ORIGINAL ARTICLE

Antimicrobial, radical scavenging, and insecticidal activity of leaf and flower extracts of *Couroupita guianensis* Aubl.

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Abstract

Objectives: The objective of the present study was carried out to investigate antimicrobial, radical scavenging, and insecticidal activity of leaf and flower of *Couroupita guianensis* Aubl. (Lecythidaceae). **Methods:** Extraction of leaf and flower was carried out by maceration process using methanol. Antibacterial and antifungal activity of extracts was carried out by agar well-diffusion method and poisoned food technique, respectively. Radical scavenging activity of extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging assays. Insecticidal activity of extracts was evaluated in terms of larvicidal and pupicidal effects against *Aedes aegypti*. **Results:** Leaf extract displayed marked antibacterial activity when compared to flower extract. Highest and least inhibitory activity of extracts was observed against *Staphylococcus epidermidis* and *Escherichia coli,* respectively. Both extracts displayed antifungal activity with highest activity exhibited by leaf extract. Highest and least susceptibility were shown by *Curvularia* sp. and *Fusarium* sp., respectively. Both extracts scavenged DPPH and ABTS radicals dose dependently. Leaf extract (IC_{50} = 19.61 µg/ml) caused marked DPPH radical scavenging potential than flower extract ((IC_{50}= 257.13 µg/ml). IC_{50} value of ABTS radical inhibition of leaf and flower extract was found to be 7.63 and 53.34 µg/ml, respectively. Larvicidal and pupicidal activity by extracts was concentration dependent. The susceptibility of larvae and pupae to extract was in the order: 2nd instar larvae > 4th instar larvae > pupae. Leaf extract displayed marked insecticidal activity when compared to flower extract as revealed by lower LC_{50} values. **Conclusion: O**verall, leaf extract exhibited marked bioactivities than flower extract. The plant can be used to treat microbial infections and oxidative damage and to manage fungal diseases. The plant can be used against mosquito vectors which transmit arboviral diseases.

Key words: 1,1-diphenyl-2-picrylhydrazyl, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, *Aedes aegypti*, agar well diffusion, *Couroupita guianensis* Aubl., free radical, maceration, poisoned food technique

INTRODUCTION

Plants are the most valuable resource of various requirements such as food, cloth, timber, dyes, and medicine. Plants are traditionally used in various parts of the world as medicine. Plant-based medicines have been extensively used by medical practitioners in developing and under-developing countries and people from remote areas. It is estimated that about 80% of population in the world depends on traditional medicine. Countries such as India, China, Thailand, and Sri Lanka use many plants as traditional medicine to treat various diseases. A good knowledge on medicinal plants and their use by indigenous population is useful for conservation of plant biodiversity and for the development of drugs. Drugs such as quinine, artemisinin, morphine, vincristine, vinblastine, digoxin, reserpine, and aspirin are of plant origin. The therapeutic properties of plants are due to the presence of various secondary metabolites such as phenolic

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compounds, flavonoids, alkaloids, terpenes, and saponins that are present in them. These phytochemicals protect the plants from microbial infections, insects and herbivores. All over world, medicinal plants have been extensively used to treat microbial diseases, inflammation, diabetes, worm infections, pain, and cancer.[1-12]

