ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
June 2016 Vol.:6, Issue:3

Mosquito Repellency Potential of the Essential Oil from *Mentha* Spp.

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Sunita Bhargava

Department of Chemistry, College of Life Sciences
Cancer Hospital &Research Institute, Gwalior
M.P.India.

Submission: 10 June 2016
Accepted: 15 June 2016
Published: 25 June 2016

www.ijppr.humanjournals.com

Keywords: Synthetic repellents, *Aedes aegypti*, and repellent properties

ABSTRACT

As natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms, their innate biodegradability. Mentha oil is extremely useful in a wide variety of industries namely food industry, the pharmaceutical industry and also in perfumery and flavoring industries. Also, constituents and derivatives of mentha oil like mint and menthol are used widely. Menthol is the major derivative product of mentha oil. The majority of the oil is converted into menthol, which is so important that it is even considered as one the basic uses of mint oil. It is extracted through a complex process that involves cooling of the oil slowly, which turns the oil into crystal form and then it is centrifuged and dried to attain a yellow colored substance called menthol. This oil like substance is often required by the medicine industry and it is used in balms, cough drops, inhalers, toothpaste, mouthwashes etc. Around 40% to 50% menthol and around 50% to 60% dementholized oil is obtained from mentha oil in the production process of menthol. Dementholized oil is used in confectioneries and peppermint oil. Keeping in view the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, the availability, low budget, and less environmental impact, this study was undertaken to assess the repellent potential of the essential oil of peppermint plant, *M. piperita* against adult stages of *Ae. aegypti*. Oil formulations were tested for mosquito repellency in laboratory condition application of oil to the upper surface of the human forearms at the rates between 0.08 to 3.33 mg/cm^2 of skin. Oils exhibited a reasonable protection time and sometimes better than synthetic repellents.The results of the present study would be useful in promoting research aiming at the development of a new agent for mosquito control based on bioactive chemical compounds from an indigenous plant source.

INTRODUCTION

The mosquitoes are a family of small, midge-like flies: Culicidae. Although a few species are harmless or even useful to humanity, most are a nuisance because they consume blood from living vertebrates, including humans. In feeding on blood, various species of mosquitoes transmit some of the most harmful human and livestock diseases. The mosquito repellent is used for warding off mosquitoes which are the most harmful insect. Arthropod bites can cause local or systemic effects that may be infectious or inflammatory in nature. Measures to curtail the impact of insect bites are important in the worldwide public health effort to safely protect patients and prevent the spread of disease. Currently, the use of synthetic chemicals to control insects and arthropods raises several concerns related to environment and human health. An alternative is to use natural products that possess good efficacy and are environmentally friendly. Among those chemicals, essential oils from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource. The history of insect repellent (IR) lends insight into some of the current scientific strategies behind newer products. Active ingredients of currently available IRs include N,N-diethyl-3-methylbenzamide (DEET), botanicals, citronella, and, the newest agent, picaridin. Currently, the Environmental Protection Agency's registered IR ingredients approved for application to the skin include DEET, picaridin, MGK-326, MGK-264, IR3535, oil of citronella, and oil of lemon eucalyptus. The essential oils whose repellent activities have been demonstrated. Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes. However, in some cases, these chemicals can work synergistically, improving their effectiveness.Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides. The plant oils have received much attention as potentially useful bioactive compounds against insects showing a broad spectrum of activity, low mammalian toxicity and degrading rapidly in the environment. Peppermint oil extracted by steam distillation from the leaves of *Mentha piperita* (*M. piperita*) has a long tradition of medicinal use. Several studies in the literature show the efficacy of antifungals and antibacterials obtained from the essential oils of various species of *Mentha*. Thus, this essential oils showed antimicrobial activity against bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella choleraesuis*[1-5], and other microorganisms, such as yeasts and periodontopathogens[2,7,8]**.**The essential oil of

Mentha piperita is also capable of exerting a direct virucidal effect on the herpes simplex virus type 1 (HSV-1). The oil was active against an acyclovir-resistant strain of HSV-1, and plaque formation was reduced by a significant 99%[9]**.** Preliminary evidence suggests that the main peppermint oil component, menthol, may protect against herpes simplex[10]**.**The action of certain species of *Mentha* against the yeast *Candida albicans* and some periodontopathogens has already been tested**.**

