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Phytochemical analysis, anti-oxidant and antimicrobial activities of Ethanolic extracts of *Acacia auriculiformis*

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Abstract

Acacia auriculiformis is an important medicinal plant and distributed throughout India. It is used as a folk medicine against derm diseases, diarrhea and fever. In the present study, ethanolic extract was prepared from *Acacia auriculiformis* and it was used to screen for phytochemical analysis, antimicrobial and antioxidant properties. Antimicrobial activity was carried out by using three bacterial strains such as *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and two fungal species such as *Aspargillus niger and Candida albican.* This ethanolic extract showed highest antibacterial activity against *Pseudomonas aeruginosa* (19.54±0.4) and as *staphylococcus aureus* (18.28±0.47). In contrast, it showed moderate activity against Bacillus subtilis (14.68±0.47). Whereas ethanolic showed best antifungal activity against *Aspargillus niger* (20.62±0.17) and ethanolic extract showed low antifungal activity against *Candida albican* (9.62±0.92) at highest concentration 100mg/ml concentration. The ethanolic extract showed better free radical scavenging (antioxidant) activity at all concentrations. Our results clearly reveal that the ethanolic extract of *Acacia auriculiformis* showed high content of phytochemicals, good antimicrobial and antioxidant activity and our results supporting the usage of the plant *Acacia auriculiformis* as folk and traditional medicine.

Keywords: *Acacia auriculiformis*, ethanolic extract, minimum inhibitory concentration, antioxidant activity, DPPH

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Introduction

Oxygen species byproducts as a result of physiological and biochemical metabolism. Free radicals can cause oxidative damage to lipids, proteins and nucleic acids such as DNA, eventually leading to many dangerous diseases, such as cancer, sugar (diabetes), aging, and other diseases in humans (Harman, 1998). Plant are endowed with free radical scavenging molecules, such as vitamins, phenolic acids, quinines, terpenoids, lignins, stilbenes, tannins, flavonoids, coumarins, alkaloids, amines, betalains, and other plant secondary metabolites which are high in antioxidant activity (Zheng and Wang 2001; Cai et al. 2003).

Materials and methods

Plant material and preparation of plant extract

The *Acacia auriculiformis* were collected from Seshachalam forests of Chittoor district, Andhra Pradesh, India. Freshly collected *Acacia auriculiformis* part were dried in shade and pulverized to a coarse powder and extracted by using ethanol. The filtrate obtained was evaporated to dryness at 50-65°C in a rotary vacuum evaporator to obtain a dark coloured molten mass.

Phytochemical analysis

The methods described by Harborne (1998) with slight modifications were used to screen the presence of the active ingredients in the bark extracts.

Test for Steroids

10 ml of the extract was evaporated to dry mass and dissolved in 0.5 ml of solvent. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added and a blue or green colour or a mixture of these two shades was an indication for the presence of steroidal compounds Harborne (1998).

Test for Terpenoids

The presence of the phytochemical such as terpenoids were determined as described for steroids except that red, pink or violet colour determine the presence of terpenoids Harborne (1998).

Test for Tannins

i) 1 cm3 of freshly prepared 10% KOH was added to 1 cm3 of the plant extract. A dirty white precipitate determines the presence of tannins in the obtained extract Harborne (1998).

ii) Powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water was added and the mixture was boiled for five minutes. To this two drops of 5% $FeCl_3$ were then added. Production of greenish precipitate confirms the presence of tannins Harborne (1998).

Test for Flavonoids

A small piece of magnesium ribbon was added to the plant extract and followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red determine the availability of flavones, red to crimson determine flavonols, crimson to magenta confirm the flavonones according to Harborne (1998).

Test for Alkaloids

The plant bark extract sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution was filtered and then 1 ml of the filtrate was treated with two drops of Mayer's reagent and these two solutions were mixed and made up to 100 ml with deionized water. Turbidity of the extract filtrate on the addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the plant extract according to Harborne (1998).

Test for Saponins

Stem bark of the plant was ground into powder form and it was introduced into a tube containing 5.0 ml of distilled water, this mixture was vigorously shaken for 2 min, formation of froth determine the presence of Saponins Harborne (1998).

