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Full Length Research Paper

Comparative mosquito repellent efficacy of alcoholic extracts and essential oils of different plants against *Anopheles Stephensi*

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Mosquitoes control and personal protection from mosquito's bites is one of the serious ways for preventing of contagious diseases distribution. Mosquitoes in addition to the local symptoms (itching, redness and irritation) can cause transmission of fatal and dangerous disease especially in tropical areas. In recent years, interest in plant-based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides and their rising cost. Various plant-based products as herbal repellents are safe and biodegradable alternatives to synthetic chemicals for use against mosquitoes. In the present study, essential oils and extracts of six plants "*Melissa officinalis*, *Rosmarinus officinalis*, *Lavandula officinalis*, *Citrus limonum*, *Eucalyptus globulus* and *Ocimum basilicum*" were evaluated compared against mosquitoes of *Anopheles Stephensi*. Finally, repellant properties of essential oils and extracts as experimental groups and of *N, N-diethyl 3 - methylbenzamide* (DEET) as a positive control group were compared. We used Duncan's multiple range tests to determine the significant differences at 1% level between the experimental group and the control group. Results of statistical analysis showed significant differences between the extracts and essential oils. Essential oils indicated more effectiveness rather than extracts.

Key words: Malaria, *Anopheles stephensi*, insect repellent, essential oil, plant extract.

INTRODUCTION

Problems with chemical insecticides and possible effect of essential oils attracted the attention of researcher. In the recent years, long term application of chemical substances for controlling, repelling and killing of hazardous insects make serious anxieties for environment and human health (Nerioa, 2010; Yang, 2002).

Therefore, uses of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention as agents for mosquitoes control (Nerioa, 2010). The years before 1940, volatile substances such as Citronella oil, Clove seed oil and Camphor were the common insect repelling substances. In addition some plants such *as Marquise*, *eucalyptus*, *fennel*, *oregano*, *pepper*, *wormwood* plant and tea tree are known to show such properties (Wilkinson, 1996; Sima, 2012).

Natural Iranian flour consists of many herbs which are traditionally used as repelling insects especially against *malaria* vectors. According to WHO report, eastern Mediterranean countries like Iran are the source of contagious disease by insects specially mosquitoes species (WHO, 2010; Jinous and Fereshteh, 2012). So far, 64 species of mosquitoes have been reported from Iran, which included 28 species of *Anopheles*, 3 species of *Ades* and 19 species of *Culexus* and 14 species are from other genus (Azari, 2007). Among all the insect

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Table 1. Six tested plants.

No	Plant name	Family	Parts used for extracts and essential oils	Common name
1	*Lavandula officinalis*	*Lamiaceae*	Flower	*Lavender*
2	*Melissa officinalis*	*Labiatae*	Leaf	*Melissa*
3	*Rosmarinus officinalis*	*Lamiaceae*	Leaf	*Rosemary*
4	*Citrus limonum*	*Rutaceae*	Peel	*Lemon*
5	*Eucalyptus globolus*	*Myrtaceae*	Leaf	*Eucalyptus*
6	*Ocimum basilicum*	*Lamiaceae*	Herb	*Basil*

vectors of human disease, *Anopheles* is the most popular and worst species of mosquitoes (Collins, 1995; Curtis, 1994).

Among the species, *Anopheles stephensi* mosquito is the most important *malaria* vector especially in Iran. This mosquito in India and some of West Asian countries are annually the reason of 40 to 50% of malaria disease. Globally, malaria kills 3 million people each year, including 1 child every 30 s (Shell, 1997). The search for effective vaccines against malaria is still in progress. Individual protecting actions, comprising repellents are extensively applied to put off the transmission of arthropod – borne diseases by decreasing access between persons and vectors (Coleman, 1993; Walker, 1996). World War II and the needs of military groups in the tropical lands promoted the scientists to extensive research for discovery and preparation of insect repellent drugs (Wilkinson, 1996). Undoubtedly, Iranian military forces are face to such disease by the insects; thus, more than ten thousands chemical compounds have been tested for the insect repelling effects. Nevertheless, long term use of chemical insecticides has side effects on health. To avoid the adverse effect, researches on repellents that are derived from plant are promising in that they are effective, safe to users and also inexpensive (Abu-Qare, 2001; Fradin, 1998; Barnard, 2004).

For this reason and achieving better results, we select the plants that can be grown in Iran and are easily propagated. An enormous amount of plant products have been stated to have mosquito larvicidal and/or repellent action against mature mosquitoes.

