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ANTIMICROBIAL ACTIVITY OF *BASELLA ALBA* FRUIT

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ABSTRACT

The antimicrobial activity of the extracts *Basella alba* fruit were evaluated by measuring the zones of inhibition using Agar well Diffusion method against eight species of microorganisms: *Bacillus subtilus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Lactobacillus, Klubsiella, Aspergillus niger* and *Aspergillus fumigatus*. The extract showed significant antibacterial activity against *Lactobacillus* and antifungal activity against *Aspergillus fumigates*, no activity was found against *Klebsiella* and moderate activity was observed for all other tested organism. The minimum inhibitory concentration of the extract against bacterial strains was found to be 25mg/ml for *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus, Escherichia coli, Aspergillus niger* and *Aspergillus fumigates* and 50mg/ml for *Klebsiella pneumonia*. The overall result of this study indicates that the extract from *Basella alba* fruit have interesting antimicrobial property and thus provide justification for the use of the plants in folk medicine to treat various infectious diseases.

INTRODUCTION: Natural products have been used for combating human diseases for thousands of years, since they exhibit a wide range of biological properties that can be exploited for medical application [1]. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases [2]. This resistance has increased due to indiscriminated use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants [3].

Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites [4, 5]. Many people in Indian rural areas depend on the traditional medicine for the treatment of their ailments and since prehistoric times, various parts of plants has been used in the treatment and prevention of various diseases [6]. Medicinal plants represent a rich source of antimicrobial agents and they are used in different countries and are a source of many potent and powerful drugs [7].

Basella alba commonly known as Indian Spinach belonging to family Basellaceae is a fast growing perennial vine native to tropical Asia, probably originating from India or Indonesia and extremely heat tolerant. Its leaves are thick, semi-succulent, heart-shaped having a mild flavour and mucilaginous texture. The mucilaginous liquid obtained from the leaves and tender stalks of this plant is a popular remedy for habitual headaches. A decoction of the leaves is a good laxative for pregnant women and children [8]. The fruits are fleshy, stalkless, 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 [9, 10]. The flowers are used as an antidote to poisons and also as diuretic and febrifug[5]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [11]. The present study is designed to evaluate the antimicrobial activity of fruit of *Basella alba*.

MATERIUALS AND METHODS

Plant materials: *Basella alba* fruit were collected from Coimbatore, Tamilnadu, India and stored in sealed polyethylene bags at -20°C until extraction. The plant was idientified and authenticated (No.BSI/SRC/5/23 /2011-12/Tech.1485) by botanical survey of India (BSI), Tamil Nadu Agriculture Unversity (TNAU), Coimbatore, Tamil Nadu, India.

Extraction: 0.5 gm of *Basella alba* fruit were treated with 10 ml acidified methanol. And the mixture was centrifuged at 10,000rpm for 10 min and supernatant was taken for analysis [12].

Micro-organisms: *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus, Aspergillus niger and Aspergillus fumigatus* were the microorganisms used and they were stored at freeze temperature until use.

Preparation of 24 hours Pure Culture: A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours for bacterial culture and 48 hours in case of fungal culture.

Preparation of test Sample: The acidifed methanolic extract was dissolved in methanol to obtain the different concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml). 0.5ml of methanol was used as negative control (solvent control). 0.5ml of streptomycin (bacterial strains) and nystin (fungal strains) was used as positive reference standard

Agar-well Diffusion Method: Using 25ml of sterile Nutrient agar medium (bacterial culture) or Sabouraud agar medium (fungal culture) was poured into sterile culture plates and allowed to set. 0.5ml of 24 hours old culture of test organism was layered onto the medium and allowed to set. The seed medium was then allowed to dry at room temperature for about 30 minutes [13]. With the aid of a sterile cork borer, wells of about 8mm in diameter were punched on the plates. About 0.5ml of each dilution of the extracts, 0.5ml of streptomycin and nystin (positive control) and methanol (negative control) was dispensed into the wells and the plates were incubated at 37ºC for 24 hours for bacterial cultures and for fungal culture it was incubated at room temperature for 48 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

Minimum Inhibitory Concentration (MIC) [14]: The experiment was according to two fold serial dilution method. The stock solution of test solution (extracts) was prepared at concentration of 100µg/ml in nutrient broth and serially diluted up to five times. Six assay tubes were taken for screening of minimum inhibitory concentration of each strain. In the first tube 1ml of the sterilized nutrient broth was inoculated and then 1ml of the test solution was added and thoroughly mixed. Further dilutions of this solution were made by inoculating 1ml from first tube into second assay tube serially and 0.1ml of each test inoculums were added in each tube and were done in duplicate.

