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Full Length Research Paper

Natural insecticides and phytochemical analysis of gaggassa (*Agarista salicifolia)* plant leaves against brown banded cockroach

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A plant of genus gaggassa belongs to the family of Ericaceae, and has been widely employed by the traditional healers to treat cockroach pest. The objective of this study was to do preliminary phytochemical screening and insecticidal activity of extracted gaggassa (*Agarista salicifolia*) plant leaves against cockroach. About 10 g of air dried powdered material of leaves was extracted with 90% ethanol, acetone and water using an electric shaker for 24 h. Thereafter, the extract material was concentrated to dryness under reduced pressure and controlled temperature (50° C) using rotary evaporator. Different leaf extract concentrations of 25, 50, 75 and 100%, respectively were implemented in triplicate experiment and mortality was assessed after 4, 8 and 12 h of treatment time interval. The toxicity test extract of plant was carried out by using adult cockroach, and obtained result showed extracts of LC_{50} of 1.44 mg/mL from water extract and 1.33 mg/mL from ethanol respectively. No mortality was observed in control treatment. Preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, flavonoids, steroids, tannins and cardiac glycosides. Finally, the high polyphenolic and flavonoid contents of the plants suggest their potential source of botanical insecticides. Overall results suggest that extracts from gaggassa plant leaves showed the highest insecticidal effects on cockroach. Considering the side effect of chemical insecticides to human health, it is suggested that the use of organic insecticides should be encouraged so as to ameliorate health problems, since it is eco-friendly in nature. The plants are good candidates to be developed as sources of natural insecticides for the pest management.

Key words: Bioactive compounds, ethanolic extracts, total phenolic content and toxicity.

INTRODUCTION

In about 20% of homes without visible evidence especially in the urban environment, cockroaches had mechanically carried and transmit many pathogenic reactions in humans, such as bacteria, viruses, fungi, protozoa and helminthes (Cochran, 1982). They also serve as potential causes of bacterial diarrhea and nosocomial infections in hospitals (Agbodaze and Owusu, 1989). There is an ample evidence which show that

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substances produced by cockroaches are involved in allergic symptoms (Kongpanichkul et al., 1997; Pumhirun et al., 1997).

Cockroaches are found all over the world with about 3500 known species. They are among the notorious pests that are found in households, supermarkets, public places, and refuse dumps. Some evidence shows that the number of immuno-compromised people and bacterial drug resistance is on the increase in Ethiopia. To prevent the parasites of cockroaches and the potential in diseases transmission, products usually rely on chemical insecticides. However, the use of synthetic chemicals to control insect pests has led to several adverse effects, including water and soil contamination, insect resistance, toxicity to man and animals, environmental pollution and toxicity to non-target species (Donahay et al., 1992; Kumar et al., 2011; Umadevi and Sujatha, 2013). Currently, botanical pesticides are a promising source of insecticidal activity, being easy to process and apply on residual activity and not accumulating in the environment because they are highly biodegradable (Berger, 1994; Kuusik et al., 1995; Zhishen et al., 1999; Carlini and Grossi-de Sá, 2002; Clemente et al., 2003; Yinebeb Tariku, 2008; Vinha et al., 2012). The utilization of botanical insecticides in cockroach pest control demonstrates to be very promising, mainly due to the environment being less harmful than synthetic pesticides and maximizing the insecticidal effect. Several plants may have insecticidal activities against cockroach and among them; the gaggassa (*Agarista salicifolia* (Comm. ex Lam.) G. Don)) plants which belong to the Ericaceae family, and potential bioactive plants extensively studied in laboratory in the field against several insects. The present study is on the preliminary phytochemical analysis and insecticidal activity of gaggassa plant leaves against Brown banded Cockroach.

MATERIALS AND METHODS

Collection and extraction of plant leaves

Fresh and healthy leaves of the gaggasa plants were collected from natural forest of Rumudaamo kebele in Arbegona Woreda near the Logita River, Sidaama Zone, SNNPR. Identification and voucher specimens of NHB 001 of the plant were prepared and deposited at the National Herbarium, Department of Biology, Addis Ababa University. The collected plant leaves were washed with tap water to remove sand, dust and other contaminants, then air dried in a Chemistry Department laboratory of Hawassa College of Teacher Education (HCTE) for two weeks ensuring sufficient air flow to avoid damping. Dried plant leaves were crushed to powder by using an electric grinder at a speed of 6000 rpm for 60 s at the Department of Animal Science, Hawassa University.

Crude ethanolic, water and acetone extract of plant leaves

10 g powder of the plant leaves was extracted by using 100 ml of ethanol, water and acetone, respectively. It was kept for 24 h at room temperature (25°C) and shaken by using an electric shaker (INSIF, blue line instrument 133001, India) to get a better extraction. Thereafter, the extract was filtered through Whatman filter paper No. 1. After filtering, ethanol, water and acetone were removed at 50°C by using a rotary evaporator (Rotavapor, R-3000, BUCHI, Switzerland) to obtain a solid extract, dried in vacuum desiccators at room temperature. Finally, dry material was stored in desiccators until required for further analysis.

Qualitative phytochemical analysis

Phytochemical analysis of the gaggasa plant leaves were carried out by using the standard procedures as described by Asawalam et al. (2009).

