The change of attitude and building trust among the research team, the herbalists and the patients must be undertaken so as to obtain maximum benefits to all the stakeholders participating in this project.

It is believed that these strategies must be integrated in the practice with the objectives of validation and value addition to the practice. Such efforts shall aid rational decisions on the formulation of policies and their implementation for the control of herbal medical practices and therefore health management and socio-economic development while exploiting the biodiversity in a sustainable manner.

The foregoing information directed the research strategies in which rural rapid appraisal and participatory research method to test the suitability of the strategy that was employed to conduct the pilot project herein described. Thus the team made up of a forester, plant taxonomist, phytochemist and a medical doctor organized a meeting with three herbalists and their patients where questionnaires and discussions were conducted to obtain information on identification of the plants, disease diagnosis and treatments. The literature on the ethnobotanical, ethnomedicinal, phytochemical and pharmacological data were collected and analysed so as to validate the use of the plants in treatment of the diseases by the herbalists. The information so far obtained are found useful for the design and future implementation of observational and clinical trials in herbal medical practice in Kenya that would lead to value addition to the traditional medicinal practice.

MATERIALS AND METHODS

The project setting

The recruitment of research team and patients

The herbalists and patients were recruited from Seme and Gem sub-locations of Kisumu and Siaya districts. A female herbalist was recruited from Gem sub-location while two male ones were recruited from Seme sub-location. The criteria for the selection of the herbalists was based on their education, age, experience and our contact with them in another project on the phytochemical and pharmacological evaluations for sustainable exploitation of *Toddalia asiatica* (Orwa et al., 2008). These herbalists recruited the patients whom they treated under the supervision of the medical doctor. The taxonomist was recruited on the basis of his experience in the identification of the plants in the fields. The recruitment of patients were done in Seme sub-location in Kombewa division in Kisumu District and South Gem sub location in Gem Division in Siaya District in Nyanza province in Kenya. Seme sub-location has no hospital but four, six and one health centres, dispensaries and clinic nursing home, respectively serving a population of about 50,000 people (GOK, 1996). This sub-location is in Siaya District where in 1990 to 1992, top ten causes of morbidity due to diseases were malaria, acute respiratory infection, skin infection, diarrhoeal diseases, intestinal worms, urinal tract infection, eye infection, rheumatism, ear infection and gonorrhoea with disease prevalence of 42, 23.8, 8.5, 6.7, 3.9, 3.2, 2.6, 1.7, 1.6 and 1.5%, respectively (GOK, 1993).

South Gem sub location is served by three and six dispensaries and health centres, respectively and has a population of 70,000 people. The disease prevalence is probably similar to the situation in Seme sub location, Siaya, Bondo and Kisumu towns have each one district hospital which is supposed to serve the two sub locations which are however in the rural areas, approximately 50 kilometres away from these towns. These rural areas have poor road networks and the health services due to poor personnel and drug availabilities as well as few all-weather roads. This indicates that the most immediate health services are accessed through traditional healers including herbalists. These factors were considered during the recruitment of the patients at random. In Seme, the herbalists were stationed at Kondik Trading centre while in Gem the recruitment was done at Ebusakami Primary School

where each reporting patient was asked to sign the treatment agreement form after the objectives of the project was discussed with them.

The discussion on the nature of herbal medicinal treatment they were to get was adequately explained to them before diagnosis and treatment processes.

Workshop for the herbalists and the patients

The recruited patients and the herbalists were called for a three day workshop at Kondik Trading centre at which the three herbalists and fifty recruited patients were taught by the facilitators on the objectives, principles, implementation and benefits of the project to each of the stakeholders as well as the nature and issues on good herbal medical practices. The herbalists were trained on simple taxonomic, preservation and preparation techniques of herbal products. They were also given lectures on some simple methods of diagnosing and identification of the common diseases including clinical management of patients. Further, the herbalists were given lectures on the general values of research to the herbal medicinal practice with respect to the government health policies on their practice.

Questionnaires were administered to the herbalists to establish the value of the research activity compared to their earlier practice. The herbalists were also given a three hour discussion in the standardisation and measurements introduced in preparation of herbal remedies as well as diagnosis of the diseases. The patients were taught the knowledge of the common diseases they usually contact in the areas. There was a lecture on the practice of herbal medicinal practice which was meant to create awareness and attitudinal change towards misconceptions held on herbalists, herbal remedies and the practice in the rural societies and expert communities. There was a general discussion session in which stakeholders were given time to give their reflection on the herbal medical practice. Questionnaires were administered to the patients to assess their knowledge and attitudes towards the herbal medical practice they might have attended before and compared to the current one.

Identification of the herbal plants

The research team visited the herbalists who took the team around to observe the plants in their habitats for taxonomic identification. The plants which were not found at Got Ramogi, Seme and Gem sub-locations and within the vicinity of the area were collected and given to the taxonomist for identification. The herbalists were taught how to preserve the specimen in a box supplied by the project. The plant specimen was kept in paper by the herbalists for future identification of the plants for collection and processing.

Literature study of the ethnobotanical, pharmacological and phytochemical infromation

The databases such as NAPRALERT, AFLORA (AFLORA (2008)), MEDFLO, PHARMEL 2 and PRELUDE (Fernsworth, 1994), textbooks, journals, conference proceedings and the internet were sourced for botanical ethnobotanical, pharmacological, toxicological and phytochemical information available in the literature about the identified plant species. The information were analysed to validate the medicinal uses of the plants by the herbalists and also to aid preparation of the remedies. The data was tabulated and analysed to guide the standardization of the preparation of the herbal products for use by the herbalists and for formulation of future research agenda.

Preparation of the herbal remedies

The remedies were standardized by weighing and measurement of the volumes of water used in the preparation of decoctions from wet plant parts. The wet plant parts were shredded by chaff-cutter before grinding them by a plate mill. The tincture of the wet plants parts were made in a mixture of 2:1 ratio of water: whisky over a period of three days. The dry plant parts were dried under shade and burned to ash. The other plant parts were similarly dried and ground using a hammer mill to fine powders which were then mixed in ratios by weights as directed by the herbalists. The weighed amounts of dry plants were put in definite ratios to be decocted in water as directed by the herbalists. The decoctions were measured by graduated cups when dispensing to the patients. Some plants were placed into a saucepan and burned to ash to be used for treatment of wounds whereas some were dried and rolled into paper to be smoked for the treatment of asthmatic cases. Both powders and ashes were standardized by passing them through a sieve. The water extracts were filtered to obtain the suspended particles and dissolved compounds as well as pH. The density of the filtrate was obtained for standardization. The water extracts were then administered to the patients in doses advised by the herbalists. The decoctions powders and ashes were labelled by the coded names and the diseases for which they are indicated.

Standardization of the prepared remedies

The decoctions of both wet and dry plant materials were done by weighing of the wet and dry plants before putting them into measured water whose final volume was measured after boiling. The mass of both suspended particles and the dissolved compounds were measured so as to know how much substance was given to the patients in a measured volume. The pH of the decoction was also taken.

The particle sizes of the ground materials were standardized by the size of the sieve of the grinder as well as sieves for 5 to 10 micron particles. The ash which was standardized by a sieve was suspended in distilled water so as to take its pH. The rolled powdered plant material was weighed and put into a paper of known size. The decoctions were filtered and density measured then freeze dried and dissolved in a mixture of 1:1 ratio of methanol and chloroform and analysed by thin layer chromatographic technique. The number of spots identified by ultra-violet lamp and 50% sulphuric acid were used for standardisation.

Diagnosis of diseases

The patients were recruited by the herbalists and taken to Kondik trading centre of Ebusakami Primary School where they were diagnosed by both the herbalists and the medical doctor at the beginning of the project to obtain pre-treatment diagnosis. The herbalists listened to and observed the medical doctors' clinical observation of patients. The same activity was done by the medical doctor as the herbalists interviewed the patients. At the end of the clinical interviews both the herbalists and the doctor harmonised the diagnosis for each patient before and after each treatment. The patients whose diagnosis required laboratory tests were taken either to Kombewa Health Centre or Maseno Hospital of Anglican Church or Kisumu District Hospital. The project paid for the costs of the tests. After the diagnosis the herbalists administered the herbal preparations. The patients were given appointments to see the herbalists every day in the evening to review their cases. Once the patients indicated that they were feeling well then they were reviewed by the medical doctor and the patients whose cases required post-treatment laboratory tests were sent to the laboratories for confirmation of successful treatment.

RESULTS AND DISCUSSION

The species of plants used by the three herbalists

In total, 95 plant species found in 41 families were identified with frequency of occurrence of 57, 54 and 51 in Got ramogi, Seme and Gem, respectively (Table 1). Only three species were obtained from Eldoret and Kisumu towns through vendors. The plants collected from Got ramogi were purchased by the project for the herbalists at half dollar per bundle of 2 kg of wet plants. The plants collected at Gem and Seme were not purchased since they were from the herbalist's home gardens or live fences in Seme and Gem or conservation sites in Seme. The percent of the plant species collected from home gardens and conservation sites were 80 and 20%, respectively. The conservation site is owned by Miguye conservation group.

The biodiversity was represented by the 12 families of the plants with the number of species indicated in the brackets: Compositae (13), Labiatae (10), Euphorbiceae (6), Solanaceae (4), Caesalpinioidea (4), Anacadiaceae (3), Bignoniaceae (3), Cucurbitiaceae (3), Meliaceae (2), Myrtaceae (2), Malvaceae (2), Convulvulaceae (2) and Verbenaceae (2), whereas the following 26 families were each represented by one species: Rutaceae, Simarombaceae, Papilinioideae, Guttiferae, Rosaceae, Urticaceae, Hypoxidaceae, Leguminosae, Combretaceae, Acanthaceae, Menispermaceae, Caricacea, Rubiaceae, Papilinioideae, Mimosoideae, Phytolacceae, Musaceae, Vitaceae, Liliaceae, Amaranthaceae, Cuppressaceae, Moraceae, Umbellifarae, Asclepidiaceae, Apocynaceae and Astraceae. The popularity of the use of the families are corroborated by the observation recorded by Kasonia et al. (1997) in their study of the ethnoveterinary plants in the Lake Victoria region. A moss plant species was not identified.

The frequencies of the plant parts used were given in brackets as seeds (1), flowers (6), fruits (30), root bark (39), whole (44), leaves (52) and stem bark (53) (Table 1). Literature review using databases such a NAPRALERT, AFLORA, PROTA, MEDFLORA, PHARML 2 and PRELUDE including textbooks and journals (Farnsworth, 1994; Kokwaro, 1994; Hans, 1996) revealed a lot of corroboration on the types of diseases treated by other herbalists in several societies in East Africa as indicated in Table 1. The herbalists treated 16 diseases using a multiple of plants prepared from fresh or dried materials in the form of decoctions or tinctures or burnt materials and administered orally, smoked or applied on affected skins of the patients. The literature survey indicated that the plants were ethnomedically indicated for a wider range of diseases (Table 1). The 60 and 32% of the species of the plants included in the study had received pharmacological and phytochemical evaluations which were used to validate some of the treatment claims given by three herbalists and those from other ethnic backgrounds (Tables 1 and 2). The plants in Table 2 were used by one of the herbalists in making the decoction called a multipurpose remedy that was orally given patients suffering from several ailments (diseases 12 to 19) in Table 3. It was also encouraging to note that *Centella asiatica, Withania somnifera, Spilanthes mauritiana, Achyranthus aspera, Mongifera indica, Psidium guajava, Lantana trifolia* and *Euphorbia hirta* which were used by the herbalists in this project had been used in clinical trials. A balm of *C. asiatica* (0.03%) and *S. mauritiana* (0.03%) applied topically in the forehead of patients reduced the symptoms of migraine headaches 19 min after applications

(Patent No. 200610658122), while essential oils from *A. aspera* were used in observational studies of asthmatic patients with positive results.

The clinical trial studies have been conducted using *P. guajava* (Xavier et al., 2006)*, E. hirta, M. indica* and *L. trifolia* for treatment of patients suffering from diarrhoea, amoebic dysentery, liver ailment and pulmonary tuberculosis, respectively with reasonable results. Clinical trials with the aqueous extracts of leaves of *A. conyzoides* (Kamboj et al., 2008) on patients suffering from arthrosis indicated that 66% of them experienced the analgesic effects while 24% of them had improved articulation and mobility without side effects. Clinical trials on human volunteers were conducted with 4% endod (*Phytolacca dodecandra*) ointment against dermotomyces and found to be effective (Jassim, 2003). Artex Mendar, a standardized multiplant Ayurvedic drug compost of *W. somnifera, B. serrata, Zinger officinale* and *Curcuma linga* was used in controlled clinical trials on 358 patients suffering from symptomatic osteoarthritis of the knees. The results indicated reasonable efficacy and safety over a period of 32 weeks (Chopra et al., 2004; Srivanasan et al., 2007). The clinical trials give evidence that the traditional herbal medical practice can be an avenue for discovery of herbal remedies which can be used to treat the people who may not be able to access government health facilities.

The physico-chemical parameters

The sizes of the fresh materials were cut with a chuff cutter to about 2 to 4 cm and 5 to 6 cm in length and width, respectively before grinding them in a plate mill. Both juice and the solid residues were transferred to the saucepan for boiling. The sample was then strained by tea strainer and the residues thrown away. The decoction was the set for treatment. A portion was then taken for measurement of physico-chemical parameters. The dry plant materials were milled by a hammer mill to obtain 5 to 10 micron particles whose sizes were confirmed by sieve measuring device. The powders were infused in boiling water and filtered. A portion of the decoctions from both fresh and dry plant materials were used to obtain physico-chemical parameters. The density was about 0.9 g/ml while the pH range was 6.0 to 7.9. The undissolved

Table 1. The species of the plants used by three herbalists.

No.	Species	Family	Place of collection	A: Parts used	B:Diseases indicated by the herbalists	C:Diseases indicated in the literature (Kokwaro, 1994)	D: Pharmacological evaluations
1	*Conyza sumatrensis.*	Asteraceae	Gem	W	11,12,13,14,15,16,17	15,	-
2	*Cassia spectabilis*	Caesalpinioideae	Got Ramogi	L,Fl,Fr,S,R	11,12,13,14,15,16,17	-	2,5,
3	*Catharanthus roseus*	Apocynaceae	Got Ramogi	L,R,S,Fl	14,15,16,17	4h,	3,6,
4	*Spathodea campanulata*	Bignoniaceae	Gem	W	14,15,16,17	1c,10b	1,2,3,5
5	*Dichondra repens*	Convolvulaceae	Gem	W	14,15,16.17	-	-
6	*Centella asiatica*	Umbellifereae	Gem	W	8,14,15,16,17	13c	1,2,5
7	*Hibiscus vitifolius*	Malvaceae	Gem, Seme	L,R,S,Fl	14,15,16,17	-	5
8	*Ricinus comminus*	Euphorbiaceae	Got Ramogi, Gem	Fr,L,R,Se,S	7,14,15, 16,17	1d, 4a, 4f, 5c, 8d, 9e, 10b, 10g.	1,2,3,4
9	*Markhamia lutea*	Bignoniaceae	Gem	L,S,R	14,15,16,17	-	4
10	*Ficus lutea*	Moraceae	Gem, Got Ramogi	S,R,LFr	4, 14,15,16,17	-	5
11	*Cupressus lusitanica*	Cuppressaceae	Gem	L,Fr	14,15,16,17	-	6
12	*Achyranthes aspera*	Amaranthaceae	Gem, Got Ramogi,Seme	L,S,R,Fr,W	14,15,16,17	1a, 4d, 9e, 10d, 10h, 17a.	1,2,5,7
13	*Solanum nigrum*	Solanaceae	Gem	Fr,L,R	14,15,16,17	1c, 4f, 10d, 10g, 12c.	1,5,6
14	*Bidens pilosa*	Compositae	Gem, Got Ramogi	W	11,12,13,14,15,16,17	1c,4f,6e,12a,	12,5,6
15	*Sida rhombifolia*	Malvaceae	Gem	S,R,Fr,L	14,15,16,17	-	2,5
16	*Bryphylum pinnatum*	Crassulaceae	Seme	LR,S	14,15,16,17	3f, 6e, 8f, 9a, 17b.	1,3,4,5
17	*Vernonia amygdalina*	Compositae	Got Ramogi	L,R,S,Fl	14,15,16,17	1a,6e	1,2,3,5
18	*Ipomea rubens*	Convulvolaceae	Seme	L	14,15,16,17	-	-
19	*Kegelia africana*	Bignoniaceae	Got Ramogi, Gem	S,R,Fr,L	4,14,15,16,17	1a, 8f, 12a, 12g.	1
20	*Mangifera indica*	Anacadiaceae	Gem, Seme	S,R,L, Fr	14,15,16,17	-	1,2,5,
21	*Persia americana*	Lauraceae	Gem	L,Fr	-	-	-
22	*Lantana trifolia*	Verbenaceae	Got Ramogi	L,Fr,S,R	14,15,16,17	1de,4e,12c15,17b	1,2,5
23	*Phyllanthus amarus*	Euphorbiaceae	Gem	W	14,15,16,17	4h.	-
24	*Psidium guajava*	Myrtaceae	Seme, Got Ramogi	L,Fr,Fl,S,R	14,15,16,17	-	2,5
25	*Momordica foetida*	Cucurbitaceae	Gem, Seme	W	14,15,16,17	1d, 6e, 8f.	1,4, 6
26	*Justicia betonica*	Acanthaceae	Gem, Seme	W	14,15,16,17	3d,19	-
27	*Flueggea virosa*	Euphorbiaceae	Got Ramogi	L,Fr,S,R	14,15,15,17	-	1,5
28	*Aloe kedogensis*	Liliaceae	Gem	L,S,R	11,12,13,14,15,16,17	-	5
29	*Bridelia micranthus*	Euphorbiaceae	Gem, Got Ramogi	W	11,12,13,14,15,16,17	1a, 4f, 6c, 21c.	1,2,5
30	*Euphorbia hirta*	Euphorbiaceae	Gem, Seme	W	9,10,14,15,16,17	1c, 3c, 4g,10h.	1,2,5
31	*Ageratum conyzoides*	Compositae	Got Ramogi	W	11,12,13	1b,1c,3f,4f, 13f,14a	1,2,5
32	*Hibiscus acetocela*	Malvaceae	Gem, Seme	L,Fr,Fl	14,15,16,17	-	-
33	*Tagetis minuta*	Compositae	Gem, Seme	W	14,15,16,17	-	-
34	*Cyphostema nodiglandulosa*	Vitaceae	Seme, Gem	L,R,Fr,Fl	10,14,15,16,17	-	-
35	*Hoslundia opposita*	Labiatae	Gem, Seme	W	14,15,16,17	1a,3d,8e,10h	1,3,5
36	*Erythrococca atrovidea*	Labiatae	Got Ramogi	W	-	-	-
37	*Sida termifolia*	Malvaceae	Gem	W	14,15,16,17	-	-
38	*Musa sapientum*	Musaceae	Gem	S,L,Fr	14,15,16,17	1a,14b	--
39	*Plectranthus barbatus*	Labiatae	Gem	W	14,15,16,17	4f, 5c, 12g.	5

Table 1. Contd.

40	*Leonotis nepetifolia*	Labiatae	Gem	W	14,15,16,17	4e,4f	1,
41	*Phytolacca dodecandra*	Phytolaccaceae	Gem, Got Ramogi	L.R,S	14,15,16,17	3d, 4h,5a,5c,6c,8f,9b	3,4
42	*Capsicum frutecens*	Solanaceae	Gem, Seme	L.Fr	14,15,16,17	-	5
43	*Albizia coriaria*	Mimosoideae	Got Ramogi, Gem, Seme	S,R,L,Fr	5,14,15,16,17	8a, 1c, 8d, 8f, 9e.	2
44	*Dichrocephala integrifolia*	Compositae	Gem, Seme	W	14,15,16,17	10k	-
45	*Senna didymobotrya*	Caesalpinioideae	Gem, Seme, Got Ramogi	Fr,L,S,R	7,14,15,16,17	1a, 4f, 5a, 5c10c, 10d, 12a, 19d.	1,5
46	*Entada abyssinica*	Caesalpinioideae	Seme, gem, Got Ramogi	Fr,L,S,R	14,15,16,17	17b,	12,5
47	*Erythrina abyssinica*	Papilionoideae	Got Ramogi, Seme, Gem	S,R,L,Fr	3,4,14,15,16,17	1a, 4a, 4h, 6e 9a, 10d, 10h, 10j, 12h.	1,2,3,4,5
48	*Rubia cordifolia*	Rubiaceae	Gem	S,L,R	14,15,16,17	1c, 4a4e, 10k, 19d.	2,5,6
49	*Croton macrostachys*	Euphorbiaceae	Gem, Seme	L.S,R	14,15,16,17	3d, 4f, 5c, 6c, 9e, 10b, 10h, 12a 14a.	6
50	*Carica papaya*	Cariaceae	Got Ramogi, Seme, Gem	L,Fr,R S	9,10	-	2,5,7
51	*Jakaranda mimosifolia*	Bignoniaceae	Gem, Seme	S,R,L,Fr	14,15,16,17	-	2
52	*Spathodea campanulata*	Bignoniaceae	Gem	W	14,15,16,17	1c, 10b.	1,2,5
53	*Stephania abyssinica*	Menispermaceae	Gem, Seme.	S,R,L,Fr	14,15,16,17	19b, 21.	1,3, 5,6
54	*Ipomea rubens*	Convulvulaceae	Seme, Gem, Got Ramogi	L	-	-	-
55	*Ocimum kilimandscharicum*	Labiatae	Seme, Got Ramogi, Gem	W	14,15,16,17	1a, 1c, 3d, 4a, 4h, 12g.	-
56	*Senna bicasularis*	Caesalpinioideae	Gem, Seme	S,R,L,Fr	14,15,16,17	-	-
57	*Terminalia brownii*	Combretaceae	Got Ramogi,Seme ,Gem	L,S,R	1,14,15,16,17	15	-
58	*Pelliploca linariifolia*	Asclepidaceae	Seme, Gem	W	14,15,16,17	-	-
59	*Lippia trifolia*	Verbenaceae	Gem	W	14,15,16,17	-	-
60	*Leucas gradis*	Labiatae	Gem	W	14,15,16, 17	-	
--61	*Eucalyptus camaldulensis*	Myrtaceae	Got Ramogi, Gem, Seme	L,S,R,Fr	14,15,16,17	-	2,5,6
62	*Crassocephalum vitellinum*	Compositae	Gem	W	14,15,16,17	-	-
63	*Tetradenia riparia*	Labiatae	Gem	W	14,15,16,17	-	1
64	*Helichysum odoratissimum*	Compositae	Gem	W	14,15,16,17	3d,5c,6e, 10h	-
65	*Mucuna gigantea*	Leguminosae	Seme	R,L,R,S	14,15,16,17	-	-
66	*Kedrostis foedsisima*	Cucurbitaceae	Kisumu	W	14,15,16,17	12d,12g	-
67	*Hypoxis obtusa*	Hypoxidaceae	Seme	R,L,	14,15,16,17	-	-
68	*Sadoxus multiflorus*		Seme	W	14,15,16,17	-	-
69	*Sphaeranthus gomphrnoids*	Compositae	Seme	W	14,15,16,17	8d	-
70	*Urtica dioica*	Urticaceae	Seme	S,L,R	7	-	-
71	*Fuerstia africana*	Rosaceae	Seme	W	-	1c, 5c,6c.8g	-
72	*Garcinia buchananii*	Guttiferae	Seme	R,S,L,Fr	-	21a,10h	5,6
73	*Gutenbergia cordifolia*	Compositae	Seme, Got ramogi	W	-	-	-
74	*Datura stramonium*	Solanaceae	Gem Seme, Got Ramogi	L,R,S,Fl	1,8,9,14,15,16,17	1d,10c,17b,	5
75	*Leonotis molissinia*	Labiatae	Seme	W	1,7	1a,1c,4b, 4d,4e,6c10h,10k	1
76	*Lannea schimperi*	Anacadiaceae	Got Ramogi	LR,S	-	1a, 1c, 3f, 4a.	-
77	*Lannea schweinfurthii*	Anacadiaceae	Got Ramogi	L,R,S	-	21c	1
78	*Withania somnifera*	Solanaceae	Seme	L,S,R,Fr	8	1a,4d,4f,4h,8c,9a,10b, 10g, 15,20a.	-
79	*Ocimum bacilicum*	Labiatae	Seme	W	8	1b, 2a,4d,8b.	2
80	*Azadirachta indica*	Meliaceae	Kisumu	L,S,R,	-	-	2,3,7

Table 1. Contd.

81	*Ajuga remota*	Labiatae	Eldoret	W	10	1a, 1c.14b.	1,
82	*BRyonia dioica*	Curcubitaceae	Seme	W	1	-	-
83	*Microglosa pyrifolia*	Compisitae	Seme, Got ramogi	W	3	17a	-
84	*Eucalyptu citodora*	Myrtaceae	Seme, Got Ramogi	L,S,R,Fr	9	-	-
85	*Erythrina excelsa*	Papilioideae	Seme	S,L,R	2	-	-
86	*Spilanthes mauritiana*	Compositae	Gem, Seme	W	14,15,16,17	1c,4a,4f 17a	1,2
87	*Tussilago vulgaris*	Compositae	Seme, Got ramogi	W	8	-	-
88	*Aspilia mossambicensis*	Compositae	Seme, Got ramogi	W	8	6a,6e,8e,10c,10h,13a15, 17b.	-
89	*Taraxacum offinalis*	Compositae	Seme, Got ramogi	W	6	-	-
90	*Viscum album*	Viscaceae	Seme	W	6	-	-
91	*Maribium vulgaris*		Seme, Got ramogi	W	9	-	-
92	*Melia azadirach*	Meliaceae	Seme, Got ramogi	L,S	2	-	-
93	*Harrisonia abyssinica*	Simaroubaceae	Seme, Got ramogi	L,S,Fr.	10	-	-
94	*Toddalia asiatica*	Rutaceae	Seme, Got ramogi	S,R,L,Fr.	3,4	-	-
95	*Moss sp.*		Seme	W	8	-	-

A. Key to parts of plants used by the herbalists in this project. W = Whole, R = root bark, L = leaves, S = stem bark, Fl = flowers, Fr = fruit, Se = seeds. B: Key to diseases indicated for treatment by plants used by the herbalists in this project. Number corresponds to the disease number in Table 3. C: Key to diseases indicated for identified plant species by other herbalists (Kowaro, 1993).1. Diseases of the head (including colds, fever, influenza and flu).1a. Headache, 1b. Nose, 1c. Eye, 1d. Ear, 1e. Mouth, tongue and teeth.2. Diseases of the throat and neck; 2a. Soar throat, 2b.Bubonic diseases, 2c. Tonsilitis.3.Diseases of the chest; 3a. Pneumonia; 3b.Tubeculosis; 3c. Asthma; 3d. Coughs; 3e. Bronchitis; 3f.General chest illness; 4. Diseases of the abdomen; 4a. Diarrhoea; 4b. Dysentery; 4c. Typhoid fever; 4d. Constipation; 4e. Indigestion; 4f. Stomach-ache; 4g. Heartburn; 4h. Haemorrhoids; 4i. Hernia; 4j. General abdominal pains; 5. Emetics and purgatives; 5a. Emetics; 5b. Antovomiting; 5c. Purgatives; 6. Intestinal worm infections; 6a. Hookworm; 6b. Roundworm; 6c. Tapeworm; 6d. Tread worm; 6e. General antihelminths; 7. Bilharzia and filarial infection; 7a. Bilharzia; 7b. Elephantiasis; 8. Female conditions and diseases; 8a. Mensturation; 8b. Pregnancy; 8c. Child birth; 8d. Postpartum; 8e. Breast lactation; 8f. Abortion; 8g. Sterility; 9. Veneral diseases; 9a. Gonorroea; 9b. Syphilis; 9c. Orchitis (swollentesticles); 9d. Yaws; 9e. General venereal diseases; 10.Skin diseases; 10a. Leprosy; 10b. Rashes; 10c. Ringworm; 10d. Abscess and boils; 10e. Warts; 10f. Pimples; 10g. Ulcers; 10h. Wounds; 10i. Whitlows; 10j. Sores; 10k. General skin diseases; 11. Systematic diseases; 11a. Malaria; 11b. Glandular swellings; 11c. Small pox; 11d. Measles; 11e. Anthrax; 11f. Leishmaniasis; 11g. Trypanosomiasis; 12. Nervous systems diseases; 12a. Back-ache and lumbago; 12b. Epilepsy; 12c. Paralysis and palsy; 12d. Polio; 12e. Mental disturbance; 12f. Captive qualms; 13. Cardiovascular diseases; 13a. Haemorrhage; 13b. High blood pressure; 13c. Palpitation and other heart diseases; 13d. Dropsy and oedema; 13e. Anaemia; 14. Liver and spleen diseases; 15. Kidney and bladder diseases; 16. Bone and joint diseases; 17. Cancer; 18. Antidotes; 18a. Anti-snake bites; 18b. Anti-scorpion and spider bites; 18c. Anti-arrow poisoning; 18d. General antidotes; 19. Anaesthetics; 20. Aphrodiasics, tonics and stimulants; 20a. Aphrodisiacs; 20b. Tonics; 21. Lassitudes, importance and Kwashiorkor. D: Key to pharmacological evaluations reported on the plants used by the herbalists in this project. 1. Antiprotozoanal activities: Plasmodium, Schistosomes, Trypanosome and Leishmania; 2. Antibacterial activity evaluations: Gram positive and Gram negative bacteria; 3. Antifungal evaluations: Spore forming and filamentous fungi; 4. Antiviral evaluation: Human and avian viruses; 5. Physiological properties: Anti-inflammatory, antioxidant, analgesic, antinoreceptive, hyperglycaemic, antiplasmodic, antispasmodic, stimulant, sedative, anticholinestrase, antitoxicity, psychotropic, hepatoprotechtive, anti-ulcercer, antiallergic, antidiarrheal, antiasthmatic, antidepressant, antiplasmolytic, antihistamine, antimutagenic, and immunosuppressive properties; 6. Anti-tumor, anticancer properties; 7. Antifertility.

