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## Screening of Secondary Metabolites and Antibacterial Activity of *Acacia concinna*

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**Abstract:** The prime purpose of this study was to screen secondary metabolites i.e., phytochemical analysis and to evaluate the antimicrobial activities of *Acacia concinna* pods. The phytochemical analysis was done to screen Alkaloids, Flavonoids, Phytosterols, Saponin, Tannins, Phenolic compounds, Gums and Mucilage. Antimicrobial activity was also done *in vitro* by agar cup diffusion method by using methanol, benzene, chloroform, petroleum ether, butanol and aqueous extracts against bacteria, viz., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and fungi like *Aspergillus niger*, *Penicillium* sp. *Candida albicans* etc. The benzene, methanol and aqueous extracts of fresh pods of *Acacia concinna* showed maximum activity against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Acacia concinna* was observed to have an antimicrobial activity and can be used for medicinal purposes. In the present study, it was noted that *Acacia concinna* has antimicrobial activity and also there is presence of Secondary metabolites in pods of *Acacia concinna*.

**Key words:** Phytochemicals, shikakai, alkaloids, phenolic compounds

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### INTRODUCTION

*Acacia concinna* Shikakai means fruit for hair has been used for hair care in India for centuries, it is now grown commercially in India and Far East Asia. The drugs used in indigenous system of medicines like Ayurveda in India has about 18,000 species of angiosperms, of which about 3,000 species are considered as important sources of medicinal and aromatic chemical compounds. The plant parts used for the dry powdered or the extract are the bark, leaves or pods. It is a common shrub found in jungles throughout India (Abelson, 1990). The bark contains high levels of Saponins, which are foaming agents that are found in several other plant species (Segelman and Farnsworth, 1969).

Antidermatophytic activity of pods of *Acacia concinna* was studied against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton violaceum*, *Microsporum nanum* and *Epidermophyton floccosum* (Natarajan and Natarajan, 2009). *Tridax procumbens*, *Ocimum sanctum* and *Vitex nigundo* are some of the important medicinal plants having antimicrobial activity and also possess secondary metabolites (Kapoor *et al.*, 1996). *Acacia concinna* is important medicinal plant belonging to family

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Acaciaceae. The saponin of the bark has spermicidal activity against human semen (Nielsen, 1992). In aqueous state it has a low pH.

## MATERIALS AND METHODS

### Plant Materials

The pods of *Acacia concinna* were collected from agricultural fields of Maharashtra, India. (December, 2009) The plant was identified and confirmed using standard manuals (Theodore Cooke, Flora).

### Preparation of Extracts for Phytochemical Analysis

The pods of *Acacia concinna* was allowed to dry and pulverized by using mortar and pestle. Five gram pulverized material was dissolved in 50 mL of solvent (petroleum ether, benzene, chloroform, butanol methanol and aqueous extracts) and kept in an orbital shaker overnight. The obtained extracts were filtered with Whatman No. 42 filter paper (125 mm) and the filtrate was collected and used for experimental analysis.

### Phytochemical Analysis

#### Test for Alkaloids

Three milliliter of each extract was evaporated to dryness and residue was heated on a boiling water bath with 2N HCL (5 mL). After cooling the mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent and the other with equal amount of Wagner's reagent (Rizk, 1982). The sample was then observed for the presence of turbidity or precipitation. + Score was recorded if the reagent indicated the presence of compound. Score was recorded for the absence of compound (Salehi *et al.*, 1992).

#### Test for Flavonoids

Five milliliter of each extract was treated with a few drops of conc. 2N HCL and Magnesium turnings (0.5 g). The presence of Flavonoids was indicated if pink or magenta red colour developed within 3 min (Somolenski *et al.*, 1972).

#### Test for Phytosterols

One gram of the extract was dissolved in a few drops of dry acetic acid; 3 mL of acetic anhydride was added, followed by a few a drops of conc. sulphuric acid. Appearance of bluish green color showed the presence of phytosterols. Phytosterols are plant sterols structurally similar to cholesterol that act in the intestine to lower cholesterol absorption.

#### Test for Saponin

About 2.5 g of dried powdered sample was extracted with boiling water. After cooling the extract was shaken vigorously to froth and then allowed to stand for 15-20 min and classified for saponin content as follows. No froth = negative; forth less than 1 cm = weakly positive; froth 1.2 cm high = positive and froth greater than 2 cm high = strongly positive (Segelman and Farnworth, 1969).

#### Test for Tannins and Phenolic Compounds

Ten milliliter of each extract was evaporated and residue was extracted by 10 mL of hot 0.9% NaCl solution, filtered and 1% gelatin salt reagent is added; precipitation with the

reagent is indicative of the presence of Tannins. Positive tests are confirmed by addition of  $\text{FeCl}_3$  solution to the extract and should result in catachrestic blue, blue black, green or blue green color and precipitate.

#### **Test for Gums and Mucilage**

About of 10 mL of various extracts were added separately to 25 mL of absolute alcohol with constant stirring and then filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

#### **Test Organisms**

Test organisms: *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and Fungal cultures *Aspergillus niger*, *Penicillium* sp. *Candida albicans* etc. were obtained from NCIM Department of National Chemical Laboratory, Pune.

#### **Antibacterial Assay**

A modified agar diffusion cup method (Baurer *et al.*, 1966; Wilkins *et al.*, 1972) was used to determine Antibacterial Assay. Twenty four hours old overnight cultures (0.2 mL) of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, were spread on respective nutrient agar plates, wells are prepared with the help of borer and to that 0.1 mL of each of these extracts were added and kept for incubation for 24 h at 37°C. Distilled water was used as negative control. Inhibition zone diameter (mm) around each of the cup was calculated for each of the plate.

