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Full Length Research Paper

Chemical composition, repellent and antimicrobial activity of *Schinus molle* L.

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The aim of this research was to determine the repellent and antimicrobial activities of the essential oil and hexanic extracts obtained from the leaves and fruits of *Schinus molle* L. (Anacardiaceae). The composition of essential oils and hexanic extracts were analyzed by GC-MS. Oriental cockroach (*Blatta orientalis* L.) adults and nine bacterial strains were used for repellent and antimicrobial activity assays, respectively. The best antimicrobial activities were determined at 30 µg/ml dosage for *Escherichia coli* 0157:H7 and *Bacillus cereus* with leaf extracts. At all doses (176, 70 and 35 µg/cm^2) the leaf essential oil showed not significant repellency than the positive control, DEET (*N,N* diethyl-*m*-toluamide), Hexanic extract of the leaf showed good repellency (83.33%) at the highest concentration (0.075 mg/L) against the oriental cockroach. The results indicated that the essential oil and hexanic extracts of *S. molle* showed potential in terms of antimicrobial and repellent activities.

Key words: Antimicrobial, *Blatta orientalis*, essential oil, *Schinus molle*, repellent.

INTRODUCTION

Schinus molle L. (Anacardiaceae), also known as Brazilian pepper tree, is a tree which is short and has thin, long leaves and it is often used in subtropical climates for landscaping. *Schinus* sp. has been traditionally used as medicine by indigenous people throughout the tropics (Erazo et al., 2006). Recent research show that extracts obtained from *S. molle* can be used as an analgesic (pain-reliever), anti-inflammatory and anti-tumorous agent (Barrachina et al., 1997; Yueqin et al., 2003; Diaz et al., 2008). It also possessesial potent antibacterial, antiviral, antifungal, insecticidal and repellent properties (Dikshit et al., 1986; Chopa et al., 2006; Ferrero and Gonzales, 2006; Ferrero et al, 2007; Padin et al., 2007; Bayramoglu et al., 2008).

Cockroaches (Blattidae) are insects that inhabit public places like hospitals, residential areas and food processing and serving facilities (Kutrup, 2003; Yoon et al., 2009). These insects have the potential to physically carry and transmit many pathogens such as bacteria, viruses, fungi, protozoa and helminthes. At present, cockroach combat is generally carried out with synthetic-based chemicals (Cochran, 1999). However, the development of resistance in cockroaches, as well as the hazardous effects on human health and the negative effects on the environment has limited the use of these chemicals. Therefore, intensive efforts are being put to find alternative environmentally friendly compounds such as plant-based repellents (Ngoh et al., 1998; Thavara et al., 2007; Yoon et al., 2009).

Up to now, no study has been reported on the evaluation of plant-based repellents against cockroaches in Turkey. The present study was initiated to study the repellent activity of extracts and essential oils from leaf and fruits (ripe and unripe) of *S. molle* against the adults of Oriental cockroach and to determine the antimicrobial activity against *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus, Staphylococcus aureus* (MRSA), *Enterococcus feacalis*, *Escherichia coli* 0157:H7, *Escherichia coli*, *Salmonella typhimurium* and *Candida albicans*. The activity of these oils and extracts were compared with control agents.

MATERIALS AND METHODS

Plant material

The leaves, unripe fruits and ripe fruits of *S. molle* were collected from Bornova, Izmir in December 2008. Leaf samples were dried at

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30˚C in an oven and all samples were stored at +4˚C.

Chemicals

Positive control, DEET (*N,N* diethyl-*m*-toluamide) was obtained from MATSAN A.Ş., Kocaeli, Turkey, at 98% purity. Hexane (Merck) was used as extraction solvent at 99% purity.

Extraction of essential oils

For the essential oil extraction, 100 g of each parts of the plant material was mixed with 600 ml distilled water and then distilled for 2 h using the Clevenger apparatus. After distillation, 3.04, 3.75 and 3 ml oils were obtained from leaves, unripe fruits and ripe fruits, respectively, dried with sodium sulphate (Na_2SO_4) and were stored at +4˚C

Preparation of hexanic extracts

For the water insoluble oils extractions hexane, which is a nonpolar compound, was chosen as the solvent. Twenty five (25) g biological material was pulverized in the mixer and placed into the Soxlet appliance containing 750 ml hexane and then left to extraction for 2 h. The hexane was removed using a vacuum evaporator and the remaining material was dissolved in ethanol at a proper ratio. As a result, 0.68, 0.44 and 0.13 g/ml oils were obtained from unripe and ripe fruits and leaves, respectively and were stored at +4˚C.

