International Journal of Botany Studies
ISSN: 2455-541X, Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 1; Issue 4; July 2016; Page No. 19-21

Evaluation of phytochemical analysis and antimicrobial activity of different solvent seed extracts of *Caesalpinia Bonduc* (L.) Roxb.

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Abstract

The present study was carried out to find out the phytochemical screening and antimicrobial activity of different solvent seed extracts of *Caesalpinia bonduc*. The phytochemical analysis carried out revealed the presence of alkaloids, flavonoids, glycosides, coumarins, saponins, tannins, terpenoids, steroids and quinones in the plants. The antimicrobial activity of three different extracts of *Caesalpinia bonduc* was evaluate using well diffusion method against *Bacillus cereus, Staphylococcus epidermidis, Escherichia coli, Salmonella paratyphi, Beauveria bassiana* and *Candida albicans*. Ethyl acetate extract presence of number of phytochemical than the other solvent extracts. Ethyl acetate extract of *Caesalpinia bonduc* has least activity against all the tested cultures. The methanol extract of *Caesalpinia bonduc* showing strong antimicrobial activity against all test species. Our findings showed that *Caesalpinia bonduc* plant extracts have higher levels of antimicrobial activity.

Keywords: *Caesalpinia bonduc*, Phytochemicals, Antimicrobial activity, Seeds

1. Introduction

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [2]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [3].

Caesalpinia bonduc (L.) Roxb. is a medicinal herb that belongs to the family Caesalpiniaceae. The root of *Caesalpinia bonduc* was used in eliminate intestinal worms and also reduce fever and cough. The leaf paste made from water is applied topically on the cheek to control toothache and skin diseases. Hence the present study mainly aims at phytochemical screening and antimicrobial activity of *Caesalpinia bonduc*.

2. Materials and Methods

Plant material

Caesalpinia bonduc plant was collected from the natural population growing in the Theni forest Area, Tamil nadu, India, during October 2015. The plant sample was taken to the Botany Research Laboratory and a Voucher specimen of the plant was deposited in the Botany research laboratory of V.H.N.S.N. College (Autonomous) for further references.

Preparation of extract

The dried seed extracts were treated to sequential extraction using five organic solvents on the basis of polarity of solvents (Hexane, Ethyl acetate and Methanol). 30g of the seed sample was taken in a separate conical flask and 200 ml of hexane was added. The conical flask was kept on mechanical shaker for 24 hours, after which the extract was filtered through Whatman filter paper 1. The pellet was allowed to dry and this pellet was used for the next solvent extraction (Ethyl acetate and Methanol). The dried extract was recovered and stored in Refrigerator for further analysis.

Extract Screening

The collected plant seed extracts were subjected to phytochemical analysis and antimicrobial activity were carried out using standard methods.

Test for Tannins (Braymer's Test)

1ml of the seed extracts were mixed with 2ml of water. To this, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [4, 5].

Test for alkaloids

To 1 ml of seed extracts, 6 drops of Mayers reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids [4, 5].

Test for Saponins (Foam Test)

1ml of seed extracts were mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins [4, 5].

Test for phenols

1ml of the seed extracts were treated with 3% ferric chloride. The appearance of deep blue color shows the presence of phenols [6, 7].

Test for flavonoids
1ml of the seed extracts were added to 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [6, 7].

Test for Quinones
1ml of the seed extracts were treated with 5 ml of HCL. Formation of yellow color precipitate indicated the presence of quinones [6, 7].

Test for Glycosides
0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides [6, 7].

Test for steroids (Salkowski Test)
To 2ml of the seed extracts, 2ml of chloroform was added followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids [5, 8, 9].

Test for terpenoids
2ml of the seed extracts were treated with 2ml acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids [5, 8, 9].

Test for Coumarins
2ml of the extracts were taken and 3ml of 10% sodium hydroxide was added. Formation of yellow coloration indicated the presence of coumarins [5, 8, 9].

Statistical analysis
All the data was reported as mean ± standard deviation of three replicates. Statistical analysis was performed using Microsoft Excel.

3. Results
The phytochemical screening of *Caesalpinia bonduc* Hexane, Ethyl acetate and Methanol extract showed the presence of alkaloids, flavonoids, glycosides, steroids and quinines (Table 1)

Table 1: Preliminary phytochemical screening of seed extracts of *Caesalpinia bonduc*

S. No.	Phytochemical constituents	Observation for different solvent extract		
		Hexane	Ethyl acetate	Methanol
1.	Alkaloids	-	++	+
2.	Coumarins	-	-	-
3.	Flavonoids	-	++	-
4.	Glycosides	+	++	+
5.	Phenolics	-	-	-
6.	Saponins	-	++	-
7.	Steroids	+	+	-
8.	Tannins	-	-	-
9.	Terpenoids	-	-	-
10.	Quinones	-	+	++

- Absent, + Present, ++ Moderate

Comparatively the plant extract showed the better results for both gram negative and gram positive bacteria and fungus. The methanol extract showed similar activity against *Staphylococcus epidermidis, Escherichia coli* (22mm) and *Bacillus cereus, Salmonella paratyphi* and *Candida albicans* showed (20mm). The hexane extract showed moderate activity against the *Staphylococcus epidermidis* (22mm). The ethyl acetate extract revealed the activity ranges between (10-18mm) which is all the extract of activity compared with commercially available synthetic antibiotic. Thus, the *Caesalpinia bonduc* seed extract showed antimicrobial activity against gram positive, gram negative bacterial strains and fungus strain (Table 2).

Table 2: Antimicrobial analysis of seed extracts of *Caesalpiniabonduc*

S. No.	Strains	Zone of inhibition (mm)			
		Neocin (10µg)	Hexane extract	Ethyl acetate extract	Methanol extract
1.	*Bacillus cereus*	21±0.4	12±0.3	10±0.8	20±0.4
2.	*Staphylococcus epidermidis*	20±0.4	22±0.5	12±0.8	22±0.3
3.	*Escherichia coli*	21±0.1	16±0.6	14±0.6	22±0.6
4.	*Salmonella paratyphi*	22±0.6	14±0.3	10±0.7	20±0.2
5.	*Beauveria bassiana*	21±0.5	10±0.4	18±0.3	24±0.7
6.	*Candida albicans*	23±0.3	14±0.5	10±0.6	20±0.8

* Values are Mean±S.E in triplicates

4. Discussion
In recent years, secondary plant metabolites have been investigated as a source of medicinal agents [10]. The explore for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [11 12]. Our preliminary investigation showed that methanol, ethyl acetate and hexane extracts of *Caesalpinia bonduc* have active compounds and were active against the locally isolated human pathogens like *Bacillus cereus, Staphylococcus epidermidis, Escherichia coli, Salmonella*

paratyphi, Beauveria bassiana and Candida albicans. In the present study, different extracts of *Caesalpinia bonduc* was evaluated for exploration of their antimicrobial activity against certain Gram negative, Gram positive bacteria and fungus which was regarded as human pathogenic microorganism.

5. Conclusion

In conclusion, of the present study *Caesalpinia bonduc* contain potential antimicrobial components. From this results of the study support the folklore claim along with the development of new antimicrobial drugs from the plants. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

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