Screening of antimicrobial activity and phytochemical analysis of Caesalpinia sappan L.

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ABSTRACT

Present study deals with the antimicrobial activity and phytochemical analysis of Caesalpinia sappan L. Antimicrobial activity of C. sappan was analyzed by using Well Diffusion (WD) and Disc Diffusion (DD) methods. Antimicrobial activity was analyzed using crude extract of C. sappan prepared by using various solvents such as methanol, ethanol, acetone, chloroform and petroleum ether against various microorganisms such as Escherichia coli, Bacillus cereus, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumonia. Phytochemical analyses were carried out for most potent methanol, ethanol and acetone extract of C. sappan using standard assays. Out of the various selected extracts, methanol, ethanol and acetone extracts showed effective antimicrobial activity against the bacteria. Petroleum ether extract of the plant showed no significant zone of inhibition against the bacteria. The phytochemical analysis of C. sappan showed the presence of flavonoids, phenolic compounds, tannins, saponin, protein, oxalic acid, carbonate, oil and fat which also exhibited the presence of antimicrobial activity. These results were confirmed using the Thin Layer Chromatography (TLC).

Key words: Antimicrobial activity, C. sappan, phytochemical analysis, TLC, Well Diffusion, Disc Diffusion.

INTRODUCTION

Caesalpinia sappan L. belongs to the family Fabaceae. It is a small thorny tree, grows up to 10 m in height and the wood spreads 15-30 cm in diameter. It bears 3-4 seeds, ellipsoid, brown to black colored. It is commonly known as Sappan wood or Brazil wood. In early days, the heart wood of the C. sappan was used in calico printing of cotton, wool and silk [1]. Later it is being used to colour wines and meat. The roots of the C. sappan called ‘yellow wood’ are also used to make yellow dye. The tree is cultivated in south-east Asia for the red (Brazilin) dye. The dye is reported to also well establish as a safe natural colouring agent with good medicinal value for food products, beverages and pharmaceuticals [2]. The pigment finds to use in manufacture of facials which are resistant to light heat and water and also nonirritating [3]. C. sappan used in traditional Chinese medicine for activating blood circulation and removing stasis [4]. In recent years, the extract of sappan lignum has been found to be a potential immunosuppressive agent [5, 6] and has many other biological activities includes hepato protective activity [7], immunomodulator[8], hypoglycemic activity[9], anti-complementary[10], anticonvulsant property[11], anti-inflammatory and antibacterial activity[12,13], xanthine oxidase inhibition[14], aldose reductase inhibition[15], antioxidant activity[16]. The various compounds were isolated from the wood of C. sappan like Flavonoids, phenolic compounds [17].

In the present study, antimicrobial activity and phytochemical analysis of crude leaf extract of Caesalpinia sappan L. were carried out. Antimicrobial activity of C. sappan was analyzed by two methods Well Diffusion (WD) and Disc
Diffusion (DD) using crude leaf extract prepared by using various solvents such as methanol, ethanol, acetone, chloroform and petroleum ether against various microorganism such as *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Klebsiella pneumonia*. Phytochemical analyses were carried out for most potent methanol, ethanol and acetone extract of *C. sappan* using standard assays.

**EXPERIMENTAL SECTION**

**Plant Material:**
Leaves of *Caesalpinia sappan* L were collected from the Jeppiaar Engineering College campus in the month of October 2012.

**Extract Preparation:**
The leaves were collected and washed thoroughly in water, chopped, air dried for 2 days at 35°-40°C and pulverized in electric grinder. 5gm of powder had taken and packed with Whatman filter paper and kept in the 50 ml of various solvents like methanol, ethanol, chloroform, acetone and petroleum ether. Sample along with the solvent was kept in the shaker for 24 Hours to get the crude extract. These crude extracts were allowed to evaporate to get the powdered extract. For the stock, 5mg of crude extract powder was dissolved with 1 ml of mother solvent for the further analysis.

**Antibacterial Activity**
For Well Diffusion (WD) method, 50µl and 100µl from the stock (5mg/ml) was taken and for Disc Diffusion (DD) method, the sterile paper disc dipped in the stock (5mg/ml) was used against various bacterial pathogens such as *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Klebsiella pneumonia* which causes diarrhea, urinary tract infection, intravenous liver infection, nosocomial blood stream infection and neonatal meningitis respectively. The Luria Britani Agar (LBA) was used as media for this study.

**Phytochemical analysis**
The phytochemical tests were carried out for the above mention plant extracts using the standard procedures to identify the components [18].

**TLC**
The TLC analyses were carried out for the *C. sappan* leaf extract stock (5mg/ml) [19]. The modified mobile phase used was Ethanol: Glacial Acetic Acid: Formic Acid: water having a ratio of 7:0.5:7:0.5:1 for the analysis.