The procedures were conducted under aseptic conditions. The inoculated tubes were kept at $37^{o}C\pm 1^{o}C$ at 24 hours for bacterial assay and kept for 48 hours for fungal assay during the incubation period. After the incubation period, tubes were removed and observed for any deposits or turbidity in the solution and shaken to suspend bacteria that might have been settled down. These concentrations were observed & assumed as minimum inhibitory concentration (MIC).

RESULT AND DISCUSSION:

Agar-well Diffusion Method: Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antimicrobial activity assay [15]. The Results obtained in the present study relieved that the extract showed highest activity against *Lactobacillus and Aspergillus fumigates* with the zone of inhibition of 1.45mm and 1.75mm at 100mg concentration (**Plate 1 and 2**), no inhibitory

was observed in *Klebsiella pneumonia* and moderate activity was observed against all other tested microbes at various concentration (**Table 1**). Most of the betacyanin extracts exhibited some kind of antimicrobial activity against both gram positive and gram negative strains.

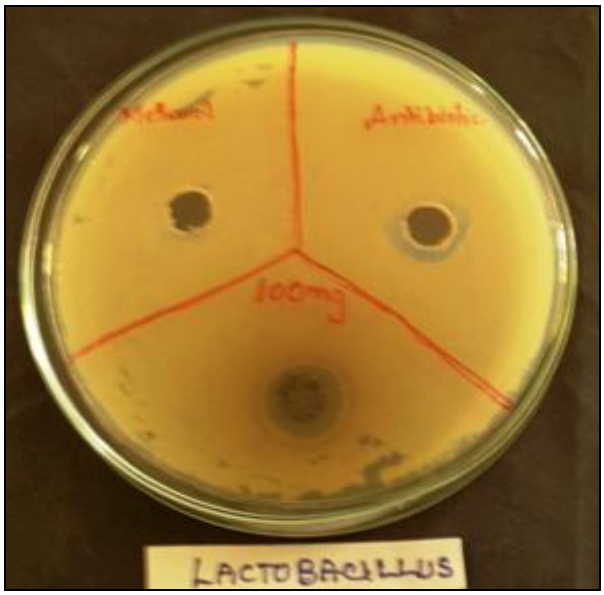

PLATE 1: ANTIMICROBIAL ACTIVITY OF *BASELLA ALBA* FRUIT BETACYANIN AGAINST *LACTOBACILLUS* AT 100mg CONCENTRATION

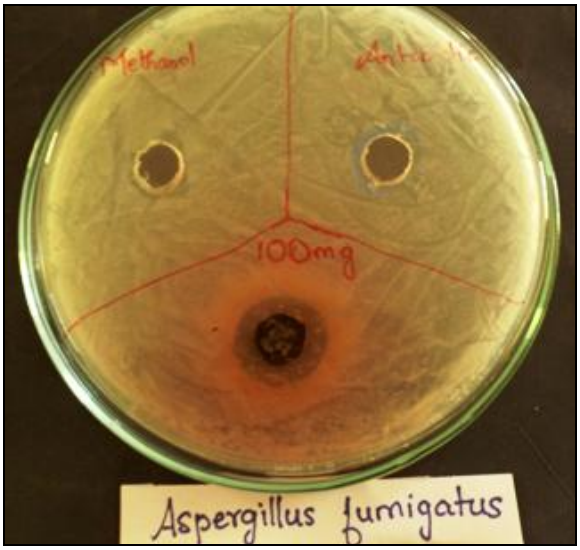

PLATE 2: ANTIMICROBIAL ACTIVITY OF *BASELLA ALBA* FRUIT BETACYANIN AGAINST *ASPERGILLUS FUMIGATUS* AT 100mg CONCENTRATION

The results was observed in betacyanin extracted from *Basella alba* fruit indicating that gram positive strain was more sensitive then gram negative. This observation can be attributed in the difference in the structure of bacterial cell wall. The less complex structure of the cell wall in the gram positive bacteria makes it more permeable to the antimicrobial compounds [16].