Table 2. The phytochemical and pharmacological evaluations reported on the plants used by the herbalists in this project.

Plant	Compounds	Pharmacological properties
Catharanthus roseous	Vincristin, vinblastin	Anticancer activity and used for treatment of cancer (Daniel, 2006).
	Raubasine, reserpine,serpentine	Antifibroidal and antihypertensive activity.

Table 2. Contd.

Erythrina abyssinica	Erythrobissin, phaseollin, abyss none, erybraedin, kolavic acid erycrystagallin, erythrassin, erycrstallin	Active against *Staphylococcus sp*. and *Plasmodium sp* (Andayi et al., 2006).
Toddalia asiatica	Nitidine, isopimpinellin, dihydrinitidine,magnoflorin 5,7-dimethoxy-8-(3-hydroxy-3-methyl-1-butene coumarin, leaf essential oils	Active against *Plasmodium* and HIV-aid virus and antipletlet and anticardiac activities: anti-inflammatory activity (Okech et al., 2002; Gakunju et al., 1995 ; Iwasaki et al., 2006; Saxena, 1999)
Stephania abyssinica	Dicentrine	Hypotensive, antihyperlipidemia, antineoplastic, antiarrythmic, antiplasmodial and anticancer and antifungal (Helen de Wet, 2005)
Solanum nigrum	Glycoprotein (150D) degalactotigenin, solanigriside	Anticancer cells (Kye-Taek Lem, 2005).
Byophyllum pinnatum	Bryophyllin A &B ,gossypin, bufadienilide orthoacetate, bryotoxin A&B Patuletin acetyl rhamnoside	Anticancer and antileishmanial, toxic, Immunosuppressive activities (Duraisami and Srinavasan, 2008; McKenzie et al 1987; Yamagishi et al., 1989)
Spathodea campanulata	3-beta-20-beta-dihydroxyurs-12-en-28-oic acid, tomentosolic, ursolic acid, urcenic acid and verminoside, quercetin	Anti-plasmodial and antidiarrheic activities (Ngouela et al., 1991; Dieter et al., 1996)
Bidens pilosa	2-beta-D-glucopyranosyl-oxy-1-hydroxy-5-(E)-tridecene-7-9-11-tryne, centaurein, ceunteidin, and 3-beta-D-glucopyranosyl-oxy-1-hydroxy-6-(E)-tetradecene-8-10-12-tryne.and other compounds	Antiplasmodial, antifungal, antihyperglycaemic, antihypertensive, immunosuppressive, anticancer (Shu-Lin et al., 2007).
Psidium guajava	Quercetin, guaijiaverin, leucocyanidin, terpenoids, phenolics, flavonoids, carotenoids, and triterpenoids.	Antispasmodic,antibacterial, antioxidant, antiallergic ,antigenotoxic, antiplasmodial,Cardioactive, anticough,antidiabetic,Anti-inflammatory, antidiahrreal cytotoxic, hepatoprotective, antinociceptive analgesic, antipyretic supporting ethnomedicinal and clinical uses, (Guitiernez et al., 2008; Khan et al., 1985).
Lantana trifolia	Sitosterol,sitosterol glycoside,stigmasterol, and umuhengerin	Active against *Mycobacterium tuberculosae* (Rwangabo et al., 1988).
Momordica foetida	Iriodictoyl-7-O-beta-D-glucopyranoside,xanthohumol, 2,3-dihydroxyhumol and its pyrano derivative and antiviral peptide	Antiplasmodial, antiviral and anti-cancer activities.(Jian et al., 2005).
Flueggea virosa	Flueggenins A &B, bergenin and neobergenin	Antiplasmodial, antitrypanosomal and anticancer activities (Nasser et al 2004)
Phytolacca dodecandra	Dodecandrin and gelonin	Antiviral and cytotoxic properties (Jassim et al., 2003).
Euphorbia hirta	Quercetrin, beta-amyrin, 24-methylcycloartenol and beta-sitosterol,tinyatoxin, euphorbins A, Band C, 12-deoxy-4-beta-phorbol.	Antidiarrheal and toxic activities. (Mariano et al., 1999; Daniel, 2006).
Hoslundia opposita	3-O-benzoyl hosloppone	Antiplasmodial activity (Achenbarch et al., 1992).
Centalla asiatica	Asiaticoside and oxy-asiaticoside	Have activity against *Mycobacterium* tuberculosis, *Bacillus leprae* and *Entamoeba histolyca* (Daniel, 2006).
Hibiscus vitifolius	Gossypin	Antiinflammatory and antiniceptive (Duraisami and Srinavasan, 2008).

Table 2. Contd.

Garcinia buchananii	3-geranyl-2,4,6-trihydroxy-benzo-phenone,1,3,5,7-tetrahydroxy-8-isoprenyl-xanthone, 1,3,5-trihydroxy-8-isoprenyl xanthone,3-gernyl-2,4,6-trihydroxybenzophenone and butinilic acid	Activities against *Candida albicans* and *Staphylococcus aureus* (Han et al., 2006).
Vernonia amygdalina	Luteolin,luteolin-7-O-beta-gluciside,vernadalin, vernonioside B 1, vernoniolB1 and vernoamygdalin.	Antioxydant, antiplasmodia, antischisotosomial, antileishmonial and anitihepatotoxic properties.(Iwalokun et al., 2006)
Entada abyssinica	Iso-kolavenol and it glycoside, kolavic acid	Anidiabetic, antitrypanosomal activities (Nyasse et al., 2004a, b)
Rubia cordifolia	Rubiadin, emodin, physion, luteolin, quercetin cyclic peptide,1-hydroxy-2-quinone, 1,4-dihydroxy-2-methyl-5-methoxyanthraquinone,1,3-dimethoxy-2-carboxyanthraquionone,6-methoxy geniposidic acid, manjistin, garasin, alizarin, rubiprasin and rubiarbonal	Antibacterial, anti-inflammatory, antiplatelet, hepatoprotective, antioxidant, anti-cancer,antihepatitic,antiallergic,anti-ulcer, antidiabetic properties (Basu et al., 2005).
Croton microstachys	Crotepoxide,neoclerodan-5,10-en-19, 6-beta-20-dienolide,3-alpha-19-dihy droxytrachylobane,3-alpha-18,19-trihydrotrachylobane	Antitumor activity (Daniel, 2006)
Markhamialutea	Luteoside A,B, & C , verbascoside and isoverbascoside	Anti viral activity against respiratory syncytial virus (Kernan et al., 1998)
Spilanthes mauritania	N-isobutyl decadienamide	Antiplasmodial activity (Jondiko, 1986; Weenen et al., 1990)
Carica papaya	Carpain and papain	Cardiotonic and antihelminthic activities (Daniel, 2006)
Leonotis nepetifolia	Lavenduliside,martinoside, verbascoside	Antioxidant activity (Atta-ur-Rahaman., 2006)
Bryonia dioica	Bryodins 1 and 2	Anticancer activity (Siegall et al.,1994)
Ageratum conyzoides	Essentialoils,6-(1-hydroxymethyl)-7, 8-dimethoxy chromene, 6-hydroxy-7,8-dimethoxy-2,2-dimethylchromene,coumarins, Isoflavones.	Antibacterial activity (Kamboj et al , 2008)
Withania somnifera	Dimeric thiowithanolide, withanoside D, sominone, withaferin A,monomeric glycoprotein(28KDa)	Anticancer activity and antibacterial activities (Subbaraju et al., 2006, Sirinivasan et al., 2007, Kobuyama et al., 2006)
Ajuga remota	Ajugarin1 ,Ajugarin 2, ergostenol-5,8-Endoperoxide	Antiplasmodial activity, anti-mycobacterium tuberculosis (Manguro et al., 2005).
Cassia spectabilis	3-O-acetylspectalin,(-)-7-hydroxy-spectalin, iso-6-spectalin	Antifungal and antinoreceptive anti activities (Viegas et al., 2008)
Datura stramonium	Hyoscine, atropine and hyoscyamine,	Stimulant,sedative,hyprotic,antiacylcholinestrase Antispasmodic (Daniel, 2006)

Table 3. The treatment of patients and administration of the prepared herbal remedies as practised by the herbalists in this project.

Diseases	Number of patients	Plant used (part used)	Preparation of herbal remedies	Administration of herbal remedies and outcome of treatment
Bronchial asthma	4	*Leonotis molissima* (leaf), *Terminalia brownii* (bark), *Bryonia dioica* (leaf), *Datura stromonium* (seed)	Ten grammes of equal ratios of *L.molissima, T.brownii* and *B.dioica* powders was infused in 150mls of water for 15 minutes and filtered. Twenty drops of tincture of *D. stromonium* in whisky steeped for seven days were put into 70mls of water	The patient drunk 150 mls of infusion and 150 mls of diluted tincture daily for two weeks. Clinical symptoms of three patients subsided while one patient did not heal
Bronchial pneumonia	3	*Melia azadirach* (leaf), *Erythrina excelsa* (bark)	Twenty grammes of equal ratio of powders of the plants were boiled in 150 ml of water for 15 minutes and filtered.	The patient was orally given 75 mls of the decoction three times daily for 8 days. All patients healed
Malaria	4	*Erythrina abyssinica* (stem bark), *Toddalia asiatica* (root bark), *Microglosa pyrifolia* (leaf)	Ten grammes of equal amounts of the powders of the plants are infused in 200 mls of boiling water for 20 min and filtered	The patient orally drunk 100 mls of the infusion three times daily for two weeks. All patients had no clinical symptoms and parasitamia
Malaria	3	*Erythrina abyssinica*(stem bark), *Toddalia asiatica*(root bark), *Kigelia africana* (fruit), *Ficus lutea* (stem bark)	One kilogram in equal amounts of fresh plant materials were cut into small pieces and ground by a plate mill and boiled in one litre of water for one hour then filtered	The patients were given 150 mls of the decoction three times daily for two weeks. Two patients showed no clinical symptoms and parasitamia while one patient had persistent symptoms of both
Intestinal worms	3	*Albizia coriaria* (stem bark)	Ten grammes of the powder was infused in 150 mls of warm water for ten minutes and filtered	The patients were orally given 75 mls three times daily for two weeks. Two patients tested negative for cists of worms with disappearance of clinical symptoms while patient did not heal
Intestinal swelling nodes with headache and dizziness	4	*Viscum album* (whole plant), *Taraxacum officinalis* (whole plant)	Twenty grammes of equal amounts of the powdered plants were decocted in boiling 600 mls of water for twenty minutes and filtered	The patient drunk 150 mls of decoction twice daily for three weeks. Clinical symptoms in two patients subsided while one did not heal
Allergy and fungal skin infection	4	*Urtica dioica* (leaf), *Senna didymobotrya* (leaf), *Leonotis mollissima* (whole plant), *Ricinus communis* (seed, leaf)	Ten grammes of equal amounts of the leaf powders of three plants together with that whole *L.mollissima* were decocted in 150mls of boiling water for ten minutes and filtered. An ointment of five grammes of the leaf powder of *S. didymobotrya* and seeds of *R. communis* in equal amounts was made in 10mls of the oil of *R. communis*.	The patient drunk 75 mls of the decoction three times daily for two weeks. The ointment was applied on affected skin twice daily for three weeks. The clinical symptoms in three patients disappeared while one did not heal

Table 3. Contd.

Pectic ulcer	6	*Datura stromonium* (leaf), *Centella asiatica* (whole), *Ocimum bacilicum* (whole), *Aspilia mosambiensis* (whole), *Withania somnifera* (leaf), *Tussilago vulgaris* (whole), *Moss sp*.(whole)	A tincture of 10 grammes of macerated fresh leaves of *D. stomonium* was made in 20mls of whisky for 7 days and strained. Twenty grammes of the powders in equal amounts *C. asiatica. bacilicum,W.somnifera, T.vulgaris, Moss sp*. and *A. mosambiensis* were boiled in 150mls of water for 20 minutes and filtered	The patient was orally given 75 mls of the decoction in which 20 drops of tincture were added three times daily for three weeks. The clinical symptoms and barium meal tests showed improvement in all patients
Bronchial asthma	4	*Carica papaya*(leaf), *Euphorbia hirta* (whole), *Eucalyptus citrodora* (leaf), *Maribium vulgaris* (leaf), *Datura stromonium*(seed, leaf)	Half gramme of equal amounts of powdered leaves of four plants and whole *E. hirta* was rolled as a cigarette in plain paper. A decoction of twenty grammes of the powder of the plants in equal amounts were made in 150mls of boiling water for twenty minutes and filtered. A tincture of 20 grammes of equal amounts of fresh leaves of the plants together with one gramme of ground seeds of *D. stromonium*. were steeped in 100 mls of 20% surgical spirit for seven days then strained.	The patient smoked the cigarette and drunk the 150 mls of the decoction containing 12 drops of tincture three times daily for three weeks. The clinical symptoms subsided in three patients while one patient did not heal
Amoebic dysentery	10	*Carica papaya* (leaf), *Euphorbia hirta* (whole), *Harrisonia abyssinica* (stem bark), *Cyphostemma nodiglandulosa* (corm,leaf), *Ajuga remota* (leaf)	Twenty grammes of equal amounts of each part of the plants were decocted in 150 mls of boiling water for 15 min and filtered	The patients drunk 150 mls three times daily for three weeks. The clinical symptoms and laboratory evidence indicated that eight patients were treated from the disease while two did not
Facial skin allergy due to cosmetics	3	*Conyza sumatrensis* (leaf), *Bidens pilosa* (leaf), *Aloe kedogensis* (leaf), *Bredelia micranthus* (leaf), *Ageratum conyzoides* (leaf), *Cassia spectabilis* (leaf)	A decoction of three kilogrammes of equal amounts of fresh plants were made in two litres of boiling water for one hour and strained. The gel from fresh leaves of *A. kedogensis* was expressed manually. 0ne kilogramme of leaves of *A. spectabilitis* was burned and ash kept for use	The patients drunk 75 mls of the decoction and applied the gel on affected skin twice daily for three weeks. The clinical symptoms subsided in all patients
Infected wounds on legs	3	The plants were the same as the ones for facial allergy due to cosmetics.	The preparation was the same as the one for the case for allergy due to cosmetics	The patient drunk 150 mls of the decoction and washed the wounds with it and also applied the gel and the ash on the affected skin three times daily for three weeks. All patients healed as evidenced from clinical observation
Fungal infection on the scalp	10	The plants were the same as those used for treatment of allergic case.	The preparations were same as the ones used in the treatment of allergic case	The patients orally drunk 150 mls of the decoction, washed the surface with it and applied the gel on the surface three times daily for three weeks. All patients did not show clinical symptoms of the skin disease

Table 3. Contd.

Gastric hyper acidity and arthritis	4	The stem bark and leaves of the plants in Table 2	Ten kilogrammes of equal amounts of fresh parts of the plants were chopped into small pieces and ground by a plate mill then boiled in ten litres of water and filtered	The patient drunk 150 mls of the concoction three times daily for three weeks. Three patients showed no clinical symptoms while one did not heal
Chronic menorrhagia	5	The plants were the same as those used for treatment of gastric hyper acidity and arthritis.	The concoction was the one used for treatment of patients suffering from gastric hyper acidity and arthritis	Administration was the same as that used for treating gastric hyper acidity and arthritis. four patients indicated no clinical symptoms of the disease while one did not heal
Elephantiasis	2	The plants were as those used in the treatment of chronic menorrhagia	The concoction was the same as that used for the treatment of chronic menorrhagia	The patient drunk 150 mls of the concoction and cleaned the lesions with it three times daily for one month. There was no improvement
Herpes zoster	2	The plants were the same those used for treatment of chronic menorrhagia.	The concoction was the same as that used for treatment of chronic menorrhagia	The patient drunk 150 mls of the concoction and applied the ash from *A. spectabilis* on the wound three times daily for three weeks. The wound and pain subsided as indicated by clinical observation and the two patient's response

substances in the decoctions ranged between 0.0002 to 0.0003 g per ml whereas the dissolved substances were between 0.0002 to 0.0004 g per ml. The thin layer chromatographic analysis indicated that most of the extracts of the decoctions had 5 to 10 spots. The parameters indicated that the decoctions contained both insoluble and soluble substances or compounds which could be responsible for the therapeutic properties of the herbal drugs. These parameters can be tentatively used for standardization of the herbal drugs as well as the quantification of the doses.

The patients and the treated diseases

Three herbalists recruited 74 patients through their usual practice in Seme and Gem sub locations (Table 3). The composition of patients by gender was 60 and 40% female and males, respectively while by age groups 10, 30, 50 and 10% were corresponding to 8 to 15, 16 to 25, 26 to 50 and 51 to 70 years old, respectively. The females were more than males whereas most age group with sickness were those in 26 to 50 years old. The diagnosis by both herbalists and the medical doctor revealed eleven diseases whose frequencies were shown by number of patients in brackets as: skin infections (fungal, bacterial, wounds allergies) (22), bronchial and asthmatic pneumonia (11), intestinal pain, swelling nodes and headache and dizziness (10), amoebic dysentery (10), malaria (7), peptic ulcer (6) and chronic monorrhagia (5), gastric hyper acidity and arthritis (4), intestinal worms (3), elephantiasis (2) and herpes zoster (2). The frequencies of the diseases are close to those recorded for Seme sub location (GOK, 1993). Gastro-intestinal and chest diseases as well as malaria are the leading maladies treated by the selected herbalists in the two sub locations.

Clinical and observational evaluations

The patient and herbalist focused approach in ethnomedicinal studies led to the identification of the listed 95 plant species in Table 1 and the diseases indicated for them by the herbalists in Table 3. The literature survey on ethnobotanical uses of these plants revealed very wide spread use of these plants thus validating their use by the chosen herbalists in the project. Further phytochemical and pharmacological literature survey confirms our conviction that despite low educational capacity and poor access of information to the herbalists, the traditional practice has a lot to guide the discovery of potential drugs for treatment of the diseases.

Table 3 indicates that half of the plants used by the herbalists had received reasonable pharmacological and phytochemical evaluations thus further validate the medicinal practice by these herbalists. The incooperation of the medical doctor in the

project also indicated that the diagnostic capability of the herbalists did not show wide variation. There was qualitatively close agreement between the diagnosis given by the herbalist and that given by the doctor in 80% of the diseases. Such diagnoses were confirmed by both pre-treatment and post-treatment clinical laboratory tests where it was applicable. The percent healings were as indicated by bracket for each skin diseases (95%), bronchial and asthmatic pneumonia (82%), intestinal disease and headache (70%), dysentery (80%), chronic monorrhagia (80), gastric acidity and arthritis (75%), pectic ulcer (100%), intestinal worms (66%), herpes zoster (100%) and elephantiasis (0%). The medical doctors' observation and laboratory diagnosis confirmed the healing rates. The questionnaires administered to the herbalists with respect to healing rates reported for same period before this intervention indicated an improvement of 10 to 20% healing rates for most of the diseases. The details of the plants species, preparation and administration of the herbal medicines are indicated Table 3. The pharmacological, phytochemical and clinical trials attributed to the species are believed to contribute to the high healing rates between 66 to 100% except for elephantiasis for which there was no healing. It is important to note that all the herbal remedies were made of multiple plants except for treatment of intestinal worms in which only one plant was used (Table 3). These plants were indicated for treatment of multiple diseases. For example, percentages of species indicated for 5, 8, 7, 6 and 4 diseases were 50, 5, 2, 3 and 1%, respectively (Table 1). A survey of ethnomedicinal literature revealed that other herbalists elsewhere in East Africa employ polyherbal and multi-disease treatment approach. For example, 20% of the species are indicated for at least 8 diseases. Fifty percent of the plant species identified by the herbalists were indicated for nearly fifty diseases thus confirming the importance of these plants in disease management.

The pharmacological evaluations using either *in vitro* or *in vivo* methods indicated that 45% of the solvent extracts of the species had antifungal, antibacterial and antiviral activities including physiological properties which validate the therapeutic or healing values observed in this project (Tables 1 and 2). The physiological properties such as anti-inflammatory, antioxidant, analgesic, hyperglycaemic and hepatoprotective activities of the crude extracts and the isolated compounds lend credence to the therapeutic observations in this project and thus confirms the hypothesis that the plants used by the herbalists have chemical, protective and medicinal principles.

Table 2 contains 31 plant species and 80% of which are used by one of the herbalist in the preparation of the multipurpose herbal remedies orally given to patients suffering from several ailments. These plants had received phytochemical and pharmacological evaluations leading to isolation and structural elucidation of antifungal, antibacterial and antiviral as well as antiplasmodial properties. The ethnomedical, ethnopharmacological and phytochemical data not only validated the traditional medical practice but opens avenue for the next research agenda as well as clinical trials which would lead to value addition to the practice and proper health management.

AKNOWLEDGEMENTS

I sincerely thank the Regional Programme for Sustainable Use of Dryland Biodiversity for the research grant as well as Dr. Jeff Odera for his support during the research activities. My sincere thanks are due to Mr. Simon Mathenge who identified the plants. The three herbalists; Mrs Mary Muga, Mr. Okaka and Mr. Obunga must be profusely thanked for their contribution in showing us the plants, recruiting the patients and the treatment they gave to the patients. I must thank Dr. Mannase Onyimbi who kindly agreed to participate in the project as an orthodox doctor against the medical ethics as an observer but also to help us diagnose the diseases. Finally, I must thank the patients and the medical laboratory technicians who took part in the project.

REFERENCES

Achenbarch H, Waibel R, Nkunya MHH, Weenen H (1992). Antimalarial compounds from *Hoslundia opposita*. Phytochemistry 92:3781-3784.

AFLORA (2008). The database of traditional plant utilization in African area studies, Kyoto University. WWW.http:/130.54.103.36/aflora.nsf. (Accessed on 15th July 2008).

Alamo M, Moral R, Perula de Torres L (2000). Evaluation of a patient-centered approach in generalized approach musculoskeletal pain/fibromyalgia patients in primary care. Patient Education Couns. 48(1): 23.

Andayi AW, Yenesew A, Derese S, Midiwo JO, Gitu MG, Jondiko OJ, Akala H, Liyala P, Wangui J, Waters NC, Heidenreich MM, Peter MG (2006) Antiplasmodial flavonoids from *Erythrina sacleuxii*. Planta Medica. 72(2):187-189.

Basu S, Ghosh A, Hazra B (2005). Evaluatiion of the antibacterial activity of *Ventilago madraspatana* Gaertn., *Rubia cordifolia* Linn.and *Lantana camara* Linn: Isolation of emodin and physion as active antibacterial agents. Phytother. Res.10:888-894.

Chopra A, Lavin P, Patwadhan B, Chitre D (2004). A 32 week randomized, placebo controlled clinical evaluation of RA/11, an ayurvedic drug on osteoarthritis of the knees. J. Clin. Rheumatol. 10(5):236-245.

Viegas Jr C, Alexandre-Moreira MS, Fraga CA, Barreiro EJ, Bolzani Vda S, de Miranda AL (2008). Antinociceptive profile of 2,3,6-trisubstituted piperidine alkaloids: 3-O-acetyl-spectaline and semi-synthetic derivatives of (-)-spectaline. Chem. Pharm. Bull. 56:407-412.

Daniel M (2006). Medicinal plants: chemistry and properties. Science publishers. London.

Duraisami R, Srinivasan D (2008). Anticonvulsant activity of bioflavonoid gossypin. Bangl. J. Pharmacol. 4:51-54.

Farnsworth NR (1994). Ethnopharmacology and drug development in Ethnobotany and the search for new drugs in Ciba Foundation Symponium 185.John Wiley and sons. Chichester, New York pp. 42-59.

Found WMC (1995). Participatory research and development: An assessment of IDRCs' experience and projects: Report to the International Development Research Centre. 000931/94-0817. ttps://idl bnc.ca/dspace/bitstream/1234556789/13765/1102546.pdf. (Accessed 25th September 2007).

Gakunju DMN, Mberu EK, Dossaji SF, Gray AI, Waigh RD, Waterman PG, Watkins WM (1995). Potent antimalarial activity of alkaloid

nitidine isolated from a Kenyan herbal remedy. Antimicrobiol Agents Chemother. 39:(12):2606-2609.
GOK (1993). Siaya District Development Plan 1989-1993. Ministry of Planning and Development. Government Press. Nairobi.
GOK (1996). Kisumu District Development Plan 1993-1996. Ministry of planning and Development.Government Press. Nairobi.
Guitiernez R, Mitchell S, Solis RV (2008). *Psidium guajava*. A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 117(1):1-27.
Han QD, Wang YL, Yang l, Tso TF, Qiao CF, Song JZ, Xu LJ, Chen SL, Young DJ, Xu HX (2006). Cytotoxic polyprenylated xathone from the resin of *Garcinia hambrury*. Chem. Pharmaceut. Bull. 54:87-89.
Hans DN (1996) African Ethnobotany: Poisons and Drugs Chemistry, Pharmacology and Toxicology. CRC Press. London.
Helen de Wet (2005). An ethnobotanical and chemotaxonomic study of South African Menospermaceae.PhD. Thesis, University of Johannesburg. S.A.
Iwalokun BA, Efededе BU, Alabi-Sofunde JA, Oduala T, Magbageeola OA, Akinunde AI (2006). Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen induced hepatic damage in mice. J. Med. Food 9(4):524-530.
Iwasaki H, Oki H, Takara R, Miyahira, H, Hanashiro, K, Yoshida Y, Kamada Y, Toyokawa T, Takara K, Inakuju M (2006). The tumor spefific cytotoxicity of dihydronitroditine from *Toddalia asiatica*. Cancer Chemother. Pharmacol. 8:1-9.
Jassim SAA, Naji MA (2003). Novel antiviral agent: Medicinal plant perspective. J. Appl. Microbiol. 961:412-427.
Jeruto P, Lukhoba C, Ouma G, Otieno D, Mutai C (2008). An ethnobotanical study of medicinal plants used by Nandi people in Kenya. J. Pharmacol. 116:370-203.
Jian CC, Ming HC, Rui LN, Geoffrey AC, Qui SX (2005). Cucurbitacins and cucurbutacane glycosides, structures and biological activities. Natural Products Report 22:386-399.
Jondiko IJO (1986). A mosquito larvicide in *Spilanthes mauritiana.* Phytochemistry 25(10):2289-2290.
Kamboj A, Saluja AK (2008). *Ageratum conyzoides*: A. review on its Phytochemical and Pharmacological Profile 2:59-68.
Kernan MR, Amarquaye Chen JL, Sesin DF, Parkinson N, Ye Z, Barrett M, Soddart CA, Sloan B, Blanc P, Limbach C, Mrisho S, Rhozhon EJ (1998). Antiviral phenylpropanol glycosides from the medicinal plant *Markhamia lutea.* J. Natural Products 61(5):564-570.
Khan MIH, Ahmad J (1985). A pharmacognistic study of *Psidium guajava*. Int. J. Crude Drug Res. 29:95-103.
Kokwaro JO (1993). Medicinal plants of East Africa. Second Edition, Kenya Literature Bureau, Nairobi.
Kye-Taek L (2005). Glycoprotein isolated from *Solanum nigrum*.L. kills HF-29 cells through apotosis. J. Med. Food. 8(2):205-226.
Lewis WH, Elvin-Lewis MP (1994). Basic quantitative and experimental research phases of future ethnobotany with reference to the medicinal plants of South America in Ethnobotany and the search for new drugs in Ciba Foundation Symposium 185. John Wiley and sons. Chichester. New York pp. 60-76.
Lozoya X, Reyes-Morales H, Chávez-Soto MA, Martínez-García Mdel C, Soto-González Y, Doubova SV (2002). Intestinal antispasmodic effect of a phytodrug of Psidium guajava folia in treatment of acute diarrhea disease. J. Ethnopharmacol. 83(1-2):19-24.
Manguro AOL, Wagai OS, Lemmen P (2006). Flavonol and iridoid glycosides of *Ajuga remota* aerial parts. Phytochemistry 67:830-837.
Mariano MV, Teresa ORA, Maria UL, Robert B (1999). Antiiflammatory active compounds from the N-hexane extract of *Euphorbia hirta.* J. Mexocan Chem. Soc. 43(3-4):103-105.
McKenzie RA, Franke FP, Duuster PJ (1987). The toxicity of cattle and butadienolide of six *Bryophyllum* species. Aust. Vet. J. 64(10):298-301.
Ngouela S, Nyasse B, Tsamo E, Sondengam BI (1991). Spathol, a new polyhydroxy sterol from leaves of *Spathodea campanulata.* J. Natural Products 54:873-876.
Nyasse B, Ngautchou I, Tchana EM, Sonke B, Dnier C, Fontaie C (2004a). Inhibition of both *Trypanosoma brucei* blood forms and related glycolytic enzymes by kolvic acid derivatives isolated from *Entada abyssinica.* Farmazie 59(11):873-875.
Nyasse B, Nono J, Sonke B, Denier C, Fontaine C (2004b). Trypanocidal activity of bergenin, the major constituents of *Flueggea virosa.* Farmazie 59(6):492-494.
Okech R, Mwangi H.A, Lisgarten J, Mberu EK (2002). A new antiplasmodial coumarin from *Toddalia asiatica* (L) Lam. Roots. Fitoterapia 71:636-640.
Orwa JA, Jondiko JI, Minja RJ, Bekunda M (2008). The use of *Toddalia asiatica* (L). Lam. J. Ethnopharmacol. 116(3):469-482.
Ostrom E (2008). Sustainable agriculture and natural resource management: Collaborative research support programme. 4BEJ: www. oired. vt. edu /sanrem. crsp/ document / semi Annual March. (Accessed 25th September 2008).
Rwangabo PC, Claey SM, Pieters L, Courthot J, Vanden BDA, Vlietinck (1988). Umuhengrin, a new antimicrobially active flavonoid from *Lantan trifolia.* J. Natural Products 51:966.
Saxena SVR (1999). Antimicrobiol activity of the essential oils of *Toddalia asiatica* (L) Lam. Fitoterapia 70: 64-66.
Shu-Lin C, Yi-Ming C, Cicer-Lee TC, Hsiu-Hua Y, Lie-Fen, S, Yue-Hsiung K, Tung-Kung W, Wen-Chin Y (2007). Flavonoids: centaurein and centaureidin from *Bidens pilosa* and stimulate of IFN-expression. J. Pharmacol. 112:232-236.
Siegall CB, Gawlak SL, Chace D, Wolff EA, Mixan B, Marquardt (1994). Characterization of ribosome -inactivating proteins isolated from *Byronia dioica* and their utility as carcinoma reactive immunoconjugates. J. Bioconjugate Chem. 5:5.
Sindiga I, Chach NC, Kanunah MP (1995). Traditional medicine in Africa. East African Educational Publisher Ltd. Nairobi.
Srivanasan S, Ranga RS, Burikhanov R, Han SS, Chendil, D (2007). Par/4 dependent apotosis by dielzeng compound, withaferinA in prostrate cancer cells. Cancer Res. 1(67):246-253.
Subbaraju GV, Vanisree M, Sivama-Krishna C, Sridhar P, Jayaprakasam B, Nair MG (2006). Ashwagathanolide, a bioactive dimeric thiowithanolide isolated from the roots of *Withania somnifera*. J. Natural Products 69(12):1790.
Weenen H, Nkunya MHH, Bray DH, Mwasumbi LB, Kinabo LS, Kilimali VAEB, Wijnberg JBPA (1990). Antimalarial compounds containing alpha-beta-unsaturated carbonyl moiety from Tanzania plants. Planta Med. 56:371-373.
Yamagishi T, Haruna M, Yan XZ, Chang JJ, Lee KH (1989). Antitumor agents 110 Bryophyllin B a novel cytotoxic bufadienolide from *Bryophyllum pinnatum.* J. Natural Products 52(5):1071-1079.

academicJournals

Vol. 8(3), pp. 158-161, 17 January, 2014
DOI: 10.5897/JMPR10.233
ISSN 1996-0875 © 2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

The medicinal characteristics of alcohol-extraction-water-precipitation fraction from *Swertia mussotii* Franch.