Test for Glycosides

Coarsely powdered plant extract (1g) was transferred into two separate beakers and among these two beakers 5 ml of sulphuric acid was added, while 5 ml of water was added to the other beaker and then these two beakers were heated for 3-5 min and the contents were filtered into labelled test tubes. The filtrate was made alkaline, with 5% sodium hydroxide and heated with Fehling's solution. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were confirmed as positive for glycosides according to Harborne (1998).

Test for Gums and Mucilage

about 10ml of extracts were added separately to 25ml of absolute alcohol with constant stirring and then filtered. The precipitate was dried in air and test for its swelling properties and in the presence of carbohydrates according to Harborne (1998).

Antimicrobial activity

Test Organisms for Antimicrobial Activity

Three bacterial strains such as *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and two fungal species such as *Aspargillus niger and Candida albican* were obtained from Institute of Microbial Technology (IMTECH),Chandigarh. The strains were maintained and tested on nutrient agar for bacteria and potato dextrose (PDA) for fungi for the Antimicrobial tests.

Antimicrobial Activity

The agar disc diffusion method was used to determine the antimicrobial activity of the different plant extracts (Cruickshank, 1968). The discs (6 mm diameter) impregnated with different concentrations of the extracts were placed on the surface of the petri plates containing 20 ml of nutrient agar media for bacterial strains and potato dextrose agar media for fungal strains respectively, cultured with 100µl of microbial cultures (5 x 10 5 CFU/ml). The plates were incubated for 24 hours at 35 ±20C for bacteria and for 72 hrs for fungi at 30°C. The inhibition zones formed around the discs were measured and revealed in millimetre. The microbial activity was confirmed by transferring a subculture from the clear zone of inhibition to a fresh broth media and observed for the growth of microbes.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined, by using the micro dilution method in 96 well micro titre plates (Cruickshank, 1968; NCCLS, 1999). From the previously prepared different microbial suspensions, cultures (105 CFU/ml) were added to each well. Plates were incubated for 18 hr at 370 C and then were examined with Elisa reader (TECAN, Sunrise, China) at 620nm and the lowest concentration of each extract showing no growth was taken as its minimum inhibitory concentrations (MIC). All the samples were tested in triplicate to confirm the activity and the values were noted.

Antioxidant activity

Evaluation of antioxidant activity was done by using 2, 2'-diphenly-1-picrylhydrazyl method described by Burits and Bucar (2000). The antioxidants present in the extract reacts with DPPH and convert into α, α -diphenyl.-β-. picryl hydrazine. One ml of plant extract was added to 4ml of 0.004% methanol solution of DPPH. After 20-30 min incubation period at room temperature and the absorbance was read and checked against blank at 517 nm. Inhibition of free radicals by DPPH in percent was calculated by using the following equation. The degree of discoloration indicates the scavenging potentials of the obtained plant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity.

% DPPH radical-scavenging = [(Absorbance of Control - Absorbance of test Simple) /(Absorbance of Control)] x 100

Results

The prepared ethanolic extract was used for screening of phytochemicals such as steroids, triterpinoids, tannins,

flavonoids, alkaloids, glycosides, saponins, carbohydrates and phenolic compounds. The ethanolic extract composed with high content of steroids, triterpinoids, flavonoids, alkaloids, saponins and phenolic compounds. Whereas moderate content of phytochemicals such as tannins and glycosides were observed in ethanolic extract. But in contrast the phytochemicals such as carbohydrates and gums and mucilages were completely absent in the ethanolic extract of Acacia auriculiformis (Table-1)

Table 1. Phytochemical analysis of water extract of *Acacia auriculiformis*

S.No	Phyto chemicals	Ethanolic extract
1.	Steroids	++++
2.0	Tri terpinoids	++++
3.0	Tannins	+++
4.0	Flavonoids	++++
5.0	Alkaloids	++++
6.0	Saponins	++++
7.0	Glycosides	+++
8.0	Gumsand Musilages	-
9.0	Carbohydrates	-
10	Phenolic compounds	++++
1.	Steroids	++++

Table 2: Antimicrobial activity of Acacia auriculiformis, Zone of inhibition (mm), minimum inhibitory concentration in mg (MIC). The data represented is the mean ± S.D of five parallel measurements.