Tannins

200 mg extracted plant material was dissolved in 10 ml distilled water, filtered. 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins.

Alkaloids

200 mg of the extracted plant material was added to 1.5 ml of 10% HCl in a test tube, heated for 20 min. It was cooled and filtered. 1 ml of the filtrate was tested with 5 drops of Draggendorff's reagents, formation of precipitates orange colored indicated the presence of alkaloids in the extracts.

Dragendorff's reagent

1.7 g of basic Bismuth nitrate was dissolved in 80 ml of distilled water. 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions were mixed in 1:1 ratio.

Saponins

0.5 ml filtrate was dissolved in 5 ml of distilled water. Frothing persistence meant Saponin was present.

Cardiac glycosides

2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + Conc. H_2SO_4. The green- blue colour indicated the presence of cardiac glycosides.

Steroids

200 mg extracted plant material was mixed with 2 ml of acetic anhydride followed by 2 ml of Sulphuric acid. The colour changed from violet to blue or green indicating the presence of steroids.

Terpenoids

0.5 ml of the extracted plant material was mixed with 2 ml of $CHCl_3$ and added 3 ml of Conc. H_2SO_4 in a test tube, reddish brown color indicate presence of terpenoids.

Flavonoids

2 ml filtrate + Conc. HCl + Magnesium ribbon. Pink-tomato red colour indicated the presence of flavonoids.

Anthocyanin

2 ml of the extract of plant material was added to 2 ml of 2N HCl and NH_3, the appearance of pink red turns blue violet indicating the presence of anthocyanin.

Coumarin

3 ml of 10% NaOH was added to 2 ml of plant extract, formation of yellow colour indicates the presence of coumarin.

Polyphenols (Phenolic compounds)

3 drops of a mixture of 1 ml each of the 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$ were added to 2 ml of the extracts material. Formation of green or blue color was taken as an indication of the presence of polyphenols.

Quantitative phytochemical determination

Total phenolic compounds analysis

Total phenolic compounds were determined by colorimetrically using Folin-Ciocalteau reagent with slight modifications (Okwu, 2004). 0.5 ml of water, and acetone extract and 0.333 ml of ethanolic extract respectively were mixed with 1.5 ml of Folin-Ciocalteau reagent and allowed to stand at 22° C for 90 min. 1.5 ml sodium bicarbonate solution (8%) was added to the mixture. After 90 min, absorbance was measured at 760 nm by using UV-Visible Spectrophotometer. Total phenolic amounts were quantified by calibration curve obtained from measuring the absorbance of a know concentrations of Gallic acid standard. The concentrations were expressed as mg of Gallic acid equivalents (GAE) per 100 g of dry weight. Gallic acid was used as a standard, and total phenolics were expressed as mg/g gallic acid equivalents using the standard curve equation: y=0.01455x + 0.02331, R^2=0.99354. *Where* y is absorbance at 760 nm and x is total phenolic content in the different extracts of plant leaves expressed in mg/gm.

Total flavonoids compound analysis

The total flavonoids content of each extract were estimated by using Chandrashekar et al. (2013) method. Based on this method, 1.0 ml of each extracted materials of water, ethanol and acetone were mixed with 1.5 ml of distilled water and subsequently, 75 ml of a 10% $NaNO_3$ solution was added. After 6th min, 150 ml 10% $AlCl_3$ solution was added and at 5th min, 1.0 ml of 1MNaOH solution was added to each sample immediately the solution of each extract forms a pink colour. The solutions were mixed well and absorbance of each solution was measured at 510 nm by using UV-Visible Spectrophotometer. Total flavonoid contents of the extracted gaggassa plant leaves were expressed as mg/ml gallic acid equivalent using the standard curve equation: y = 0.02395x + 0.11164, R^2=0.898729, *Where* y is absorbance at 510 nm and x is total flavonoids content of water, acetone and ethanol extracts, respectively. Quercetin was used as the standard for the calibration curve.

Mortality test of Brown banded Cockroach

Ten (10) adult cockroaches were collected from resident home and brought to the laboratory. The culture was established using Petri dishes of 25cmx10 cm and maintained at room temperature (25°C). Laboratory studies have been carried out to ascertain the insecticidal properties of the candidate plant species against cockroach. Concentrations of extracts residue 25, 50, 75 and 100 mg/mL was prepared in distilled water and ethanol respectively. One milliliter (1ml) of the solution was spread with the help of the pipette, over a filter paper (Whatmann no.1) of diameter 9 cm, in Petri dish. Laboratory reared adult insects of 10 cockroach were released in the container (Kundu et al., 2007). The container was closed with the holed lid. Mortality counts were undertaken after 4, 8 and 12 h and expressed as percentage mortality. Three replicates were set up for the treated and controls. Percentage insect mortality was calculated by using the formula (Umadevi and Sujatha, 2013).

$$(\%)\ \text{Mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \text{x} 100$$

Statistical analysis

The mortality (%) was corrected by Abbott's formula (Finny, 1971). Probit analysis was used to estimate LC_{50} values by using statistical package for the social sciences (SPSS) software (V.20). Least significant differences (LSD) at $P \geq 0.05$ were applied to determine differences between treatments.

