Anti-bacterial properties of fractions isolated from Couroupita guianensis and Atalantia monophylla

S Premalatha and G Ramar

Abstract
In the present study different fractions from ethanol extracts of Couroupita guianensis and Atalantia monophylla were isolated and tested for their antibacterial bioassay on selected pathogens. Antibacterial activity was determined against three gram positive bacteria Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilius and four Gram negative bacteria Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Proteus vulgaris was carried in well diffusion method. The results obtained fractions from ethanol crude extracts of Couroupita guianensis and Atalantia monophylla had inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract fractions was significantly greater than the fraction II and fraction III. Gram negative bacteria Escherichia coli and the Gram positive bacteria Staphylococcus aureus showed the highest zone of inhibition in ethanol extracts fractions II and III of Couroupita guianensis and Atalantia monophylla.

Keywords: Anti-bacterial activity, Couroupita guianensis, Atalantia monophylla, Staphylococcus aureus

Introduction
Plants play a major role in our ecosystem. By nature we have abundant medicinal plants in the world balancing life in the ecosystem. Indians used food as medicine and vice versa, but after few decades food became one of the source to cause the diseases. Ancestral methods are still being followed to an extent but the plants of medicinal value are forgotten over a period of time. Our ancestors and various tribes in the world were living alongside with these medicinal plants keeping their health in good condition by using the right plants to cure diseases of different nature. Some methods and treatments are still remembered but we have to accept the fact that the value of most medicinal plants are long forgotten as they are not scripted anywhere. Newer generation are in the hunt to research on these medical plants to extract medicines to cure newly arising diseases caused by micro-organisms day by day. Though advanced medicines have been found to cure various diseases, we are still unable to stop the death rate due to diseases caused by micro-organisms. So most developed and under-developed nations have turned towards these medicinal plants and started spending more on the research works to find cure for these diseases which is the right direction to go. E.g., Recently, Nilavembu Kashayam (Nilevembu in liquid form) has been found as a source to cure severe viral fevers in India especially in the state of Tamil Nadu. Plant based medicines always prove to be cheaper with less side effects. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase of new and re-emerging infectious due to variety of bacterial agents. The natural products such as plant extracts provide unlimited opportunities for new drug discoveries, mostly because of excessive of varieties of phytochemicals (Sasidharan et al., 2011) [1]. Thus natural products have been a major source of drugs for centuries. Couroupita guianensis Aubl is belonging to family Lecythidaceae and its found extensively all over the Indian subcontinent and North-eastern South Africa. The plant has regional synonyms such as cannon ball tree, Shivalingam and Kailashpati in English, Kannada and Hindi respectively (Sumathi and Anuradha 2017) [2]. In India, this tree is sacred to Hindus, who believe its hooded flowers look like the Naga, it is found in Thanjavur big temple and it is grown at most of the Shiva temples in Tamil Nadu. Atalantia monophylla Linn belongs to the family Rutaceae, comprises of 11 species which are closely-related. The leaves are used for the treatment of snakebite by local Malayali tribes of Kolli hills.
As a result of indiscriminate use of antimicrobial drugs in the
treatment of infectious diseases, microorganisms have
developed resistance to many antibiotics. There is a need to
develop alternative antimicrobial drugs. One of the approach
is to screening the local medicinal plants which represent a
rich source of novel antimicrobial agents (Reddy et al., 2010)
[19]. The present study aimed to analyse the antibacterial
properties of fractions from Couroupita guianensis and
Atalantia monophylla.

Materials and Methods
Collection of plant Materials
The leaves of Couroupita guianensis and Atalantia
monophylla was collected from in and around Tiruchirapalli
District, Tamil Nadu, India. Plant specimen was identified by
Dr. S. John Britto, Director, The Rapanit Herbarium and
Centre for Molecular Systematics, St’ Joseph’s College,
Tiruchirapalli, Tamil Nadu, India. The voucher specimen of
Couroupita guianensis and Atalantia monophylla was prepared
and deposited in PG and Research Department of Botany,
Government Arts College, Coimbatore, Tamil Nadu, India.

