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EVALUATION OF ANALGESIC ACTIVITY OF LEAF PARTS OF *BASELLA ALBA LINN*

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ABSTRACT

Methanol was prepared from dried leaf parts of *Basella alba* by cold maceration method. Anxiolytic activity of the extract of *B. alba* was evaluated by tramedol HCl induced by tail flick and hot plate method test in mice. Methanol extract (100 and 200 mg/kg, oral rout) showed highly significant ($p<0.001$) analgesic activity. All the results were compared with reference drug tramadol HCl. The presence of various phytoconstituents in the extracts was identified by preliminary phytochemical studies. The results of this study suggested that *B. alba* methanol extract possesses analgesic activity.

Keywords: *Basella alba*, analgesic activity, tail flick method, hot plate method, tramadol HCl, etc.

1. INTRODUCTION

Basella alba (Family: Basellaceae), is commonly known as Indian spinach. The paste of root of red *B. alba* along with rice washed water is taken in the morning in empty stomach for one month to cure irregular periods by the rural people of Orissa, India **[1]**. Leaves of *B. alba* is used for the treatment of hypertension by Nigerians in Lagos **[2]**, and malaria in cameroonian folk medicine **[3]**. A literature survey revealed that the plant has

reported its antifungal **[4]**, anticonvulsant, analgesic, anti-inflammatory **[5]** and androgenic **[6]** activities and for the treatment of anaemia **[7]**. Also the leaves of *B. alba* is traditionally used in ayurveda system of medicine for to bring sound refreshing sleep when it is applied on head about half an hour before bathing **[8]**. Hence, the present investigation was aimed to scientifically evaluate anxiolytic and analgesic activity of the leaf parts of *B. alba*.

2. Plant Material: The leaf parts of *B. alba Linn*. were collected from Azamgarh, Jaunpur,varanasi and Allahabad. It was authenticated by Prof. N.k Dubey FNASc, FNAAS centre of advanced study in Botany Institute of Science, Banaras Hindu University Varanasi. A voucher specimen was deposited in the departmental herbarium.

2.1 Preparation of Extract: The collected leaf parts of *B. alba* were shade-dried at room temperature. The dried materials were size reduced to coarse powder and macerated with methanol and distilled water separately for seven days. Methanol extract (MEBA) of *B. alba* was collected separately, filtered and concentrated under vacuum using rotary vacuum evaporator **[9]**. The colour, consistency and the % yield of the extracts was noted in **Table 1**. All the extracts was kept in desiccators until further use.

2.2 Preliminary Phytochemical Analysis: Preliminary phytochemical investigation of the extracts for identification of phytoconstituents such as alkaloids, carbohydrates, and flavanoids, proteins, was carried out **[10]**.

3. Pharmacognostical Evaluation

3.1 Macroscopic Examination

Colour

Untreated samples were examined under diffuse day light. An artificial light source with wavelength similar to those of day light may also be used. The colour of sample was observed. Surface Characteristic, Texture and Fracture Characteristics Materials was touched to determine if it is soft or hard bend and ruptured it to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough, granular etc.

Odour

A small portion of the sample was placed in the palm of the hand and slowly and repeatedly, the air was inhaled over the material. Taste A small amount of drug powder was kept over the tongue and the taste was observed.

3.2 Physicochemical Evaluation

Loss on Drying

For estimation of loss on drying, it was dried at 105°C for 5 hours in a hot air oven, cooled

in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material.

Determination of Foreign Matter

Determination of Foreign Matter about 10 gm of sample has been weighed and spread on a white tile uniformly, without overlapping. Then the sample was inspected by means of 5X lens and the foreign organic matter has been separated. After complete separation, the matter was weighed and percentage w/w was determined.

3.2.1 Determination of Solvent Extractive Value

Determination of Water-Soluble Extractive Value

Five gm of powdered drug was macerated with 100ml of water closed flask for 2hr and was occasionally shakes with 6hr time period and was allowed to stand for 18hr. After filtration the 25ml of the filtrate evaporated to dryness in a tarred flat-bottomed shallow dish. Dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Determination of Ethanol-Soluble Extractive Value Ethanol is an ideal solvent for extraction of various chemicals like tannins, alkaloids, resins etc. Ethanol (95% V/V) was used for determination of ethanol soluble extractive. Five gm of powdered drug was macerated with 100ml of ethanol closed flask for 24 hours and was occasionally shakes with 6 hours' time period and was allowed to stand for 18 hours. After filtration the 25 ml of the filtrate evaporated to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