RESULTS

Insecticidal activity against cockroach

Extracts of the gaggassa plant leaf was tested and caused significant adult mortality of *Brown banded* cockroach at high concentrations. Mean mortality of the plant extract with concentration and exposure-period dependent was presented in Figure 1 and Figure 2 respectively. The highest mortality was obtained at 4 h exposure time and mean mortality is increase with increasing the concentration as shown in Table 1. There was no significant difference (P>0.05) between mortality recorded in water and ethanol treated experiments. Preliminary test of 10 adult cockroach per dose level was conducted to establish the range of toxicity so that the proper dose level could be established for LC_{50} determinations. Observed result show that, all adult cockroach was killed at 0.1 mg/mL of 1.44 mg/mL from water and 1.33 mg/mL at 0.05, 0075 and 0.1 mg/mL concentration of ethanol extract as presented in Table 2.

Phytochemical analysis

The preliminary phytochemical analysis of the ethanol,

Table 1. Effect of ethanol and water plant extracts concentrations on mortality of adult cockroach at 4, 8 and 12 hours after treatment.

	Mean mortality														
Time	**4 h**					**8 h**					**12 h**				
Concentration	Cont.	0.025	0.05	0.075	0.1	Cont.	0.025	0.05	0.075	0.1	Cont.	0.025	0.05	0.075	0.1
Ethanol	0.00	8.67	10	10	10	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Distill water	0.00	3.33	5.67	6.00	9.67	0.00	0.00	1.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00

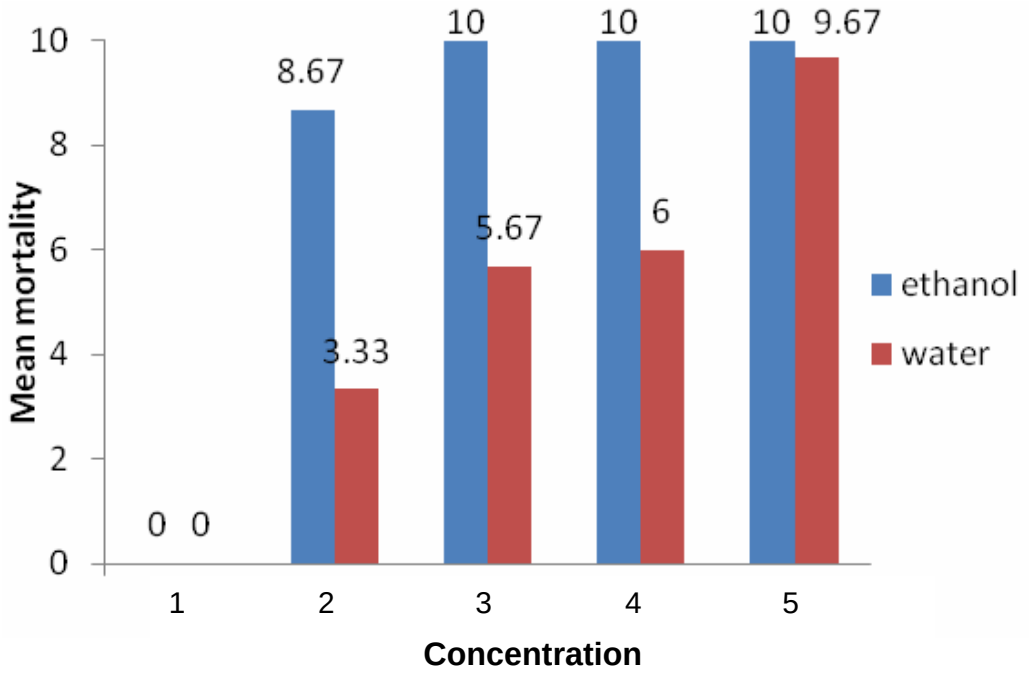

Figure 1. Concentration effect of gaggassa plant extract on mortality of adult cockroach.

water and acetone extracts of the selected plants showed the presence of alkaloids, Polyphenols, terpenoids, flavonoids, steroids, tannins and cardiac glycosides as presented in Table 3.

Quantitative analysis

The analyses are presented in Tables 4 and 5.

DISCUSSION

The results of the present study are interesting. The findings indicate the importance of traditional knowledge in science. As laboratory experiment shows the leaf of gaggassa plant has been shown to possess insecticidal activity. Since the yield of active secondary substances is high, it would be possible to produce enough quantities for field application in farms especially at the coast. Another advantage of extracting the material from leaf is that this part of the plant is easy to process during extraction due to its softness.

Today, the environmental safety of an insecticide is considered to be of paramount importance. Therefore, experimental evidence shows that high insecticidal activity of gaggassa plant leaf extracts was tested against cockroach at different concentration and different exposure time. The different concentration of plant extracts was tested against the cockroach and mean mortality increased with increase in concentrations of extract. During the treatment, no cockroach showed swirls movements in the Petri dishes at high concentration. This may be due to the fact that gaggassa based pesticides rapidly knock down insects, this might be an alternative pesticides for control of vector borne diseases without any side effects, and are environmentally safe. Best result was observed from 4 h