Several studies prove their effectiveness as antifungal agents against Candida spp.[2-3,7-8,12-13]**.**The essential oil from *Mentha* spp. may be considered a safe ingredient for the development of antibiofilm agents that could find a role in the pharmaceutical industry[6]**.** This is especially relevant at a time when there is increasing interest in finding more natural alternatives to many existing preservatives[5]**.**

Seasonality crop calendar:

Planting -: Second week of Jan –Feb

Flowering -: June- October

Harvesting -: July-August

Oil properties

Peppermint oil has a fresh, sharp, menthol smell, is clear to pale yellow in color and watery in viscosity.

Origin of peppermint oil

It is a native of the Mediterranean.It is a perennial herb that grows up to 1 meter (3 feet) high and has slightly hairy serrated leaves with pinkish-mauve flowers arranged in a long conical shape. It has underground runners by which it easily propagates. This herb has many species, and *peppermint piperita* is a hybrid of water mint (*M. aquatica*) and spearmint (*M. spicata).*

Extraction

Peppermint oil is extracted from the whole plant above ground just before flowering. The oil is extracted by steam distillation from the fresh or partly dried plant and the yield is 0.1 - 1.0 %.

General Characteristics-

- Mentha oil is obtained by steam distillation of Mentha arvensis leaves.
- Mentha oil and its constituents and derivatives are used in food, pharmaceutical and perfumery, and flavouring industry.
- Menthol is the main constituent of mentha oil and slow cooling through refrigeration, crystallization,centrifugation, and drying are done to obtain it. It is used in the manufacture of lozenges, toothpaste, pain balms, cold balms etc.
- Mentha is widely cultivated in India .
- India cultivates about eight species of mentha, however, three species are approved / recognized by the purpose of quality standards and international marketing.
- The natural oil yields on an average 40-50% menthol and 50-60% dementholised oil, which can be used both in confectionery and medicine in place of imported peppermint oil. Japanese mint oil is not distinguished from the peppermint oil in the Indian trade.

EVALUATION

Relative density : 0.900 to 0.916.

Refractive index : 1.457 to 1.467.

Optical rotation : -10° to -30°.

Acid value : maximum 1.4, determined on 5.0 g diluted in 50 ml of the prescribed mixture of solvents.

STANDARDS

Peppermint Oil contains not less than 4.5 percent w/w and not more than 10.0 percent w/w of esters, calculated as menthyl acetate, $C_{12}H_{22}O_2$, not less than 44.0 percent w/w of free alcohols, calculated as menthol, $C_{10}H_{20}O$, and not less than 15.0 percent w/w and not more than 32.0per cent w/w of ketones, calculated as menthone, $C_{10}H_{18}O$.

Chemical composition

The chemical composition of the oil from *Mentha* spp. varies according to the age of the plant, variety of species, geographic region and conditions of processing[14]**.** The plant is sensitive to latitude and climate and grows principally in the mid-Western states of the USA where 75% of the world's fresh supply originates[15].The main elements identified in the volatile essential oil of *Mentha* spp. Are menthol (33-60%), menthone(15-32%), iso menthone (2-8%), 1.8 cineol (eucalyptol)(5-13%), menthyl acetate (2-11%) menthofuran (1-10%), limonene (1-7%), β-myrcene (0.1-1.7%), β-caryophyllene (2-4%), pulegone (0.5-1.6%) and carvone.The main active component of peppermint oil is menthol, which is responsible for its medicinal properties, whilst esters, such as menthyl acetate, provide the familiar minty taste and associated aroma.