Determination of Moisture Content

The percentage of active constituents in crude drug is mentioned on air dried bases. Hence, the moisture content of the crude drugs should be determined and should also be controlled. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

Procedure: The powdered sample of leaves of S. asper weighed 5gm accurately and kept in IR moisture balance. The loss in weight was recorded as percentage (%) moisture with respect to air-dried sample of crude drug

Determination of Ash Value

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drugs or adhering to it or deliberately added to it as a form of adulteration. Many a time the crude drugs are admixed with various mineral

substances like sand, soil, calcium oxalate, chalk powder or other drugs sample of crude drugs with different inorganic content. Ash value is a creation to judge the purity of crude drugs. Generally, either ash value or acid-insoluble ash value or both is determined. Total ash usually consists of phosphates, silicates and silica. On the other hand, acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid, contains adhering dirt and sand .

Determination of Total Ash Total ash was determined by weighing 2 gm of the air-dried crude drug in the tarred platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then was cooled and weighed.

Determination of Acid Insoluble

Ash The ash obtained from the previous process was boiled with 25ml of 2M HCI for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of Foreign Matter

Weigh 100-500 gm of the drug sample to be examined or the maximum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be totally free from insect, moulds, harmful and poisonous matters and detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and calculate the amount and percentage present.

2.3 Experimental Animals: Swiss albino mice of either sex (20 - 30g) were used for this investigation and kept at the Laboratory Animal House. They were kept in well ventilated room for 1 week before and during the experiments. Animals were provided with commercial rodent pellet diet and water ad libitum. All the experiments were performed according to current guidelines for the care of the laboratory animals and the ethical guidelines. The standard orogastric cannula was used for oral drug administration.

2.4 Determination of Acute toxicity studies (LD 50) [10]

It is further planned to study the acute toxicity of solvent extract of *Basella alba* leaves. In albino mice of either sex (20-30gm).Fixed dose method (OECD guideline number 420) of CPCSEA will be adopted for toxicity studies to obtained dose range of extracts of *Basella alba* leaves.

2.5 Experimental design:

For all experiments, the animals are randomly divided into nine groups of (n = 2) animals each. Group I: Control

Group II: Treated With tramadol HCl

Group III: Treated With *basella alba* extract

2.6 Analgesic Activity

Tail Immersion Model

Albino mice (170–210 g body weight) are used. They are placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the mice reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after either oral or subcutaneous administration of the test substance, e.g., after 0.5, 1, 2, 3, 4 and 6 h. The cut off time of the immersion is 15 s. The withdrawal time of untreated animals is between 1 and 5.5 s. A withdrawal time of more than 6 s, therefore, is regarded as a positive response.

Figure 1: Tail Immersion Model

Both chloroform and methanolic extracts of *Basella alba* leaves was tested for antinociceptive activity using tail immersion model followed by Vogel HG (2002) with some modification. The animals are kept into separate means one animal in one restraining cages for leaving the tail hanging out freely and adopt for 30 min before testing. They are randomly selected and divided into seven groups for methanolic extract and eight groups in case of methanolic extract six group in each. The I group served as control and received vehicle (distilled water 5ml/kg). Group II used to serve as reference standard and received tramadol HCl (10mg/kg) and III group received standard drug mix with methanol in case of methanolic extract study. The next five groups III, IV, V, VI and VII received methanolic plant extract respectively at the five different doses 50, 100, 150, 200 and 250 mg/kg p.o. respectively. For the experiment, the rat was the lower 5 cm portion of all groups animals was marked and immersed in cup of freshly filled water. The temperature of water that are used was maintained exactly at 55 ºC. The reaction time was noted by withdrawing the tail by the animal. The tail was carefully dried after each determination. The result were evaluated by calculating the mean reaction time of per group and antinociceptive activity was exhibited by withdrawing of the tail by animal **[11-14]**.

Hot plate method

Groups of 10 mice of either sex with an initial weight of 18 to 22 g are used for each dose.The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56° C . This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. The latency is recorded before and after 20,60 and 90min following oral or subcutaneous administration of the standard or the test compound.