#### **Antifungal Assay**

For the evaluation of antifungal effect PDA, medium was inoculated with fungal cells. The plates were incubated for 3 days at 25°C and further process was repeated as mentioned above.

## **RESULTS**

The results for phytochemical analysis are reported as above. Phytochemical analysis shows that methanol, benzene, chloroform, petroleum ether, butanol and aqueous extracts of pods of *Acacia concinna* contains alkaloids, flavonoids, phytosterols, saponin, tannins, phenolic compounds, gums and mucilage.

Table 1 shows the analysis of phytochemical screening of various solvent extracts of pods; *Acacia concinna*. The benzene, methanol, aqueous, chloroform extracts shows presence of all photochemical constituents except for tannins in petroleum ether and butanol extract. The presence of these compounds may show antibacterial property.

Table 2 shows the antimicrobial activity of methanol, benzene, chloroform, petroleum ether, butanol and aqueous extracts of *Acacia concinna*. The benzene, methanol and aqueous extracts of fresh pods of *Acacia concinna* has maximum activity against all types of microorganisms. The inhibition was found to be maximum against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Table 3 shows the antifungal activity of methanol, benzene, chloroform, petroleum ether, butanol and aqueous extracts of *Acacia concinna*. The butanol, methanol and aqueous extracts of fresh pods of *Acacia concinna* has maximum activity against all types of fungal

Table 1: Qualitative analysis of phytochemical screening of various solvent extracts of pods; *Acacia concinna*

Phytochemical constituents	Aqueous extract	Benzene extract	Chloroform extract	Petroleum ether extract	Butanol extract	Methanol extract
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phytosterols	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	-	-	+
Phenolic compounds	+	+	+	+	+	+
Gums and Mucilage	+	+	+	-	-	+

+ indicates presence of compound, - indicates absence of compound

Table 2: *In vitro* antibacterial activity of different extracts of pods; *Acacia concinna*

Organisms	Extract (mm)					
	Aqueous	Benzene	Chloroform	Petroleum ether	Butanol	Methanol
<i>Klebsiella pneumoniae</i>	12.5	14.2	6.8	6.8	6.2	11.4
<i>Bacillus subtilis</i>	10.4	11.2	7.4	6.4	5.5	10.2
<i>Escherichia coli</i>	10.2	10.4	6.4	6.8	4.2	7.8
<i>Staphylococcus aureus</i>	5.4	6.4	6.3	4.6	4.2	6.2
<i>Pseudomonas aeruginosa</i>	5.4	5.0	5.4	4.2	4.0	5.2

Values are mean of three replicates

Table 3: Antifungal activity of different extracts of pods; *Acacia concinna*

Organisms	Extract (mm)					
	Aqueous	Benzene	Chloroform	Petroleum ether	Butanol	Methanol
<i>Aspergillus niger</i>	12.5	6.6	7.6	6.8	10.2	10.4
<i>Penicillium</i> sp.	10.4	5.5	-	6.4	9.2	-
<i>Candida albicans</i>	8.2	4.2	6.4	-	8.4	7.8

Values are mean of three replicates

species. The inhibition was found to be maximum against *Aspergillus niger*, *Penicillium* sp., followed by *Candida albicans*.

## DISCUSSION

The screening of Secondary metabolites and antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (Cowen, 1999; Kelmanson *et al.*, 2001; Srinivasan *et al.*, 2001). The aqueous extraction of plants showed greater activity than the organic extraction. Britto (2001) reported that most of the antibacterial principles were either polar or non-polar and were extracted only through the organic solvent medium. Hence the present investigation suggested that the organic solvent extraction method is also suitable to verify antibacterial activity.

The result of the present study reveals that the employed extracts of plants exhibited potential antibacterial activity against the tested pathogens. The present study supports the view that several medicinal plants might be useful as antibacterial agents. In this study we used only crude extracts of pods of *Acacia concinna*. Identification of active principle and use of pure compounds will help us to compare the activity of known antimicrobial agents. Annapurna *et al.* (2003) have already reported the wound healing and chemo protective effects of *I. coccinea* flowers. In this study, the maximum activity was observed against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This showed that the plant can be used for medicinal purposes. Earlier studies on phytochemicals reported that the antibacterial activity of terpenoids (Mahmoud *et al.*, 1999), saponins (Soetan *et al.*, 2006), tannin (Hamilton-Miller,

1995), alkaloids (Rizk, 1982) and flavonoids (Tsuchiya *et al.*, 1996) isolated from the plant materials. Thus the present study supports that some of medicinal plants have antibacterial activity, along with that it also contains some important secondary metabolites and this study supports the previous work which had lots of attention towards antimicrobial activity and findings and use of secondary metabolites. The presence of phytochemicals in this study might be a factor for the antibacterial activity of *Acacia concinna*. Methanol, benzene and aqueous extracts of pods of *Acacia concinna* showed maximum activity against *Aspergillus niger* followed by *Penicillium* sp. and *Candida albicans*.

### CONCLUSION

The study reveals the usefulness of medicinal plant in the control of disease caused by bacterial and fungal pathogenic species. Plant extracts have assumed an increased importance in medicine and in healthcare industry and further works on the above suggested aspects may be undertaken. Thus, it can be very useful and seems to be a potential source for arresting the growth and metabolite activities of various general bacteria and fungi. Nevertheless, our present study suggests further study of plant extract for their therapeutic efficacy is essential.

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