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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *BIDENS BITERNATA* (LOUR.) MERR. & SHERIFF - OF SOUTHERN WESTERN GHATS

Pradeesh S

Guest Lecturer, Department of Botany, Christian College, Kattakada, Thiruvananthapuram - 695 572, Kerala.
pradeeshnair10@gmail.com,

Abstract: *Bidens biternata* (Lour.) Merr. & Sheriff, belongs to the family Asteraceae, is an erect annual herb, up to 1 m height, a wide spread weed of cultivated areas. This plant is common, particularly in Western Ghats regions of Kerala state. It is used as a leafy vegetable by Paniya, Chetti, Kani and Kattunaayika tribes of Waynadu Districts in Kerala and also to cure hepatitis, cold, cough, dysentery, asthma etc. Phytochemical constituents are responsible for medicinal activity of ethno medicinal plant *B. biternata*. Hence in the present study preliminary phytochemical screening and antimicrobial activity of *B. biternata* a medicinal plant was carried out. Qualitative phytochemical analysis of these plants confirm the presence of various secondary metabolites like reducing sugar, glycosides, flavonoids, alkaloids, tannins, steroids, terpenoids, coumarins and saponins. Antimicrobial activity of the crude methanolic plant extract was evaluated by disc-diffusion method and revealed a low antimicrobial property.

Key words: *Bidens biternata*, glycosides, flavonoids, alkaloids and saponins.

I INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs (Ncube *et al.*, 2008). Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug (Vinoth *et al.*, 2011). There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Turger and Usta (Turker and Usta, 2008), screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases,

including cancer (Sheeja and Kuttan, 2007) and Alzheimer's diseases (Mukherjee *et al.*, 2007). Phytochemicals are responsible for medicinal activity of plants (Savithamma *et al.*, 2011). These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. Primary metabolites are comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of glycosides, alkaloids, saponins, terpenoids, steroids, flavonoids, tannins and so on (Kumar *et al.*, 2009). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (Savithamma *et al.*, 2011). Though the plant and its extracts have been extensively used in the tribal medicine, information from organized search of published literature does not provide evidences for the secondary metabolites and its antimicrobial potentiality of *B biternata*. So the present study aimed to analyse the preliminary phytochemicals and antimicrobial activity of crude methanolic extracts of *B. biternata*.

II MATERIALS AND METHODS

Collection and preparation of sample

B. biternata were collected fresh from Western Ghats of Kerala, mature leaves, young leaves, stem and roots were separated and shade dried, ground well using mechanical blender to fine powder and transferred to airtight containers for further phytochemical and antimicrobial analyses.

Extraction from plant parts

The fine powder was used for extraction by using solvents like methanol, acetone, petroleum ether, chloroform and distilled water. Fifty gram of sample powder was covered with cotton cloth and kept into the soxhlet apparatus for distillation. Three hundred ml of solvent was taken into the round bottom flask and heated in a mantle for 8 hours at 70°C. After completing the process, extract was collected in beaker and was kept in oven at 37°C-40°C for evaporation. Methanol, acetone, petroleum ether and chloroform were obtained as residues and were green, black solid, respectively. The crude concentrated extract was again weighed and used for further preliminary phytochemical and antimicrobial activity investigations.

Phytochemical Screening

The phytochemicals like reducing sugar, glycosides, flavonoids, alkaloids, tannins, steroids, terpenoids, coumarins, saponins, anthraquinones, phlobatannins and iridoids were tested (Harborne, 1977).

Reducing Sugar (Fehling's test)

The extract was dissolved in distilled water and added with boiling Fehling's solution, brick red precipitate denoted the presence of reducing sugar.

Glycosides (Keller-Killani test)

The extract was dissolved in distilled water and added with 2 ml of glacial acetic acid containing one drop of ferric chloride solution followed by 1 ml of concentrated sulphuric acid along the side of the test tube. Brown ring at the interface denoted the presence of glycosides.