Use-

Peppermint oil can assist in nervous disorders and is dramatically effective in stimulating the mind and focusing concentration, for treating the respiratory tract, muscular aches, and pains and for some skin problems.Adding few drops of peppermint essential oil in a glass of water and drinking it after a meal gives relief from indigestible properties. This oil acts as a carminative and helps effectively in removing the gas. It was also reported that peppermint oil is effective against type I allergic reactions.[16 -17]1:20 dilution (5.0%) of concentrated peppermint water has now been shown to exhibit considerable fungistatic but not fungicidal activity against strains of *Aspergillus niger* and *Penicillium ckysogenum*.[18]

· One interesting study concluded that peppermint oil can indeed reduce daytime sleepiness. However, the mechanisms by which peppermint oil has its effect and the applicability of these findings to situations in everyday life will require further empirical investigation.[19]

· Peppermint oil was reported to have a relaxing effect in patients with colonic spasms.[20]

· One recent study by J. A. Reed *et al.* results that peppermint scent can be used as an effective adjunct to decrease appetite, decrease hunger cravings, and consume fewer calories, which may lead to weight reduction and greater overall health.[21]

· The use of peppermint oil given orally can cure certain internal ailments such as gallstones or ureteric stones. The doses of them sometimes exceed 45 ml/day in France and Germany.[22]

· Headlice: Phenols, phenolic ethers, ketones, and oxides (1, 8-cineole) appear to be the major toxic components of these essential oils *when* used for lice.Aldehydes and sesquiterpenes may also play a role.[23]

· In vapor therapy, peppermint oil can help to increase concentration and to stimulate the mind, as well as sorting out coughs, headaches, nausea and also has value as an insect repellant.[24]

· External usage of peppermint oil gives relief from pain. The existence of calcium antagonism in peppermint oil helps in removing the pain. It has wonderful cooling properties and reduces the fever also.[24]

· A mouthwash with peppermint oil included can help with bad breath and gum infections. [24]When included in a cream or lotion, it will help to ease the sting of sunburn, reduce redness of inflamed skin, reduce itchiness and cools down the skin with its vasoconstrictor properties.[24]

· The oil gives a cooling effect on your head and helps in removing dandruff and lice. Larvicidal and mosquito repellent action.

Oil of *Mentha piperita* L. (Peppermint oil), a widely used essential oil, was evaluated for larvicidal activity against dierent mosquito species: *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatu*s by exposing IIIrd in star larvae of mosquitoes in enamel trays 6´ 4 inch2 size filled to a depth of 3 inches with water. The oil showed strong repellent action against adult mosquitoes when applied on human skin. Percent protection obtained against *An. annularis*, *An. culicifacies*, and *Cx. quinquefasciatus* was 100%, 92.3% and 84.5%, respectively

Keeping in view the recently increased interest in developing plant origin repellent as an alternative to chemical repellents , the availability, low budget and less environmental impact, this study was undertaken to assess the repellent potential of the essential oil of peppermint plant, *M. piperita* against of *Ae. aegypti*. The results of the present study would be useful in promoting research aiming at the development of a new agent for mosquito control based on bioactive chemical compounds from an indigenous plant source.

MATERIALS AND METHODS

Extraction of plant material

Fresh plants were purchased from a local hypermarket. A voucher specimen was submitted to the Herbarium unit, The procured plant samples were washed and dried at room temperature (30°C) for a week, grounded into a powdered using an electrical blender. 200 g of the finely powdered material were macerated and soaked in 80% methanol for 4 days. The extracts were clarified by filtration with Whatman No. 1 paper and concentrated in vacuo in a rotary evaporator. Finally, the crude extracts were obtained.

Various scientists have used a different procedure for repellency testing of synthetic and natural materials, thereby making it compare these results are obtained with the same substance when tested in a different way. The most acceptable method as described by.[25] has used during the present studies. An appropriate quantity of the test material dissolved in ethanol was applied on the external surface of the human forearm which has a surface area of nearly 150 cm2.250, 300, 350, 400, 450 and 500 mg respectively. The above values after calculation using standard scale for synthetic chemicals i.e. 1 mg/cm^2 will correspond to the values i.e. 0.04, 0.08, 0.16, 0.33, 0.50, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00 and 3.33 mg/cm2 respectively. The other forearm of the vehicle acted as a control. The treated forearm was exposed to about 5-7 days old 200 starved female mosquitoes held in a female cage, the number of mosquitoes landed/bitten was recorded for 5 minutes. The protection time was worked out by the same procedure. Exposure of hands for 5 minutes. At an interval of 30 min. until 5 or more bites were observed less than 5 bites in 5 min. was considered to be indicative of repellency for each formulation at least 3 replicates were taken. The recorded data were subjected to the one-way analysis of variance (ANOVA) and the level of significance was calculated as described by .[26]

Phytochemical analysis

Qualitative phytochemical tests of the crude extracts were done to identify the presence of sugar, flavonoids and alkaloids [27].

