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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *BASELLA ALBA* LINN

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ABSTRACT

Medicinal plants contain some organic compounds which provide definite physiological action on the human body. The present study was aimed at physico chemical and preliminary phytochemical screening and antimicrobial activity of *Basella alba.* The invitro antimicrobial activity of crude chloroform and ethnolic extracts of various plant parts of *Basella alba* was investigated. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 10 to 40 mm. All the extracts exhibited appreciable activity against all the clinically important bacterial and fungal species clinically investigated. Maximum Inhibition zone (40mm) was observed in seeds against Streptomyces and minimum in stem (10mm) against Bacillus. Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids and flavonoids in the extracts. The antimicrobial activity of the extract was compared with the standard drugs. The ability of the crude extracts of *Basella alba* plant parts to inhibit the growth of various bacteria and fungi showed its broad spectrum antimicrobial potential, which may be employed in the management of microbial infections. Hence this study offers a base of using *Basella* species as herbal alternative for the synthesis of antimicrobial agent.

Keywords: Antimicrobial, *Basella alba* ,Phytochemical screening,Antifungal and Antibacterial.

INTRODUCTION

Phytochemistry or plant chemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structured of these substances, their biosynthesis, turnover and metabolism, their natural distribution and biological function[1].This plant is mainly used as a leafy vegetable because of it high nutrient qualities. It is good source of vitamin A and C, folic acid, and also rich in minerals like Ca, Mg, Fe, and several antioxidants such as β carotene besides other use phytochemicals[2], betacyanine, phenols[3], basella saponins, essential amino acids such as arginine, isoleucine,leucine,lysine,threonine and tryptophan[4].Saponins, tannins, flavonoids,terpinoids. Anthocyanins are present which are responsible for the blue, purple, violet and red colours in fruits, flowers, stem and leaves[5] *Basella alba* show different properties like aperients ,demulcent, diuretic, laxative, emollient, rubefacient, in cosmetics. Many plants are nutritionally important and having high medicinal properties *Basella alba* which is a nutritionally and medicinally important leafy vegetable is taken as an experimental plant.Dietary photochemical are involved in enhancing long-term health[6]. Antioxidant secondary metabolites and other phytochemicals are very important in preventing various nutrient and stress related diseases[7]. Because of this importance research was undertaken to find out comparative antimicrobial activity of different parts of *Basella alba*.

INTRODUCTION OF PLANT

It is a native of tropical Asia. It is fast growing perennial climber reaching up to 10 meter in length, with thick semi succulent ovate or

chordate shaped leaves having dark green or purple colour. Size of leave range from 4 to 10 cm in length with lot of mucilage. Flowers small bisexual born on axillary spikes or peduncles. Fruits are fleshy, stalk less pigmented ovoid or round shaped 5 to 6 mm in size.

Fig. 1: *Basella alba*

MATERIAL AND METHODS

Collection and Identification of Plant MATERIAL

Various plant parts of *Basella alba* (seed and stem) were collected from the fields at Ajmer and authenticated. The voucher (RUBL* No. 211570) of experimental plant was Deposited in the Herbarium of Department of Botany, University Of Rajasthan, Jaipur. Plant parts were separated, cleaned and Oven dried at 35ºc for 30 min and then at 250 gms till constant Weight was achieved and powdered.

Quantification of Primary Metabolites

Quantification of Primary Metabolites were carried out by using following methods of carbohydrates, proteins, lipids and phenols.

1. Extraction and Quantification of Carbohydrates

Total Soluble Sugars

The dried experimental plant material (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the method of[8].

Starch

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5 mL of 52% perchloric acid[9]. Later, 6.5 mL of water was added to each sample and the mixture was shaken vigorously for 5 minutes.1mL of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid[10].

2. Extraction and Quantification of Proteins

The test sample (50mg each) were separately homogenized in 10 mL of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^0C for 24 hours. These mixtures were centrifuged separately and supernatants were discarded. Each of the residues was again suspended in 10 mL of 5% TCA and heated at 80^0 on a water bath for 30 minutes. The samples were cooled, centrifuged and supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature[11].Each of the above samples (1 mL) was taken and the total protein content was estimated using the spectrophotometer[12].

3. Extraction and Quantification of Lipids

The test sample were dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol (2/1:v/v)[13]. The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. Two layers were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the preweighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried *in vacuo* and weighed. Each treatment was repeated thrice and their mean values were calculated.

4. Extraction and Quantification of Phenols

The deproteinized test materials (200mg each) were macerated with 10 mL of 80% ethanol for 2 hours, and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 mL by adding 80% ethanol[15].Total phenol content in each sample was estimated by spectrophotometer[14].

Phytochemical analysis of the plant extract

All the sequentially extracted fractions obtained from Various organic solvents were subjected to phytochemical tests For the presence of different metabolites following methods of Harborne[15] and established protocols.

