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Antimicrobial and Antioxidant Activity of the Leaf Extract of *Basella alba*

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ABSTRACT

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacturing of traditional and modern medicine. Medicinal plants are rich sources of antimicrobial agents. *Basella alba* leaves were collected and dried. Powder was used for the antibacterial activities, which were determined by disc diffusion method and antioxidant activity was also determined. In antimicrobial activity, the concentration of 100mg/ml of *Basella alba* leaf extract showed highly active against bacterial strains. Antioxidants were determined by two methods in that 100mg/ml of the extract showed highly active to remove free radicals by DPPH (72.3±5.98) and ABTS (78±4.04). The aim of study was to evaluate the role of *Basella alba* extraction on antimicrobial and antioxidant activity. Leaves extracts of this plant showed admirable *in vitro* activity.

1. INTRODUCTION

Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs (1). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antibacterial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of world (2). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (3).

Description

B. alba is a widely cultivated, cool season vegetable with climbing growth habit. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Stem are Purplish or green. Leaves are fresh, ovate or heart-shaped, 5 to 12 cm long, stalked, tapering to a pointed tip. Spikes are auxiliary, solitary, 5-29 cm long, and purple when mature. Mainly leaves and stems are used for the medicinal purpose **Figure 1**.

Figure 1: *Basella alba*

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies (4). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (5). In this study, methanolic extract of leaves of *Basella alba L.*, which has been described in herbal books and

folklore medicine, were screened for their antimicrobial activity. The drug resistant bacteria and fungal pathogens have further complications in treatment of infectious diseases. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antibacterial substances from other sources including plants (6). The methanolic extracts exhibited marked antimicrobial activity against gram positive and gram negative bacteria and fungi. *Basella alba* showed good inhibitory activity against *Aspergillus niger* (7).

The large generation of free radicals, particularly reactive oxygen species and their high activity plays an important role in the progression of a great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, parkinson's disease, Alzheimer's disease etc. (8,9). Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power) (10,11).

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases (12). In this study, ethanol extract of leaves of *Basella alba L*., which has been described in herbal books and folklore medicine, were screened for their antimicrobial and antioxidant activity.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The plant leaves (fresh leaves) used in the study was collected from natural populations of the plants around Kolathur (Salem District), Tamil Nadu in 2012. Authentication was carried out at the Department of Botanical Survey of India, Southern Regional Centre and Tamil Nadu

Agricultural University Campus in Coimbatore, where voucher specimens were deposited. The plant material were washed thoroughly with running tab water, chopped into small pieces and then dried under shade for a period 15 days. The dried plant materials were then ground into fine powders using a grinding machine. The powders were placed in sealed airtight bottles, well labeled and stored in the dark room temperature until extraction.

2.2 Preparation of Solvent Extracts

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal (13). One gram of the dried and powdered plant material (Leaves) was soaked separately with 10 ml of the methanol in a shaker until complete extraction of the material at the end of 24 hrs. Each extract was filtered through Whatmann filter paper No.1 and filtrates concentrated at room temperature in order to reduce the volume. The sample was concentrated using rotary evaporator and freeze dried to paste like form. The paste like extract was weighed 40mg, 60mg, 100mg and it was diluted in methanol for the further process. Four bacterial species were collected from the Department of Microbiology, KSRCAS, for the study. The microbial strains were used such as *Staphylococcus aureus, Bacillus subtilis* (gram-positive) and *Escherichia coli, Klebsiella pneumoniae* (Gram-negative).

2.4 Maintenance of Microorganisms

The test bacteria's were maintained in Nutrient Agar (Himedia Laboratories Pvt. Ltd., Mumbai) slants. The microbial cultures were sub-cultured, cultured strains were allowed two days for bacterial growth and they were stored at 5°C for further studies.

2.4 Determination of Antimicrobial Activity of *Basella alba*

2.4.1 Preparation of Sample

The sample was prepared to determine the antibacterial activity of *Basella alba* extract, for that three sterile capped tubes were arranged in a row of 40mg, 60mg and 100mg concentrations, which were diluted in 1ml of methanol to obtain working solution (14).

2.4.2 Disc Diffusion Method

The antimicrobial activity for methanolic extracts was determined by the disc diffusion method Bauer (15). Solutions of known concentrations (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized Whatmann filter papers No.1discs were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Blank discs impregnated with methanol were used as a control. These plates were then incubated at 37°C for 24 hrs to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter.

2.5 Antioxidant Activity of *Basella alba*

The antioxidant activity was determined by the following two methods.