Flavonoids (Shinoda test)

The extract was dissolved in methanol and added a few pinch of magnesium turnings followed by concentrated hydrochloric acid drop by drop. Presence of pink colour confirmed the presence of flavonoids.

Alkaloids (Dragendorff's method)

The extract was warmed with 10 ml of 2% sulphuric acid for 2 minutes and filtered. A known quantity of aliquot was treated with a few drops of Dragendorff's reagent (glacial acetic acid in a solution of bismuth nitrate and potassium iodide). Orange brown precipitate denoted the presence of alkaloids.

Tannins (Ferric salt test)

The extract was added with two drops of 2% ferric chloride, dirty green colour indicated the presence of tannins.

Terpenoids and Steroids (Liebermann-Burchard method)

A little of the extract was dissolved in dry chloroform and added three drops of acetic anhydride followed by the addition of two to three drops of concentrated sulphuric acid. Appearance of green colour for steroids while pink colour indicated the presence of terpenoids.

Coumarins (Alcoholic sodium hydroxide-hydrochloric acid test)

The extract was dissolved in a few drops of alcoholic sodium hydroxide. Appearance of yellow colour on the addition of concentrated hydrochloric acid through the sides of the test tube indicated the presence of coumarins.

Saponins (Chloroform and H₂SO₄ test)

The extract was dissolved in distilled water mixed well and heated to the boiling point. Froth formation indicated the presence of saponins.

Anthraquinones

A known quantity of powdered material was mixed with 10 ml of 1% hydrochloric acid and boiled for 5 minutes. The mixture was filtered, cooled, added with equal volume of chloroform. Separated the chloroform layer, mixed well and added with equal volume of 10% ammonia solution and allowed the layer to separate. A delicate pink colour indicated the presence of anthraquinones.

Phlobatannins

The extract was warmed with 5 ml of 1% hydrochloric acid and red precipitate showed the presence of phlobatannins.

Iridoids

The extract was added to 1 ml of reagent (10 ml acetic acid, 0.2% copper sulphate solution and 0.5 ml concentrated hydrochloric acid). The mixture was heated over a small flame. Development of a light blue colour indicated the presence of iridoids.

Antimicrobial studies by in vitro methods

Crude methanolic extract of leaves of *B. biternata* was used for evaluation of antibacterial and antifungal activity *in vitro* by disc-diffusion method.

Antibacterial activity in *B. biternata*

Standard strains of pathogenic and industrially important bacteria selected for antibacterial studies includes *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Paracoccus denitrificans* and *Klebsiella pneumonia* which were procured from NIIST (National Institute of Interdisciplinary Science and Technology), Thiruvananthapuram.

Media preparation and inoculation

Petridishes, test tubes, forceps and glass rods were washed with mild detergents and rinsed with sterile distilled water and autoclaved for sterilization at 121°C and 1.05 kg cm⁻² for 20 minutes.

Preparation of the media for the subculturing the Bacteria

Peptone water was made by dissolving peptone in distilled water (13.6 g⁻¹). The solution was then autoclaved 121°C and 1.05kg cm⁻² for 20 minutes. The spores of each bacterium from the isolated colony were introduced into the media taken in test tubes. The test tubes were kept in an incubator at a temperature of 37°C for an hour for maximum growth.

Preparation of filter paper disc for inhibition study

Disc of 6 mm diameter were cut out from Whatmann No.1 filter paper. They were autoclaved for sterilization at 121°C and 1.05kg cm⁻² for 20 minutes. The disc was then soaked in various concentrations of methanol extracts till it was completely saturated. Control discs were prepared by using equal volume of solvent only.

Preparation of nutrient agar medium

Nutrient agar medium was prepared by dissolving nutrient agar in distilled water (37.5 g l⁻¹). After dissolving the agar completely, the nutrient medium was autoclaved and after cooling to 50°C, 20 ml of molten medium was poured into sterile petridish and allowed to solidify under aseptic conditions and stored.