TABLE 1: ANTIMICROBIAL ACTIVITY OF *BASELLA ALBA* FRUIT

Extract	Micro organism	Concentration (mg/ml)	Zone of inhibition (mm)	Streptomycin (1mg/ml)	Nystin (1mg/ml)
Basella alba extract	*Bacillus subtilus*	25	0.75 ± 0.070	1.35 ± 0.353	-
		50	1.15 ± 0.070		
		100	1.25 ± 0.070		
	Lactobacillus	25	1.05 ± 0.212	1.5 ± 0.141	-
		50	1.15 ± 0.212		
		100	1.45 ± 0.070		
	Staphylococcus aureus	25	0.55 ± 0.070	1.75 ± 0.212	-
		50	0.9 ± 0.141		
		100	1.1 ± 0.141		
	Escherichia coli	25	0.65 ± 0.070	1.6 ± 0.282	-
		50	0.85 ± 0.070		
		100	1.1 ± 0.141		
	Pseudomonas aeruginosa	25	0.95 ± 0.070	1.7 ± 0.282	-
		50	1.15 ± 0.070		
		100	1.35 ± 0.070		
	Klebsiella pneumonia	25	-	1.3 ± 0.282	-
		50	-		
		100	-		
	Aspergillus niger	25	0.6 ± 0.141	-	1.6 ± 0.282
		50	0.85 ± 0.070		
		100	1.2 ± 0.141		
	Aspergillus fumigates	25	0.9 ± 0.141	-	1.85 ± 0.070
		50	1.2 ± 0.141		
		100	1.7 ± 0.070		

*Mean ± S.D. (n=2)

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration of the extract against bacterial strains was found to be 25mg/ml for S*taphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus, Escherichia coli, Aspergillus niger* and *Aspergillus fumigates* and 50mg/ml for *Klebsiella pneumonia* (**Table 2**) which clearly indicates its strong inhibition potential. It was already reported that the minimum inhibitory concentration of *Basella alba* leaf was found to be 6.25µg/ml against S*taphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa & Bacillus subtilus* and 12.5µg/ml against *Escherichia coli* [14]. Phenolic compounds possess high levels of antimicrobial activity [17], e.g.carvacrol, oxygenated derivatives (thymol methyl ether) and its precursors *p*-cymene and *γ*-terpinene [18]. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes, altering their function and in some instances their structure, causing swelling and increasing their permeability. The increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, decreased ATP levels, and the loss of the proton motive force, which lead to cell death. According to the existing literature, there are several phenolic acids, such as chlorogenic, caffeic, p-caumaric, ferulic p-hydroxy benzoic, vanillic, protocatechuic, syringic [19, 20] well as some other phenolic compound like Quercetin, hydroxyl tyrosol, resveratrol [19, 21, 22] identified to have antimicrobial activities.

TABLE 2: THE MINIMUM INHIBITORY CONCENTRATION OF *BASELLA ALBA* FRUIT ON VARIOUS STRAINS

Microorganism	Serial dilution(mg/ml)					
	100	50	25	12.5	6.25	3.12
Bacillus subtilus	-	-	-	+	+	+
Lactobacillus	-	-	-	+	+	+
Staphylococcus aureus	-	-	-	+	+	+
Escherichia coli	-	-	-	+	+	+
Pseudomonas aeruginosa	-	-	-	+	+	+
Klebsiella pneumonia	-	-	+	+	+	+
Aspergillus niger	-	-	-	+	+	+
Aspergillus fumigates	-	-	-	+	+	+

- No growth; + Growth

CONCLUSION: The present study confirms the potential antimicrobial activity of the extract *Basella alba.* Anyway, 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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