

Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF STEM OF THE HERB *BASELLA ALBA L. VAR RUBRA (L.) STEWART (FAMILY-BASELLACEAE)*

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ABSTRACT

Objective: To evaluate the antimicrobial activity ethanolic extracts of *Basella alba L. var. rubra (L.) stewart* (family-basellaceae) along with phytochemical analysis.

Methods: The ethanolic extract of stems of the herb *Basella alba L. var. rubra (L.) stewart* (family-basellaceae) were prepared and analysed for phytochemical constituents using standard methods. The antimicrobial activity of the plant extracts were examined against 2 bacterial strains among one is gram positive and other is gram negative and 2 fungal strains using agar well diffusion method.

Results: The present study describes the phytochemical profile and antimicrobial activity of *Basella alba L. var. rubra (L.) stewart*. Preliminary phytochemical screening of the extract were carried out and it revealed the presence of carbohydrates, tannins, terpene, saponin. The presence of these bioactive constituents is associated with the antimicrobial activity of the plant. The extract solvated by ethanol showed varying levels of antimicrobial activity against the tested bacteria namely *E. coli*, *S. aureus*, and fungi namely *Candida albicans* and *Trichophyton rubrum*. The study showed that the extract has a marked Sensitivity towards antibacterial strains namely *E. coli*, *S. aureus*, and antifungal strain namely *Candida albicans* and *Trichophyton rubrum*.

Conclusion: The present study concluded ethanolic extract of stem of the *Basella alba L. var. rubra (L.) Stewart* reveals many phytochemicals such as carbohydrate, tenin, saponin etc. This extract was found to possess promising antimicrobial activity when compared with the standards.

Keywords: *Basella alba L var rubra*, Antimicrobial, Zone of Inhibition, Agar disc diffusion method

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INTRODUCTION

Medicinal plants since from prehistoric times played a most important character in world health. The use of traditional system of herbal medicine is the ancient form of healthcare and it has been used by all traditions and cultures throughout history [1].

Plants have formed the basis of traditional system of medicine that have been in existence from ancient years and continue to provide mankind with new remedies. The majority of world's population on this planet are depended on their traditional material medica (medicinal plants and other materials) for their everyday health care needs. It is likely that the herbal remedies in traditional cultures developed through trial and error over many centuries, and that the most important cures were carefully passed on verbally from one generation to another [2].

Since from a long ago natural products which were used for combating human diseases for thousands of years, since they exhibit a wide range of biological properties which can be induced for medical application [3]. Microorganisms have developed resistance to many antibiotics and this resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [4].

Medicinal plants are used locally for the treatment of microbial infections caused by fungi, bacteria, viruses and parasites and many people in worldwide depend on the traditional medicine for the treatment of their ailments. Since from ancient times, various parts of plants has been used in the treatment and prevention of various diseases [5]. Medicinal plants having a rich source of antimicrobial agents and they are used in different countries and are a source of many potent and powerful drugs [6].

According to the reports of World Health Organization (WHO), about 70-80% of the world populations, depends on non-conventional

medicine mainly of herbal sources in their primary healthcare in spite of the great advances observed in modern medicine in present days, medicinal plants are still plays a major role by making a vital contribution to global health care [7].

Medicinal plants were promoted and dispensed worldwide, but they are most profuse in tropical countries. It is assessed that majority of all modern medications are directly or indirectly derived from higher plants. It is primary fact that one quarter of all medical preparations formulations are founded on substances derived from plants or plant-derived synthetic products [8].

Medicinal plants are species of plant material which is having therapeutic properties and it is used for clinical trial or which can be used as precursors for the synthesis of various drugs. Since from long ago there has been an increasing interest among the scientists in the use of traditionally naturally occurring medicinal plants of having biologically active chemical constituents. Therefore traditional practice of herbal remedies in several parts of the world with many of the herbal remedial being incorporated into approved medical practice. There are many plant species available all over the world which has been used for the multi valuable activities. India and China are the two key countries that are richer in many of the medicinal plant species. In spite of incorporation of millions of chemically produced drug natural products of plant origin has achieved prominence and has remained the most significant source of new drugs [9].