Extraction of Plant material
The plants leaves were thoroughly washed with tap water and
shade dried under room temperature (27.0 ± 2°C and 75 ± 5%
RH). After complete drying the leaves was powdered using
electric blender and sieved through a kitchen strainer. 1000g
of plant powder was extracted by soxhlet extraction (Plate I)
methods with ethanol of solvents and filtered through What
Man’s No. 1 filter paper. The solvent from the crude extract
were condensed with Rotary Vacuum Evaporator (Plate II)
and condensed extracts were collected in clean borosil vials
(Plate II) and stored in the refrigerator.

Thin Layer Chromatography (TLC)
Thin layer chromatography was done on pre-coated plates
Silica Gel G (0.2mm thick; Merck, India) were trimmed with
strips and the position of the origin marked by a straight line.
Ethanol extracts of Couroupita guianensis and Atalantia
monophylla and their fractions were dissolved in hexane and
were spotted on the plate with fine capillary tube at the height
of 0.8-1.0cm from the base. In the present study, different
solvent mixtures viz; hexane and ethanol, were used for
developing the TLC plates to get better results. After putting
the plates in solvent system, the appropriate distance moved
d by the solvent was measured to find the retention factor
(nearly 2/3 of plate). The plates were air dried and developed
by the solvent was measured to find the retention factor
(Plate II) and stored in the refrigerator.

Column Chromatography (CC)
The ethanol extracts of Couroupita guianensis and Atalantia
monophylla for the isolation of fractions used by Column
chromatography. Silica gel -100 - 200 mesh (Molychem) was
packed in a column (size 60cm x 4 cm) with hexane using the
wet slurry method. This involves preparing a solution of silica
gel, with hexane in this case, and a beaker and subsequently
adding this unto the column till it is about three fourths filled.
The solution was stirred for dispersal and quickly added to the
column before the gel settles. This method was used to
prevent the trapping of air bubbles. A ball of wool was pushed
into the column to settle a top the packed silica gel. For the
elicitation of components, the polarity of the solvent (mobile
phase) was increased using a combinations of hexane, hexane:
chloroform (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9),
then chloroform and Similarly the column was run over
chloroform, then chloroform: ethanol (9:1, 8:2 and 2:8) and
then ethanol respectively. The volume of the collected
fractions was 10ml each test tubes. Totally 300 fractions of
Couroupita guianensis and Atalantia monophylla were collected.
TLC plate was run on every column fraction and it
was exposed to iodine vapour to investigate the spots to
calculate Rf values. Fractions in which similar spots appeared
were collected in one pool. The fractions with similar Rf
values were pooled and isolates designated as three fractions
for Couroupita guianensis and Atalantia monophylla were
obtained. All the isolated fractions were stored in solid form
for further experimentation.

Test microorganisms
The test organisms used were clinical isolates viz
Staphylococcus aureus, Streptococcus epidermidis, Bacillus
subtilius, Escherichia coli, Pseudomonas aeruginosa,
Salmonella typhi, Vibrio cholerae and Proteus vulgaris
The bacteria were cultured were maintained on Muller Hinton agar
medium. Standard antibiotics of Gentamycine for S. aureus,
S. epidermidis Bacillus subtilius, E. coli P. aeruginosa,
S. typhi, V. cholerae and P. vulgaris was used as reference
Control.

Anti-bacterial Activity
The fractions from ethanol extracts of Couroupita guianensis
and Atalantia monophylla with three different concentrations
were tested for antibacterial activity using agar disc diffusion
assay method (Chung et al., 1990) [3].

