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Original Article

PHYTOCHEMICAL SCREENING AND ANALGESIC EFFECTS OF ETHANOLIC EXTRACT OF PLANT *MURDANIA NUDIFLORA (L) BRENAN* (COMMELINACEAE) IN ALBINO MICE USING HOT PLATE METHOD

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ABSTRACT

Objective: *Murdania nudiflora (L) Brenan* (Commelinaceae) has long been used in folk medicine in treatment of many diseases. In this study, attempts have been made for pharmacological screening of the plant *Murdania nudiflora (L) Brenan* (Commelinaceae) for analgesic activity and presence of different phytochemicals.

Methods: To this end, ethanolic extract of *Murdania nudiflora (L) Brenan* (Commelinaceae) was evaluated for analgesic properties using plate reaction time in mice and phytochemical screening of the plant was done by different methods.

Results: The analgesic study showed that the ethanolic extract of the leaves have significant analgesic effects ($P < 0.05$; $P < 0.001$) as compared to morphine sulphate (10 mg/kg) used as a standard drug. The result of the preliminary phytochemical studies revealed the presence of tannins, flavonoids, saponins, alkaloids as a whole and which are reported to be responsible for the analgesic and anti-inflammatory activities in many medicinal plants of this family.

Conclusion: From these studies, it may be concluded that ethanol extracts of *Murdania nudiflora* (L) Brenan may contain novel bioactive principles with analgesic activity. Further study is required for evaluation of active principle(s) in different animal models.

Keywords: *Murdania nudiflora (L) Brenan* (Commelinaceae), Morphine sulphate, Plate reaction time, pain and inflammatory conditions.

INTRODUCTION

The World health Organization estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary healthcare needs. In all most all the traditional medical systems, the medicinal plants play a major role and constitute their backbone. Nearly 50% of medicines in the market are made of natural basic materials. Interestingly, the market demand for medicinal herbs is likely to remain high because many of the active ingredients in medicinal plants cannot yet be prepared synthetically [1].

Currently, available drugs for the management of pains, fever and inflammation conditions are either opoids or non- opoids and these drugs have been reported to posses potential toxic effect such as gastrointestinal bleedings [2]. On the other hand drugs of plant origin have been used for management of diseases for many centuries and have not been reported of any deleterious effects to their hosts.

Murdannia nudiflora(L) Brenan is a herb belonging to family Commelinaceae. It is a slender, nearly smooth, creeping perennial herb. The stem is simple to branched 15-30 cm long, reclining on the ground with rooting at the nodes. The roots are fibrous, the leaves are rather thick, linear to linear oblong, alternate, narrowed into a base sheath, entire, acute, tapering to a point with sides incurved, measuring 3-10 cm long and 4-10 mm wide. Stems decumbent below and ascending above, branch lets reddish with white nodes, flowers clustered in terminal or axillary cymes, blue or pinkish. The plant is traditionally being used in the treatment of asthma, leprosy and piles, stomach complaints, giddiness, and astringent. Root paste with goat milk is prescribed orally to cure asthma, whole plant paste with common salt is applied on the affected area to cure leprosy [3]. But, there is no scientific investigation report available in view of analgesic activity of these plants so far. In this study, attempts have been made for pharmacological screening of the plant for analgesic activity and presence of different phytochemicals.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The plants *Murdania nudiflora* under the investigation shown in Figure 1 have been collected in the month of Jan-Feb from the Belsor area of Nalbari district of Assam, India. The plant was authenticated at Department of Botany, Gauhati University by Prof. G. C. Sharma. The report of authentication as per voucher specimen (A/N 17708 dated, 6th May, 2013) was presented in Figure 2.

Fig. 1: *Murdania nudiflora* whole plant

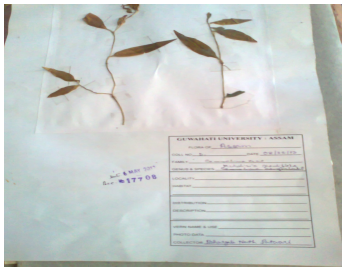

Fig. 2: Herbarium sheet of *Murdania nudiflora*

Screening of Physico-Chemical parameters of *Murdania nudiflora*

Physico-Chemical parameters were determined for the powdered drug according to the method described in W.H.O guidelines [4].