GC/MS analysis

Essential oils and extracts were analyzed by GC/MS. The analyses were performed with an Agilent GC 6890 MS 5973. The gas chromatography equipped with a HP-Innowax (60 m × 0.32 mm × 0.5 µm). The carrier gas was helium at 11.4 psi, constant pressure and the split ratio was 1/100, whereas the temperature of the column was programmed from 70 - 210˚C at 7˚C/min. The injection port was set at 150˚C and detector at 250˚C. The injection volume was 1 µL. Components of the oil were identified by comparison of their mass spectra and retention indices and contained in the NIST ’98 MS computer library (Wiley).

Antimicrobial activity

Microorganisms

B. cereus CCM99, *M. luteus* ATTC 9341, *S. aureus* ATCC 6538, methicillin-resistant *S. aureus* (MRSA) RSSK 95047, *E. feacalis* ATCC 29212, *E. coli* 0157:H7 RSSK 234, *E. coli* ATCC 11230, *S. typhimurium* CCM 5445, *C. albicans* ATCC 10239 were used as test organisms to determine antimicrobial activity.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Agar dilution susceptibility assay was performed according to National Committee for Clinical Laboratory Standards (NCCLS 2000), for the determination of minimum inhibition concentration (MIC) of the samples. Bacterial strains were cultured to activation for 24 h at 37˚C in Mueller Hinton agar (MHA) and the yeast was cultured 24 h at 30˚C in Saboraud dextrose agar (SDA). Test media were prepared containing different concentrations of extracts ranging from 0.03 - 8 mg/ml, the suspension of the test microorganisms were adjusted to 0.5 McFarland standard. Finally, 3 - 4 µl 10^6 cfu/ml of the test organism suspension was spotted onto agar media. Petri dishes were incubated at 37˚C for 24 h for bacterial cultures and at 30˚C for 48 h for the yeast. The same test was performed using gentamycin as a reference positive control compound MBCs were confirmed by reinoculating the cultures on agar plates containing no extract and/or oil.

Repellent activity

Test cockroaches

The cockroach species used in repellency assays, *Blatta orientalis* L., were collected in Trabzon, Turkey in 2008 and laboratory-reared in the Department of Zoology, Ege University, Turkey at 24(±2)°C and 60(±5)% relative humidity with a photoperiod of 12:12 (L:D) h. They were kept in glass jars and fed with mouse food pellets. Adult cockroaches selected for the tests were separated from the master colony 48 h prior to the bioassays. Both sexes of *B. orientalis* were used for the repellency tests, since no significant difference was determined in preliminary experiments (unpublished data) for sensitivity to olfactory stimuli regarding sexes.

Repellency assays

The choice-test assay was used for repellency test by slight modification of the method of Chopa et al. (2006). Circular filter papers (15 cm diameter) were cut in two halves and one was treated with 1 ml of the test compound solution; the other half was treated with 1 ml of either petroleum ether (solvent for essential oil treatments) or hexane (solvent for extract treatments). After the treatments, the filter papers were aired for 60 min for extract solutions and for 5 min for essential oil solutions in order to evaporate the solvent. Both halves were fitted together and placed at the bottom of the Petri dish as a single layer. To eliminate any effect resulting from the residual solvents another control experiment was designed where, one of the halves was treated with solvents (petroleum ether or hexane) and the other one was left untreated. The concentrations tested for each essential oil solution were 176, 70 and 35 µg/cm^2 and for the extract solution, 0.075, 0.05 and 0.025 mg/L. The essential oils and extract concentrations were determined according to previous studies made by Chopa et al. (2006) and Ferrero et al. (2007). DEET was dissolved in acetone and used as positive control. Ten cockroaches of mixed sexes were released at the center of each plate. They were kept in a dark and isolated environment to prevent disturbances from surroundings. After 24 h, their distribution was carefully observed and recorded. Repellency against the cockroaches was calculated with the following equation:

Repellency (%) = 100 – {(T x 100) / N}

Where; T: Number of cockroaches located in the treated zone, N: Total number of cockroaches used,

Each experiment was repeated three times and the average repellency was calculated from the values obtained from these replicates. Repellency values of 60 - 70, 70 - 80, 80 - 90 and 90 - 100% were ranked as weak, moderate, good and high repellencies, respectively.

Data analysis

ANOVA was carried out with SPSS 16.0. Twenty four hours repellent activity means were compared with the Duncan Multiple

Table 1. The compositions of the leaf extract and essential oil.