Inoculum Preparation
Inoculums of each bacterial strain was suspended in 10 ml
Muller-Hinton agar medium and incubated overnight at 37°C
cultures were tested for antibacterial activity using agar disc diffusion
assay method (Chung et al., 1990) [3] which is widely used for the antibacterial
susceptibility testing (Barry and Thornsberry, 1985) [3].

Result
Considering the evolution of resistance genes to antibiotics of
microbial origin and non-antibiotic chemicals (Lee et al.,
2003) [8], plant materials have become the subject of public
attention and therefore the pharmaceutical industry is moving
away from drug discovery or screening towards compounds
isolated by medicinal plants. The different fractions from
ethanol crude extracts plants Couroupita guianensis and
Atalantia monophylla leaves were evaluated for their
antibacterial bioassay on selected pathogens and results are
tabulated 1 & 2.

The plants Couroupita guianensis and Atalantia monophylla
leaves fractions were evaluated for their antibacterial activity
against nine clinical bacterial isolates namely Staphylococcus
aureus, Streptococcus epidermidis, Bacillus subtilius, three
Gram positive bacteria and Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera and Proteus vulgaris six Gram negative bacteria. The screening of the three fractions reveals that, fraction I, II, and III and these fractions were subjected to disc diffusion assay against the bacterial pathogens the zones of inhibition obtained for the various micro-organisms are showing remarkable activity against the micro-organisms tested.

The data pertaining to the above experiments are shown in table 1. Fraction I, II, III are promising in controlling the growth of bacterial strain in the nutrient agar (Muller Hilton) medium and their values ranging from 9-39 zone of inhibition (mm) in Couroupita guianensis leaves fractions I, II, III were performed at 10 µl/ml, 20 µl/ml, and 30 µl/ml concentration. Besides, fraction I. showing least activity against the Vibrio cholera 9mm zone of inhibition at 10 µl/ml concentration, 15mm in 20 µl/ml and 19mm at 30 µl/ml concentration and Klebsiella pneumoniae 10mm at 10 µl/ml, 15mm and 20 mm at 30 µl/ml concentration. While the maximum values of zone of inhibition are recorded from Staphylococcus aureus and Escherichia coli bacteria, 29 mm and 28 mm at 30 µl/ml concentration from fraction I respectively.

The Couroupita guianensis leaves fractions II, showed potent activity against all the organisms tested and minimum values of zone of inhibition showed 13mm zone of inhibition at 10 µl/ml, 17mm at 20 µl/ml and 19mm at 30 µl/ml. And the maximum values of zone of inhibition are recorded from Staphylococcus aureus 25mm at 10 µl/ml, 33mm at 20 µl/ml and 39mm at 30 µl/ml, Bacillus subtilis 19mm at 10 µl/ml, 23mm at 20 µl/ml and 37mm at 30 µl/ml and Escherichia coli bacteria, 19mm and 21 mm and 35mm at 10, 20, 30 µl/ml concentration from fraction II were recorded. Similarly fraction III showed 9 mm zone of inhibition against Vibrio cholerae 13 mm at 30 µl/ml followed by Salomonella typhi 15mm and Pseudomonas aeruginosa 19mm 30 µl/ml. The maximum zone of inhibition was noticed in Staphylococcus aureus 15mm at 10 µl/ml, 21mm at 20 µl/ml and 31mm at 30 µl/ml, Bacillus subtilis 13mm at 10 µl/ml, 19mm at 20 µl/ml and 29mm at 30 µl/ml and Escherichia coli bacteria, 15mm at 10 µl/ml, 17mm at 20 µl/ml and 27mm at 30 µl/ml.