Basella alba L. var. rubra (L.) stewart belongs to family Basellaceae is a fast growing perennial vine native to tropical Asia, probably initiating from India or Indonesia and exceedingly heat tolerant. Its leaves are thick, semi-succulent, heart shaped having a mild flavor and mucilaginous texture. A decoction of the leaves is a good laxative for pregnant women and children. The fruits are fleshy,

stalk less, ovoid or spherical, 5-6 mm long, and purple when mature. The roots are used in the treatment of diarrhea, the cooked leaves and stems are used as laxatives. The flowers are used as an antidote to poisons and also as diuretic and febrifuge [10].

So the present study is to determine phytochemical screening and evaluate the antimicrobial activity and phytochemical screening of stem of *Basella alba* L. var. *rubra* (L.) Stewart.

MATERIALS AND METHODS

Collection of plant materials

The whole plant with leaves, stems and roots was collected from Chan sari area of Kamrup district of Assam in January 2017 and the plant was thoroughly washed with water; roots and leaves were discarded and the stems were dried in shade for 2 w and finally dried in a thermostatic oven at considerably low temperature not exceeding 30 °C for 24 h. The plant was authenticated by Prof. Dr. G. C. Sharma, Department of Botany, Gauhati University. A voucher specimen (Acc-18215, Dated: 22/12/2016) was kept in Department of Botany, Gauhati University for future reference.

Chemicals and reagents

Ethanol (ET), Petroleum Ether (PE), Dimethyl sulfoxide (DMSO), Dilute hydrochloric acid, Dragondroff reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, Sulphuric acid, Fehling's solution A and B, Dilute iodine solution, Ferric chloride, Sodium hydroxide, lead acetate, Ninhydrin, Benzene, Chloroform, Ammonia, Millon's reagent, Glacial acetic acid, Potassium dichromate, Acetic anhydride, Sodium chloride, Nutrient agar medium. All the chemicals and solvents used were of standard analytical grades.

Preparation of the plant extracts

The entire fresh plant materials were collected washed, shade dried and then kept at tray drier at 37 °C for 48 h. The dried plant materials were grinded to a fine powder using an electric grinder. The dried and powdered plant material was defatted by using Petroleum Ether and then extracted with Ethanol in the order of their increasing polarity by maceration process until it became colourless according to the standard methods. Each extract was concentrated by using rotary vacuum evaporator and stored in the refrigerator for further analysis.

The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material using the formula:

$$\% \text{ Yield} = \left(\frac{\text{Dry weight of the extract}}{\text{Dry weight of leaf sample}} \right) \times 100.$$

The colour and consistency of the extracts were also noted.

Phytochemical screening

The screening of the plant extract was carried out for the purpose of detecting active components like tannins, glycosides, alkaloid, terpene, steroids, phenolics, saponins, carbohydrates, proteins and flavonoids [11].

Test for alkaloids

A small portion of the extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidal reagents such as Mayer's reagent, Dragondroff's reagent, Hager's reagent and Wagner's reagent.

(a) Dragondroff's reagent test: To the filtrates few drops of dilute Dragondroff's reagent (Potassium bismuth iodide solution) was added. An orange brown precipitate indicates presence of alkaloids.

(b) Wagner's reagent test: To the filtrates few drops of Wagner's reagent (Iodine-potassium iodide solution) was added. Reddish brown precipitate indicates presence of alkaloids.

(c) Mayer's reagent test: To the filtrates few drops of Mayer's Reagent (Potassium mercuric iodide solution) was added. Cream precipitate indicates presence of alkaloids.

