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Synergistic effect of two medicinal plants in cream formulation on repellency of vector mosquito and rove beetle

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Abstract

Domestic pests are major arthropod vectors responsible for several pathogenic diseases. In recent years, repellents of botanical origin, particularly essential oils, have been used against mosquitoes and have been found effective and safe. In this study, four different repellent cream formulations (I-IV) were prepared using combinations of essential oils, including *Matricaria chamomilla*, *Rosmarinus officinalis* and their repellency was tested using *Culex pipiens*, *Anopheles stephensi*, and *Paederus fuscipes* under laboratory conditions and compared to the standard synthetic repellent N,N-diethyl-meta-toluamide (DEET-12%, w/w). Among the four cream formulations, IV at a dose of 5 mg/cm2 showed the longest protection time of 289 and 275 min against *C. pipiens* and *A. stephensi*, respectively, under laboratory conditions. The formulations IV had 91% repellency against *P. fuscipes* that was near to chemical repellent DEET. The antibacterial effects of essential oils in three kinds of cream formulation were tested that mixed formulation had more antibacterial activity. The cream formulation IV showed the best mosquito biting symptom healing. Thus, formulations IV could be used in developing an effective natural repellent, antibacterial and healing the bite symptoms as an alternative to the existing synthetic repellents.

Keywords: Repellency, *Culex pipiens*, *Anopheles stephensi*, *Paederus fuscipes.*

1. Introduction

Insect-transmitted diseases remain a major cause of illness and death worldwide [1]. Among the vector insects, mosquitoes alone transmit diseases to more than 700 million people annually [2]. Mosquito-borne diseases like malaria, encephalitis, dengue, chikungunya, West Nile virus, and yellow fever cause significant morbidity and mortality in humans and livestock globally [3]. *Culex pipiens* L. (Diptera: Culicidae) is one of the most widely distributed mosquitoes in the world. The species, commonly referred to as "house mosquito", can be found in urban and suburban areas and lives near people, but feeds primarily on birds [4]. The *Anopheles stephensi* Liston (Diptera: Culicidae) is another major vector in the word as well as in some of the West Asian countries and has been shown to be directly responsible for about 40–50% of the annual malarial incidence [5, 6]. *Paederus* dermatitis is a peculiar, irritant contact dermatitis characterized by a sudden onset of erythema to bullous lesions on exposed areas of the body. The disease is provoked by *Paederus fuscipes* (Col: Staphylinidae) the common name of this insect is rove beetles [7]. This beetle does not bite or sting, but accidental brushing against or crushing the beetle over the skin provokes the release of its coelomic fluid, which contains paederin, a potent vesicant agent [8]. Various outbreaks of dermatitis attributed to the Paederus beetle have been reported in southern Turkey, central Africa, Okinawa, India and north of Iran [9].

Plants and plant-derived substances have been used to try to repel or kill mosquitoes and other domestic pest insects for a long time before the advent of synthetic chemicals [5]. A review on the uses of botanical derivatives against mosquitoes has been presented by Sukumar *et al.* [10]. Moreover, they are rich sources of bioactive compounds, including larvicides, repellents, insect growth regulators, antifeedants, ovicides, oviposition deterrents, and compounds that reduce fecundity and fertility [11-15]. Repellent properties of essential oils from many plants have been well documented. For example, eucalyptus oil from *Eucalyptus polybractea* [16, 17], clove oil from *Eugenia caryophyllus* [18], and citronella oil from *Cymbopogon nardus* [19] were individually tested for their repellency to mosquitoes. Essential oils of a large number of plants have been found to have repellent properties against various haematophagous arthropods;

some have formed the basis of commercial repellent formulations [5]. The repellency of these oils appears to be generally associated with the presence of one or more volatile mono-terpenoid constituents. Although they are effective when freshly applied, their protective effects dissipated relatively rapidly [20]. Many repellents that are currently available in the market are synthetic chemicals, such as N, N-diethyl-meta-toluamide (DEET), picaridin, and ethyl butylacetylaminopropionate. Synthetic chemical repellents aimed to protect humans from mosquito bites are fairly effective [21]. However, concerns have been raised regarding the side effects of synthetic chemical repellents, thus interest in botanical-based repellents has increased. Economically feasible plant essential oils and secondary metabolites are considered to be potential alternatives to chemical repellents in protecting against various species of mosquitoes because of their availability and environmental safety. Oils extracted from plants are widely used as fragrances in cosmetics, food additives, household products and medicines. The US Food and Drug Administration (FDA) generally recognize these as safe. They has registered some essential oils, including citronella, lemon, and eucalyptus oils, as insect repellent ingredients suitable for application to the skin [22, 23].