Ping Lv[1,2], Lixin Wei[1], Yuzhi Du[1], Yuanchan Xiao[1] and Min Peng[1]*

[1]Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining Qinghai, 810001, China.
[2]Graduate University of Chinese Academy of Sciences, Beijing, 100049, China.

Accepted 10 June 2010

The alcohol-extraction-water-precipitation fraction of *Swertia mussotii* Franch. (SME-d) had been proved to be hepatoprotective without toxicity in previous report. In this article, high performance liquid chromatography (HPLC), rat experiment and P450 model tests were employed for studying the pharmacology characteristics of SME-d. The results showed that the contents of sweroside, swertiamarin, mangiferin, gentiopicroside, and isoorientin were 0.24, 3.96, 12.30, 13.53, 16.85 mg/g in SME-d, respectively. SME-d could reduce the CCl_4-induced exaltation of alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), total bile acid (TBA) significantly in rat, and the protective activity showed dose-dependent in 0.9 and 1.8 g/kg body weight (BW). The hepatoprotective activity of SME-d was different to positive drug bifendate, which only did on ALT value significantly. Bifendate could inhibit 66.17 ± 2.12% of CYP3A4 activity, while SME-d showed 99.0 ± 0.267% reductions on CYP1A2. The different medicinal characteristics of SME-d to bifendate, which are used widely to cure hepatitis in china, can give more choices for hepatitis.

Key words: *Swertia mussotii* Franch, the alcohol-extraction-water-precipitation fraction, pharmacology characteristic, hepatoprotective activity.

INTRODUCTION

Liver is an important organism for the metabolism and detoxification of various components entering into the body, and is hurt usually by the toxins and drugs, viral infections (Hepatitis A, B, C, D, etc.) and microbial infections (Sharma and Ahuja, 1997). Hepatitis is a big challenge to the modern medicine always. Plant-based traditional medicines were widely and successfully used in the treatment of liver disorders, for example, *Picrorhiza kurroa* (Chander et al., 1992), *Phyllanthus emblica* (Gulati et al., 1995), *Silybum marianum* (Flora et al., 1998). Bifendate, coming from herb *Schisandra chinesis*, had been the common drug for hepatitis in China (Pan et al., 2006).

Swertia mussotii Franch., referred to as "Zang Yin Chen" in Chinese, is a biennial herb of the family Gentianeceae that has been widely used in Tibetan folk medicine. *S. mussotii* is often used to remedy diseases in liver (Yang, 1991). Inventing a new medicine for hepatitis is always a big objective and challenge to plant chemist from *S. mussotii* Franch..

The alcohol-extraction-water-precipitation fraction from *S. mussotii* Franch. (SME-d) had been proved to be hepatoprotective without obvious toxicity in mice (Lv et al., 2010). If we wanted to devise a new medicine in china, 50% composition should be identified for injection medicine, its function should be proved in rat, the dose relationship should be illustrated, and the effects on P450 activity should be clarifed. So high performance liquid

*Corresponding author. E-mail: Pengm@nwipb.ac.cn. Tel: +86 971 6143668. Fax: +86 971 6143765.

chromatography (HPLC), hepatoprotection evaluation in rat and P450 model test were employed for answering these questions.

MATERIALS AND METHODS

The preparation of SME-d

The whole plant of *S. mussotii* Franch. (SM) was collected from Sichuan province, China, and at the full-blooming stage in July, 2008. It was authenticated by the Centre of Tibetan Medicine, Northwest Institute of Plateau Biology, Chinese Academy of Sciences. 1 g crude SM was subjected to 10 ml ethanol (75% v/v) in hot water bath for three times, and the ethanol was removed by distillation under reduced pressure. Then the ethanol electuary was dissolved in distilled water (1:8 v/v) for 24 h, and centrifuged (8000 rpm/min) for 10 min. At last, the sedimentation was taken as SME-d.

Chemicals

Carbon tetrachloride (CCl_4), olive oil and other solvents were purchased from XinXin Glass & Reagent Co. (Xining, China). Bifendate was supplied as a positive control sample by Zhejiang Wanbang Pharmaceutical Co., Ltd (Wenling, China). The references of swertiamarin, gentiopicroside, sweroside, mangiferin, and isoorientin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Chemical analysis with high performance liquid chromatograph (HPLC)

The chemical profile of SME-d was recorded by high performance liquid chromatography (Agilent 1100) with diode array detection (DAD). Samples were dissolved by methanol, and the solutions were filtered with 0.45 μm Millipore filters. A reverse phase C18 column (Agilent Eclipse XDB-C18，250 mm × 4.6 mm, 5 μm) was eluted with the gradient phase (0 min, 18% methanol → 25 min, 55% methanol → 47 min, 80% methanol → 60 min, 100% methanol) at the flow rate of 1 ml/min. The eluate was monitored at the wavelength of 210, 230 and 254 nm, and the column temperature was kept at 25°C. Swertiamarin, gentiopicroside, sweroside, mangiferin and isoorientin were used as the reference standard.

Hepatoprotective evaluation in rats

KM rats (male and female) 20 to 25 g, were purchased from Laboratory Animal Center, Gansu College of Traditional Chinese Medicine. The animals were maintained at a constant temperature of 23 ± 2°C and fed with tap water and standard laboratory chow (Beijing Ke-Ao-Xie-Li feed. Co., LTD, Beijing, China). Normal group and control group were fed with 20 ml/kg BW distilled water for 8 days orally. Test groups were fed with SME-d at doses of 0.9 and 1.8 g/kg BW for 8 days orally, and positive group was fed with 80 mg/kg BW bifendate for 8 days. Each group contained eight rats. At the 8th day, 10 ml/kg BW CCl_4 (0.1%, v/v in olive oil) was administrated by intraperitoneal injection to all groups except normal group. At 22 h after the last dose, all rats were sacrificed. Serum was separated by centrifuging at 3000 rpm for 10 min and used for the measurement of the alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (TBIL), bile acid (TBA) value (Drotman and Lawhorn, 1978). The ALT and AST activities were measured with the ALT and AST Elisa Kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The TBIL and TBA contents were determined by the TBIL and TBA Test Kits (Kehua, Shanghai, China).

The effects of SME-d on CYP1A2 and CYP3A4 activities

CYP1A2 and CYP3A4 belonged to the cytochrome P450 superfamily. They are plentiful in liver and responsible for catalyzing the oxidation of organic substances. For drug discovery, researchers need to determine how drug candidate alter P450 activity. In this experiment, all samples were analyzed by P450-GloTM CYP1A2 Screening System (Cat.#V9970) and P450-GloTM CYP3A4 Screening System (Cat.#9910) (Promega, America). CYP1A2 or CYP3A4 enzymes were incubated with their substrates, or and sample for 10 to 30 minutes at 37°C. Reactions were initiated by the addition of a nicotinamide adenine dinucleotide phosphate regenerating solution. P450 activity was stopped and luminescence was initiated by adding luciferin detection reagent. Luminescence was read directly on the FLUOstar OPTIMA microplate reader (BMG LABTECH, Germany) in luminescence mode. 1 μM α-naphthoflavone (sigma, America) were positive control in CYP1A2 screening experiment, and 5 μM Ketoconazole (sigma, America) was in CYP3A4 screening experiment. Firstly, samples were tested in 20 μg/ml dose. If the inhibition of some sample was bigger than 50%, the half maximal inhibitory concentration (IC_{50}) was measured in seven gradients.

Statistical analysis

All statistical analyses were performed by using Microsoft Excel 2000 (Guo, 2000) or the SPSS 10.0 (Mo, 2004) for windows software package. The date were analyzed by Student's t-test to assess the significance of the differences between two means or by one-way analysis of variance (ANOVA) followed by least-significant-difference (LSD) test for more than two means (Milton and Tsokas, 1983). Statistical significance was considered at $p < 0.05$.

RESULTS

The contents of five hepatoprotective chemical compounds

The contents of sweroside, swertiamarin, mangiferin, gentiopicroside, isoorientin were 0.24, 3.96, 12.30, 13.53, 16.85 mg/g in SME-d, respectively (Table 1).

The hepatoprotective activity in rat

Compared with normal group, CCl_4 induced serum ALT and AST activity in control group significantly ($P < 0.01$). High and low dose of SME-d and bifendate could inhibit the exaltation of the serum ALT activity induced by CCl_4. The inhibition effect of 0.9 g/kg SME-d < 80 mg/kg bifendate < 1.8 g/kg SME-d (Table 2). High and low dose of SME-d could also inhibit the exaltation of the serum AST activity induced by CCl_4, but the inhibition of bifendate was not obvious statistically. The effect of 0.9 g/kg SME-d < 1.8 g/kg (Table 2). CCl4 also induced the serum TBIL and TBA content in control group significantly

Table 1. The contents of five hepatoprotective chemical compounds (mg/g).

Parameter	Sweroside	Swertiamarin	Mangiferin	Gentiopicroside	Isoorientin
SME-d	0.24	3.96	12.30	13.53	16.85

Table 2. The ALT and AST, TBA and TBIL value (mean ± s, n = 6).

Treatments	Dose	ALT (U/L)	AST (U/L)	TBIL (μmol/L)	TBA (μmol/L)
Normal	-	48.67±5.72**	154.33±15.33**	2.65±0.10**	19.84±3.74**
Control	-	1952.96±771.75	3130.67±1598.88	6.65±2.71	257.90±95.16
SME-d	0.9 g/kg	842.33±467.94*	1143.33±493.11*	3.95±0.62*	141.36±13.09*
	1.8 g/kg	605.54±185.45**	1050±253.24*	3.32±0.35*	103.87±24.64**
Bifendate	80 mg/kg	648.05±177.48**	2222.5±857.93	8.40±2.49	193.38±65.21

*P < 0.05, **P < 0.01 vs control.

Table 3. The effects of SME-d on CYP1A2 and CYP3A4 activity.

Samples	CYP1A2		CYP3A4	
	% Inhibition (20 μg/ml)	IC_{50} (μg/ml)	% Inhibition (20 μg/ml)	IC_{50} (μg/ml)
SME-d	99.0±0.267	0.79±0.040	17.38±5.50	>20
Bifendate	5.3±6.76	-	66.17±2.12	5.703±0.072
α-naphthoflavone (1 μM)	99.5±0.267	-	-	-
Ketoconazole (5 μM)	-	-	93.89±1.14	-

(P < 0.01). High and low dose of SME-d could inhibit significantly the exaltation of the serum TBIL and TBA content induced by CCl_4 (Table 2). The inhibition effect of SME-d showed dose-dependent in 0.9 g kg and 1.8g/kg (Table 3). Bifendate showed no significant effect on the serum TBIL and TBA content (Table 2).

The effects of SME-d on P450 (CYP1A2 and CYP3A4) activities

SME-d could decrease 99.0 ± 0.267% activity of CYP1A2 in liver cell, and did little influences on CYP3A4. On the contrary, bifendate could inhibit 66.17 ± 2.12% activity of CYP3A4, but affected the activity of CYP1A2 hardly.

DISCUSSION

Some constituents of *S. mussotii* have been proved to be hepatoprotective (Sun et al., 1991), which contain swertiamarin (Singh, 2008), gentiopicroside (Li et al., 2001), sweroside (Singh, 2008), mangiferin (Liao et al., 2005), isoorientin (Orhan et al., 2003). Measuring these compounds contents in SME-d was a shortcut for understanding its hepatoprotective mechanism. The contents of sweroside, swertiamarin, mangiferin, gentiopicroside, isoorientin were 0.24, 3.96, 12.30, 13.53, 16.85 mg/g in SME-d, respectively. The extract procedure had enriched isoorientin, because the isoorientin content had been measured to be between 2.46 and 7.4 mg/g in *S. mussotii* (Bao et al., 2006; Li et al., 2008). Moreover, isoorientin could show obvious hepatoprotective function in 15 mg/kg BW (Orhan et al., 2003), and 1.5 mg/kg BW isoorientin should be given in 0.9 g/kg BW of SME-d, so isoorientin should be take parted in the hepatoprotective activity of SME-d. Of course, more works should be done for understanding the hepatoprotective mechanism of SME-d.

Carbon tetrachloride-induced liver injury model was the common model for studying the hepatoprotective medicine. ALT and AST were the sensitive index for the acute liver injury (Fu and Wei, 2005), and TBIL and TBA were the sensitive index for jaundice of liver injury (Zhang et al., 1989). Therefore, the serum ALT, AST, TBA and TBIL were taken as liver injury indicators in rats. CCl_4 could induced the ascension of the serum ALT and AST activity, TBA and TBIL content significantly (P < 0.01), which showed the CCl_4-induced liver injury model was constructed successfully. SME-d could cut down the exaltation of the ALT, AST, TBA and TBIL induced by CCl_4 significantly, which could be deduced that SME-d can perform the hepatoprotective function in rat. Bifendate could decrease the serum ALT activity significantly, and did no obviously influence on the serum AST activity, the serum TBA and TBIL contents, which was similar to

previous results (Liu, 2006). The difference of SME-d and bifendate should give SME-d more chance to be a new medicine.

The CYP3A4 and CYP1A2 belonged to the cytochrome P450 superfamily. They are plentiful in liver and often employed for evaluating the possibility of the interactions in medicines, especially for new medicine. SME-d could inhibit the CYP1A2 activity without effects on CYP3A4, which should give some suggestions for using SME-d correctly. Its difference to bifendate would give hepatitis more choices.

ABBREVIATIONS

HPLC, High performance liquid chromatography; **ALT,** aspartate transaminases; **AST,** alanine transaminases; **TBIL,** total bilirubin; **TBA,** total bile acid; **CYP1A2,** cytochrome P450 1A2; **CYP3A4,** cytochrome P450 3A4; **SME-d,** the alcohol-extraction-water-precipitation fraction of *Swertia mussotii* Franch.; **BW,** body weight.

REFERENCES

Bao Y, Ji WH, Ma YH, Ji LJ (2006). Simultaneous determination of six main constituents in *Swertia* of Qinghai province and Sichuan province by HPLC. China J. Chin. Mater. Med. 31:2036-2038.

Chander R, Kapoor NK, Dhawan BN (1992). Picroliv, picroside-I and kutkoside from Picrorhiza kurrooa are scavengers of superoxide anions. Biochem. Pharmacol. 44:180-183.

Drotman RB, Lawhorn GT (1978). Serum enzymes are indicators of chemical induced liver damage. Drug Chem. Toxicol. 1:163-171.

Flora K, Hahn M, Rosen H, Benner K (1998). Milk thistle (Silybum marianum) for the therapy of liver disease. Am. J. Gastroenterol. 93:139-143.

Fu CL, Wei HJ (2005). The Interpretation of Liver Function Test Results. Clin. Med. 25:79-81.

Gulati RK, Agarwal S, Agrawal SS (1995). Hepatoprotective studies on Phyllanthus emblica Linn. and quercetin. Indian J. Exp. Biol. 33:261-268.

Guo WJ (2000). Variance Analysis by Microsoft Excel. J. Yunnan Agric. Univ. 15:9-12.

Li YL, Ding CX, Wang HL, Suo YR (2008). Separation and determination of flavone and xanthone glycosides in Tibetan folk medicinal species *Swertia mussotii* and *S. franchetiana* by capillary electraophoresis. J. Anal. Chem. 63:574-579.

Li YQ, Zhao DH, Pan BR, Li BG, Sun WJ, Tian Q, Jia M (2001). Effect of gentiopicroside on liver injury of rats. J. Fourth Military Med. Univ. 22:1645-1649.

Liao HL, Wu QY, Ye GM, Cai LZ (2005). Advanced research on the pharmacology of mangiferin. Tianjin Pharm. 17:50-52.

Liu GT (2006).The pharmacological research and drug discovery and traditional chinese medicine. Peking Union Medical College Press, Beijing p. 322.

Lv P, Wei LX, Du YZ, Yang HX, Peng M (2010). Hepatoprotective and toxic characteristics of the whole herb of traditional Tibetan folk medicine Swertia mussotii Franch. J. Med. Plants Res. 4:706-709.

Milton JS, Tsokas JO (1983).Statistical methods in the biological and health sciences. In: International Student Edition. McGraw-Hill, London pp. 281-290.

Mo JK (2004). The applications of SPSS 10.0 for Windows In the medical field. international medicine and health guidance news 10:60-61.

Orhan DD, Aslan M, Aktay G (2003). Evaluation of hepatoprotective effect of *Gentiana olivieri* herbs on subacute administration and isolation of active principle. Life Sci. pp. 2273-2283.

Pan S, Yang R, Han Y, Dong H, Feng X, Li N, Geng W, Ko K (2006). High doses of bifendate elevate serum and hepatic triglyceride levels in rabbits and mice: animal models of acute hypertriglyceridemia. Acta Pharmacol. Sin. 27:673-678.

Sharma M, Ahuja V (1997). Amoebic liver abscess: clinician's perspective. Bombay Hosp. J. 39:615-619.

Singh A (2008). Phytochemicals of Gentianaceae: A Review of Pharmacological Properties. International Journal of Pharmaceut. Sci. Nanotechnol. 1:33-36.

Sun HF, Hu BL, Ding JY, Fan SF (1991). The glucosides from *Swertia mossotii* Franch. Acta Bot. Sin. 33:31-37.

Yang YC (1991).Tibetan Medicines. Qinghai People's Publishing House, Xining p. 111

Zhang GZ, Li BL, Yang L (1989). The Experimental Model of hepatocellular jaundice in rats. J. Guiyang Med. Col. 14:174-178.

academicJournals

Vol. 8(3), pp. 162-166, 17 January, 2014
DOI: 10.5897/JMPR10.717
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Protective effects of *Launaea procumbens* against oxidative adrenal molecular, hormonal and pathological changes in rats

Rahmat Ali Khan[1]*, Muhammad Rashid Khan[1,2], Sumaira Sahreen[1] and Jasia bokhari[1]

[1]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan.
[2]Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology Bannu, KPK, Pakistan

Accepted 23 December, 2010

The aim of the study was to investigate the protective effects of Launaea procumbens methanolic extract (LM) against CCl4-induced molecular, hormonal and pathological abnormalities in rats. Male Sprague Dawley rats were provided by National Institute of Health (NIH) Islamabad and orally fed with 100, 200 mg/kg body weight of LM after 48 h of CCl_4 treatment (3 ml/kg body weight, 30% in olive oil) biweekly for 4 weeks. The results showed that the administration of LM significantly improved the CCl_4-induced serum level of hormones, argyrophilic nucleolar organizer regions (AgNORs) and DNA damages. Histopathology showed that LM reduced the incidence of adrenal lesions induced by CCl_4 in rats. These results suggest that LM could protect adrenal against the CCl_4-induced oxidative damage in rats.

Key words: Carbon tetrachloride, *Launaea procumbens*, adrenal histopathology.

INTRODUCTION

The imbalance between the reactive oxygen species and the ability of biological system to detoxify these reactive intermediate or easily repair the resulting damage causes by them is called oxidative stress. All living organisms maintain a reducing environment within their cells by a system of antioxidant enzymes. This imbalance causes toxic effects through the rapid production of peroxides and free radicals that damage cell and macromolecules including proteins, lipids and nucleic acids. Carbon tetrachloride has molecular formula CCl_4, and its molecular weight is 153.8 g/mol, has been used as solvent in varnishes, resins and as starting material of many industrial organic compounds, and it is estimated that the average daily intake of CCl_4 for a general population is 0.1 µg (Abraham et al., 1999; ATSDR, 2003). Exposure to such toxic chemical through inhalation, ingestion or skin absorption is distributed throughout the body with high concentration in liver, muscles, fat tissue brain, kidney and blood (Ogeturk et al., 2004), and damages various tissues especially liver (Khan and Ahmed, 2009).

Carbon tetrachloride induces reactive oxygen species (ROS) and oxidative DNA damages, with the formation of DNA adducts, genetic mutation, strand breakage and chromosomal alterations. DNA strand breaks are especially important in inducing mutations, such as deletions and translocations in affected cells undergoing replication with error-prone repair or without proper repair. Moreover, extensive DNA strand breaks without prompt repair may cause cell death and compensatory cell regeneration (Khan et al., 2010a, b).

Nuclear morphology can be evaluated histologically using a newly developed silver (argyrophilic) staining method for nucleolar organizer regions (NORs), the so-called AgNOR technique. NORs are composed of

*Corresponding author. E-mail: rahmatgul_81@yahoo.com. Tel: +92 928 633425

chromosomal sites endowed with ribosomal DNA (rDNA) and complexes with a set of non-histone proteins characterized by high affinity for silver (Trere et al., 1996; Khan et al., 2010c) used for identification of normal cells from neoplastic cells (Cheah et al., 1996). Medicinal plant play crucial role in improving various pathogensis (Sahreen et al., 2010; Khan et al., 2009; Khan et al., 2010c). *Launaea procumbens* is locally used in Pakistan in adrenal dysfunction. Therefore the present study was arranged to evaluate the protective function of *L. procumbens* versus carbon tetra chloride induced oxidative damages in rats.

MATERIALS AND METHODS

Plant collection and extraction

L. procumbens at maturity was collected from Wah Cantt District Rawalpindi (Pakistan), identified and its ariel parts (leaves, stem, flowers and seeds) were shade dried at room temperature, grinded mechanically and extracted with methanol to get crude methanolic extract. Methanolic extracts were stored at 4°C for *in vivo* screening.

Animals

Six week old, 30 Sprague Dawley male rats (190 to 200 g) were provided by National Institute of Health Islamabad and were kept in ordinary cages at room temperature of 25 ± 3°C with a 12 h dark/light cycle. They were allowed to standard laboratory feed and water. The study protocol was approved by Ethical Committee of Quaid-i-Azam University Islamabad for laboratory animal feed and care.

Experimental design

To study the protective effects of LM, rats were equally divided into 5 groups (6 rats). Group 1 received only raw water and free access to food materials. Group 2 received olive oil intraperitoneally (Monday and Thursday) and dimethyl sulphoxide (DMSO) intragastric (Wednesday and Saturday) at a dose of 3 ml/kg body weight. Group 3 received CCl_4 3 ml/kg (30% in olive oil) intraperitoneally (Monday and Thursday). Group 4 and 5 received 100, 200 mg/kg body weight of LM after 48 h of CCl_4 (Wednesday and Saturday), respectively. Experimental period was of four weeks. After 24 h of the last treatment, all the animals were weighted, sacrificed; with their blood collected, weighted and perfuse adrenal in ice-cold saline solution. Half of adrenal tissues were treated with liquid nitrogen for further enzymatic and DNA damage analysis while the other portion was processed for histology.

Assessment of serum markers

Serum hormonal analysis of adrenal gland was carried through kits.

Histopathalogical studies

For microscopic evaluation adrenal glands were fixed in a fixative (absolute ethanol 60%, formaldehyde 30%, glacial acetic acid 10%) and embedded in paraffin, sectioned at 4 µm and subsequently stained with hematoxylin/eosin. Sections were studied under light microscope (DIALUX 20 EB) at 40 and 100 magnifications. Slides of all the treated groups were studied and photographed.

DNA fragmentations

DNA fragmentation (%) assay was conducted using the procedure of Wu et al. (2005) with some modifications. The adrenal tissue was homogenized in TE solution pH 8.0, centrifuged and separates the intact chromatin (pellet, B) from the fragmented DNA (supernatant, T). The pellet and supernatant fractions were assayed for DNA content using a freshly prepared DPA (Diphenylamine) solution for reaction. Optical density was read at 620 nm.

AgNORS analysis

Silver staining technique was used according to Trere et al. (1996) with some modifications. The cells were examined under light microscope at 100× magnification and number of NORs was counted per cell.

DNA ladder assay

DNA was isolated by using the methods of Wu et al. (2005) to estimate DNA damages. 5 µg of rats DNA was loaded in 1.5% agarose gel containing 1.0 µg/ml ethidium bromide including DNA standards (0.5 µg per well).

Statistical analysis

Data were expressed as mean and standard error (SE) and analysis of variance (ANOVA) test was used to analyze the difference among various treatments, with least significance difference (LSD) at 0.05 and 0.01 as a level of significance. SPSS ver. 14.0 (Chicago, IL, USA) and Microsoft Excel 2007 (Roselle, IL, USA) were used for the statistical and graphical evaluations.

RESULTS

Effect of *L. procumbens* on serum level of adrenalin, nor adrenalin and cortisol in rat

The protective effects of *L. procumbens* against CCl_4 intoxication on serum level of adrenalin, nor adrenalin and cortisol in rat are shown in Table 1. Administration of CCl_4 significantly ($P < 0.01$) elevated the serum level of adrenalin, nor adrenalin and cortisol as compared to the control group. Serum level of adrenalin, nor adrenalin and cortisol was reversed towards the control group in a dose dependent manner by the treatment of methanolic fraction of *L. procumbens*.

Effect of AgNORs count and DNA fragmentation

Changes in the effect of *L. procumbens* against the CCl_4 on AgNORs count and DNA fragmentation in adrenal gland of rat are shown in Table 2. CCl_4 treatment significantly ($P < 0.01$) AgNORs count and DNA damages

Table 1. Effect *L. procumbens* on serum level of adrenalin, nor adrenalin and cortisol in rat.

Treatment	Adrenalin (mg/dl)	Nor adrenaline (mg/dl)	Cortisol (mg/dl)
Control	35±3.8^{++}	17.8±0.45^{++}	63.±3.65^{++}
Olive oil+DMSO	36±2.93^{++}	18.86±0.50^{++}	64±3.23^{++}
3 ml/kg CCl_4	61±3.26**	27.3±0.58**	103±2.12**
100 mg/kg LM+CCl_4	48±3.1**$^{++}$	21±0.41*$^{++}$	81±3.12**$^{++}$
200 mg/kg LM+CCl_4	36±2.81^{++}	18±0.18^{++}	66±5.25^{++}

Mean ±SE (n=6 number). *, **indicate significance from the control group at P<0.05 and P<0.01 probability level, respectively. +, ++ indicate significance from the CCl_4 group at P<0.05 and P<0.01 probability level, respectively.

Table 2. Effect of various fractions of L. procumbens on adrenal AgNORs count and DNA damages.