2.5.1 DPPH Spectrophotometric Assay

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical of 2, 2-diphenyl-1-picryl hydrazyl-hydrate (DPPH) was measured by the method (16). The leaf extracts were added to 0.5 ml methanol solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as blank and DPPH in methanol, without the leaf extracts, served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = \frac{A\ 518(\text{Sample}) - A\ 518(\text{blank})}{A\ 518(\text{blank})}$$

2.5.2 ABTS Radical Scavenging Assay

The radical scavenging capacity of antioxidant for the ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6 sulphonate) radical action was determined by (17). ABTS was generated by mixing a 7mM aqueous solution of ABTS with 2.5mM Potassium ferrous sulphate (final concentration) followed by storage in the dark at room temperature for 12 hours before use. The mixture was diluted with ethanol to give an absorbance of 0.70±0.02 units at 734 nm using spectrophotometer. For the study, 10 µl of the diluted extracts or fractions (100 µg ml^{-3} in methanol) was allowed to react with 990 µl of fresh ABTS solution and the absorbance was taken 15 min after initial mixing. Ascorbic acid was used as standard (y =0.032x+0.0634: R^2 =0.09996) and the capacity of free radical scavenging was expressed as µ mol Ascorbic Acid Equivalent (AAE)/g extract or fraction.

2.6 Statistical Analysis

The biochemical results were subjected to mean ± standard deviation using statistical package to test the level of statistical significance. The results were obtained for the various parameters analyzed during the different phases of the study are presented in the next chapter.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Screening of Methanolic Extract of *B. alba*

Plants have been used since time immemorial for their antimicrobial traits shown by the various secondary metabolites (phytochemicals) synthesized and deposited in specific parts or all parts of plant. Screening of these compounds and identification of the bioactive molecules and their antimicrobial properties is the need of the time. Methanol was found to be the most effective solvent enabling maximum separation of the different phytochemicals, and preliminary analysis of the extracts revealed the presence of secondary metabolites in leaves as well as extract of *B. alba*. After the qualitative identification of the phytochemicals from the plant, each phytochemical was extracted and then subjected for testing its antibacterial activity against all pathogens of both Gram Positive and Gram Negative. In the study, the zone of inhibitions (mm) of methanolic extracts of *B. alba* on *S. aureus, B. subtilis, E. coli, and K. pneumoniae* at concentrations of 40mg/ml, 60mg/ml and 100mg/ml and control (methanol) showed a strong antimicrobial activity **Table 1, Figure 2**. The results suggest that aqueous extract has a

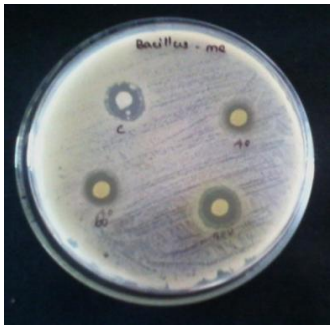
significant activity against bacteria both Gram positive (*Bacillus subtilis and Staphylococcus aureus*) and Gram negative (*Escherichia coli, Klebsiella pneumoniae*) organisms and the same results were observed in the antimicrobial and antifungal activity of *Basella alba*. It is very necessary to introduce new, biologically safe and active drugs, which are eco-friendly in nature and effective antimicrobial agents. Both gram positive and gram negative bacterial strains were used for the test (18).

TABLE: 4 Zone of Inhibition for Methanolic Extract of B*asella alba* against Bacterial Species

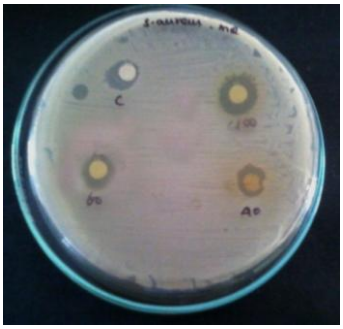
Test microorganism	**Diameter of the zone of inhibition (cm)**		
	40(mg/ml)	**60(mg/ml)**	**100(mg/ml)**
Gram positive bacteria			
Staphylococcus aureus	0.8±0.40	1.2±0.50	1.6±0.70
Bacillus subtilis	0.9±0.45	1.1±0.55	1.3±0.50
Gram negative bacteria			
Escherichia coli	0.9±0.60	1.5±0.60	1.8±0.70
Klebsiella pneumonia	0.9±0.26	1.0±0.40	1.2±0.35

Values are mean ± SD of three replicates

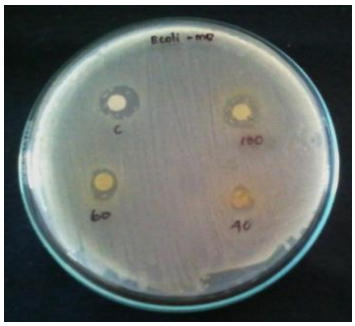
Figure: 2 Antibacterial Activity of Methanolic Extract of *B. alba*



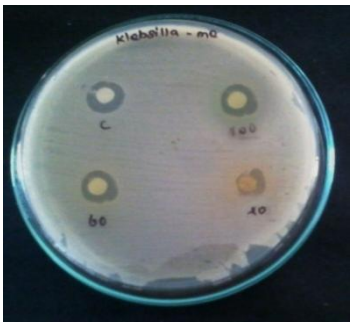
Bacillus subtilis



Staphylococcus aureus



Escherichia coli



Klebsiella pneumoniae

Antibacterial activity of methanolic extract of *Basella alba* were studied by measuring the zone of inhibition formed around the discs. Depending on the measured values of the complete inhibition diameter of the circle including the disc the millimeter, the antibacterial activity can be classified into highly sensitive (100mg) moderately sensitive (60mg), less sensitive (40mg) and resistant (<6mm). From above findings the extracts of *Basella alba* showed high sensitivity to gram negative bacteria and moderate sensitivity to gram positive bacteria

