

Full Length Research Paper

In vitro* antimicrobial and antioxidant activities of bark extracts of *Bauhinia purpurea

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Bark extracts of *Bauhinia purpurea* were phytochemically analyzed and evaluated for antimicrobial and antioxidant activities. The phytochemical analysis of the bark extracts revealed the presence of alkaloids, flavonoids, steroids, saponins, triterpenes and carbohydrates. While most of them were present in methanolic and aqueous extracts, one or a few of them were present in other solvent extracts. Among different solvent extracts, aqueous extract exhibited a broad spectrum of antimicrobial activity. It showed strong antibacterial activity against Gram positive bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative strains like *Escherichia coli* and *Klebsiella pneumonia* and antifungal activity against *Candida albicans*. While methanolic extract showed moderate to strong antibacterial activity against *B. subtilis*, *E. coli* and *K. pneumonia*, the extracts of hexane, chloroform and ethyl acetate did not show any anti bacterial or antifungal activity against the tested fungal and bacterial strains. Antioxidant activity of the bark extracts were evaluated in terms of inhibition of free radicals by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH). Aqueous extract followed by methanolic extract exhibited strong to moderate antioxidant activity. The antioxidant property and antimicrobial activity of the extracts of *B. purpurea* against the tested microbial strains therefore, supports that there is scientific basis for their utilization in traditional medicine for wound healing and also in treatment of some infectious diseases.

Key words: *Bauhinia purpurea*, phytochemical analysis, antimicrobial activity, antioxidant property.

INTRODUCTION

The use of plants for medicinal purposes dates back to antiquity because they contain components of therapeutic value (Charaka and Sofovora Basu, 2000). Medicinal plants are cheaper and more accessible to most of the population in the world. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate various therapeutic uses of medicinal plants (Benignant et al., 1996). Therefore, the quest for plants with medicinal properties continues to receive attention as scientists are in need of plants, particularly of ethno botanical

significance for a complete range of biological activities, which ranges from antibiotic to anticancerous.

Bauhinia purpurea is a shrub or small tree of Fabaceae family. It is found in most types of vegetation ranging from evergreen lowlands, rain forests to mountain forests up to 2000 to 3000 m altitude and also in savanna, scrub and dry deciduous forests to swamp forests on various soils. This plant is used traditionally in dropsy, pain, rheumatism, convulsions, delirium and septicemia (Asolker et al., 2000). The bark of the plant is used as an astringent and its decoctions are recommended for ulcers as a useful wash solution (Kirthikar and Basu, 2000). The leaves and roots are used for the treatment of catarrh, infection of children, boil, glandular and swelling (Chakre et al., 2000). The aerial parts of the plant are reported to contain flavone glycosides, foliar flavonoids, 6-butyl-3-hydroxy flavanone, amino acids, phenyl fatty ester, lutine and β -sitosterol (Yadava and Tripathi, 2000). These active

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constituents have been attributed in the therapeutic Zactivity of the plant (Salantino and Blatt, 1999). Therefore, this study was undertaken to evaluate the antimicrobial and antioxidant properties of bark extracts of *B. purpurea*.

MATERIALS AND METHODS

Collection of plant material

B. purpurea bark was used in this study. The plant material was collected from local areas of Tirupati, Chittoor district, in Andhra Pradesh, at India. The bark was allowed to be dried in shade to retain the active compounds present in the bark for three days at room temperature. The shade dried bark was taken for further studies and then powdered with the help of warring blender.

Preparation and isolation of plant extracts

Hexane extract was prepared by taking 100 g of *B. purpurea* dried bark powder in a separate container, to this 400 ml of hexane was added and kept for 24 h in a shaker. Filtered through eight layers of muslin cloth and extract was collected, the extraction process was repeated twice. The collected extracts were pooled. Chloroform, ethyl acetate and methanolic extracts were prepared as that of hexane extract. Aqueous extract was prepared by taking 100 g of *B. purpurea* bark powder in a separate container, to this 400 ml of water was added and boiled for 2 h in a mild heat and kept for 24 h. Then filtered and extract was collected. The extraction process was repeated twice. Then the collected filtrates were pooled (Akpulu et al., 1994). The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Phytochemical screening

Phytochemical screening of plant extract was carried out qualitatively for the presence of sterols, triterpenes tannins, flavonoids, saponins, alkaloids, carbohydrates, glycosides and steroids (Harborne, 1998).

Anti microbial assay

The micro organisms used for this study include bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and fungal species, *Candida albicans* were obtained from Department of Microbiology, Sri Venkateswara University Tirupati and from IMTECH, Chandigarh, India.

In vitro determination of antimicrobial activity

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of colonies from the stock culture to peptone water and incubated for 4 h at 37°C. Antibacterial activity was determined by agar disc diffusion method (Bauer et al., 1966). The discs (6 mm diameter) impregnated with known concentrations of the standards and 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, seeded with 100 µl of microbial cultures (5×10^5 CFU/ml).