Table 1.

Phytochemical analysis of the plant extracts

Plant	Benedict's test	Frothing test	Borntrager's Test	Flavonoid test	Ferric chloride test	Alkaloid test
M. piperita	+	-	-	+	-	+

+ indicates the presence

- indicates the absence of the organic compounds tested by the qualitative color reaction.

(Benedict's test - reducing sugar, frothing test - saponins, Borntrager's test - anthraquinones, flavonoid test - flavonoids, ferric chloride test - tannins, alkaloid test-alkaloids)

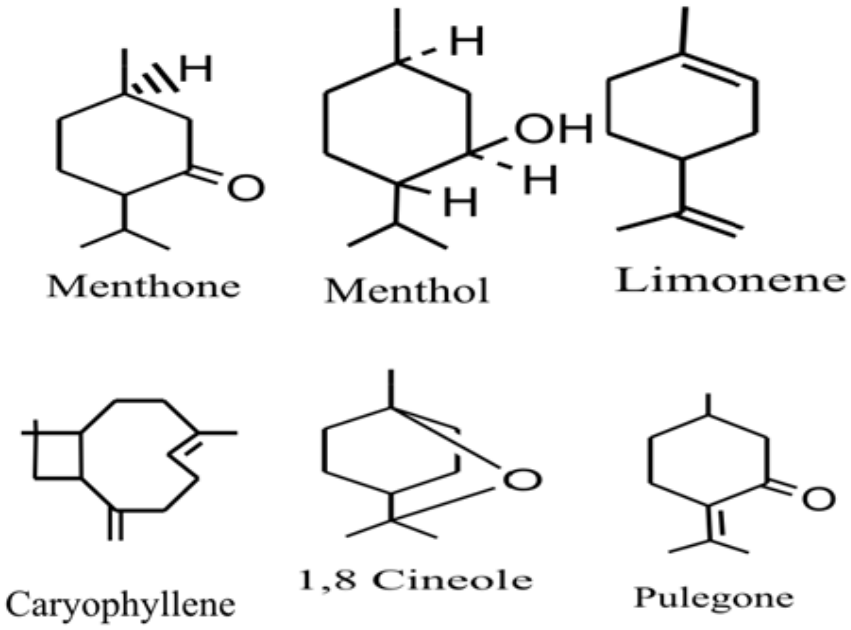

FIGURE 1. Various chemical constituents of peppermint oil.

RESULTS

The mosquito repellent is used for warding off mosquitoes which are the most harmful insect. Nowadays mosquito repellent is used for controlling mosquito and is replacing other mosquito destroyers gradually. Repellency of oils of lemon, eucalyptus, geranium, and lavender have also been recorded against *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field.[28]However, plants whose essential oils have been reported to have repellent activity include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cinnamon, rosemary, basil, thyme, and peppermint. Most of these essential oils provided short-lasting protection usually lasting less than 2 h. Many essential oils and their monoterpenic constituents are known for their mosquito repellent activity against *Culex* species[29-30] The mosquito repellent activity of 38 essential oils was screened against the mosquito *A. aegypti* under laboratory conditions using human subjects.[31] The oils of *Cymbopogon nardus* (citronella), *Pogostemon cablin* (patchuli), *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* were the most effective and provided 2 h of complete repellency. Among three essential oil constituents namely eugenol, cineole and citronellal, the later was found to be most effective against *A. aegypti* mosquito Lemongrass oil ointment containing 15% v/w citral exhibited 50% repellency which lasted for 2–3 h[32]. It has now been reported that a component of the essential oil of the catnip plant (*Nepeta cateria*), the nepetalactone repels mosquitoes 10 times more effectively than DEET as it takes about one-tenth as much nepetalactone as DEET to have the same effect. *Tagetes erecta* is a potential plant whose essential oil from flowers has been an effective repellent against insects[33]. Accordingly ocimene from *T. minuta* has also repellent properties which need to be exploited in detail. Cinnamaldehyde, eugenol, cinnamyl acetate and essential oils from different *Cinnamomum* species are effective mosquito larvicides.[34-35] Several monoterpenoidal constituents evaluated for their insect repellent activity show that linalool and nerol in linear monoterpenoids and carvone, pulegol, pulegone and isopulegol in monocyclic monoterpenoids are the most effective space repellents; some others have been found effective as repellents against the German cockroach, *B. germanica.*[36]Two monoterpenes namely menthol and citral have been reported to be toxic against tracheal mites.[37] Thus such essential oil compounds may play a pivotal role in the control of mosquito driven dengue and malaria outbreaks through lure and kill technique. In recent years, several monoterpenoids have been considered potential alternatives to conventional insecticides as a natural means of pest control. Since oxygenated essential oil constituents are