Treatment	AgNORs (NORs/cell)	% DNA fragmentation
Control	1.4950±0.0448 $^{++}$	46.67±1.28^{++}
Olive oil+DMSO	1.6017±0.0744^{++}	47.67±1.09^{++}
3 ml/kg CCl_4	6.595±0.367**	67.333±0.803**
100 mg/kg LM+CCl_4	3.835±0.159**$^{++}$	57.500±0.885**$^{++}$
200 mg/kg LM+CCl_4	2.102±0.215**$^{++}$	48.17±1.30^{++}

Mean ±SE (n=6 number). *, ** indicate significance from the control group at P<0.05 and P<0.01 probability level, respectively. +, ++ indicate significance from the CCl_4 group at P<0.05 and P<0.01 probability level, respectively.

as compared to the control group. Treatment of *L. procumbens* significantly (P < 0.01) ameliorated the CCl_4 intoxication and reduced the number of NORs per cell and DNA fragmentation in a dose dependent manner

Effect of *L. procumbens* on DNA damages

Protective effects of different fractions of *L. procumbens* versus CCl_4 induced DNA damages in the adrenal tissues of rats are shown by DNA ladder assay in Figure 1. Extensive DNA breakages in adrenal gland were depicted by the treatment of CCl_4 to rats. Post-administration of *L. procumbens* reduced the DNA damages dose dependently as shown by DNA bands of different groups as compared to CCl_4 group.

Effect of different fractions of *L. procumbens* on histopathology of adrenal glands in rat

The microscopic evaluation of adrenal gland sections in control rats showed normal architecture having uniform basophilic nuclei and lack pleiomorphism. CCl_4 treatment caused necrosis of adrenal cortex, degradation of modularly cells, accumulation of fatty droplets, damage of structural proteins and the breakage of nuclear membrane. Adrenal medulla showed hypertrophy, hyperplasia and dilation of blood vessels with CCl_4 treatment. Post-administration of *L. procumbens* in the CCl_4 intoxication and reversed necrosis cortex started to attain the normal shape and size and the amount of lipid droplets were also decreased. The nuclear membrane started to repair, medulla size was normal and blood vessels were less dilated than CCl_4 group as shown in Table 3 and Figure 2.

DISCUSSION

Our results show that CCl_4 treatment causes significant increase in the secretion of epinephrine, nor epinephrine and cortisol. These changes are markedly restored by treatment of plant extracts. Stern and Brody (1963) reported that the oral administration of 2.5 ml/kg CCl_4 in peanut oil to rats elevated free epinephrine and nor epinephrine levels in plasma and urine, which supports our results. Similarly, results of Rubinstein (1962) are in accordance with our investigations, and reported that intraduodenal administration of carbon tetrachloride to rats for 2 h caused increase in serum epinephrine level.

According to the Marnett (2000), the product of lipid peroxidation, MDA react with DNA to form the adduct M1G, the mutagenic pirimedopurinone adduct of deoxyguanosine. According to Shimoda et al. (1994), it is very important to identify risk factors for genomic instability

Table 3. Effect of *L. procumbens* on histopathology of adrenal glands in rat.

Treatment	Adrenal cortex necrosis	Fatty changes	Accumulation of cells	Blood vessels dilation
Control	-	-	-	-
Olive oil+DMSO	-	-	-	-
3 ml/kg CCl_4	+++	+++	++	++
100 mg/kg LM+CCl_4	-/+	-/+	-/+	-/+
200 mg/kg LM+CCl_4	-	-	-/+	-

-, normal; -/+, mild; ++, medium; +++, severely damaged.

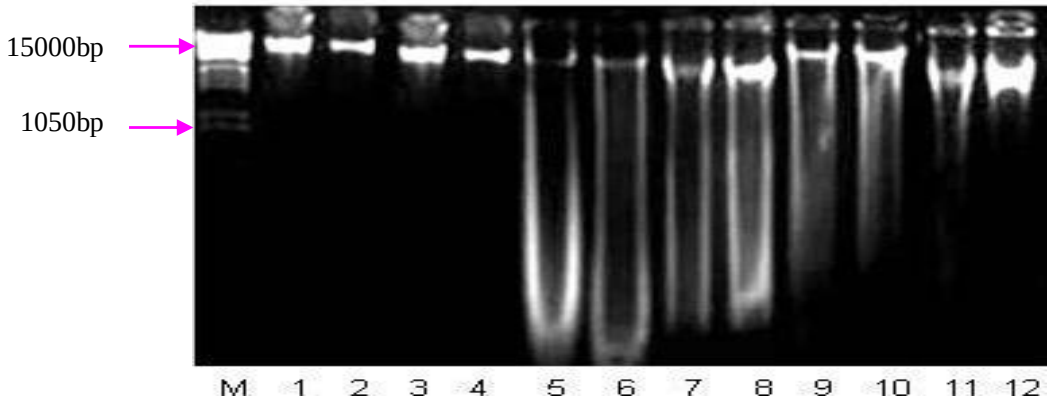

Figure 1. Agrose gel showing DNA damage by CCl_4 and preventive effect of *Launaea procumbens* extracts in different groups. Lanes (from left) DNA marker (M), Control (1- 4), CCl_4 (5, 8), 100 mg/kg LM (9, 10) 200 mg/kg LM (11, 12).

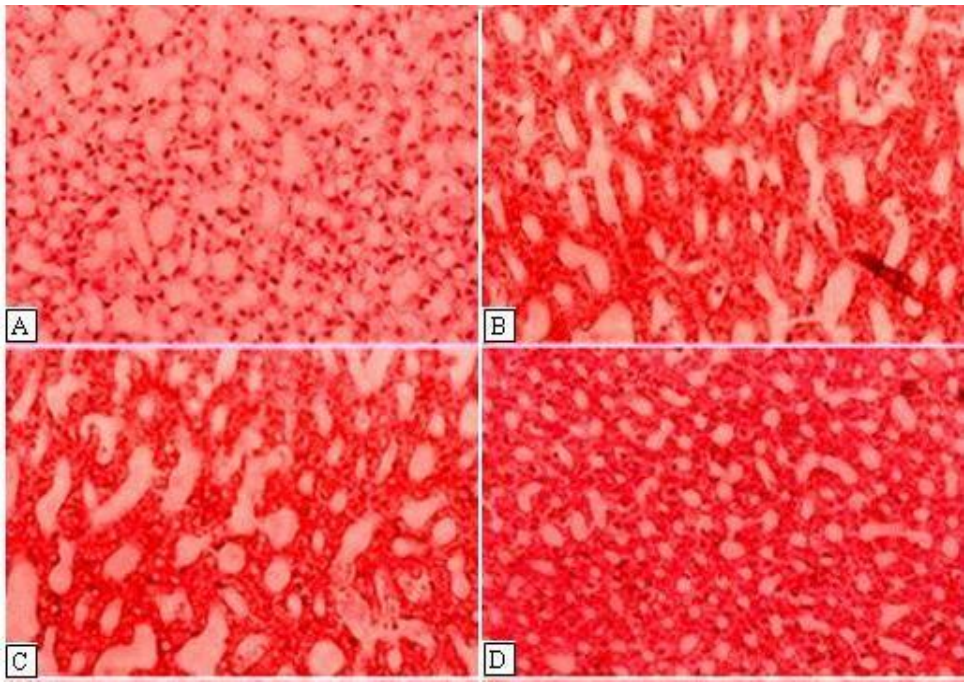

Figure 2. Histopathological changes caused by CCl_4 and preventive effect of *Launaea procumbens* extracts in different groups. Slides (from left) Control (A), CCl_4 (B, C), 200 mg/kg LM (D).

which is responsible for the occurrence of genetic alterations for carcinogenesis. The data of the present study revealed that the treatment of CCl_4 causes significant oxidative DNA damage in adrenal gland which are visualized on agarose gel by staining with ethidium bromide. Treatment with *L. procumbens* plant extracts significantly reduces these damages. Similar investigation was reported by Khan et al. (2009) during study of protective effects against carbon tetrachloride induced toxicity in rats.

The microscopic evaluation of adrenal glands showed that CCl_4 treatment caused necrosis of adrenal cortex, degradation of cells, accumulation of fatty droplets, modularly hypertrophy, hyperplasia and blood vessels dilation. Similar histopathological changes were reported that carbon tetrachloride causes necrosis to the adrenal cortex after initiation of lipid peroxidation, which requires a CYP-catalysed bioactivation (Rosol et al., 2001).

Conclusion

From data it was inferred that protective effects are due to the presence of bioactive compounds in the extract, which might be responsible in modulating the effects of CCl_4-induced toxicity and concomitantly near to normal rats as *L .procumbens* treated groups.

REFERENCES

Abraham P, Wilfred G, Cathrine SP (1999). Oxidative damage to lipids and proteins of the lungs, testis and kidney of rats during CCl_4 intoxication. Clinical Chimica Acta 289:177-179.

ATSDR-Agency for Toxic Substances and Disease Registry, (2003). Toxicological profile for carbon tetrachloride, U.S. Department of Health and Human Services, Public Health Services; Atlanta, GA.

Cheah PL, Looi LM, Chan LL (1996). Immunohistochemical expression of p53 proteins in Wilms tumour: a possible association with the histological prognostic parameter of anaplasia. Histopathol. 28:49-54.

Khan MR, Ahmed D (2009). Protective effects of *Digera muricata* (L.) Mart. On testis against oxidative stress of carbon tetrachloride in rat. Food Chem. Toxicol. 47:1393-1399.

Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S (2009). Carbon tetrachloride induced nephrotoxicity in rats: Protective role of *Digera muricata*. J. Ethnopharmacol. 122(1):91-99.

Khan RA, Khan MR, Sahreen S (2010a). Evaluation of *Launaea procumbens* use in renal disorders: A rat model. J. Ethnopharmacol. 128:452-461.

Khan RA, Khan MR, Sahreen S, Bokhari J (2010b). Prevention of CCl_4-induced nephrotoxicity with *Sonchus asper* in rat. Food Chem. Toxicol. 48(8-9):2469-2476.

Khan RA, Khan MR, Sahreen S, Bokhari J (2010c). Antimicrobial and Phytotoxic screening of various fractions of *Sonchus asper.* Afr. J. Biotech. 9(25):3883-3887.

Marnett JL (2000). Oxyridicals and DNA damage. Carcinogenesis 21:361-370.

Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M (2005). Caffeic acid phenyl ester protects kidney against carbon tetrachloride toxicity in rats. J. Ethanopharmacol. 97:273-280.

Rosol TJ, Yarrington JT, Latendresse J, Capen CC (2001). Adrenal Gland: Structure, Function, and Mechanisms of Toxicity. Toxicol. Pathol. 29:41-48.

Rubinstein D (1962). Epinephrine release and liver glycogen levels aftercarbon tetrachloride administration.American. J. Physiol. 203:1033-1037.

Sahreen S, Khan MR, Khan RA (2010). Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chem. 122(4):1205-1211.

Shimoda R, Nagashima M, Sakamoto M, Yamaguchi N, Hirohashi S, Yokota J, Kasai H (1994). Increased formation of oxidative DNA damage, 8-Hydroxydeoxyguanosine, in human livers with chronic hepatitis. Cancer Res. 54:3171-3172.

Stern PH, Brody TM (1963). Catecholamine excretion following carbon tetrachloride administration. J. Pharm. Exp. Ther. 141:65-73.

Trere D (1993). Critical analysis of the methods commonly employed in the assessment of cell proliferation: advantages of NOR silver staining technique in routine cytohistopathology. Anal Cell Pathol 5:191-201.

Wu B, Ootani A, Iwakiri R, Sakata Y, Fujise T, Amemori S, Yokoyama F, Tsunada S, Fujimoto K (2005). T cell deficiency leads to liver carcinogenesis in Azoxymethane-treated rats. Exp. Biol. Med. 231:91-98.

academicJournals

Vol. 8(3), pp. 167-177, 17 January, 2014
DOI: 10.5897/JMPR10.737
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Review

A review on therapeutic potential of *Nigella sativa* (kalonji) seeds

S. V. Tembhurne[1]*, S. Feroz[2], B. H. More[3] and D. M.Sakarkar[1]

[1]Sudhakarrao Naik Institute of Pharmacy Pusad, Dist: Yavatmal 445204. (M.S), India.
[2]Bombay College of pharmacy, Kalina Santacruz (East) Mumbai 400098, India
[3]Tasgaonkar college of Pharmacy Bhivpuri Dist. Raigarh, India.

Accepted 14 April, 2011

***Nigella sativa* name as black seed or Kalonji seed belongs to family of rananculacea. It is widely grown in different part of world and is an annual herb cultivated in India and Pakistan, Phytochemically; it contains fixed oil, protein, alkaloids saponin and essential oil. *N. sativa* has been reported to possess potent antioxidant, hepatoprotective, antiparasitic, anticancer, antidiabetic, antimicrobial, antiparasitic, analgesic and anti-inflammatory, anti-nociceptive, anti-ulcer, anti-histaminic etc. The present article reviews on morphology, cultivation, chemical constituent and therapeutic potential as well as clinical aspect and toxicity of *N. sativa* seed.**

Key words: *Nigella sativa,* morphology, cultivation, chemical constituents, therapeutic potential, clinical aspects and toxicity.

INTRODUCTION

Amongst the promising medicinal plant, Kalonji (*Nigella sativa*) a dicotyledonous of rananculacea is an amazing herb with a rich historical and religious background. The seeds of *N. sativa* are the source of the active ingredient of this plant. The actual importance of *N. sativa* to the Muslims came from the holy saying of the Prophet Mohammed “Prayers and peace be upon him” in the black seed is the medicine for every disease except death (Ghaznavi, 1991). It is the same black seed referred by Prophet Mohammed as a panacea (universal healer), that is a remedy for all ailments but cannot prevent ageing or death (Ghaznavi, 1991). Historical use of black seeds has been mentioned in various religious and ethnic books. Black seeds are identified as the curative black cumin in the holy bible; it is also described as the melanthion of Hippocrates and Dioscordes. In the Greco Arab/ Unani-Tibb system of medicine which originate from Hippocrates, his contemporary Galen and Ibn- sina has regarded black seed as a valuable remedy in hepatic and digestive disorder. The famous book of medicine by Ibn-sina “The cannon of medicine (980-1037) revealed historical importance of this Black seeds as the seeds “That stimulates the body’s energy and help recovery from fatigue (Ghaznavi, 1991; Chevallier, 1996).

Through thousand of years, until the time being, millions of people in the mediterranean region and Far East countries use the oil of *N. sativa* seeds daily as a natural protective and curative remedy. Historically, it has been recorded that *N. sativa* seeds were prescribed by ancient Egyptian and Greek physicians to treat headache, nasal congestion, toothache and intestinal worm, as well as a diuretic to promote menstruation and milk production (Hajhashemi et al., 2004). In Ayurverdic system of medicine, the seeds are given with butter-milk to obstinate hiccups and are also used in loss of appetite, vomiting, dropsy. They are also used as emmenagogue and galactogogue and as an abortificient in large doses.

In different combinations, the seeds of *N. sativa* have

*Corresponding author. E-mail: stembhurne@gmail.com. Tel: 09922070123.

been used in obesity and dyspnoea. They have antibilious property and are administered internally in intermittent fever. Constant inhalation of fried seeds releases cold and catarrh. The seeds have also been used in mercury poisoning, sores and leprosy (Ahmad et al., 2004).

Synonym of black seeds in various languages

English: Black cumins, Love-in-a-mist.
Arabic: Habatut Barakah; Sonez ; Habatut – sauda; Kamune-asvad.
Hindi: Kalonji.
Sankrit: Krishana – Jiraka.
Persian: Siyadanah (Ahmad et al., 2004; Chevallier, 1996).

MORPHOLOGY OF THE PLANT

N. sativa is a bushy, self branching plant of about 50 to 60 cm in height. Leaves are divided into linear segment 2 to 3 cm long; they are apposite in pairs on either side of the stem. Its lower leaves are small, and petiolate and upper leaves are long. The plant has finely divided foliage and pale bluish or white flowers. The flowers grow terminally on its branches. *N. sativa* reproduces with itself and forms a fruit capsule which consist of many white trigonal seeds, once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air becoming black in colour (black seeds), seeds are triangular in shape, black in colour and possess a severe pungent smell, contains considerable amount of oil (Chevallier, 1996).

SCIENTIFIC CLASSIFICATION OF THE PLANT

Kingdom: Plantae.
Subkingdom: Tracheobionata that is, vascular plant.
Supervision: Spermatophyte.
Order: Ranunculales.
Family: Ranunculaceae-Butter cup family.
Genera: *Nigella.*
Species: *sativa*.

CULTIVATION AND COLLECTION

The Plant is widely grown in different part of the world and is an annual herb cultivated in India and Pakistan. *N. sativa* is cultivated during winter season in much the same way as wheat. The areas where maize, green gram or black grams are grown can be used after harvesting these crops. Before sowing the seeds, 2 to 3 times plouging is enough for good crops and weed control.

Heavy soils need more plouging than light soils. The seeds are sown 30 cm apart. The seeds should not be sown deep because the germination is delayed. About 12 to 15 kg seeds per hectare are sown. Three to five irrigation are required that is, presowing, seeding stage, flowering stage, and fruit formation stage and seeds development stage. Crop matures during April and May. It should be harvested early in the morning. The crop is harvested when the fruit/capsule turn yellowish. The late harvesting may result in shattering the seeds. After harvesting and proper drying it can be threshed by trampling the crop with tractor or proper thresher. After threshing, the seeds should be properly stored in bags or containers (Ahmad et al., 2004).

Chemical constituents

In view of its wide range of medicinal uses, the plant has under gone extensive phytochemical studies. *N. sativa* seeds contain 36 to 28% fixed oil, proteins, alkaloid, saponin and 0.4 to 2.5% essential oil. The fixed oil is mainly composed of unsaturated fatty acid that includes arachidonic, eicosadienoic, linoleic and linolenic acid. The saturated fatty acid present in the oil are palmitic, stearic and myristic acid (Hajhashemi et al., 2004).

The essential oil present in the seeds was analyzed by gas chromatography-mass spectrometry (GC–MS). Many components were characterized but the pharmacologically active constituent of volatile oil are thymoquinone (Figure 1a), dithymoquinone, thymol (Figure 1b) and thymohydroquinone (Figure 1c). Dithymoquinone is the dimerised form of Thymoquinone (Ghosheh et al., 1999; Hajhashemi et al., 2004). The crystalline active principle, nigellone is the only constituent of the carbonyl fraction of the oil. The other constituents of the volatile oil of the seed are p-cymene carvacrol, t-anethole, 4-terpineol and longifoline. Four alkaloids have been reported as constituent of *N. Sativa* seeds. Nigellicine (Figure 1d) and nigellidine have an indazole nucleus whereas nigellimine (Figure 1e) and N-oxide of nigellimine are isoquinolines (Atta-ur-Rehman, 1985a, b, 1995). Recently, a triterpene saponin Alfa herein was isolated from the seeds of *N. sativa*. α-heredin (Figure 1f) is known to have antitumor activity (Kumara and Haut, 2001).

The ethanolic extract of the seeds was found to contain three flavonoids namely quercetin and kaempferol 3-glucosyl (1-2) galactosyl (1-2) glusoside and quercitin –3-(6-ferulolyl glucosyl) (1-2) galactosyl (1-2) glucoside (Merfort et al., 1997). Other than those triglycoside quercetin 3-glucoside, kaempferol 3-glucoside and rutin were also isolated from the seeds of *N. sativa.*

N. sativa seeds contain other ingredient including nutritional components such as carbohydrates, fats vitamins mineral elements and proteins including eight or nine essential amino acid. Fractionation of whole *N. sativa* seeds using sodium dodecyl sulfate polyacrylamide gel

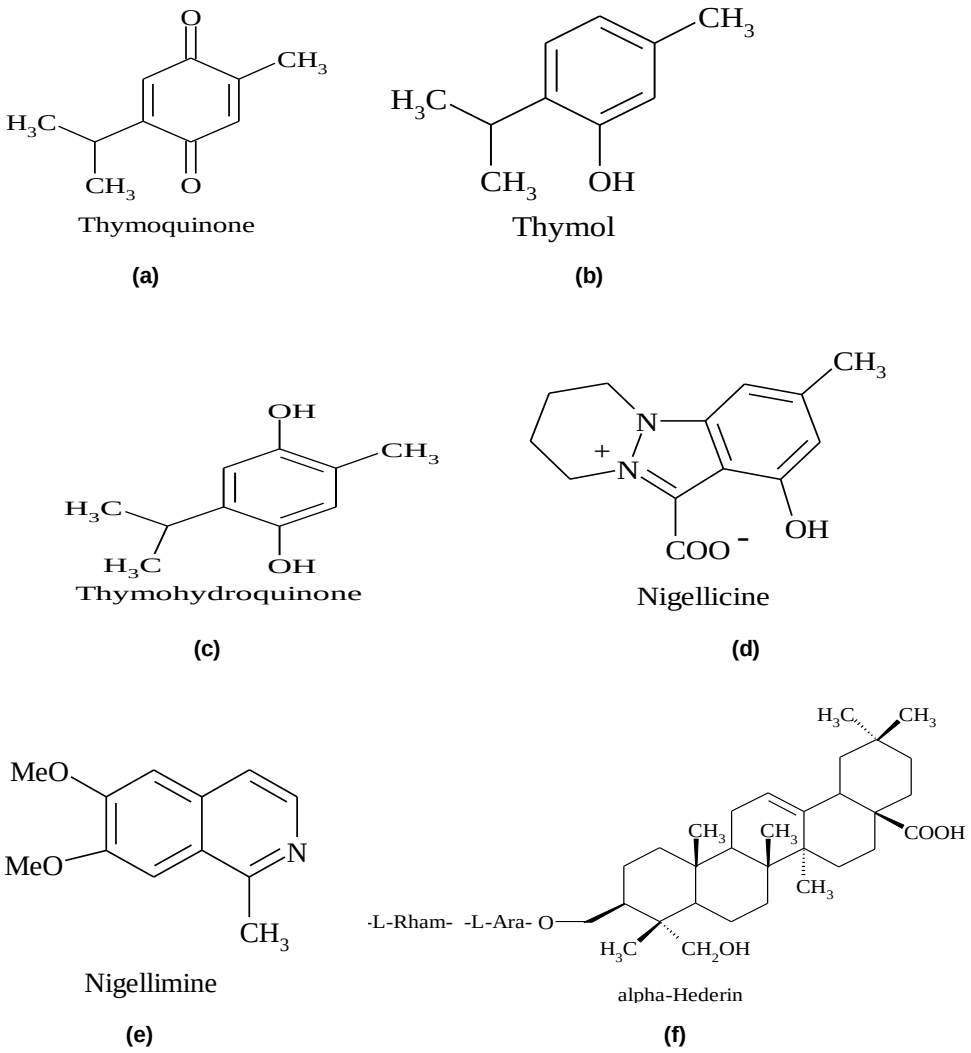

Figure 1. Chemical structure of active ingredient of *Nigella sativa* essential oil.

electrophoresis (SDS-PAGE) shows bands ranging from 94 to 100 KDa molecular mass (Haq et al., 1999). Monosaccharide in the form of glucose rhamnose, xylose and arabinose are also found. The seeds also contain carotene, which is converted by liver to vitamin A, the *N. sativa* seeds are also a source of calcium, irons and potassium (Salem et al., 2000). The summary of the all chemical composition and active principle in *N. sativa* are given in Table 1.

PHARMACOLOGICAL PROPERTIES OF *N. SATIVA* SEEDS

Many studies have been conducted particularly during the last two decades on the effect of *N. sativa* seeds extracts or its active compounds on the various body systems *in vivo* or *in vitro*. The following is the selection of some of these studies.

Antioxidant activity

Generation of free radicals may be at least partially the basis of many human diseases and conditions. Therefore the antioxidant action of *N. sativa* may explain its claimed usefulness in folk medicine. The essential oil of *N. sativa* was tested for a possible antioxidant activity. The essential oil, thymoquinone and other components like carvacrol, anethole and 4-terpineol demonstrated respectable radical scavenging property. The free radical scavenging effect of thymol, thymoquinone and dithymoquinone were studied on the reactions generating reactive oxygen species such as superoxide anion radical, hydroxyl radical and singlet oxygen using the chemiluminescence and spectrophotometer methods (Kruk et al., 2000). Thymoquinone and fixed oil of *N. sativa* were also reported to inhibit non-enzymatic peroxidation in ox brain phospholipid liposomes (Houghton et al., 1995). The antioxidant effect of thymoquinone (TQ)

Table 1. Chemical composition, including active principles, of *N. Sativa* seed.

Group	Sub-group	Components
Fixed oil (32-40 %) (Gad et al., 1963; Babayan et al., 1978; Salama 1973; Staphylakis and Gegiou 1986)	Unsaturated fatty acids	Arachidonic, eicosadienoic linoleic, linolenic, oleic and almitoleic acid. Palmitic, stearic and myristic acid. Beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides
Volatile oil (0.4-0.45 %) (Enomoto et al., 2001; El-Dakhakhany 1963; Ghosheh et al., 1999)	Saturated fatty acids	Nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α & β-pinene, d-limonene, d-citronellol, p-cymene and 2-(2-methoxypropyl)-5-methyl-1,4-benzenediol6,16-18
Proteins (Babayan et al., 1978) (16-19.9 %)*	Amino acids	Arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline and threonine, etc.13
Alkaloids (Atta-ur-Rehman et al., 1985; Atta-ur-Rehman et al., 1995)	-	Nigellicine, nigellidine, nigellimine-N-oxide
Coumarins (Atta-ur-Rehman et al., 1985; Atta-ur-Rehman et al., 1995; El-Zawahry, 1964; Drozed et al., 1973)	-	6-methoxy-coumarin, 7-hydroxy-coumarin, 7-oxy-coumarin
Saponins (Kumara and Haut 2001; Ansari et al., 1988)	Triterpenes, Steroidal	Alpha-Hedrin, Steryl-glucosides, acetyl-steryl-glucoside
Minerals (1.79-3.74 %) (El-Zawahry, 1997; Babayan et al., 1978)	-	Calcium, phosphorous, potassium, sodium and iron
Carbohydrates (33.9%) Fiber (5.5 %), Water (6 %) (Haq, et al., 1999; El-Zawahry, 1997)	-	-

and a synthetic structurally related ter-butyl thymoquinone (TBHQ) were examined *in vitro*. Interestingly, both TQ and TBHQ efficiently inhibited iron dependant microsomal lipid peroxidation in a concentration dependent manner (Badary et al., 2003).

Hepatoprotective activity

Hepatotoxicity is associated with alteration in the levels and activities of certain enzymes such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), oxidant scavenger enzymes system including glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT).

The protective action of thymoquinone against the hepatotoxin: terbutyl hyderoperoxide has been demonstrated using isolated rat hepatocytes (Daba et al., 1998). In this study, the hepatoprotective activity of thymoquinone (TQ) was compared with that of silybin a known hepatoprotective agent. The mechanism of hepatoprotection of TQ is not certain but may be related to the preservation of intracellular gluathione (GSH), the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury. It has also been shown that pretreatment of rats with *N. sativa* oil for 4 weeks was effective in protection against CCl_4 and D-galactosamine induced hepatic damage. No ill effects on liver function were observed when the oil was green orally at a dose of 100 mg/kg/day for 4 weeks. In mice thymoquinone, 8 mg/kg/day for 5 days before and 1 day after CCl_4 treatment was found to protect against the biochemical and histological markers of liver damage (Nagi et al., 1991). Recently, it is also found to show protective effects against ischemia reperfusion injury on liver (Fahrettin et al., 2008).

Anti nephrotoxic activity

Administration of seed extract with cysteine, Vitamin E and *Crocus sativa* before administrating the nephrotoxic drug cisplatin was effective in ameliorating the biochemical and physiological indices of nephrotoxicity (El-Dally et al., 1996).

This was also confirming with our previous results and reported results of Nephroprotective activity of *N. sativa* seed oil in nephrotoxicity induced by Cisplatin and Gentamycin (Tembhurne et al., 2008; Ali, 2004). The reason for the protective action is not certain but may be related to the antioxidant action of the drug and the fact that the neprotoxic drug may induce its effects via generation of free radicals (El-Dally et al., 1996). Fanconi syndrone (FS) induced by ifosfamide is characterized by wasting off glucose, electrolyte and organic acids along with elevated serum creatinine and urea as well as decreased creatinine clearance rate. Administration of thymoquinone with the drinking water before and during ifosfamide treatment ameliorated the severity of ifosfamide induced renal damage and improved most of the alteration of biochemical parameters (Badary et al., 1996)

Anti cancer activity

Salomi et al. (1992) have shown that the crude methanolic extract of the seeds of this plant exhibited a strong cytotoxic action on Elrich ascites carinoma, Dalton's ascites lymphoma and sarcoma 180 while exerting minimal cytotoxicity to the normal lymphocytes. In another study, the aqueous and alcoholic extract of *N. sativa* alone or in combination with H_2O_2 as an oxidative stressor were found to be effective in *in vitro* inactivating MCF-7 breast cancer cells (Farah and Begum, 2003). The antitumor effect of thymoquinone and β-elemene were investigated both *in vivo* and *in vitro* in male albino rats on fibrosarcoma induced by 20-methyl cholanthrene and it was found to inhibit tumor incidence and tumor burden significantly. The possible modes of action were discussed as its antioxidant activity and interference with DNA synthesis coupled with enhancement of detoxification process (Badary and Gamal-el-Din, 2001; Zhou et al., 2003; Gali-Muhtasib et al., 2006; Amr, 2009).