S. No	Organism Name	Zone of Inhibition 25mg/ml	Zone of Inhibition 50mg/ml	Zone of inhibition 75mg/ml	Zone of inhibition 100mgs/ml	MIC (mg)
1	*Pseudomonas aeruginosa*	07.35±1.61	11.28±0.59	15.87±0.58	19.54±0.17	09.21 ±1.72
2	*Staphylococcus aureus*	05.61±1.47	08.36±1.28	14.58±2.82	18.28±1.17	25.67 ± 1.07
3	*Bacillus subtilis*	08.48±1.74	10.58±1.17	12.05±1.89	14.68±1.17	16.28 ±2.71
4	*Aspergillus niger*	010.25±1.29	13.58±1.5	17.97±1.87	20.62±1.58	10.17 ±1.78
5	*Candida albicans*	03.57±2.45	05.58±1.28	09.52±2.85	9.62±2.58	32.68 ±4.28

In our study, we performed antimicrobial activity by using ethanolic extract. Antimicrobial activity was measured in terms of the Zone of inhibition (mm) and Minimum inhibitory concentration (mg) to various antibacterial and antifungal species such as staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and two fungal species such as Aspergillus Niger and Candida albican. This ethanolic extract was showed the highest antibacterial activity against Pseudomonas aeruginosa (19.5) and as Staphylococus aureus (18.28). In contrast, it showed moderate activity against Bacillus subtilis (14.68). Whereas ethanolic showed best antifungal activity against Aspergillus niger (20.62) and ethanolic extract showed low antifungal activity against Candida albican (9.62) at the highest concentration 100mg/ml concentration. In contrast remaining low concentrations showed moderate activity against with all microbes (Table-2).

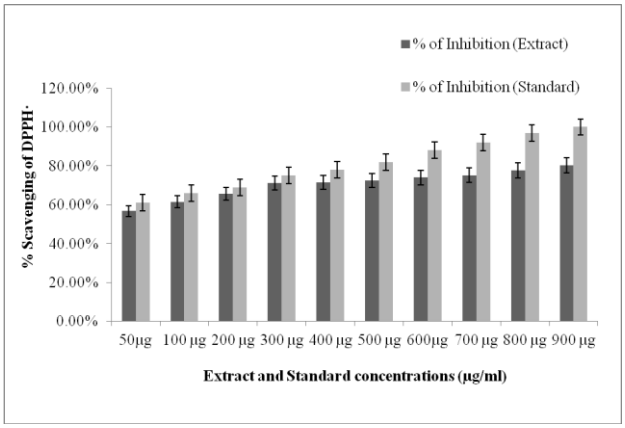

Fig-1.Hydroxy radical scavenging activities of *Acacia auriculiformis* ethanolic extract and reference standard Ascorbic acid. The data represented is the mean ± S.D of five parallel measurements.

The ethanolic extract of Acacia auriculiformis was checked for free radical scavenging by using DPPH method. Different dilute concentrations (µg/ml) of the methanol extract were used for antioxidant activity. With increased concentration of extract and standard the antioxidant activity was also increased (Fig-1). Reference standard ascorbic acid showed good antioxidant activity than ethanolic extract at all concentration (Fig-1). The presence of phytochemicals such as polyphenols and remaining phytochemicals may be attributed the highest antioxidant activity showed by the extract.

Discussion

The ethanolic extract of Acacia auriculiformis was checked for free radical scavenging by using DPPH method. Different dilute concentrations (µg/ml) of the methanol extract were used for antioxidant activity. With increased concentration of extract and standard the antioxidant activity was also increased (Fig-1). Reference standard ascorbic acid showed good antioxidant activity than ethanolic extract at all concentration (Fig-1). The presence of phytochemicals such as polyphenols and remaining phytochemicals may be attributed the highest antioxidant activity showed by the extract.

Conclusion

The phytochemical compounds such as steroids, triterpinoids, tannins, flavonoids, saponins, glycosides and phenolic compounds were rich in the ethanolic extract of the *Acacia auriculiformis*. This ethanolic extract showed good antimicrobial activity against tested bacterial and fungal species such as *Staphylococus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and two fungal species such as *Aspargillus niger and Candida albican* and also showed good antioxidant activity. These results were authenticating the use of *Acacia auriculiformis* as folk medicine for local people for treating various diseases caused by bacterial and fungi.

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