Inoculation

Pure cultures of each bacterium from the peptone water were spread evenly on the nutrient agar plates with sterile swabs in highly aseptic conditions. Prepared disc containing the concentrated extract were placed over each bacterial colony. The inoculated petridishes were labeled and kept in an incubator at 37°C for 24 hours. Antibacterial activity was observed as zone of inhibition in milli meters (mm). The experiments were repeated thrice to confirm the findings.

Antifungal activity in *B. biternata*

Antifungal activity studies against common phytopathogenic forms of fungi were done with crude methanol extract of leaves of *B. biternata*. The fungal strains were procured from the School of Life Sciences, M. G. University, Athirampuzha, Kottayam. Fungal strains used were *Aspergillus niger*, *Alternaria solani*, *Mucor ramosissimus*, *Rhizopus nigricans* and *Fusarium oxysporum*.

Media preparation and inoculation

Test tubes, conical flask, petridishes, glass rod and needles were washed with mild detergent and rinsed with distilled water. They were sterilized for 20 minutes in an autoclave. Sabouraud Dextrose Agar (SDA) media was used for fungal culture. SDA were prepared by dissolving SDA (65 g l⁻¹) and agar (10 g l⁻¹) in distilled water by heating. The media was sterilized and poured in petridishes in a manner that each tube contained 4-5 ml of media. Then mixed 50, 100 and 150 µg of crude methanol extract in petridishes containing the media. Pure culture of *A. niger*, *A. solani*, *M.*

ramosissimus, *R. nigricans* and *F. oxysporum* were inoculated by using inoculation needle and loop under sterilize condition. After inoculation the petridishes were incubated at room temperature ±29°C for three to five days. The experiments were repeated thrice to confirm the results.

III RESULTS AND DISCUSSION

Phytochemical screening of *B. biternata*

Phytochemical screening of *B. biternata* showed that glycosides, flavonoids, alkaloids, steroids and tannins were present actively in methanol extract of mature leaves, young leaves, stem and root, but the presence of anthraquinones, phlobatannins, iridoids etc. were not detected (Table 1). The medicinal values of herbs are mainly due to the presence of secondary metabolites that produces a definite physiological action on the human body (Tirupathi *et al.*, 2011). The most important of these metabolites include glycosides, alkaloids, steroids, tannins, saponins, terpenoids and flavonoids (Premkumar *et al.*, 2011). The alkaloids have been investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic (Akindele and Adveyemi, 2007) and anti-inflammatory (Malairajan *et al.*, 2006) properties. The saponins and glycosides occur widely in plants. Saponins also known to have antifungal properties (Argal and Pathak, 2006) and are used as mild detergents. In medicine, they are used for hypercholesterolemia, hyperglycemia to enhance central nervous system activities (Malairajan *et al.*, 2006) and also for weight loss. They also have antioxidant, anti-inflammatory and anticancer activities. Tannins exhibit antidiabetic, anti-inflammatory, antibacterial and antitumor activities (Argal and Pathak, 2006). It was reported that certain tannins were able to inhibit HIV replication selectively (Argal and Pathak, 2006). The herbal tannins have been recognized globally for their pharmacological properties.

Glycosides were reported to exhibit anti-diabetic characteristics (Ogbonnia *et al.*, 2008). Cardiac glycosides are known to hamper the Na⁺/K⁺ pump (Ogbonnia *et al.*, 2008) and results in an increase in the level of sodium ions in the myocytes which then enhance the level of calcium ions. This consequently increases the amount of Ca²⁺ ions available for contraction of the heart muscle, which improves cardiac output and reduces distention of heart and thus used in the treatment of congestive heart failure and heart cardiac arrhythmia. Phenolic compounds such as lignans, alkyl resorcinols and phenolic acids are potential bioactive compounds due to their antimicrobial, antioxidative and anticarcinogenic effects (Pratt, 1992). Phenolic and polyphenolic compounds constitute an important class of secondary metabolites that act as free radical scavengers and inhibitors of LDL (low density lipoprotein), cholesterol oxidation and DNA breakage (Shahidi, 2004) but it form a complex with