Couroupita guianensis Aubl. [Figure 1] belongs to the family *Lecythidaceae*. It is a tree and is a native of South America; often planted in gardens. Flowering occurs more or less throughout the year. It is commonly known as Naagalinga pushpa in Kannada and cannon-ball tree in English. Leaves are alternate, crowded toward the ends of the branches, up to 20 cm × 7 cm, oblong or oblong-ovate, blunt at apex, almost acute basally with a short petiole. Flowers are 8-10 cm across, in racemes on trunk and lower branches. Sepals are short and six in number. There are 6 petals which are broad, yellow or red outside, pink inside. Stamens are numerous, basally connate on a basal ring and extended on one side into a curved fleshy androphore. Ovary is 5-7 celled and the cells are many-ovuled. Fruit is globose up to 20 cm across, hard outside, and brown. It has an unpleasant smell when ripe. Seeds are numerous.[13] Different parts of *C. guianensis* are used traditionally to treat human and veterinary ailments across the world. The plant is used to treat ailments such as skin infections, digestive infections, malaria, hypertension, tumors, pain, inflammation, cold, wound snake bite, stomach ache, and stroke.[14-18] Various parts of the plant are reported to exhibit bioactivities such as cytotoxic,[19] anthelmintic,[20] insecticidal,[21,22] enzyme inhibitory,[23] analgesic,[24] antiinflammatory,[24] allelopathic,[25] anticoagulant,[26] antiulcer,[27] wound-healing,[28] antimicrobial,[28,29] and antioxidant[30,31] activities. A compound called isatin isolated from flowers was shown to exhibit cytotoxic and antioxidant[32] properties. The present study was carried out to investigate antimicrobial, radical scavenging, and insecticidal potential of leaf and flower of *C. guianensis*.

Figure 1: *Couroupita guianensis* Aubl

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The plant was collected at outskirts of Shikaripura, Shivamogga district, Karnataka, during February 2017. The plant was identified on the basis of its characteristics.[13] The leaves and flowers were separated and washed well to remove adhering matter. The plant materials were dried under shade and powdered in a blender. The extraction of powdered leaf and flower of *C. guianensis* was carried out using methanol by maceration process in which the powdered material was left in methanol for 48 h in a stoppered container with occasional stirrings. After filtration through Whatman No. 1 filter paper, the filtrates were evaporated to dryness and stored in refrigerator.[12,33]

Test Bacteria

A total of five bacteria which included three Gram-positive bacteria (*Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493 and *Bacillus cereus* NCIM 2016 and two Gram-negative bacteria (*Escherichia coli* NCIM 2065 and *Salmonella typhimurium* NCIM 2501) were used to assess their susceptibility to leaf and flower extracts of *C. guianensis*. The bacteria were maintained on nutrient agar slants in refrigerator.

Preparation of Bacterial Inoculum

The test bacteria were seeded into tubes containing sterile nutrient broth, and the tubes were incubated at 37°C for 24 h. The 24 h old broth cultures of test bacteria were used to determine antibacterial activity of leaf and flower extracts.

Antibacterial Activity of Leaf and Flower Extract

Agar well-diffusion method was conducted to evaluate antibacterial activity of leaf and flower extract. In this method, the broth cultures of test bacteria were swab inoculated on sterile nutrient agar plates followed by punching wells of 8 mm diameter using a sterile cork borer. The respective wells were filled with 100 µl of leaf and flower extracts (20 mg/ml of dimethyl sulfoxide [DMSO]), reference antibiotic (chloramphenicol; 1 mg/ml of sterile distilled water) and DMSO. The plates were incubated at 37°C for 24 h, and the zones of inhibition were measured using a ruler.[12,34]

Test Fungi

Three fungi, namely, *Fusarium* sp., *Alternaria* sp., and *Curvularia* sp. isolated previously from moldy grains of sorghum were used to assess their susceptibility to leaf and

flower extract of *C. guianensis*. The fungi were maintained on potato dextrose agar (PDA) slants in refrigerator.

Antifungal Activity of Leaf and Flower Extract

The antifungal potential of leaf and flower extracts was evaluated by poisoned food technique. The well sporulated test fungi were inoculated, aseptically on control (without extract) and poisoned (1 mg extract/ml of medium) PDA plates and incubated at room temperature for 96 h. The diameter of fungal colonies developed on control and poisoned plates was measured in mutual perpendicular directions. Antifungal activity of extracts in terms of inhibition of mycelial growth of test fungi was determined using the formula:

Inhibition of fungal growth (%) = (A−B/A) × 100, where "A" and "B" refers to colony diameter in control and poisoned plates, respectively.[33,35]

Radical Scavenging Activity of Leaf and Flower Extract

2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays were employed to investigate the radical scavenging potential of leaf and flower extract of *C. guianensis*.

