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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL POTENTIAL OF *COUROUPITA GUIANENSIS* AUBL AND *ERYTHROXYLUM MONOGYNUM* ROXB

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ABSTRACT

Couroupita guianensis Aubl and *Erythroxylum monogynum* Roxb. are medicinal plants popularly used in traditional medicine to cure many ailments. The aims of this research were to screen the extracts of the leaves of the two medicinal plants for phytoconstituents, and to approve the antibacterial property of the extracts against four strains of bacterial species, namely, *Escherchia coli, Klebsiela pneumoniae, Pseudomonas putida* and *Staphylococcus aureus.* The highest inhibition zone noted for aqueous extract of *E.monogynum* leaves against *E.coli* was 14.66 mm followed by ethanol extract (13.80 mm). The highest activity of acetone extract against *K.pneumoniae* was (12.0 mm). The ethanol leaf extracts of *C.guianensis* showed maximum antibacterial activity against *E.coli* (13.60 mm). The acetone leaf extracts showed highest activity against *P.putida* (12.80 mm). The aqueous leaf extracts showed significant activity against *E.coli* (12.28 mm). The results of the phytochemical screening various solvent namely acetone and methanol were taken which revealed that the leaves extract of *C.guianensis* contained tannins, saponins, flavonoids, terpenoids, phytosterols, cardiac glycosides and carbohydrates. Phytochemical analysis of *E.monogynum* showed the presence of saponins, tannins, flavonoids and alkaloids. In conclusion, the present results of the study evidence the utilize of the plants in traditional biomedicine for the curing of ailments caused by the microorganisms.

INTRODUCTION

Medicinal plants are notable principle of producing valuable bioactive phytoconstituents which are large significance for the health of persons and communities. The medicinal worthiness of the plants are due to the chemical substances that yield a exact physiological action on human body (Uddin *et al.,* 2011; Qaisar, 2012). Bacterial ailments are a main health difficulty because they are capable of enormous death per day worldwide. In India and other countries of the world, biotherapeutic medicines have been utilized since time immemorial to cure many diseases long before the introduction of recent medicine. Herbal drugs are still largely utilized in various parts of the world particularly in areas where people do not have access to recent medicines (Ajibad *et al.,* 2005). Plants are utilized medicinally in various countries and are a source of several effective and powerful medicines. According to World Health Organization, more than 80 percentage of the world's population relies on traditional drug for their primary healthcare demands. Plants are the gifts of nature utilized to treat number of human ailments (Deepa *et al.,* 2012). Medicinal plants are the highest productive source of novel compounds and medicines of natural origin. Most of the natural products isolated from medicinal plants are the phytoconstituents, which include tannins, alkaloids, steroids, terpenoids, flavonoids, phenylpropanoids (Harvey, 2008) and anthraquinones (Ayo, 2010). The phytochemical is popularly considered an effective approach in the discovery of new anti-infective agents from angiospermic plants (Duraipandiyan *et al.,* 2006). Antibacterial properties of many plants parts, such as leaves, stem, root seeds and fruits have been well documented for some of the medicinal plants for the past two decades (Leven *et al.,* 1979). Antibiotic principles are distributed widely among angiospermic plants. Antimicrobial medicines are used in medicinal practices for treating food-borne ailments (Abramouics, 1990). *Couroupita guianensis* Aubl. belonging to the family Lecythidaceae, commonly known as cannon ball tree in English. In Tamilnadu, it is called Nagalingam (Tamil) and Shivalingam, Kailaspathi (Hindi) is a large tree of 25-35 m height with large spreading branches bearing a peculiar flower. *Couroupita guianensis* has been used for treatment of tumours, hypertension, pain and inflammatory process (Sanz *et al.,* 2009). It is also used in the treatment of antibacterial activity (Khan *et al.,* 2003; Kavitha *et al.,* 2011; Regina and Uma Rajan, 2012; Shah *et al.,* 2012). It has stomachache, cold, intestinal gas formation and also used for immunomodulatory activity and larvicidal activity against vector (Anonymous, 1950; Desal *et al.,* 2003; Pradha *et al.,* 2009). Leaves of *C.guianensis* are widely used as an analgesics medicine by the Brazilian rural population (Mariana *et al.,* 2010). It is used to cure antifungal, antibiotic and analgesic qualities. Juice made from the leaves is used to treat skin disorders and the Shamans of South America have even utilized tree parts for curing malaria. The inside of the fruit and disinfect wounds and young leaves treat toothache (Geetha *et al.,* 2004). *Erythroxylum monogynum* Roxb. belonging to the family of Erythroxylaceae, commonly called Bastard Sandard, Red Cedar in English and locally known as Sembulichan, Devadara in Tamil. Its medicinal claims have included treats skin diseases, diuretic, diaphoretic and stomach problems (Ayyanar and Ignacimuthu, 2005; Senthilkumar *et al.,* 2006). Leaf juice given orally as a cooling beverages and jaundices and stem bark decoction is used for treatment of hiccups (Ravi Prasad Rao and Sunitha, 2011). The aims of this research were to decide the phytochemical profile and antibacterial potential of *C.guianensis* and *E.monogynum* leaf extracts. The screening of these plants was based on the demands of their efficient utilize in traditional medicine for the manage of various ailments in the area. Phytochemical analysis and the testing of antibacterial activity of the extracts were carried out against four bacterial strains.