3. RESULTS AND DISCUSSION

The results of the present work showed that there was significant CNS depressant activity in mice pre-treated with MEBA as compared to control. MEBA exhibited a dose-dependent reduction of the onset time A dose-dependent reduction was observed up to 60min in MEBA treated mice and no movement was observed at 120min respectively. The maximum activity was observed at 120min in MEBA treated animals.

Table 1: Physiochemical parameters of *Basella alba*

S.No.	Parameters	Results
1.	Foreign matter	None
2.	Total ash (mg/g)	21.33
3.	Acid insoluble ash (mg/g)	12.58
4.	Water insoluble ash (mg/g)	18.13
5.	Loss on drying (mg/g)	9.23
6.	Swelling index	3.8 ml
7.	Foaming index	>1000

Table 2: Analgesic effect of MEBA by Tail Immersion method (mean ± SEM)

Group	Dose (mg/kg)	Reaction time (in second)									
		Pre Treatment	I 0.30hr	II 1.0hr	III 1.30hr	IV 2.0hr	V 2.30hr	VI 3.0hr	VII 3.30hr	VIII 4.0hr	IX 24.0hr
I	50	2.01±0.01**	5.57±0.53*	3.02±0.01**	3.56±0.47*	2.03±1.01*	5.50±1.50*	2.02±0.01**	1.06±0.01**	1.04±0.01*	2.01±0.01**
II	100	1.59±0.50*	6.07±0.99*	4.03±0.01**	3.02±1.0*	2.52±0.50*	3.07±0.03*	2.05±0.03**	2.02±0.15**	1.56±0.48*	2.04±0.04**
III	150	3.52±0.50*	6.55±1.47*	5.55±0.47*	5.04±0.01*	4.53±1.49*	2.55±0.49*	2.03±0.01**	2.52±0.52*	2.52±0.52*	4.01±1.0*
IV	200	5.02± 1.0*	5.05±0.03*	5.59±0.49*	5.52±0.50*	3.52±0.48*	2.55±0.49*	3.02±0.01*	2.52±0.52*	3.01±0.10*	5.0±1.0*
V	250	3.51±1.51*	4.03±1.03*	4.57±0.49*	5.02±0.99*	3.58±0.50*	3.57±0.49*	2.55±0.47*	2.01±0.01**	1.56±0.47*	3.52±1.52*
Std.	10	2.53± 0.48	5.02±1.01	6.52±0.49	5.51±1.50	5.15±0.96	5.51±0.50	4.55±0.54	3.54±0.54	3.51±0.49	3.01±0.01
Cont.	10	4.53± 0.53	4.06±1.97	4.54±0.48	4.50±1.50	4.52±1.50	5.56±1.47	5.52±0.50	4.02±0.01	4.00±1.00	4.54±0.52

All values are expressed in mean ± standard error mean (n=7).
All data were found to be significant at 5% level of significance and non-significance where **p<0.05 and *p>0.05 respectively

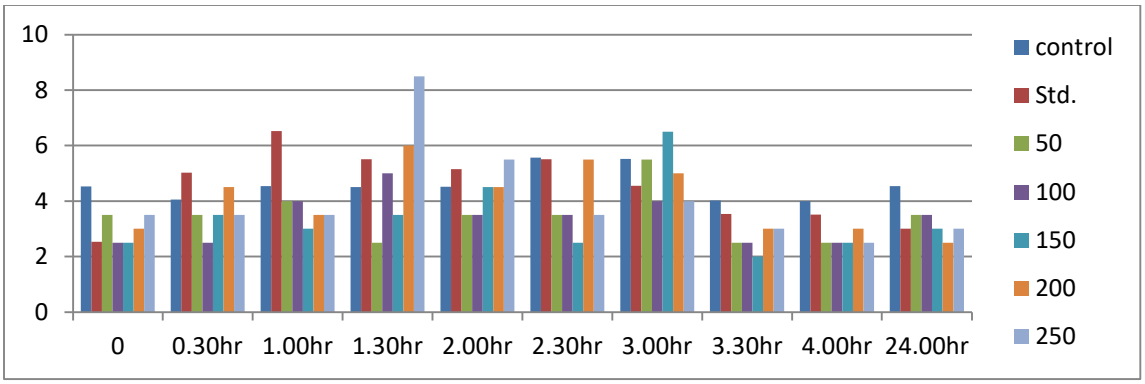

Figure 2: Effect of MEBA in Immobility time

Table 3: Analgesic effect of MEBA by Hot plate method (mean ± SEM)