DPPH-free Radical Scavenging Assay

Various concentrations (12.5-200 µg/ml of methanol) of extracts and ascorbic acid (standard antioxidant) were prepared in methanol, and 1ml of each concentration of extract/standard was mixed with 3 ml of DPPH radical solution in clean and dry test tubes. The tubes were incubated for 30 min in dark, and the absorbance was measure spectrophotometrically at 517 nm. Methanol replacing the extract/ascorbic acid served as control. The radical scavenging potential of extracts and ascorbic acid was calculated using the formula:

Scavenging activity (%) = (C −T/C) × 100, in which "C" and "T" denotes the absorbance of DPPH control and absorbance of DPPH in the presence of extracts/standard. IC_{50} value was calculated by linear regression analysis.[35,36]

ABTS Radical Scavenging Assay

Various concentrations (12.5-200 µg/ml of methanol) of extracts and ascorbic acid (standard antioxidant) were prepared in methanol. In clean and dry test tubes, 1 ml of each concentration of extract/standard was mixed with 3 ml of ABTS radical solution. The tubes were incubated for 30 min in dark, and the absorbance was measure spectrophotometrically at 730 nm. Methanol replacing the extract/ascorbic acid served as control. The ABTS radical scavenging potential of extracts and ascorbic acid was calculated using the formula:

Scavenging activity (%) = (C − T/C) × 100, in which "C" and "T" denotes the absorbance of ABTS control and absorbance of ABTS in presence of extracts/standard. IC_{50} value was calculated by linear regression analysis.[12,35]

Insecticidal Activity of Leaf and Flower Extract

The insecticidal activity of extracts, in terms of larvicidal and pupicidal activity, was assessed against *Aedes aegypti*. 20 larvae (2^{nd} and 4^{th} instar), and pupae were introduced into conical flaks containing 50 ml of dechlorinated water with different concentrations of leaf and flower extracts (0.0-2.0 mg/ml) and the flaks were left for 24 h (12 h light and 12 h dark). The number of dead larvae and pupae were counted after 24 h and the mortality (%) was calculated using the formula:

Mortality (%) = (number of dead larvae or pupae/total number of larvae or pupae) × 100.[37,38] The LC_{50} value was calculated by linear regression analysis.

RESULTS AND DISCUSSION

Antibacterial Activity of Leaf and Flower Extract

The discovery of antibiotics is considered as one of the major milestones in the field of chemotherapy. The use of antibiotics has saved millions of deaths worldwide due to infectious microbes. However, the success of therapy by antibiotics is challenged by the development of resistance in pathogens. The resistance development in pathogenic bacteria against antimicrobial agents seems to be a global problem and is mainly due to indiscriminate use of antibiotics. The tendency of pathogens to transmit the resistance trait to susceptible strains is making the therapy more difficult. These resistant microbes are of serious concern in community as well as hospital settings. Furthermore, high cost and adverse health effects of many of the antibiotics are other serious limitations of antibiotics. Hence, there is a greater need for developing antimicrobial agents from other resources. Natural products including plants are known to be promising resources of antimicrobial agents. Higher plants have shown to exhibit antibacterial activity against a wide range of pathogenic bacteria including drug-resistant strains.[2,4,8,11,12,39] In the present study, we evaluated antibacterial activity of leaf and flower extract of *C. guianensis* by agar well-diffusion assay. Positive result is indicated by the presence of zone of inhibition (absence of growth) around the wells. The result of antibacterial activity of leaf and flower extract of *C. guianensis* is shown in Table 1. Both the extracts were effective in inhibiting test bacteria. Among extracts, marked antibacterial activity was displayed by leaf extract when compared to flower extract. Highest and least inhibitory

