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Comparative preliminary phytochemical and analgesic activity on methanolic extract of leaf and bark of *Aphanamixis polystachya* (Wall.) Parker

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Keywords

Aphanamixis polystachya (wall.) Parker, phytochemical and analgesic.

Abstract

The present study was designed to investigate as well as compare the possible phytochemical and analgesic activity on methanolic extract of Leaf and Bark of *Aphanamixis polystachya* (wall.) Parker (Family: Meliaceae). The phytochemical investigations were carried out following standard chemical procedure. The crude extracts of Leaf and Bark were tested for the presence of different chemical groups and the following groups were identified in Leaf extract– Alkaloid, Flavonoid, Tannin, Glycoside, Saponin whereas Flavonoid, Reducing Sugar, Tannin, and Steroid are present inBark extract. Analgesic activity test was also performed with these extracts using Swiss Albino mice and Diclofenac Sodium as standard chemical .The bark extract showed remarkable analgesic activity (67.71%) than that of leaf extract(52.18%). The plant is available in Bangladesh and could be a prominent source of medically important natural compounds.
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Introduction

The plants are indispensable to man for his life. Man's dependence on plant kingdom for the essentials of his existence has been of paramount importance in his life. Since disease, decay and death have always co-existed with life, the early man had to think about disease and its treatment at the dawn of human intellect (Kiritikar & Basu, 1999). Thus the human race started using plants as a means of treatment of diseases and injuries from the early days of civilization on earth and its long journey from ancient time to modern age the human race has successfully used plants and plant products as effective therapeutic tools for fitting against diseases and various other health hazards. (Ghani, 2003).Nature always has been a valuable source of drugs and despite the unprecedented opportunities afforded by medicinal chemistry continues to deliver lead or bioactive compounds. There is a growing focus on the importance of medicinal plants in the traditional health care system.

The plant *Aphanamixis polystachya* (wall.) Parker which belongs to the Meliceae family locally known as Pithraj, Royna, Tiktaraj, Baddiraj, a large evergreen tree found to grow in Rajshahi, Chittagong, Khulna and Sylhet regions. So far the literature survey revealed is based on the biological activity (Antioxidant, Laxative, Antineoplastic, Antimicrobial, Repellent, Antifeedant, Cytotoxicity, Hepatoprotective) of leaf and bark extracts. Several phytochemical works were carried out with this plant and number of chemical constituents have been reported in this plant such as Rohitukin,

Limonoids, Amooranin, Aphanamixoid, Saponin, Tetranotriterpene, Aphanamixinin, and Polystachyol (Chatterjee *et al*., 1970; Mulholand and Naidoo, 1999; Ghani 2003; Rabi *et al.*, 2003, Sadhu *et al.*, 1980;Jie-Yun Cai andYu Zhang 2012; Bhatt *et al.*, 1981).But the aim of this study was to enlighten the comparative study on methanolic extract of Leaf and Bark of this plant for phytochemical screening and analgesic activity

Materials and Methods:

Plant Material Collection and Identification

Healthy and disease free leaf and bark of the plant *Aphanamixis polystachya (*wall.) Parker was collected during summer in the month of May, 2014 from Paikgachha, Khulna, Bangladesh. The plant was identified by the experts of Botany Department, University of Rajshahi and was preserved in Phytochemistry lab, Department of Pharmacy, Khulna University, Bangladesh.

Preparation of Extracts

The collected plant materials were washed to separate from undesirable plant parts and sun-dried for one week .The dried parts were cut into small pieces and ground into a coarse powder with the help of a suitable laboratory grinder.200gm of Leaves and Barks powder of *Aphanamixis polystachya (*wall.) Parker was macerated in 850 ml of 80% methanol at 37°C for 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by clean, white cotton, followed by a filtration through Whatmann filter paper. The filtrate (methanol extract) was then evaporated through rotary evaporator followed by desiccation to get the dried crude extract (yield:8% for Leaf and 9% for Bark). These extracts were used for subsequent phytochemical screening and analgesic activity.

Phytochemical Screening

Methanolic extracts of *Aphanamixis polystachya* (wall.) Parker Leaves and Bark were tested for different chemical groups according to the described methods (Evans, 2002; Ghani, 2003).

Study of analgesic activity by acetic acid induced writhing method (Whittle 1964)

The test consists of injecting the 0.7 % acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between control, positive control (Diclofenac Sodium), and test samples. Diclofenac Sodium was used as the positive control in this method that acts by inhibition of prostaglandin synthesis which has been reported to be responsible for pain sensation (Rang HP, 1993).

Experimental animal

Young Swiss-albino mice aged 4-5 weeks, average weight 25-30 gm were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) for the experiment. They were kept in standard environmental condition (RH 55% to 60%, room temperature 25± 2^{o}C and 12 h light/ dark cycle) for one week for adaptation after their purchase and fed ICDDR,B formulated rodent food and water.

Preparation of Sample

To prepare suspension of test samples at the dose of 500mg/kg per body weight 125 mg of samples was measured. The extracts were triturated in unidirectional manner by the addition of small amount of tween-80 with distilled water to make the final volume of about 2.5 ml which reserve in separate container.

To stabilize the suspension, it was shaken well by vortex mixer**.** For the preparation of Diclofenac Sodium at the dose of 25 mg/kg-body weight, 6.25 mg of Diclofenac was taken and a suspension of 2.5 ml was made.