Retention time	component name	Extract %	Oil %
2.56	Sabinen	-	2.56
6.95	Alpha-pinene	0.6	0.87
7.44	Camphene	-	0.4
7.94	Beta Pinene	-	0.13
8.42	Myrcene	0.58	0.47
8.63	Alpha-phellandrene	3.53	6.94
9.13	Limonene, Cinene, Cajeputene	4.09	2.98
9.19	Beta-Thujone	4.36	-
10.3	P-cymene	6.16	1.56
15.28	Alpha gurjunene	3.28	1.38
15.94	Endobornyl acetate	0.69	-
16.09	Elemene	0.72	-
16.28	Beta Elemene	-	0.48
16.32	Beta-Ceryophyllene	13.48	4.3
17.31	Alpha copaene	2	0.47
17.31	Trans Caryophyllene	2.12	-
17.52	(+) Aromadendrene	-	0.54
17.96	Alpha-humulene	3.63	2.04
18.65	Germacrene D	20.77	6.54
19.07	Bicyclogermacrene	2.04	1.82
19.33	Delta Cadinene	4.09	11.28
20.01	Alpha Cadinene	-	0.59
24.77	(+) Fenchon	-	3.24
25.19	Elemol	-	5.11
25.6	Ledene	-	5.21
27.41	Calarene	-	4.68
28.9	Alpha Eudesmol	-	2.38
29.05	Alpha cadinol	-	10.77

Range test ($p < 0.05$).

RESULTS AND DISCUSSIONS

Chemical composition of *S. molle* leaf essential oil and extract

When the plant extracts and essential oils obtained from different parts (leaves, unripe fruits and ripe fruits) of test plant are compared in terms of antimicrobial and repellent activities leaves extracts and essential oils were found more effective than unripe fruits and ripe fruits. Therefore, Table 1 show only the leaf essential oil and extracts compositions. Sixteen components were identified comprising 72.14% of the total components in the extracts. The major components of the leaf extract were Germacrene D (20.77%) and Beta-ceryophyllene (13.48%). Essential oil of leaf contained 24 components; mainly delta-cadinene (11.28%) and alpha-cadinol (10.77%) (Table 1). Rossini et al. (1996) reported delta-cadinene as major component of leaf oil. The results show that the composition of *S. molle* exhibit significant differences. The composition may differ by season and the region that the plant material was collected (Abdel-Sattar, 2010).

Antimicrobial activity

Antimicrobial activities of the extracts and essential oils were evaluated by MIC and MBC values (Table 2). The results showed that the extracts have better antimicrobial activity than essential oils. The essential oil of unripe fruits did not show any antimicrobial activity, whereas MIC values the essential oil of ripe fruits were determined as 4 ≥ mg/ml for *E. coli* 0157:H7 and 8 ≥ mg/ml for *E. coli*. Essential oil of the leaves exhibited more promising results. The MIC values were 2 ≥ mg/ml for *E. coli* 0157:H7, *B. cereus*, *S. aureus*, 4 ≥ mg/ml for *S. aureus* (MRSA) and *M. luteus* and 8 ≥ mg/ml for *E. coli*, respectively. MBC results of the essential oils of leaves were determined as ≥ 4 mg/ml for *S. aureus, S. aureus* MRSA and *M. luteus.* The antimicrobial activity against *S. aureus* was also reported previously by Hayouni et al.

Table 2. MIC and MBC values of extracts and essential oils of *S. molle*.

Microorganisms	Extracts			Essential oils			
	Leaf	ripe fruits	unripe fruits	Leaf	ripe fruits	unripe fruits	G
	mg/ml			mg/ml			µg/ml
E. coli ATCC 11230	8> / NA	4≥ / 8>	8> / 8>	8≥ / 8>	8> / NA	8> / NA	10
E. coli 0157:H7 RSSK 234	0.03 ≤ / 0,03 ≤	0.03< / 8>	0,03< / 8>	2≥ / 8>	4≥ / 8>	8> / NA	5
E. feacalis ATCC 29212	8≥ / 8>	8≥ / 8>	8> / NA	8> / NA	8> / NA	8> / NA	10
S. typhimurium CCM 5445	8> / NA	8> / NA	8> / NA	8> / NA	8> / NA	8> / NA	20
S. aureus ATCC 43300 (MRSA)	1≥ / 2 ≥	8> / NA	2≥ / 8>	4≥ / 4≥	8> / NA	8> / NA	5
S. aureus ATCC 6538	0.5≥ / 1 ≥	2≥ / 8>	1≥ / 2 ≥	2≥ / 4 ≥	8> / NA	8> / NA	2.5
M. luteus ATTC 9341	1≥ / 1 ≥	2≥ / 8>	2≥ / 8 ≥	4≥ / 4 ≥	8> / NA	8> / NA	1.25
B. cereus CCM99	0.03< / 8>	0.12≥ / 8>	0.03< / 8>	2≥ / 8>	8> / NA	8> / NA	5
C. albicans ATCC 10239	8≥ / 8>	8> / 8>	8≥ / 8≥	8> / NA	8> / NA	8> / NA	----

NA: not applicable, G: gentamycin, MRSA: methicillin resistant *S. aureus*.