Table 1: Antibacterial activity of leaf and flower extract

Extracts	Zone of inhibition in cm				
	S. aureus	***S. epidermidis***	***B. cereus***	***E. coli***	***S. typhimurium***
Leaf extract	1.67±0.06	2.33±0.06	1.77±0.06	1.00±0.00	1.13±0.06
Flower extract	1.37±0.06	2.07±0.06	1.70±0.10	1.00±0.00	1.07±0.06
Antibiotic	3.53±0.12	3.77±0.06	3.33±0.12	2.73±0.12	2.93±0.06
DMSO	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, B. cereus: Bacillus cereus, E. coli: Escherichia coli, S. typhimurium: Salmonella typhimurium, DMSO: Dimethyl sulfoxide

Table 2: Mycelial growth of test fungi in control and poisoned plates

Treatment	Colony diameter in cm		
	***Curvularia* sp.**	***Alternaria* sp.**	***Fusarium* sp.**
Control	4.53±0.06	5.13±0.12	4.27±0.06
Flower extract	1.17±0.06	1.73±0.06	2.03±0.06
Leaf extract	1.03±0.06	1.33±0.06	1.83±0.06

activity of extract was observed against *S. epidermidis* and *E. coli,* respectively. Next to *S. epidermidis*, *B. cereus* was inhibited to high extent. Overall, Gram-positive bacteria were inhibited to higher extent when compared to Gram-negative bacteria. Reference antibiotic inhibited test bacteria to high extent when compared to leaf and flower extracts. DMSO did not cause inhibition of any of the test bacteria. In our study, leaf extract exhibited marked antibacterial activity. However, in an earlier study by Abdullah *et al*.,[40] the methanolic extract of flower inhibited *Bacillus subtilis* to higher extent when compared to leaf extract. Moreover, inhibitory activity of methanol extract was not observed against *E. coli*. In our study, the leaf and flower extracts inhibited Gram-positive bacteria to higher extent when compared to Gram-negative bacteria. The lower susceptibility of Gram-negative bacteria to leaf and fruit extracts could be attributed to the presence of an outer membrane which might have acted as an additional barrier for the entry of extract. No such observations were made in the study of Umachigi *et al*.[28] The study carried out by Bhuvaneswari *et al*.[41] showed the effectiveness of methanol extract of leaf of *C. guianensis* against a panel of bacteria with maximum activity against *E. coli*. In another study, Patel *et al*.[29] observed highest and least activity of methanol extract of leaf of *C. guianensis* against *S. aureus* and *B. subtilis,* respectively.

Antifungal Activity of Leaf and Flower Extract

Plants are vulnerable to infections caused by infectious agents such as bacteria, fungi, viruses, and nematodes. Among these, fungi are known to cause remarkably high number of diseases in plants. The fungal infections of plants are associated with significant reduction in yield leading to huge economic loss to farmers. In severe cases, >50% loss in yield often occurs. Management of fungal infections of plants is usually carried by the use of synthetic fungicides. However, the use of chemicals is associated with drawbacks such as high cost, emergence of resistant pathogens, residual effects on environment, effects on nontarget organisms, and health hazards in humans. The high cost of these fungicides is not affordable by many farmers. Hence, there is a greater need for developing antifungals from other sources. Higher plants are shown to be promising resources with antifungal activity against a variety of fungi, and many studies have shown the potential of plants and plant-based formulations to inhibit fungi.[9,12,35,42-47] The effect of leaf and flower extract to inhibit three fungi was evaluated by poisoned food technique. Reduction in mycelial growth of test fungi in poisoned plates was taken as positive for antifungal activity. The extracts displayed marked inhibition of test fungi as evidenced by reduced colony growth on poisoned plates when compared to control plates. Among fungi, marked susceptibility was recorded in case of *Curvularia* sp. while *Fusarium* sp. was inhibited to least extent. An inhibition of >50% of test fungi was exhibited by both extracts [Table 2 and Figure 2]. In an earlier study, various solvent extracts of flower of *C. guianensis* was found to inhibit fungi namely *Candida albicans*, *Aspergillus niger,* and *Saccharomyces cerevisiae* with maximum activity displayed by ethyl acetate extract.[48] In another study, Shivashankar *et al*.[30] observed the inhibition of *C. albicans* by hydromethanolic extract of bark of *C. guianensis*. The study of Al-Dhabi *et al*.[49] revealed the inhibitory activity of fruit extract of *C. guianensis* against *C. albicans* and *Malassezia pachydermatis*. The study by Lavanya and John[50] showed the potential of solvent extracts of leaf of *C. guianensis* against human pathogenic fungi.