Moisture content

The powdered material (10gm) was placed in a moisture disc and dried to a constant weight in a oven at 100-105ºC.The loss of weight (in mg/g) of air dried was calculated as follows.

$$\% \text{ Moisture content} = \frac{\text{Weight loss}}{\text{Weight of the sample}} \times 100$$

Total Ash

3 gm of drug was weighed and incinerated in a China dish at a temperature not exceeding 450ºC until free from carbon, cooled and weighed, until a constant weight was obtained for three successive readings. Percentage of total ash was calculated with reference to air dried drug.

$$\text{Total Ash} = \frac{\text{Weight of ash}}{\text{Weight of drug}} \times 100$$

Acid-Insoluble Ash

The total ash was obtained by boiling for 5 min with 25 ml of dilute hydrochloric acid; the insoluble matter was collected in a Gooch crucible, the insoluble matter was wash with hot water and ignites to constant weight. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

Alcohol-soluble extractive

5 gm of accurately weighed powdered drug was taken in a stoppard conical flask and add 100 ml of 90% alcohol, and shake constantly for 6hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:

$$\text{Alcohol-soluble extractive} = \frac{\text{Weight of extractive}}{\text{Weight of drug}} \times 100$$

Water Soluble extractive

5 gm of accurately weighed powdered drug was taken in a stoppard conical flask and add 100 ml of chloroform water, and shake constantly for 6hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:

$$\text{Water-soluble extractive} = \frac{\text{Weight of extractive}}{\text{Weight of drug}} \times 100$$

Fluorescence Analysis [5, 6]

Fluorescence characteristics of the powdered fruit with different chemicals and the difference were observed in daylight and ultraviolet light. The powder was treated with neutral solvents like water and acids like 1M hydrochloric acid, 80% sulphuric acid, 50% nitric acid, 50% FeCl3, 5% sodium hydroxide, and glacial acetic acid and picric acid solutions were subjected to daylight and ultraviolet light for its fluorescence characteristics.

Preliminary phytochemical Screening [7-9]

About 100gm of dried ground powder and bushes of the plant were extracted with ethanol by cold maceration technique with constant shaking, for about 7 days. After the extraction period the solute was separated from the solvent by gradual distillation and later was evaporated to dryness on water bath. The extracts were subjected to qualitative preliminary phytochemical screening for identification of phytochemical constituents for total Tannin content, Flavonoids, Alkaloids and Carbohydrates.

Pharmacological evaluation for analgesic effects

Experimental Animals

Swiss albino mice aged 8-10 weeks (weight 20-30 gm) were maintained at 25 ± 2ºC temperature, 50 ± 15ºC relative humidity and normal photoperiod(12h dark/12h light) in plastic cages. The animals were fed standard pellet diet and water *ad libitum*. All the animal experiments were carried out in accordance with the guidelines of CPCSEA and were approved by the Institutional Animal Ethical Committee of Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati, India (*GIPS/IAEC/BPH/2013/2).*

Hot Plate Test

This test was carried out based on the method described by Eddy and Leimback (1953) [10]. The experimental animals of either sex were randomly selected and divided into four groups consisting of five mice each to serve as control, (positive control) and test sample group respectively. The first group (control group) received normal saline (0.9% w/v NaCl Solution), while groups 2 and 3, 100 mg and 200 mg extract/kg body weight *p.o* respectively, and group 4 received Morphine sulphate (positive control) at a dose of 10 mg/kg body weight *p.o*.The animals were positioned on a hot plate kept at a temperature of (55 ± 0.5ºC). A cut off period of 15 seconds was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws or jumped prior to and 30 min, 60 min and 90 min after oral administration of the samples. The maximum possible effect (MPE) was calculated as:

% MPE = (test latency-control latency)/ (cut off time- control latency) × 100 [11-12].

Statistical analysis

All data were expressed as mean ± S.E.M. and analyzed with Student's t-test.

RESULTS

Table1 showed the physicochemical parameters of whole plant. Table 2 showed the results of different chemical test of the powdered drug, Table 3 showed the Phytochemical constituents present in the ethanolic extract of the crude leaves of *Murdania nudiflora* and Table 4 showed the results of analgesic activity of crude aqueous extract of the *Murdania nudiflora* leaf using Hot plate method.

DISCUSSION

Physical evaluation of the powder showed 12% w/w moisture, 26.7% w/w total ash, 8.7% w/w acid insoluble ash, 44% w/w alcohol soluble extractive, 45% water soluble extractive. The powdered drugs were subjected to chemical analysis, fluorescence analysis which showed different color in different reagent.

