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Phytochemical Screening, Antioxidant Activities and Antibacterial Potential of Leaf Extracts of *Buchanania axillaris* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Author MVV wrote the protocol and performed the statistical analysis. Author SAM managed the literature searches and wrote the first draft of the manuscript. Author KR designed the study and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Buchanania axillaris* L. *(B. axillaris)* is a traditional medicinal plant distributed in India and other Asian countries. It is well known as 'Cuddapah Almond' and belongs to the family of Anacardiaceae. The aim of the present study was to determine the phytochemicals present, quantify total phenols, total flavonoids, total tannins, *in vitro* antioxidant activity, catalytic activity and antibacterial potential of aqueous, methanol and n-butanol leaf extracts of *B. axillaris*.
Materials and Methods: The dried leaf was extracted with different solvents and screened qualitatively and quantitatively for phytochemicals. The antioxidant property was evaluated by free radical scavenging activity by 2, 2-diphenyl-l-picrylhydrazyl (DPPH). The catalytic activity was demonstrated by using Methyl orange (MO) as a degrading agent and *in vitro* antibacterial activity was performed by agar well diffusion on selected five human pathogens.
Results: Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids,

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carbohydrates, phenols, tannins, steroids, glycosides, proteins and diterpenes. Total phenols, total flavonoids and total tannins were all found to be highest in n-butanolic extracts. DPPH % scavenging activity was observed more in butanolic leaf extract. The result of the antibacterial activity of n-butanolic extracts showed good inhibitory activity against all the tested pathogens and showed comparatively better antibacterial activity than other solvent extracts.

Conclusions: The extracts exhibited inhibitory activity against all the pathogens and n-butanolic extracts showed relatively better antioxidant and inhibition activities which may be assigned to the greater bioactive compounds present in it.

Keywords: Buchanania axillaris L.; phytochemicals; free radical scavenging activity; anti-microbial activity; methyl orange.

1. INTRODUCTION

Medicinal plants are a source of great economic value in the Indian subcontinent. In India, thousands of species are known to have medicinal value and the use of various parts of several medicinal plants to cure specific aliments has been in vogue since ancient times. According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [1]. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant [2]. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Natural products isolated from higher plants and microorganisms have been providing novel clinically active drugs [3]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. Herbal medicines are becoming popular in the modern world as people resort to natural therapies [4].

Today about 300 species of medicinal and aromatic plants are used worldwide in the pharmaceutical, food, cosmetics and fragrance industries [5]. Plants are a rich source of secondary metabolites with interesting biological activities. Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs [6]. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [7]. Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, polyphenols etc. Synthetic dyes are extensively used in a variety of industries such as textiles, paper, polymers, adhesives, ceramics, construction, cosmetics, food, glass, paints, ink, soap, and pharmaceuticals [8]. The catalytic reduction studies of these dyes assume greater significance in the present context as most of these dyes are known to be toxic when inhaled or ingested orally and pose health hazards such as skin and eye irritation in humans. The adverse effects of these synthetic dyes to the environment include the ability to deplete oxygen in the surface waters and streams thereby affecting the very sustenance of aquatic flora and fauna and causing an inhibitory effect on the photosynthetic activity of plants.

In the present work, we report the phytochemical analysis, antioxidant activity, dye degradation and anti bacterial activities of the different solvent extracts of leaves of *B. axillaris* The leaf extract has been reported to possess anti-inflammatory [9]. The aerial parts are used to cure itch of the skin and to remove blemishes from the face. The kernels are used in Indian medicine as a brain tonic. The gum is anti-diagonal and it's used internally for rheumatism. In addition, the ethanol extract of the aerial parts showed central nervous system depressant activity in mice. Further, the leaves are reported to be cooling, digestive, expectorant, purgative, depurative and aphrodisiac and are useful in hyperpiesia, burning sensation, cough, bronchitis, dyspepsia, leprosy and constipation [10].

2. MATERIALS AND METHODS

2.1 Selection and Collection of Plant Material

Healthy leaves of *B. axillaris* were collected from Palakondalu and the hills near Rayachoti, Kadapa, Andhra Pradesh, India. The taxonomic

identification and authentication of the specimen were performed in Laboratory of Botany, YVU University, Kadapa. The gathered leaves of *B. axillaris* were washed with tap water, rinsed with distilled water and allowed to shade dry at room temperature for 15 days and then ground to fine powder.