The oils which have been reported as potential sources of insect repellents include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint. Medicinal plants other than insect repellency have been used as medicines since centuries and recently they are used in the formation of various medicines, drugs and food [24]. Pharmacological properties of essential oils are well known for their possible antimicrobial properties [25]. Moreover, these oils are also believed to be useful against breathing and topical infections, as well as in cold and fever [26]. However, synergistic phenomena between the diverse components of the essential oils are less studied, although they may provide better repellent response (e.g., increased repellent duration) than the individual components [27, 28].

In the present study, attempts have been made to evaluate the repellency, synergistic effect, antibacterial and bite irritation healing effects of two essential oils based on cream formulation against. The characterize the relationship between different concentrations of 2 selected essential oils in cream formulation against *C. pipiens* and *A. stephensi* mosquitoes using human subjects with caged mosquitoes the direct choice method carried for first time against *P. fuscipes*. The more promising of the oils were also studied for their bite irritation healing effects activities against *C. pipiens* bitting.

2 Material and methods

For the present investigation, plants and insects samples were collected during spring and summer seasons from May, 2015- to September, 2015.

2.1 Essential oils

The *Matricaria chamomilla* and *Rosmarinus officinalis* essential oils and their mixed formulation were selected for this study because the plants are commonly available in Iran and the oils are available commercially. These oils were purchased from Giah essence Industry Co, Ltd, Golestan Province, Iran.

2.2 Insects

2.2.1 Mosquitoes: Laboratory colonies of different species of mosquitoes (*C. pipiens* and *A. stephensi*) were reared continuously for several generations in a laboratory free of exposure to pathogens and insecticides. They were maintained at 26 ± 20 ^{0}C and 60-80% relative humidity in the insectory University of Tehran. Larvae were fed on a mixture of commercial dog pellets and yeast powder (3:2 ratio) as nutrient. Adult mosquitoes were reared in humidified cages and fed with 10% glucose. Female mosquitoes were periodically blood-fed on rabbits for egg production.

2.2.2 Rove beetles: Adults of the rove beetle, *P. fuscipes*, were collected from rice fields in Rasht (Guilan province, north Iran). The insects kept in a laboratory free of exposure to pathogens and insecticides. They were maintained at 26 ± 20 ^{0}C and 60-80% relative humidity in the insectary University of Tehran.

2.3 Preparation of oil formulations

Four different essential oil formulations were prepared by mixing the above-mentioned oils. Chamomile (0.5%), Rosemary (0.5%), formulation I. Formulation II was prepared by mixing, Chamomile (1%), Rosemary (1%). Formulation III was a mixture of Chamomile (2.5%), Rosemary (2.5%). Formulation IV was prepared by mixing Chamomile (5%), Rosemary (5%).These four essential oil formulations were used for the repellent, antibacterial and bite irritation healing experiments.

2.4 Preparation of cream formulations

Cream was prepared with Phase A (40% Emulsifying wax, 2% Cetyl alcohol, 1% Bee's wax, 1.5% Lanolin at 75°C), phase B(40 mL Distilled Water) and Phase C (5.5% Glycerine and 10% essential oil of Chamomile and Rosemary each essential oil with 5% = adjusted for formulation IV). For each concentration preparation, amount of water and essential oils portion was changed. The ingredients of phase A were mixed and heated at 75°C. The ingredients of phase B were mixed separately. The mixture of phase B was mixed with phase A and stirred well at room temperature till the complete mixing. The ingredients of phase C were mixed with mixture of phase A and B and stirred well till the complete mixing. The mixture was then stirred homogeneously at a speed of 50 rpm using a mechanical stirrer. This method was followed to prepare cream formulations (I, II, III and IV) for all four essential oil mixtures. The prepared repellent creams were stored at room temperature for 24 h prior to use.

