Cytotoxic and Antibacterial Activity of *Basella Alba* Whole Plant: A Relatively Unexplored Plant

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Summary

Aim of the study: To evaluate the Cytotoxic and antibacterial activity of *Basella alba* whole plant extract.
Materials and methods: The phytochemical screening of the prepared plant extract was carried out by chemical, thin-layer chromatography and spectroscopic methods. The Cytotoxic activity was carried out using different dilutions of methanolic extract against Jurkat and lung cancer cell lines. The antibacterial activity was carried out using different dilutions of methanolic extract against gram positive strains (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilus)* and gram negative ones (*Pseudomonas aeruginosa)* by the cup-plate assay method and minimum inhibitory concentrations (MICs).
Results: The TLC fingerprinting reveals the presence of β-Sitosterol & Lupeol in the methanolic extract. The BAE showed significant dose dependant cytotoxicity on Jurkat cell lines as compared with the reference standard. The different concentrations of extract showed moderate activity against *Pseudomonas aeruginosa, Bacillus subtilis* while weak response against S*taphylococcus aureus, Micrococcus luteus &* Escherichia *coli*. The minimum inhibitory concentration of methanolic extract was 6.25µg/ml against S*taphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa & Bacillus subtilus* and 12.5µg/ml against *Escherichia coli*.
Conclusion: The methanolic extract shows the significant growth inhibition on human cancer cell lines & momentous zone of inhibition for microorganisms studied. The overall result of this study indicates that the methanolic extract from *Basella alba* have interesting anticancer & antibacterial properties and the traditional use of this plant may also derive from its antibacterial & anticancer properties.

Keywords: Cytotoxicity; antibacterial activity; *Basella alba;* Cell lines

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]. Naturally occurring substances play an increasing role in drug discovery and development. In fact, the majority of anticancer and antiinfectious agents are of natural origin. In the present study we tried to investigate the anticancer and antibacterial potential of relatively unexplored plant *Basella alba* Linn. Synonym- *Basella rubra* Roxb. commonly known as Indian Spinach belonging to family Basellaceae is a fast growing perennial vine found in the tropics where it is widely used as a leaf vegetable reaching 10 m in length. Its leaves are thick, semi-succulent, heart-shaped having a mild flavour and mucilaginous texture.

In Chinese traditional medicine, the leaves or the aerial parts of *B. rubra* have been used for the treatment of constipation and also as a diuretic, toxicide, and anti-inflammatory [2]. *Basella alba* is an important medicinal plant in ethnoveterinary for treatment of retained afterbirth and anaplasmosis. It is administered in gonorrhea and balanitis. 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 [3]. It is extensively cultivated as an ornamental and the aerial parts such as the leaves, stems, and young shoot with buds are consumed as a vegetable and health food. The roots are used in the treatment of diarrhea, the cooked leaves and stems are used as laxatives [4-5]. The flowers are used as an antidote to poisons and also as diuretic and febrifuge [6]. A paste of the root is applied to swellings and is also used as a rubefacient, leaf juice is used in Nepal to treat catarrh and is applied externally to treat boils [7]. The sap is used to anoint any part of the body affected by acne in order to diminish the irritation. The plant is also used in treatment of aphthae.

Chemical constituents: Plant is reported to have Betacyanins, Carotenoids and Organic acids [8-10]. Triterpene oligoglycosides, Basellasaponins A, B, C, and D [11] having a dioxolanetype substituent, along with Betavulgaroside I [12-16], Spinacoside C [17], Momordins IIb, and IIc, [18] had been isolated from the aerial parts of *B. rubra*. The leaves also contain carotenoids, organic acids and water soluble polysaccharides, bioflavonoids and vitamin K [19]. β-Sitosterol & Lupeol are also reported to be present in the plant and are reported to have anticancer, anti-inflammatory and antioxidant activities [20-30].