more active, efforts have been made to improve bioefficacy of one such oxygenated essential oil constituent fenchone (LC50 = 3.8 mg/l for house flies and 14.2 mg/l for red flour beetles;[38] by its chemical modification and structure-activity relationship studies. Turmerone and *ar*-turmerone (dihydro turmerone), the major constituents of turmeric rhizome powder oil are strong repellents to stored grain pests. The turmeric oil has been reported to provide protection to wheat grains against red flour beetle, *T. castaneum*[39]. The fruit oil of *Piper retrofractum* has also shown high repellency (52–90%) against *T. castaneum* at 0.5–2% concentration.

Mentha oil exhibited protection time 15,26.6 38.3, 67.6 and 85 min. Higher doses of mentha oil show irritant and allergic effects on skin, therefore, higher concentration of this oil was not screened. In ANOVA calculation, we find that the value at minimum dose level was doubled the values were significant.(Table-2).

Table -2

Protection time of essential oil against the bite of *Aedes aegypti*.

S.N.	Dosage mg/cm^2	Mentha oil (MO)
	Control	0.000
1.	0.08	0.000
2.	0.16	0.000
3.	0.33	0.000
4.	0.50	15.00[a]
5.	1.00	26.60[a]
6.	1.33	38.30[a]
7.	1.66	61.60[a]
8.	2.00	85.00[a]
9.	2.66	------
10.	3.33	------
Variance ratio 'F'		
For replicate		0.175
For treatment		5.100
Standard error of Mean SEm		12.43
Standard error of deviation SEd		17.40
Least significance difference LSD		40.12

Values shown by the same letter are not significantly different P<0.001

Phytochemical analysis:

Results from the phytochemical analysis indicated the presence of reducing sugar, flavonoids, and alkaloids (Table 1.).Phytochemical analysis indicated the presence of reducing sugar, flavonoids, and alkaloids. Mint extracts had good flavonoid content and total phenolic content [40]. GC profiling indicated the presence of fatty acid methyl esters (hexadecane,heptadecane, octadecane) terpenoids, terpenoid alcohol,caryophyllene, and glycosides. A recent report on GC-MS analysis indicated the presence of menthone, isomenthone, and hexadecanoic acid in Mentha extracts [41]. Bioassay guided separation of the ethyl acetate soluble portion of Mentha extracts afforded 13 compounds including flavonoids, glycosides and lower alcohols and rosmaniric acid [42].Another study stated that the essential extracted from M.spicta contained mainly carvone (50-70%) and menthone [43-44].Monoterpenes are the major essential components of the mint including peppermint [45]. The chief components of the essential oil from M. longifolia from South Africa were found to be them on oterpene ketone, menthone. Some other chemotypes had carvone, piperitone, U-pinene, cineole, pulgone, limonene, germacrene, and U-caryophyllene[47-48]. External lipophilic methylated flavonoids have been extracted from dried leaves of *Mentha aquatica*, *Mentha spicata* and *M. x piperita* have been identified by mean of spectrophotometric methods (UV, NMR). The main element identified in the volatile essential oil of Mentha are menthol (33-60%) menthone (15-32%), isomenthone (2-8%), 1,8cineol (eucalyptol), (5-13%), methyl acetate (2-11%), menthofuaran(1-10%), limonene (1-7%), B-myrcene, B-caryphyllene, pulegone, carvone[42].There are thus the opportunities like (i) changing consumer preferences towards the use of 'natural' over synthetic products; (ii) existence of and growth in niche markets, where quality is more important than price; (iii) strong growth in demand for essential oils and plant extracts; (iv) potential to extend the range of available products including new product development through biotechnology; (v) production of essential oils and plant extracts from low-cost developing countries.

