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Evaluation of antimicrobial activity and *Bidens biternata* ehrenb Leaves



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ABSTRACT

The present study aims to determine the antimicrobial activity of *Bidens biternata*. Ethanolic extract of leaves were used at the concentration of 0.5 and 1 gm/ml. The ethanolic extract was screened for antimicrobial activity against Gram positive and Gram negative bacteria by using agar cup plate method. The screening results showed that the ethanolic extract of leaves of *Bidens biternata* was found to be effective against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *proteus vulgaris*. The study suggests that the leaves of *Bidens biternata* ehrenb were promising in the development of antimicrobial properties.



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INTRODUCTION

The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains. Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects [1,2].

According to WHO, medicinal plants would be the best source for obtaining variety of drugs [3].

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of species have not been adequately evaluated. There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials.

Bidens biternata, belonging to the family Asteraceae, is an erect annual herb, up to 1 cm in height, and a widespread weed of cultivated areas. This plant is common, particularly in the Western Ghats regions of Kerala state in India. It is used as a leafy vegetable by the Paniya and Kattunaayika tribes of Waynadu Districts in Kerala and also to cure hepatitis, cold, cough, dysentery, etc. The multiplication and utilization of this leafy vegetable will help to overcome the nutritional deficiency problem and also to maintain the biodiversity. For effective biochemical analysis, plant extract was taken using different solvents. Various phytochemicals like reducing sugar, glycosides, flavonoids, alkaloids, tannins, steroids, terpenoids, coumarins, saponins, anthraquinones, phlobatannins and iridoids were estimated. Different nutritional factors like total carbohydrates, total proteins, total reducing sugar, different amino acids, free

fatty acids, crude fibres, lipids, total moisture content, vitamins, etc. were tested by standard estimation methods. Anti-nutritional factors like phytic acid, total phenol, tannic acid, etc., were also estimated. Micronutrients and different pigments were quantified. The present studies revealed that this wild leafy plant has numerous nutritional factors with a low level of anti-nutritional factors. Therefore, this nutritive herb with diverse health-promoting compounds can be effectively utilized to overcome the nutritional deficiency problem around the globe [4]. The determination of the antibacterial activity (microbicidy) of surfaces is described in the following norms: ISO 22196^[5] and JIS Z 2801^[6]. The Japanese norm JIS Z 2801 was published in 2000 and published again in 2007 as the internationally valid norm ISO 22196. Therefore, ISO 22196 and JIS Z 2801 are identical. In the test, both a surface system coated with sporicide and an identical surface system without an antibacterial coating is charged with selected microorganisms.

MATERIALS AND METHODS

Collection authentication

Bidens biternata ehrenb leaves were collected from Narsapur, Medak district and authenticated by D.Venkateshwara Rao, Deputy Director, A.P. Forest Academy, Dullapally, Hyderabad, Ranga Reddy Dist.

Microbial strains used: Two strains of gram positive (*Bacillus subtilis* MTCC 736, *Staphylococcus aureus* MTCC 96) and three gram negative strains of (*Escherichia coli* MTCC 443, *Proteus vulgaris* MTCC 1171, *Pseudomonas aeruginosa* MTCC 424) bacteria were used as test organisms.

Chemicals used

Dimethyl sulfoxide, nutrient agar medium, alcohol, ethanol

Methods

Preparation of extracts

Freshly collected plant material was cleaned to remove adhering dust and then dried under shade. Maceration process was done for 6 days .On the first maceration the 300 grams of plant material

was taken in three round bottom flask (each R.B.F with 100 grams) each filled with 300 ml of ethanol and macerated for 3 days. On the 4th day the macerated product was filtered. The filtered product was distilled at 20°C in heating mantle. The ethanol was condensed. The condensed ethanol was used for next maceration and the filtrate was again distilled and green residue was obtained which was stored in desiccator.

Preparation of samples

Ethanolic extract of leaves were used at the concentration of 0.5 and 1 g/ml dissolved in DMSO (dimethyl sulphoxide).

Mueller hinton agar medium

Mueller hinton agar medium is used for determination of susceptibility of microorganism to antimicrobial agents. Mueller hinton agar medium with 5% sheep blood and Mueller hinton agar with hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumonia* and *Haemophilus influenza* [7]. Mueller hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species [8].

Composition

Gms/Ltre

Ingredients

Peptone	9.4 gm
Yeast extract	4.7 gm
Beef extract	2.4 gm
Agar	23.5 gm
Water	1000 ml
Final pH (25 ⁰ C)	6.1+0.1

Determination of Antimicrobial activity

The various strains of gram positive and gram negative bacteria were used for testing the antimicrobial activity of ethanolic extract of leaves of *Bidens biternata*. Mueller Hinton agar

medium was used as the nutrient medium for testing the antimicrobial activity. It was sterilized by autoclaving at 120°C at pressure 15 lb/inch². 20 ml of agar medium inoculated with the respective strains of bacteria and was transferred aseptically into each sterile Petri plate. Complete procedure was carried under laminar airflow chamber under aseptic conditions. The plates were left at room temperature to consent for solidification. In each plate wells of 8 mm were made using sterile cork borer. The extracts were freshly reconstituted with sample dimethyl sulfoxamide (DMSO)) and tested at various concentrations. 4 cavities were made in each Petri plate. Alternative cavities were filled with sample solution. The remaining two cavities were filled each with alcohol and DMSO respectively. These plates were kept in the refrigerator for half an hour for pre-diffusion of the extract into agar layer. After pre-diffusion these plates were incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