Antimicrobial Activity

Chloroform and ethnolic extracts was used for determination of antimicrobial activity of *Basella alba*. Four bacterial and four fungal were selected for the antimicrobial screening. Clinical laboratory isolates of bacteria viz *Streptococcus viridians, Staphylococcus aureus, Escherichia coli, Bacillus* and fungi viz *Tricoderma ressie , F.fuserium , Pennicilium funicellosum* and *Aspergillus niger* were procured from the Microbiology Laboratory, SMS Medical College, Jaipur.

Preparation of Extract

The chloroform and ethnolic extracts was obtained by macerating 100 g of dried powder of different plant parts in 95% ethanol and kept on a rotary shaker for 24 h, separately. Each of the extract was filtered, centrifuged at 5000rpm for 15 min, dried under reduced pressure and stored at 4 ºC in airtight bottles.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude chloroform and ethanol extracts were studied against gram +ve and --ve bacterial strains by the agar well diffusion method[16]. Mueller Hinton Agar No.2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% dimethylsulphoxide at the concentrations of 5 mg mL-1. The Mueller Hinton agar was melted and cooled to 48-50 ºC and a standardized inoculum (1.5×108 CFU mL-1, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (40 μl) was introduced in the well (6 mm). The plates were incubated overnight at 37oC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotic streptomycin and ampicillin. For each bacterial and fungal strain, controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed in triplicate to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Antifungal activity of the experimental plant was investigated by agar well diffusion method[17]. The yeasts and saprophytic fungi were subcultured on Sabouraud's Dextrose Agar (SDA; Merck, Germany) medium and respectively incubated at 37 °C for 24 h and 25 °C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS (phosphate buffered saline) and adjusted to a concentration of 106 cells mL-1. Dipping a sterile swab into the fungal suspension was rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plateswere incubated at 37 °C. After incubation of 24 h, bioactivities were determined by measuring the diameter of inhibition zone (mm). The diameters of zone of inhibition produced were with those of standard clotrimazole used as standard antifungal agent. All the experiments were performed in triplicate and mean values were taken.

RESULTS

Phytochemical screening Investigations on the phytochemical screening of *Basella alba* extracts revealed the presence of carbohydrates, lipids, proteins, phenols, phytosterols, alkaloids and flavonoids, which are known to be biologically active. These metabolites can exert antimicrobial activity through different mechanisms (Table 1). The antimicrobial activity of chloroform and ethanolic extracts of different plant parts of *Basella alba* were tested against 4 bacterial strains (S. aureus, Bacillus , E. coli and S. viridians) and 4 fungal strains (A. niger , F. fuserium P. funicellosum and T.ressie). The Inhibition Zone (IZ) was measured by antibiotic zone reader (Table 2). Individually Staphylococcus aureus against maximum IZ was in extract of seed (40mm) and minimum IZ was in stem (10mm) In case of P.funicellosum maximum IZ was observed in stem ethnolic (16mm) and minimum in seed (12mm) and Among the fungal strains against P.funicellosum and T. ressie it was observed and A. niger and F. fuserium did not show any activity.

DISCUSSION

Plant scientists and natural products chemists are combing the flora for the phytochemicals and lead compounds, which could be developed for treatment of various diseases. In 2010 a survey of 1000 plants was done out of which, 156 clinical trials for evaluation of their pharmacological

activities and therapeutic applications gave encouraging results[18]. This led to the new search for drugs and dietary supplements derived from plants. During the last 10 years pace of development of new antimicrobial drugs has slowed down, while prevalence of resistance has increased multifold[19]. The problem of microbial resistance of growing and outlook for the use of antimicrobial drugs in future is still uncertain therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for better understanding of genetic mechanism of resistance. This prompted to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethnomedicinal use[20]. In the present investigation initial screenings of the experimental plant for possible antimicrobial activities was done using crude methanolic extracts. Nearly all of the identified components from plants that are active against microorganisms are aromatic or saturated organic compounds and most often obtained through ethanol or methanol extractive. In the present study *Basella alba* showed antimicrobial potent activity against bacterial strains as compared to fungal strain.