DPPH Free Radical Scavenging Activity of Leaf and Flower Extract

DPPH is a stable, organic, nitrogen centered free radical (by virtue of the delocalization of the spare electron over

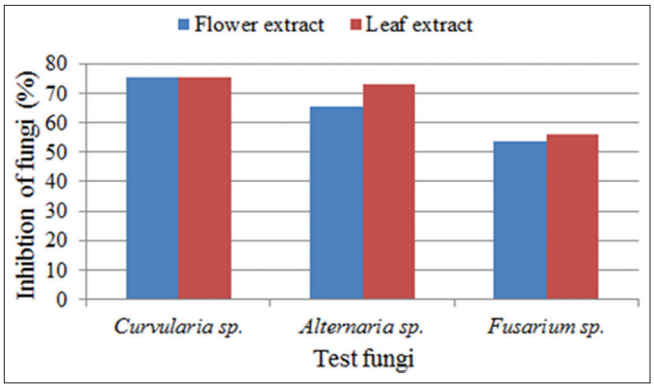

Figure 2: Inhibition of test fungi (%) by leaf and flower extract

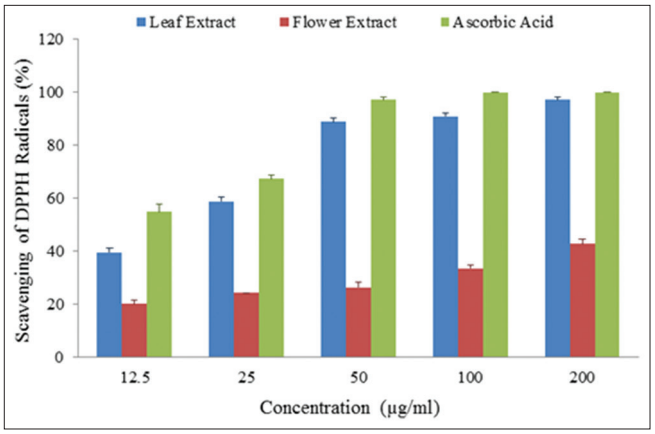

Figure 3: Scavenging of 1,1-diphenyl-2-picrylhydrazyl radicals by leaf and flower extract

the molecule as a whole) and is purple in color. It shows an absorption maximum at 517 nm in alcoholic solution. The radical readily undergoes scavenging by compounds (antioxidants) which can donate proton and gets converted into a nonradical form (DPPHH) which is yellow. The method of scavenging of DPPH radicals was developed by Blois (1958). The method is rapid, inexpensive, simple, and the results are reproducible. This method has been widely used by various researchers to evaluate free radical scavenging activity of plants.[12,35,51-60] The result of scavenging potential of leaf and flower extract against DPPH free radicals is shown in Figure 3. The extracts exhibited concentration dependent scavenging of free radicals as evidenced by bleaching of color of radical solution in the presence of varying concentrations of extracts. Among extracts, marked scavenging effect was displayed by leaf extract (IC_{50} value 19.61 µg/ml) when compared to flower extract (IC_{50} value 257.13 µg/ml). The scavenging potential of ascorbic acid (IC_{50} value 8.89 µg/ml) was higher than that of leaf and flower extracts. It is evident from the result that the leaf and flower extracts of *C. guianensis* possess hydrogen donating ability, and therefore, these extracts can serve as free radical scavengers, acting possibly as primary antioxidants. In similar studies, various parts such as flower,[31,48] bark,[30] and leaf[41] of *C. guianensis* were shown to exhibit scavenging of DPPH radicals.