Plant extracts and compounds are of new interest as antimicrobial agents. As a result, the antimicrobial activity of Atalantia monophylla plant leaves fraction was screened against the most common pathogens such as Gram positive (Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilis) and Gram negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salomonella typhi, Vibrio cholerae, Proteus vulgaris). The activity was measured in terms of zone of growth inhibition in mm. It is interesting to note that Klebsiella pneumoniae 11mm at 10 µl/ml, 15mm at 20 µl/ml and 19mm zone of inhibition at 30 µl/ml, Escherichia coli showed 12mm at 10 µl/ml, 15mm at 20 µl/ml and 19mm zone of inhibition at 30 µl/ml and in the case of Staphylococcus aureus, 9mm at 10 µl/ml, 13mm at 20 µl/ml and 17mm zone of inhibition at 30 µl/ml of the Atalantia monophylla plant leaves fractions I. The Proteus vulgaris 7mm at 10 µl/ml, 11mm at 20 µl/ml and 12mm zone of inhibition at 30 µl/ml are recorded as the minimal concentration which inhibits the growth of the bacteria. Whereas, not high but moderate activity is obtained from the plants of Couroupita guianensis and Atalantia monophylla leaves fractions against the Gram-negative Pseudomonas aeruginosa, Salmonella typhi and Proteus vulgaris bacteria. The maximum zone of inhibition clearly seen in Klebsiella pneumoniae, Staphylococcus, aureus, Escherichia coli at 28mm, 26mm and 26mm at 30 µl/ml of fraction II and the similar trends are also reflected in fraction III. Escherichia coli 14mm at 10 µl/ml, 17mm at 20 µl/ml and 21mm zone of inhibition at 30 µl/ml followed by Klebsiella pneumoniae 10mm at 10 µl/ml, 14mm at 20 µl/ml and 19mm zone of inhibition at 30 µl/ml. Staphylococcus aureus shows highly sensitive at 14mm at 10 µl/ml, 19mm at 20 µl/ml and 21mm zone of inhibition at 30 µl/ml of the Atalantia monophylla plant leaves fraction III respectively. Among the three fractions, fraction II shows promising bacterial growth inhibition activity in both Couroupita guianensis and Atalantia monophylla against all the species.

Discussion

Some of these metabolites particularly some flavonoids that were detected were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Yoshi’s et al., 2008). In addition, some alkaloids and tannins are well documented for antimicrobial activity (Tringali C, 2005; Mansouri S et al., 2001) [9, 10].

We conducted prior research on Couroupita guianensis Preliminary phytochemical and the antimicrobial activity in Dichloromethane, Chloroform and Ethyl acetate extracts. Among the crude extracts tested, Dichloromethane extract showed the presence of protein, terpenoids tannins and ethyl acetate extract showed the presence of alkaloid, flavonoid, and carbohydrate and saponin compounds. Antibacterial activity of Dichloromethane, Chloroform and Ethyl acetate extracts of Couroupita guianensis tested against five important human pathogenic bacteria viz., Salmonella typhi, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae at 25, 50, 75 and 100µl concentrations and the data pertaining to the experiments are shown in table 2. Among the tested bacteria the results showed the highest zone of inhibition against Staphylococcus aureus is 23 mm in 100 µl /ml Dichloromethane extract and Escherichia coli also showed 23 mm in 100 µl /ml chloroform extract. Whereas, ethyl acetate extracts showed the maximum zone of inhibition against Staphylococcus aureus and Escherichia coli is 21mm in 100 µl/ml (Karthi and Premalatha, conference proceedings, 2017) [18].

In our previous studies, preliminary phytochemical and antibacterial studies of Ethanol, Chloroform and Ethyl acetate extract obtained from the leaves of Atalantia monophylla for its medicinal potentials. The effect exhibited by ethanol extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts. Gram negative bacteria Escherichia coli and the Gram positive bacteria Staphylococcus aureus showed the highest zone of inhibition in all the three ethanol, chloroform and Ethyl acetate extracts. The phytochemical screening revealed the presence Carbohydrates, alkaloids, flavonoids, Cardiac glycosides, Protein and Phenolic compounds found in Atalantia monophylla ethanol leaves extracts. The experimental data clearly revealed that the effect exhibited by ethanol extract of leaves was significantly greater than the Chloroform and Ethyl acetate leaves extracts (Premalatha and Karthi, 2017) [18]. The purpose of this study was to evaluate the antibacterial activity of the fractions of C. guianensis and A. monophylla against nine clinical bacterial isolates namely Staphylococcus aureus, Streptococcus epidermidis, Bacillus subtilis, three