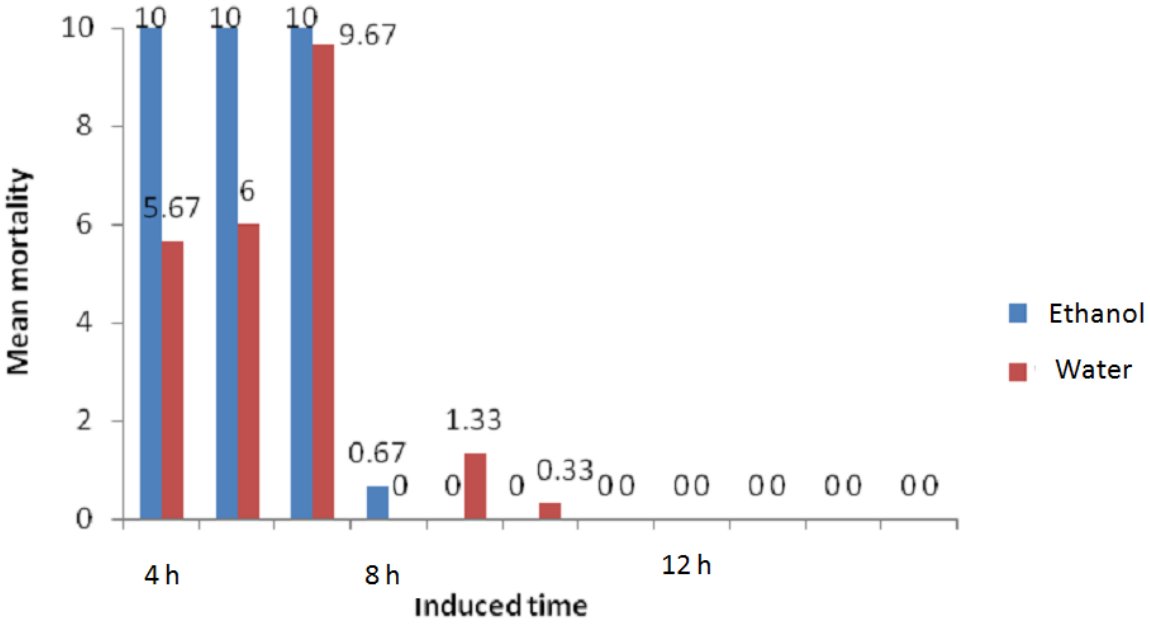

Figure 2. Time effect of gaggassa plant extract on mortality of adult cockroach.

Table 2. LD_{50} calculated for mortality of adult cockroach different concentrations plant extracts for 4, 8 and 12 h.

Doses	Extract conc.	Distill water			Ethanol		
mg/mL	(mg/mL)	4 h	8 h	12 h	4 h	8 h	12 h
0.025	0.02	33.33	0.00	0.00	86.67	6.67	0.00
0.05	0.02	56.67	13.33	0.00	100	0.00	0.00
0.075	0.02	60.00	3.33	0.00	100	0.00	0.00
0.1	0.02	96.67	0.00	0.00	100	0.00	0.00
LD_{50}	-	1.44	3.14	-	1.33	18.04	-

exposure time, which causes the highest mortality value of 10 (numbers) for ethanol extract at concentration of 0.05, 0.075 and 0.1 mg/mL, respectively and 9.67 value for water extract at concentration of 0.1 mg/mL.

This may be due to the presence of potential active compounds in the highest concentration. This study confirms that the mortality of insect depends on both extract and concentration. No mortality was recorded in the control treatment. Preliminary test with 10 adult cockroach per dose level was conducted to establish the range of toxicity so that the proper dose level could be established for LC_{50} determinations. With the toxicity test, it was possible to establish the highest dose of the extract that killed all adult cockroach (1.44 and 1.33 mg/mL from water and ethanol extract) respectively as shown in Table 2. There was no significant difference ($P>0.05$) between mortality recorded in water and ethanol treated experiments. This result inferred that 50% mortality can best be achieved if gaggassa plant extract are used.

The phytochemical analysis of the gaggassa plant leaves extracts of water, acetone and ethanol reveal that the presence of several bioactive secondary metabolites such as alkaloids , Polyphenols, terpenoids, flavonoids, steroids, tannins and cardiac glycosides that singly or in combinations may be responsible for the insecticidal activity and antioxidant activity. As a result, gaggassa plant leaves extracts with three different solvents (water, ethanol and acetone) to test the availability of biochemical compounds which gave positive results and the others gave negative results as presented in Table 3. From these secondary active metabolites, phenolic compounds are one of the largest and most ubiquitous groups of plants. The beneficial effects derived from phenolic compounds have been attributed to their higher insecticidal activities. Therefore, plants have higher

Table 3. Phytochemical analysis of gaggassa crude extracts.

S/N	Tests	Acetone	Ethanol
1	Tannins	+	+
2	Alkaloids	+	+
3	Saponins	-	
4	Cardiac Glycosides	+	+
5	Steroids	+	-
6	Flavonoids	-	-
7	Anthocyanin	+	+
8	Coumarin	-	-
9	Polyphenols	+	+
10	Terpenoids	+	+

*(+): Presence of active compound, (-): Absence of active compound.

Table 4. Total phenolic content of gaggassa in different plant extracts.

Sample	Concentration (Mean) (µg/ml)	Absorbance (Mean) λmax=760 nm	Mean±SD
Water	115.214	1.699667	115.214±0.06007773
Acetone	149.944	2.205	149.944±0.095504
Ethanol	213.839	3.134667	213.839±0.02386071

Values are expressed as mean ± SE of three replicates.