CONCLUSION

In the present study, we have found that the potential plants based essential oils tested against mosquitoes. The peppermint essential oil is proved to be efficient larvicide and repellent against

dengue vector. Further studies are needed to identify the possible role of oil as adulticide, oviposition deterrent and ovicidal agent. The isolation of active ingredient from the oil could help in formulating strategies for mosquito control. The essential oils appeared to have no toxic effects in this study and a history of herbal medicine use indicates safety, The present results have clearly demonstrated the possibility of transforming the potential natural products into commercial repellent. Finally, although from an economical point of view synthetic chemicals are still more frequently used as repellents than essential oils, these natural products have the potential to provide efficiently, and safer repellents for humans and the environment.

REFERENCES

1. Sivropoulou A, Kokkimi S, Lanaras T, Arsenakis M.Antimicrobial activity of mint essential oils. J AgricFood Chem. 1995; 43:2384-8.
2. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM,Duarte MCT, Rehder VLG. Composition and antimicrobialactivity of essential oils from aromatic plants used in Brazil.Braz J Microbiol. 2004; 35:275-80.
3. Duarte MCT, Rehder VLG. Composition and antimicrobialactivity of essential oils from aromatic plants used in Brazil.Braz J Microbiol. 2004; 35:275-80.
4. Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Biochemical activities of Iranian *Mentha* piperita L. and Myrtus communis L. essentialoils. Phytochemistry 2006; 67(12):1249-55.
5. Fazlara A, Najafzadeh H, Lak E. The potentialapplication of plant essential oils as natural preservatives against Escherichia coli O157:H7. Pak J Biol Sci. 2008;11(17):2054-61.
6. Rasooli I, Shayegh S, Taghizadeh M, Astaneh SD. Phytotherapeutic prevention of dental biofilm formation.Phytother Res. 2008; 22(9):1162-7.
7. Tampieri MP, Galuppi R, Macchioni F Carelle MS,Falcioni L, Cioni PL, Morelli I. The inhibition ofCandida albicans by selected essential oils and their major components. Mycopathologia 2005; 159:339-45.
8. Lee SB, Cha KH, Kim SN, Altantsetseg S, Shatar S,Sarangerel O, Nho CW. The antimicrobial activity ofessential oil from Dracocephalum foetidum against pathogenic microorganisms. J Microbiol. 2007;45(1):53-7.
9. Schuhmacher A, Reichling J, Schnitzler P. Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 in vitro. Phytomedicine.2003; 10 (6-7):504-10.
10. Melzer J, Rösch W, Reichling J, Brigmoli R, Saller R.Meta-analysis: phytotherapy of functional dyspepsia with the herbal drug preparation STW 5 (Iberogast).Aliment Pharmacol Ther. 2004; 20:1270-87.
11. Oumzil H, Ghoulami S, Rhajaoui M, Ilidrissi A, Fkih-Tetouani, Faid M, Benjouad A. Antibacterial and antifungal activity of essential oils of *Mentha* suaveolens.Phytother Res. 2002; 16:727-31.
12. Freitas MSM, Martins MA, Vieira IJC. Produção equalidade de óleos essenciais de *Mentha* arvensis emresposta à inoculação de fungos micorrízicos arbusculares.Pesqui Agropecu Bras. 2004; 39(9):887-94.
13. Soković MD, Vukojević J, Marin PD, Brkić DD, VajsV, van Griensven LJ. Chemical composition of essentialoils of Thymus and *Mentha* species and their antifungalactivities. Molecules. 2009;14(1):238-49.
14. Xu P, Jia W, Bi L, Liu X, Zhao Y. Studies on componentsand quality of essential oil from *Mentha* piperita L.produced in Xinjiang,China. Chem Ind Forest Prod.2003; 23:43-5.
15. Spirling LI, Daniels IR. Botanical perspectives on health peppermint: more than just an after-dinner mint. J R Soc Health. 2001; 121(1):62-63.
16. Arakawa T, Ishikawa Y, and Ushida K: Volatile sulfur production by pig cecal bacteria in batch culture and screening inhibitors of sulfate reducing bacteria. J Nutr SciVitaminol 46: 193–198, 2000.