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Gram positive bacteria and Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera and Proteus vulgaris six Gram negative bacteria. The screening of these three fractions reveals that, fraction I, II and III and these fractions were subjected to disc diffusion assay against the bacterial pathogens the zones of inhibition obtained for the various micro-organisms showed remarkable activity against the micro-organisms tested. The increasing prevalence of antimicrobial drug-resistant microorganisms recovered from hospitalized patients is a major concern worldwide (Struelens, 1998) [11]. However, with so many antibacterial agents available for the treatment of systemic infections, treatment of bacterial infection is no problem at present, but the ability of microorganisms to acquire the capacity for multi-drug resistance can negate the therapeutic effect of an entire family of antibacterial agents. Therefore, the search for new, safe and effective antibacterial agents is necessary. Plants could be a good source for this purpose, and many plant extracts are reported to have anti-microbial activities (Naqvi et al., 1991; Sabahi et al., 1987; Almagboul et al., 1988) [12, 13, 14]. In the present study both the fractions of C. guianensis and A. monophylla were effective in all the tested bacteria. Among the fractions, marked antibacterial activity was displayed by leaf fractions II. Infectious diseases of microbial origin caused by Staphylococcus aureus, Bacillus cereus, Shigella spp constitute the major causes of morbidity and mortality in several countries (Kloos and Zein, 1993) [15]. Such microbial infection and pathophysiology in water and electrolyte transport could lead to diarrhoea. The fractions of C. guianensis and A. monophylla showed effective repressing activity against Staphylococcus aureus bacteria. Ramalakshmi et al., 2013 [16] conjointly showed antimicrobial property of methanolic extract Couroupita guianensis flowers. However, the therapeautic potentials of some of these botanicals have not been scientifically evaluated (Havagiray et al., 2004) [17]. It would be interesting therefore to search for plants with antimicrobial activities that could be used against infectious diseases.

**Table 1:** Antibacterial activity of fractions of Couroupita guianensis tested against the important selected human pathogenic bacteria

<table>
<thead>
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<th>Fractions tested</th>
<th>Type</th>
<th>Pathogens tested</th>
<th>Concentrations tested</th>
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<td>Bacillus subtilis</td>
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<td>Gram negative</td>
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<td>Vibrio cholerae</td>
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Values showing in the table are zone of inhibition obtained through disc diffusion method; Control = commercially available chemical drug: *Gentamycin (30µg/ml) for E. coli, S. aureus, B. subtilis and P. aeruginosa (Hailu Tadege et al., 2005) [17], Ofloxacino (10µg/ml for P. vulgaris, K. pneumoniae and S. typhi; Karman et al., 2002) and Chlorephenicol (30µg/ml for P. vulgaris, V. cholerae and S. epidermidis; Nancy et al., 2000) [8] were used as reference standards.

**Table 2:** Antibacterial activity of fractions of Atalantia monophylla tested against the important selected human pathogenic bacteria

<table>
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<th>Fractions tested</th>
<th>Type</th>
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<td>Bacillus subtilis</td>
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<td>Gram negative</td>
<td>Klebsiella pneumoniae</td>
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**Conclusion**

Based on the studies, it may be concluded that *C. guianensis* and *A. monophylla* possess a wide range of antibacterial activities, in both crude and fractionated extracts. Also, new antimicrobial drugs can be developed for treating various diseases from the selected plant. Further, the individual active compounds can be isolated by chromatographic techniques and the fractions shall be evaluated separately for TLC, NMR to identify the compound functional group, nature and structure for converting as a new active drug.

**Acknowledgement**

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