2.2 Extract Preparation

25 g of dried powdered leaf material was used for each extract preparation (methanol, n-butanol) through cold extraction at 4°C for an overnight while the aqueous extract was prepared using warm water at 70°C. All extracts were taken in 1:6 ratios and concentrated using rotary evaporator at 40°C and dry residues were preserved at in 4°C air tight containers until further use [11].

2.3 Qualitative Analysis of Phyto-chemicals

Aqueous, methanolic and butanolic leaves leaf extracts of *B. axillaris* were subjected to preliminary phytochemical screening to qualitatively determine some of the secondary metabolites: phenols, carbohydrates, proteins, tannins, alkaloids, flavonoids, steroids, glycosides and diterpenes by the following method as described in Prasad MV et al. [11].

2.4 Quantitative Analysis of Phytochemicals

Quantitative analysis of total phenol, total flavonoid and total tannin content was determined by the spectrophotometric method as per the modified methodology is given by Ashok et al. [12]. Gallic acid, Rutin and catechin (1 mg/ml) were used as a standard positive control for determination of total phenol, total flavonoid and total tannin content [11].

2.5 DPPH Radical Scavenging Activity

The free radical scavenging activity of the fractions was measured *in vitro* by 2,2′- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [13,14]. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98 ± 0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100µl of the sample at various concentrations (10-100 µl/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. The absorbance was taken at 517 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] × 100

2.6 Catalytic Experiment

The catalytic activity of *B. axillaris* was demonstrated by degrading methyl orange (MO) dye as described by Salem et al. [15].

Percentage of dye degradation was estimated by the following formula:

$$\% \text{ Decolourization} = 100 \times \frac{(C_0 - C)}{C_0}$$

Where C_0 is the initial concentration of dye solution and C is the concentration of dye solution after photo catalytic degradation.

2.7 Antibacterial Activity (Agar Well Diffusion Method)

The antibacterial activities of the extracts were tested against the selected bacterial strains by the agar well diffusion method. The bacterial strains were grown in nutrient broth at 37°C until the bacterial suspension has reached 1.5x10 ^{8} CFU/ml. 20 ml of molten nutrient agar was poured into the Petri dishes and cooled. All the bacterial suspension was swapped over the medium and 5 wells of 0.5 cm deep were made by using a sterile tip. 25, 50, 75 and 100 µl of aqueous, methanolic and n-butanolic leaf extracts were added into wells and Gentamycin (1 µg /ml, Sigma) was added to one well as control. After these, plates were incubated at 37°C for 24 hours. Then antibacterial activities were recorded by measuring the diameter of the zones of inhibition around each well and compared with the standard antibiotic (Gentamycin). Bacterial strains used were *Bacillus subtilis* (G^{+ve}), *Escherichia coli* (G^{-ve}), *Klebsiella pneumonia* (G^{-ve}), *Staphylococcus aureus* (G^{+ve}) and *Pseudomonas aeruginosa* (G^{-ve}). The tested microorganisms were collected from the Dept. of Microbiology, Yogi Vemana University, Kadapa.

2.8 Statistical Analysis

A minimum of three replicates was involved in each experiment of quantification and antibacterial activity of three leaf extracts of *B. axillaris* Two-way ANOVA (analysis of variance) was performed with Duncan's multiple range test using Graph Pad Prism 5 software.

3. RESULTS

3.1 The Yield of the Extracts

25 g of leaf powder yielded 8% of aqueous, 8.4% of methanolic and 5.2% of butanolic B. axillaries leaves extracts. Dilutions were made with respective solvent and used for qualitative and quantitative analysis of phytochemicals and antibacterial activity.

3.2 Qualitative Analysis of Phytochemicals

The results of preliminary phytochemical screening of *B. axillaris* (aqueous, methanol and butanol) confirmed the presence of various classes of secondary metabolites. The leaf extract of three solvents revealed the positive results to the following tests of alkaloids, flavonoids, carbohydrates, phenols, tannins, steroids, glycosides, proteins and diterpenes (Table 1).

Table 1. Phytochemical analysis of different leaf extract of *B. axillaris* L.

Name of the tests	*B. axillaris* leaf extract		
	Aqueous	Methanolic	n-butanolic
Alkaloids	+	+	+
Flavonoids	+	++	+++
Carbohydrates	+++	+++	+++
Phenols	++	+++	+++
Tannins	++	++	+++
Steroids	+++	+++	+++
Glycosides	++	++	+
Proteins	+++	++	+
Diterpenes	++	++	++

+ = present, ++ = more quantity, +++ = more than quantity

3.3 Quantitative Analysis of Phytochemicals

3.3.1 Total flavonoid content

The total flavonoid content among the three extracts was expressed in term of rutin equivalent using the standard curve. The total flavonoid content in *B. axillaris* leaf extracts increased with increasing concentration of extracts (Fig. 1A). The butanolic extract showed highest flavonoid levels.