MATERIALS AND METHODS

The fresh leaves of *C.guianensis* and *E.monogynum* were collected in December, 2012 from Shevaroy Hills and Kondalampatti, Salem

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district, Tamilnadu, India. The plants were confirmed and authenticated by Botanist, Department of Botany, Government Arts College (Autonomous), Salem, Tamilnadu, India. The voucher specimen numbers for *C.guianensis* and *E.monogynum* deposited at the Herbarium are Nos. 117 and 125, respectively. The leaves were thoroughly washed through tap water and air dried under shade for 7-10 days. The dried leaves were ground to fine powder and kept in polythene bags for further use.

Extractions

The powdered leaves (100 g) of each plant were extracted exhaustively with 500 ml of ethanol, acetone and aqueous, respectively using Soxhlet extractor. Each extract was concentrated in vacuo at 40°C using rotary evaporator.

Phytochemical screening

The phyotchemical screening of the extracts of the leaves of *C.guianensis* and *E.monogynum* was carried out using standard procedure to identify the constituents as described by Harbourne (1984), Sofowara (1993), Kokate *et al.,* (1995) and Evans (1996). The phytochemicals tested were tannins, saponins, flavonoids, alkaloids, terpenoids, phytosterols, anthoquinones, cardiac glycosides and carbohydrates.

Test microorganisms

Four bacterial strains were used in this study: *Escherichia coli, Pseudomonas putida, Staphylococcus aureus* and *Klebsiella pneumoniae.* The pathogenic microorganisms were obtained from the Biomedical Engineering Research Foundation, Salem, Tamilnadu, India. All the test bacterial strains were maintained on nutrient agar media at 4°C and sub cultured for 24 h before use.

Antibacterial tests

Antibacterial activity of extracts was determined by agar well diffusion method (Bauer *et al.,* 1966; Chew *et al.,* 2011). The agar well diffusion method was employed for the determination of antibacterial property of the extracts. The pteriplates containing 20 ml of Mueller Hinton agar medium were seeded with 24 h culture of the microorganism. The wells (6 mm in diameter) were cut from the agar and the extract solution (5 mg/ml) was then added into it. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader) in millimeters for the organisms and comparing to the control (Anjum *et al.,* 2013). 10 µg/ml of Ampicillin served as control. Each experiment was performed in triplicates, repeated twice and were tabulated.