A fraction of the ethanolic extract of *N. sativa* seeds was studied in mice against intraperitoneally implanted murine P388 leukemia and subcutaneously implanted Lewis lung carcinoma cells. The life span of treated mice increased by 153% as compared to directly sulphoxide treated control mice. α- Hederin, a triterpene isolated from this fraction, produced significant tumor inhibition rates while the underline mechanism(s) of antitumor activity of hederin remained to be established. Topical application of *N. sativa* and *C. sativa* extracts inhibited two-stage skin carcinogenesis in mice induced by dimethylbenzanthracene and croton oil. The *in vivo* and *in vitro* inhibitory effect of thymoquinone against benzo (a) pyrene induced stomach carcinogineses are also reported in mice (Salomi et al., 1991). Worthen et al. (1998) have tested *in vitro* a crude gum, a fixed oil and two purified components of the seeds thymoquinone (TQ) and dithymoquinone (DTM) for their cytotoxicity to several parental and multi-drug resistant tumor cell lines. The gum and the oil were devoid of cytotoxicity while both TQ and DTM were cytotoxic to all of the cell lines. Both the parental cell lines and their corresponding MDR variant (that were resistant to several) standard antineoplastic drugs were equally sensitive to TQ and DTM. The study was also conducted on the structural activity relationship of 27 different analog of TQ. Among these compounds, TQ-2G, TQ-4A1 and TQ-5A1 were found to be more potent than TQ in terms of inhibition of cell growth, induction of apoptosis and modulation of transcription factor-NF-κB. The novel analogs were also able to sensitize gemcitabine and oxaliplatin-induced apoptosis in MiaPaCa-2 (gemcitabine resistant) PC cells, which was associated with down-regulation of Bcl-2, Bcl-xL, survivin, XIAP, COX-2 and the associated Prostaglandin E_2 (Banerjee et al., 2010).

Antidiabetic activity

Al-Awadi and Gumma (1987) have reported the use of a plant mixture containing *N. sativa*, myrrh, gum, asafoetida and aloe by diabetics in Kuwait. They studied the effect of these drugs for their glucose lowering effect in rats and found it to be effective. Further studies on the plant mixture containing *N. sativa* revealed that the blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis and the plant extract mixture may prove to be useful therapeutic agent in the treatment of non-insulin dependent diabetes mellitus (Al-Awadi et al., 1991; Mohamed et al., 2009). The volatile oil of *N. sativa* alone also produced a significant hypoglycemic effect on normal and alloxan induced diabetic rabbits without changes in insulin levels (Al-Hader et al., 1993).

In a more recent study, the seed extract when given orally decreased the elevated glucose levels in alloxan induced diabetic rabbits after 2 mouths of treatment. Another study was designed to investigate the possible insulinotropic properties of *N. sativa* oil in streptozotocin plus nicotinamide induced diabetes mellitus in hamsters. After four weeks of treatment with *N. sativa* oil significant decrease in blood glucose level together with significant increase in serum albumin level were observed (Farah et al., 2002). The study was also confirmed for it protective effects in diabetes for crude extract and n-Hexane extract of *N. sativa* seed (Matira et al., 2008). The clinical study of *N. sativa* on 60 diabetic patients demonstrates significant improvement with reference to total cholesterol, low density lipoprotein cholesterol (LDL- C), and fasting blood glucose indicating effective as an add-on therapy in patients of insulin resistance syndrome (Najmi et al., 2008).

In another study, Nadia and Taha (2009) evaluated the effect of *N. sativa* seed oil and thymoquinone on oxidetive stress and neuropathy in Streptozotocin induced diabetic rats. The results indicated to marked increase in norepinephrine and dopamine concentrations and a marked decrease in serotonin concentration compared to

the control group. These findings were partly reversed by oral administration of either NS oil or TQ.

Antimicrobial activity

The antibacterial effect of the phenolic fraction of *N. sativa* oil was first reported by Topozada et al. (1965). The extract and the oil have been reported to have a broad spectrum of activity against a number of microbes. *In vitro* antibacterial effects of the essential oil showed pronounced activity even in 1:1000 dilutions against several organisms that include *Staphylococcus albus, E. coli, Salmonella typhi, Vibrio cholera*. The oil was more effective against gram positive than gram negative organism. El-Kamali et al. (1998) using the plate diffusion method confirmed the report and showed that essential oil was effective against gram positive (*Bacillus substilis* and *Staphylococcus aureus*) and gram negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) the antibacterial effect was maximal when *Bacillus substilis* was used. The oil was found to have excellent antifungal activity particularly against *Aspergillus* species. In a study using murine *cytomegalovirus* as a model intreaperitoneal administration of oil substantially decreased the viral load in liver and spleen (Salem et al., 2000).

Antiparasitic activity

N. sativa oil has been shown to possess anticestodal and antinematodal properties. In a recent study *N. sativa* oil was shown to be effective in reducing the number of *Schistosoma mansoni* worms in the liver and decreased the total number of ova deposited in both the liver and the intestine (Mahmoud et al., 2002; ElShenawy et al., 2008). *Nigella* has also recently been shown to be effective against other helminths such as *Hymenolepis nana* (Ayaz et al., 2007). It performs this function by augmenting host immunity. Similar protective effects were seen against other worms such as *Trichinella spiralis* and *Aspiculuris* (AbuElEzz, 2005).

Antimalarial

Various extracts of *N. sativa* found to show antiplasmodial activity against both *in vivo* and *in vitro* plasmodia infections. It shows 100% inhibition of the parasite growth (*Plasmodium falciparum*) at concentration 50 ug/ml. *N. sativa* shows dose dependant activity against parasite (Abdulelah et al., 2007; El-Hadi et al., 2010).

Analgesic and Anti-inflammatory activity

Houghton et al. (1995) reported that crude fixed oil of *N. sativa* and an active principle thymoquinone (TQ) inhibits cycloxygenase and 5-lipooxygenase pathway of arachidonate metabolism in rat peritoneal leukocytes. The effect was demonstrated via the dose dependant inhibition of the formation of thromboxane B_2 and leukotrienes B_4. This effect was later confirmed in experimental animal studies conducted using aqueous suspension of *N. sativa* crushed seed by Al-Ghamdi (2001). In this study, formation of edema in rat hind paw was inhibited and these effects were comparable with Aspirin used as a standard antiflammatory drug. Khanna et al. (1993) using three antinociceptive tests in rats and mice (hotplates test, tail pinched test, acetic acid induced writhing) conclude that the fixed oil of the seeds is endowed with strong antinociceptive actions and these actions were due to an opioid principle in the oil as they were antagonized by naloxone. Abdel Fattah et al. (2000) have used four different models of analgesia (hot plate test, tail pinched test, acetic acid induced writing and formalin induced pain) for studying the analgesic activity of the drug. The mechanism of anti-inflammatory and analgesic effect seems to be related to the inhibition of eicosanoid synthesis as suggested by the study of Houghton et al. (1995).

Antinociceptive effects

Study showed that the oral administration of *N. sativa* oil extracted from Egyptian *N. sativa* seeds produces a suppressive effect on nociceptive responses caused by thermal, mechanical and chemical nociceptive stimuli in mice, and that the antinociceptive effect of *N. sativa* oil is partly attributable to its component, thymoquinone. It also revealed that at least the supraspinal opioid systems are involved in the antinociceptive effect of thymoquinone (Abdel Fattah et al., 2000; Al-Shebani and Al-Tahan, 2009).

Anti-ulcer activity

The aqueous extract of *N. sativa* seeds was effective in reducing the ulcer index induced by Aspirin by about 36% (Rajkapoor et al., 1996). In other study oil of seed of *N. sativa* found to show protective effects on the formation of stress gastritis in hypothyroidal rats (Khaled et al., 2009). Recent clinical study is also supported with eradication of *Helicobacter pylori* in patient with non-ulcer dyspepsia (Salem et al., 2010).

Anti-histaminic action

The antihistaminic effect was first investigated by El-Dakhakhany et al. (1982) who reported the protective action of thymoquinone and carbonyl fraction of *N. sativa* against histamine-induced bronchospasm in guinea pigs. Furthermore, an *in vitro* study demonstrated that

nigellone, isolated from *N. sativa*, effectively inhibited the release of histamine from mast cells, possibly through decrease in intracellular calcium and inhibition of protein kinase C (Chakarvarti et al., 1993). These effects together with analgesic and anti-inflammatory actions, perhaps can be correlated with the use of *N. sativa* in eczema and asthma, for scorpion and spider stings and for the bites of cat, dog and snake, recommended in the folk medicine (Al-Jishi et al., 2003).

Effect on cardiovascular system

N. sativa alone or in combination with honey or garlic are promoted for the treatment of hypertension which drew the attention of El-Tahir et al. (1993) to investigate the action of the volatile oil of *N. sativa* and its active constituent thymoquinone on the arterial blood pressure and heart of anaesthetized rats. Both agents produce a dose dependent decrease in the arterial blood processor and heart rates. These effects were significantly antagonized by atropine, cyproheptadiene and hexamethonium. This suggests that these effects were centrally antagonized mainly via the involvement of 5-hydroxy tryptaminergic and muscarinic mechanism. Oral dose of 0.6 ml/kg/day of *N. sativa* extract produced a significant hypotensive effect in spontaneously hypertensive rats. These findings were significantly comparable with the standard anti-hypertensive drug nifedipine (Zaoui et al., 2002). The effect of the drug was concluded to be partially due to its diuretic effect which was comparable to 0.5 mg/kg/day furosemide. In one of study, two-month dietary supplementation with *N. sativa* extract to normal rats has shown a homogenous cardiac hypertrophy and enhanced cardiac contractility at baseline conditions. The hearts of *Nigella*-treated rats developed a moderate but significant hypertrophy that was evident by an increase in the heart weight to body weight ratio. The observed *Nigella*-induced cardiac hypertrophy was associated with an increase in the baseline cardiac inotropic properties (Yar et al., 2008).

Antihyperlipedemic effects

Seeds of *N. sativa* were evaluated in several animals' models for lipid lowering activity in which orally administered extract of seed showed promising activity. It reduces the serum cholesterol and lipoprotein level significantly (Le et al., 2004; El Dakha Khani et al., 2000; Muhammad et al., 2007; Khadiga et al., 2009; Bahram et al., 2009; Ghanya et al., 2010). The study was also conducted on human being by administering the powder of seeds of *N. sativa* before breakfast for two months and was found to reduce the total cholesterol, triglycerides, LDL-cholesterol to a highly significant extent (Inayat et al., 2009; Datau et al., 2010).

Effect on gastro-intestinal tract

In Unani medicine *N. sativa* is used for stomachache and as a digestive, carminative, laxative and anti-jaundice (Chopra et al., 1956). Oral *N. sativa* powder was reported to relieve flatulence. While Nigellone, an active principle of *N. sativa* was found to antagonize histamine induced contractions of guinea pig intestine. In addition, to this a choleretic effect of *N. sativa* oil and its active principles (thymoquinone, thymohydroquinone and dithymoquinone) reported, respectively (Mahfouz and El-Dakhakhany, 1960). El-Dakhakhani et al. (1965, 2000) investigated the effect of *N. sativa* oil on gastric secretion and ethanol-induced ulcer in rats. Reported to significant increase in mucin content, glutathione level as well as a significant decrease in mucosal histamine content and ulcer formation, with a protection ratio of 53.56%, was found in the *N. sativa* oil pretreated group. More recently, the crude extract of *N. sativa* was shown to cause a dose dependent (0.1 to 3.0 mg/ml) relaxation of spontaneous contractions of rabbit jejunum as well as inhibition of K^+-induced contractions in a similar dose range, suggestive of calcium channel blockade (Gilani et al., 2001). Recently, Abdel-Sater (2009) investigated the protective effects of *N. sativa* on hypothyroidism induced development of acute cold restraint stress gastritis in rats.

Effect on respiratory system

El-Tahir et al. (1993) reported that volatile oil of *N. sativa* seeds produce dose dependent increases in the respiratory rate and intratracheal pressure of guinea pig. When the study was conducted only using thymoquinone, in the active principle of volatile, it was found that it only increased the intratracheal pressure without having a significant effect on the respiratory rate, thus the author suggest that volatile oil could be used as potential respiratory stimulant if thymoquinone is removed from the oil. Thus the oil then can be used in Asthma. Gilani et al. (2001) studied the effect of a crude extract of *N. sativa* seed on isolated rabbit jejunum and guinea pig tracheal preparation. The extract was found a dose dependant relaxation of spontaneous contraction in the rabbit jejunum and inhibition of KCl induced contractions. These actions were similar to those produced by verapamil, a Ca^{++} - channel antagonist. The above pharmacological activities of the petroleum ether fraction of the extract were about 10 times higher than those of the crude extract. In an *in vitro* experiment carried out by Chakravarti et al. (1993) it is suggest that nigellone, a carbonyl polymer of thymoquinone isolated from seeds of *N. sativa* was found to inhibit effectively the histamine release from the mast cells thus showing the basis for its traditional use in Asthma. The results of clinical study of *N. sativa* conducted in children showed to manage the wheeze associated with lower respiratory tract illness

(Jameel et al., 2009). In another clinical study on forty (40) chemical war victims, Mohammad and Javed (2008) investigated the effect of *N. sativa* on respiratory symptoms. They were recorded symptoms score in three different visits and found significant improvement in all respiratory symptoms score and wheezing in second and third visits compared to first visits.

Effect on nervous system

N. sativa seeds revealed promising narcotic analgesic activity mediated possibly through opioid receptors (Khanna et al., 1993). The oil from the seeds exhibited central nervous system (CNS) depressant and potential analgesic effect. It was also found to potentiate pentobarbitone induced sleeping time. The study conducted on cultured cortical neurons and influence of neurotransmitters release showed to indicate increased secretion of neurotransmitters. It also modulates amino acid release in cultured neurons. There was increased in GABA activity while secretion of glutamate, aspartate and glycine was found to decrease. All the results represented the sedative and depressive effects of *N. sativa* seed extract (Tarek et al., 2010). Repeated administration of *N. sativa* was also found to decrease the turnover of 5HT and produces anxiolytics activity (Perveen et al., 2009). Thymoquinone is the major constituent of *N. sativa* seeds. In one of the study conducted in mice, thymoquinone reported to show the anticonvulsant activity (Hosseinzadeh et al., 2004; Hosseinzadeh et al., 2005).

Effect on immune system

As a natural remedy, people take *N. sativa* seeds or oil is a promoter of good health and for the prophylaxis of common cold and Asthama. In view of that, El-Kadi et al. (1986) investigated the effect of *N. sativa* on immune system and found that the drug has immuno potentiating properties in human T-cells *in vitro*. This was confirmed by Haq et al. (1995) who showed that *N. sativa* seeds activate T-lymphocyte to secrete the interleukin, IL-3 and IL-1B production. In further experiment, they purified the proteins in the whole *N. sativa* seeds and it should be noted that some proteins have suppressive and others have stimulatory properties in lymphocyte culture (Haq et al., 1999).

Effect on genitourinary system

The study showed that the volatile oil of *N. sativa* inhibited spontaneous contraction of rats guinea pig uterine smooth muscle induced by oxytocin (Aqel et al., 1996). It was also reported that *N. sativa* crude oil induced uterine contractions both *in vivo* in pregnant rabbits and *in vitro* of non-pregnant rat uteri (El-Naggar and El-Deib, 1992). Similarly, it was found that the hexane extract of *N. sativa* exhibited mild uterotropic activity and prevented pregnancy in rats when given on day 1 to 10 post-coitum (Keshri et al., 1995).

Effect on reproductive system

Sixty days study of *N. sativa* seeds shows to increase in the weight of reproductive organs, sperm motility and count in cauda epidydimides and testicular ducts. Spermatogenesis was found to increase at primary and secondary spermatocyte. While in fertility, there was increase in number of female pregnant rats (Mukhallad et al., 2009; Al-Sa'aidi et al., 2009).

Effect on blood

In view of that the petroleum ether extract of *N. sativa* was studied for its action on blood coagulation and was reported to shorten the whole blood clotting time, plasma clot time and kaolin-cephalin clotting time of male rabbits when compared to control. In addition, a significant shortening of bleeding time in rats was also observed. However, there were no significant effects on the thrombin time or prothrombin time but the partial thromboplastin time was shortened while euglobulin time was prolonged (Ghoneim et al., 1982).

TOXICOLOGICAL REPORT

The seed extract and its constituent appear to have a low level of toxicity. The toxicity of fixed oil (10 ml/kg for 12 weeks) of *N. sativa* seeds in mice and rats were investigated through the determination, of LD_{50} values and examination of possible biochemical, hematological and histopathological changes. The low toxicity of *N. sativa* fixed oil was evidenced by high LD_{50} values (11.915 ml/kg), key hepatic enzyme stability and organ integrity values. This suggests a wide margin of safety for therapeutic doses of fixed oil and *N. sativa* seeds. The LD_{50} value of thymoquinone was found to be 2.4 g/kg. Inclusion of thymoquinone in the drinking water of mice at concentration of 0.03% for 90 days resulted in no signs of toxicity except for significant decrease in fasting plasma glucose concentration (Zaoui et al., 2002).

In a recent study of diazinon induced organ toxicity, with *N. sativa* seeds extract given orally for three and six weeks, the study observed attenuated extensive changes of hematological and biochemical parameters in diazinon-treated rats. Based upon these results, they suggested *N. sativa* seeds can be considered as a promising therapeutic agent against hematotoxicity, immunotoxicity,

hepatoxicity, nephrotxicity and cardiotoxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors (Atef and Wafa, 2010). Some other studies also demonstrate that treatment with *N. sativa* resulted in significant decrease of haematological disorders induced by aflatoxin (Abdel-Wahhab and Aly, 2005) and cadmium (Demir et al., 2006). No remarkable pathological changes were recorded in bone marrow of animals treated with suspension of *N. sativa* in carbon tetrachloride induced bone marrow toxicity *(*Abou et al., 2007)*.*

CONCLUSION

N. sativa seed and its components are frequently used as a natural remedy for many ailments. A lot of work has been done to evaluate the pharmacological basis of these uses. Most studies confirm its value in folk medicine as analgesic, anti-inflammatory, anti-oxidant, and anti-cancer, anti-microbial, anti-parasitic, antihypertensive and as an immune stimulant. However, controversial results have been reported for its effect on the respiratory system, blood coagulation and uterine motility. More work is needed to determine the pharmacokinetics, biochemical, pharmacodynamic and therapeutics of active components and their interactions with modern drugs and importance to human health with sufficient detail. The ethnobotanical approach, if combined with biochemical or physiological methods, would provide useful pharmacological leads.

REFERENCES

Abdel-Fattah AM, Matsumoto K, Watanabe H (2000). Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. Eur. J. Pharmacol. 14(1):89-97.

Abdel-Sater KA (2009). Gastroprotective effects of *Nigella sativa* oil on the formation of stress gastritis in hypothyroidal rats. Int. J. Physiol. Pathophysiol. Pharmacol. 1:143-149.

Abdel-Wahhab MA, Aly SE (2005). Antioxidant property of *Nigella sativa* (black cumion) and syzygium aromaticum (colve) in rats during of latoxicosis. J. Appl. Toxical. 25:218-223.

Abdulelah HAA, Zainal-Abidin BAH (2007). *In vivo* anti-malarial tests of *Nigella sativa* (Black Seed) different extracts. Am. J. Pharmacol. Toxicol. 2:46-50.

Abou Gabal AA, Essawy AE, Abdel-Moneim AM, Hamed SS, Elzergy AA (2007). The protective effect of black seed (*Nigella sativa*) against carbon tetrachloride-induced chromosomal aberrations and ultrastructural changes of bone marrow cells. Arab J. Biotech. 10(2):275-288.

AbuElEzz NM (2005). Effect of *Nigella sativa* and *Allium cepa* oils on *Trichinella spiralis* in experimentally infected rats. J. Egypt. Soc. Parasit. 35:511-523.

Ahmad Z, Gafoor A, Aslam M (2004). *Nigella sativa* – A potential commodity in crop diversification traditionally used in health care. Project on Introduction of Medicinal herb and species as crop. Ministry of food, agriculture and livestock, Pakistan.

Al-Awadi FM, Fatania H, Shamte U (1991). The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin-induced diabetic rats. Diab. Res. 18(4):163-168.

Al-Awadi FM, Gumma KA (1987). Studies on the activity of individual plants of an anti-diabetic plant mixture. Acta. Diabetol. Lat. 24(1):37-41.

Al-Ghamdi MS (2001) Anti-inflammatory, analgesic and anti-pyretic activity of *Nigella sativa*. J. Ethnopharmacol. 76:45-48.

Al-Hader A, Aqel M, Hasan Z (1993). Hypoglycemic effect of volatile oil of *Nigella sativa* seeds. Int. J. Pharmacog. 31(2):96-100.

Ali BH (2004). The effect of *Nigella sativa* in gentamicin nephrotoxicity in rats. Am. J. Chin. Med. 32(1):49-55.

Al-Jishi SA, Hoziafa BA (2003). Effect of *Nigella sativa* on Blood hemostatic functions in rats. J. Ethnopharmacol. 85:7-14.

Al-Sa'aidi JAA, Al-Khuzai ALD, Al-Zobaydi NFH (2009). Effect of alcoholic extract of *Nigella sativa* on fertility in male rats. Iraqi. J. Vet. Sci. 23:123-128.

Al-Shebani WH, Al-Tahan FJ (2009). Antinociceptive effect of watery suspension of *Nigella sativa* Linn. seeds in mice. Iraqi. J. Vet. Sci. 23:245-248.

Amr EE (2009). Anti-Cancer Properties of *Nigella* spp. Essential Oils and their Major Constituents, Thymoquinone and β-Elemene. Cur. Clin. Pharmacol. 4:43-46.

Ansari AA, Hassan S, Kenne L, Atta-ur-Rehman S, Wehler T (1988). Structural studies on a saponin isolated from *Nigella sativa*. Phytochem. 27(12):3977-3979.

Aqel M, Shaheen R (1996). Effect of the oil of *Nigella sativa* seed on the uterine smooth muscle of rat and guinea pig. J. Ethnopharmacol. 52(1):23-26.

Ata-ur-Rehman S, Malik S, Ahmed I, Habib-ur-Rehman (1985). Nigellimine-N-Oxide, a new isoquinoline alkaloid from seeds of *Nigella sativa*. Heterocycles. 23:953-955.

Atef M, Al-Attar, Wafa'a A, Al-Taisan (2010). Preventive Effects of Black Seed (*Nigella sativa*) extract on Sprague Dawley Rats Exposed to Diazinon. Austr. J. Basic Appl. Sci. 4(5):957-968.

Atta-ur-Rehman S, Malik S, Cun-Hung H, Clardy J (1985a). Isolation and structure determination of nigellicine, a novel alkaloid from seeds of *Nigella sativa*. Tetrahedron Lett. 26:2759-2762.

Atta-ur-Rehman S, Malik S, Sadiq H, Choudhary MI, Clardy J (1995). Nigellidine, a new indazole alkaloid from seeds of *Nigella sativa*. Tetrahedron Lett. 36:1993-1996.

Ayaz E, Yilmaz H, Ozbek H, Tas Z, Orunc O (2007). The effect of *Nigella sativa* oil against *Aspiculuris tetraptera* and *Hymenolepis nana* in naturally infected mice. Saudi Med. J. 28:1654-1657.

Babayan VK, Koottungal D, Halaby GA (1978). Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* L seeds. J. Food Sci. 43(4):1314-1315.

Badary A, Taha RA, Ayman M, El-Din G, Abdel-Wahab MH (2003). Thymoquinone is a potent suprroxide anion scavenger. Drug Chem. Toxicol. 26(2):87-98.

Badary OA (1996). Thymoquinone attenuates afosfamide-induced Fanconi syndrome in rats and enhances its antitumor activity in mice. J. Ethnopharmacol. 67:135-142.

Badary OA, Gamal-el-Din AM (2001). Inhibitory effect of thymoquinone against 20-methylcholanthrene–induced fibrosarcoma tumorigenesis. Cancer Detect. Prev. 25:362-368.

Bahram PG, Vahideh EA, Maryam R, Abolfazl G (2009). Effect of dietary supplementation with *Nigella sativa* L. on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits. J. Med. Plants Res. 3(10):815-821.

Banerjee S, Azmi AS, Padhye S, Singh MW, Baruah JB, Philip PA, Sarkar FH, Mohammad RM (2010). Structure-Activity Studies on Therapeutic Potential of Thymoquinone Analogs in Pancreatic Cancer. Pharm. Res. 27(6):1146-1158.

Chakarvarti N (1993). Inhibition of histamine release from mast cells by nigellone. Ann. Allergy. 70(3):237-242.

Chevallier A (1996). Encyclopedia of medicinal plants. New York, NY: DK Publishing. p. 237.

Chopra RN, Nayyer SL, Chopra IC (1956). Glossary of Indian medicinal plants. India: CSIR. pp. 176-188.

Daba MH, Abdel-Rehman MS (1998). Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol. Lett. 95:23-29.

Datau EA, Eko E, Surachmanto, Pandelaki K, Langi FA (2010). Efficacy of *Nigella sativa* on Serum Free Testosterone and Metabolic Disturbances in Central Obese Male. Acta. Med. Indones. 42(3):130-34

Demir H Kanter, Coskun O, Uz YH, Koe A, Yildiz A (2006). Effect of black cumin (*Nigella sativa*) on heart rate, some hematological value,

and pancreatic beta-cell damage in cadmium-treated rats. Biol. Trace. Elem. Res. 110:151-162.
Drozed AG, Komissarenko FN, Litvinenko EA (1973). Coumarins of some species of Ranunaulaceae family. Farm ZH. 25(4):57-60.
El-Dakhakhani M, El-Halim MA, Aly SM (2000). Effect of *Nigella sativa* oil on gastric secretion and ethanol-induced ulcer in rats. J. Ethnopharmacol. 72(1-2):299-304.
El-DakhaKhani M, Mady NL, Halim MA (2000). *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittelforschung. 50(9):832-836.
El-Dakhakhany M (1963). Studies on the chemical constitution of Egyptian N. sativa L. seeds. Planta Med. 11(4):465-470.
El-Dakhakhany M (1965). Studies on the Egyptian *Nigella sativa* L: Some pharmacological properties of its seed's active principle in comparison to its dihydro-compound and its polymer. Arzneim Forsch. Drug Res. 15:1227-1229.
EL-Dakhakhany M (1982). Some pharmacological properties of some constituents of *Nigella sativa* L seeds: The carbonyl fraction of essential oil. Proceeding of the 2nd International conference on Islamic Medicine, Kuwait. pp. 426-431.
El-Dally ES (1996). Protective effect of cysteine, Vitamin E, C. Sativus and *N. sativa* extract on cisplatin induced toxicity in rats. J. Isl. Acad. Sci. 9(4):47-55.
El-Hadi MA, Bakri YM, Yousif G (2010). Mohammed and Hassan S. Khalid. Antiplasmodial Activity of Some Medicinal Plants Used in Sudanese Folk-medicine. Environ. Health Insights. 4:1-6.
El-Kadi, Kandil O (1986). Effect of *Nigella sativa* (the black seed) on immunity. Proceeding of the 4th International Conference on Islamic Medicine, Kuwait. Bull. Islamic Med. 4:344-348.
El-Kamali HH, Ahmad AH, Mohammad AS, Yahia AAM (1998). Antibacterial properties of essentials oils from *Nigella sativa*. Fitoterapia. 69:77-78.
El-Naggar ARM, El-Deib AEM (1992). A study of some biological activities of *Nigella sativa* (black seeds) "Habbat El-Barka". J. Egypt Soc. Pharmacol. Exp. Ther. 11(2):781-800.
ElShenawy NS, Soliman MF, Reyad SI (2008). The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. Rev. Inst. Med. Trop. 50:29-36.
El-Tahir KE, Ashour M, Al-Harbi MM (1993). The respiratory effects of the volatile oil of black seed (*Nigella sativa*) in guinea pigs: elucidation of the mechanism(s) of action. Gen. Pharmacol. 24(5):1115-1122.
El-Tahir KE, Ashour MM, Al-Harbi MM (1993). The cardiovascular effects of the volatile oil of black seed (*Nigella sativa*) in rats: elucidation of the mechanism(s) of action. Gen. Pharmacol. 24(5):1123-1131.
El-Zawahry BH (1964). Isolation of new hypotensive fraction from *Nigella sativa* seed. Kongr. Pharm. Wiss. 23:193-203.
El-Zawahry BH (1997). Chemical composition of *Nigella sativa* Linn seed. In: effect of *Nigella sativa* on certain aspects of metabolism after feeding normal and hyperlipedemic diet in adult and old animals [MD thesis in medical basic sciences]. Cairo, Egypt: Al-Azhar University. pp. 16-28.
Enomoto S, Asano R, Iwahori Y, Narui T, Okada Y, Singab AN, Okuyama T (2001). Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. Biol. Pharm. Bull. 24(3):307-310.
Eyad MS, Talay Y, Abdullah OB, Abdulaziz Al-Quorain, Mohamed IY, Raed M A, Muhammad AR (2010). Comparative study of *Nigella sativa* and triple therapy in Eradication of helicobacter pylori in Patient with Non-ulcer dyspepsia. Saudi J. Gastroenterol. 16(30):207-214.
Fahrettin Y, Sacit C, Alpaslan T, Mustafa A, Nurten A, Hale C, Ali RO, Muharrem B (2008). *Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. World J. Gastroenterol. 14(33):5204-5209.
Farah IO, Begum RA (2003). Effect of *Nigella sativa* and oxidative stress on the survival pattern of MCF-7 breast cancer cells. Biomed. Sci. Instrum. 39:359-364.
Farah KM, Atoji Y, Shimizu Y, Takewaki T (2002). Insulinotropic properties of *Nigella sativa* oil in streptozotocin plus nicotinamide diabetic hamsters. Res. Vet. Sci. 73:279-282.
Gad AM, El-Dakhakhany M, Hassan MM (1963). Studies on the chemical constitution of Egyptian *Nigella sativa* L oil. Planta Med. 11(2):134-138.
Gali-Muhtasib H, Roessner A, Schneider-Stock R (2006). Thymoquinone: a promising anti-cancer drug from natural sources. Int. J. Biochem. Cell Biol. 38(8):1249-1253.
Ghanya Al-Naqeep, Adel S, Al-Zubairi, Maznah I, Zulkhairi HA, Norhaizan ME (2010). Antiatherogenic Potential of *Nigella sativa* Seeds and Oil in Diet-Induced Hypercholesterolemia in Rabbits. Evid. Based Complement Alternat. Med. 2011:8.
Ghaznavi KM (1991). Tibbe-e-Nabvi aur Jadid Science, Al-Faisal Nasheeran wa Tajeera-e- Kutab. Urdu Bazar Lahore, Pakistan. 1:228-236.
Ghoneim MT, El-Gindy AR, El-Alami R, Shoukry E, Yaseen S (1982). Possible effects of some extracts of *Nigella sativa* L seeds on blood coagulation system and fibrinolytic activity. Proceeding of 2nd International Conference on Islamic Medicine 12th Apr, Kuwait. pp. 528-535.
Ghosheh OA, Houdi AA, Crooks PA (1999). High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). J. Pharm. Biomed. Anal. 19(5):757-762.
Gilani AH, Aziz N, Khurram IM, Chaudhary KS, Iqbal A (2001). Bronchodilator, spasmolytic and calcium antagonistic activities of *Nigella sativa* seed (Kalonji): a traditional herbal product with multiple medicinal uses. J. Pakistan Med. Assoc. 51(3):115-120.
Hajhashemi V, Ghannadi A, Jafarabadi H (2004). Black cumin seed essential oil as a potential analgesic and anti-inflammatory. Phytother. Res. 18(3):195-199.
Haq A, Abdullatif M, Lobo PI, Khabar KS, Sheth KV, Al-Sedairy ST (1995). *Nigella sativa*: Effect on human lympocytes and polymorphonuclear leucocyte phagocytic activity. Immunopharmacol. 30(2):147-150.
Haq A, Lobo I, Al-Tufail M, Rama NR, Sedairy ST (1999). Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. Int. J. Immunopharmacol. 21:283-285.
Haq A, Lobo PI, Al-Tufail M, Rama NR, Sedairy ST (1999). Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. Int. J. Immunopharmacol. 21:283-285.
Hosseinzadeh H, Parvardeh S (2004). "Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice." Phytomed. 11(1):56-64.
Hosseinzadeh H, Parvardeh S, Nassiri-Asl M, and Mansouri MT (2005), "Intracerebroventricular administration of thymoquinone, the major constituent of *Nigella sativa* seeds, suppresses epileptic seizures in rats," Med. Sci. Monitor 11(4):106-110.
Houghton PJ, Zarka R, Heras B, Hoult JR (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 61:33-36.
Inayat UB, Fazal UR, Muhannad AK, Sarfaraz Khan (2009). Effect of Prophetic medicinal Kalonji (*Nigella sativa* L.) on Lipid Profile of Human Being: An In vivo Approach. World Appl. Sci. J. 6(8):1053-1057.
Jameel A, Rahat AK, Ashraf MM (2009). Study of *Nigella sativa* oil in the management of wheeze associated lower respiratory tract illness in children. Afr. J. Pharm. Pharmacol. 3(5):248-251.
Keshri G, Singh MM, Lakshami V, Kamboj VP (1995). Post-coital contraceptive effect of the seeds of *Nigella sativa* in rats. Indian J. Physiol. Pharmacol. 39(1):59-62.
Khadiga A, Abdel Ati AE, Mustafa HE (2009). The effect of dietary *Nigella sativa* seeds on the blood cholesterol and lipoprotein levels of rabbits. J. Animal Plant Sci. 3(3):227-230.
Khaled A, Abdel-Sater (2009). Gastroprotective effects of *Nigella sativa* oil on the formation of stress gastritis in hypothyroidal rats. Int. J. Physiol. Pathophysiol. Pharmacol. 1:143-149.
Khanna T, Zaidi FA, Dandiya PC (1993). CNS and analgesic studies of *Nigella sativa* . Fitoterapia. 5:407-410.
Khanna T, Zaidi FA, Dandiya PC (1993). CNS and analgesic studies of *Nigella sativa*. Fitoterapia. 5: 407-410(1993)
Kruk I, Michalska T, Klanda A (2000). The effect of thymol and its derivatives on reaction generating reactive oxygen species.

