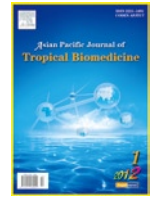




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## Document heading

*In vitro* antimicrobial activity of *Caesalpinia sappan* L.

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## ABSTRACT

**Objective:** To identify the antimicrobial activity of *Caesalpinia sappan* (*C. sappan*). **Methods:** Antimicrobial activity of *C. sappan* was tested with various solvent ethanol, aqueous and petroleum ether against various microorganisms (*Salmonella typhi*, *Streptococcus faecalis*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aerogenosa*, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albican*). Preliminary phytochemical analysis was also performed with the most potent ethanolic extract. **Results:** Out of the selected various solvent extracts, the ethanolic extract showed maximum average zone of inhibition [ $34.0 \pm 2.7$  mm] against *Pseudomonas aeruginosa* and minimum [ $6.0 \pm 0.3$  mm] zone of inhibition was observed with petroleum ether extract against *Escherichia coli coli*. In addition, the preliminary phytochemical analyses showed the presence of steroids, tannin, phenol, saponins and flavonoids in the ethanolic extract. **Conclusions:** The ethanolic extract of *C. sappan* can be used as a potential antibacterial source for various infections.

## 1. Introduction

*Caesalpinia sappan* (*C. sappan*)L. belongs to family of Caesalpinaceae and its cultivated in South–East Asia for the production of red dye, which is obtained from its heart wood. 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

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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 (1 000 mL) repeatedly for 48 hr. Then the extract was concentrated by lyophilization to yield dark brown substances (4 g, 10.52%)<sup>[18]</sup>.

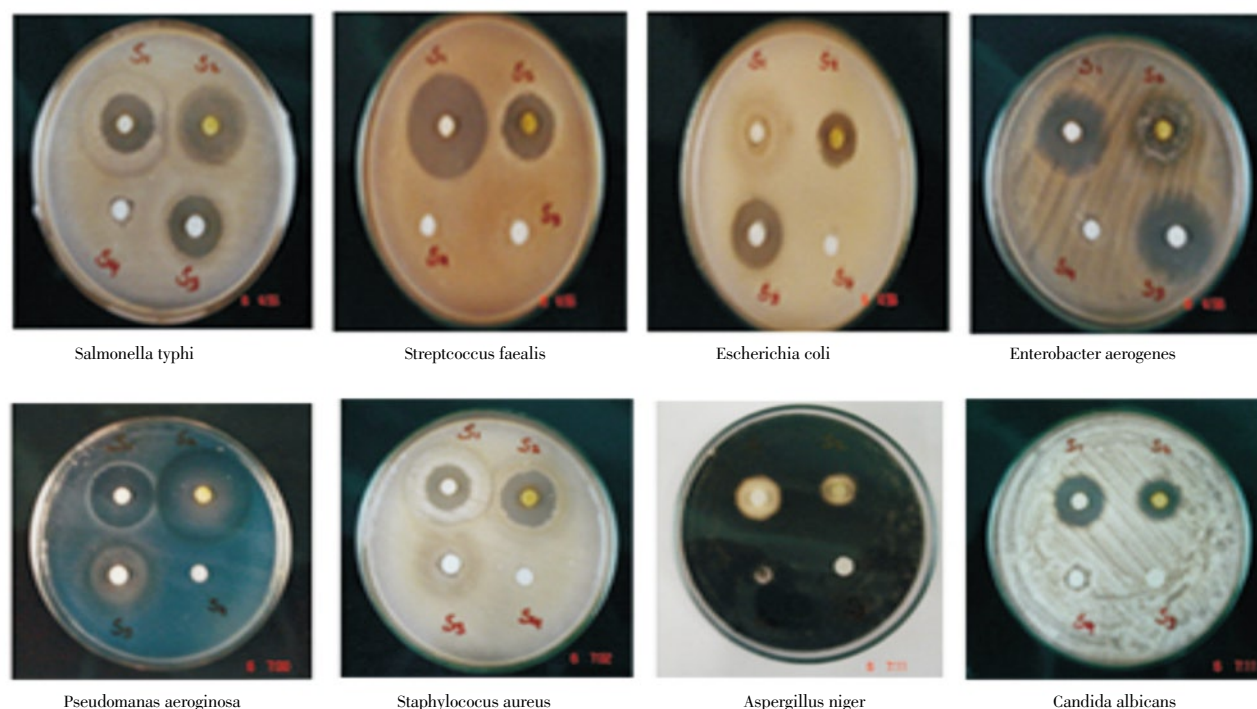
## 2.2. Antimicrobial assay

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<sup>[19]</sup>.