In the previous studies, 10 medicinal herbs were used as repelling and killing of ova, larva and mature insects of *Anopheles stephensi*, *Aedes Aegypti* and *Culex quinquefasciatus.* Finally, ginger and rosemary essential oils were introduced respectively as a killer and repellent of three mentioned mosquitoes. Also, repellent effects of either essential oil and extract of lemon and Melissa against *A. stephensi* were examined and the results showed better effect of essential oil rather than extract, but differences were not significant (Veena, 2005; Vatandoost, 2004).In another study, the essential oil of five different plants leaves and their repellent effects was investigated against the Anopheles mosquito. Other scientists also were introduced essential oils as repelling of the malaria-carrying insect (Oshaghi, 2003; Rajkumar, 2007). Essential oils repellency of aromatic plants that grow in Argentina against *Aedes aegypti* have also evaluated: *Acantholippia seriphioides*, *Achyrocline satureioides*, *Aloysia citriodora*, *Anemia tomentosa*, *Baccharis spartioides*, *Chenopodium ambrosioides*, *Eucalyptus saligna*, *Hyptis mutabilis*, *Minthostachys mollis*, *Rosmarinus officinalis*, *Tagetes minuta* and *Tagetes pusilla*. Most essential oils were effective (Gillij, 2008; Muhammad, 2012).

In recent years, several plants extracts including neem (*Azadirachta indica, A. bJuss*), Citronella grass (*Cymbopogan nardus Rendle*), basil (*Ocimum basilicum L.*, *Ocimum gratissimum L.*, *Ocimum americanum L.*), clove (*Syzygium aromaticum L.*), prickly straggler (*Solanum trilobatum L.*), musk basil (*Moschosma polystachyum L.*) and thyme (*Thymus vulgaris L.*) have been studied as possible mosquito repellents (Gillij, 2007). Consequently, the aim of this study is to compare the repellent activity of plant essential oils and plant extract of six plants against the *A. stephensi*.

MATERIALS AND METHODS

Plant selection

In this study plants according to ancient data bases for insect repelling were selected. Also tried to choose plants that are easily propagated and using of them be safe for human and does not induce toxicity in person. Table 1 showed six plants that were tested. The plants from the farm of Karaj Agriculture Faculty (Tehran University) were prepared. Leaves and spire of the plants which contain the effective substances were selected and in a proper condition with suitable air circulation and far from direct sun light were dried. Dried herbal parts were milled. Then from powder by the maceration method and use of 70% ethanol, alcoholic extract were prepared. In this method 100 g powder in 1 L of ethanol as a solvent macerated by vacuum machine and in temperature of 40°C were concentrated. For essential oil preparation, for each 100 g of herbal powders, 1 L double distilled water was added and essential oil extraction was done in Clevenger machine (Tyler, 1988). Then repellent effects of both herbal extracts and essential oils separately on Anopheles mosquitoes were investigated (Buescher, 1985; Klun, 2000).

To prepare different concentrations, the products were further diluted using alcohol as diluents. Extract and essential oil solutions were formulated on a volume- volume basis at a concentration of 3 and 1%, respectively. The compounds were applied as 4-ml aliquots of ethanol solution and were spread evenly over the animal skin as explained previously (Buescher, 1985; Klun, 2000).

Table 2. Relative repellent effectiveness of 3% extracts and 1% oils of *Lavandula officinalis*, *Melissa officinalis*, *Rosmarinus officinalis*, *Citrus limonum*, *Eucalyptus globolus*, *Ocimum basilicum* laboratory mosquitoes of *Anopheles stephensi* on guinea-pigs in the laboratory. Efficacy of each experimental substance was repeated three times. Highest efficacy were supposed for *Lavender oil* and lowest efficacy for Ethanol.

Repellent	Replicate I*	Replicate II	Replicate III	Mean	Mean ± SE (%)
Lavender oil	100	97	94.5	97.1667[a]	97.16±2.75
Melissa oil	93.5	84.5	100	92.6667[a]	92.66±7.78
Rosemary oil	84.4	93	97.5	91.6333[a]	91.63±6.65
Lemon oil	95.9	90	92	92.6333[a]	92.63±3.00
Eucalyptus oil	96	95.45	100	97.1500[a]	97.15±2.48
Basil oil	96.4	93.4	94.2	94.6667[a]	94.66±1.55
DEET(standard)	97	100	94	97.0000[a]	97.00±3.00
Lavender extract	95	100	94.73	96.5767[a]	96.57±2.96
Melissa extract	71.42	83.3	74.2	90.0667[a]	76.30±6.21
Rosemary extract	79.16	71	66.8	76.3067[b]	72.32±6.28
Lemon extract	29.41	58.8	62.15	72.3200[b]	50.12±18.01
Eucalyptus extract	93.7	92.5	84	73.9333[b]	90.06±5.2
Basil extract	73.3	82.5	66	50.1200[c]	73.93±8.2
Ethanol (control)	0	0	0	0	0

Averages that have the same letters are not statistically significant at 5%. *Efficacy of each experimental substance was repeated three times and finally we calculate the mean.

Insect selection

The mosquitoes of *A. stephensi* was obtained from a well-established laboratory colony from school of Public Health and Institute of Health Research, Tehran University of Medical Sciences. Mosquitoes were reared and maintained at 27±3°C and 80±10 relative humidity (RH) under a 12:12 (L:D) photoperiod. Larvae were reared on a diet of floating catfish food. Female mosquitoes are only the *malaria* vectors, so they were selected. The adults were maintained in screen cages on 10% sucrose solution but 24 h before experiments the sucrose solution was removed from cages. Repellency was tested against 3 to 5-day-old, blood-starved mosquitoes, and for each test 25 mosquitoes were used (Barat, 2012; Norashiqin, 2008).

