In vitro antimicrobial activity of *Caesalpinia sappan* L.

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1. Introduction

*Caesalpinia sappan* (C. sappan) L. belongs to family Caesalpiniaceae and is cultivated in South-East Asia for the production of red dye, which is obtained from its heartwood. The tree wood also contains water soluble dyes such as protosappanins, sapanchalcone and haematoxylin[1]. *C. sappan* is a small thorny spreading tree, grows up to 10m in height and the wood reaches 15–30 cm in diameter. It bears 3–4 seeds, ellipsoid, brown to black colored. Traditionally plant parts have been used as curative agents for skin infections and anemia[2]. Decoction prepared from the heartwood is commonly used for the treatment of arthritis, blood purifier, antidiabetic and improvement of complexion[3]. Many biological activity of *C. sappan* have been reported anti-complementary activity[4], anticonvulsant compounds[5], antibacterial[6], antimicrobial[7], anti-oxidant[8–11], anti-carcinogenic[12], hepatoprotective properties[13], antioxidant and hepatoprotective[14], flavonoids and phenol[15–17]. But, studies related to the scientific validation of the extracts against various pathogens are still lacking the information. In this view an identify the antimicrobial activities against various bacterial and fungal species.

2. Materials and methods

2.1. Collection and extraction

Fresh leave samples of *C. sappan* (heartwood) were collected from Coimbatore (Lat 28° 42' N; Long 77° 37' E). The samples were cut into small pieces and dried at room temperature (37°C). The air–dried sample was ground...
into fine powder and then directly subjected for Soxhlet extraction by using ethanol/petroleum ether (500 mL) solvents and then the extract was concentrated by using reduced pressure to yield a light brown colored mass. The aqueous was prepared by C. sappan of heartwood (38 g) with water (1000 mL) repeatedly for 48 hr. Then the extract was concentrated by lyophilization to yield dark brown substances (4 g, 10.52%) [18].

2.2. Antimicrobial assay

Bacterial suspension Staphylococcus aureus, Salmonella typhi, Escherichia coli, Streptococcus faecalis, Enterobacter aerogenes and Pseudomonas aeruginosa (10^6 cells/mL) were spread over the surface of Muller Hinton agar (HiMedia Laboratories private limited Mumbai, India) using sterile cotton swabs, disc impregnated with extracts (5 mg/disc) were applied on the solid agar medium by pressing slightly and incubated at 37°C for 24 h. But for antifungal activities (Aspergillus niger and Candida albicans) potato dextrose agar was used. Triplicate samples were maintained for each strain. Discs with the various solvents alone were used as a control. Further, preliminary phytochemical analysis was also performed with the most potent ethanolic extract of C. sappan based on the standard procedure [19].

3. Results

Antimicrobial activity suggested that, the maximum zone of inhibition (34.0±2.7) mm was observed in ethanolic extract against Pseudomonas aeruginosa followed by Staphylococcus aureus (31.0±2.7 mm), Salmonella typhi (24.0±2.1 mm), Enterobacter aerogenes (21.0±1.5 mm), Candida albicans (2.01±2.2 mm), Escherichia coli (15.0±1.4 mm) and minimum zone of inhibition (14.0±1.1) mm was observed in ethanolic extracts against Aspergillus niger. Further the maximum zone of inhibition (28.0±2.3) mm was observed in aqueous extract against Staphylococcus aureus, followed by Salmonella typhi (20.0±1.3 mm), Streptococcus faecalis (19.0±1.2) mm, Enterobacte aerogenes (18.0±1.4) mm, Candida albicans (18.0±1.7 mm), Aspergillus niger (10.0±0.8) mm, Escherichia coli (9.0±0.7) mm and minimum zone of inhibition (7.0±0.7) mm was observed in aqueous extract against Pseudomonas aeruginosa. In addition the maximum zone of inhibition (18.0±1.1) mm was observed in petroleum ether extract against Salmonella typhi followed by Streptococcus faecalis (17.0±1.8) mm, Pseudomonas aeruginosa (16.0±1.6) mm, Enterobacter aerogenes (15.0±1.3) mm, Aspergillus niger (13.0±1.3) mm, Escherichia coli (5.0±0.3) mm, Candida albicans (5.0±0.2) mm and minimum zone of inhibition (2.00±0.08) mm was observed in Staphylococcus aureus (Figure 1). Within adding up preliminary phytochemical analysis showed the presence of steroids, tannin, phenol, saponin, flavonoids, and absence of alkaloids, fixed oil and fats was observed in ethanolic and petroleum ether extracts. Further the presence of phenol, tannin and absence of steroids, saponin, flavonoids, alkaloids, fixed oil and fats was observed in petroleum ether extracts (Table 1).

Figure 1. Antimicrobial activity of heartwood extracts of C. sappan. S1: Ethanolic extract; S2: Aqueous extract; S3: Petroleum ether extract; S4: Control.
Table 1.
The preliminary phytochemical screening of the heartwood extracts of Caesalpinia sappan L.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Ethanol extracts</th>
<th>Petroleum ether extracts</th>
<th>Aqueous extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
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<tr>
<td>Steroids</td>
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<td>Tannin</td>
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<td>Phenol</td>
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<tr>
<td>Fixed oil and Fats</td>
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<td>-</td>
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<tr>
<td>Saponins</td>
<td>+++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Flavonoids</td>
<td>+++</td>
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</tbody>
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+++ High; ++ average; + low; - Absence.

4. Discussion

The results of the maximum antimicrobial activity was identified with ethanolic extract of C. sappan against Pseudomonas aeroginosa and the antimicrobial activity of the ethanolic extract might be due do the presence of unique phytochemical constituents. Also Mohan et al.[20] reported the antimicrobial activity with C. sappan (bark) and Mimosa pudica L against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginos, Klebsiella pneumonia, Proteus vulgaris, Candida albicans and Aspergillus niger. Similarly, Ghaleb Adwan et al.[21] reported the antimicrobial activity with Ecballium elaterium against Staphylococcus aureus and Candida albicans and Hasson et al.[22] also reported the antibacterial activity with the three medicinal plants Boswellia (Luban) species against Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli, Salmonella typhi, Proteus vulgaris, Klebsiella pneumoniae, Bacillus subtilis, Streptococcus pneumoniae, Corynebacterium diptheriae. The present study also made an attempt to identify the phytochemical constituents analysis and the results showed the presence of steroids, tannin, phenol, saponins and flavonoids and this phytochemical constituents previously reported with several biological properties phenolic compounds[23,24]. In addition Mohan et al.[20] reported the phytochemical analysis with the C. sappan (bark) and Mimosa pudica L. Similarly, Vij and Murugesan[25] reported the phytochemical analysis with the Cardioperum halicacabum and Mbaebie et al.[26] also reported the phytochemical analysis with Schotia latifolia Jacq. It can be concluded from the present findings that, the ethanolic extract of C. sappan collected from the Combator was showed potential antimicrobial activity and it can be used as a potential antibacterial drug after completing the in vivo and clinical trials.

Conflict of interest statement

We declare that we have no conflict of interest.

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References