Material and Methods

Selection of plant: The plant was selected on the basis of traditional claims for its anti-inflammatory and anti diarrhoeal activity. As inflammation plays a significant role in cancer metastasis, so based on this hypothesis we tried to found out the cytotoxic potential of the plant having anti-inflammatory activity. Chemical constituents (Betacyanins [31], Carotenoids, Sterols & Triterpenes) present in this plant were also reported to have antioxidant, anti-inflammatory & anticancer activities. Since the plant is extensively used traditionally for diarrhoea by locals, this further strengthens our selection for the work on this plant.

Collection of plant material: The *Basella alba* whole plant was collected freshly from Jamnagar (Gujarat) in the month of August-September, 2008 depending upon its easy availability. It was authenticated by our taxonomist against voucher specimen BA-1. The plant was subjected to shed drying and further crushed to powder and then the powder was passed through the mesh 40.

Preparation of extract: The dried and ground plant material (1.0 kg) was first defatted with petroleum ether and then successively extracted with methanol using Soxhlet apparatus for 12 hours and filtered to yield the extract. The extract was then concentrated in rotavapour and finally dried to a constant weight. The extract obtained was stored in a refrigerator at 4^0C until use. The dried extract was used for the evaluation of cytotoxic and antibacterial activity.

Phytochemical screening: The Liebermann-Burchard's and Salkowski reactions were carried out to detect the sterols nucleus. It was further confirmed by TLC fingerprinting and UV-Spectrophotometry. The extract was examined for β-Sitosterol & Lupeol by TLC on silica gel 60 F254 pre-coated TLC glass plates (Merck) developed with Toluene: methanol (9:1). The spraying reagents used were anisaldehyde-H_2SO_4 reagent and heated at 105^0 for 5 min. The Rf values and colour of the resolved bands were noted and matched with the standards. Further HPTLC was performed to estimate the percentage of β–Sitosterol & Lupeol in the methanolic extract by calibration curve using peak height and peak area ratio. In addition, since both, β-Sitosterol & Lupeol possess a characteristic UV absorption (λ max at 206 & 202, 320 nm respectively in Methanol); the UV absorbance of extracts for β-Sitosterol & Lupeol was measured.

Cytotoxicity studies

Cell lines and culture: Jurkat and lung cancer (A549) cell lines were procured from National Centre for Cell Science (NCCS), Pune. Cells were maintained in DMEM (Dulbecco's Modified Eagle Medium, Himedia) and 10% FBS with Penicillin (100 U/ml) and Streptomycin (100 lg/ml) at 37^0C, 5% CO_2.

Preparation of sample: Immediately before assays were performed, the stored extract was dissolved in small amount of dimethylsulfoxide (DMSO, Qualigens) upto 0.5% before diluting with medium.

Cytotoxicity assay (MTT assay): The cytotoxicity of *Basella alba* whole plant extract (BAE) was determined by tetrazolium (MTT, Sigma) assay [32]. Cells (2×10^3/well) were plated in 100 µL of medium/well in 96- well plates. After overnight incubation, BAE extract was dissolved in DMSO and the concentrations were adjusted to 25µg/ml, 50µg/ml, 75µg/ml & 100µg/ml and were added to each well. Paclitaxel was used as positive control in concentration (5µM). After treatment for 48 hours 20 µl of 5 mg/ml MTT (pH 4.7) was added to each well and cultivated for another 4 hours. The supernatant was removed and 100 µl DMSO was added per well. Samples were then shaken for 15 min. The absorbance at 570 nm was measured with microplate reader (Bio-Rad). All experiments were performed in triplicate and all tests were performed 5 times individually. The effect of BAE on the proliferation of cancer cells was expressed as relatively cell viability, using the following formula: Percent viability = OD of drug treated sample/OD of none treated sample) × 100 [33].

Antibacterial studies

Microbial strains & culture media: Four strains of bacteria were procured from the Microbial Type Culture Collection (MTCC, IMTECH), Institute of Microbial Technology, Chandigarh, and were tested: *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC 106), *Escherichia* coli (MTCC 443). All the strains were stored at freeze temperature until use. Nutrient agar (NA, Himedia) containing bromocresol purple was used for the activation of *Bacillus* species, while NA was used for other bacteria. The NA was used in sensitivity assay. Nutrient broth was used for MIC determination.

