ORIGINAL ARTICLE

Excito-Repellency Effects of *Salvia sclarea* L. (Lamiaceae) Extracts on Adult House Flies, *Musca domestica* L. (Diptera: Muscidae)

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Abstract

Background: Medicinal plant extracts such as those obtained from *Salvia* species have a wide variety of chemical compounds in their essential oils. The repellency of a number of essential oils including those from the labiates like *Salvia* against several insect species including the common house flies is reported.

Objective: The aim of this investigation was to find out the excito-repellency effects of *Salvia sclarea* L. (Lamiaceae) extracts against adult house flies, *Musca domestica* L. (Diptera: Muscidae).

Methods: Air-dried plant material from the aerial parts of *S. sclarea* was subjected to hydro-distillation in a Clevenger type glass apparatus model Soxhlet with acetone, benzene, petroleum ether, chloroform, and aqueous solvents. Only adult house flies were inserted into an exposure chamber and their behavior was monitored for feeding tendency, repellency rate and deterrence rate. Statistical analyses were carried out by one-way analysis of variance (ANOVA) with computation of the significance of differences in the outcome of various treatments.

Results: There were significant differences among most of the various extracts with their controls in the sequential effects of feeding (P=0.04), deterrent (P=0.023) and repellency (P=0.01) rates of house flies. The order of potency for various extracts with a concentration of 100 g/l was as follows: petroleum ether> benzene> water> acetone> chloroform.

Conclusion: It is thus conceivable to search for native means of combating house flies by fractionating the active ingredients in the Clary sage in the light of its excito-repellency effects.

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Keywords: Medicinal plant; House fly; *Musca*; *Salvia* extracts; Repellency

Introduction

Plants and animals, particularly insects, exhibit an intimate integrated relationship on an evolutionary time scale. Medicinal plants are rich sources of pesticides discovery, especially insecticides, partly due to the long association between the coexistence of insects, humans and species of plants. Herbal extracts have long been used for centuries as remedies in ailments ranging from headaches to microbial infections; yet, only in the last 2-3 decades have researchers begun to evaluate whether traditional plant-based remedies are effective and, if so, discover their mode of action. Of ~250 000 world's flowering plant species, <10% have so far been tested for their pharmaceutical properties but almost 25% of active medical compounds currently prescribed in some

western countries were isolated from higher plants.[1]

Insect infestation and microbial contamination naturally account for a wide variety of diseases in human and his domesticated animals.[2-6] They cause significant economic losses as well as health problems. The use of synthetic insecticides against pest insects has been a major part of an integrated pest management strategy.[7] Insect resistance, environmental pollution, ozone depletion, mammalian toxicity and other unfavorable effects have been reported.[8] Alternative pest control methods should thus be looked for. Of the known alternative control strategies, the use of plant-derived essential oil products instead of the conventional chemical insecticides has attracted much attention.[9,10]

The flowering plant family of Lamiaceae or Labiatae (Order: Tubiflorae) is very important since it is a highly diverse and rich source of secondary metabolites including essential oils which are volatile natural compounds with a strong odor. The medicinal plant genus, *Salvia* (sage), has about 800 species worldwide.[11] This genus is represented by 58 species of sage plants, of which 17 are native in Iran.[12] Many *Salvia* species have a wide variety of chemical compounds in their essential oils comprising mono- and sesquiterpenes, carbonylic and phenolic compounds, acids/esters, and hydrocarbons.[13] The increasingly acceptable use of essential oils is due to their low toxicity to warm-blooded animals, high volatility and also toxicity to insect pests.[14] These botanical compounds are effective, easily-biodegradable, environmentally-friendly, and often cheaper than the synthetic ones. The aerial parts of the herbaceous plant, *Salvia sclarea* (also known as Clary sage), have been used for different purposes in rural areas in many parts of Iran.[15]

