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An Overview on Phytochemical, Anti-Inflammatory and Anti-Bacterial Activity of *Basella alba* Leaves Extract

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Abastract: Inflammation is the complex biological response of vascular tissue to harmful stimuli such as pathogens, damaged cells, or irritants. In the present investigation an attempt was made to screen the anti-inflammatory activity of *Basellaalba* leaf. From this study, it can be concluded that, *Basellaalba* possesses a good anti-inflammatory activity. In the cotton pellet induced inflammation and In the carrageenan induced inflammation the animals treated with the plant extract have been shown a significant activity at 500 mg/kg dose (p<0.001) which was comparable with the standard drug. In view of the percentage inhibition also the plant was studied, in which the plant extract was found effective. 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). The different concentrations of extract showed moderate activity against *Pseudomonas aeruginosa, Bacillus subtilis*while weak response against *Staphylococcus aureus, Micrococcus luteus* and *Escherichiacoli.* The minimum inhibitory concentration of methanolic extract was 6.25µg/ml against *Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa* and *Bacillussubtilus* and 12.5 µg/ml against *Escherichia coli*. The overall result of this study indicates that the methanolic extract of *Basellaalba* have interesting anti-inflammatory and antibacterial properties.

Key words: Antibacterial Activity · Inflammation · Basellaalba · Cotton Pellet Granuloma · Carrageenan · Phenyl Butazone

INTRODUCTION

Medicinal plant is defined as any substance with one or more of its organ containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs [1]. Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc. *Basellaalba L., (Basellaceae)* commonly has known as "Poi (Hindi), Potaki (Sanskrit) and poi shak (Bengali) [1]. *Basellaalba*is a wildly cultivated, cool season vegetable with climbing growth habit. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Stems are purplish or green. Leaves are fleshy, ovate or heart-shaped, 5 to 12 cm long, stalked, tapering to a pointed tip with a cordate base. Spikes are axillary, solitary, 5-29 cm long. Fruit is fleshy, stalk less, ovoid or spherical, 5-6 mm long and purple when mature. Mainly leaves and stems are used for the medicinal purpose [2]. Taxonomy of the plant is.

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Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Caryophyllales

Family: Basellaceae

Genus: *Basella*

Species: *Alba*

The present study is focused towards compiling the ethanobotanical and scientific importance of above mentioned plant. India, due to its geographical and environmental positioning has traditionally been a good source for such products among the Asian countries. In Ayurveda, it is used for hemorrhages, skin diseases, sexual weakness and ulcers and as laxative in childrenand pregnant women. The plant is febrifuge, its juice is a safe aperient for pregnant women and a decoction has been used to alleviate labour. It is also an astringent and the cooked roots are used in the treatment of diarrhea. The leaf juice is a demulcent, used in cases of dysentery [3]. This plant serves as a Thai traditional vegetable. The fruit provides dark violet color for food colorant. *Basella*mucilage has been used in Thai traditional medicine as topical application for irritant, bruise, ringworm and laboring. Stem and leaves are used as mild laxative, diuretic and antipyretic [4]. In India, it has been used for antipruritic and burn [5] and has been used in Bangladesh for acne and freckle treatment [6]. The tracking of phyto constituents is important step which lead to isolation of biologically active compounds [7]. The Ayurveda treatment in India has been used *B. alba*leaves and stem for anticancer such as melanoma, leukemia and oral cancer [8]. Root and leaves has been used for the removal of after birth, stomach pains and increase milk production [9]. *Basellaalba* is administered orally for the treatment of anal prolapsed or hernia. Ground leaves of *Basellaalba*are rubbed on the human hand to introduce the whole preparation into the animal vagina every morning for the treatment of sterility [10] The leaf juice is used in Nepal to treat dysentery, catarrh and applied externally to treat boils. The mucilaginous qualities of the plant make it an excellent thickening agent in soups, stews, etc [11]. The purplish sap from fruits is used as a coloring agent in pasteries and sweets [12] *Basellaalba*has been used for the treatment of Anemia in women, coughs, cold (leaf with stem), cold related infections [13]. Maceration is taken orally for infertility, pelvic inflammatory disease, orchitis, epididymytis, threatened abortion, spurious labour [14]. Leaves are used in constipation, poultice for sores, urticaria and gonorrhea. It is also used in poultice local swellings, intestinal complaints etc [15]. The mucilaginous liquid obtained from the leaves and tender stalks of plants is popular remedyfor headaches [16]. In the present investigation an attempt was made to screen the anti-inflammatory activity of *Basella alba* leaf in experimentally induced inflammations in rats [17, 18].