Table 5. Total flavonoids Content of gaggassa plant leaves with different extracts.

Sample	Concentration (Mean) (µg/ml)	Absorbance (Mean) λ_{max}=510 nm	Mean±SD
Water	5.930132	0.253667	5.930132±0.017502
Acetone	25.95797	0.7166667	25.95797±0.035572
Ethanol	32.16534	0.882	32.16534±0.004583

Values are expressed as mean ± SE of three replicates.

biological activities and shows great impact on insecticidal activities against a host of insect pests. Total phenolic compound of gaggassa plant leaves extracts was determined by using Folin Ciocalteu reagent as shown Table 4.

Therefore, the maximum phenolic content was found in the ethanol extract (213.839±0.14 mg/g) of gaggassa plant leaves. A concentration of 0.333, 0.333 and 0.5 mg/ml of plant extract were prepared with water, acetone and ethanol, respectively, and each sample were introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The test tubes were covered and allowed to stand for 90 min at room temperature. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour solution was measured at 760 nm by using UV-Visible spectrophotometer. As a result, total flavonoids contents of 32.16534±0.004583 mg Quercetin mg/gm have been observed in the ethanol extract as compared to acetone and water extract as shown in Table 5. The study observations revealed that ethanolic extracts of gaggassa *plant* leaves contain the highest amount of flavonoid and phenolic compounds, which shows that *A. salicifolia* has very rich source of important bioactive constituents to defensive, providing protection against insect, fungal, and viral attacks.

Conclusion

Gaggasa plant *(Agarista salicifolia)* offers potential insecticidal activity against cockroach. Preliminary phytochemical analysis during the present study also ascertains the presence of some potential group of

bioactive substances.

Conflict of Interests

The author have not declared any conflict of interests.

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Full Length Research Paper

Essential oil composition and antioxidant activity of aerial parts of *Asperula oppositifolia* collected from Darkesh, Iran

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The chemical compositions of the essential oil of *Asperula oppositifolia* aerial parts were examined by gas chromatography/mass spectrometry (GC/MS); forty-two compounds were identified. Analysis of this essential oil revealed the presence of 2-(6,6-Dimethyl bicyclo[3.1.1]hepta-2en-2-yl) ethanol (17.16%), Decane (8.47%) , Dibuthyl phthalate (5.59%) and 1-Bromo Naphthalene(4%). The antioxidant activity of aerial parts of methanolic extract was studied by *in vitro* 2'2'-diphenylpicrylhydrazyl (DPPH) radical – scavenging activity, revealing that this plant could be used as new medicinal resource for antioxidant agent.

Key words: Chemical composition, essential oil, 2-(6,6-Dimethyl bicyclo[3.1.1]hepta-2en-2-yl)ethanol, *Asperula oppositifolia* ,antioxidant activity.

INTRODUCTION

Asperula oppositifolia rechingeri is described from North-Khorasan Province, Iran. *A.oppositifolia* comprises six subspecies mainly distributed in E. Afghanistan, Pakistan, and Middle Asia. Describing these taxa originating from Iran demonstrates the extent of diversity of *Asperula* species in this country.

Morphological evidence supports taxonomic position of these taxa in *A.oppositifolia*, and the subspecies appear to be most closely related to subsp pseudo-cynanchica Ehrend (Ghahramaninezhad et al., 2006).

Phytochemical studies on this plant have been carried out, and flavonoids were identified (Borisov et al., 1972). This present communication, for the first time, did an analysis of arial parts essential oil and antioxidant activity of methanolic extract of A. oppositifolia is reported.

EXPERIMENTAL

Plant material

Aerial parts of A.oppositifolia were collected at the flowering stage from Darkesh protected area (Iran), in June 2010 and identified at the Research center for plant sciences, Ferdowsi university of Mashhad. Collected specimen has been deposited in the