	Dose (mg/ kg)	Reaction time (in second)									
		Pre Treatment	I 0.30hr	II 1.0hr	III 1.30hr	IV 2.0hr	V 2.30hr	VI 3.0hr	VII 3.30hr	VIII 4.0hr	IX 24.0hr
	50	3.50± 0.50*	3.50± 0.50*	4.00± 1.00*	2.50± 0.50*	3.50± 0.50*	3.50± 0.50*	5.50± 1.50*	2.50± 0.50*	2.50± 0.50*	3.50± 0.50*
II	100	2.50± 0.50*	2.50± 0.50*	4.00± 1.00*	5.00± 2.00*	3.50± 0.50*	3.50± 0.50*	4.00± 1.00*	2.50± 0.50*	2.50± 0.50*	3.50± 0.50*
III	150	2.50± 0.50*	3.50± 0.50*	3.00± 0.00**	3.50± 0.50*	4.50± 0.50*	2.50± 0.50*	6.50± 3.50*	2.00±0 .0**	2.50± 0.50*	3.00± 0.00*
IV	200	3.00± 0.40*	4.50± 0.50*	3.50± 0.50**	6.00± 2.00*	4.50± 2.50*	5.50± 1.50*	5.00± 3.00*	3.00± 1.00*	3.00± 0.00*	2.50± 0.50*
V	250	3.50± 0.50*	3.50± 0.50*	3.50± 0.50**	8.50± 0.50*	5.50± 0.50*	3.50± 0.50*	4.00± 0.00*	Grou p	2.50± 0.50*	3.00± 1.00*
Std.	10	2.53± 0.48	5.02± 1.01	6.52± 0.49	5.51± 1.50	5.15± 0.96	5.51± 0.50	4.55± 0.54	3.54± 0.54	3.51± 0.49	3.01± 0.01
Cont .	10	4.53± 0.53	4.06± 1.97	4.54± 0.48	4.50± 1.50	4.52± 1.50	5.56± 1.47	5.52± 0.50	I	4.00± 1.00	4.54± 0.52

All values are expressed in mean ± standard error mean (n=7)
All data were found to be significant at 5% level of significance and non-significance where **p<0.05 and *p>0.05 respectively

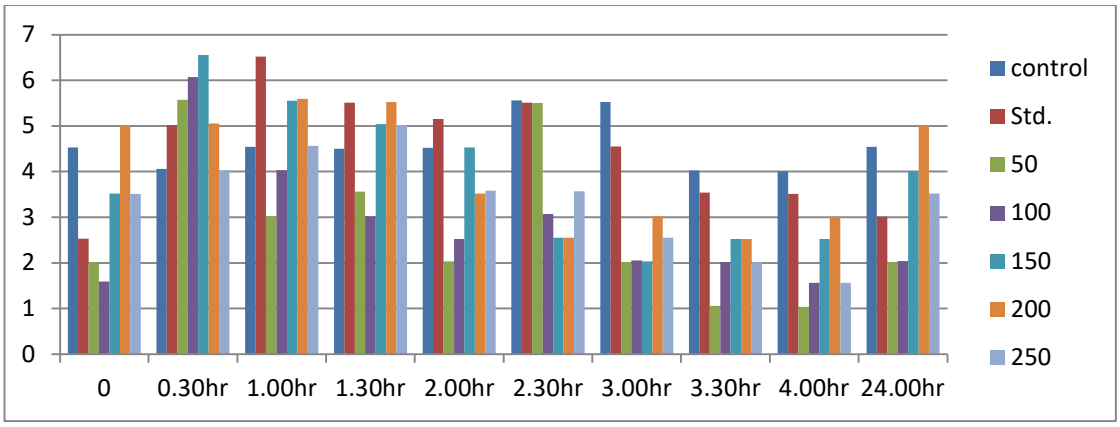

Figure 3: Effect of MEBA in Immobility time

Flavonoids have shown analgesic activity in various studies. Further, the analgesic effect of flavonoids has been attributed to its effect on central nervous system and benzodiazepine

receptors. Therefore, flavonoids of extracts of *B. alba* may be responsible for the analgesic activity.

4. CONCLUSION

These findings establish the potential of the selected plant as CNS activity and scientifically proved its traditional claim. Hence, the present study concludes that the selected plant directs the importance of further development of some potential analgesic drugs as well as their mechanism of action.

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