MATERIALS AND METHODS

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 [1, 2, 6]. The extract was then concentrated in Rotavapour and finally dried to a constant weight. The extract obtained was stored in a refrigerator at 4°C until use. The dried extract was used for the evaluation of cytotoxic and antibacterial activity [5, 9, 12, 14].

Phytochemical Screening: The phytochemical screening of the prepared plant extract was carried out by chemical, thin-layer chromatography and spectroscopic methods. The Phytochemical examination of the both extracts was performed by the standard methods [19] and shows the presence of various phytochemical constituents tabulated in Table-2.

Phytochemical Group Analysis: The testing of different chemical groups present in extract represents the preliminary phytochemical studies. The chemical group test which are perform as follows. In each test 10% (w/v) solution of extract in methanol was taken unless otherwise mentioned in individual test. The following tests were performed of identifying different chemical groups.

Tests for Alkaloids

Mayer's Test: 2ml solution of the extract and 0.2 ml of dilute di-hydrochloric acid were taken in a test tube. Then 1ml of Mayer's reagent was dissolved. Yellow color precipitate was formed and that was indicated as the presence of alkaloids [20].

Table 1: Anti-inflammatory activity of leaves extracts of *Basellaalba L.*

S.NO	Groups	Concentration(µg/ml)	(% protection Mean ± S.E.M [N=6]
1.	Control	-	-
2.	M.E.B.A	200	57.10 ±2.18**
		400	68.38±1.18**
3.	A.E.B.A	200	58.46 ±1.61**
		400	71.89 ±1.22
4.	Standard	50	70.17 ±0.22***
		100	83.54 ±0.61***

The results were expressed as mean□ ± S.E.M□□ [n=6].

Dragendroff's Test: 2ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1ml of Dragendroff; s reagent was added. Orange brown precipitate was formed and that was indicated as the presence of alkaloids [21].

Tests for Tannins

Ferric Chloride Test: 5ml solution of the extract was taken in a test tube. Then 1ml of 5% ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins [22].

Test for Flavonoids: Added a few drops of concentrated hydrochloride acid to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoids [23].

Tests for Saponins: 1ml solution of the extract was dilute with water to 20ml and shaken in a graduated cylinder for 15minutes. No one-centimeter layer of foamindicates the absence of saponins. Following reagents are used for different chemical group test [24].

Test for Mucilage: 0.5gm of mucilage was hydrolyzed with 50ml of 0.1N sulphuric acid. The solution was neutralized using barium carbonate and filtered. The filterate was concentrated and subjected to thin layer chromatography on silica gel G plate (Merck). Mobile phase were used n-butanol, ethanol and water (10:2:2) resp. The spots were visualized with aniline phthalate reagent as dark brown spot [25].

***In-vitro* Anti-Inflammatory Activity**

The Human Red Blood Cell (HRBC) Membrane Stabilization Method: The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alseversolution (2%dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (200 and 400 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac (100 and 200 µg/ml was used as reference standard and a control was prepared by omitting the extracts [26].

Antibacterial Studies: 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 [14, 18].

Microbial Strains and Culture Media

Microbial Strains and 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), *Bacillussubtilis*(MTCC 441), *Micrococcus luteus* (MTCC 106), *Escherichia coli* (MTCC 443) [20]. 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 Agar well diffusion method was used in sensitivity assay. Nutrient broth was used for MIC determination. The culture plates were incubated at 37°C for 24hr [27, 28] h and the zones of inhibition measured in diameter (mm).

Table 2: Preliminary Phytochemical screening of M.E. and A.E. of leaves of *Basellaalba*(L.)

