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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY ON BASELLA ALBA L

J. Rosaline Vimala*, S. Keerthana

Department of Chemistry, Holy Cross College, Tiruchirappalli, Tamil Nadu, India

***Corresponding Author: Email** rosalinevimalaj@yahoo.co.in

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ABSTRACT

The present study describes the phytochemical profile and antimicrobial activity ofBasellaalba L. Leaves of Basella alba L. were shaded, dried, powdered and were extracted using solvent ethanol. Preliminary phytochemical screening of the extract were carried out and it revealed the presence of alkaloids, carbohydrates, pseudo tannins, chlorogenic acids and steroidal glycosides. The presence of these bioactive constituents is associated with the antimicrobial activity of the plant. The extract solvated by ethanol showed varying levels of activity against the tested bacteria namely Staphylococcus aureus, salmonella paratyphi, vibrio cholera and Escherichia Coli and fungi namely Aspergillus fumigates, Aspergillesniger and Candida albicans. The study showed that the extract has a marked sensitivity towards antibacterial strains namely Salmonella paratyphi and Vibrio Cholera and antifungal strain namely Aspergillus fumigates.

Keywords: Medicinal plants, Phyto-compounds, Anti-bacterial activity, Anti-fungal activity,Basellaalba L and Pathogens.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds 1. 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 strategies2. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side ef¬fects and have an enormous therapeutic potential to heal many infectious diseases 3. In this study, ethanolic extract of leaves ofBasellaalba L., which has been described in herbal books and folklore medicine, were screened for their antimicrobial activity.

MATERIALS AND METHODS

Plant materials

The plant leaves used in this study were collected in and around Trichy District.The disease free plant part (leaf) was spread out and dried in the laboratory at room temperature for 5-8 days until they were easily broken by hand. Once completely dried, leaves of the plant were grounded to a fine powder using an electronic blender. Plants were stored in a closed container at room temperature until required.

Preparation of Solvent Extracts

Fifty grams of the dried and powdered plant material (leaves) were soaked separately with 300 ml of the solvent ethanol, in a soxhlet apparatus for 48 hrs at 31°C until complete extraction of the materials. At the end of 48 hrs, each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The sample was concentrated

using rotary evaporator and freeze dried to powdered form. The paste like extract was stored in pre-weighed screw scrapped bottle and the yield of extracts was weighed. These screw scrapped bottle was kept in refrigerator at 4°C. The extract was reconstituted using minimal amounts of the extracting solvent prior to use.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Chemical tests were also conducted on the aqueous extract of each plant sample and also of the powdered form of the plant sample by using standard methods of Harborne and Edeoga.4,5

GAS CHROMATOGRAPHY - MASS SPECTRUM STUDY (GC-MS)

The components of test sample were evaporated in the injection port of the GC equipment and segregated in the column by adsorption and absorption technique with suitable temperature programme of the oven controlled by software. Different components were eluted from the column based on the boiling point of the individual components. The GC column was heated in the oven between 60 to 270°C. The time at which each component eluted from the GC column was termed as Retention Time (RT).

Interpretation of mass spectrum (GC MS) was conducted using database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library6. The retention time, molecular weight, molecular formula and composition percentage of the sample material was recorded.

Micro-organisms tested:

Four bacterial species were collected from Microbial Type Culture Collection (MTCC), from the Institute of Microbial Technology (IMT), Chandigarh in Punjab, for the study. The Microbial strains used were Escherichia coli MTCC 443, Salmonella paratyphi; Vibrio cholerae; 441and Staphylococcus aureusMTCC 87.

Aspergillusfumigatus, Aspergillusniger and Candida albicanswere also collected from Microbial Type Culture Collection, the Institute of Microbial Technology, Chandigarh in Punjab, India.

Maintenance of Microorganisms The test bacteria's were maintained in Nutrient Agar (Himedia Laboratories Pvt. Ltd., Mumbai) and test fungi were maintained in Potato Dextrose Agar (PDA) slants (Himedia Laboratories Pvt. Ltd., Mumbai). The microbial cultures were sub-cultured and the cultured strains were allowed to grow one week for fungi and two days for bacteria and they were stored at 5°C for further studies.

