Phytochemical Standardization and analgesic activity of *Murdannia nudiflora* (L) Brenan

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Abstract

Our present communication deals with physicochemical parameters, preliminary phytochemical studies and pharmacological impact of *Murdannia nudiflora* (L) Brenan as analgesic plant which is used in folklore uses of Assam. No reports are available on standardization parameters hence, the present attempt was undertaken to investigate the standardization parameters to establish its authenticity. The study revealed the presence of wide range of phytoconstituents. The determination of these characters will aid future investigators in their Pharmacological analysis of this species.

Key word

Murdannia nudiflora, Standardization, Analgesic activity, TLC

Introduction

The history of herbal medicines is as old as human civilization. Almost one fourth of pharmaceutical drugs are derived from botanicals.[1] The World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary health care. *Murdannia nudiflora(L) Brenan* is a herb belonging to family Commelinaceae. It is a slender, nearly smooth, creeping annual or 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. Widely distributed in India; found in wet places, paddy fields, marshes, along ditches and in shady, grassy places, an aggressive weed in parts of West Indies;

Stems decumbent below and ascending above, branch lets reddish with white nodes, flowers clustered in terminal or axillary cymes, blue or pinkish. Used locally 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.[2] (Panda and Misra, 2011) The present investigation deals with the study of some physicochemical characteristic and its establishment as analgesic plant.

Materials and Methods

Collection and Authentication of Plant Material

The plant *Murdannia nudiflora* has been collected in the month of Jan-Feb from the Belsor area of Nalbari district of Assam. The plant was authenticated by Prof. Dr. G. C. Sharma, Department of Botany, Gauhati University. A voucher specimen (A/N 17708 dated, 6th May, 2013) was submitted in Department of Botany, Gauhati University for future reference.

Determination of physicochemical parameters

The Moisture content, ash values, extractive values with various reagents and were determined as per the Indian Pharmacopoeia.[3] The fluorescence characters of the powder with various reagents were observed under visible light and UV light (254 & 366 nm) as per the standard procedure.[4-5]

Preliminary phytochemical screening

Preparation of extract

About 110gm of dried grinded powder and bushes of the plant were taken in 1000ml RBF. To that above flask 750ml Ethanol was poured and was kept aside under cold maceration technique with constant shaking. This process is continued for about 7 days. After that the liquid extract was filtered through filter paper and was kept aside. The liquid extract was then distilled off and the remaining solvent was allowed to evaporate in a water bath at constant temperature. Then it was poured in a tarred petridish was dried for several days. After drying again weigh the petridish and calculate the % yield of extract of the crude drug. The extract was subjected to following preliminary phytochemical screening for the identification of various active constituents.[6-7]

Chromatographic screening

Thin layer chromatography

Thin layer chromatography (TLC) can successfully be employed for *Murdannia nudiflora extract*. TLC analysis was performed on silica gel G 60 F254 TLC plates as stationary phase and plates were eluted in solvent system acetone: water: conc. ammonia (90 : 7 : 3) and toluene: methanol (86:14). After development, the plates were dried and developed TLC plates were sprayed by 1% H_2SO_4 and observed under Iodine chamber and UV light.

Sample preparation

2 g of extracts of *M. nudiflora,* was dissolve in 25ml of ethanol separately and used for TLC analysis. 10 µl of the test solution was applied and the plates were then developed in above said solvent system up to $3/4^{th}$ of its length. The plate was then dried at room temperature by keeping on a flat surface.

Pharmacological evaluation

Animals

Healthy adult Albino Mice weighing (15-20gm) were selected for the studies. Mouse were housed in polypropylene cages (3 animals per cage), maintained under standard laboratory conditions (i.e. 12:12hr light and dark sequence; at an ambient temperature of 25 ± 1ºC). The animals were fed with standard pellet diet and water.

Analgesic activity

Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances, which decrease pain sensation (pain - killers) by increasing by increasing threshold of painful stimuli. The commonly used analgesics are Aspirin, Paracetamol (non - narcotic type) and Morphine (narcotic type). Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as (i) thermal (radiant heat as a source of pain), (ii) chemical (irritants such as acetic acid and bradykinin) and (iii) physical pressure (tail compression). In the present study the attempt has been focused to evaluate the Analgesic activity of extracts of whole plant of plant *Murdannia nudiflora* against hind paw licking or jump response in mice.

Study Design: The adult Albino Mice were divided into 3 groups of 6 animals and maintained for the analgesic activity.