3.3.2 Total tannin content

The total tannin content in *B. axillaris* extracts was expressed in term of catechin equivalent (□g/ml). The results reported in (Fig. 1B) revealed that the extract contains very low levels of tannins. The total tannin content was maximum in butanolic extract.

3.3.3 Total phenolic content

The amount of total phenol was estimated by Folic Ciocalteu reagent. Gallic acid was used as a standard compound. The total phenol was estimated at different concentration of leaf extract with different solvents. There was a variation of total phenolic levels with different extract. The total phenolic content was more in butanolic leaf extract than in aqueous and methanolic leaf extracts (Fig. 1C).

3.4 DPPH Radical Scavenging Activity

The antioxidant activity of the leaves of *B. axillaris* extracted with different solvent viz., aqueous, methanol and n-butanol was determined by DPPH free radical scavenging assay. DPPH % scavenging activities of the three extracts of *B. axillaris* are presented in Fig. 1D. The maximum concentration of DPPH scavenging activity was compared with the ascorbic acid standard. In our investigation, butanolic extract showed highest scavenging activity than methanolic, followed by aqueous, which proves that butanolic extract of *B. axillaris* exhibited highest antioxidant capacity than methanolic and aqueous extracts.

3.5 Degradation of Methyl Orange

Catalytic degradation was investigated using the three extracts of *B. axillaris* using MO dye. Dye degradation was initially identified by colour change. Initially, the colour of the dye was deep orange which changed into light yellow after 1 h of incubation; absorbance was measured and calculated the dye degradation percentage. Among the three extracts, methanolic extract showed the highest catalytic reduction of 71.55% followed by aqueous 65.21% and butanolic extract 64.5% (Fig. 2).

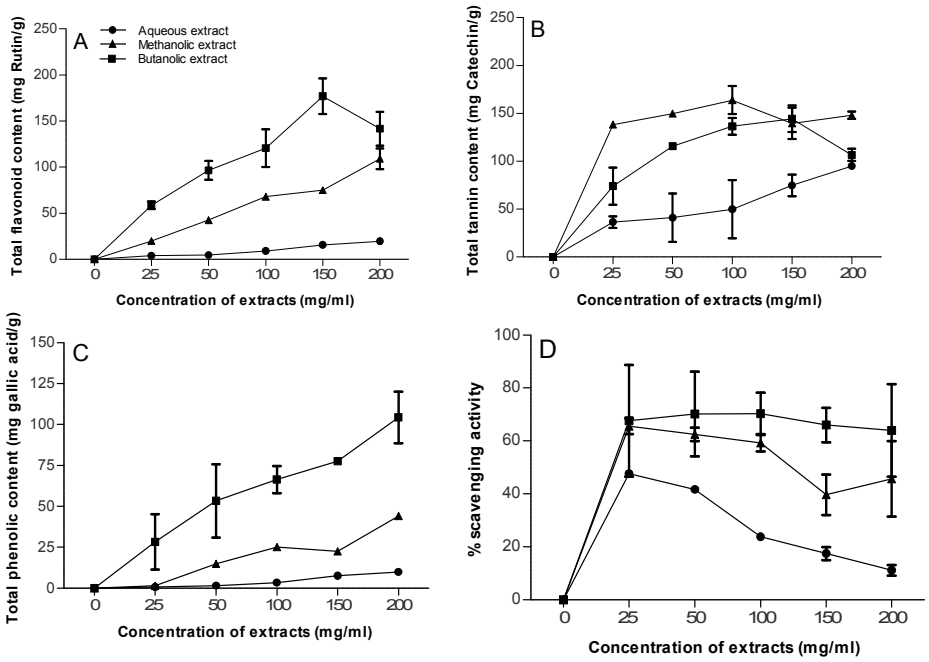

Fig. 1. Quantitative analysis of phytochemicals A) Total Flavonoids, B) Total Tannins, C) Total Phenols and D) % Scavenging activity of different leaf extract of *B. axillaris* L. (p<0.001)