2.5 Repellent assay (mosquitoes)

Repellent bioassays were carried out in cages of dimension 45 × 45 × 40 cm based on the World Health Organization (WHO) guidelines with slight modifications [29]. The cages were built using nets and had a cotton socket sleeve on the front for forearm introduction. Females (4–6 d old, n = 100) that had not been blood-fed were released into each cage. They were provided with 10% aqueous sucrose solution as food 4 h before starting the repellent bioassay experiments. The cream formulations were used at concentrations of 0.5, 1, 2.5, and 5 mg/cm2. Both hands were covered using gloves, which had a small cut (5 cm2) on the upper side, where we applied cream formulation only in the right hand. The left arm served as the control. N, N-diethyl-meta-toluamide (12%, w/w), a commercial mosquito repellent prepared and marketed was purchased from a local general store and was used as a standard reference control. At the beginning of each test, the readiness of the mosquitoes to bite was confirmed by inserting the untreated bare hand (control hand) into the cage.

The test was then carried out by exposing the treated hand to the mosquitoes for a period of 120 s, and mosquito landings and/or probing were observed. This procedure was repeated in 15-min intervals until mosquito bites occurred. The deterrent effect of the cream formulation was considered to have worn off when two or more bites occurred during the same 120-s exposure period, or when one bite occurred in each of two consecutive exposure periods. The protection time was calculated as the time elapsed between the cream application and the first observed mosquito bite. Volunteers were told to introduce the test hand without touching their sleeves to the treated skin in order to prevent the cream from being wiped off. Each cream formulation was tested with five replicates.

$$\text{Repellency (\%)} = \frac{100 - \text{No.of bitees on treated area}}{\text{CNo.of bitees on control area}} \times 100$$

2.6 Repellent assay (rove beetle)
The repellency of rove beetle conducted by direct choice method [30]. The test arena consisted of an open glass petri dish (5.5 cm diameter) with two moistened filter paper disks (1.7 cm diameter) placed in the center 1 cm apart. In the choice test, one cream formulation treated and one cream base treated paper disk were offered to 10 insect As blank controls, two untreated paper disks were placed in the petri dish, as solvent (cream base) controls an untreated and a solvent treated disk were used The existing formulation I-IV were used. Eight replicates for each test and control experiment were performed. For 60 min the number of *P. fuscipes* in contact with each filter paper was counted every 5 min (*n* 12 counts). The average numbers of *P. fuscipes* on test-paper (cream formulation treated) and control-paper (cream base treated) for each 60-min period were compared by a paired comparisons *t*-test (*P* 0.05) [31]. In addition, the mean numbers of *P. fuscipes* in contact with filter paper in direct-choice test experiments were summed separately for each *P. fuscipes*. Mean percentage data were calculated from the total numbers of *P. fuscipes*.

$$\text{\% Repellency} = \frac{\text{No.of rove beetle on treated filter paper}}{\text{No.of rove beetle on untreated filter paper}} \times 100$$

2.7 Antibacterial Activity
*Escherichia coli, Lactobacillus acidophilus, Staphylococcus aureus, Micrococcus luteu*s and *Pseudomonas aeroginosa* were selected for evaluation of the antimicrobial activity. Disc method was opted for antimicrobial assay [32]. *Lactobacillus acidophilus* were inoculated on MRS (De Man's Rogosa and Sharpe) broth, *Escherichia coli* were inoculated on N.A. (nutrient agar) while mould. Sterile 4 mm paper discs were impregnated with essential oil of Chamomile, Rosemary and their mixture in cream formulation placed on the newly seeded bacterial lawns. One control plate for each bacterium was also prepared. After 24 hours; the inhibition zones were measured with the help of a scale to the nearest mm. The Gentamicin 1% cream was prepared as a positive control and the cream bases were also used as negative controls. The tests were replicated four times. Bacterial plates were incubated at 25 ^{0}C for 48 h.

