Phytochemical investigation and antimicrobial activity of *Caesalpinia bonduc* (Linn) Roxb seeds

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Abstract
The aim of the study was to investigate phytochemical properties, antimicrobial activity and trace metal concentrations of *Caesalpinia bonduc*. The phytochemical screening of the extracts of leaves of *C. bonduc* revealed the presence of bioactive compounds such as Steroid, Triterpenoids, Reducing Sugar(A), Reducing Sugar(B), Sugars, Flavonoids, Saponin, Amino acids with absence of Alkaloids, Phenolic Compounds, Catachins, Tannins, Anthroquinones. The ethanol solvent was used for extraction and was used to screen the antimicrobial activity of *C. bonduc* leaves against certain pathogens by disc diffusion method. In the antimicrobial study, in bacteria, the test sample was most effective against *Escherichia coli* NCIM 2931 (B2) while smaller effect was noticed from *Staphylococcus aureus* NCIM 5021 (B3). In fungi, this was effective against *Epidermophyton floccosum* var. *nigricans* MTCC 613 (F2) whereas smaller effect was observed in *Candida glabrata* MTCC 3984 (F1). All the microbial strains depict higher sensitivity to the higher concentration (1.2 mg / disc) for the test sample when compared to the positive control except bacterial strains such as *Bacillus subtilis* NCIM 2920 (B1) and *Staphylococcus aureus* NCIM 5021 (B3). The result was supported the view that *C. bonduc* is a potent antimicrobial agent compared with the conventional antibiotic. The concentrations of trace metals in plants were not cross the standard level. Hence, it is signified that *Aloe vera* plant extract is safe to be used as an antimicrobial agent.

Keywords: *Caesalpinia bonduc*, Phytochemistry, Antibacterial activity, Trace metals

1. Introduction
*Caesalpinia bonduc* (L.) Fleming (Syn. *Caesalpinia bonduc* (L.) Roxb, Syn. *Caesalpinia crista* Linn.), belonging to the family *Fabaceae / Caesalpinaceae*, is a prickly shrub widely distributed all over the world specially, in India, Sri Lanka and Andaman and Nicobar Islands, in India specially present in tropical regions. All components of the plant have medicinal properties so it is a valuable medicinal plant which is utilized in traditional system of medicine. *A Bonduxela* the denomination of the species is derived from the Arabic word a Bonducaea meaning a little ball which betokened the globular shape of the seed. The seed is claimed to be styptic, purgative and anthelmintic and remedies inflammations, utilizable in colic, malaria, hydrocele, skin diseases and leprosy. In Madras (Chennai) an ointment is made from the powdered seeds with castor oil and applied externally in hydrocele and orchitis. In Guinea, the pounded seeds are contemplated vesicant in inflammations, utilizable in colic, malaria, hydrocele, skin diseases and leprosy. In Malignant malaria, they did not do any lovely. The seeds are ground in dihydrogen monoxide and given internally in snake bite. The seeds are not an antidote to snake-venom. Seed and long pepper powders taken with honey gives lovely expectorant effect. Burnt seeds with alum and burnt arecanut are a lovely dentifrice utilizable in spongy gums, gum boils, etc. In West Indies, the roasted seeds are utilized as antidiabetic.

2. Materials and Methods
2.1 Collection of plant material
The plant material was collected from the Kolli hills of Tamil Nadu in India during the period from January 2014 – February 2014.

2.2 Morphology
The seeds are almost globular in shape, grey in colour, hard with a smooth shiny surface. The shell, which is thick and brittle, encloses a yellowish-white, bitter, fatty kernel. Leaves are bipinnate, large, stipules foliaceous, pinnate 7 pairs, leaflet 3-8 pairs with 1-2 small recurved prickles between them on the underside. Flowers are yellow, in dense long peduncled supra-axillary racemes at the top. Fruits are inflated pods, covered with wiry prickles. Pods are oblong, densely armed with sharp wiry prickles, dehiscent. Branches are armed with recurved prickles.