The common house fly, *Musca domestica* L., is a famous cosmopolitan pest and an important mechanical vector of various pathogens. It is a major synanthropic pest near human dwellings. Over 100 different pathogens are associated with the house fly and they could cause disease in humans and animals, including anthrax, typhoid, trachoma, cholera, leprosy, tuberculosis, Q-fever, bacillary dysentery and infantile diarrhea.[16] These pathogens are picked up by flies from garbage, sewage and other sources of filth and carried on their mouthparts, through vomits, feces and contaminated external body surfaces to human and animal food.[17]

The repellency of a number of essential oils, including those from the labiates like *Salvia,* against several insect species is known. These have previously been reported in mosquitoes such as *Aedes albopictus,*[9,18] *Ae. aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus,*[19,20] and *C. pipiens,*[21,22] as well as the moth *Spodoptera littoralis,*[23] the aphid *Lipaphis pseudobrassicae,*[24] and the house fly *M. domestica* [25,26] The main aim of this investigation was to find out the excito-repellency effects of *Salvia sclarea* extracts against adult house flies, *M. domestica*, in Iran.

Materials and Methods

Plant identification and Preparation

In May 2012, Clary sage, *S. sclarea*, during blossoming stage were collected from the foothills of Cheram district (County of Dehdasht), 160 km to the west of the capital city of Yasuj in the southwest mountainous Iranian province of Kohgiluyeh and Boyer-Ahmad (30°45'N, 50°45'E, at an altitude of about 780 m above the sea level) (Figure 1).

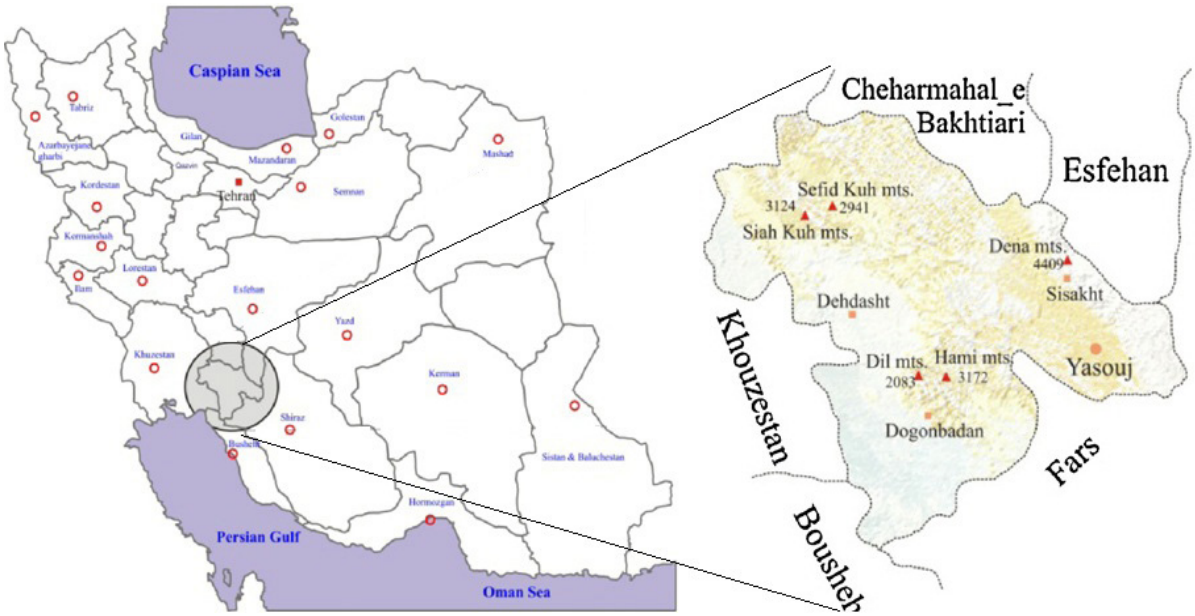

Figure 1: Map of the study area in Kohgiluyeh and Boyer-Ahmad province, Iran, shows the location of Dehdasht County in the mid-western part of the province (with slight modifications by courtesy of Mosadegh[15]).