## 3. Results

Antimicrobial activity suggested that, the maximum zone of inhibition [ $34.0 \pm 2.7$  mm] was observed in ethanolic extract against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* [ $31.0 \pm 2.7$  mm], *Salmonella typhi* [ $24.0 \pm 2.1$  mm], *Enterobacter aerogens* [ $21.0 \pm 1.5$  mm], *Candida albicans*



**Figure 1.** Antimicrobial activity of heartwood extracts of *C. sappan*. S1: Ethanolic extract; S2: Aqueous extract; S3: Petroleum ether extract; S4: Control.

[( $2.01 \pm 2.2$ ) mm], *Escherichia coli* [( $15.0 \pm 1.4$ ) mm] and minimum zone of inhibition [( $14.0 \pm 1.1$ ) mm] was observed in ethanolic extracts against *Aspergillus niger*. Further the maximum zone of inhibition [( $28.0 \pm 2.3$ ) mm] was observed in aqueous extract against *Staphylococcus aureus*, followed by *Salmonella typhi* [( $20.0 \pm 1.3$ ) mm], *Streptococcus faecalis* [( $19.0 \pm 1.2$ ) mm], *Enterobacter aerogens* [( $18.0 \pm 1.4$ ) mm], *Candida albicans* [( $18.0 \pm 1.7$ ) mm], *Aspergillus niger* [( $10.0 \pm 0.8$ ) mm], *Escherichia coli* [( $9.0 \pm 0.7$ ) mm] and minimum zone of inhibition [( $7.0 \pm 0.7$ ) mm] was observed in aqueous extract against *Pseudomonas aeruginosa*. In addition the maximum zone of inhibition [( $18.0 \pm 1.1$ ) mm] was observed in petroleum ether extract against *Salmonella typhi* followed by *Streptococcus faecalis* [( $17.0 \pm 1.8$ ) mm], *Pseudomonas*

*aeruginosa* [( $16.0 \pm 1.6$ ) mm], *Enterobacter aerogens* [( $15.0 \pm 1.3$ ) mm], *Aspergillus niger* [( $13.0 \pm 1.3$ ) mm], *Escherichia coli* [( $5.0 \pm 0.3$ ) mm], *Candida albicans* [( $5.0 \pm 0.2$ ) mm] and minimum zone of inhibition [( $2.00 \pm 0.08$ ) 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).

**Table 1.**

The preliminary phytochemical screening of the heartwood extracts of *Caesalpinia sappan* L.

Phytochemical test	Ethanol extracts	Petroleum ether extracts	Aqueous extract
Alkaloids	–	–	–
Steroids	+++	++	–
Tannin	+++	++	+
Phenol	+++	+	++
Fixed oil and Fats	+++	+	–
Saponins	+++	+	–
Flavonoid	+++	+	–

+++ High; ++ average; + low; – Absence.

#### 4. Discussion

The results of the maximum antimicrobial activity was identified with ethanolic extract of *C. sappan* against *Pseudomonas aeruginosa* and the antimicrobial activity of the ethanolic extract might be due to the presence of unique phytochemical constituents. Also Mohan *et al*<sup>[20]</sup> reported the antimicrobial activity with *C. sappan* (bark) and *Mimosa pudica* L against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*. Similarly, Ghaleb Adwan *et al*<sup>[21]</sup> reported the antimicrobial activity with *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans* and Hasson *et al*<sup>[22]</sup> also reported the antibacterial activity with the three medicinal plants *Boswellia* (Luban) species against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Streptococcus pneumonia*, *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<sup>[23,24]</sup>. In addition Mohan *et al*<sup>[20]</sup> reported the phytochemical analysis with the *C. sappan* (bark) and *Mimosa pudica* L. Similarly, Viji and Murugesan<sup>[25]</sup> reported the phytochemical analysis with the *Cardiospermum halicacabum* and Mbaebie *et al*<sup>[26]</sup> 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 Coimbatore 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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