2.3 Ethanol extraction
The five grams of air-dried seed powder of *Caesalpinia bonduc* Linn. Roxb. was macerated with 100 ml of ethanol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of ethanol. Evaporated 25 ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105 ºC and weighed. Percentage ethanol soluble extractive was calculated with reference to the air-dried seeds.
2.4 Preliminary phytochemical screening

The solvent extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in them. Steroids: A 2 ml of test solution and minimum quantity of chloroform was added with 3–4 drops of acetic anhydride and one drop of concentrated H₂SO₄. Purple color thus formed changes into blue or green color indicating the presence of steroids. Triterpenoids: A 2 ml of test solution was added with a piece of tin and 2 drops of thiol chloride. Formation of violet or purple colour indicates the presence of triterpenoids. Reducing Sugars: A 2 ml of test solution was added with a 2 ml of Fehling’s reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar. Sugars: A 2 ml of the test solution was added with very small quantity of Anthrone reagent and a few drops of concentrated H₂SO₄ and heated. Formation of green or purple color indicates the presence of sugars. Alkaloids: A 2 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer’s reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids. Phenols: A 2 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Flavonoids: A 2 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids. Saponins: A 2 ml of test solution was added with H₂O and shaken. Formation of foamy lather indicates the presence of saponins. Polysaccharides: A 2 ml of test solution was added with 1% ninhydrin in alcohol. Colour formation of violet or blue indicates the presence of polysaccharides. Anthraquinones: A 2 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthraquinones. Amino Acids: A 2 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

Catechins: A 2 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

2.5 Testing of antimicrobial activity

The test strains were: Bacillus subtilis NCIM 2920 (B1), Escherichia coli NCIM 2931 (B2), Staphylococcus aureus NCIM 5021 (B3), Candida glabrata MTCC 3984 (F1), Epidermophyton floccosum var. nigricans MTCC 613 (F2) and Microsporum canis MTCC 3270 (F3). The cultures were obtained from MTCC (Microbial Type Culture Collection), Chandigarh and NCIM (National Collection of Industrial Microorganisms), Pune, India. Microbial strains were tested for minimum inhibitory concentration using the disc diffusion method. The antibacterial and antifungal activity of test samples was analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The 0.60 and 1.20 mg/disc of sample coated disks were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48–72 h (for fungus). After incubation, the zone of inhibition was measured with ruler. The assays were performed in triplicate and the average values are presented.

In the present study, the plant of Caesalpinia bonduc Linn. Roxb was collected in the local area of Tiruchirappalli. Seeds were collected, air dried and subjected to size reduction to get coarse powder and subjected to physicochemical investigation and quality control tests of various parameters, which are prescribed in the Pharmacopoeias/Literature.

Preliminary phytochemical analysis of ethanol extracts of the Caesalpinia bonduc indicated the presence of certain secondary metabolites in a different manner (Table 1).

Table 1: Preliminary phytochemical analysis of Caesalpinia bonduc Linn. Roxb

<table>
<thead>
<tr>
<th>Test</th>
<th>Ethanol extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugar(A)</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugar(B)</td>
<td>+</td>
</tr>
<tr>
<td>Sugars</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic Compounds</td>
<td>-</td>
</tr>
<tr>
<td>Catachins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Anthroquinones</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Presence
- = Absence

Reports were revealed the Identification of major chemical constituents such as Steroid, Triterpenoids, Reducing Sugar (A), Reducing Sugar (B), Sugars, Flavonoids, Saponin, Amino acids in ethanol extract of seeds of Caesalpinia bonduc. Linn. Roxb rest of the metabolites were absent such as Alkaloids, Phenolic Compounds, Catachins, Tannins, Anthroquinones. This shrub contains a number of bioactive phytochemicals and is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. Tannins are dietary anti-nutrients that are responsible for the astringent taste of foods and drinks. Tannins bind to both proteins and carbohydrates which has several implications for commodities containing tannins. The presence can cause browning or other pigmentation problems in both fresh foods and processed products. The presence of tannin in the plants imply may have astringent properties and in addition, could quicken the healing of wounds and burns. This justifies their usage in herbal medicine.
The antimicrobial activity of *Caesalpinia bonduc* seed (ethanol extraction) was examined with gram positive, gram negative and fungal pathogenic microorganisms using the disk diffusion test. The results of the antimicrobial activities are summarized in table 2.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacteria/ Fungi</th>
<th>Zone of inhibition (mm) (Sample 0.60 &amp; 1.20 mg / disk)</th>
<th>Diseases</th>
<th>Route of Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus subtilis B1</td>
<td>10, 11, 21 &gt; PC</td>
<td>Nausea / Vomiting / Abdominal cramps</td>
<td>Water / Food / Soil</td>
</tr>
<tr>
<td>2.</td>
<td>Escherichia coli B2</td>
<td>11, 13, 0 &gt; PC</td>
<td>Gastroenteritis, Urinary tract infections / Neonatal meningitis / Hemolytic-uremic syndrome</td>
<td>Water / Food / Fecal matters</td>
</tr>
<tr>
<td>3.</td>
<td>Staphylococcus aureus B3</td>
<td>09, 10, 16 &lt; PC</td>
<td>Ritter's disease / staphylococcal scalded skin syndrome (SSSS) / Fruncles / Carbuncles</td>
<td>Water / Food / Fecal matters</td>
</tr>
<tr>
<td>4.</td>
<td>Candida glabrata F1</td>
<td>9, 10, 9 &gt; PC</td>
<td>Skin (Integument) Infections / Gastrointestinal tract Infection</td>
<td>Airways / Wound / Soil / Water</td>
</tr>
<tr>
<td>5.</td>
<td>Epidermophyton floccosum var. nigricans F2</td>
<td>10, 11, 8 &gt; PC</td>
<td>Tinea corporis / Tinea cruris / Tinea pedis</td>
<td>Airways / Wound / Soil / Water</td>
</tr>
<tr>
<td>6.</td>
<td>Microsporum canis F3</td>
<td>9, 11, 9 &gt; PC</td>
<td>Tinea capitis / Ringworm</td>
<td>Airways / Wound / Soil / Water</td>
</tr>
</tbody>
</table>