Animal testing

The extracts and essential oils were tested on animal guinea pigs. The animals were laboratory reared albino males with average 400 to 450 g weight. A 4 × 6 cm of animal abdomen hairs was removed then washed and cleaned by ethanol. Treatments were 4 ml of either the extracts containing 0.12 and 0.04 g of active ingredients of extracts and oils, respectively, and 0.4 ml DEET (N, N-diethyl-3-methylbenzamide) in the same way were used as a positive control. After treatment, the animal was bound on top of the cage in which the treated position was exposed to mosquitoes for 30 min. Each test was repeated three times replacing new mosquitoes and new animal, and number of bites through the fabrics was recorded. Animal experimentation was approved by the Animal Research Committee of Tehran University.

Statistical analysis

To compare the repellent efficacy of the compounds, we used Duncan's multiple range tests to determine the significant differences at 1% level between the experimental group and the control group.

RESULTS

Results of laboratory study on animals comparing extracts and oils of *Melissa officinalis*, *R. officinalis*, *Lavandula officinalis*, *Citrus limonum*, *Eucalyptus globulus*, *Ocimum basilicum* and DEET against *A. Stephensi* are presented in Table 2. Results showed that oils were significantly more effective than extracts. *L. officinalis* and *E. globulus* oils have most effectiveness. In this study, there was no significant differences between oil and extract of *Lavandula* (97.16 and 96.57%, respectively) and *Eucalyptus* (97.15 and 90.06%, respectively) that could indicate the better effectiveness of these two herbs in repelling of *A. Stephensi*. There are significant differences in the effectiveness rate of oils and extracts of *M. officinalis*, *R. officinalis*, *O. basilicum* and especially *C. limonum*, which repelling effect of the extracts were less than oils (Table 3). In this study, comparing repellent effect of oils rather than chemical control (DEET) was acceptable but comparing of *DEET* with extracts was not significant.

DISCUSSION

Present study shows herbal essential oils have better repellent efficacy rather than herbal extracts. Hence, essential oils could be also used as a better and safer substitution of chemical repellent substances such as *DEET* (Barat, 2012).

Previous studies regarding the extracts and essential oils of *Melissa*, *Rosemary*, *Lavender*, *lime* and *ginger* that have been done by others support our study and they

Table 3. Influence of variance analysis of extracts and essential oils of six herbs on the repellent rate of *Anopheles stephensi* in guinea pigs.

Source of variant	Mean squares	Sum of square	df	$F_{\%5}$	$F_{\%1}$	F_C
Treatment (between groups)	601.6	7219.23	12	2.15	2.96	12.09**
Error (within groups)	49.75	1293.567	26			

**Significant at 1% probability.

have also proposed the essential oils as a suitable substitution of chemical repellent (Oshaghi, 2003; Rajkumar, 2007; Barat, 2012; Kweka, 2009). In the animal experiments*, Lavender* and *Eucalyptus* oils rather than other oils had a better repellent effectiveness, 97.16 and 97.15% respectively, against *anopheles* (Gillij, 2007). Therefore, they could be recommended as a safe and suitable substitution of chemical repellent. In this work, we have tested the repellents against only one species and do not know if these compounds are protective against other mosquito species or medically important insects. By more clinical trial we may introduce the essential oils in the insect's repellent herbal cream formulation. Different factors may interfere in insect's repellent efficacy that the main factor could be effective substances of essential oils and extracts. Therefore, analysis of different fractions of herbal oils and extracts and its effect on the insects is recommended.

Most of these essential oils are highly volatile and this contributes to their poor longevity as mosquito repellents. However, this problem can be addressed by using fixatives or careful formulation to improve their longevity. For example, oils from turmeric and hairy basil with addition of 5% vanillin repelled 3 species of mosquitoes under cage conditions for a period of 6 to 8 h depending on the mosquito species (Tawatsin, 2001). The exception to this is para-menthane 3, 8 diol which has a lower vapour pressure than volatile monoterpines found in most plant oils (Barasa, 2002) and provides very high protection from a broad range of insect vectors over several hours (Carroll, 2006).

The plants can be used alone or combined for effective protection against mosquitoes. They can also be used for control of mosquito breeding (Barnard, 2004; Trongtokit, 2005). They also offer safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. However, toxicity tests of the active plants need to be done to ascertain their safety in administration (Robert, 1991; Rutledge, 1978).

Conclusion

The identification of these potential repellent plants from the local flora will generate local employment and stimulate local efforts to enhance public health. However, pilot studies are indicated to evaluate the epidemiological impact and cost-effectiveness of the natural oils which are reported to be effective in mosquito control or provide protection against mosquito bites. Further investigations are needed to elucidate the six essential oils against a wide range of mosquito species and also to identify active compounds responsible for repellent activity and to be utilized if possible, in preparing a commercial product/ formulation to be used as insecticidal.

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