Chemosphere. 41:1059-1064.
Kumara SS, Huat BT (2001). Extraction, isolation and characterization of anti-tumour principle, alpha-hedrin, from the seeds of *Nigella sativa*. Planta Med. 67:29-22.
Kumara SS, Huat BT (2001). Extraction, isolation and characterization of anti-tumour principle, alpha-hedrin, from the seeds of *Nigella sativa*. Planta Med. 67:29-22.
Le PM, Benhaddou-Andaloussi A, Settaf A, Cherrah Y, Haddad PS (2004). The petroleum ether extract of *Nigella sativa* exerts lipid lowering action in the rats. J. Ethanopharmacol. 94(2-3):251-259.
Mahfouz M, El-Dakhakhany M (1960). Some chemical and pharmacological properties of the new antiasthmatic drug "Nigellone". Egypt Pharmacol. Bull. 42:411-424.
Mahmoud MR, El-Abhar HS, Salh S (2002). The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. J. Ethnopharmacol. 79(1):1-11.
Matira K, Zesmin FD (2008). Effects of the crude and the n-hexane extract of *Nigella sativa* Linn. (kalajira) upon diabetic rats. Bangladesh J. Pharmacol. 4:17-20.
Merfort I, Wary V, Barakat H, Hussain A, Nawwar AM (1997). Flavonoltriglycosides from seeds of *Nigella sativa*. Phytochem. 46(2):359-363.
Mohamed AM, EL-Sharkawy FZ, Ahmed SAA, Aziz WM, Badary OA (2009). Glycemic Control and Therapeutic Effect of *Nigella sativa* and Curcuma longa on Rats with streptozotocin-induced Diabetic Hepatopathy. J. Pharmacol. Toxicol. 4(2):45-57.
Mohammad H, Boskabady, Javad F (2008). The Possible Prophylactic Effect of *Nigella sativa* Seed Aqueous Extract on Respiratory Symptoms and Pulmonary Function Tests on Chemical War Victims: A Randomized, Double-Blind, Placebo-Controlled Trial. J. Altern. Complement. Med. 14(9):1137-1144.
Muhammad AB, Muhammad T (2007). Effect of *Nigella sativa* on Lipid Profile in Albino rats. Gomal J. Med. Sci. 5(1):28-31.
Mukhallad AM, Mohamad MJ, Mohamad P, Hatham D (2009). Effects of Black Seeds (*Nigella sativa*) on Spermatogenesis and Fertility of Male Albino Rats. Res. J. Med. Med. Sci. 4(2):386-390.
Nadia MH, Taha RA (2009). Effects of *Nigella sativa* Oil and Thymoquinone on Oxidative Stress and Neuropathy in Streptozotocin-Induced Diabetic Rats. Pharmacology 84:127-134.
Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, AL-Bekairy AM (1999). Thymoquinone protects against carbon tetracholide hepatotoxicity in mice via an antioxidant mechanism. Biochem. Mol. Biol. Int. 47:153-159.
Najmi A, Nasiruddin M, Khan RA, Haque SF (2008). Effect of *Nigella sativa* oil on various clinical and biochemical parameters of insulin resistance syndrome. Int. J. Diab. Dev. Ctries. 28:11-14.
Perveen T, Haider S, Kanwal S, Haleem DJ (2009). "Repeated administration of *Nigella sativa* decreases 5-HT turnover and produces anxiolytic effects in rats," Pak. J. Pharm. Sci. 22(2):139-144.
Rajkapoor B, Anandan R, Jayakar B (1996). Anti-ulcer effect of *Nigella sativa* and Pongamia pannata in rats. Fitoterapia. 67:195-199.
Salama RB (1973). Sterols in the seed oil of *Nigella sativa*. Planta Med. 24(4):375-377.
Salem EM, Yar T, Bamosa AO, Al-Quorain A, Yasawy MI, Alsulaiman RM, Randhawa MA (2010). Comparative study of *Nigella sativa* and triple therapy in eradication of *Helicobacter Pylori* in patients with non-ulcer dyspepsia. Saudi J. Gastroenterol. 16:207-214.
Salem ML, Hossain MS (2000). Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. Int. J. Immunopharmacol. 22:729-740.
Salomi MJ, Nair SC, Panikkar KR (1991). Inhibitory effect of *Nigella sativa* and Saffron (Crocus sativus) on chemical carcinogenesis in mice. Nutr. Cancer. 16(1):67-72.
Salomi NJ, Nair SC, Jayawardhanan KK, Varghese CD, Panikkar KR (1992). Antitumour principles from *Nigella sativa* seeds. Cancer Lett. 63(1):41-46.
Staphylakis PK, Gegiou D (1986). The sterols of *Nigella sativa* seed oil. Phytochem. 25:761-763.
Tarek El-Naggar, Mar´ıa Pilar G´omez-Serranillos, OlgaMar´ıa P, Carmen A, Mar´ıa EC (2010). *Nigella sativa* L. Seed Extract Modulates the Neurotransmitter Amino Acids Release in Cultured Neurons *in Vitro*. J. Biomed. Biotechnol. 2010:398312.
Tembhurne SV, Firoj S, Jagtap AG (2008), Nephroprotective activity of *Nigella sativa* seeds against cisplatin induced Nephrotoxicity in Mice. Indian J. Nat. Prod. 24(1):13-16.
Topozada HH, Masloum H, El-Dakhakhany M (1965). The anti-bacterial properties of *Nigella sativa* seeds: Active principle with some clinical application. J. Egypt Med. Assoc. 48:187-202.
Worthen DR, Ghosheh OA, Crooks PA (1998). The *in vitro* anti-tumour activity of some crude and purified components of black-seed, *Nigella sativa* L. Anticancer Res. 18(3A):1527-1532.
Yar T, El-Hariri M, EL-Bahai MN, Bamosa AO (2008). Effects of *Nigella sativa* supplementation for one month on cardiac reserve in rats. Indian J. Physiol. Pharmacol. 52(2):141-148.
Zaoui A, Cherrah Y, Aloui K, Mahassine N, Amarouch H, Hassar M (2002). Effect of *Nigella sativa* fixed oil on blood homeostasis in rat. J. Ethnopharmacol. 79(1):23-26.
Zaoui A, Cherrah Y, Mahassine N, Alaoui K, Hassar M (2002). Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomed. 9:69-74.
Zhou Y, Shen K, Hou S, Qiu M, Luo Z (2003). Experimental study on apoptosis induced by elemene in glioma cells. Ai Zheng. Chin. J. Cancer 22(9):959-963.

academicJournals

Vol. 8(3), pp. 178-185, 17 January, 2014
DOI: 10.5897/JMPR11.1589
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Effects of aluminum toxicity on the growth and antioxidant status in *Jatropha curcas* seedlings

Chao Ou-yang[1], Shun Gao[2], Lan-ju Mei[1], Tsair-Wang Chung[2], Lin Tang[1], Sheng-hua Wang[1] and Fang Chen[1]*

[1]College of Life Sciences, Sichuan University, 610064, Chengdu, China.
[2]R&D Center for Membrane Technology, Chung-Yuan Christian University, Chungli 320, Taiwan.

Accepted 23 December, 2013

In the present study, the effects of aluminium (Al) concentrations on growth, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and phenylalanine ammonia-lyase (phenylalanine ammonia-lyase (PAL), EC 4.3.1.5) activities in *Jatropha curcas* L. seedlings were investigated. To carry out such investigations, *J. curcas* embryos were germinated and grown *in vitro* under Al concentrations of 0, 0.5, 1, 2 and 3 mM over a 7-day period. Biomass and the activities of antioxidant defense enzymes, such as SOD, POD, CAT and PAL in Jatropha curcas seedlings were observed. Results indicated that with the increasing Al concentrations, the biomass of cotyledons increased initially and then decreased but the biomass of hypocotyls and radicles decreased gradually. The SOD, POD, CAT and PAL activities in the cotyledons, hypocotyls and radicles were mainly increased, but the change trends were different.

Key words: Aluminium (Al) toxicity, *Jatropha curcas*, antioxidative, plant defense system.

INTRODUCTION

Aluminum (Al) is a light metal that makes up 7% of the earth's crust and is the third element. Al is a major component of the soil and most exists in a fixed status and has no hazard to plants. However, when soils become acidic as a result of natural processes or human activities, the fixed Al turns to soluble forms and the soluble Al could do harm to plants. About 40% of the world's arable soils are acidic and therefore present Al toxicity hazards (Uexküll and Mutert, 1995). Al toxicity has been considered to be a main limiting factor of crop productivity on acid soil (Foy et al., 1978; Uexküll and Mutert, 1995). The most distinct and earliest symptoms of Al toxicity in plants is the inhibition of root growth, which occurs within hours or even minutes of exposure to Al (Blamey et al., 2004; Dipierro et al., 2005; Kochian et al., 2005; Llugany et al., 1995; Ma, 2007; Ryan et al., 1993). The root meristem has been considered to be the primary site of Al accumulation and toxicity, suggesting that Al interacts with actively dividing and elongating cells (Delhaize and Ryan, 1995), but the mechanism of inhibition of root elongation is not yet well understood (Kopittke et al., 2008; Ryan et al., 1993). In fact, Al can interact with the root cell walls, apoplastic and/or symplastic constituents, disrupt the normal function of plasma membrane and plasma membrane transport system (Ahn et al., 2001; Blamey et al., 2004; Horst et al., 2010; Ishikawa and Wagatsuma, 1998; Jones and Kochian, 1997; Kopittke et al., 2008).

Some reports have shown that the common feature of several metal toxicity symptoms is the enhanced production of reactive oxygen species (ROS) and this results in oxidative stress (Cheng, 2003; Mithöfer et al., 2004; Valko et al., 2005). In order to alleviate oxidative damage, plants have developed comprehensive and integrated antioxidant enzyme and non-enzyme systems. The antioxidant enzyme systems include a series of

*Corresponding author. E-mail: chenfangscu@gmail.com.

enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), which, together with other enzymes, promote the scavenging of ROS (Alscher et al., 2002; Mittler et al., 2004; Veljovic-Jovanovic et al., 2006). If ROS is in excessive accumulation, it makes the antioxidant enzyme systems and non-enzyme systems of plant disorderly and leads to the oxidation of biomolecules (Boscolo et al., 2003) or even cell death (Cargnelutti et al., 2006).

Al stress, like other metal stress in plants, could lead to oxidative stress (Giannakoula et al., 2010; Schuch et al., 2010; Xu et al., 2011). In most cases, Al was considered to be toxic and had negative effects on plant development (Horst et al., 2010; Sun et al., 2007), but under some conditions, low concentrations can increase growth or produce other desirable effects. Plants that have shown positive growth to Al in nutrient cultures include sugar beet (Keser et al., 1975), tea shrub (Matsumoto et al., 1976), *Pinus radiate* D. Don (Huang and Bachelard, 1993), *Melastoma malabathricum* (Watanabe et al., 2005).

Jatropha curcas L. is a drought resistant shrub or tree belonging to the family Euphorbiaceae. Because of the high content of oil in the seeds, *Jatropha* has been investigated mainly as a potential source of oil and has been recognized as an adequate substitute for fossil oil (Debnath and Bisen, 2008). Before this study, there is little data about Al stress in *J. curcas* L. (de Macedo et al., 2011). Keeping in view the effects of aluminum toxicity on the growth and antioxidant status in *J. curcas* seedlings, the present study aimed to investigate the relationship between the concentrations of aluminum (Al) in *J. curcas* seedlings and the growth, as well as antioxidant enzymes.

MATERIALS AND METHODS

Plant materials and chemicals

Mature *J. curcas* seeds were collected in August, 2010 from more than 10 individual wild trees in Panzhihua, Sichuan province, China. Seeds were oven dried, selected and stored in a plastic box (Labeled, No. 20100822) and were deposited at 4°C until processing. Other reagents used were of reagent grade or higher.

Embryo germination and seedlings growth

J. curcas seeds were surface sterilized in 70% ethanol for 30 s, and then in 0.1% mercuric chloride for 8 min. Seeds were rinsed with distilled sterile water several times and soaked in sterile water for 24 to 36 h in a culture room. Each embryo was dissected from the seeds on a clean bench. Murashige and Skoog (MS) medium pH was adjusted to 5.8 ± 0.1 prior to autoclaving at 121 ± 2°C for 15 min, with 30 g/L sucrose and 6 g/L agar powder. Culture mediums (25 ml) were dispensed into Wide-neck bottles (100 ml), containing 0, 0.5, 1, 2 and 3 mM Al concentrations. Al was supplemented as $AlCl_3$. The embryos were placed for germination and growth in *in vitro* culture for 7 days. The cultures were incubated at 30 ± 2°C under a 12-h photoperiod in cool, white fluorescent light. When the cotyledons of seedlings had developed, cotyledons were washed with double distilled water, blotted and immediately frozen in liquid nitrogen or stored at -80°C for analysis. Three sets of seedlings were analyzed for each Al concentration, with 15 embryos per set.

Protein extraction

Protein extraction of fresh tissues was performed as previously described (Gao et al., 2010). The supernatant was used immediately or frozen and stored at -80°C for assaying of enzyme activity at a later date. Protein was quantified by the Lowry method using bovine serum albumin as standard.

Assay of antioxidant enzymes

POD activity was determined by the Sakharov and Ardila method (Sakharov and Ardila, 1999). One enzyme unit was defined as the amount of enzyme that produced a change of 1 absorbance per min at 470 nm. SOD activity was determined by measuring its ability to inhibit photochemical reduction of NBT (Chen and Pan, 1996). One unit of SOD was defined as the amount of enzyme that caused 50% inhibition of the photo-reduction of NBT under the assay condition. CAT activity was determined by the Montavon method (Montavon et al., 2007). One unit of CAT activity was defined as the amount of enzyme needed to reduce 1 µmol of H_2O_2 per minute. The activities were expressed as unit per gram fresh weight (U/g fw).

Polyacrylamide gel electrophoresis (PAGE)

Native gel electrophoresis for isoenzymes was carried out with 10% acrylamide gel. SOD isoenzyme activity was determined by the Beauchamp and Fridovich method (Beauchamp and Fridovich, 1971). Gels were equilibrated with 50 mM phosphate buffer (pH 7.5) containing 28 µM riboflavin, 28 mM *N*,*N*,*N*,*N*-tetramethyl ethylenediamine (TEMED) for 30 min, then washed in distilled water for 1 min and resubmerged in the same buffer containing 2.45 mM NBT for 10 to 20 min with gentle agitation in the presence of light. Enzyme bands appeared as colorless bands on a purple background. For the POD isoenzymes activity assay, the gel was soaked in deionized water for 5 min, and then incubated in 0.03% H_2O_2, 0.2% (w/v) benzidine and 0.1% (v/v) acetic acid for 3 to 5 min. When maximum contrast was achieved, the reaction was stopped by rinsing the gel with deionized water (Gao et al., 2009).

Assay of phenylalanine ammonia-lyase (PAL) activity

Enzyme extraction for the PAL activity assay was carried out as previously described (Gao et al., 2010). PAL activity was determined by assaying the reaction L-Phe decomposition product *trans*-cinnamate, as measured by the increase of absorbance at 290 nm (Hahlbrock and Ragg, 1975). One unit of enzyme activity was defined as the amount of enzyme needed to decrease in absorbance of 0.01 per min. PAL activity were expressed as unit per gram fresh weight (U/g fw).

Statistical analysis

All treatments were arranged in a completely randomized design with three replicates. All data were expressed as means ± standard deviation (SD). Statistical significance was evaluated with a Student's t-test, and differences were considered significant if P values were less than 0.05.

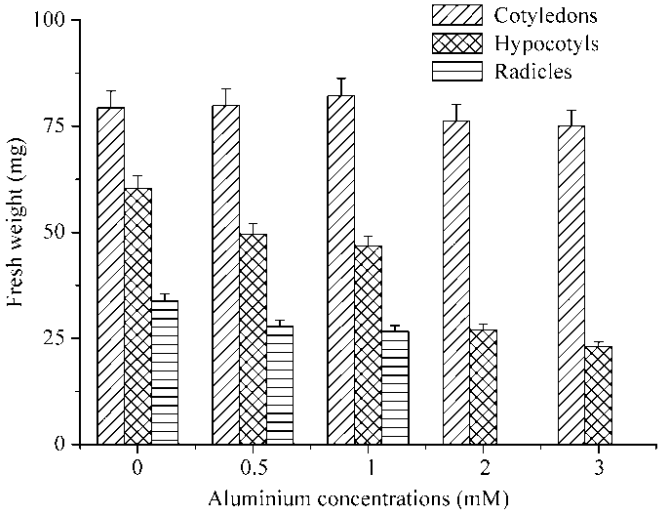

Figure 1. Effects of Al on the fresh weights in the cotyledons, hypocotyls and radicles of *J. curcas* seedlings grown in MS medium containing 0.5, 1, 2 and 3 mM Al. Values are the means ± SD (*n* = 3).

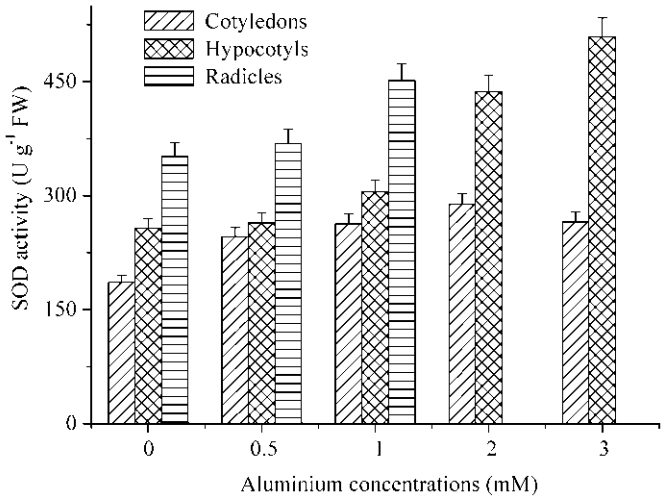

Figure 2. Effects of Al on superoxide dismutase (SOD) activity in the cotyledons, hypocotyls and radicles of *J. curcas* seedlings grown in MS medium containing 0.5, 1, 2 and 3 mM Al. Values are the means ± SD (*n* = 3).

RESULTS AND DISCUSSION

Effects of Al on plant growth

Figure 1 showed the changes of the fresh weights of cotyledons, hypocotyls and radicles in *J. curcas* seedlings. With the increasing of $AlCl_3$ concentration up to 1 mM, the fresh weight of cotyledons had a little increase. When $AlCl_3$ concentration was up to 2 and 3mM, the fresh weight of cotyledons, compared to the control, was slightly decreased and the changes were not significant. The fresh weight of hypocotyls decreased gradually with increasing Al concentration up to 3 mM and the fresh weight of radicles showed a similar trend, but when Al concentration was higher than 1 mM, the development of radicles was completely suppressed and could be observed, significant morphological aberrations included impaired radicles development, coarser hypocotyls and cotyledons chlorosis (data not shown). Al, at low concentrations, increased the fresh weight of cotyledons. This could not exclude the reason that low Al concentrations might have positive effect to cotyledons, as reported by other researchers (Ma, 2007; Watanabe et al., 2005). With the inhibition of Al toxicity on radicles development, it inhibited the absorption of nutrients and affected the photosynthesis, thereby suppressed the growth of hypocotyls and cotyledons. At the same time, high Al concentrations possibly enhance the ROS production, which led to the oxidative damage to plant cells and blocked the growth. On the base of these results, our findings suggested that high Al concentrations (> 1 mM) can inhibit the normal growth and development of *J. curcas* seedling.

Effects of Al on SOD activities

Al stress, like other abiotic and biotic stress, can induce oxidative stress reactions (Giannakoula et al., 2010). Effects of Al on SOD activity in *J. curcas* seedlings were shown in Figures 2 and 3. Compared to the control, SOD activity in the cotyledons, hypocotyls and radicles was all enhanced by Al stress. SOD activity in the hypocotyls increased significantly with increasing Al concentrations, and the maximal levels increased by 98.1% when Al concentration was 3 mM. In the cotyledons and radicles, the SOD activity increased by 55.1 % and 28.2 % at Al concentrations of 2 and 1 mM compared to the control, respectively. Many research have also indicated that Al could increase SOD activity in plants (Schuch et al., 2010; Du et al., 2010; Li et al., 2011) and this may be due to Al inducing the cell to initiate SOD synthesis to remove the superoxide radicals (Giannakoula et al., 2010). However, when the amount of free radicals exceeds cell's capacity, enzymatic activities start decreasing and if unchecked could ultimately lead to DNA damage (Meriga et al., 2004). The significant increase of SOD activity in our study may be induced by the increased production of ROS and can be a defensive mechanism developed by *J. curcas* seedling against stress. The pattern of SOD isoforms was analyzed by native PAGE, and activity staining revealed that at least four SOD isoenzyme bands in the cotyledons, hypocotyls and radicles were detected, respectively (Figure 3A to C).

The staining intensities of isoenzyme I and II in the cotyledons, hypocotyls and radicles were induced with increasing Al concentrations, but isoenzyme III and IV had virtually no change. The different expression of SOD genes may due to the subcellular location of the enzyme, the upstream sequences in the genomic sequences and

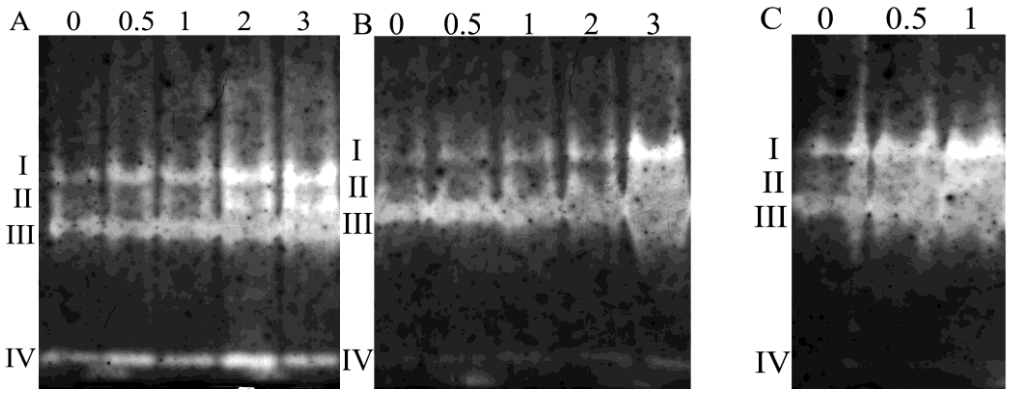

Figure 3. Patterns of SOD isoenzymes of cotyledons, hypocotyls and radicles in *J. curcas* seedlings. A: patterns of SOD isoenzymes in the cotyledons; B: patterns of SOD isoenzymes in the hypocotyls. C: patterns of SOD isoenzymes in the radicles. Lanes from left to right were 0, 0.5, 1, 2 and 3 mM, respectively.

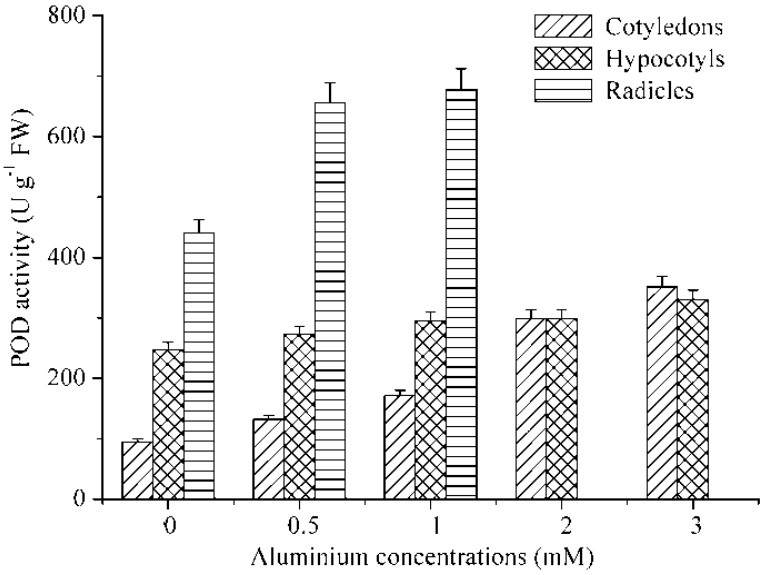

Figure 4. Effects of Al on peroxidase (POD) activity in the cotyledons, hypocotyls and radicles of *J. curcas* seedlings grown in MS medium containing 0.5, 1, 2 and 3 mM Al. Values are the means ± SD (n = 3).

the environmental stress (Mittler et al., 2004). The staining intensities of these isoenzymes showed a similar change compared to the changes of SOD activity assayed in solutions (Figures 2 and 3). With the increase of Al concentrations, the SOD activities were induced and this may promote the tolerance of *J. curcas* seedlings against Al stress.