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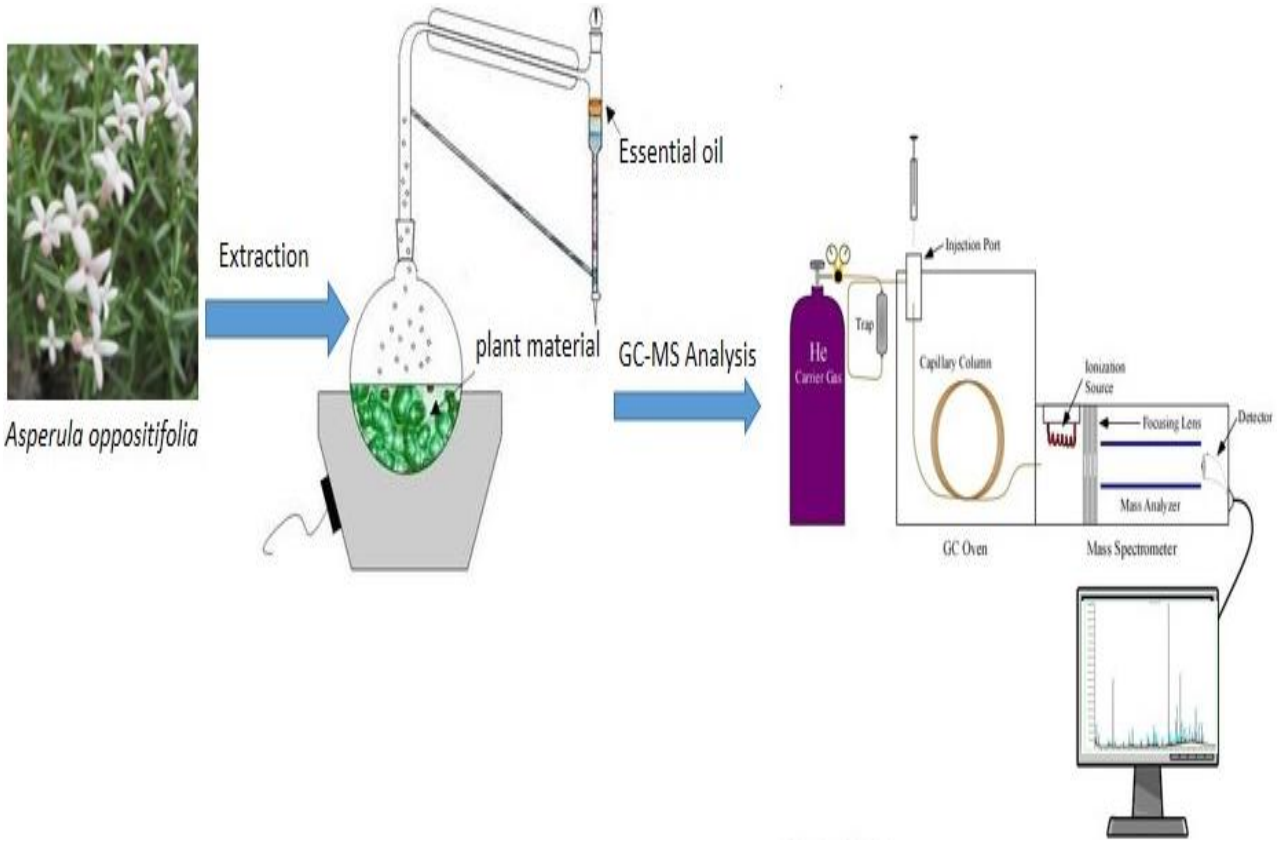

Figure 1. Aerial parts of *A.oppositifolia* collected at the flowering stage from Darkesh protected area, Iran.

Herbarium of Research Center (Figure 1).

Isolation of the essential oil

Aerial parts of *A. oppositifolia* were air-dried for 3 days before essential oil distillation. The plant material (100 gr) was cut into small pieces and the essential oil was obtained by hydrodistillation method, using a Clevenger apparatus. The temperature and pressure of hydrodistillation were 120ºC and 560 mmHg respectively. The distillation time was five hours. The resulting pale yellow oil was then dried over anhydrous sodium sulphate and 30 µL was solubilized in 1 mL of dichloromethane before the GC/MS (Gas Chromatography and Mass Spectroscopy) injection. 1 µL of this solution was directly used for analysis (Boland et al., 1991).

Gas chromatography and mass spectrometry (GC/MS)

Gas chromatographic analyses were performed using a HP 5890 series II gas chromatograph (Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a HP-5 (5% phenyl/95% dimethylpolysiloxane) fused silica capillary column (30 m× 0.25 mm; film thickness 0.25 Mm). Hydrogen was the carrier gas (1.0 mL min^{-1}) (Bianchi et al., 2007).

The injector temperature was kept at 250°C and the oven temperature program was from 60 to 240°C at a rate of 3°C min^{-1}. Detector (FID) was operated at 280°C. Pure oils (1 µL) were injected in split mode (100:1). The GC-MS analyses were performed in an Agilent 5973N mass selective detector coupled to an Agilent 6890 gas chromatograph (Palo Alto, CA), equipped with a HP5-MS capillary column (30 m × 0.25 mm × 0.25 µm). It operated in electronic ionization mode at 70eV, with transfer line maintained at 260°C; while quadrupole and ion source temperature were held at 150 and 230°C, respectively. Helium (1.0 mL min^{-1}) was used as carrier gas. Oven temperature program, injector temperature and split rate were the same as stated for GC analyses (Kohl et al., 2001; Dos Santos et al., 2001).

A standard solution of *n*-alkanes (C_8-C_{24}) was used to obtain the retention indices (Vandendool and Kratz, 1963). Individual volatile components were identified by comparison of their mass spectra (MS) and retention indices (RI) with those reported in literature (Adams, 2001; Davies.,1990) and also to the Wiley Registry of Mass Spectral Data,6th Edition (Wiley Interscience, New York).Component relative percentages were calculated based on GC/MS peak areas without using correction factors (Pino et al., 2005; Bianchi et al., 2007).

Antioxidant activity

The antioxidant potential of the methanolic extract was evaluated in term of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability. The determination was performed in triplicate.

DPPH radical scavenging activity

The scavenging effect on the DPPH radical was determined according to the methods reported previously (Singh et al., 2005).