minerals which reduce the bioavailability of minerals. Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel, 1995). The flavonoids showed antioxidant activity and their effects on human nutrition and health is of considerable significance. The mechanism of action of flavonoids are through scavenging or chelating process (Kessler *et al.*, 2003) and they also inhibit microbes which are resistant to antibiotics (Linuma *et al.*, 1994). Flavonoids are free radical scavengers, super antioxidants and potent

water soluble compounds which prevent oxidative cell damage and have strong anticancer activity (Salah *et al.*, 1995). As antioxidants flavonoids provide anti-inflammatory actions (Okwu, 2001). Quercetin is found to be the most active of the flavonoids and many medicinal plants owe much of their activity due to their high quercetin content (BBC, 2007). Munmi *et al.*, (2013) reported the presence of secondary metabolites like alkaloids, saponins, terpenoids, tannins, glycosides, flavonoids, steroids and phenolics in Asteraceae members like *Ageratum conyzoides*, *Eupatorium odoratum* and *Mikania micrantha*.

Table 1: Preliminary phytochemical evaluation of *B. biternata* in different solvents

Phytochemicals	Mature leaves					Young leaves					Stem					Roots				
	M	A	PE	C	DW	M	A	PE	C	DW	M	A	PE	C	DW	M	A	PE	C	DW
Reducing sugar	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
Glycosides	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	-	+
Alkaloids	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+
Tannins	+	+	-	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	-	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
Terpenoids	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	+	+	-	+	-
Coumarins	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+
Saponins	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	-	-	-	-	+
Antraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Iridoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

M-methanol, A-acetone, PE-petroleum ether, C-chloroform, DW-distilled water

Antimicrobial studies by *in vitro* methods

Table 2: Antibacterial activity of the methanolic leaf extract of *B. biternata*

Bacterial species	Gram +/-	Minimum inhibitory concentration (MIC) in mm				
		50 µg / disc	100 µg / disc	150 µg / disc	Standard (Ampicillin) 25 µg / disc	Control
<i>Staphylococcus Aureus</i>	+	6	11	14	32	Nil
<i>Escherichia coli</i>	-	7	9	11	30	Nil
<i>Pseudomonas Aeruginosa</i>	-	8	11	13	33	Nil
<i>Paracoccus denitrificans</i>	-	Nil	Nil	Nil	20	Nil
<i>Klebsiella pneumonia</i>	-	8	10	11	28	Nil

Antibacterial activity in *B. biternata*

Antimicrobial activity of *B. biternata* against selected bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Paracoccus denitrificans* and *Klebsiella pneumonia* was evaluated. Results showed that the methanolic extract of *B. biternata* showed a mild action against bacteria such as *S. aureus*, *E.*

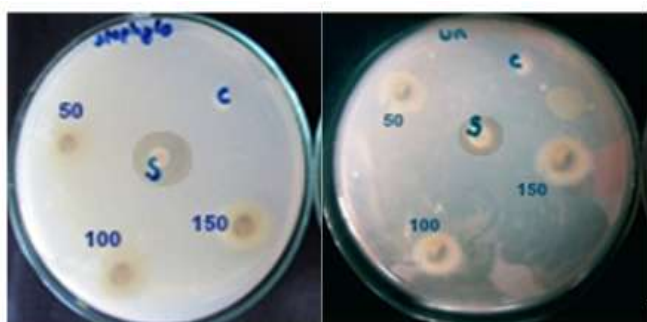
coli, *P. aeruginosa* and *K. pneumonia*, but not very effective against *P. denitrificans* (Table: 2 and Plate 1).

Antifungal activity *B. biternata*

Methanolic extract of *B. biternata* leaves is active against fungi such as *Aspergillus niger*, *Alternaria solani*, *Mucor ramosissimus* and *Rhizopus nigricans* but no zone of inhibition against *Fusarium oxysporum* (Table: 3 and Plate 2).

