

Antimicrobial activity and Screening of Secondary metabolites from *Acacia concinna*

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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 spp.* *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: *Acacia concinna*, Antibacterial activity, Secondary metabolites.

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 (Rajasekaran, 2001).

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. The bark contains high levels of Saponins, which are foaming agents that are found in several other plant species.

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.) *Acacia concinna* is important medicinal plant belonging to family Acaciaceae. The saponin of the bark has spermicidal activity against human semen. (Nielsen 1992.) It has a natural low pH, is extremely mild, and doesn't strip hair of natural oils. Usually no rinse or conditioner is used since Shikakai also acts as a detangle.

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MATERIAL AND METHODS

Plant Materials

The pods of *Acacia concinna* were collected from agricultural fields of Maharashtra, India. The plant was identified and confirmed using standard manuals.

Preparation of Extracts for Phytochemical Analysis

The pods of *Acacia concinna* was allowed to dry and pulverized by using mortar and pestle. 5 gm pulverized material was dissolved in 50ml 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 (125mm) and the filtrate was collected and used for experimental analysis.

Phytochemical Analysis

Test for Alkaloids

3 ml of each extract was evaporated to dryness and residue was heated on a boiling water bath with 2N HCL (5ml). 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

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

Test for Phytosterols

1gm of the extract was dissolved in a few drops of dry acetic acid; 3 ml of acetic anhydride was added, followed by a few drops of conc. Sulphuric acid. Appearance of bluish green color showed the presence of phytosterols.

Test for Saponin

About 2.5 gm 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; froth less than 1cm = weakly positive; froth 1.2cm high = positive; and froth greater than 2 cm high = strongly positive (Segelman and Farnworth 1969).

Test for Tannins and Phenolic Compounds

10 ml 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 FeCl₃ 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 spp.* *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; and Wilkins *et al.*, 1972) was used to determine Antibacterial Assay. 24 hours old overnight cultures (0.2ml) 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 0.1ml of each of these extracts were added and kept for incubation for 24 hours 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 in Table 1. 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 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 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	+	+	+	-	-	+

Note: + indicates presence of compound. – indicates absence of compound.

Table 2. In vitro Antibacterial activity of Different extracts of pods; *Acacia concinna*

Phytochemical Constituents	Aqueous Extract mm	Benzene Extract mm	Chloroform Extract mm	Petroleum ether Extract	Butanol Extract mm	Methanol Extract mm
<i>Klebsiella pneumoniae</i>	12.5	14.2	6.6	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

Note: Values are mean of three replicates;

Table 3. Antifungal Activity of Different extracts of pods; *Acacia concinna*

Phytochemical Constituents	Aqueous Extract	Benzene Extract	Chloroform Extract	Petroleum ether Extract	Butanol Extract	Methanol Extract
<i>Aspergillus niger</i>	12.5	6.6	7.6	6.8	10.2	10.4
<i>Penicillium spp</i>	10.4	5.5	-	6.4	9.2	-
<i>Candida albicans</i>	8.2	4.2	6.4	-	8.4	7.8

Note: Values are mean of three replicates;

DISCUSSION

The screening of Secondary metabolites and antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (De Smet, 1997; Cowen, 2001; Kelmanson *et al*, 2001; Srivasan *et al*, 2001). The aqueous extraction of plants showed greater activity than the organic extraction. John (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*, 1992), saponins (Soetan *et al*, 2006), tannin (Hamilton-Miller, 1995), alkaloids (Rizk, 1982) and flavonoids (Tsuchiya *et al*, 2006) isolated from the plant materials. 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 spp.* 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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