**RESULTS AND DISCUSSION**

**Screening of antimicrobial activity through the disc and well diffusion method**
The antimicrobial activity of solvent extract *C. sappan* against human pathogenic bacteria, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis* were measured by measuring the zone of inhibition in disc diffusion and well diffusion method. Test sample per disc was about 100 µg/disc and per well 50µl (250µg/50µl), 100µl (500µg/100µl). The organisms used and zone of inhibition to the corresponding extracts are shown in Table 1. The Zone of inhibition ranged from 6 – 14 mm for methanol, 5-15mm for ethanol, 7-14mm for acetone 5-13mm for chloroform and no zone formation in petroleum ether respectively. The highest zone of inhibition, for methanol against *E. coli* 10mm (DD) and *E. faecalis* 14mm (WD-100µl/well), for ethanol against *E. coli* 9mm (DD) and 13mm (WD-100µl/well), for acetone against *B. cereus* 10mm (DD) and *E. faecalis* 14mm (WD-100µl/well), for chloroform against *E. coli* 9mm (DD) and 13mm (WD-100µl/well). Petroleum ether extract of the plant shows no significant zone of inhibition against the bacteria.

Different extracts of leaves of *C. sappan* were examined for their antimicrobial property activity. Out of the various selected extracts, the methanol, ethanol and acetone extracts have the good effective against the bacteria. It is evident from this study, that as the concentration of the various extracts of leaves sample were increased; there is increase in the diameter of the inhibition zone irrespective of the organism used. The methanol extracts were used in this study as they were found to give better effect against the microorganisms, the methanol extracts of the plant provide more consistent antimicrobial activity compared to those extracted by other solvents.
Table 1: Antibacterial activity of leaf crude extract of Caesalpinia sappan L.

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Methanol Extract</th>
<th>Ethanol Extract</th>
<th>Acetone Extract</th>
<th>Chloroform Extract</th>
<th>Petroleum Ether Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc 50µl 100µl</td>
<td>Disc 50µl 100µl</td>
<td>Disc 50µl 100µl</td>
<td>Disc 50µl 100µl</td>
<td>Disc 50µl 100µl</td>
</tr>
<tr>
<td>B. cereus</td>
<td>9 9 12</td>
<td>7 7 12</td>
<td>10 11 14</td>
<td>8 9 11</td>
<td>- - -</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>- - 8</td>
<td>- - 15</td>
<td>5 12</td>
<td>- -</td>
<td>- - -</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>4 8 10</td>
<td>- - 8</td>
<td>- - 10</td>
<td>5 7 11</td>
<td>- - -</td>
</tr>
<tr>
<td>E. coli</td>
<td>10 10 12</td>
<td>10 12 15</td>
<td>- 10</td>
<td>9 10 13</td>
<td>- - 5</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>6 9 14</td>
<td>5 10 11</td>
<td>7 9 14</td>
<td>5 10 13</td>
<td>- - -</td>
</tr>
</tbody>
</table>

Figure 1: Antimicrobial Activity of C. sappan for Well diffusion method (50µl/well) against various bacteria.

Figure 2: Antimicrobial Activity of C. sappan for Well diffusion method (100µl/well) against various bacteria.

Figure 3: Antimicrobial Activity of C. sappan for Disc diffusion method against various bacteria.

Phytochemical Analysis
The different phytochemicals tests performed on the extracts of C. sappan leaves showed Table 2, the presence of flavonoids, phenolic compounds, tannins, saponin, proteins, oxalic acid, carbonate, oil and fat, and absence of alkaloids, glycosides and sulphate also shows the presence of antimicrobial activity of the C. sappan.

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Table 2: Phytochemical constituents of methanol, ethanol and acetone extract of C. sappan

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical Test</th>
<th>Methanol Extract</th>
<th>Ethanol Extract</th>
<th>Acetone Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Organic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Saponin (foth test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Organic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TLC analysis also suggests the presence of different kinds of phytocompounds in leaves extract. Table 3 reports the Rf values for various extracts and plate 6 shows photographs of the studied TLC analyses. TLC analyses of plant extract in Methanol and Ethanol extracts reports two spots for various phytocompounds at the resolution value of Rf = 0.67, Rf = 0.90 for methanol extract and Rf = 0.6, Rf = 0.95 for ethanol extract. The reported spots are separated with enough space and having various Rf values showing the presence of at least one phytocompounds in Chloroform and acetone solvent extracts at the resolution of Rf = 0.78 and Rf = 0.82.

CONCLUSION

Though there are a number of antibiotic drugs available in the market, they may produce many side effects. In this study, it is found to be Methanol and Ethanol extract of leaves of Caesalpiniasappan have high potency to act against the human pathogens. The different phytochemicals tests performed on the extracts of C.sappan confirms the presence of flavonoids, phenolic compounds, tannins, saponin protein, oxalic acid, carbonate, and oil and fat also exhibited antimicrobial activity. The TLC analyses also confirmed the presence of active compounds in the crude extract of C. sappan. Thus, CaesalpiniasappanL could be used as an alternative source of production of antibiotic drug against the pathogens.
REFERENCES