This province is covered mostly with oak forests. A voucher specimen was deposited in the herbarium of Eram botanical garden of Shiraz University, Shiraz, Iran. Aerial parts (leaves and flowers) of the plants were dried in the shade at ambient temperature. Morphological features of *S. sclarea* were determined using Flora Iranica key[27] and a Stereomicroscope. Its broad mint-like triangularly-shaped mutual leaves on the cross-sectional squared stem are representative (Figure 2). The leaf margin is serrated. The height of this plant amounts to 60-100 cm with bi-lobed lilac flowers on the extremities of the shoot.

Isolation of the Extracts

The plants were subjected to automated grinding in an electric stainless steel grinder. Air-dried plant material (50g) from the aerial parts of *S. sclarea* was subjected to hydro-distillation for 3 h in a Clevenger type glass apparatus model Soxhlet with acetone, benzene, petroleum ether, chloroform and aqueous solvents. After extraction, the resultant samples were dried over anhydrous magnesium sulfate to delete water and kept in amber vial at 4 degree centigrade prior to the biological assays. The sample yielded 4% of solvent extracts and 18% of aqueous extract on a dry weight basis of 100 g.

House flies Rearing

Since only adult flies were to be used in this investigation, a representative sample of puparia of *Musca domestica* together with some cow pats were collected from animal pens and dens around Cheram district. These were brought to the lab and kept under optimal conditions with a temperature of 30±2°c and relative humidity of 70±5%. The emerging adults from puparia were transferred to predesigned cages with water and sugar cubes *ad libitum*.

Exposure Chamber for House Flies

Following earlier tests on malaria vector mosquitoes,[28] an exposure chamber was prepared with dimensions of 70×70×70 cm and a small (radius=5cm) entry hole equipped with a short (15 cm long) netting sleeve on the outside for leading the house flies into the system and a window trap cage on the opposite side measuring 30×30×30 cm, all made up of fiberglass (Figure 3).

Figure 2: The Clary sage, *Salvia sclarea*, grows naturally in its native habitat of Cheram district, Iran.

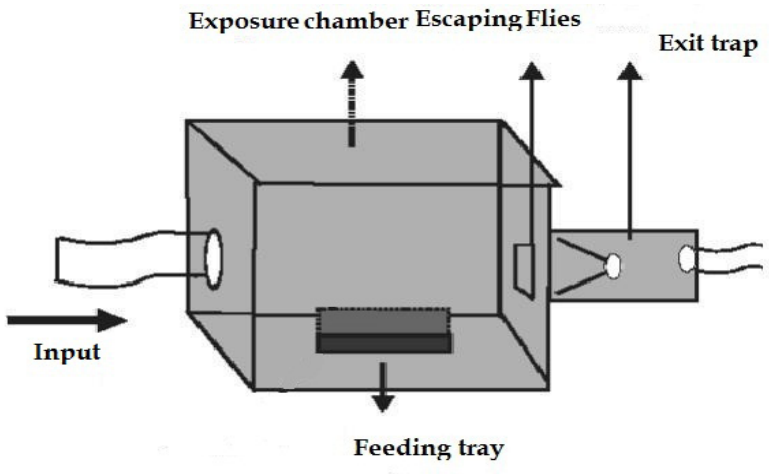

Figure 3: Exposure chamber shows the arrangement of different parts in the excito-repellency of house flies under various conditions.

To prevent the loss of extract odor from the exposure chamber, its sides were equipped with glass walls to make the interior part visible. After preparation of each extract, a small piece of white polyester net (mesh size=2 mm, area=0.1 m^2) was impregnated by standard dipping with the herbal extract (the control was left untreated) and placed over the Petri dish containing sugar cubes with water inside it on the centre stage of the exposure chamber. The house flies (25 in each trial) were starved 48 h prior to the start of each test. They were then released into the chamber following our earlier method.[28] Each trial was followed up for one hour and the behavior of the house flies was investigated. The data were recorded in specific log books.

Repellent Activity Bioassay

This study was done with two distinct doses (0.5 and 1.0 g/10 ml solvent) each replicated four times. Five extracts (aqueous, acetone, petroleum ether, benzene, chloroform) were separately tested. To fulfill the aims of this research, the tests were performed to compare the different extracts at different doses and repellency versus deterrent rates among house flies.