ABTS Radical Scavenging Activity of Leaf and Flower Extract

Unlike DPPH assay, the ABTS radical scavenging assay needs the generation of ABTS radicals. This is usually done by mixing ABTS stock (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture for 16 h. The resulting blue-green radical solution is diluted to an absorbance of 0.7 and used for assay. An electron donating compound (antioxidant) reduces the blue-green colored radical solution to colorless neutral form which is indicated by the suppression of its characteristic long wavelength absorption spectrum. The assay involving scavenging of ABTS radicals has been widely used to investigate the radical scavenging potential of various plants.[12,52,55,56,57,59,61-66] The result of scavenging potential of leaf and flower extract against ABTS free radicals is shown in Figure 4. The extracts exhibited concentration dependent scavenging of free radicals as evidenced by change color of radical solution in the presence of varying concentrations of extracts. Among extracts, marked scavenging effect was displayed by leaf extract (IC_{50} value 7.63 µg/ml) when compared to flower extract (IC_{50} value 53.34 µg/ml). The scavenging potential of ascorbic acid (IC_{50} value 3.59 µg/ml) was higher than that of leaf and flower extracts. It is evident from the result of this study that the leaf and flower extracts possess electron donating potential which makes them to scavenge free radicals. In an earlier study, Shivashankar *et al*.[30] showed ABTS radial scavenging activity of hot and cold hydromethanolic extract of *C. guianensis* bark. It was found that the cold extract from bark scavenged ABTS radicals to higher extent when compared to hot extract.

Insecticidal Activity of Leaf and Flower Extract

Mosquitoes are the vectors transmitting dreadful human diseases such as malaria, filariasis, dengue, chikungunya, Japanese encephalitis, and yellow fever. Among mosquitoes, species of *Anopheles*, *Culex,* and *Aedes* are known to transmit several diseases. The prevention of these diseases can be carried out by preventing the mosquitoes. *A. aegypti* is one of the important mosquito vectors, and it transmit diseases, namely, dengue and chikungunya. The prevention and control of mosquitoes involves various strategies such as use of mosquito repellants, prevention of egg hatching, killing of larvae, pupae, and adult mosquitoes. Interest in botanicals with insecticidal activity has been intensified due to the drawbacks associated with the use of synthetic insecticides. Synthetic chemicals are costly, pollutes environment, cause adverse effects on non-target, and the insect vectors have been shown to develop resistance against them. The use of plants offers a safer and cheaper way for mosquito control, and it is shown that plants extracts and plant metabolites exhibit insecticidal activity against several mosquitoes such as species of *Aedes*, *Culex* and *Anopheles*.[34,37,38,67-71] The result of insecticidal potential of *C. guianensis* is shown in Figures 5 and 6. Both leaf and flower extracts exhibited

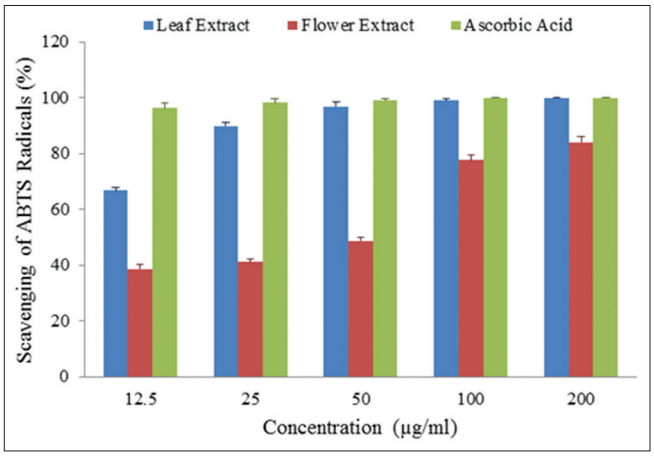

Figure 4: Scavenging of 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid radicals by leaf and flower extract

