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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 LC50 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
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; Yinebe Tarku, 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 gagassa (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 gagassa plant leaves against Brown banded Cockroach.

MATERIALS AND METHODS

Collection and extraction of plant leaves

Fresh and healthy leaves of the gagassa plants were collected from natural forest of Rumudaamo kebele in Arbégona 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 gagassa 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 FeCl₃, 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 Dragendorff’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 + FeCl₃ + Conc. H₂SO₄. 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 CHCl₃ and added 3 ml of Conc. H₂SO₄ 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 NH3, 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% FeCl₃ and 1% K₃Fe (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: \( y = 0.01455x + 0.02331 \), \( R²=0.99354 \). 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% NaNO₃ solution was added. After 6th min, 150 ml 10% AlCl₃ 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: \( y = 0.02395x + 0.11164 \), \( R²=0.898729 \). 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 hoid 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).

\[
\% \text{ Mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100
\]

**Statistical analysis**

The mortality (%) was corrected by Abbott’s formula (Finny, 1971). Probit analysis was used to estimate LC₅₀ 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 LC₅₀ 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,
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 gagasss 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 gagasss 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 gagasss 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.

<table>
<thead>
<tr>
<th>Doses (mg/mL)</th>
<th>Extract conc. (mg/mL)</th>
<th>Distill water</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 h</td>
<td>8 h</td>
</tr>
<tr>
<td>0.025</td>
<td>0.02</td>
<td>33.33</td>
<td>0.00</td>
</tr>
<tr>
<td>0.05</td>
<td>0.02</td>
<td>56.67</td>
<td>13.33</td>
</tr>
<tr>
<td>0.075</td>
<td>0.02</td>
<td>60.00</td>
<td>3.33</td>
</tr>
<tr>
<td>0.1</td>
<td>0.02</td>
<td>96.67</td>
<td>0.00</td>
</tr>
<tr>
<td>LD$_{50}$</td>
<td></td>
<td>1.44</td>
<td>3.14</td>
</tr>
</tbody>
</table>

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 gaggasa crude extracts.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Tests</th>
<th>Acetone</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Anthocyanin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Coumarin</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (Mean) (µg/ml)</th>
<th>Absorbance (Mean) λmax=760 nm</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>115.214</td>
<td>1.699667</td>
<td>115.214±0.06007773</td>
</tr>
<tr>
<td>Acetone</td>
<td>149.944</td>
<td>2.205</td>
<td>149.944±0.095504</td>
</tr>
<tr>
<td>Ethanol</td>
<td>213.839</td>
<td>3.134667</td>
<td>213.839±0.02386071</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE of three replicates.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (Mean) (µg/ml)</th>
<th>Absorbance (Mean) λmax=510 nm</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>5.930132</td>
<td>0.253667</td>
<td>5.930132±0.017502</td>
</tr>
<tr>
<td>Acetone</td>
<td>25.95797</td>
<td>0.7166667</td>
<td>25.95797±0.035572</td>
</tr>
<tr>
<td>Ethanol</td>
<td>32.16534</td>
<td>0.882</td>
<td>32.16534±0.004583</td>
</tr>
</tbody>
</table>

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 gaggasa plant leaves extract 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 gaggasa 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 gaggasa 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.

**REFERENCES**