Group I- Contol (1% CMC)

Group II- Standard Diclofenac sodium (3mg/kg body weight, orally)

Group III- Ethanolic extract of *Murdannia nudiflora* (100 & 200 mg/kg body weight, orally)

Hot plate method

The method originally described by Woolfe and Mac Donald (1944) has been modified by several investigators. The hot plate, which is commercially available, consists of an electrically heated surface. This can be a copper plate or a heated glass surface. Reaction time of animals was noted down in hot plate at 0, 30, 60, 90, 120 and minutes after the treatment. The basal reaction time taken by observing hind paw licking or jump response (whichever appear first) in animals while placed on hot plate, which was maintained at constant temperature 55^0C.[8]

Result

Physicochemical parameters

Physicochemical parameters like foreign matter, percentage of moisture content, total ash, acid insoluble ash, water soluble ash were determined and depicted in Table 1. The results of fluorescence analysis of the powder drug are mentioned in Table 2.

Table 1: Physicochemical parameter of *M. nudiflora* (In %)

S.No.	Parameter	Percentage (% w/w)
1	Foreign matter	Nil
2	Moisture content	12
3	Total ash	26.7
4	Water soluble ash	8.7
5	Acid insoluble ash	8.7

Table. 2: Fluorescence analysis of powdered drug

Reagents	Fluorescence Observed
Powder as such	Green
Powder + Concentrated HCL	Green
Powder + Concentrated HNO_3	Blue
Powder + Concentrated H_2SO_4	Blue
Powder + Glacial acetic acid	Blue
Powder + 5% NaOH solution	Blue
Powder + 5% KOH solution	Light yellow
Powder+5%Ferricchloride solution	Green
Powder + Picric acid	Green
Powder + Ammonia	Blue

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of phenolic compounds, phytosterols, alkaloids, flavonoids in ethanolic extract. (Table 3)

Table 3: Preliminary phytochemical screening of the ethanolic extract of *M. nudiflora*

S. No.	Constituents	Tests	Ethanolic extract
1.	Carbohydrate	Molish's test	-
		Fehling's test	-
2.	Fixed oil & fats	Spot test	_
		Saponification test	_
3.	Proteins & amino acids	Million's test	_
		Ninhydrin test	_
		Biuret test	_
4.	Saponins	Foam test	_
5.	Phenolic compounds	$FeCl_3$ test	-
		Gelatin test	_
		Lead acetate test	-
6.	Phytosterol	Salkowiski test	+
		Libermann burchard test	+
7.	Alkaloids	Dragendroff's test	+
		Mayer's test	+
		Wagner's test	+
		Hager's test	_
8.	Gum & mucilage	Swelling test	_
9.	Flavonoids	Aqueous NaOH test	+
		Con. H_2SO_4 test	+
		Shinoda's test	+

Chromatographic screening

Many of the modern herbal pharmacopoeias and other regulatory agencies like WHO included TLC as a powerful and most economical tool for true identification of the plant material, especially in terms of its chemical constituents. The TLC of plant extract was performed and number of spots and their Rf values has been tabulated in table no. 4.

Table 4. TLC Profile of extracts of *Murdannia nudiflora*

SOLVENT SYSTEM	ALCOHOLIC EXTRACT
Acetone: Water: Conc.NH_3 (90:7:3)	1 spot, Rf:- 0.93
Toluene: Methanol (86:14)	1 spot, Rf:- 0.6

Pharmacological evaluation

Table 5: After performing the animal study the following results are observed and they are listed bellow-

Time interval (Minutes)	Reaction time in Seconds			
	Control (CMC 1%)	Standard (3mg/Kg body wt)	Test	
			100mg/kg bw	200mg/kg bw
0	3.19	4.41	3.67	3.89
30	3.53	5.71	3.78	4.01
60	1.55	3.46	2.89	2.92
90	2.11	2.84	2.52	2.57
120	1.21	3.62	2.19	2.21

Discussion: Physicochemical and fluorescence characters of *Murdannia nudiflora (L) Brenan*., is used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guide lines. Preliminary phytochemical screening of different plant extracts revealed the presence of phenolic compounds, phytosterols, alkaloids and flavonoids. TLC profile of ethanolic extract was confirmed the presence of phyto-constituents, thus further research on *Murdannia nudiflora* is necessary for isolation and characterization of important bioactive constituents which have wide medicinal values in view of its allied species.[9] The results of analgesic activity of ethanolic extract of *Murdannia nudiflora* (L) Brenan showed significant analgesic activity which is comparable to the standard drug diclofenac sodium. From the data it was confirmed that ethanolic extract of *Murdannia nudiflora* (L) Brenan has analgesic activity. **Conclusion:** In the present study, various phytochemical and pharmacological parameters were screened .It shows that the plant *Murdannia nudiflora is* having significant analgesic effect. Here ethanolic extract of the plant was screened on albino mice using hot plate method. Further

experiment for evaluation of the same activity can be carried away by using different analgesic activity screening methods. Hence, the claim made by the traditional Indian systems of medicine regarding the use of this plant in the treatment of analgesic stands confirms.

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