Effects of Al on POD activities

Effects of Al on POD activity in the cotyledons, hypocotyls and radicles were shown in Figure 4. Al stress significantly affected the POD activity in the cotyledons with an increase of 270% when Al concentration was up to 3 mM. At the same time, POD activity in the hypocotyls and radicles was also induced, with the maximum increases of 33.4 and 37% compared to the control when Al concentration was 3 and 1 mM, respectively. As an important enzyme in plant defense system, POD can play an important role when plant is in adverse condition, and multiple POD isoforms have been found in many plant species (Passardi et al., 2005). The expression pattern of *J. curcas* seedling was shown in Figure 5. On the activity gels, at least six bands in the cotyledons, hypocotyls and radicles were observed. POD isoenzyme (II and III) in the

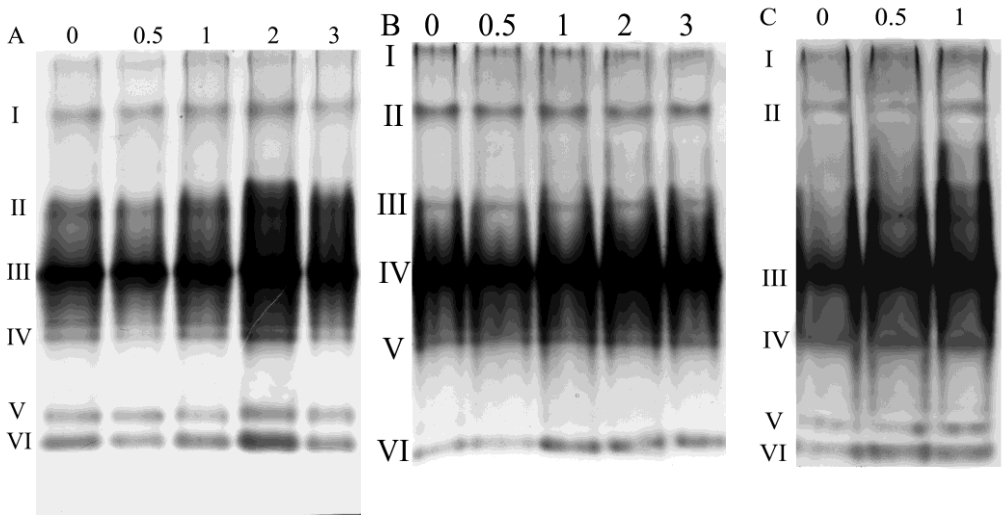

Figure 5. Patterns of POD isoenzymes of cotyledons, hypocotyls and radicles in *J. curcas* seedlings under Al stress condition. A: patterns of POD isoenzymes in the cotyledons; B: patterns of POD isoenzymes in the hypocotyls. C: patterns of POD isoenzymes in the radicles. Lanes from left to right were 0, 0.5, 1, 2 and 3 mM, respectively.

cotyledons showed an increase in the staining intensities with the increasing of Al concentration. In the hypocotyls and radicles, the main increase in the staining intensities was isoenzyme IV and III, respectively.

According to Figure 4 and 5, the changes of total staining intensities on activity gels and POD activity assayed in solutions were similar. In Al stress response, POD isozymes might consist in scavenging the toxic lipid hydroperoxides generated by the peroxidation of membrane lipids and they could participate in lignin biosynthesis to build up the physical barrier against toxic metals entering the cell (Ezaki et al., 1996; Hegedüs et al., 2001). Our findings suggested that POD, together with SOD and CAT, can increase their activities when *J. curcas* seedlings were exposed to Al stress, and they could scavenge ROS and reduce the damage caused by Al stress.

Effects of Al on CAT activities

CATs and PODs are the two major systems for the enzymic removal of hydrogen peroxide (H_2O_2) in plants, and CATs have mainly been associated with the removal of H_2O_2 in peroxisomes (Willekens et al., 1995). The changes of CAT activities in *J. curcas* seedlings exposed to Al stress were shown in Figure 6. Compared to control, CAT activities in hypocotyls and radicles were all increased, with the maximum increases of 46.4 and 10.7% when Al concentration was 2 and 1 mM, respectively. In cotyledons, CAT activities were increased first and then decreased with the increasing Al concentration. When the Al concentration was 0.5 mM, the CAT activity in cotyledons was the highest with increase by 58% but when concentration increased to 3 mM, the activity was lower than control. The CAT activity in the hypocotyls and radicles was very low; this might indicate that H_2O_2 degradation occurred due to POD rather than CAT. Similar results have already been observed in maize exposed to Al stress (Boscolo et al., 2003).

According to Figures 4 and 6, at low Al concentration, the removal of H_2O_2 in the cotyledons was mainly due to CAT rather than POD and at high Al concentration, this situation was just opposite. The decrease in CAT activity at highly Al stressed seedlings might be due to inhibition of enzyme synthesis or due to a change in the assembly of enzyme subunits under such conditions (Sharma and Dubey, 2007). Our findings suggested, at least here, that CAT appeared not to be an effective ROS-scavenger exposed to Al toxicity.

Effects of Al on PAL activities

PAL, a key enzyme involved in the metabolism of phenolics and lignification of cell walls, was mainly involved in defense mechanisms (Kovacik and Backor, 2007). Effect of Al on PAL activity in the cotyledons, hypocotyls and radicles are shown in Figure 7. Compared to the control, the PAL activities were all increased but the change trends were different. In the cotyledons and radicles, PAL activities were increased first and then decreased with the increasing Al concentration, and when exposed to 0.5 mM Al, the activity was the highest which increased by 37.2 and 23.4%, respectively. In the hypocotyls, the maximum activity was observed at 3 mM Al and the increase was 149.9%. Similar changes were also observed in the previous studies (Kovacik and

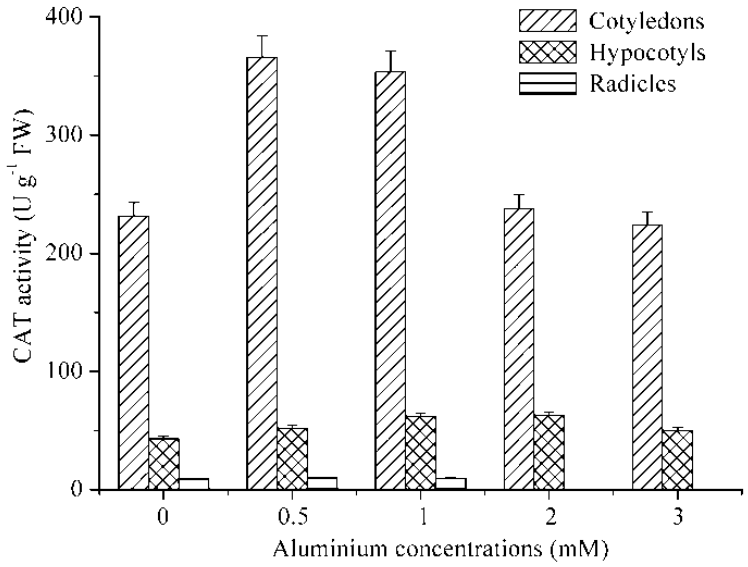

Figure 6. Effects of Al on catalase (CAT) activity in the cotyledons, hypocotyls and radicles of *J. curcas* seedlings grown in MS medium containing 0.5, 1, 2 and 3 mM Al. Values are the means ± SD (*n* = 3).

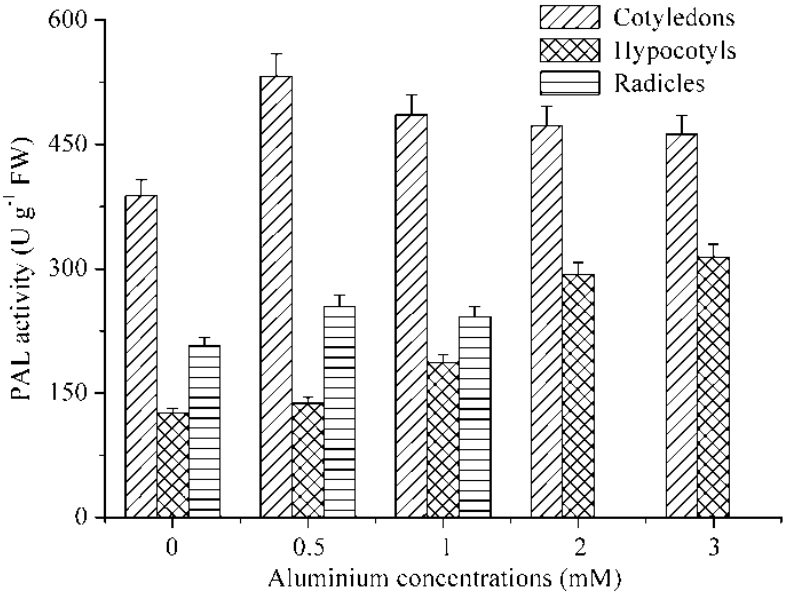

Figure 7. Effects of Al on phenylalanine ammonia-lyase (PAL) activity in the cotyledons, hypocotyls and radicles of *Jatropha curcas* seedlings grown in MS medium containing 0.5, 1, 2 and 3 mM Al. Values are the means ± SD (*n* = 3).

Backor, 2007; Dai et al., 2006). Some researchers indicated that PAL enhancement in the environmental stressed conditions is due to H_2O_2 generation which occurs as primary reaction in response to stress (Dorey et al., 1999). So, our results suggested that increased PAL activities may be related to *J. curcas* seedlings response to Al stress.

Conclusion

The changes of SOD, POD, CAT and PAL activity were studied when *J. curcas* seedlings were exposed to different Al concentration and the expression patterns of SOD and POD were also shown based on *in vitro* embryo germination and culture. The results in this study showed

that the increases of SOD, POD, CAT and PAL activity might be an important part of *J. curcas* seedlings resistance mechanisms to Al stress and the synergistic effects might help to reduce the accumulation of ROS and the oxidative damage. This research might provide some evidences for further study into the response mechanisms of *J. curcas* to Al stress.

ACKNOWLEDGEMENTS

This work was supported by grants from a grant from “Eleventh Five Years” Key Program of the State Science and Technology Commission of China (General Program, 2007BAD50B05) and the Key Project of Chinese Ministry of Education (General Program, 307023).

REFERENCES

Ahn SJ, Sivaguru M, Osawa H, Chung GC, Matsumoto H (2001). Aluminum inhibits the H^+-ATPase activity by permanently altering the plasma membrane surface potentials in squash roots. Plant Physiol. 126:1381–1390.

Alscher RG, Erturk N, Heath LS (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 53:1331–1341.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276–287.

Blamey FPC, Nishizawa NK, Yoshimura E (2004). Timing, magnitude, and location of initial soluble aluminum injuries to mungbean roots. Soil Sci. Plant Nutr. 50:67–76.

Boscolo PRS, Menossi M, Jorge RA (2003). Aluminum-induced oxidative stress in maize. Phytochemistry 62:181–189.

Cargnelutti D, Tabaldi LA, Spanevello RM, Jucoski GO, Battisti V, Redin M, Linares CEB., Dressler VL, Flores EMM, Nicoloso FT (2006). Mercury toxicity induces oxidative stress in growing cucumber seedlings. Chemosphere 65:999–1006.

Chen CN, Pan SM (1996). Assay of superoxide dismutase activity by combining electrophoresis and densitometry. Bot. Bull. Acad. Sin. 37:107–111.

Cheng S (2003). Effects of heavy metals on plants and resistance mechanisms. Environ. Sci. Pollut. Res. 10:256–264.

Dai LP, Xiong ZT, Huang Y, Li MJ (2006). Cadmium-induced changes in pigments, total phenolics, and phenylalanine ammonia-lyase activity in fronds of *Azolla imbricata*. Environ. Toxicol. 21:505–512.

de Macedo FL, Pedra WN, *da* Silva SA, Barreto MCD, Silva-Mann R (2011). Effect of aluminum in plants of *Jatropha curcas* L. grown in nutritive solution. Semin.-Cienc. Agrar. 32:157–163.

Debnath M, Bisen PS (2008). *Jatropha curcas* L., a multipurpose stress resistant plant with a potential for ethnomedicine and renewable energy. Curr. Pharm. Biotechnol. 9:288–306.

Delhaize E, Ryan PR (1995). Aluminum toxicity and tolerance in plants. Plant Physiol. 107:315–321.

Dipierro N, Mondelli D, Paciolla C, Brunetti G., Dipierro S (2005). Changes in the ascorbate system in the response of pumpkin (*Cucurbita pepo* L.) roots to aluminium stress. J. Plant Physiol. 162:529–536.

Dorey S, Kopp M, Geoffroy P, Fritig B, Kauffmann S (1999). Hydrogen peroxide from the oxidative burst is neither necessary nor sufficient for hypersensitive cell death induction, phenylalanine ammonia lyase stimulation, salicylic acid accumulation, or scopoletin consumption in cultured tobacco cells treated with elicitin. Plant Physiol. 121:163–171.

Du B, Nian H, Zhang Z, Yang C (2010). Effects of aluminum on superoxide dismutase and peroxidase activities, and lipid peroxidation in the roots and calluses of soybeans differing in aluminum tolerance. Acta Physiol. Plant 32:883–890.

Ezaki B, TsugUa S. Matsumoto H (1996). Expression of a moderately anionic peroxidase is induced by aluminum treatment in tobacco cells: possible involvement of peroxidase isozymes in aluminum ion stress. Physiol. Plant 96:21–28.

Foy CD, Chaney RL, White MC (1978). The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol., 29: 511–566.

Gao S, Li Q, Ou-Yang C, Chen L, Wang S, Chen F (2009). Lead toxicity induced antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. radicles. Fresenius Environ. Bull. 5:811–815.

Gao S, Ou-yang C, Tang L, Zhu J, Xu Y, Wang S, Chen F (2010). Growth and antioxidant responses in *Jatropha curcas* seedling exposed to mercury toxicity. J. Hazard. Mater. 182:591–597.

Giannakoula A, Moustakas M, Syros T, Yupsanis T (2010). Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. Environ. Exp. Bot. 67:487–494.

Hahlbrock K, Ragg H (1975). Light-induced changes of enzyme activities in parsley cell suspension cultures: Effects of inhibitors of RNA and protein synthesis. Arch. Biochem. Biophys. 166:41–46.

Hegedüs A, Erdei S, Horváth G (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci. 160:1085–1093.

Horst WJ, Wang Y, Eticha D (2010). The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Ann. Bot., 106:185–197.

Huang J, Bachelard EP (1993). Effects of aluminium on growth and cation uptake in seedlings of *Eucalyptus mannifera* and *Pinus radiata*. Plant Soil 149:121–127.

Ishikawa S, Wagatsuma T (1998). Plasma membrane permeability of root-tip cells following temporary exposure to Al ions is a rapid measure of Al tolerance among plant species. Plant Cell Physiol. 39:516–525.

Jones DL, Kochian LV (1997). Aluminum interaction with plasma membrane lipids and enzyme metal binding sites and its potential role in Al cytotoxicity. FEBS Lett. 400:51–57.

Keser M, Neubauer BF, Hutchinson FE (1975). Influence of aluminum ions on developmental morphology of sugarbeet roots. Agronomy J. 67:84–88.

Kochian LV, Pineros MA, Hoekenga OA (2005). The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274:175–195.

Kopittke PM, Blamey FPC, Menzies NW (2008). Toxicities of soluble Al, Cu, and La include ruptures to rhizodermal and root cortical cells of cowpea. Plant Soil 303:217–227.

Kovacik J, Backor M (2007). Phenylalanine ammonia-lyase and phenolic compounds in chamomile tolerance to cadmium and copper excess. Water Air Soil Pollut. 185:185–193.

Li C, Xu H, Xu J, Chun X, Ni D (2011). Effects of aluminium on ultrastructure and antioxidant activity in leaves of tea plant. Acta Physiol. Plant 33:973–978.

Llugany M, Poschenrieder C, Barceló J (1995). Monitoring of aluminium-induced inhibition of root elongation in four maize cultivars differing in tolerance to aluminium and proton toxicity. Physiol. Plant 93:265–271.

Ma JF (2007). Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants. Int. Rev. Cytol. 264:225–252.

Matsumoto H, Hirasawa E, Morimura S, Takahashi E (1976). Localization of aluminium in tea leaves. Plant Cell Physiol. 17:627–631.

Meriga B, Krishna Reddy B, Rajender Rao K, Ananda Reddy L, Kavi Kishor PB (2004). Aluminium-induced production of oxygen radicals,lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). J. Plant Physiol. 161:63–68.

Mithöfer A, Schulze B, Boland W (2004). Biotic and heavy metal stress response in plants: evidence for common signals. FEBS Lett. 566:1–5.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004). Reactive oxygen gene network of plants. Trends Plant Sci. 9:490–498.

Montavon P, Kukic KR, Bortlik K (2007). A simple method to measure effective catalase activities: optimization, validation, and application

in green coffee. Anal. Biochem. 360:207–215.
Passardi F, Cosio C, Penel C, Dunand C (2005). Peroxidases have more functions than a Swiss army knife. Plant Cell Rep. 24:255–265.
Ryan PR, Ditomaso JM, Kochian LV (1993). Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44:437–446.
Sakharov IY, Ardila GB (1999). Variation of peroxidase activity in cacao beans during their ripening, fermentation and drying. Food Chem. 65:51–54.
Schuch MW, Cellini A, Masia A, Marino G (2010). Aluminium-induced effects on growth, morphogenesis and oxidative stress reactions in *in vitro* cultures of quince. Sci. Hort. 125:151–158.
Sharma P, Dubey R (2007). Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. Plant Cell Rep. 26:2027–2038.
Sun P, Tian QY, Zhao MG, Dai XY, Huang JH, Li LH, Zhang WH (2007). Aluminum-induced ethylene production is associated with inhibition of root elongation in *Lotus japonicus* L. Plant Cell Physiol. 48:1229–1235.
Uexküll HR, Mutert E (1995). Global extent, development and economic impact of acid soils. Plant Soil 171:1–15.
Valko M, Morris H, Cronin MTD (2005). Metals, toxicity and oxidative stress. Curr. Med. Chem. 12:1161–1208.
Veljovic-Jovanovic S, Kukavica B, Stevanovic B, Navari-Izzo F (2006). Senescence- and drought-related changes in peroxidase and superoxide dismutase isoforms in leaves of *Ramonda serbica*. J. Exp. Bot. 57:1759–1768.
Watanabe T, Jansen S, Osaki M (2005). The beneficial effect of aluminium and the role of citrate in Al accumulation in *Melastoma malabathricum*. New Phytol. 165:773–780.
Willekens H, Inze D, Van Montagu M, Van Camp W (1995). Catalases in plants. Mol. Breed. 1:207–228.
Xu FJ, Jin CW, Liu WJ, Zhang YS, Lin XY (2011). Pretreatment with H_2O_2 alleviates aluminum-induced oxidative stress in wheat seedlings. J. Integrat. Plant Biol. 53:44–53.

academicJournals

Vol. 8(3), pp. 186-194, 17 January, 2014
DOI: 10.5897/JMPR12.1298
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro antibacterial activity and phytochemical analysis of some medicinal plants

Mohammadi Abolfazl[1], Amrollahi Hadi[2], Malek Frhad[3] and Nazari Hossein[1]*

[1]Department of Biochemistry, Faculty of Medicine, Semnan University of Medical Science, Semnan, Iran.
[2]Department of Microbiology, Faculty of Medicine, Semnan University of Medical Science, Semnan, Iran.
[3]Department of Internal Medicine, Faculty of Medicine, Semnan University of Medical Science, Semnan, Iran.

Accepted 19 November, 2013

Essential oils of medicinal plants have been used traditionally against pathogenic bacteria that caused infectious disease in human and microbial spoilage of food and have been used safely in herbal medicine as antibacterial compounds. In the present study, the antibacterial activities of the oils were evaluated against human and animals pathogenic bacteria. In this assay, the selective plants reported ethnobotanical uses traditionally and also were referenced in some herbal medicine text. The essential oil of *Stachys pubescens*, *Mentha piperita, Clinopodium vulgare* and *Satureja hortensis* were prepared by hydrodistillation and were analyzed by gas chromatography/mass spectrometry (GC/MS). The number 23, 22, 21 and 21 components were identified in *S. pubescens, S. hortensis*, *M. piperita* and *C. vulgare*, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oils were determined with broth microdilution and agar diffusion method on bacterial strains. Results from the antibacterial testing indicated that *S. pubescens, M. piperita* and *C. vulgare* essential oils showed high activities and inhibited the growth of all the selected bacteria. While the essential oil of *S. hortensis* displayed the moderate potential activity. Our finding supported the notion that plant essential oils composition or total extract may have a role as pharmaceuticals and preservatives effects as safely and effective drugs with low resistance against microorganisms. Therefore, these essential oils could be used for management of these pathogens as a potential source of sustainable eco-friendly botanical bactericides.

Key words: Antibacterial, *Stachys pubescens*, *Mentha piperita*, *Clinopodium vulgare*, *Satureja hortensis*, gas chromatography/mass spectrometry (GC/MS).

INTRODUCTION

Herbal medicine has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources as a good choice, because these natural resources have ordinarily fewer side effects (Zargari, 1996). The medicinal plants have been proved effective in the treatment of infectious diseases and simultaneously decrease many of the side effects (Iwu et al., 1999). Also, they are costless and effective against a broad spectrum of antibiotic resistant microorganisms and they have very potent natural biologically active agents (Nychas et al., 2003). In many parts of the world, the extracts and essential oil of medicinal plants with active biological compounds are used for their antimicrobial and antiviral properties (Hassawi and Kharma, 2006) that have been used in folk medicine. The increasing occurrence of

*Corresponding author. E-mail: hossen253@yahoo.co.uk. Tel: +98 -231–3353170–3. Fax: +98-231-3354161.

antimicrobial resistance represents a worldwide major concern for both human and veterinary medicine (Lorian, 1996). For this reason, there is a growing interest in the antimicrobial screening of extracts and essential oils from plants in order to discover new antimicrobial agents. Nowadays, about 25% of the drugs prescribed worldwide come from plants and 252 of them are considered as basic and essential by the World Health Organization (WHO). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs in developing countries. Infectious diseases are the second leading cause of death worldwide (Fazly-Bazzaz et al., 2005). From the time of the ancient Iranian, the plants were considered to protect against diseases. Iran has a very honorable past in traditional medicine, which goes back to the time of Babylonian, Assyrian civilization. One of the most significant ancient heritages is sophisticated experience of people who have tried over millennia to find useful plants for health improvement, with each generation adding its own experience to this tradition (Naghibi et al., 2005). Based on literature search, 18% of the plant species are used for medicinal purposes in Iran. Treatment of infections continues to be a problem in modern time, because of side effects of some drugs and growing resistance to antimicrobial agents. To investigate for novel, safer and more potent antimicrobials is a pressing need. Herbal medicines have received much attention as a source of new antibacterial with low side effect and significant activity (Fazly-Bazzaz et al., 2005). In the present study, the biological activities of four plants: *Stachys pubescens, Mentha piperita, Satureja hortensis* and *Clinopodium vulgare* were evaluated. Nowadays, there is a considerable research interest towards the compositional analysis of essential oil and extract. It has been reported that essential oil yield and their components in plants is related to genetic (Mohammed and Al-Bayati, 2009), climate, elevation, topography (Pourohit and Vyas, 2004; Rahimmalek et al., 2009a) and genotype (G), growing conditions (E) and their interaction (G × E) (Basu et al., 2009; Shafie et al., 2009). Previous studies have shown that these selected plant species have potential medicinal activity (Iscan et al., 2002; Mimica-Dukić et al., 2003; Saeed and Tariq, 2005; Andoğan et al., 2002; Mathur et al., 2011; Hammer et al., 1999; Adam et al., 1998; Sahin et al., 2003; Gulluce et al., 2003; Razzaghi-Abyaneh et al., 2008; Adinguzel et al., 2007; Boyraz and Özcan, 2006; Dikbas et al., 2009; Azaz et al., 2005; Chorianopoulos et al., 2004; Mihajilov-Krstev et al., 2010; Mihajilov-Krstev et al., 2009; Karami-Osboo et al., 2010). The antibacterial activity of essential oil of *S. pubescens* and *C. vulgare* has never been evaluated and was carried out for the first time in this study. The widespread use of antibiotics both inside and outside medicine is playing a significant role in the emergence of bacterial resistant (Goossens et al., 2005). Although, there were low levels of preexisting antibiotic resistant bacteria before the widespread use of antibiotics; evolutionary pressure from their use has played a role in the development of multidrug resistance varieties and the spread of resistance between bacterial species (Hawkey and Jones, 2009). Biological cost or metabolic price is a measure of the increased energy metabolism required to achieve a function. Drug resistance has a high metabolic price (Steven and Timothy, 2010) in pathogens for which this concept is relevant (Wichelhaus et al., 2002). Although several strategies have been proposed to overcome and control this situation. However, a clear solution has not yet been elucidating due to the antibiotic resistance, consequences, and side effects of antimicrobial drugs. Many plants are used in Iran in the form of oils and crude extracts, infusion or plaster to treat common infections without any scientific evidence of efficacy. Pharmacological studies carried out on essential oils of some aromatic plants' species that were obtained in central regions of Iran, have shown antimicribial activity which is coherent with the use of these plants in folk medicine. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. In the present study, three medicinal plants were selected which are widely used in the folk medicine in our region. All the plants have been used in the treatment of infectious diseases with different geographical area (Rechinger, 1982a, b; Chevallier, 1996). The aim of this study was to evaluate the antibacterial potential of the essential oils derived from *S. pubescens*, *M. piperita, S. hortensis* and *C. vulgare* that grows in the wild in the central part of Iran against standard strains. The selected strains: *Staphylococcus aureus* (PTCC:1431), *Listeria monocytogenes* (PTCC:1163), *Streptococcus pneumoniae* (PTCC:1240), *Pseudomonas aeruginosa* (PTCC:1430), *Klebsiella pneumoniae* (PTCC:1053*), Escherichia coli* (PTCC:1329) and *Salmonella typhi* (PTCC:1609) were purchased from Iranian Research Organization for Science and Technology (IROST). The antimicrobial potential was performed by disc diffusion (DD) and broth microdilution method (BMD) to determine the minimum inhibitory concentration (MICs) and maximum bactericidal concentration (MBCs).

MATERIALS AND METHODS

Collection of plant and essential oil extraction

The plants were collected from their wild habitat in Semnan city in the central part of Iran between April and June, 2011 which are shown as geographical and environmental conditions in Table 1. Plants were identified by experts of the University of Applied Science and Technology (UAST) Education Center in Semnan branch. The leaves of *S. pubescens, M. piperita*, *S. hortensis* and *C. vulgare* in full flowering stage were collected to determine antibacterial activity. A voucher specimen for each plant has been deposited in the herbarium of Medicinal Plants Research, UAST.

Table 1. Geographical and environmental conditions.

S/N	Plant	Region	Altitude (m asl[1])	Latitude	Longitude
1	Mentha *piperita*	Garmsar,North Eyvanakey	2100	35.432670	53.256050
2	*Satureja hortensis*	Shahrood Mayamey Bekran	2150	36.43520	54.376050
3	*Clinopodium vulgare*	Semnan Fullad mahaleh	2650	35.78527	53.32405
4	*Stachys pubescens*	Shahrood, Semnan	2315	36.32415	54.35316

Air-drying of plant material was performed in a shady place at room temperature for 4 days. Ground and dried leaves of plants (100 g) were subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis

The essential oils were analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. A fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) was used for the separation. Helium was used as a carrier gas at a flow rate of 1 ml/min. The oven temperature was programmed at 60°C (4 min), and then rising to 300°C at 4°C min^{-1}. The injector and detector temperature were kept at 250 and 300°C, respectively. The mass spectrometer was operated in electron-impact ionization (EI) mode with 70 eV energy with MS transfer line at temperature of 300°C was used. Ion source and interface temperatures were 200 and 250°C, respectively. The split ratio was 1:50. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250°C. The column was programmed as follows: 60°C for 2 min and then increased by 3°C min^{-1} up to 300°C. Volume of injected samples was 0.5 µl. Identification of components was based on the comparison of retention times (RT) and the computer mass spectra libraries using Wiley 275 GC/MS Library (Wiley, New York), those found in the literature (Adams, 2001; McLafferty, 1993) and the mass spectrometry data bank (NIST). The percentage composition of the essential oil was computed by the normalization method from the GC peak areas measurements (Table 4).

Microorganisms, inoculums and antibacterial assay

Bacterial strain

In the present study, a total of 7 standard isolates were obtained from IROST in 2011. Bacterial strains used in this study were four Gram-negative bacteria: *P. aeruginosa* (PTCC: 1430), *K. pneumoniae* (PTCC: 1053), *E. coli* (PTCC: 1329), *S. typhi* (PTCC: 1609) and three Gram-positive bacteria: *S. aureus* (PTCC: 1431), *L. monocytogenes* (PTCC: 1163), *S. pneumonia*e (PTCC: 1240), that were grown in Müeller–Hinton (MH) agar (Oxoid) and incubated for 24 h at 37°C. Cultures were used for making bacterial suspensions, and turbidity was adjusted to 0.5 McFarland and confirmed using a spectrophotometer (UV-VIS 1650, Shimatzu, Japan).

Preparation of inoculums

Bacterial strains were prepared by suspending one isolated colony from MH agar plates in 5 ml of MH broth and overnight broth cultures. The suspensions were adjusted in 0.5 McFarland standard turbidity to obtain final inoculums of 5×10^5 to 5×10^6 CFU/ml after 24 h of growth at 37°C and confirmed using a spectrophotometer. The essential oils were dissolved in dimethyl sulfoxide (DMSO, 25 mg/ml) and diluted to MH broth for antibacterial tested. All strains were tested by BMD and disk diffusion (DD) techniques according to the National Committee for Clinical Laboratory Standards (NCCLS, 2003a, b).