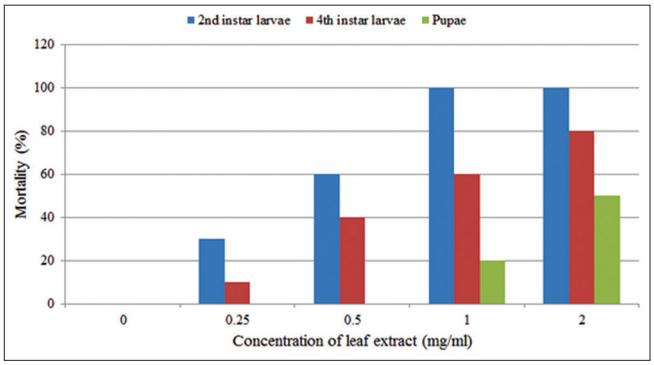

Figure 5: Larvicidal and pupicidal activity of leaf extract of *Couroupita guianensis*

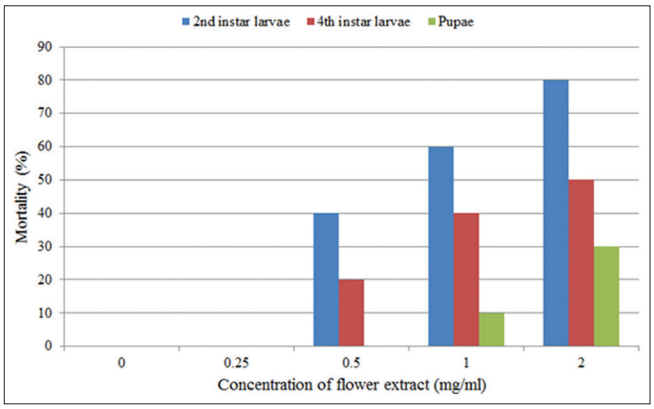

Figure 6: Larvicidal and pupicidal activity of flower extract of *Couroupita guianensis*

concentration-dependent insecticidal activity in terms of larvicidal and pupicidal effect. The susceptibility of larvae and pupae is in the order: 2nd instar larvae > 4th instar larvae > pupae i.e., early developmental stages of mosquito are more susceptible to extracts than later stages. Leaf extract was more effective against larvae and pupae when compared to flower extract. The LC_{50} of leaf extract for 2nd instar larvae, 4th instar larvae, and pupae was 0.66, 0.79, and 2.08 mg/ml, respectively. The LC_{50} of fruit extract for 2nd instar larvae, 4th instar larvae and pupae was 1.03, 2.12, and 2.73 mg/ml, respectively. Extract concentrations 0.25 and 0.50 mg/ml were ineffective in causing mortality of pupae. The plant is shown to exhibit ovicidal activity against cotton bullworm *Helicoverpa armigera*.[21] The leaf extract of *C. guianensis* showed insecticidal activity against silver leaf whitefly *Bemisia tabaci*.[72]

CONCLUSIONS

Both leaf and flower extracts of *C. guianensis* have shown antimicrobial, antiradical, and insecticidal potential. Among extracts, leaf extract was found to display greater activity when compared to flower extract. In suitable form, the plant can be used to treat infections caused by pathogenic bacteria and oxidative damage caused by free radicals, to manage fungal diseases of plants, and to control mosquito vectors which transmit dreadful diseases. The observed bioactivities could be attributed to the presence of phytochemicals in leaf and flower. Further studies on isolation and characterization of bioactive principles from the plant and their bioactivity determinations are to be conducted.

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