RESULTS AND DISCUSSION

The data of the phytochemical analysis of the methanol and acetone leaf extracts of *C.guianensis* and *E.monogynum* are presented in Table 1. The results showed that the plants contained tannins, saponins, flavonoids, alkaloids, terpenoids, phytosterols, cardiac glycosides and carbohydrates. The antibacterial potentialities exhibited by the extracts of the leaves of the *C.guianensis* and *E.monogynum* may be due to the presence of the secondary metabolites revealed in their phytochemical analysis. The secondary metabolites obtain pharmacological properties, accountable for the utilize of plants in traditional biotherapeutic medicine to cure ailments caused by pathogenic microorganisms (Wang *et al.,* 2009; Olajuyigbe and Afolayan, 2012). The finding of this research conforms with the result procured by Olajuyigbe and Afolayan (2012) that phenolic compounds including the flavonoids, and tannins and alkaloids reveal a extensive measure of antimicrobial, including antifungal and antibacterial potentialities. The advantageous medicinal results of plant materials familiarly effect from the secondary metabolites present in the plant although and it is regularly not attributed to a particular compound but a combination of the metabolites (Janaikaraman *et al.,* 2012). The result suggests that this plants is containing phytoconstituents such as tannins, saponins, flavonoids, alkaloids, terpenoids, phytosterols, cardiac glycosides and carbohydrates as secondary metabolites which can be utilized in several pharmacological utilities. The results of antibacterial activities of ethanol, acetone and aqueous extracts of the leaves of *C.guianensis* are summarized in Table 2. All the extracts demonstrated wide spectrum of activity. When the three extracts were compared with each other and with that of standard antibiotic Ampicillin, the ethanol leaf extract showed the highest potentialities compared to that of the acetone and aqueous extracts. The extract obtained using ethanol showed highest effective against *E.coli* (13.60 mm), *P.putida* (10.20 mm) and *K.pneumoniae* (9.40 mm) and minimal inhibition zone was noted against *S.aureus* (8.52 mm). The study made on acetone extract accounted highest activity against *P.putida* (12.80 mm), *E.coli* (10.45 mm) and *S.aureus* (10.0 mm) and minimum inhibition zone was noted

Table 1. Phytochemical analysis of extracts of the leaves of *C. guianensis* and *E.monogynum*

Secondary metabolites	Couroupita guianensis		Erythroxylum monogynum	
	CGAE	CGME	EMAE	EMME
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	-	-	+	+
Terpenoids	+	+	-	-
Phytosterols	+	-	-	-
Anthoquinones	-	-	-	-
Cardiac glycosides	+	+	-	-
Carbohydrates	+	+	-	-

CGAE = Acetone extract of *Couroupita guianensis*, CGME = Methanol extract of *Couroupita guianensis*, EMAE = Acetone extract of *Erythroxylum monogynum,* EMME = Methanol extract of *Erythroxylum monogynum,* + = Positive, - = Negative.

Table 2. Antibacterial activity of the various leaf extracts of *Couroupita guianensis* and *Erythroxylum monogynum* by agar well diffusion method

Plant name	Part used	Plant extract	Zone of inhibition (in mm)			
			Escherchia coli	*Staphylococcus aureus*	*Pseudomonas putida*	*Klebsiella pneumoniae*
C.guianensis	Leaves	Ethanol	13.60±0.27	8.52±0.16	10.20±0.13	9.40±0.12
		Acetone	10.45±0.38	10.0±0.11	12.80±0.47	8.70±0.38
		Aqueous	12.28±0.60	8.70±0.30	9.13±0.05	-
E.monogynum	Leaves	Ethanol	13.80±0.20	10.28±0.15	11.70±0.15	9.20±0.02
		Acetone	10.15±0.17	8.30±0.19	-	12.0±0.17
		Aqueous	14.66±0.31	9.10±0.12	10.35±0.01	8.15±0.07
		Ampicillin 10µg/ml	21.37±0.19	20.60±0.28	22.15±0.05	19.51±0.15

Data given are mean of triplicates ± Standard Error. – indicates no activity, concentration used 50 µg/ml.

against *K.pneumoniae* (8.70 mm). Aqueous extract showed highest activity against *E.coli* (12.28 mm) and *P.putida* (9.13 mm) and the minimal activity against *S.aureus* (8.70 mm). No activity was recorded against *K.pneumoniae* (Fig.1).