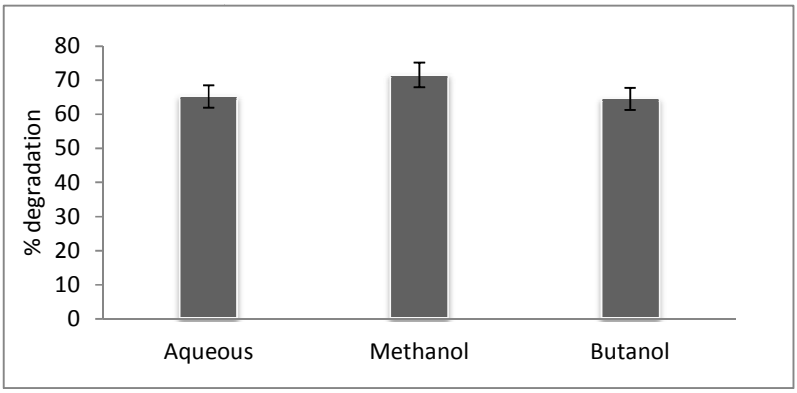

Fig. 2. Effect of leaf extracts of *B. axillaris* on the reduction of MO dye (p<0.001)

3.6 Antibacterial Activity

In the present investigation, the antibacterial activity of leaf extracts of *B. axillaris* was screened *in vitro* by agar well diffusion method using Gentamycin as the standard positive control against different Gram positive and Gram negative bacterial strains. The antibacterial activities of three different solvent extracts of *B. axillaris* leaves are reflected in the graphical representation (Fig. 3).

Among the three solvents, maximum antibacterial activity was exhibited by butanolic extract of *B. axillaris* against all the tested pathogens at the following concentrations i.e. 25, 50, 75, 100 µg. (Fig. 3). The extracts of *B. axillaris* showed maximum antibacterial activity on both Gram positive and Gram-negative bacteria. The antibacterial studies revealed the following sequence of inhibitory action *E. coli* > *K. pneumonia* > *B. substilis* > *P. aeruginosa* > *S. aureus* in butanolic extract. The highest

activity was against *E. coli* (27 mm). The lowest activity was observed in methanolic extract; the sequence of inhibition was *E. coli* > *B. substilis* > *K. pneumonia* > *S. aureus* > *P. aeruginosa*. In methanolic extract lowest activity was observed against specific bacteria *P. aeruginosa* (16 mm). Whereas aqueous extract exhibited the following sequence of antibacterial activity i.e. *B. substilis* > *E. coli* > *S. aureus* > *P. aeruginosa* > *K. pneumonia*.