Chemicals for antimicrobial assay: Ciprofloxacin (Central Drug House (P). LTD., New Delhi) was used as positive reference standard for all bacterial strains. The DMSO was used as solvent for the tested samples.

Preparation of inoculums: Bacterial inoculums were prepared by growing freeze-dried cells in Nutrient Broth for 24 hours at 37^0C. Slants were prepared by streaking of these cell suspensions and sub culturing was done by using the same broth to provide initial cell counts of about 104 CFU/ml and then sub culturing was done, incubated at 37^0C for required time.

Preparation of test sample: The methanolic extract was dissolved in 10% aq. DMSO to obtain the different concentrations (10 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml). 10% aq. DMSO was used as negative control (solvent control). Ciprofloxacin was used as positive reference standard having a concentration of 5µg/ml for all bacterial strains.

***Antibacterial assay*:** Antibacterial activity was determined by cup plate method. Petri plates were prepared by pouring 30 ml of Nutrient Agar Medium for all the bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spreaded and allowed to dry for 10 min. Three wells or cavities were made in agar containing each petri dish by a sterilized steel borer. To these cavities standard and test solutions were filled. All the work was carried out under aseptic conditions for microbial assay. The plates for the bacteria were incubated at 37^0C ± 1^0C for 24 hours. The antibacterial potential of test solution was determined on the basis of mean diameter of zone of inhibition around the wells in millimeters. Each assay was carried out in the form triplicate three times.

Minimum Inhibitory Concentration **(MIC):** 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 to concentration of 50µg/ml. Further dilutions of this solution were made by inoculating 1ml from first tube into second assay tube serially and 0.1 ml 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 37^0C ± 1^0C at 24 hours for bacterial 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).

Results and Discussion

TLC fingerprinting profile: The colorimetric reactions and UV absorption spectrum confirmed the presence of β- Sitosterol & Lupeol in the extract. In the TLC plates one blue (visible) and one purple (UV 525 nm) spots were present at Rf value of 0.37 & 0.60 after spraying with anisaldehyde- H_2SO_4 reagent, respectively. The colours and Rf values above mentioned were the same as those obtained for standard reference compounds under the same conditions. Though a TLC densitometric method was reported for the quantification of lupeol, we modified the method so that both the compounds (β-Sitosterol & Lupeol) could be quantified simultaneously. The contents estimated by HPTLC were found to be 0.07 & 0.05% w/w respectively.

Cytotoxicity assay: The results of preliminary screening showed that the BAE exhibited cytotoxic activity against both cancer cell lines but the activity was very much significant against Jurkat cell lines. The data also showed that the BAE exhibited cytotoxicity comparable with positive control Paclitaxel on Jurkat cell lines. The percentage of growth inhibition of BAE at various concentrations on human cancer cells were determined as the relatively cell viability of viable treated cells in comparison with viable treated cells of positive control. It showed a dose dependent inhibitory effect on cell growth (Fig. 1 & Table 1).