Data analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) with computation of the significance of differences in the outcome of various treatments. These were determined by using arcsine-transformed data (Y = Arc Sine vP) in SPSS. A *P*-value of <0.05 was considered statistically significant.

Results

The present results indicated that there were significant differences among most of the various extracts with their controls in the sequential effects of feeding (*P*=0.04), deterrent (*P*=0.023) and repellency (*P*=0.01) rates of house flies. It was shown that there was a significant difference between the repellency rate (entry into the exit trap) of house flies using aqueous extract in relation to the other extracts (*P*=0.01). There was also a significant difference between the two concentrations used (*P*=0.05), so that the entry index of the house flies at 100 g/l herbal extract was more than at 50 g/l, indicating that the higher concentration gave a larger value of repellency (Table 1).

The excito-repellency indices of house flies in the top entry cage under both concentrations indicated that the petroleum ether and benzene extracts were more effective in the deterrence of house flies than the other extracts (Table 2). The order of potency for various extracts with a concentration of 100 g/l was as follows: petroleum> benzene> water> acetone> chloroform (Table 2).

Discussion

There was an overall excito-repellency effect of *S. sclarea* extracts against adult house flies, *M. domestica*, in the southern parts of Iran. Although in comparison to the other extracts, the aqueous extract clearly revealed a better repellent activity against house flies, the petroleum and benzene extracts exhibited a marked deterrent activity against these insects. Even though the number of reports on aromatic plants used in different countries for their

Table 1: The excito-repellency index (shown as %) of the common housefly, *Musca domestica*, on exposure to different concentrations in various extracts of Clary sage, *Salvia sclarea*

	100 g/l H_2O			50 g/l H_2O		
Extract type	**Tendency to feed**	**Entry to exit trap**	**Deterrence in top entry cage**	**Tendency to feed**	**Entry to exit trap**	**Deterrence in top entry cage**
Aqueous	12	48	40	25	37	38
Acetone	27	30	43	41	23	36
Petroleum	16	32	52	31	19	51
Benzene	19	32	49	36	20	44
Chloroform	34	34	32	46	24	30
Control	82	4	14	86	3	11

Table 2: The average rates of houseflies' tendency to feed, repellency rate and deterrent rate in relation to the extract types and tested concentrations (Concn.)

Rates (Concn.)/ Extracts	Aqueous	Acetone	Petroleum	Benzene	Chloroform	**Control**
Feeding tendency (100 g)	3	6.5	4	4.7	8.2	19.3
(50 g)	6.2	10.2	7.7	7.7	11.5	18.9
Repellency rate (100 g)	12	7.5	8	8	8.2	1.2
(50 g)	9.2	5	4.7	5	6	1.3
Deterrent rate (100 g)	10.7	10.5	13	12.2	8	4.4
(50 g)	10.5	9.7	12.2	12.2	7.5	4.2

house fly-repellent activities continues to increase, to the best of our knowledge, no studies have been published on the repellent activity of *Salvia sclarea* against the common house fly, *Musca domestica* adults in Iran.

This research focused solely on excito-repellent activity with instant effectiveness after the exposure, of which the highest was within one hour of exposure to petroleum ether extract. Effectiveness is generally likely to be the most real factor in the search for noble herbal compounds. Apart from the direct impact of these compounds on repellency of house flies, secondary impacts may culminate in reduced fecundity and eggs delivery.[26] The essential oils (like β-pinene in balsamic sage) of some *Salvia* species have revealed significant insecticidal activities which vary in different insects depending on the developmental stage and the species of the insect and the plant origin of the essential oil.[10] The Clary sage is native to Iran. This plant is not only traditionally used in folk remedies but also it was found to have remarkable excito-repellent activity against house flies.

Conclusions

As a result of the strong excito-repellency effects of various extracts, particularly the petroleum and benzene extracts, of *S. sclarea* on adult house flies, it is thus conceivable to search for native means of combating these insects by fractionating the active ingredients in the Clary sage.

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Conflict of Interest: None declared

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