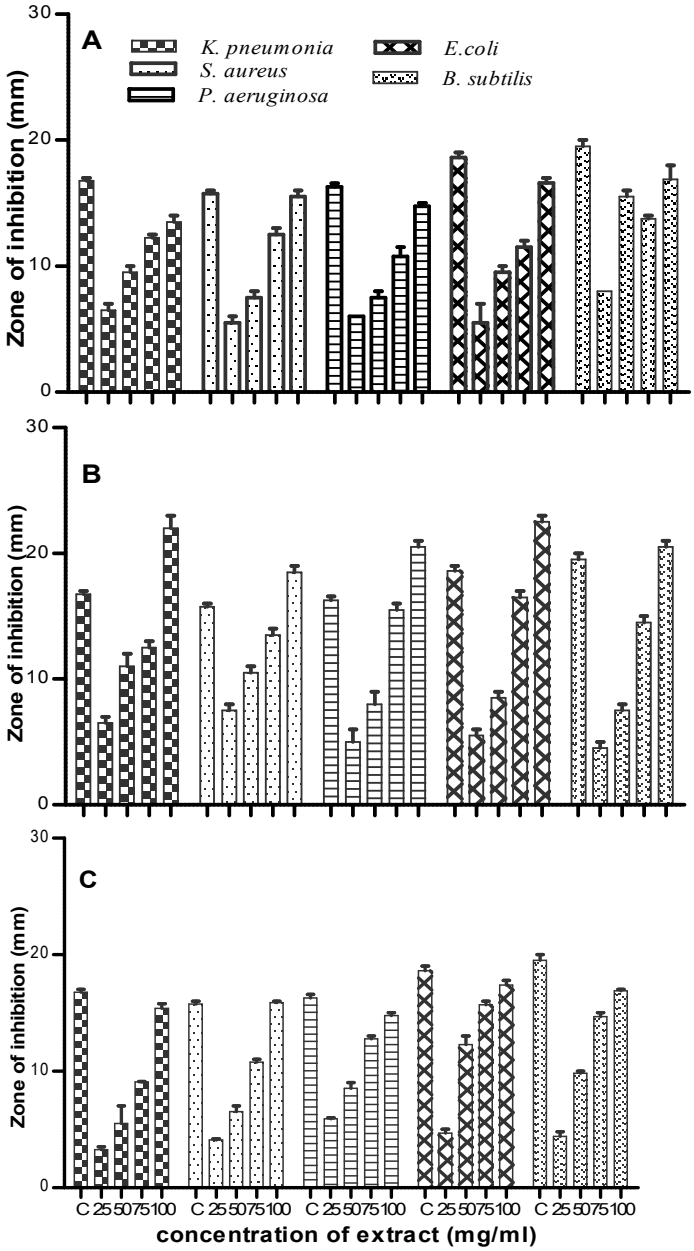

Fig. 3. Antibacterial activity of *B. axillaris* A. Aqueous, B. Butanolic, C. Methanolic leaf extracts). Values are mean inhibition zone (mm) ± S.E ($p<0.001$). Positive control 'C'– Gentamycin

4. DISCUSSION

Natural products are in great demand owing to their extensive biological properties and bioactive components which have proved to be useful against a number of diseases. It is proved that present extracts of *B. axillaris* leaves showed a wide array of activities like antibacterial, antioxidative and dye degradation. A wide variety of other phytochemicals may also have an influence on the antioxidant potentials in leaves of *B. axillaris* using successive solvents such as aqueous, butanolic and methanol in increasing polarity.

Phytochemicals or secondary metabolites in plant samples are known to be biologically active compounds and they are responsible for different activities, such as antioxidant activity. The different extracts obtained by using aqueous, methanolic and butanol as solvents were screened for the presence of alkaloids, carbohydrates, glycosides, phenols, tannins, flavonoids, proteins, diterpenes, and steroids with standard phytochemical tests (Table 1). Phenolic and flavonoid compounds are commonly reported in plants and they are known to exert various biological activities, including antioxidant activity [16,17] as well as possess antibacterial properties [18,19].

The aqueous, methanolic and n-butanol extracts of *B. axillaris* leaf extract have shown significant medical uses in all the analytical experiment in this study. Our study clearly supports the view that medicinal plants are a great source of potential antioxidants and may be used as a potent natural antibacterial and antioxidant agents. Total phenol, flavonoid and tannin content in the leaf extract were found to be higher in n-butanolic followed by methanolic and aqueous extract simultaneously (Fig. 1). Several studies have reported a strong and significant correlation between the scavenging activity and total phenolic compound, as well as the flavonoid content and its significant contribution toward the total antioxidant activity [17] % scavenging activity, was highest in case of n-butanolic followed by a methanolic and aqueous extract of *B. axillaris* (Fig. 1D).

Plant phenolic compounds impart colour, flavour and are associated with health benefits such as reduced risk of heart and cardiovascular diseases due to their antioxidant properties. Flavonoids are hydroxylated phenolic substances synthesized by plants and have been found *in vitro* to be effective antimicrobial substances against a wide range of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins as well as bacterial cell walls. Presence of reducing agents like alkaloids, polyphenols and flavonoids, the major phytoconstituents in leaf extract of *B. axillaris*, are responsible for the degrading activity in three different solvents by MO dye. Catalytic activity was highest in methanolic leaf extract followed by aqueous and butanolic extracts. Among the three solvents, maximum antibacterial activity was exhibited by butanolic extract of *B.axillaris.* against all the tested pathogens (Fig. 2). The bioactive molecule thought to be responsible for antibacterial activity is sterol which has been obtained in large quantities in *B. axillaris* extracts. It has been reported that sterol works through the disruption of the permeability barrier of microbial membrane structures [20,21].

5. CONCLUSION

The results of preliminary phytochemical screening suggest that the aqueous, methanolic, and n-butanol extracts of *B. axillaris* leaf extracts are an excellent source of valuable phytochemicals. In our study, n-butanolic leaf extract exhibited a broad spectrum of antibacterial activity against selected test microorganism. Our study clearly supports the view that medicinal plants are a great source of potential antioxidants and may be used as a potent natural antibacterial and antioxidant agents. It is concluded that the leaves of *B axillaris*. With rich source of secondary metabolites may attribute to the pharmacological properties. Further studies are necessary to isolate and characterize the bioactive compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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