17. Inoue T, Sugimoto Y, Masuda H, *et al:* Effect of peppermint*Mentha piperita L.*) extracts on experimental allergic rhinitis in rats. Biol Pharm Bull 24: 92–95, 2001.
18. Hugbo PG. An evaluation of antifungal properties of peppermint water. International Journal of Pharmaceutics.10 (1982) 193-198.
19. Mark Ian Keith Norrish and Katie Louise Dwyer.Preliminary investigation of the effect of peppermint oil on an objective measure of daytime sleepiness. InternationalJournal of Psychophysiology. 55 (2005) 291– 298.
20. Masato Ai *et al.* Assessment of the Antispasmodic Effect ofPeppermint Oil and Shakuyaku-Kanzo-To (TJ-68): A Chinese Herbal Medicine on the Colonic Wall.Gastrointestinal Endoscopy. 61(5) AB107.
21. J. A. Reed *et al.* Effects of peppermint scent on appetite control and caloric intake. Appetite. (2008), doi:10.1016/ j.appet.2008.04.196.
22. Balchin ML. Essential oils and 'aromatherapy': their modern role in healing. J R Soc Health 1997;117:324– 9.
23. Lowana Veal. The potential effectiveness of essential oils as a treatment for head lice *Pediculus humanus capitis*. Complementary Therapies in Nursing & Midwifery. (I 996)2, 97-101.
24. . http://www.hc-sc.gc.ca/home-accueil/text-eng.php. As assessed on 20/08/08.
25. Rao,S.S.,Rao,K.M.and Ramachandran,P.K.Efficacy,Safty and use of N,N-Diethyl phenyl acetamide, a multi – Insect repellent. *J.Sci.Ind.Res*. 47: 722-735, 1988.
26. Snedecore G.N.and Cocharan,W.G. "Statistical Methods",8th edition 1989
27. Harborne. J. B. 1993. Phytochemistry. Academic Press, London,pp 89-131.
28. Jaenson, T.G.T., Garboul, S. and Pålsson, K. (2006) Repellency of oils of lemon, eucalyptus, geranium, and lavender and the mosquito repellent MyggA natural to *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field. *J. Med. Entomol.*, **43**, 731– 736.
29. Choi, W.I., Lee, S.G., Park, H.M. and Ahn, Y.J. (2004) Toxicity of plant essential oils to *Trialeuroides vaporariorum* (Homoptera:Aleyrodidae). *J. Econ. Entomol*., **96**, 1479–1484.
30. Traboulsi, A.F., Taoubi, K., El-Haj, S., Bessiere, J.M.and Rammal, S. (2002) Insecticidal properties of essential plant oils against the mosquito *Culex pipiens molesters* (Diptera : culicidae). *Pest Manag.Sci.*, **58**, 491–495.
31. Trongtokit,Y., Rongsrivam, Y., Komalamisra, N. andApiwathnasorn, C. (2005) Comparative repellencyof essential oils against mosquito bites. *Phytother.*
Res., **19**, 303–309.
32. Oyedela, A.O., Gbolade, A.A., Sosan, M.B.,Adewoyin, F.B., Soyely, O.L. and Orafidiya, O.O.2002). Formulation of an effective mosquito repellent topical product from lemon grass oil.*Phytomedicine*, **9,** 259–262.
33. Ray, D.P., Walia, S., Dureja, P and Singh, R.P. (2000)Composition and repellent activity of the essential oil of marigold (*Tagetes erecta*) flower. *Ind. Perf*.,**44**, 267–270.
34. Huang, Y. and Ho, S.H. (1998) Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects *Tribolium castaneum* (Herst) and*Sitophillus zeamais* Motsch. *J. Stored Prod. Res.* **34,** 11–17
35. Cheng, S.S., Liu, J.Y., Tsai, K.H., Chen, W.J. andChang, S.T. (2004) Chemical composition andmosquito larvicidal activity of essential oils fromleaves of different *Cinnomomum osmophloeum*provenances. *J. Agric. Food Chem.*, **52**, 4395–4400.
36. Inazuka, S. (1983) Monoterpenoids as repellents against the German cockroach (*Blattela germanica* L.). *J.estic. Sci.*, **8,** 293–299.
37. Ellis, M.D. and Baxendale, F.P. (1997) Toxicity of seven monoterpenoids to tracheal mites (Acari: Tarsonemidae)and their honey bee (Hymenoptera:Apidae) hosts when applied as fumigants. *J. Econ.Entomol.*, **90**, 1087–1091.
38. Rice, P.J. and Coats, J.R. (1994) Insecticidal properties of several monoterpenoids to the house fly(Diptera : Muscidae), red flour beetle (Coleoptera :Tenebrionidae) and southern corn root-worm(Coleoptera : Chrysomelidae). *J. Econ. Entomol.*,**87,** 1172–1179.
39. Chahal, K.K., Arora, M., Joia, B.S. and Chhabra, B.R. (2005) Bioefficacy of turmeric oil against *Tribolium castaneum* (Herbst) under laboratory conditions. *In* V.K. Dilawari, G.S. Deol, B.S. Joia and P.K. Chuneja (eds.), *Proc. 1st Congress on Insect Science*: *Contributed Papers*, PAULudhiana, pp. 147–148.