ANTI-MICROBIAL TESTING

Disc Diffusion Method:

The freeze dried extract was reconstituted with DMSO to obtain a stock solution of 50 µg/ml, 250 µg/ml, 500µg/ml and 1000µg/ml. Nutrient agar (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective microbial strains. Discs of (6 mm were punched from Whatman No.1 filter paper. Upto 10 µl of each concentration of the extract were respectively introduced in the discs using sterile automatic pipettes. The discs were allowed to dry at room temperature for 2 hours and were placed at equidistance in each of the plates using a sterile forceps. The plates were incubated to 37°C for 24 hours. The control antibiotic Kanamycin (10 µg) and Nystatin were used for gram-positive bacteria and fungi (Hi Media Laboratories Pvt. Ltd. Mumbai). Diameters of the inhibition zones were measured. The anti-microbial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract. 7-10

RESULTS AND DISCUSSION

Preliminary phytochemical screening:

Phytochemical screening of the extract of the leaves of Basella alba L. revealed the presence of alkaloids, carbohydrates, pseudo tannins, chlorogenic acids, steroidal glycosides, saponins and flavonoids (table-1).

GC-MS Analysis:

The GC separated compounds are identified from the recorded mass spectra by comparison with the mass spectra from the database of National Institute of Standard Technology (NIST) library.GC-MS chromatogram of the ethanolic extract of Basellaalba L. showed 6 peaks indicating the presence of 6 chemical constituents (Figure - 1). The 6 active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the ethanolic extract of Basellaalba L. are presented in Table – 2. On comparison of the mass spectra of the

Table – 1: Qualitative analysis of Phytochemical constituents in the ethanolic extract of the leaves of *Basella alba L.*

Phytochemical constitutents	Extract
Alkaloids	++
Carbohydrates	+
Saponins	-
Tannins	-
PseudoTannins	+
Chorogenic acid	+
Anthocyanin	+
Steroidal Glycosides	+
Saponins Glycosides	-
Flavonoids	+
Flavones	-
Phenols	-
Coumarin	+
Anthracene Glycoside	-

(+) = Detected; (-) = Not detected

Table – 2: Phytocomponents identified in the ehanolic extract of the leaves of *Basellaalba L.* by GC-MS

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
1	11.59	10-Undecen-1-ol	$C_{11}H_{22}O$	170	5.31
2	13.41	Heptanoic acid, 2-ethyl-	$C_9H_{18}O_2$	158	2.80
3	14.91	Phytol	$C_{20}H_{40}O$	296	41.12
4	15.37	1,4-Cyclohexanedimethanol	$C_8H_{16}O_2$	144	7.48
5	20.79	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	28.04
6	24.62	Squalene	$C_{30}H_{50}$	410	14.95

Table – 3: The Structure and nature of the predominant compounds

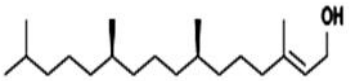
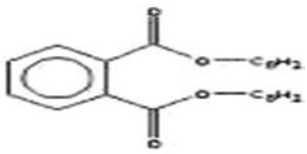
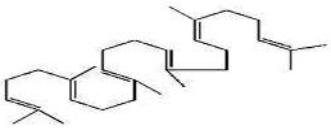
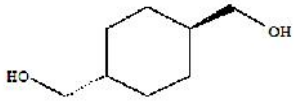
S. No.	Name of the compound	Structure	Nature
1.	Phytol		acyclic diterpene alcohol
2.	1,2-Benzenedicarboxylic acid, diisooctyl ester		Ester compound
3	Squalene		Triterpene
4	1,4-Cyclohexanedimethanol		Alcoholic compound
5.	10-Undecen-1-ol		Alkenols

Table – 4: Effect of Anti-bacterial Activity of *Basella Alba L. by* Disc Diffusion Method

Name of the extract	Concentration of the extract µg/ml	Zone of inhibition (mm)			
		Gram positive bacteria		Gram negative bacteria	
		Escherichia coli	*Salmonella paratyphi*	*Vibrio cholera*	*Staphylococcus aureus*
Ethanol	250	10	15	10	10
	500	12	16	11	12
	1000	13	18	18	13
Kanamycin	10 mg/ml	20	40	30	25

Table – 5: Effect of Anti-fungal Activity of *Basellaalba L.* by Disc Diffusion Method