Along with this 30 µg ampicillin disc, 30 µg tetracycline disc and 30 µg Ketoconazole discs (Himedia standard) were studied for

Gram positive, Gram negative bacterial and fungal isolates, respectively as a positive control for antimicrobial activity. The plates were incubated for 24 h at $35 \pm 2^\circ\text{C}$ for bacteria and for 48 h for fungi at 30°C . At the end of incubation, inhibition zones formed around the disc were measured with Himedia zone scale. The study was performed in triplicate and the mean values were presented.

Minimum inhibitory concentration (MIC)

MIC means the lowest concentration of an antimicrobial agent that appears to inhibit the growth of microorganism. Minimum inhibitory concentration (MIC) was determined by micro-broth-dilution method (Bonjar Shahidi, 2004). Bark extracts with a concentration ranging 0.1 to 2 mg/ml were evaluated for their MIC efficacy. 0.1 ml of standardized inoculum ($1-2 \times 10^7$ CFU/ml) was added to each plate. The plates were incubated at 28°C for 48 h. The study was performed in triplicate and the mean values were presented. The lowest concentration (highest dilution) of the extract that produced no visible signs of bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

DPPH radical scavenging activity

Free radical scavenging activity was determined by using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) method described by Burits (2000). 1 ml of plant extract was added to 4 ml of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated by using the following equation:

$$I \% = [(A \text{ control} - A \text{ sample}) / A \text{ Control}] \times 100$$

Where, A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Reducing power assay

The reducing power was determined in accordance with the procedure of Oyaizu et al. (2003). Methanolic and aqueous extract of *B. purpurea* were prepared in methanol and mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min and 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl_3 (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a standard.

RESULTS AND DISCUSSION

Phytochemical analysis

In this study, steroids were present in all extracts of *B. purpurea* except in hexane and ethyl acetate extracts. While triterpenes were present in chloroform and methanolic extracts, they were absent in other extracts. Flavonoids, alkaloids and carbohydrates were found to be present in methanolic and aqueous extracts and absent in other extracts. Saponins were present only in

Table 1. Secondary metabolites present in bark of *B. purpurea*.

Secondary metabolite	Test	HE	CE	E.AE	ME	AE
Steroids	Salkowski test	-	+	-	+	+
	Liebermann test	-	+	-	+	+
Triterpenes	Salkowski test	-	+	-	+	-
	Liebermann test	-	+	-	+	-
Saponins	Foams test	+	-	+	+	-
Alkaloids	Meyers test	-	-	-	+	+
	Wagner's test	-	-	-	+	+
Carbohydrates	Fehling's test	-	-	-	+	+
	Molisch test	-	-	-	+	+
	Benedicts test	-	-	-	+	+
Flavonoids	Shinoda test	-	-	-	+	+
	FeCl ₃ test	-	-	-	+	+
	Lead acetate test	-	-	-	+	+
Tannins	FeCl ₃ test	-	-	-	WP	WP
	Gelatin test	-	-	-	WP	WP
Glycosides	Bal jest test	-	-	-	WP	WP
	Legal's test.	-	-	-	WP	WP

HE = Hexane extract; CE = chloroform extract; E.AE = ethyl acetate extract; ME= methanol extract; AE = aqueous extract; WP = weakly positive; (+) = indicates presence of secondary metabolites; (-) = indicates absence of secondary metabolites.

hexane, ethyl acetate and methanolic extracts. Tannins and glycosides were weakly present in methanolic and aqueous extracts but absent in other extracts (Table 1).

***In vitro* antimicrobial activity and MIC**

The stem bark powder of *B. purpurea* was checked for both antibacterial and antifungal activity with all the extracts. However, the results of only methanolic and aqueous extracts are mentioned here just because these two extracts were active against the tested microorganisms. While the aqueous extract was active against all the tested microorganisms, methanolic extract was active against all except *S. aureus* and *C. albicans*. The maximum zone of inhibition (20 mm) was observed with aqueous extract (100 µg/ml) and methanolic extract (100 µg/ml) against *B. subtilis*. The minimum inhibitory concentrations and the diameter of the zone of inhibitions of the tested microbes are shown in Table 2. Ampicillin (20 µg/ml), tetracycline (20 µg/ml) and ketoconazole (20 µg/ml) were used as positive controls.

Antimicrobial activity of flavonoids, saponins and cardiac glycosides has been reported with various plant

extracts (Soetan et al., 2006). Similar reports of antimicrobial activity were observed with plant extracts of *Terminalia* sp., *Withania somnifera*, *Cassia auriculata* and *Morinda citrifolia* (Burapadaja and Bunchoo, 1995; Usha et al., 2010). The antimicrobial activity of *B. purpurea* bark suggests that the extract contains the effective active phytochemicals responsible for the elimination of microorganisms.

