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Research Article

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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 CaesalpiniasappanL. Antimicrobial activity of C. sappanwas analyzed by using Well Diffusion (WD) and Disc Diffusion (DD) methods. Antimicrobial activity was analyzed using crude extract of C. sappanprepared 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. sappanusing 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*is 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 *Caesalpiniasappan*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^{o}-40^{o}C and pulverized in electric grinder. 5gm of powder had taken and packed with Whattman 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, Klepsiella 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 7:0.5:7:0.5:1for 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* 10mm(DD) and *B. subtilis* 15mm(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.

Plant Extract	Zone of inhibition (millimeter)														
	Methanol Extract			Ethanol Extract			Acetone Extract			Chloroform Extract			Petroleum Ether Extract		
	Disc	50µl	100µl	Disc	50µl	100µl	Disc	50µl	100µl	Disc	50µl	100µl	Disc	50µl	100µl
B. cereus	9	9	12	7	7	12	10	11	14	8	9	11	-	-	-
B. subtilis	-	-	8	-	-	15	5	-	12	-	-	-	-	-	-
K. pneumonia	4	8	10	-	-	8	-	-	10	5	7	11	-	-	-
E. coli	10	10	12	10	12	15	-	-	10	9	10	13	-	-	5
E. faecalis	6	9	14	5	10	11	7	9	14	5	10	13	-	-	-

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

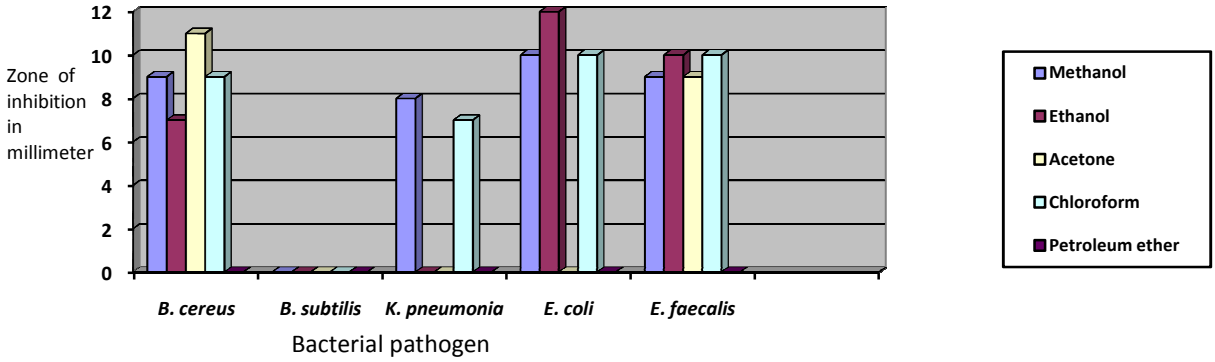


Figure 2: Antimicrobial Activity of *C. sappan* for Well diffusion method (100µl/well) against various bacteria.

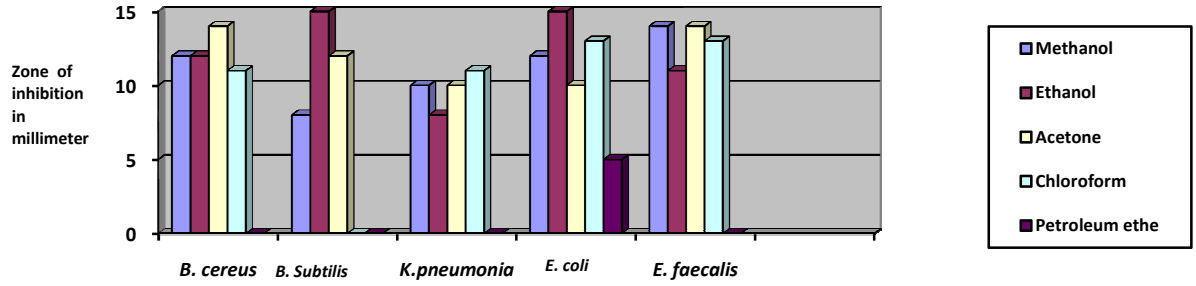
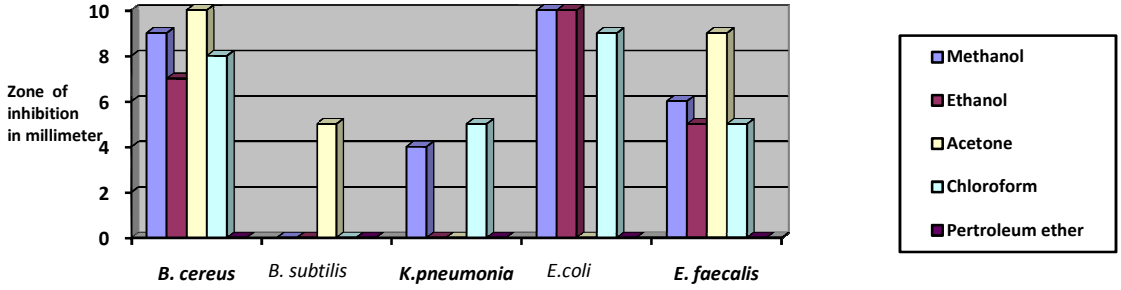


Figure 3: Antimicrobial Activity of *C. sappan* for Disc diffusion method against various bacteria



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*.

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

S. No	Phytochemical Test	Methanol Extract	Ethanol Extract	Acetone Extract
1.	Flavonoids			
	a. Alkaline reagent test	+	+	-
	b. Ferric chloride test	+	+	+
2.	Alkaloids	–	–	–
	Wagner's test			
3.	Phenolic compounds			
	a. Lead acetate test	+	+	+
	b. Ferric chloride test	+	+	+
4.	Tannins			
	a. Lead acetate test	+	+	-
	b. Ferric chloride test	+	+	+
5.	Glycosides			
	a. Keller killiani test	-	-	-
6.	Amino acids			
	a. Ninhydrin test	+	+	+
7.	Proteins			
	a. Biuret test	+	+	+
8.	Oils and fats	+	+	+
9.	Organic acid			
	a. Oxalic acid test	+	+	+
	b. Malic acid test	-	-	-
10.	Carbohydrate			
	a. Molisch's test	+	+	+
	b. fehling's test	+	+	+
11.	Saponin(froth test)	+	+	-
12.	Organic acid			
	a. Sulphate test	-	-	-
	b. Carbonate test	+	+	+

TLC

Table 3: *Rf*values of various extracts of *C. sappan*leaves

Plant Extract	***Rf* value**
	Ethanol : Glacial Acetic Acid:Formic Acid : water 7:0.5:0.5:1
Methanol	R_f = 0.67 R_f = 0.90
Ethanol	R_f = 0.6 R_f= 0.95
Acetone	R_f= 0.78
Chloroform	R_f = 0.82

TLC analysis also suggests the presence of different kinds of phytocompouds in leaves extract. Table 3 reports the Rfvalues 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 R_f = 0.67, R_f = 0.90 for methanol extract and R_f = 0.6, R_f= 0.95 for ethanol extract. The reported spots are separated with enough space and having various $R_f$values showing the presence of at least one phytocompounds in Chloroform and acetone solvent extracts at the resolution of R_f= 0.78 and R_f = 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*L have high potency to act against the human pathogens. The different phytochemicals tests performed on the extracts of *C.sappan*leaves 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, *Caesalpiniasappan*L could be used as an alternative source of production of antibiotic drug against the pathogens.

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