The mean value of the replicates (3) were presented PC: Positive Control (Bacteria – Methicillin (10mcg/disk); Fungi – Itraconazole (10mcg/disk))

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Caesalpinia bonduc</em> L</td>
<td>0.10</td>
<td>0.02</td>
<td>0.30</td>
<td>0.64</td>
<td>BDL</td>
<td>0.06</td>
<td>0.72</td>
</tr>
</tbody>
</table>

BDL – Below detectable limit

Fortunately, the tested concentrations (0.60 and 1.20 mg/disk) produce zone of inhibition on selective media plates. In the present study, higher (1.20 mg) concentration of sample got greater sensitivity when compared to the lower (0.60 mg) concentration in all the tested strains. Billah and his co-workers (2013) stated that the ethanol extraction of *C. bonduc* seed were highly affects the gram positive than gram negative organisms. But in our study, the gram negative strains were highly susceptible to the test samples than gram positive. In this study, all the bacterial and fungal strains were moderately affected and nil effect was not observed in the test samples. In bacteria, the test sample was more effective against *Escherichia coli* NCIM 2931 (B2) while smaller effect was noticed from *Staphylococcus aureus* NCIM 5021 (B3). In fungi, this was effective against *Epidermophyton floccosum var. nigricans* MTCC 613 (F2) whereas smaller effect was observed in *Candida glabrata* MTCC 3984 (F1). All the microbial strains depict higher sensitivity to the higher concentration (1.2 mg / disc) for the test sample when compared to the positive control except bacterial strains such as *Bacillus subtilis* NCIM 2920 (B1) and *Staphylococcus aureus* NCIM 5021 (B3). But, the fungal strains were highly sensitive to test samples than the positive control. There is no antimicrobial activity in negative control such as ethanol.

Toxic chemicals and trace metals are very important a pollutants which affect all ecosystem especially plants. Lithogenic and anthropogenic sources are plays a vital in the trace metal pollution. Chemical leaching of bedrocks, water drainage basins and runoff from banks are the lithogenic contribution of heavy metals while discharge of urban/ industrial waste water, combustion of fossil fuels, mining and smelting operations, processing and manufacturing industries and waste disposal including dumping are anthropogenic sources of metal pollution. The results of the trace metal concentrations are summarized in table 3. The mean concentration of trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) were 0.10, 0.02, 0.3, 0.64, BDL, 0.06 and 0.72 mg kg⁻¹, respectively. The higher concentration of metals in the plants could be entered into the various food chains and concentrations become elevated to levels which can prove to be toxic to human, animal, plant and other living organisms. Certain metals (Zn, Mn and Fe) are essential for plant growth and some are not play an important role in the plant physiology which can able to accumulate in plants in large extent. These metals were easily transferred from plants to animal and human food chain, and were caused to harmful effects on humans.

### 4. Conclusion

Preliminary phytochemical analysis of ethanolic seed extract of *Caesalpinia bonduc* indicated that the presence of steroid, triterpenoids, reducing sugar (a), reducing sugar (b), sugars, flavonoids, saponin and amino acids are responsible for good antibiotic applications against the certain pathogens. All the bacterial and fungal strains were moderately affected and nil effect was not observed in the test samples. Therefore, it can be recommended for high throughput research in the process of drug development for several diseases.

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### References