(d) Hager's reagent test: To the filtrates few drops of Hager's reagent (Saturated solution of Picric acid) was added. Yellow color precipitate indicates presence of alkaloids.

Test for carbohydrates

The minimum amount of the extracts were dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates.

(a) Molisch's test: To the filtrate, add 2-3 drops of Molisch's reagent shake and add few drops of concentrated sulphuric acid from sides of the test tube. Violet ring at the junction of two liquids indicates presence of carbohydrates.

(b) Fehling's test: The filtrate was treated with 1 ml of Fehling's A and B solutions, and heated in a boiling water bath for 5-10 min. First yellow, then brick red precipitate shows the presence of carbohydrates.

(c) Test for starch: Mix 3 ml of test solution and few drops of dilute Iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling.

Test for proteins

Various extracts were dissolved in few ml of water and treated with

(a) Millon's reagent: Appearance of red color shows the presence of proteins and free amino acids.

(b) Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple color indicates the presence of proteins and free amino acids.

Test for flavanoids

(a) Shinoda's test: The extracts were dissolved in alcohol, to that a piece of magnesium and followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoid.

(b) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Test for phenolic compounds

Ferric chloride test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for steroids

Salkowski reaction

To 2 ml extract was mixed with 2 ml of chloroform and 2 ml conc. Sulphuric acid. Shake well. Chloroform layer appears red and acid layers shows greenish yellow fluorescence.

Test for tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins. Chapter 5 Experimental Work Dept. of Pharmaceutical Chemistry, GIPS

Test for saponin

Foam Test—The extract and powder were mixed vigorously with water. Persistent foam observed.

Test for glycosides

Modified borntreger's test: To 3 ml extract dilute sulphuric acid was added, boiled and filtered. To the cold filtrate equal volume benzene or chloroform was added. The organic layer was separated and ammonia was added. Ammonical layer turns pink or red.

Microbial strains

Two bacterial strains [*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922)] and two fungi [*Candida albicans*]

(ATCC 10231), and *Trichophyton rubrum* (ATCC 28188)] were used. Stains were obtained from Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati.

Antibacterial activity

The Susceptibility test of the test organisms to ethanolic extracts of plant at concentrations of 100 mg/ml, 50 mg/ml, and 25 mg/ml was carried out using agar cup plate technique. Gentamycin was used as standard. Nutrient agar was prepared according to the standard concentration and autoclave at 121 °C for 15 min. It was then poured on to plates and allowed to solidify after which wells were made on the agar media using a sterile cup borer. Standardized inoculum of each test organisms was spread on to agar plates so as to achieve a confluent growth. Different concentration of the extract was introduced into the wells equidistant from one another. The plates were then incubated at 37 °C for 24 h. [12].

Antifungal activity

The potato dextrose agar plates were prepared and inoculated with a fungal culture. Wells of approximately 10 mm was bored using a well cutter and samples of different concentration was added. The zone of inhibition was measured in millimeters after overnight incubation and compared with that of standard antifungal

(Fluconazole) which was used as positive control and DMSO (10%) as the negative control.

RESULTS

The present investigation shows the phytochemical analysis, antimicrobial activity of the different extract of the plant *Basella alba L var rubra (L) stewart*. The yield % of the successive extraction of ethanol was 3.31%.

Phytochemical analysis

Table 1 shows the phytochemical screening of the ethanolic extracts of *Basella alba L. var. rubra (L.) stewart* The result indicates the presence of tannin, terpene, steroids, saponins, anthraquinone, carbohydrate in the stem.