***In vitro* antioxidant assay**

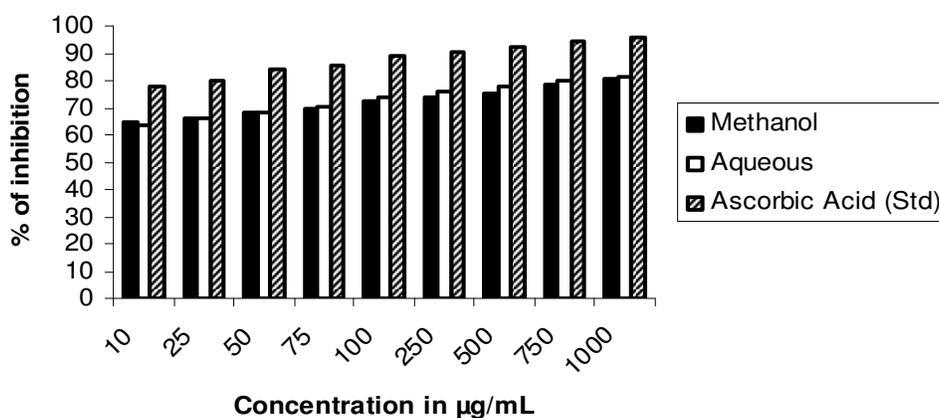
Antioxidant efficacy of methanolic and aqueous extracts increased with increase in concentration of the plant extract. Both the extracts showed 80 to 90% antioxidant activity in terms of DPPH radical scavenging activity when compared with the standard (Ascorbic acid) (Figure 1). In reducing power assay, methanolic and aqueous extracts showed 40 to 50% and 55 to 65% antioxidant activity, respectively in comparison to ascorbic acid standard (Figure 2). The presence or absence of particular component(s) plays a major role in deciding the antioxidant property of medicinal plant extracts. Potent antioxidant activity was earlier reported in several

Table 2. *In vitro* antimicrobial activity of bark extracts of *B. purpurea*.

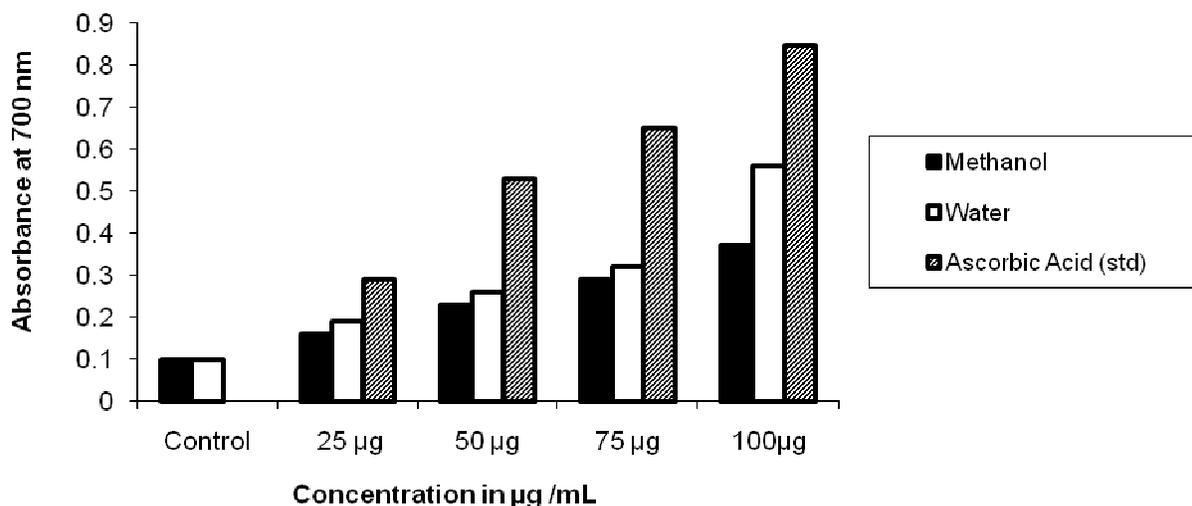
S/N	Name of the test organism	Methanol extract		Aqueous extract		Standard antibiotic
		ZI (mm)	MIC	ZI (mm)	MIC	
1	<i>Bacillus subtilis</i>	16	100	20	100	22 ^A
2	<i>Staphylococcus aureus</i>	-	-	14	100	21 ^A
3	<i>Escherichia coli</i>	14	150	16	100	22 ^B
4	<i>Klebsiella pneumonia</i>	12	150	17	100	22 ^B
5	<i>Candida albicans</i>	-	-	12	150	24 ^C

ZI, Zone of inhibition in mm; MIC, minimum inhibitory concentration ($\mu\text{g/ml}$); A, ampicillin 20 $\mu\text{g/ml}$; B, tetracycline 20 $\mu\text{g/ml}$; C: ketoconazole: 20 $\mu\text{g/ml}$.

DPPH radical scavenging activity

**Figure 1.** DPPH radical scavenging activity of *Bauhinia* bark extracts.

Reducing power assay

**Figure 2.** Reducing power assay of *Bauhinia* bark extracts.

vegetables, fruits and medicinal plants (Cao et al., 1996; Jadhav and Bhutani, 2002).

Conclusion

This study evaluated the presence of various secondary metabolites in the bark extracts of *B. purpurea*, which may be responsible for the antimicrobial and antioxidant efficacy of aqueous and methanolic extracts of this medicinal plant. Other studies are underway to find out its antilipidemic and anti obesity properties.

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