Name of the extract	Concentration of the extract µg/ml	Zone of inhibition (mm)		
		Aspergillusniger	*AspergillusFumigatus*	*Candida albicans*
Ethanol	50	10	11	10
	250	11	13	11
	500	12	14	13
	1000	14	16	15
Nystatin	10 mg/ml	25	25	26

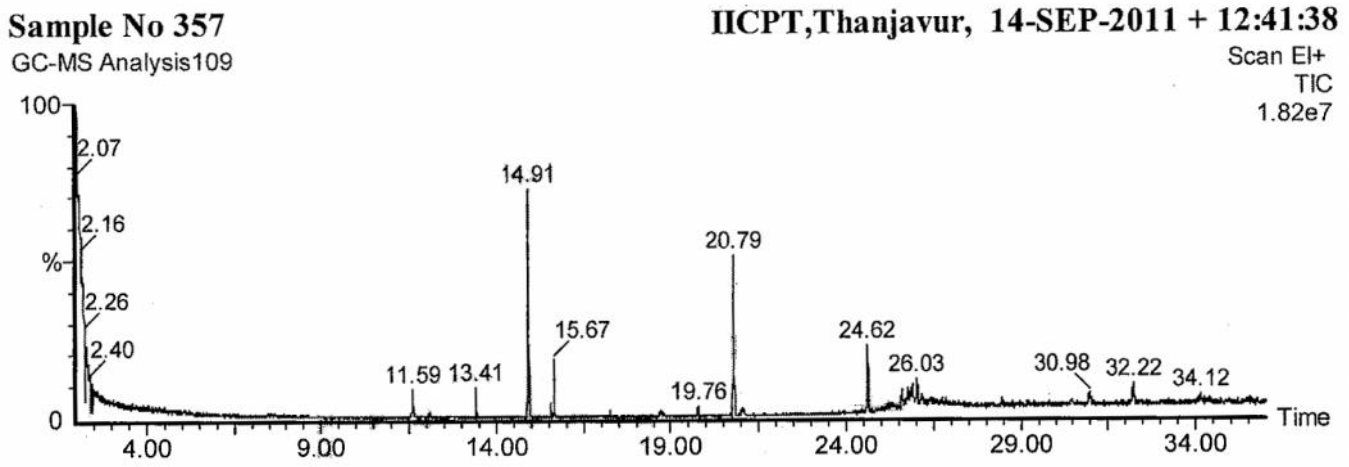


Figure – 1: GC-MS for *Basellaalba L.* Leaves

constituents with the NIST library the 5 predominant constituents were characterized and identified. The structure and nature of the compound are presented in Table – 3.

Anti-microbial Studies

Anti-bacterial Assay in Basella alba L.- Disc Diffusion Method

The sensitivity pattern of the four selected antibacterial strains (Escherichia coli, Salmonella paratyphi, Vibrio cholera and Staphylococcus aureus)used for the study is shown in Table - 4. The most pronounced activity with inhibition zones is shown at a concentration of 1000 µg/ml against the corresponding bacteria. The extract has a marked sensitivity towards Salmonella paratyphiand Vibrio cholera with inhibition zones 18.0 mm at concentration 1000 µg/ml respectively against the standard which has 40.0 mm and 30.0 mm of inhibition efficiency respectively.

Anti-fungal Assay in Basella alba L.

Disc Diffusion Method

The sensitivity pattern of the three fungal pathogens (Aspergillusniger, Aspergillusfumigatus, Candida albicans) used for the study is shown inTable - 5. The most pronounced activity with inhibition zone is shown at a concentration of 1000 µg/ml against the corresponding fungus. The extract has a marked sensitivity towards Aspergillusfumigatus, with 16.0 mm at concentration 1000 µg/ml respectively against the standard which has 26.0 mm of inhibition efficiency at a concentration of 10 mg/ml.

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective antimicrobial agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the micro organisms. In this work Basellaalba L. leaf extract has good activity against antibacterial strains namely Salmonella paratyphi and Vibrio Cholera and antifungal strain namely Aspergillus fumigates.Therefore, those might be utilized for the development traditional medicines and further investigation should be necessary for the development of novel lead compound. This study suggests that further research will be needed for pharmacological aspects of this taxon.

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