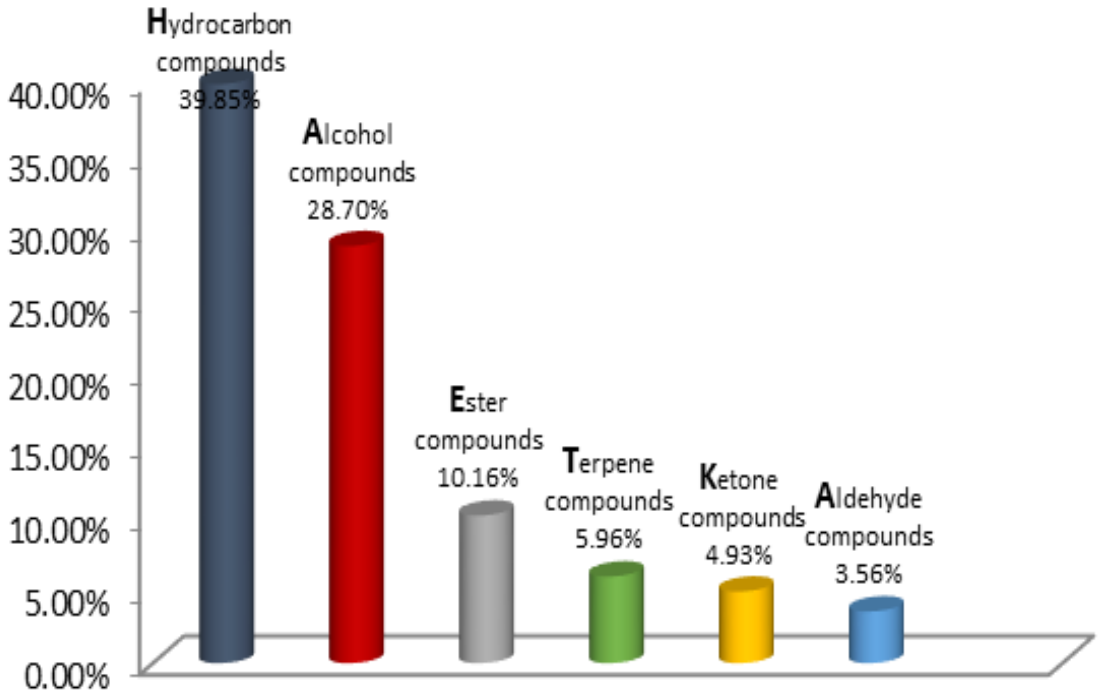

Figure 2. Distribution of *Asperula oppositifolia* compounds.

50 µl of various amounts of methanolic extracts (10, 5, 2.5, 1.125, 0.625, 0.312, 0.156 and 0.078 mg/mL) was mixed with 5 mL of 0.004% methanolic solution of DPPH. Each mixture was incubated for 30 min in the dark and the absorbance of the samples was calculated at 517 nm using the UV-Vis spectrophotometer. The DPPH solution was freshly prepared and kept in the dark at 4°C in between the measurements. Both control and standard were subjected to the same procedure except that of the control.

A lower absorbance indicated a higher radical scavenging power and these data were calculated according to the equation: DPPH scavenging activity (%I)=[1–(A_t /A_o)]x100, where A_t is the absorbance of the sample at 517 nm and A_o is the absorbance of the control at 517 nm.

RESULTS AND DISCUSSION

Essential oil analysis

The average yield of essential oil obtained after hydrodistillation of the leaves of *A.oppositifolia* was about 0.3%. Table 1 reports the chemical composition. Forty-two components were identified, accounting for 67.94% of the total oil.

The various compounds were identified by comparison of their Kováts retention indexes, determined utilizing a non-logarithmic scale on nonpolar (Rtx-5MS) columns , and by comparison of the mass spectra of each GC component with those of standards and reported literature (Jennings et al.,1980).

High resolution gas chromatography-mass spectrometric (HP GC-MS) analysis and Kováts Index values showed that its principal components are:2-(6,6-Dimethyl bicyclo[3.1.1]hepta-2en-2-yl)ethanol (17.16%), Decane (8.47%), Dibuthyl phthalate (5.59%) and 1-Bromo naphthalene(4%) and Dodecane (3.08%). Figure 2 shows the distribution of *A. oppositifolia* essential oil compounds.

Antioxidant activity

DPPH radical scavenging activity

Figure 3 shows the dose-response curve of 2'2'-diphenylpicrylhydrazyl radical scavenging activity of the methanolic extract of *A.oppositifolia* .It was observed that highest concentration showed the highest inhibitory effect.

Conclusion

Owing to the undesirable problems and side effects arising from the consumption of artificial chemical compounds, essential oils from various plant species, especially those edible and medicinal ones, have attained appreciable interest among the research community. This is the first study on the essential oil compounds and antioxidant activity of ethanolic extract of *A.oppositifolia.* Our data indicate that the polar compounds are the major ones in essential oils and possess a moderate antioxidant activity. These results suggest that essential

Table 1. Percentage composition of the essential oil distillated from aerial parts of *Asperula oppositifolia.*