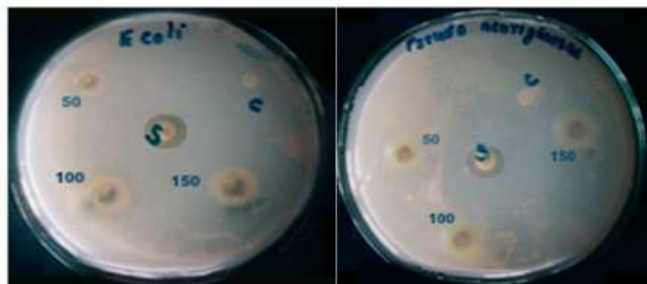
Table 3: Antifungal activity of the methanolic leaf extract of *B. biternata*

Fungal species	Minimum inhibitory concentration (MIC) in mm				
	50 µg / disc	100 µg / disc	150 µg / disc	Standard (Bavistin) 25 µg / disc	Control
<i>Aspergillus niger</i>	10	12	14	30	Nil
<i>Alternaria solani</i>	8	10	11	25	Nil
<i>Mucor ramosissimus</i>	9	11	12	27	Nil
<i>Rhizopus nigricans</i>	8	9	11	32	Nil
<i>Fusarium oxysporum</i>	Nil	Nil	Nil	35	Nil



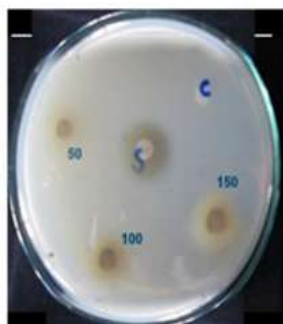
Staphylococcus aureus

Klebsiella pneumonia



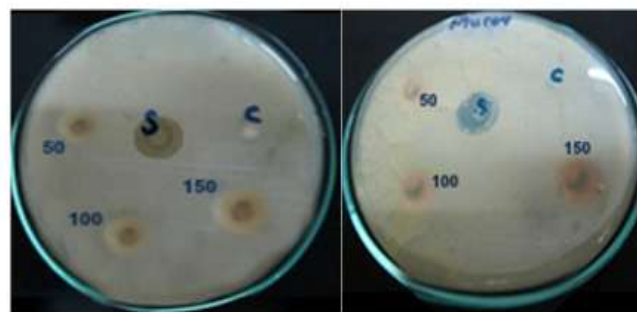
Escherichia coli

Pseudomonas aeruginosa



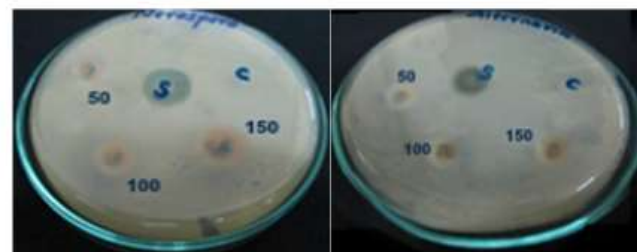
Paracoccus denitrificans

PLATE 1: Antibacterial activity in *Bidens biternata*



Aspergillus niger

Mucor ramosissimus



Fusarium oxysporum

Alternaria solani



Rhizopus nigricans

PLATE 2: Antifungal activity in *Bidens biternata*

Antimicrobial testing of medicinal plant *B. biternata* with various bacterial and fungal species indicated a mild action. This means that, *B. biternata* have high nutritive value although they were not much effective against the microorganisms tested.

IV CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic can be attributed to their high alkaloids, phenols, tannins and flavonoids. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation of techniques like extraction, purification, separation, crystallization and identification.

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BIOGRAPHY



Dr. Pradeesh. S has graduated from Dept. of Botany, Christian College, Kattakada, Thiruvananthapuram, PG and Ph. D from Dept. of Botany, University College, Palayam, Thiruvananthapuram, under University of Kerala. He has also worked as Botanical Assistant at

Botanical Survey of India (BSI) Southern Regional Centre, Coimbatore, Tamil Nadu. Currently he is working as Guest Lecturer at Dept. of Botany, Christian College, Kattakada, Thiruvananthapuram. He has published over 20 research papers in International and National Journals and Conferences.