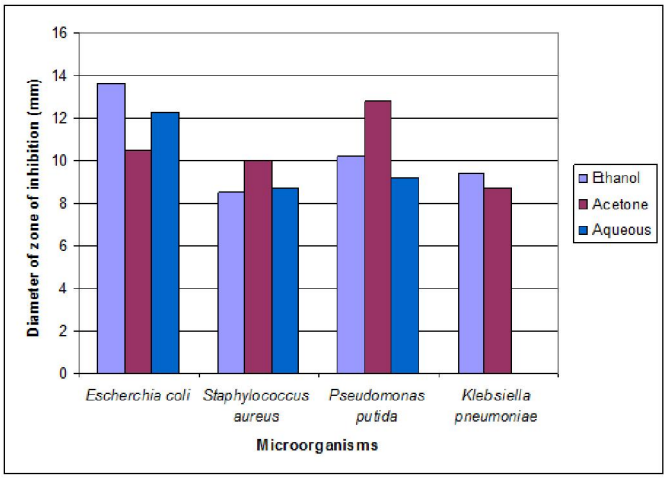

Figure 1. Graphical representation of antibacterial activity of various leaf extracts of *Couroupita guianensis* against test microorganisms

The results of the present study on the antibacterial potentials of ethanol, acetone and aqueous extracts of the leaves of *E.monogynum* are showed in Table 2. The ethanol, acetone and aqueous extracts of the leaf of *E.monogynum* showed considerable antibacterial activities. All the extracts exhibited a large spectrum of activity. When the three extracts were compared with each other and with that of standard antibiotic Ampicillin, the aqueous leaf extract showed the highest activity compared to that of the ethanol and acetone extract. The inhibitory zone for aqueous extracts of the leaf showed maximum antibacterial activity *E.coli* (14.66 mm), *P.putida* (10.35 mm) and *S.aureus* (9.10 mm). Least inhibition zone was noted against *K.pneumoniae* (8.15 mm). The ethanol extract of the leaves showed notable and highest antibacterial property against *E.coli* (13.80 mm), *P.putida* (11.70 mm) and *S.aureus* (10.28 mm), while decrease in potential against *K.pneumoniae* (9.20 mm). The leaf extracts of acetone showed maximum activity against *K.pneumoniae* (12.0 mm) and *E.coli* (10.15mm). Further, it showed minimal activity against *S.aureus* (8.30 mm). No activity was observed against *P.putida* (Fig.2).

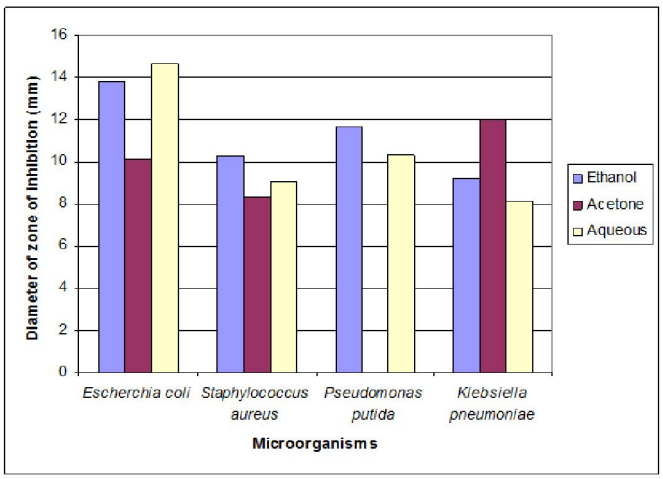

Figure 2. Graphical representation of antibacterial activity of various leaf extracts of *Erythroxylum monogynum* against test microorganisms

The results of the present study revealed that antibacterial potentialities of acetone, ethanol and aqueous extracts varied in usefulness which may be attributed to the presence of tannins and saponins. The presence of phenolic compounds in the plants indicates the antibacterial activities. In the present study, the author also notable the antibacterial activity, which agrees with the findings of Divya *et al.,* (2011). The phytochemicals are recognized to have antibacterial activities (Gupta *et al.,* 2010).

Conclusion

The presentation of the antibacterial potential of the leaf extracts of the plants, *C.guianensis* and *E.monogynum* against pathogenic microorganisms is confirmation that the extracts are potential source of antibiotics with a wide spectrum of properties. Results of this research confirm the utilize of the plants in traditional biomedicine to cure ailments caused by the pathogenic bacterial species. Further study will be required to bioassay indicated isolation to isolate, identify and characterize the structure of the biologically active compound accountable for pharmacological properties.

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