2.8 Dermal bite irritation healing test
Occluded dermal irritation test method was adapted to determine the degree of skin irritation in human [33]. The volunteer were divided into three groups. Group 1 was left untreated, group 2 was treated with cream formulation I-IV and group 3 was treated with cream base without essential oils. The cream formulations applied on the upper side, where we applied cream formulation only in the right hand that area, before bit with mosquito. The thin layer of cream formulation IV and cream base as control were applied evenly and covered then after 5, 10, 15, 20, 25 volunteer were observed for the presence of erythema and edema as described by Draize dermal irritation scoring system [34] and the itching feeling asked from volunteer.

2.9 pH Value
Cream was mixed with deionized water in 1:2 and pH was determined at room temperature [35, 36].

2.10 Statistical analysis
Results are presented as means ± SD. All data obtained in repellent activity trials were evaluated using Tukey's test (SPSS Program; Version 11.5). The differences were considered significant at $p \leq 0.05\%$. Mean comparisons were performed using one way analysis of variance (ANOVA). Tukey test at $\alpha = 0.01$ was used to determine the difference between the mean of mortalities.

3 Results
3.1 Repellency of mosquitoes
The repellency of essential oils formulation showed in figure 1 and 2. The formulation IV with high concentrations essential oils showed the most effectiveness and provided at least 289 min complete repellency against *C. pipiens*. The protection times of these oils were less when they were diluted. The formulation I, II, III and IV with 5 g/cm2, showed 95,120, 152 and 289 min protection, respectively against *C. pipiens* and, the repellent activity decreased to 30, 59, 102 and 133 min or less when diluted to 5% (formulation I). The results showed that (Table 3, 4) the formulation IV was more effective on *C. pipiens* in compare with *A. stephensi*. The IV formulation showed the highest protection time against all three species of insects. The mean durations of protection from bites for formulation I, II, III and IV in 2.5 g/cm2 application were 29, 56, 101and 133 min and 41, 89, 92 and 153 min against *C. pipiens*, respectively (Table 3).
The mean protection time of cream formulations screened against *C. pipiens* and *A. stephensi* in the laboratory. All four cream formulations showed repellency to the tested insects species with variable protection times. Among the four different cream formulations, formulation IV at the highest dose of 5 mg/cm2 showed the highest protection time up to 230 min against *A. stephensi*, (Tables 4), with 81% repellency. The standard reference control DEET (12%, w/w) showed the highest protection time against *C. pipiens* (401 min) and *A. stephensi* (361 min) under laboratory conditions

3.2 Repellency of rove beetle
In both blank and solvent controls formulation I-IV equally contacted two simultaneously offered filter paper disks. For each control, the mean numbers of *P. fuscipes* counted on both control disks in the test arena gave no significant difference (Fig. 2). The *P. fuscipes* showed significant avoidance behavior toward an extract-treated paper disk. During the 60-min test period the number of *P. fuscipes* in contact with the control disk significantly exceeded the number of *P. fuscipes* on the corresponding extract-treated disk (Fig. 2). The relative numbers of *P. fuscipes* counted on filter papers during a 60-min test period significantly differed among formulation were applied on filter papers. Repellency for formulation I-IV was 19, 43, 61 and 91% respectively and for DEET was 95%.

3.3 Antimicrobial Activities of the Essential Oils
The antimicrobial activities determined using the agar diffusion assays are given in Table 1 for the novel essential oils of *M. chamomilla* and *R. officinalis*. Except for *P. aeruginosa* with 5.6, 6.1, 8.2 mm inhibition against Chamomile, Rosemary and formulation IV respectively, which showed little or no sensitivity, all the remaining test organisms displayed a high level of sensitivity towards mixed formulation and Rosemary oil. *Escherichia coli*, another isolate, were particularly sensitive to this oil. Rosemary oil showed a moderate antimicrobial activity against all the test organisms, with *P. aeruginosa* being the least sensitive (with 6.9 mm inhibition). However, the oil has a unique aroma, which makes it a good candidate for use as a fragrance ingredient and the Chamomile showed the lowest inhibition with 00.0, 5.2, 15.2, 7.5 and 5.6 mm inhibition against *Lactobacillus acidophilus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa*. In general, oils of the aromatic plants, showed remarkable antimicrobial activities against the test organisms (Table 1). However, the intensity of the activity varied with the type of the oil and the concentration used. Formulation IV was the most efficient. Rosemary had better antibacterial activity in compare to Chamomile (Table 3).