Table 3. Percentage repellency (±SEM) of DEET and *S. molle* essentials oil from fruits and leaf against *B. orientalis* in a choice-test assay.

Doses(µg/cm^2)	Essential oils			DEET
	Ripe fruit	Unripe fruit	Leaf	
35.38	70.0 aB	46.6 aA	93.3 aC	90.0 aC
70	80.0 aB	53.3 abA	96.6 aC	96.6 bC
176	83.3 aB	63.3 bA	100 aC	100 bC

Means followed by the same letter are not significantly different (Duncan P ≤ 0.05). Capital letter for each row, small letters for each column.

Table 4. Percentage repellency (±SEM) of DEET and *S. molle* hexanic extracts from leaf to *B. orientalis* in a choice-test assay.

Concentration (mg/l)	Leaf extract	DEET
0.025	53.3 aA	86.6 aB
0.05	66.6 bA	96.6 bB
0.075	83.3 cA	100 bB

Means followed by the same letter are not significantly different (Duncan P ≤ 0.05). Capital letter for each row, small letters for each column.

(2008).

Although, the leaf extract gave the best results in terms of antimicrobial activity amongst the three different types of plant materials, it did not show any activity against *E. coli* and *S. thyphimurium.* It showed weak activity against *E. feacalis* and *C. albicans*. Correspondingly, the best MIC and MBC results for leaf extracts were determined as 30 µg/l for *E. coli* 0157:H7. While unripe and ripe fruits extracts exhibited lower MIC values, MBC values were observed to be outside the range of concentrations tested. MIC results of leaf extract were also determined against *B. cereus* (0.03 mg/ml), *S. aureus* (0.5 mg/ml), *S.* also effective at low concentrations against *E. coli* 0157:H7, *S. aureus* MRSA, *S. aureus*, *M. luteus* and *B. cereus*.

Repellent activity

The repellent activity rates of all essential oils and leaf extract against the oriental cockroach (*B. orientalis*) are shown in Tables 3 and 4. All materials showed repellency in varying degrees against *B. orientalis* adults. At all doses, both leaf essential oil and DEET were found to be equally effective, while fruit oils and leaf extract were not as effective as the leaf essential oil was found more effective than the leaf extract. There were no significant differences among the all doses from fruit oils and leaf oil

in terms of repellent activity. The leaf extract showed dose dependant activity.

Chopa et al. (2006) reported high repellency of the essential oils from ripe fruits and no repellency from leaves of *S. molle* against the German cockroach. Their study agrees with the former results. However, unlike this study, we have shown that the essential oil from the leaves of *S. molle* has high repellency against the oriental cockroach. This discrepancy between the two studies may be due to the different cockroach species used in the experiments and their sensitivity to olfactory stimuli. In a previous study, it was shown that both petroleum ether and ethanol extracts of *S. molle* leaves exhibited a high repellency effect against the German cockroach (Ferrero et al., 2007). Their findings agree with the results of this study, although for the extracts in this study hexane was used as solvent instead of petroleum ether or ethanol. The same study reported the extract of *S. molle* fruits as highly repellent to German cockroaches. This is in contradiction with our results, as our preliminary tests did not indicate any repellence to the oriental cockroach (unpublished data). This discrepancy may be due to the maturity of the fruit used in the study, which was not stated, and/or the different sensitivity to olfactory stimuli of two different cockroach species.

Germacrene D, one of the two major components identified from the leaf extract, has been reported as showing repellent activity against cattle ticks and aphids (Bruce et al., 2005; Birkett et al., 2008). The other major component identified from the leaf extract, beta-caryophyllene, was reported as being repellent to aphids as well (Liu et al., 2010). Also, delta-cadinene and alpha-cadinol, the two major components identified from the essential oil of the leaf, have been reported as repellents against some arthropods (He et al., 1997; Yatagai et al., 2002). The repellent effect of the *S. molle* against the oriental cockroach can be attributed to these components of the extract in this research.

Conclusion

The results presented herein indicate that essential oil and hexanic extracts of *S. molle* show potential results in terms of antimicrobial and repellent activity. Further studies are required in order to confirm whether these components can act individually as repellents or antimicrobial agents. These findings are promising for the non-chemical control of the Oriental cockroach and further experiments are needed to determine the efficiencies and effectiveness of these biological compounds. With respect to antimicrobial activity, hexanic extract of leaves of *S. molle* showed the highest effect on *E. coli* 0 157:H7 indicating that the extract can be used as a preservative or food additive or even as a mild disinfectant in various places where *E. coli* infection is a threat. Also, new studies are needed on both antimicrobial and repellent activities of the extracts and oils for proper formulation and commercialization as a natural, environmentally safe product.

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