The preliminary photochemical screening of ethanolic extract of the plant, showed the presence of Alkaloid, Saponin, Tanin and flavanoids. Flavonoids compounds are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception; hence its presence in the ethanolic extract of the plant may be contributory to the analgesic effects of the plant [13-14] There are also reports on the role of tannins in anti-nociceptive activity [15]**;** On the other hand alkaloids are well known for their effects to inhibit pain perception [16]. The pharmacological evaluation result showed that there was no significant difference in the pain reaction time (PRT) before drug administration in all the mice. However, 30 mins after administration the PRT was significantly ($p<0.05$ and $p<0.001$) increased by the extract and the reference drug (Morphine sulphate) but in a dose-independent manner when compared to the normal saline treated group. At doses of 100 mg/kg was much higher than the standard drug used which compared relatively with the treatment at 200 mg/kg. Invariably in this experiment the extracts at 100 mg/kg was more effective than the standard drug.

The, overall result obtained suggested that the ethanolic extract of *Murdania nudiflora* (L) Brenan showed significant analgesic activity in the hotplate test that may be in part mediated by opoid receptors.

Table 1: Physicochemical parameters of plant *Murdania nudiflora*

S. No.	Parameters	*Murdania nudiflora* (%w/w)
1	Moisture content	12%
2	Total ash	26.7%
3	Acid insoluble ash	8.7%
4	Alcohol soluble extractive	44%
5	Water soluble extractive	45%

Table 2: Test results of powdered drug with different reagents/solvents

Reagents	Colour observed	Fluorescence Observed
Powder as such	Green	Green
Powder + 1M HCL	Green	Green
Powder + 50% HNO_3	Brown	Blue
Powder + 80% H_2SO_4	Yellowish-Brown	Blue
Powder + Glacial acetic acid	Yellowish-Green	Blue
Powder + 5% NaOH solution	Greenish-Yellow	Blue
Powder + 5% KOH solution	Greenish-Yellow	Light yellow
Powder + 50% Ferric chloride solution	Brown	Green
Powder + Picric acid	Greenish-Yellow	Green
Powder + Ammonia	Yellowish-Green	Blue

Table 3: Phytochemical constituents of ethanolic extract of the plant *Murdania nudiflora*

Constituents	Tests	Ethanolic extract
Tannins	Lead acetate	+
	Ammonia Solution	+
	Ferric Chloride	+
Flavanoides	Shinoda test +	+
	Ferric chloride +	+
	Lead acetate +	+
	Sodium hydroxide+	+
Alkaloides	Dragendoff's reagent	+
	Wagner reagent	+
	Mayer reagent	+
		+
Carbohydrates	Molich test	_
	Fehlings' solution	_
Cardiac glycosides	Keller-kilian (cardenoides)	_
	Salkowski test	_
	Liberman-burchard test	
Saponins	Frothing test	_
Phlobatannins	1% HCl	_
Anthraquinones	Borntrager's test	_
Phenolics	Ferric chloride	_
Resins	10% KMnO4	_

Note: +: Detected; -: Absent

Table 4: Analgesic activity of crude ethanolic extract of the *Murdania nudiflora* Leaf using Hotplate method

Groups	Dose(mg/Kg)	Time interval (Min)	Ethanolic extract Mean latency ±SEM	Inhibition (%)
Normal saline	10	0	1.44±0.16	-
(-ve control)		30	1.25±0.21	-
		60	1.51±0.19	-
		90	1.23±0.52	-
1	100	0	1.67±0.21	15.97
		30	1.50±0.22	20.0
		60	1.94±0.23*	28.47
		90	1.68±0.19*	36.58
2	200	0	1.72±0.21	19.44
		30	2.11±0.23**	68.8
		60	2.43±0.18**	60.92
		90	1.55±0.11	26.01
Morphine sulphate	10	0	1.73±0.15	20.13
(+ve control)		30	1.80±0.06**	44.0
		60	2.52±0.09**	66.88
		90	1.53±0.12	24.39

Note: Values are means ± S.E.M. *$p < 0.05$; **$P < 0.001$, significantly different from control; Student's t-test (n = 5).

CONCLUSION

It may be concluded that ethanol extracts of *Murdania nudiflora* (L) Brenan may contain novel bioactive principles with analgesic activity. Further study is required for evaluation of active principle(s) in different animal models.

CONFLICT OF INTERESTS

Declared None

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