The ethanolic extract of leaves of *Bidens biternata* were tested for antimicrobial activity against *Escherichia coli* (Fig. 1), *Staphylococcus aureus* (Fig. 2), *Proteus vulgaris* (Fig. 3), *Pseudomonas aeruginosa* (Fig. 4), *Bacillus subtilis* (Fig. 5). The result has been shown in Table 1. The sample showed effective zone of inhibition against gram negative (*Escherichia coli*) and positive (*Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) strains. The zone of inhibitions were *Escherichia coli* (10 mm) *Staphylococcus aureus* (0.9 mm), *Proteus vulgaris* (0.9 mm), *Pseudomonas aeruginosa* (10 mm) and *Bacillus subtilis* (0.8 mm).

The result on various strains of bacteria was shown in Figure 1.

Table 1

Zone of inhibition in mm for following bacterial strains						
Extracts	Concentrations (g/ml)	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>P. vulgaris</i>
Leaves (ethanolic extract)	1	10	10	0.9	0.9	0.9
	0.5	0.9	0.8	0.8	0.8	0.9



Figure 1. *Escherichia coli*

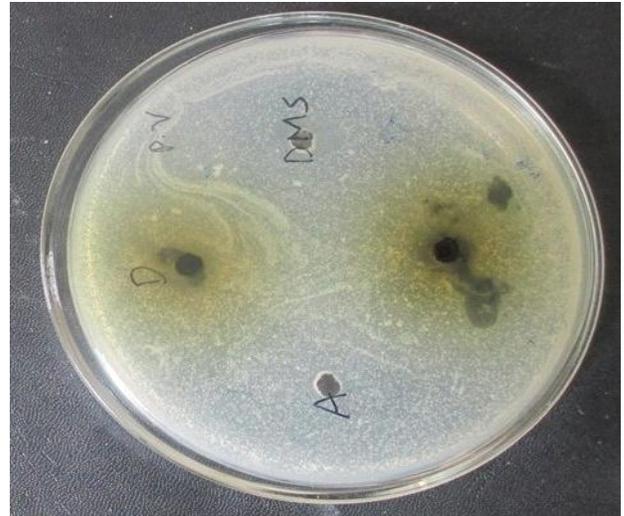


Figure 3. *Proteus vulgaris*

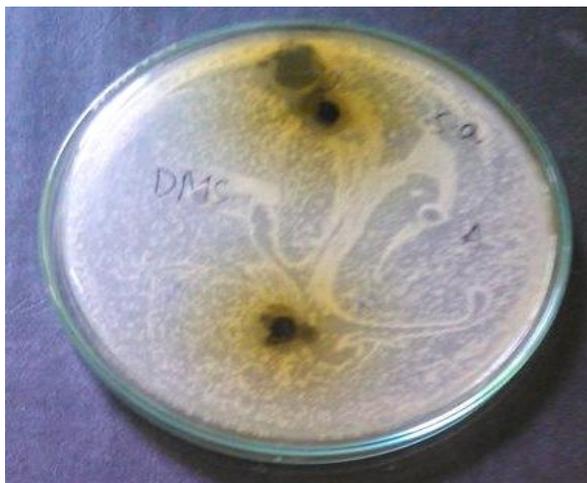


Figure 2. *Staphylococcus aureus*

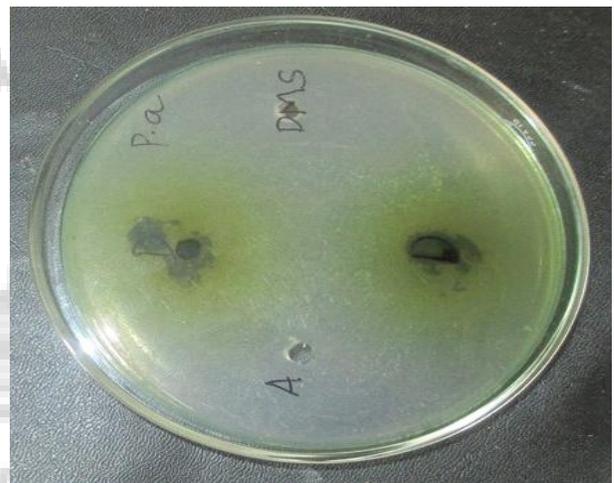


Figure 4. *Pseudomonas aeruginosa*



Figure 5. *Bacillus subtilis*

CONCLUSION

From the results it was concluded that the ethanolic extract of *Bidens biternata* showed antimicrobial activity. Further work on the profile and chemical constituents of leaves of *Bidens biternata* will provide valuable information on the therapeutically active constituents responsible for pharmacological properties.

Phytochemicals present in leaves of extracts *Bidens biternata* indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested for antispasmodic and antihelmentic activities. Furthermore, isolation, purification and characterization of the phytochemicals will construct interesting studies.

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