Serial dilution method

MICs and MBCs of essential oils were determined by using BMD method as described by NCCLS (2003a) in flat-bottomed 96-well clear plastic tissue-cultured plates (Greiner, 650161). The MIC was assayed using two-fold BMD method in MH Broth in 96-well plates. Plates contained two-fold dilutions of antibacterial agents at the concentration ranges: 0.5 to 64 µg/ml (25%, v/v). These dilutions were used to dispense 100 µl into each of the sterile 96-wells and an equal volume of bacterial inoculums was added to each well on the microtiter plate. After incubation for 24 h at 37 °C, the micro-dilution trays were checked with unaided eyes to detect the growth inhibition of the bacteria, and then the MICs were determined with spectrophotometer. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The final concentration of DMSO in the assays did not interfere with the bacterial proliferation which is used as a control. Negative controls were prepared with non-inoculated medium with oils, and one non-inoculated well, without antimicrobial agents, was also included to ensure medium sterility. The commercial antimicrobials Ciprofloxacin (Sigma) and Gentamicin (Merk) were included as positive controls. One inoculated well was included to allow control of the broth suitability for organism growth. To determine the MBCs, the suspensions (20 µl) were taken from each well without visible growth and inoculated in MH agar for 24 h at 37°C. The MBC was defined as the lowest concentration of the essential oil at which incubated micro-organisms are completely killed. Tests were performed in triplicate for each test concentration ($P > 0.05$).

Disc diffusion method

Agar diffusion method was carried out for the assessment of the essences antibacterial activity as recommended by NCCLS (2003b). The potential activity of oils were confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition zone (IZ) compared to known standard antibiotics. Essential oils were diluted in DMSO to different concentrations (0.5, 1, 2, 4, 8, 16, 32 and 64 µg/ml). 50 µl of standardized inoculums according to 0.5 McFarland turbidity standard solutions (10^5 to 10^6 CFU/ml) of the selected strains were spread onto the surface of Mueller Hinton (MH) agar and kept for 2 h at 4°C for absorption. Sterilized paper discs (Whatman, 6 mm diameter) containing approximately 20 µl of the essential oils were impregnated with different amount of essential oils. The prepared discs of the oils and standard antibiotics were placed on the surface of MH agar media. The inoculated plates were incubated at 37°C for 24 h and the resulting

Table 2. MIC and MBC (µg/ml) values for different essential oils of plants.

Bacteria	Gentamicin		Ciprofloxacin		*M. piperita*		*C. vulgare*		*S. hortensis*		*S. pubescens*	
	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]
S.p	1	2	0.5	0.5	0.5	0.5	1	1	1	2	1	1
S.a	0.5	1	1	2	0.5	1	0.5	1	4	4	0.5	1
P.a	1	1	0.125	0.5	1	2	2	4	1	2	2	2
E.c	2	4	0.5	1	2	2	8	8	8	8	1	2
S.t	1	2	0.5	0.5	4	8	4	4	16	32	4	8
L.m	2	2	1	1	8	16	16	32	32	32	8	8
K.p	2	4	1	2	8	16	16	64	-	-	8	16

MIC=Minimum inhibitory concentration; MBC= minimum bactericidal concentration; "-" No growth inhibition. E.c=*Escherichia coli*, P.a= *Pseudomonas aeruginosa*, S.a=*Staphylococcus aureus*, S.t=*Salmonella typhi*, S.p=*Streptococcus pneumonia*, K.p=*Klebsiella pneumoniae*, L.m=*Listeria monocytogenes*.

Table 3. Antibacterial activity screening of antibacterial agents by zone of inhibition (mm diameter) in disc diffusion method.

Bacterial strains	D.D of NC	D.D of PC		*M. piperita*	*C. vulgare*	*S. hortensis*	*S. pubescens*
		G	C	$D.D_T$	$D.D_T$	$D.D_T$	$D.D_T$
**S. pneumonia*	-	18	25	27	25	21	26
***S. aureus*	-	24	28	25	27	18	25
P. aeruginosa	-	19	22	23	15	20	21
E. coli	-	14	16	17	10	13	20
****S. typhi*	-	17	21	16	19	11	13
L. monocytogenes	-	16	15	10	8	5	11
K. pneumoniae	-	15	18	8	7	-	8

D.D= Diameter of inhibition zone (mm) including of disc diameter of 6mm. T=tested at a concentration of 20 µg /disc. NC=Negative Control. PC=Positive Control (G=Gentamicin, C=Ciprofloxacin,). "-" No growth inhibition. *S=Streptococcus, **S=Staphylococcus, P=Pseudomonas, E=Escherichia, ***S=Salmonella, L=Listeria, K=Klebsiella.

zone of inhibition (diameter) was measured in millimeters by comparing the different concentrations of oils and the standard antibiotics. The MIC was defined as the lowest concentration, resulting in a clear zone of growth inhibition around the disc after incubation period. Gentamicine (Merk) and Ciprofloxacin (Sigma) discs were applied over the test plates as a positive control. Negative controls were prepared using the solvent to dissolve the essential oil solution. All experiments were performed in triplicate.

Statistical analysis

Comparison of data was performed using the one way analysis of variance (ANOVA) or the unpaired Student's t-test and is presented as mean ± standard deviation. Comparison of MIC and MBC values, tests were made in triplicate for quantification. Values of $p < 0.05$ were considered significant.

RESULTS

All essential oils showed effective antibacterial activities on the selected pathogenic bacteria. Antibacterial activities of essential oils were investigated by broth microdilution and the disc diffusion method. The MICs and MBCs and diameter of inhibition zone (DD) of the selected oils on the bacteria are shown in Tables 2 and 3. The results showed that essential oil of the plants were active against all the pathogenic bacteria species with different degree in the following range of concentrations: essential oil of *S. pubescens* and *M. piperita* had the best antibacterial effect and its MIC value was between 0.5 and 8 µg/ml. *C. vulgare* is the second degree with MIC values between 0.5 and 16 µg/ml. Otherwise, *S. hortensis* had a lowest antibacterial effect comparison to the earlier essences and its MIC value was 1 to 32 µg/ml. Ciprofloxacin and Gentamicin used as positive control as well as DMSO as a negative control which did not show any inhibition against the pathogens bacteria. MIC range of standard antibiotics "Ciprofloxacin and Gentamycin" were 0.5 to 1 µg/ml and 0.5 to 2 µg/ml, respectively. Even at low concentrations, the plant's species showed antibacterial activity more or nearly equal to the commercial bactericidal agents. All of the oils had the best inhibitory activities against *S. Pneumonia*, *S. aureus* and *P. aeruginosa*. The weakest activity was observed against *L. monocytogenes* and *K. pneumoniae* with the highest MIC and MBC, and *K. pneumoniae* was resistance against *S. hortensis*. The results of the chemical analyses using GC/MS of the essential oils were listed in Table 4. Number of indentified constituents

Table 4. Chemical analyses of essential oils.

S/N	*M. piperita*			*C. vulgare*			*S. hortensis*			*S. pubescens*		
	Component	**P.A**	**RT**	**Component**	**P.A (%)**	**RT**	**Component**	**P.A (%)**	**RT**	**Component**	**P.A (%)**	**RT**
1	α-Pinene	0.83	5.473	Terpinen-4-ol	4.84	5.479	α- Terpinene	0.58	6.760	β-Pinene	1.8	4.840
2	Sabinene	0.57	6.085	β-pinene	16.04	5.627	o-Cymene	16.91	6.880	1,4-Cyclohexadiene	0.4	5.412
3	β-Pinene	1.04	6.154	Sabinene	10.19	6.159	1,8-Cineole	0.93	7.018	Myrcene	0.9	6.124
4	dl-Limonene	3.09	6.944	Cymen-8-ol	2.24	6.972	γ-Terpinene	3.56	7.413	α- Terpinene	2.7	6.529
5	1,8-Cineole	5.12	7.001	Limonene	5.84	7.012	Terpineol	1.06	7.567	Benzene	0.9	6.790
6	Trans-abinene Hydrate	0.61	7.561	Terpinolene	1.52	7.212	2-Butoxyethy lacetate	1.69	7.853	Limonene	6.3	6.893
7	l-Menthone	18.68	8.923	1.8-cineole	6.76	7.319	Linalool	2.73	8.013	(*E*)-β-Ocimene	2.8	7.115
8	Menthofuran	25.70	9.066	β-(Z)-ocimene	3.61	7.364	Borneol	2.42	9.112	γ-terpinene	1.2	7.430
9	Menthol	23.17	9.198	γ- Terpinene	1.95	7.480	3-Cyclohexen -1-ol	0.64	9.267	3-Cyclohexen-1-ol	1.5	9.280
10	Cyclohexen	0.74	9.272	α-Copaene	3.27	8.114	α- Terpineol	1.06	9.455	Linalool	9.7	9.987
11	Cyclohexanol	0.43	9.358	linalool	2.26	8.049	Benzene, 1-methoxy-4-(2-propenyl)	0.64	9.541	2,6-Octadien	11.5	10.420
12	3-Cyclohexene-1-methanol	0.42	9.450	Camphor	1.72	9.112	1-Isopropyl	2.05	10.039	Octen-1-ol acetate	1.6	10.560
13	Pulegone	6.72	10.182	Pulegone	2.45	10.375	2,6-Octadien-1-ol	1.27	10.302	2,6-Octadienal	2.1	10.602
14	2-Cyclohexen-1-one	0.26	10.394	Thymol	5.32	10.743	Phenol	3.06	10.634	Linalyl acetate	1.2	10.742
15	3-Memthene	1.11	10.634	Camphene	0.48	11.802	Thymol	48.58	10.817	δ-Elemene	5.4	11.124
16	Camphane	7.40	10.897	Carvacrol	6.04	11.894	Carvacrol	5.19	11.526	β-Bourbonene	0.2	12.247
17	Cyclohexene	0.30	11.126	Isosativene	0.27	12.014	Nerol acetate	0.45	12.047	δ-Cadinene	19.7	13.905
18	trans-Caryophyllene	1.47	12.706	Caryophyllene	1.76	12.695	trans-Caryo phyllene	3.72	12.705	Naphthalene	1.2	13.926
19	Germacrene	1.16	13.478	Calarene	0.63	13.207	Naphthalene	1.05	13.867	β-Gurjunene	0.3	13.945
20	Mint furanone	0.47	13.690	γ-Cadinene	3.52	13.659	delta-Cadinene	0.69	13.947	Bicyclogermacrene	1.8	14.364
21	Hexahydrochrysene	0.67	14.308	Germacrene D	18.12	14.826	Nerolidol	0.90	14.342	Caryophyllene oxide	1.3	14.821
22	-	-	-	-	-	-	Caryophyllene oxide1	0.79	4.760	Spathulenol	0.8	14.834
23	-	-	-	-	-	-		-	-	Germacrene	22.4	15.248
Total	-	99.96	-	-	98.83	-	-	99.97	-	-	97.7	-

TR = Retention Time; PA = Peak Area

in *S. pubescens, S. hortensis*, *M. piperita* and *C. vulgare* were 23, 22, 21 and 21, respectively. Also, analysis of data with creditable library shows that, the main components of *S. pubescens* were: Germacrene (22.4%), δ-Cadinene (19.7%), 2,6-Octadien(11.5%), Linalool (9.7%); *M. piperita* were: Menthofuran (25.70%), Menthol (23.17%), Menthone (18.68%) and Camphane (7.40%), *C. vulgare* were: Germacrene D (18.12), β-pinene (16.04), Sabinene (10.19) and 1.8-cineole (6.76); and *S. hortensis* were Thymol (48.58%), o-Cymene (16.91%), Carvacrol(5.19), trans-Caryophyllene (3.72%) and γ-Terpinene (3.56%).

DISCUSSION

This study was attempted to purify the selected plant's oils that were native in our region in order to identify their essential oils as antibacterial properties. In addition, components of plants were determined and the result was compared with other studies. This is due to several reasons, namely, conventional medicine can have side effects, high coast, abusive or incorrect usage of synthetic drugs result in complications, and the large percentage of the world's population do not have access to conventional pharmacological treatment. The best antibacterial activities were seen in *M. piperita, S. pubescens* and *C. vulgare*,

while *S. hortensis* displayed a moderate response against bacterial species. In comparison to the standard drugs, these data showed *M. piperita* and *S. pubescens* had the highest activity; *C. vulgare* had the lower activity but with the lowest different, while the different properties of *S. hortensis* was more. The results confirmed the antibacterial potency of these plants. In other studies concerning the antimicrobial activity of these plants, inhibition effects of *M. piperita* on some microorganisms such as *S. paratyphi*, *Proteus mirabilis, Proteus vulgaris, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes*, *Lactobacillus acidophilus, Bacillus subtilis, Enterobacter aerogenes, Shigella dysenteriae* and *Yersinia enterocolitica* growth was studied and this plant showed the highest antimicrobial activity (Iscan et al., 2002; Mimica-Dukić et al., 2003; Saeed and Tariq, 2005; Mathur et al., 2011; Andogan et al., 2002). In previous studies, *S. hortensis* showed antimicrobial activity against some of the standard and clinical microorganisms (Iscan et al., 2002; Sahin et al., 2003; Gulluce et al., 2003; Adinguzel et al., 2007; Boyraz and Özcan, 2006; Azaz et al., 2005; Chorianopoulos et al., 2004; Mihajilov-Krstev et al., 2010; Mihajilov-Krstev et al., 2009; Karami-Osboo et al., 2010; Baser et al., 2004). Other study concerning the extract *of C. vulgare*, inhibited the growth of some bacterial species with different degrees (Opalchenova and Obreshkova, 1999). Our result with other studies confirmed that a variety of bacterial species are affected by essential oil of selected plants, especially about the essential oils of *S. pubescens* and *C. vulgare* evaluated for the first time. The aforementioned finding supports their traditional usage of these oils as antibiotic and antiseptic (Riley, 2005). Briefly, the results of this study showed that the essential oils of these plants have a very broad spectrum of antibacterial activities with notable MICs and MBCs, which are near or lower than dose synthetic drugs. These plants could safely be used as organic preservatives to replace synthetic antibiotics in the prevention and cure of some human and animal infectious disease as well as food industrial preservatives. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. In comparison to the other studies, the composition of the plants showed little difference. Similar to our result, previous studies on *M. piperita* showed that the main components of the oils and extracts were menthol, menthofuran, menthone and menthyl acetate (Iscan et al., 2002; Maffei et al., 1999; Soković et al., 2009; Rohloff, 1999). There are three numbers of these components in our main components, but in the other study in Iran, the main components were very different: α-terpinene, isomenthone, *trans*-carveol, pipertitinone oxide (Rasooli et al., 2006). Menthone and menthol has been reported to be responsible for the antimicrobial activity of *M. piperita* (Gupta and Saxena, 2010; Bassolé et al., 2010; Kizil et al., 2010). But, the antibacterial activity of menthofuran has not been determined. Since *M. piperita* showed the highest antibacterial activities, then it was suggested the menthol, menthofuran or menthone alone or mix together (synergic effect) play a major antibacterial role. Since, each of menthol, menthofuran or menthone alone do not have considerable amount in the total extract; therefore, its antibacterial activity also belongs to the mixture of the whole components. With attention to other, its components were less than 8%. But, a point of consideration is the study that showed the good antimicrobial activity of *M. piperita*, while there are any these three components in their chemical composition (Rasooli et al., 2006). Concerning the *S. pubescens*, there are a few studies which determined the chemical composition. Many studies have not been conducted so far; Iranian researcher reported (*Z*)-β-Ocimene, Germacrene D and Bicyclogermacrene as the main components (Baher and Mirza, 2006). Previous studies related to the chemical composition of *S. hortensis,* thymol, Carvacrol, γ-terpinene and p-cymene reported as main components which are similar to our result (Gulluce et al., 2003; Adinguzel et al., 2007; Azaz et al., 2005; Sefidkon et al., 2006). Since, in these studies, *S. hortensis* display the high antimicrobial activity, therefore, it is concluded that the main components like thymol and carvacrol have an antimicrobial activity. But, the important point in these studies confirmed the antimicrobial activity of *S. hortensis* (Gulluce et al., 2003; Adinguzel et al., 2007; Mihajilov-Krstev et al., 2009) which is the lack of thymol or carvacrol as part of the main compounds, and the amount of these components was inconsiderable against whole extract. Therefore, it can be said that the total extract of *S. hortensis* has better antimicrobial activity than pure components, because in this status, there is synergistic effect between components as was observed in this study. Concerning the *C. vulgare*, in the previous study, the constituents of sabinene, germacrene D, E-caryophyllene, (Z)-β-ocimene and γ-terpinene was reported as the main components (Nurzyn´ska-Wierdak, 2009). The main components of their plants were similar to our results, but in the other study the main components were different to our result, in that the study of germacrene-D, b-caryophyllene and b-caryophyllene oxide was reported as the main components (Kökdil, 1998). In the present study, the similarity of composition to other studies is low. Nonetheless, all of them have a good antimicrobial activity and this subject describes that the special compound cannot have the major antimicrobial potency and the antibacterial of this plant yielded the mixture of their components. Although, these plants are in the same family, but their main component is different, and this variety in biological activity is related to their composition. The two Gram positive: *S. aureus, S. pneumonia* and two Gram negative: *P. aeruginosa, E. coli* bacteria showed high sensitivity against the essential oils with the lowest MICs and MBCs. Some studies reported that these plants

inhibited both Gram positive and Gram negative bacteria (Dikbas et al., 2006; Hoferl et al., 2009). It was suggested that these differences in components could be due to the variety of the ecotype system reported by other scientists and references (Asbaghian et al., 2011). Since the essential oils are complex mixtures of several compounds, it is difficult to attribute their biological activity to a particular constituent. Usually, major compounds are the ones responsible for the antimicrobial activity of the essential oils. However, some studies showed that minor components may have a crucial role in the biological activity of the oils (Koroch et al., 2007). Further studies are needed to determine the antibacterial activities of the bioactive compounds responsible for the observed potential value. Natural plant-derived bactericidal may be a source of a new alternative active compounds. In attention to, in the present study, most isolates showed a less difference concentrations of essential oils between bacteriostatic and bactericidal values. Suggesting that the essential oils of the selected plants could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms and also in the search for novel antibacterial agents with the potential application of some major or minor constituents alone, mixture of the extract or in combination with antibiotics for the treatment and prevention of pathologies associated with multi resistant bacteria. However, the mechanism of inhibitory effects of these plant's oils against infectious bacteria is still unclear. Further investigations regarding the *in vitro* and *in vivo* should be conducted in order to clear mechanisms pathway and develop such products.

REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography /quadrupole mass spectroscopy, *third ed.* Allured Publishing Corporation: Carol Stream, Illinois, USA.

Adinguzel A, Ozer H, Kilic H, Ctin B (2007). Screening of antimicrobial activity of essential oil and methanol exteract of *Satureja hortensis* on foodborne bacteria and fungi. Czeck J Food Sci. 25: 81-89.

Andoğan BC, Baydar H, Selçuk K, Demirci M, Özbaşar D, Mumcu E (2002). Antimicrobial activity and chemical composition of some essential oils. Pharmacol. Res. 25:860-864.

Asbaghian S, Shafaghat A, Zarea K, Kasimov F, Salimi F (2011). Comparison of volatile constituents, and antioxidant and antibacterial activities of the essential oils of *Thymus caucasicus*, *Mentha piperita* and *Satureja hortensis*. Nat Prod. Commun. 6:137-140.

Azaz AD, Kürkcüoglu M, Satil F, Can BKH, Tümen G (2005). *In vitro* antimicrobial activity and chemical composition of some *Satureja* essential oils. Flavour Fragr. J. 20:587–591.

Baher NZ, Mirza M (2006). Chemical composition of the essential oils of *Stachys pubescens* Ten. Flavour Fragr. J. 21:757–759.

Baser KHC, Özek T, Kirimer N, Tümen G (2004). A Comparative Study of the Essential Oils of Wild and Cultivated *Satureja hortensis* L. J. Essent. Oil Res. 16:422-424.

Bassolé IHN, Lamien-Meda A, Bayala B, Fran STC, Novak J, Nebié RC, Dicko MH (2010). Composition and Antimicrobial Activities of *Lippia multiflora* Moldenke, *Mentha* x *piperita* L. and *Ocimum basilicum* L. Essential Oils and Their Major Monoterpene Alcohols Alone and in Combination. Molecules 15:7825-7839.

Basu S, Chaplin WJ, Elsworth Y, New R, Serenelli AM (2009). Fresh insights on the structure of the solar core. Astrophys. J. 699:1403-1417.

Boyraz N, Özcan M (2006). Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. Int. J. Food Microbiol. 107:238–242.

Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitaku S, Nychas GJ, Haroutounian SA (2004). Essential Oils of *Satureja*, *Origanum*, and *Thymus* Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens. J. Agric. Food Chem. 52:8261–8267.

Dikbas N, Kotan R, Dadasoglu F, Karagöz K, Çakmakc R (2009). Correlation between Major Constituents and Antibacterial Activities of Some Plant Essential Oils against Some Pathogenic Bacteria. Turk. J. Sci. Technol. 4:57-64.

Fazly-Bazzaz BS, Khajehkaramadin M, Shokooheizadeh HR (2005). *In vitro* antibacterial activity of *Rheum ribes* extract obtained from various plant parts against clinical isolates of Gram-negative pathogens. Iranian J. Pharmacol. Res. 2:87-91.

Goossens H, Ferech M, Vander Stichele R, Elseviers M (2005). Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet Group Esac Project 365:579-587.

Gulluce M, Sökmen M, Daferera D, Ağar G, Özkan H, Kartal N, Polissiou M, Sökmen A, Şahin F (2003). *In Vitro* Antibacterial, Antifungal, and Antioxidant Activities of the Essential Oil and Methanol Extracts of Herbal Parts and Callus Cultures of *Satureja hortensis* L. J Agric. Food Chem. 51:3958–3965.

Gupta N, Saxena G (2010). Antimicrobial activity of constituents identified in essential oils from Mentha and Cinnamomum through GC/MS. Int. J. Pharm. Biol. Sci. 1:715-720.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86:985–990.

Hassawi D, Kharma A (2006). Antimicrobial activity of some medicinal plants against *Candida albicans*. J. Biol. Sci. 6:109-114.

Hawkey PM, Jones AM (2009). The changing epidemiology of resistance. J. Antimicrob. Chemother. 64:3–10.

Hoferl M, Buchbauer G, Jirovetz L, Schmidt E, Stoyanova A, Denkova Z, Slavchev A, Geissler M (2009). Correlation of Antimicrobial Activities of Various Essential Oils and Their Main Aromatic Volatile Constituents. J. Essent. Oil Res. 21:459-463.

Iscan G , Kirimer N , Kürkcüoğlu M , Can BKH , Demirci F (2002). Antimicrobial Screening of *Mentha piperita* Essential Oils. J. Agric. Food Chem. 50:3943–3946.

Iwu MW, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin. In: J Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. pp 457–462.

Karami-Osboo R, Khodaverdi M, Ali-Akbari F (2010). Antibacterial Effect of Effective Compounds of *Satureja hortensis* and *Thymus vulgaris* Essential Oils against *Erwinia amylovora*. J. Agric. Sci. Technol. 12:35-45.

Kizil S, Tolan V, Kilinc E, Yuksel U (2010). Mineral cntent, essential oil components and biological activity of two Mentha species (*M. piperita* L., *M. spicata* L.). Turk. J. Field Crops 15:148-153.

Kökdil G (1998). Composition of the essential oil of Clinopodium vulgare L. ssp. arundanum (Boiss.) Nyman collected from two different localities in Turkey. Flavour Fragr. J. 13:170-172.

Koroch AR, Juliani HR, Zygadlo JA (2007). Bioactivity of essential oils and their components, R.G. Berger, Editor, Flavour. Fragr. Springer-Verlag, Berlin, Heidelberg. pp 87–115.

Lorian V (1996). Antibiotics in Laboratory Medicine, fourth ed. Williams and Wilkins, Baltimore.

Maffei M, Canova D, Bertea CM, Scannerini S (1999). UV-A effects on photomorphogenesis and essential-oil composition in *Mentha piperita*. J. Photochem. Photobiol. 52:105–110.

Mathur A, Prasad, GBKS, Rao N, Babu P, Dua VK (2011). Isolation and identification of antimicribial compound from *Mentha pipirita*. Rasayan J. 4:36-42.

McLafferty FW (1993). Registry of Mass Spectral Data. Wiley, New York.

Mihajilov-Krstev T, Radnović D, Kitić D, Zlatković B, Ristić M, Branković S (2009). Chemical composition and antimicrobial activity of *Satureja hortensis* L. essential oil. Cent. Eur. J. Biol. 4:411-416.

Mihajilov-Krstev T, Radnovic D, Dusanka K, Stojanovic-Radic Z, Zlatkovic B (2010). Antimicrobial activity of *Satureja hortensis* L. essential oil against patho*Genic*. Arch. microbial Biol. Sci. 62:159-166.

Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M (2003). Antimicrobial and Antioxidant Activities of Three *Mentha* Species Essential Oils. Planta Med. 69:413-419.

Mohammed MJ, Al-Bayati FA (2009). Isolation and identification of antibacterial compounds from *Mentha piperita* aerial parts and *Dianthus caryophyllus* flower buds. Phytomed. 16:632-637.

Naghibi F, Mosaddegh M, Motamed MS, Ghorbani A (2005). Labiatae family in folk medicine in Iran from Ethnobotany to Pharmacology. Iran. J. Pharm. Res. 2:63-79.

National Committee for Clinical Laboratory Standards (NCCLS) (2003a). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. Wayne PA, USA.

National Committee for Clinical Laboratory StandardS(NCCLS) (2003b). Performance Standards for Antimicrobial Disc Susceptibility Tests. 8th ed. Approved Standard M2-A8. Wayne PA, USA.

Nurzyn´ska-Wierdak R (2009). Herb yield and chemical composition of common oregano (Origanum vulgare L.) essential oil according to the plant's developmental stage. Herba Polonica 55:55-62.

Nychas GJE, Tassou CC, Skandamis P (2003). Antimicrobials from herbs and spices (eds Roller, S.) in "Natural antimicrobials for the minimal processing of foods" CRC Press Woodhead Publishers, New York pp. 176–200.

Pourohit SS, Vyas SP (2004). Medicinal plants cultivation. Agrobios Press, India.

Rahimmalek M, Bahreininejad B, Khorami M, Sayed TBE (2009a). Genetic diversity and geographical differentiation of *Thymus daenensis*, an endangerd medicinal plant, as revealed by Inter Simple Sequence Repeat (ISSR) markers. Biochem. Genet. 47:831-842.

Rasooli I, Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Alipoor AS (2006). Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochem. 67:1249–1255.

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Yoshinari T, Rezaee MB, Jaimand K, Nagasawa H, Sakuda S (2008). Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. Int J. Food Microbiol. 123:228–233.

Rechinger KH (1982a). *Labiatae* In: Flora Iranica. No. 150, Akademische Druch-u. Verlagsanstat, Austria.

Rechinger KH (1982b). Flora Iranica. No. 139. Graz: Akademische Druck. Verlagsanstalt.

Riley AP (2005). Food Policy, Control and research. Nova Science Publisher, Inc.

Rohloff J (1999). Monoterpene Composition of Essential Oil from Peppermint (*Mentha* × *piperita* L.) with Regard to Leaf Position Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry Analysis. J. Agric. Food Chem. 47:3782–3786.

Saeed S, Tariq P (2005). Antibacterial activities of *Mentha piperita*, Pisum sativum and Momordica charantia. Pak J Bot. 37:997-1001.

Sahin F, Karaman I, Güllüce E, Öğütçü H, Şengül M, Adıgüzel A, Öztürk S, Kotan R (2003). Evaluation of antimicrobial activities of *Satureja hortensis* L. J. Ethnopharmacol. 87:61–65.

Sefidkon F, Abbasi KH, Bakhshi KGH (2006). Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. Food Chem. 99:19–23.

Shafie MSB, Zain HSM, Shah MS (2009). Study of genetic variability of Wormwood capillary (Artemisia capillaris) using inter simple sequence repeat (ISSR) in Pahang region, Malaysia. Plant Omics J. 2: 127-134.

Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, Van Griensven LJLD (2009). Chemical Composition of Essential Oils of *Thymus* and *Mentha* Species and Their Antifungal Activities. Molecules 14:238-249.

Steven HG, Timothy DM (2010). Antibiotic Resistance Protocols 2nd Edition. Springer-Verlag LLC, New York.

Wichelhaus TA, Böddinghaus B, Besier S, Schäfer V, Brade V, Ludwig A (2002). "Biological Cost of Rifampin Resistance from the Perspective of Staphylococcus aureus". Antimicrob. Agents Chemother. 46:3381–3385.

Zargari A (1996). Medicinal Plants, Tehran University Publications, Tehran, Iran, fourth edition.

UPCOMING CONFERENCES

The International Symposium on Ocular Pharmalogy and Therapeeutics
June 19-22, 2014, Reykjavik

6th International Workshop on Advance in the Molecular Pharmaclolgy and Therapeutics of Bone and other Musculoskeletal Diseases

28 June - 2 July 2014

Conferences and Advert

June 2014
The International Symposium on Ocular Pharmalogy and Therapeeutics
June 19-22, 2014, Reykjavik

June 2014
6th International Workshop on Advance in the Molecular Pharmaclolgy and Therapeutics of Bone and other Musculoskeletal Diseases
28 June - 2 July 2014


Journal of
Medicinal Plant Research
Related Journals Published by Academic Journals
Journal of Medicinal Plant Research
African Journal of Pharmacy and Pharmacology
Journal of Dentistry and Oral Hygiene
International Journal of Nursing and Midwifery
Journal of Parasitology and Vector Biology
Journal of Pharmacognosy and Phytotherapy
Journal of Toxicology and Environmental Health Sciences
academicJournals