S/N	Compound	Experimentally determined KI[a]	HP GC-MS Peak area [%]	Method of identification
1	Ethylbenzene	868	0.62	GC-MS,Ms
2	Z-3-Hexene-1-ol	849	1.03	GC-MS,Ms
3	Deca111 1,3-Dimethyl benzen	883	2.28	GC-MS,Ms
4	1,4-Dimethyl benzene	883	1.48	GC-MS,Ms
5	5-Methyl nonane	960	0.39	GC-MS,Ms
6	3-Methyl nonane	971	0.44	GC-MS,Ms
7	Decane	999	8.47	GC-MS,Ms
8	p-Cymene	1076	0.6	GC-MS,Ms
9	Linalool	1100	0.7	GC-MS,Ms
10	Nonanal	1108	0.27	GC-MS,Ms
11	α- Terpineol	1055	0.49	GC-MS,Ms
12	Dodecane	1095	3.08	GC-MS,Ms
13	Decanal	1206	0.29	GC-MS,Ms
14	Pulegone	1217	0.4	GC-MS,Ms
15	Thymol	1298	0.96	GC-MS,Ms
16	β – Damascenone	1382	0.36	GC-MS,Ms
17	Tetradecane	1399	1.07	GC-MS,Ms
18	Neryl acetone	1454	0.37	GC-MS,Ms
19	1-Bromo naphthalene	1463	4	GC-MS,Ms
20	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadienone	1473	0.9	GC-MS,Ms
21	β- Ionone	1485	0.77	GC-MS,Ms
22	Pentadecane	1499	0.54	GC-MS,Ms
23	2,6-di(t-butyl)phenol	1511	1.6	GC-MS,Ms
24	Hexadecane	1599	0.78	GC-MS,Ms
25	Z-14-methyl-8-hexadecane-1-ol	1668	0.35	GC-MS,Ms
26	Cyclotetradecane	1684	0.4	GC-MS,Ms
27	2-(6,6-Dimethyl bicycle[3.1.1]-2en-2-yl)ethanol	1694	17.16	GC-MS,Ms
28	Heptadecanal	1716	2	GC-MS,Ms
29	Nonyl phenol	1727	0.5	GC-MS,Ms
30	Anthracene	1765	0.53	GC-MS,Ms
31	Octadecane	1799	0.64	GC-MS,Ms
32	6,10,14-trimethyl -2-pentadecanone	1847	1.83	GC-MS,Ms
33	Nonadecane	1898	0.55	GC-MS,Ms
34	(E,E)-6,10,14-trimethyl -5,9,13-pentadecatrien-2-one	1919	0.44	GC-MS,Ms
35	Methyl hexadecanoate	1928	0.35	GC-MS,Ms
36	Dibuthyl phthalate	1966	5.59	GC-MS,Ms
37	Eicosane	2000	0.63	GC-MS,Ms
38	n-Heneicosane	2098	1.22	GC-MS,Ms
39	(Z,Z,Z)-9,12,15-Octa-decatrienoic acid methyl ester	2101	1.35	GC-MS,Ms
40	Docosane	2200	0.64	GC-MS,Ms
41	Tricosane	2300	1.14	GC-MS,Ms
42	Tetracosane	2400	0.73	GC-MS,Ms
	Total	-	67.94	-

a: Retention; indices on RTX-5MS(based on homologous series of n-alkanes;C_8-C_{24}).

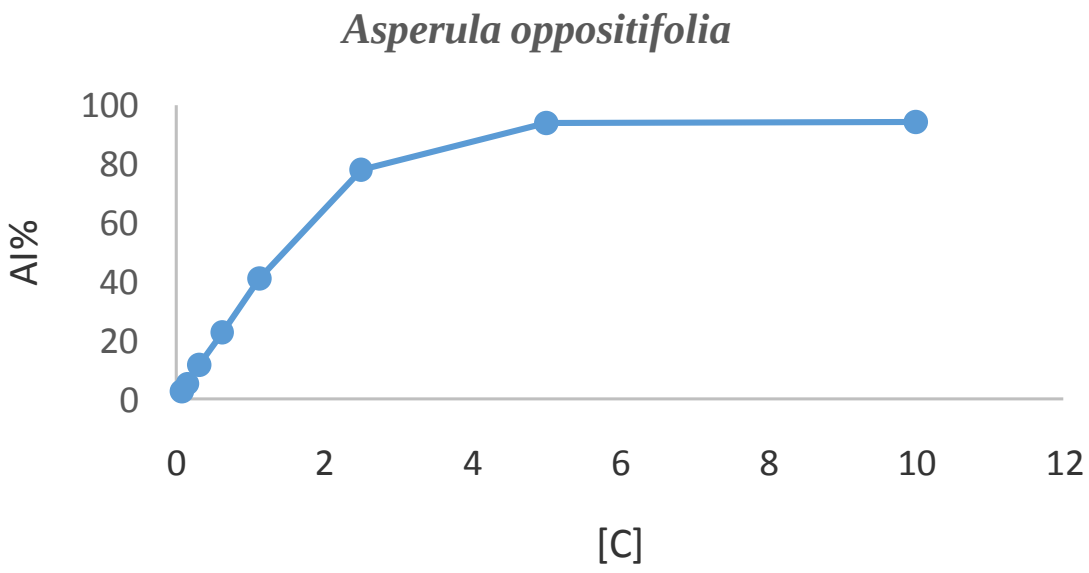

Figure 3. Radical scavenging of methanolic extract of *A. oppositifolia* on DPPH.

oil of *A. oppositifolia* could be used as new medicinal resource for antioxidant agent.

Conflict of Interests

The authors have not declared any conflict of interests.

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