ANTIBACTERIAL STUDIES WITH METHANOL EXTRACT OF 
COUROUPIA GUIANENSIS FLOWERS AGAINST METHICILLIN-
RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT
The present study was aimed at evaluating the anti-MRSA effect of Couroupita guianensis flower methanol extract. These flowers are used to treat microbial infections in traditional medicinal practices of India. The methanolic extract of Couroupita guianensis flowers were subjected to preliminary antimicrobial activity by agar well diffusion method and found to be active against both Gram-negative Escherichia coli as well as Gram-positive methicillin-sensitive and methicillin-resistant Staphylococcus aureus strains. Biochemical property of the extract was established by phytochemical analysis. The preliminary biochemical tests showed the presence of glycosides, tannins, and phenolics, which group of compounds have previously been reported to exhibit anti-microbial effects.

Key Words: Couroupita guianensis, flower, Staphylococcus aureus, MRSA.

INTRODUCTION
Couroupita guianensis Aubl. belongs to the family Lecythidaceae and is native to Central and South America. In English it is known as the Cannonball tree. The plant is considered to be
of medicinal importance among traditional medicinal practitioners of the Indian sub-continent and elsewhere in the world. Roots of the tree are used by the native Amazonian groups residing by the Nanay River in Peru for treatment of malaria.\[1\] The tribal people of Kailasagirikona Forest Range, and the Yanadi tribes in Seshachalam Biosphere Reserve Forest of Chittoor District, Andhra Pradesh, India, use leaves of the plant to vitalize hair.\[2,3\] Folk medicinal practitioners of Gaurnadi Upazila in Barisal district, Bangladesh use leaves and bark of the plant to treat snake bite. \[4\] The local herbalists and rural people of various areas in Tamil Nadu, India have been observed to treat microbial infections with various parts of the plant, suggesting that the plant parts may have antimicrobial properties.

Methanol and aqueous extracts of leaves of the plant have been tested and found to be effective in varying degrees against bacterial strains like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Leuconostoc lactis*, and fungal strains like *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus*. \[5\] Methanol extract of flowers of the plant reportedly showed effective inhibitory activities against *S. aureus*, *Plesiomonas shigelloides*, *Vibrio mimicus* and *Proteus vulgaris*, and moderate inhibitory activity against *E. coli*, *S. typhi* and *Klebsiella pneumoniae*. \[6\] Chloroform extract of fruits have been shown to be effective against *P. aeruginosa*. \[7\] Various solvent extracts of fruit rind have been tested against *S. aureus*, *E. coli*, *Micrococcus*, *Corynebacterium diphtheriae*, and *Candida albicans*. \[8\] Acetone and dichloromethane extracts of leaves of the plant reportedly showed effectiveness against *Staphylococcus* sp. \[9\]

Various antibiotics have been the drugs of choice in allopathic medicine against different microbial infections. However, it was quickly found out that microbes develop antibiotic resistance. The increasing prevalence of multi-drug resistant organisms with few or no treatment options such as methicillin resistant *S. aureus* (MRSA), vancomycin resistant *Enterococci* (VRE) and the extended spectrum β-lactamase (ESBL) producing Gram-negative bacilli both in hospitalized patients and, to a lesser extent, in the community are a serious cause for concern and have become a global problem. \[10\] Methicillin resistant *S. aureus* (MRSA) and methicillin resistant coagulase negative *Staphylococcus* sp (MR-CoNS) is perhaps the pathogen of the greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections and its capacity to adapt to different environmental conditions.\[11\] To combat these antibiotic-resistant microorganisms,
particularly MRSA, new drugs need to be found, and the plant kingdom has always been a good source for finding lead compounds which can be useful as antibiotics. The objective of the present study was to evaluate the antibacterial potency of various extracts of *C. guianensis* flowers against MRSA.

**METHODS**

**Collection of plant materials**

Fresh flowers of *C. guianensis* were collected from their natural habitat in and around Bangalore, Karnataka, India. A voucher specimen of *C. guianensis* was submitted at Botanical survey of India (BSI), Central National Herbarium, Howrah, Voucher no: CNH/67/2014/Tech.II/134.

**Surface stérilisation of flowers**

The petals were separated from the other parts of the flower and washed thoroughly with tap water to obtain petals free of dust and soil. The petals were next washed with 5% sodium chloride solution to remove the surface contaminants. Then the petals were rinsed with distilled water to remove the excess sodium chloride solution. The water was drained and the flowers dried in shade on a clean filter paper.

**Methanol extraction**

Cleaned petals were dried using liquid nitrogen and then powdered. 5g of the powder was extracted with 33.3 ml methanol for 24h on a rotary shaker at ambient temperature at 25 rpm and then allowed to stand for 5 h. The extract was filtered and the residue re-extracted with methanol as before. The two filtrates were combined and the methanol evaporated to dryness. The extract was dissolved in 1% DMSO for further uses.

**Test organisms**

Wild type strains of *S. aureus* [methicillin-resistant (MRSA) and methicillin-sensitive (MSSA)] were collected from pus samples of patients at the Department of Microbiology & Serology, Lab Medicine, NH Health City, Bangalore, India. MSSA ATCC 29213, MRSA ATCC BAA-1026, and *E. coli* ATCC 25922 were used as control. The investigation was approved and a written informed consent was obtained from the Institution Ethical Committee.
Agar well diffusion method
Gram negative bacilli *Escherichia coli* and Gram positive cocci *Staphylococcus aureus* overnight cultures adjusted with 0.5 Mac Farland’s standard were swabbed on the Muller-Hinton media plates using a sterile swab and allowed to stand for 15 min. A sterile 6 mm well borer was used to make wells in the media and extract was added into the well with sterile micro-pipettes and incubated overnight at 37°C and checked for the development of zone of inhibition around the well. The experiment was done in triplicate. The zone of inhibition was calculated as described before.  