3.4 pH Value
The pH of formulation were 5.7-6.1 volume in all cases.

3.5 Healing the bite irritation
Formulation IV had the highest rash healing between other formulations we showed that with increasing the concentration of essential oils the bite healing rate increased. The recovery time for formulation I and II were near the control with 12-15% irritation complain after 25 min, formulation III with 2% irritation complain after 25 min was better than formulation I and II. Formulation IV had the best recovery activity after 20 min the volunteer completely recovered and in 15 min there was 7% irritation symptom.

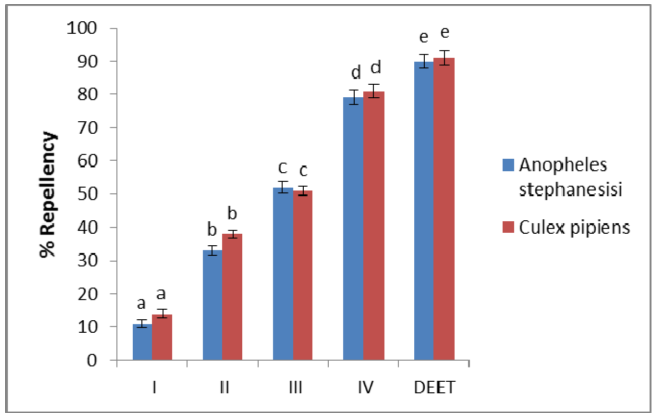

Fig 1: Repellency of *R. officinalis*, *M. chamomilla* and their mixed formulation I-IV against mosquito, DEET were used as positive control.

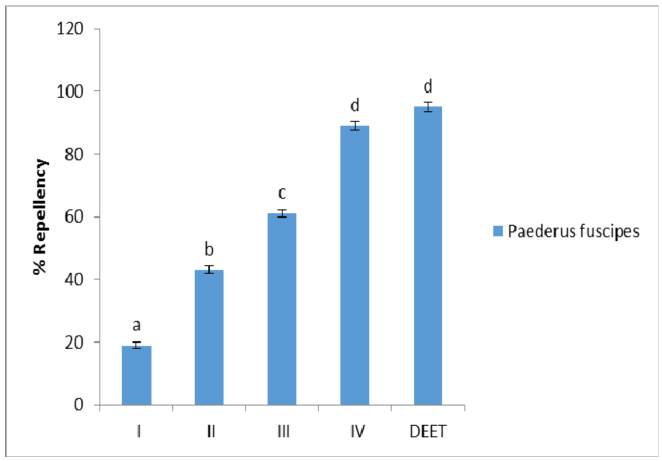

Fig 2: Repellency of *R. officinalis*, *M. chamomilla* and their mixed formulation I-IV against *P. fuscipes*, DEET were used as positive control.

Table 1: Antibacterial activity of the *R. officinalis*, *M. chamomilla* in cream formulation and formulation IV expressed as diameter of zone of inhibition in millimetres (including disc diameter of 6 mm) against selected bacteria, Gentomycin were used as positive control.

Bacteria	Diameter of zone of inhibition (mm)±SDV			
	Matricaria chamomilla (5%)	*Rosmarinus Officinalis* (5%)	Formulation IV (5+5%)	Gentamicin cream 1%
Lactobacillus acidophilus	0.00 ± 0.0	2.3 ± 0.5	2.4 ± 0.7	20
Micrococcus luteus	5.2± 0.3	9.2± 0.3	12.2± 3.3	22
Staphylococcus aureus	15.2± 3. 4	18.4± 2. 1	21.2± 2. 7	27
Escherichia coli	7.5± 0. 6	12.3± 2. 2	15.9± 1. 4	25
Pseudomonas aeroginosa	5.6± 0.6	6.9± 1.3	8.2± 0. 4	24

The values are averages of four replicates standard deviation (SD).

Table 2 The bite irritation healing activity of formulation I- IV in human that bited with *C. pipiens*, table represents the time of volunteer with rash and irritation complain after biting.