S. No	Nameof the test constituents	Reagent/Methods adopted	Methanolic extracts of Basella alba	Aqueous extracts of Basella alba
1	Alkaloids	Picric acid	+	_
		Dragendroffs	+	_
		reagents	+	_
		Mayer's reagents	+	+
2	Carbohydrates	Molish test	+	
		Fehling's test	+	
		Benedicts test	+	
3	Tannins	Ferric chloride test	_	+
4	Flavonoids	Shinoda test	+	+
5	Mucilage	Ruthenium red	_	+
6	Saponin	Heamolysis test	+	+

"+"=Presence, "_"=Absence

Table 3: Antibacterial activity of BAE on various strains

		Cup Plate method (Inhibition Zone, mm)				
Extracts	Conc. (Mg/ml)	S.A	M.L	B.S	P.A	*E.coli*
Basella albaExtract	10	_	_	3.32±0.28	5.3±0.058	_
	25					
	50	5.0±0.1	3.3±0.058	6.02±0.036	10.7±0.12	4.0±0.1
	100	10.3±0.058	7.7±0.12	13.67±0.058	15.1±0.1	7.7±0.058
		16.3±0.058	10.7±0.12	21.02±0.1	22.2±0.1	15.3±0.058
Ciprofloxacin	5µg/ml	26±0.051	14±0.068	32±0.024	25±0.035	22±0.056

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

RESULTS AND DISCUSSION

The present results provide evidence that the extracts of *Basella alba* contains substances with anti-inflammatory and 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 and antibacterial properties. The results are reported in Table 1. The petroleum ether, chloroform, ethylacetate, methanol and aqueous extracts of the leaves of *Basellaalba L.*were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. The *in vitro* anti-inflammatory activity of the extracts were concentration dependent, with the increasing concentration, the activity is also increased. Among both the extracts, aqueous extract at a concentration of 400 µg/ml showed 71.89% protection of HRBC in hypotonic solution. All the results were compared with standard Diclofenacwhich showed 83.54 % protection. Aqueous extract showed significant *in-vitro* anti-inflammatory activity as compared to standard. The aqueous extract showed significant anti-inflammatory activity (71.89 %) at the dose of 400 µg/ml. On the basis of the above results it can be concluded that the *Basellaalba* L. have an anti-inflammatory activity. An anti-inflammatory activity of leaves extracts of *Basellaalba L*is given bellow in Table 1.

Anyway, further studies are necessary to isolate andcharacterize the active constituents of the plant to evaluate their modes of action and render this species interesting for future research.In the present work a humble attempt was made to detect, by using various standard qualitative chemical tests, the presence of reported compounds and to look for possible presence of other chemical constituents in the root and stem bark of the *Basellaalba* (L.). The phytochemical screening of the extracts showed the presence of alkaloids, flavonoids, Carbohydrate, Saponin in Methanolic extracts and and tannin, mucilage, saponnin and Carbohydrate are present in aqueous extracts. The phytochemical studied results are reported in Table 2.

Methanolic extract was screened for antibacterial activity. The different concentrations of extract showed moderate activity against *Pseudomonasaeruginosa, Bacillus subtilis*while weak response against *Staphylococcus aureus, Micrococcusluteus and Escherichia coli* [Table 3]. The results of different concentrations of extract were correlated with standard drug and activity was found to be dose dependent against all bacteria.

Table 4: The MIC values of BAE on various bacterial strains

	Serial dilution (µg/ml)					
Microorganisms	50	25	12.5	6.5	3.12	1.56
Staphylococcus aureus	_	_	_	_	+	+
Micrococcus luteus	_	_	_	_	+	+
Pseudomonas aeruginosa	_	_	_	_	+	+
Bacillus subtilus	_	_	_	_	+	+
Escherichia coli	_	_	_	+	+	+

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

The minimum inhibitory concentration of methanolic extract against bacterialstrains was found to be 6.25 µg/ml for *Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa and Bacillus subtilus*and 12.5 µg/ml for *Escherichia coli* (Table 4) which clearly indicates its strong inhibition potential.

CONCLUTION

From the above obtained results, it can be concluded that the extract of *Basellaalba*shows a significant anti-inflammatory activity which was demonstrated in above methods which it can be under stood that *Basella alba* showing a dose depending activity. It can be stated that, *Basellaalba*will promise a significant and effective an anti-inflammatory and antibacterial agent in future.

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