Table 1: Phytochemical evaluation from different plant parts of *Basella alba*

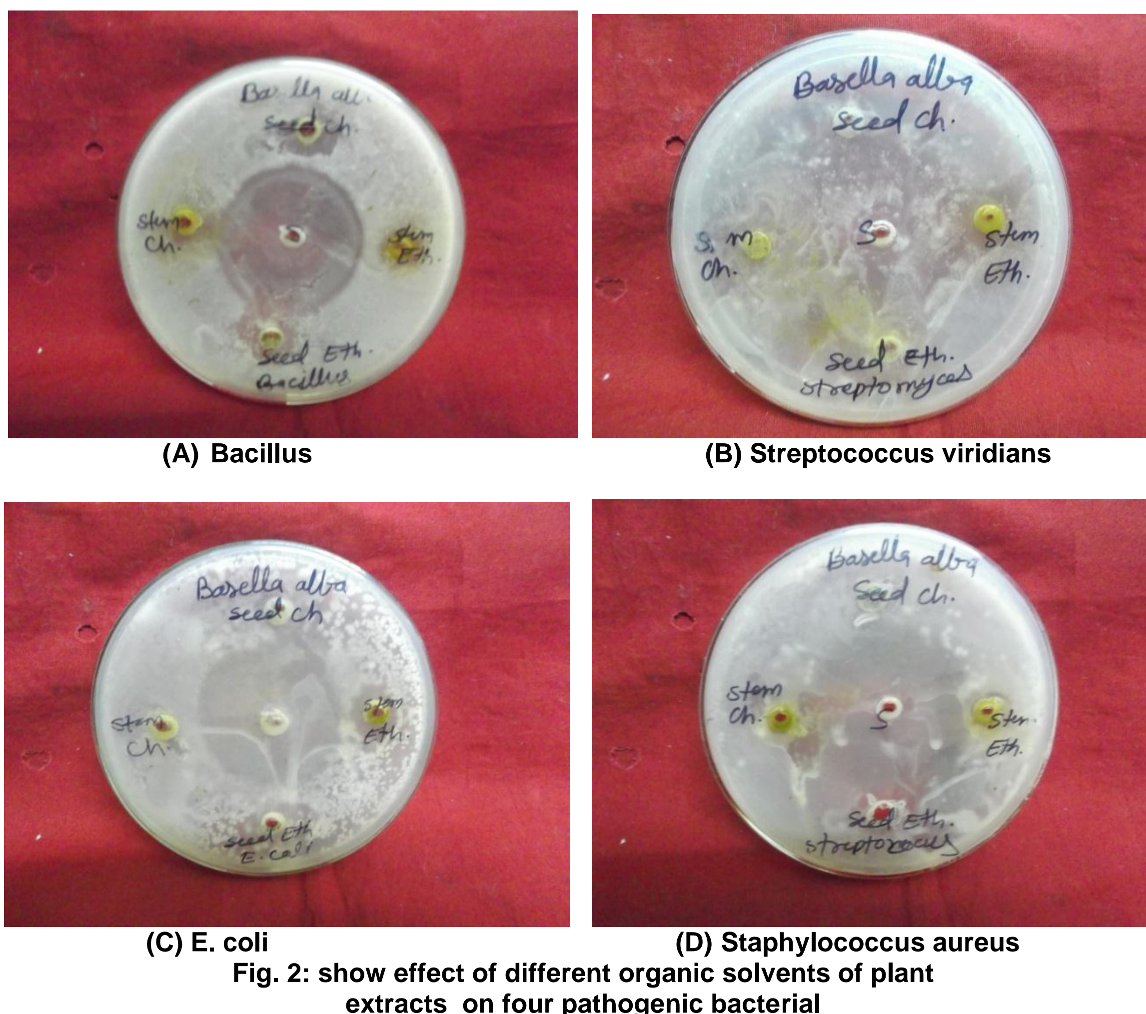
Phytochemical Name		Stem (mg/gdwt)	Seed (mg/gdwt)
Proteins		120±1.29	820±8.20
Lipids		2±0.02	14±0.08
Phenols		5.50±0.02	5.0±0.02
Carbohydrates	TSS	7.4±0.009	2.4±0.07
	Starch	8.1±0.04	6.2±0.03

Values are the mean ± SEM (n = 3 strains in each group). *P < 0.05; **P < 0.001 compared with the control ; P < 0. 001

Table 2: Antimicrobial activities of ethanolic and choloforma extracts of *Basella alba*

S. No.	Fungus and bactetial stains	Ethanolic extract		Chloroform extract	
		Seed(mm)	Stem(mm)	Seed(mm)	Stem(mm)
1	Fusarium oxysporium	NA	NA	NA	NA
2	Penicillium funicullosum	NA	ZI=16 AI=.72	ZI=12 AI=.54	NA
3	Trichoderma ressie	ZI=12 AI=.54	NA	NA	NA
4	Aspergillus niger	NA	NA	NA	NA
5	Streptococcus viridians	ZI=20 AI=.90	ZI=10 AI=.45	ZI=12 AI=.54	ZI=10 AI=.45
6	Escherichia coli	ZI=25 AI=1.13	ZI=20 AI=.90	ZI=20 AI=.90	ZI=12 AI=.54
7	Bacillus	ZI=18 AI=.81	ZI=10 AI=.45	ZI=20 AI=.90	ZI=18 AI=.81
8	Staphylococcus aureus	ZI=32 AI=1.45	ZI=30 AI=1.5	ZI=40 AI=1.18	ZI=20 AI=.90

Zone of Inhibition = ZI , Area of Inhibition = AI and No Activity = NA



(A) Bacillus **(B) Streptococcus viridians**

(C) E. coli **(D) Staphylococcus aureus**

Fig. 2: show effect of different organic solvents of plant extracts on four pathogenic bacterial

CONCLUSION

Basella Alba, used for the present investigation is a very important leafy vegetable commonly used in India. The result concluded that the Basella alba is not only used as vegetable but also in health preservation ,in prevention of various nutrient deficiency diseases and stress related diseases. Basella Alba is having many important antioxidant phytochemicals , antioxidant enzymes proved to be potential plant , which can prevent protect and progress of various diseases and disorders caused by the free radicals.

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