Group (g/cm2)	Time (min)				
	5	10	15	20	25
Control	75	53	40	32	15
Cream bases without essential oils	79	57	41	31	14
Cream formulation I	71	55	37	30	12
Cream formulation II	65	32	21	18	8
Cream formulation III	46	27	16	9	2
Cream formulation IV	21	15	7	-	-

Table 3: The repellent activity time of topical cream formulation of mixed essential oil in different dose against *C. pipiens* bite

Group (g/cm2)	Time (min)				
	0.5	1	2.5	5	Control
Cream formulation I	10	17	29	95	1.4± 3.3
Cream formulation II	19	25	56	120	1.3± 3.3
Cream formulation III		41	101	152	1.5± 3.3
Cream formulation IV	23	69	133	289	1.2± 3.3
DEET	43	89	221	401	1.3± 3.3

Table 4: The repellent activity time of topical cream formulation of mixed essential oil in different dose against *A. stephensi* bite

Group (g/cm2)	Time (min)				
	0.5	1	2.5	5	Control
Cream formulation I	9	14	41	104	1.4± 3.3
Cream formulation II	22	20	89	150	1.3± 3.3
Cream formulation III	39	33	92	193	1.2± 3.3
Cream formulation IV	19	52	153	275	1.4± 3.3
DEET	32	68	169	361	1.5± 3.3

4. Discussion

Many plant extracts and essential oils possess repellent properties against various insect pests, and several plant oils have been identified as potential botanical sources of insect repellents [37-39]. Essential oils from various plant species have been tested on different species of mosquitoes worldwide. However, the repellent activity of essential oils against vector mosquitoes differs with many factors, including the organic properties of plants, plant parts used, distillation method, environmental factors, and the species and age of the targeted mosquitoes [40, 41]. Plants containing polyphenols have been used for their wound healing properties, instead of using the current methods like antibiotics which has unwanted side effects. These compounds are capable of promoting rapid reepithelialisation of acute wounds and burns and have antimicrobial properties. In this study the mean protection time of four different cream formulations screened against *C. pipiens* and *A. stephensi* that all four cream formulations showed repellency to the tested insects species with variable protection times. Among the four different cream formulations, formulation IV at the highest showed the highest protection. Our result showed greater activity compared to that in an earlier study by Oyedele *et al* [42], who recorded 50% repellency for 2–3 h to 2-day starved *A. aegypti* when using a 1% v/v solution concentration of cream and ointment (15% v/w) prepared from lemongrass oil. The repellent activity of formulation IV to *C. pipiens* and *A. stephensi* in the present study was comparable to the results of Tawatsin *et al* [38]; they mixed *Cymbopogon winterianus* oil with 5% vanillin and achieved 100% protection for 6 h against *C. quinquefasciatus*, *A. aegypti*, and *Anopheles dirus*. In all treatments, the protection time increased with an increase in concentration. Our results showed that formulation III was moderately effective and formulation I and formulation II were less effective, Choochote *et al*. [43] investigated the repellent activities of essential oils obtained from ten plant species and found that oils of *Zanthoxylum piperitum*, *Anethum graveolens*, and *Kaempferia galangal* offered protection against *A. aegypti*, with median complete protection times of 1 h, 0.5 h, and 0.25 h, respectively. None of the volunteers indicated hypersensitivity, uneasiness, or discomfort while using the prepared cream formulations and DEET cream. Chio and Yang [44] reported the repellent activity of neem seed (*Azadirachta indica*) oil and djulis (*Chenopodium* spp.) seed extract; activity was higher in djulis seed extract (0.930%) than in neem oil (0.579%) against the Asian tiger mosquito *A. albopictus*. In another report, *Baccharis spartioides*, *Rosmarinus officinalis*, and *Aloysia citriodora* oils at 12.5% w/w provided the longest protection time of 90 min against *A. aegypti* [28]. In our study, the standard reference control DEET (12%, w/w) showed the highest protection times against *C. pipiens* (401 min) and *A. stephensi* (361 min) under laboratory conditions. However, at the lowest concentration (1 mg/cm2), no difference was recorded in the effects of DEET (12%, w/w) and formulation IV by Tukey's test ($p = 0.05$) against *C. pipiens*. Curtis *et al.* (1987) showed that Anopheles mosquitoes were less sensitive to DEET and other repellent chemicals than *A. aegypti*. For oils manifesting insects repellency, the protection time generally increased with increasing oil concentration. Our results also showed that the relative repellency increased as the dose was increased. Furthermore *A. aegypti*, the traditional test species for repellent studies, was an exceptionally poor predictor for the responses of *A. stephensi* to repellents. The present results showed that of the four formulations tested, the formulation III and IV provided better protection against all tested insects. The mean duration of repellency on *C. pipiens* was slightly greater than from the others.