Antimicrobial activity

Table 2 shows the zones of inhibitions (mm) of ethanolic extracts of *Basella alba L. var. rubra (L.) stewart* on two bacterial strain *E. coli*, *S. aureus* and two fungal strain *C. albicans* at concentrations 25 mg/ml, 50 mg/ml and 100 mg/ml and control (20 mg/ml). The control had a higher antimicrobial effect on the tested organisms than the ethanolic extract of the *Basella alba L. var. rubra (L.) stewart*

Table 1: Phytochemical screening of Ethanolic (ET) e stem extract of *Basella alba L. var. rubra (L.) stewart*

S. No.	Phytochemical constituent	Test performed	Result
1	Alkaloids	Mayer's test Wagner's test	- -
2	Carbohydrate	Dragendroff's test Molisch's test Benedict's test Fehling's test	- + + +
3	Protein and Amino acid	Ninhydrin solution	+
4	Flavonoids	Lead Acetate test	-
5	Phenol	FeCl ₃ solution	-
6	Steroids	Salkowski's test	+
7	Tannin	gelatin test	+
8	Saponin	Foam test	+
9	Glycoside	Modified Borntrager's test	-

+sign indicates presence and -sign indicates absence

Table 2: Antimicrobial activity of *Basella alba L. var. rubra (L.) stewart*

Name of the Compounds	Concentration(mg/ml)	Antibacterial activity diameter of inhibition zone (mm)		Antifungal activity diameter of inhibition zone (mm)	
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Trichophyton rubrum</i>
Standard Gentamycin	20	29	23		
Standard Fluconazole	20			24	22
<i>Basella Alba L. var Rubra(L) Stewart extract</i>	25	8.58	6.57	13.27	11.25
	50	9.85	8.57	17	13.65
	100**	15.65	17.67	20.2	15.25

** Significant activity

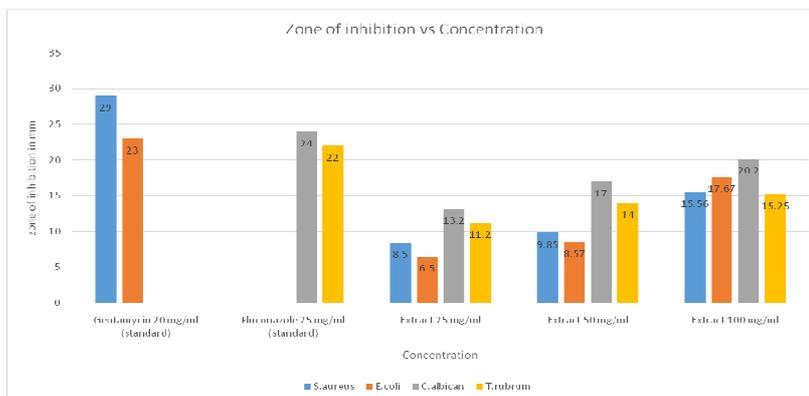


Fig. 1: Zone of inhibition vs concentration

DISCUSSION

The antimicrobial activity of stem of plant has been evaluated to determine the Phyto-constituents present in plants namely tannin, terpene, steroid, saponin, carbohydrate. In the present study, a variety of were bacteria like Gram positive *S. aureus* and Gram negative *E. coli* and fungus like *Candida albicans*, and *Trichophyton rubrum* for screening antimicrobial effect to determine antimicrobial activity by agar well diffusion method. The results suggest that ethanolic extract has a significant activity against bacteria both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) organisms and the fungi strains of *Candida albicans*, and *Trichophyton rubrum* and significant results were observed in the antimicrobial and antifungal activity of *Basella alba* L. var. *rubra* (L.) stewart.

CONCLUSION

The present study confirms the significant antimicrobial activity of the extract *Basella alba* L. var. *rubra* (L.) stewart In this study, the antibacterial activity of the stem of *Basella alba* L. var. *rubra* (L.) stewart was evaluated by using disk diffusion method. The microorganisms chosen to be studied were bacteria like Gram positive *S. aureus* and Gram negative *E. coli* and fungus like *C. albicans*, and *T. rubrum* further studies are necessary to isolate and characterize the active constituents of the plant to evaluate their modes of action and render this species interesting for future.

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CONFLICT OF INTERESTS

Declare none

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