Essentially, the diameter of the inhibition zone was measured around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were determined.

Preliminary phytochemical screening of methanolic and ethanolic extracts
Analysis of various groups of phytochemicals was conducted according to procedures as previously described.  

1. Test for Carbohydrates (Benedicts Test)
Equal volumes of Benedict’s reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 min. Solution appearing green was taken as an indication of the presence of reducing sugars.

2. Test for Proteins (Biuret Test)
A small quantity of extract was dissolved in a few ml of water. To this test solution 4% NaOH solution and a few drops of 1% CuSO₄ solution were added. Appearance of violet color indicated the presence of proteins.

3. Test for Steroids (Salkowski Test)
To 2 ml of extract, 2 ml of chloroform and 2 ml of concentrated H₂SO₄ was added. The solution was shaken well. Chloroform layer turning red and acidic layer showing greenish yellow fluorescence indicated the presence of steroids.

4. Test for Alkaloids (Hager’s Test)
To 2-3ml of extract, Mayer’s reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.
5. Test for Flavonoids (Lead acetate Test)
To a small quantity of extract, lead acetate solution was added. Formation of yellow precipitate indicated the presence of flavonoids.

6. Test for Tannins and Phenolic compounds (FeCl₃ Test)
On addition of 5% FeCl₃ solution to the extract, appearance of a bluish black color indicated the presence of tannins and phenolic compounds.

RESULTS
Preliminary phytochemical screening
Preliminary phytochemical screening showed the presence of glycosides, tannins, and phenolics in the methanol extract.

Preliminary screening for antibacterial activity
A preliminary antibacterial screening was done with 20 mg/ml of the methanol extract against Gram positive *S. aureus* (various strains) and Gram negative *E. coli* using the agar well diffusion method. The various zones of inhibition obtained are shown in Table 1 and represent the mean value of three replicate experiments. The results show that the methanolic flower extract is active against both Gram-positive and Gram-negative bacterial strains.

<table>
<thead>
<tr>
<th>Organism and Zone of inhibition (mm)</th>
<th>MRSA from pus (wild strain)</th>
<th>S. aureus from pus (wild strain)</th>
<th>S. aureus ATCC 29213</th>
<th>E. coli ATCC 25922</th>
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Concentration-dependent zone of inhibition against different *S. aureus* strains
The zone of inhibition of various concentrations of extract (10, 12, 14, 16, 18, 20, 30, 40 and 50 mg/ml) was checked against MSSA ATCC 29213, MRSA (wild type) and MRSA ATCC BAA-1026. The results are shown in Table 2 and represent the mean values of 3 replicate experiments. The results suggest that the methanolic extract was equally active against both MSSA and MRSA strains, including wild type MRSA strain.
Table 2. Concentration-dependent zone of inhibition of *C. guianensis* methanolic flower extract against different *S. aureus* strains

<table>
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<tr>
<th>Concentration of the extract (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
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<tr>
<td></td>
<td>MSSA ATCC 29213</td>
<td>MRSA (Wild strain)</td>
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DISCUSSION

The number of reports \(^5\text{-}^9\) on the antimicrobial activities of various parts of *C. guianensis* suggests that the plant may have the potential for discovery of new antimicrobial components. These reports present for the first time, to our knowledge, the antibacterial efficacy of methanol extract of flowers of the plant against both methicillin-sensitive and methicillin-resistant *S. aureus*. Although the active principle(s) were not identified in this preliminary study, phytochemical screening showed the presence of glycosides, tannins, and phenolics in the methanolic extract.

Hot aqueous extract of *Acacia nilotica* leaves have been found to inhibit *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *Bacillus cereus*, *S. aureus*, and *Streptococcus uberis* and fungal pathogens *Aspergillus niger* and *Aspergillus fumigatus*. Phytochemical screening of the extract showed the presence of glycosides and phenolic compounds among the constituents. \(^13\) Chloroform and methanol fractions of *Euphorbia milii* have been shown to be active against *S. epidermis* and *K. pneumoniae*; glycosides and tannins were present in the fractions. \(^14\) Thus glycosides, tannins, and phenolic compounds present in methanol extract of flowers of *C. guianensis* can account for the observed antibacterial activities.

CONCLUSION

What is interesting in the present study is that the methanol extract demonstrated antibacterial activity against MRSA. Not many reports are available for anti-MRSA activity of plant
extracts or phytochemicals. Erypostyrene (a cinnamylphenol), isolated from *Erythrina poeppiginia*, has been shown to demonstrate anti-MRSA activity. \(^{15}\) (6E, 12E)-tetradecadiene-8, 10-diyne-1, 3-diol, isolated from roots of *Atractylodes japonica* have also been reported for anti-MRSA activity. \(^{16}\) New compounds may thus be present in flowers of *C. guianensis*, which can act as effective antibacterial agent(s) against MRSA, which has rapidly become of great concern because of its virulence and antibiotic-resistance properties.

ACKNOWLEDGEMENT

REFERENCES