The essential oil of Rosemary and their mixture with Chamomile displayed significantly inhibitory activity against all bacteria used in the study (Table 1). The inhibition of Gram-negative test bacteria, namely *E. coli, P. aeruginosa* (during the challenge test in four cream formulation is displayed in Table 1. The population of *E. coli* was progressively reduced by rosemary formulation tested during the challenge test in the cream formulation (Figure 1). The population of this test microorganism seemed to be effectively controlled by Rosemary oils and their mixed formulation with Chamomile, respectively. A similar trend in the control suggested that combined factors intrinsic to the formulation also contributed to the reduced viability observed. Overall, Rosemary oils and their mixed formulation with Chamomile showed some antimicrobial reduction abilities, although the inhibition varied with the test organism species, the type and the concentration of the oil used, as well as the time of incubation. Rosemary oils and their mixed formulation with Chamomile seemed to give more inhibition than Chamomile oil. Except for *Pseudomonas* strains that displayed less inhibition in both the control and the test, the population of all the other microorganisms tested were remarkably controlled by the oils tested. The plants' essential oils showed remarkable preservative capabilities and could therefore be considered as alternative preservatives for cream formulations. The mixture of essential oils formulation, proved to be the most effective of all the oils tested with regard to protecting the cream against microbial contamination. The inhibition was stronger to gram positive than gram negative bacteria. These results showed that mixed formulation oil was very active against those dermatophytes. Factors that affect the release of an active principle from a base include its affinity for the base and the viscosity of the preparation [42]. Moreover, the good appearance with pH similarly to the skin and highly stable should be considered for the suitable base. The color of all cream bases freshly prepared in this study was white. The pH and average viscosity of the bases varied between 6-7 and 500-50,000 centipoise depending on the amount and kinds of ingredients in each base. The pH of all formulations showed an optimum value, similar to the healthy skin pH, of 5-6, lower than that of the bases. This could be due to the low pH of the oil. The freshly prepared creams exhibited the antimicrobial activity whereas the bases showed no activity. The antibacterial effect of the creams increased with increase in essential oil content, we found that Rosemary oil had more antibacterial effects in compare to Chamomile but in mixed formulation the data showed some synergistic effects against tested bacteria (Table 1). The nature of base in which a drug is formulated has considerable effect on its efficacy [45]. Gentamycin showed markedly higher activity than our cream formulations containing 10% oil. The above-mentioned results of antibacterial activity of creams demonstrated that the higher content of the oil, the higher activity of the cream. Therefore, the concentration of 10% oil in cream base was considered to be suitable for cream formulations for further studies. The present work revealed the synergistic effect of the tested essential oil-based cream formulations on repellency. We obtained the best repellent activity with formulation IV, which contained a combination of oils of Rosemary and Chamomile, against the *A. stephensi* as well as the *C. pipiens* and *P. fuscipes* compared to other formulations under laboratory conditions. Further, our antibacterial activity study indicated that formulation IV was effective against some important bacteria. Our results may contribute to a reduction in the application of synthetic repellents and will provide a basis for developing new, effective, safe, and affordable insect repellents from plant-derived essential oil formulations. Further studies are needed to develop more appropriate formulations including a fixative, which would increase their efficacy and cost effectiveness. Field trials should be carried out, particularly to evaluate the operational feasibility and dermal toxicity over a long period, especially to infants and children. It is important to determine whether widespread use of one of these repellents would produce an overall reduction of vector biting in a community or would simply divert biting from repellent users to non-users.

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