40. Sweetie, R., Kanatt, Ramesh Chander., Arun Sharma. 2007.Antioxidant potential of mint (Mentha spicata L.) in radiation processed lamb meat. Food Chemistry 100: 451-458.
41. Padmini E, A. Valarmathi M, Usha Rani. Comparative analysis of chemical composition and antibacterial activities of Menthaspicata and Camella sinensis. Asian .J. exp. Biol. Science, 1(4) 2010: 772-781.
42. Satoshi, Yamamura., Koichiro, Ozawa., Kazuhiro, Ohtani,, Ryoji,Kasai and Kazuo, Yamasaki. 1998. Antihistaminic flavones and aliphatic glycosides from Mentha spicata. Phytochemistry.Vol 48 (1):131-136.
43. Gulluce M, F. Sahin, M. Sokmen, H. Ozer, D.Daferara . A.Sokmen, M Poliisiou , A Adiguzel, H. Ozkan. 2007.Antimicrobial and antioxidant properties of the essential oils and methanol extract form Mentha Longifolia L. ssp. Longifoila.Food Chemistry 103: 1449-1456.
44. Aflatuni. A. 2005. The yield and essential oil content of mintMentha spp in Northern Ostrobonthia. (http://herkules.oulu.fi).Oulu University Press, Finland, pp 52 .
45. Hussian, A.I. 2009. Characterization and biological activities of essential of some species of Lamiaceae: Ph. D Thesis,Faisalbad, Pakistan, pp 257
46. Soheil, S., Mahmoud, Mathew, Williams., Rodney, Croteae. 2004.Co-suppression of limoene-3-hydroxylase in peppermintpromoted accumulation of limonene in the essential oil.Phytochemistry 65: 547-554.
47. Younis, Y. M. H., and Beshir, S. H. 2004. Carvone-rich essential oils from Mentha longifolia (L.) Huds. ssp. Schimperi Briq andMentha spicata L., Grown in Sudan. Journal of Essential oil research, 16(6):539-541.
48. Karousou, R. A., Lanaras, T. B and Kokkini, S. 1998. Piperitoneoxide-rich essential oils from Mentha longifera subsp. Petioleand M. x. villoso-nervata grown wild on the Island of Crete(S.Greece). Journal of essential oil research, 10(4), 375-379.