3.2 Antioxidant Activity of Methanolic Extract of *B .alba*

Antioxidant properties and other bioactivities of secondary metabolites of plants are of great interest in many fields such as pharmacology and the food nutrition industry. It is a growing tendency that natural antioxidant compounds are being used to replace synthetic antioxidants due to their side effects (19). In recent years, there has been an increasing trend towards the exploration of safer and effective antioxidants and functional ingredients from natural dietary sources like fruits, vegetable, oilseeds, cereals, grains and herbs (20). Antioxidant activity is the common assay used and widely accepted by researchers as an anticancer indicator (21). Therefore, these substances have been proposed as health promoting natural products (22-24).

3.2.1 DPPH Radical Scavenging Activity of Methanolic Extract of *B. alba*

Free radical mediated oxidative stress is believed to be the primary cause of many diseases and disorders. Hence, therapy using free-radical scavengers (antioxidants) has a potential to prevent, delay or ameliorate many of these disorders. In the present study, the methanolic extract of 100 mg/ml concentration showed a higher radical scavenging activity (72.3 %) corresponding increase in absorbance is noted in extract as well as standard when the concentrations of extract and standard (ascorbic acid) were increased **Table 2**. DPPH is a stable nitrogen centered free

radical, the color of which changes from violet to yellow upon reduction by either hydrogen or electron donating. Substances that are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (25). Many studies have been reported the use medicinal plants as radical scavengers. Our findings are in line with previous findings (26), who showed higher DPPH, reducing power, hydrogen peroxide, nitric oxide and lipid peroxidation scavenging ability of acetone and ethanolic leaves extract of *Hippobromus pauciflorus*.

Table: 2 DPPH Radical Scavenging Activity of Methanolic Extract of *B. alba*

Concentration(mg/ml)	DPPH inhibition (%)	
	Ascorbic acid	Methanol
20	58.16 ± 4.31	8.7 ± 1.16
40	70.14 ± 3.16	20.6 ± 2.84
60	78.25 ± 7.01	38.1 ± 3.96
800	81.01± 2.06	56.87 ± 5.04
100	93.32±1.02	72.3±5.98

Values are mean ± SD of the 3 replicates.

DPPH assay is the most widely reported method for screening antioxidant activity of many plant drugs, based on the reduction of coloured free radical DPPH in methanolic solution by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH which is proportional involves to concentration of free radical scavenger added to DPPH reagent solution (27).

3.2.2 ABTS Radical Scavenging Activity of Methanolic Extract of *B. alba*

The present study gives some scientific evidence on effect of extraction solvents, was made to find out the therapeutically better efficacious extract. Among comparative significance of various extracts, the methanolic extract of *B. alba* leaves having better efficacy and significant antimicrobial and antioxidant activity. Therefore, the present study support the traditional believes of this plant and highlighted profound potential of *Basella alba* to be investigated for bioactive compounds responsible for antimicrobial and antioxidant effect. It was observed that

the percentage ABTS scavenging activity of the methanolic extract of *B.alba* was higher in 100 mg/ml concentration (78%). The antioxidant capacity of methanolic extract of *B. alba*as determined by DPPH and ABTS were lower when compared with ascorbic acid standard **Table 3**. We have estimated the total antioxidant activity of dried leaves of *M. spicata* in different fractions (hexane, chloroform, ethyl acetate and water) of ethanolic extract, using $ABTS^{+}$ (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) discoloration method. Additionally the total phenolic compounds are also estimated in these ethanolic fractions. ABTS with potassium per sulfate generates blue/green ABTS+. The radical shows maximum absorbance at 645 nm, 734 nm and 815 nm, as per previously reported studies (28). This method can be used for both pure compounds and biological samples (29). Antioxidants transfer a hydrogen atom to radical cation and causes discoloration of the solution (30).

Table: 3 ABTS Radical Scavenging Activity of Methanolic Extract of *B. alba*

Concentration(mg/ml)	ABTS scavenging (%)	
	Ascorbic acid	**Methanol**
20	58.16 ± 4.31	38 ± 1.01
40	70.14 ± 3.16	46.42 ± 2.14
60	78.25 ± 7.01	57.07 ± 3.06
80	81.01± 2.06	64.37 ± 3.94
100	89.15±1.01	78±4.04

Values are mean ± SD of the 3 replicates.

CONCLUSION

In the study, there is necessity to introduce new, biologically safe and active drugs. Naturally the plants possess biologically effective antimicrobial and antioxidant agents. The methanolic leaf extract of *Basella alba* L. showed good activity against the bacterial strains of namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and antioxidant studies namely DPPH and ABTS. It indicates that the plant leaf contains phytochemical (medicinal) compounds for curing the different human diseases and further

investigation should be needed to screen the phytochemicals which are useful for pharmacological studies.

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