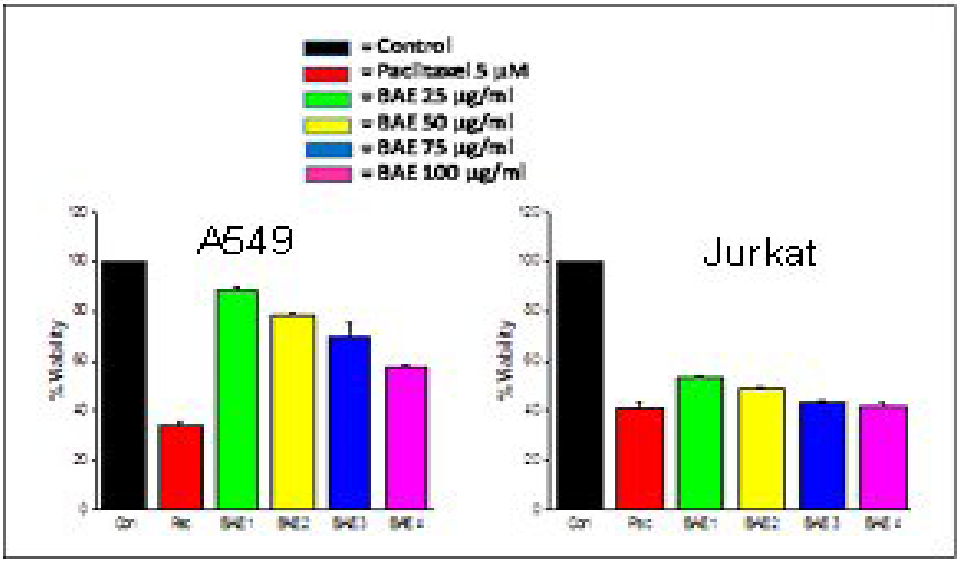

Fig. 1: % Growth inhibition of BAE on Jurkat & lung cancer cell lines

Table 1: Effect of different doses of BAE on cell viability of human cancer cell lines

Sample	Concentration	% Cell Viability*	
		A549	Jurkat
Paclitaxel	(5 µM)	34.09 ± 0.93	40.72 ± 2.34
Basella alba whole plant Extract (different conc.)	(25 µg/mL)	88.33 ± 1.20	53.32 ± 0.70
	(50 µg/mL)	78.35 ± 0.99	48.70 ± 0.79
	(75 µg/mL)	69.73 ± 5.51	43.28 ± 0.72
	(100 µg/mL)	57.12 ± 1.11	41.78 ± 1.25

*Mean ± S.D. (n=3)

Antibacterial assay: Methanolic extract was screened for antibacterial activity. The different concentrations of extract showed moderate activity [34] against *Pseudomonas aeruginosa, Bacillus subtilis* while weak response [34] against S*taphylococcus aureus, Micrococcus luteus &* Escherichia *coli* [Table 2]. The results of different concentrations of extract were correlated with standard drug and activity was found to be dose dependant against all bacteria. The minimum inhibitory concentration of methanolic extract against bacterial strains was found to be 6.25µg/ml for S*taphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa and Bacillus subtilus* and 12.5µg/ml for *Escherichia coli* [Table 3] which clearly indicates its strong inhibition potential [35].

In summary, the present results provide evidence that the extracts of *Basella alba* contains substances with cytotoxic & antibacterial activity, and, therefore, suggest that the traditional use of this plant for the treatment of diarrhoea and anti-inflammatory properties can be linked to cytotoxic & antibacterial properties. 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 research.

Table 2: Antibacterial activity of BAE on various strains

Extracts	**Conc. (mg/ml)**	**Cup-plate method (inhibition zone, mm)**				
		S. A.	**M. L.**	**B. S.**	**P. A.**	**E. Coli**
Basella alba extract	10	-	-	3.32 ± 0.28	5.3 ± 0.058	-
	25	5.0 ± 0.1	3.3 ± 0.058	6.02 ± 0.036	10.7 ± 0.12	4.0 ± 0.1
	50	10.3 ± 0.058	7.7 ± 0.12	13.67 ± 0.058	15.1 ± 0.1	7.7 ± 0.058
	100	16.3 ± 0.058	10.7 ± 0.12	21.02 ± 0.1	22.2 ± 0.1	15.3 ± 0.058
Ciprofloxacin	5 µg/ml	26 ± 0.051	14 ± 0.068	32 ± 0.024	25 ± 0.035	22 ± 0.056

*Mean ± S.D. (n=3)
S. A. -Staphylococcus aureus, M. L. - Micrococcus luteus, B. S. - Bacillus subtilus,
P. A. - Pseudomonas aeruginosa, E. coli - *Escherichia coli*;
- Sign shows no zone of inhibition

Table 3: The MIC values of BAE on various bacterial strains

Microorganisms	**Serial dilution (µg/ml)**					
	50	25	12.5	6.25	3.12	1.56
Staphylococcus aureus	-	-	-	-	+	+
Micrococcus luteus	-	-	-	-	+	+
Pseudomonas aeruginosa	-	-	-	-	+	+
Bacillus subtilus	-	-	-	-	+	+
Escherichia coli	-	-	-	+	+	+

- No growth; + Growth; Stock solution = 100 µg/ml

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