Methodology

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control, the Leaf and Bark extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Test samples, control and Diclofenac Sodium were given orally by feeding needle. 30 minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7 %) was administered intraperitoneally to each of the animals of selected groups. After an interval of 5 minutes, which was given for absorption of acetic acid, number of squirms

(writhing) was counted for 15 minutes and % of writhing inhibition was calculated as follows:

% Inhibition of writhing = 100 –(Treated mean/control mean) × 100

Results

1. Phytochemical Screening

Phytochemical analysis showed that *Aphanamixis polystachya* Bark contains Flavonoid, Reducing Sugar, Tannin,and Steroid whereas Leaf contains Alkaloid, Flavonoid, Tannin, Glycosides, and Saponin among others. The results are as given in Table1.

Table 1. Test result for chemical groups of *Aphanamixis polystachya* leaf and bark extract

Sl. No.	Compound groups	APLE	APBE
01.	Flavonoid	+	+
02.	Alkaloid	+	_
03.	Saponin	+	-
04.	Tannin	+	+
05	Steroid	_	+
06.	Reducing Sugars	_	+
07.	Glycosides	+	+

APLE= *Aphanamixis polystachya* leaf extract
APBE=*Aphanamixis polystachya bark extract*

2. Acetic Acid Induced Analgesic Activity:

Statistical Analysis

Student's t-test was used to determine significant differences between the control group and test groups.

Table 2: Statistical evaluation of the results of analgesic activity on methanolic extracts of *Aphanamixis polystachya*(wall.) Parker Leaf and Bark

Animal group	Total Writhing	Mean	% Writhing	Standard deviation (SD)	Standard error (SE)	% Inhibition	T-test (value of p)
Controll (Tween 80, 10ml/kg)	161	32.2	100	3.7	1.85	-	-
Diclofenac Sodium(25mg/kg)	39	7.8	24.22	2.16	1.08	75.78	11.39 (P< 0.001)
APLE(500mg/kg)	77	15.4	47.82	2.4	1.2	52.18	7.61 (P< 0.001)
APBE(500mg/kg)	52	10.4	32.29	2.6	1.3	67.71	9.64 (P< 0.001)

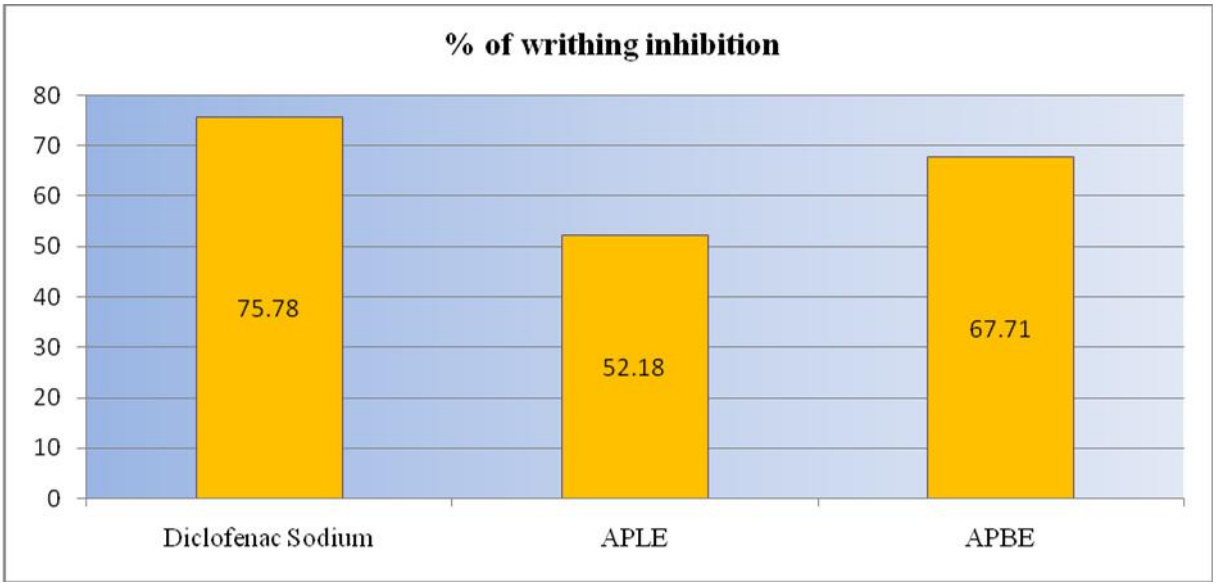

Fig 1: Effect of *A. polystachya* Leaf and Bark on acetic acid induced writhing of mice

APLE= *Aphanamixis polystachya* Leaf extract; **APBE**=*Aphanamixis polystachya Bark extract*

Significance:

Control VsDiclofenac sodium: Significant (P< 0.001)
Control Vsbark Extract (500 mg/kg): Significant (P< 0.001)
Control Vs leaf Extract (500 mg/kg): Significant (P< 0.001)

Discussion

Algesia (pain) is an ill-defined warning signal, unpleasant sensation, usually evoked by an external or internal noxious stimulus. An Analgesic neutralizes pain sensation as a symptom, without affecting its cause. It selectively relieves pain by acting on CNS or on peripheral pain mechanisms, without significantly altering consciousness. Intraperitoneal administration of acetic acid (0.7%) causes induction of writhing or algesia by liberation of eicosanoids(mainly prostacyclin (PGI_2) and prostaglandin-E) from free arachidonic acid resulting from tissue phospholipid by the action of phospholipase A_2 and other acyl hydrolases (Ranolds *et al.*,1982). Diclofenacsodium used as the positive control in this method acts by inhibition of prostaglandin synthesis. Methanolic extract of Leaf and Bark of *Aphanamixis polystachya* (wall.) Parker lowers the number of writhing demonstrating analgesia by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.The methanolic extract of Bark and Leaf of *Aphanamixis polystachya* (wall.) Parker produced 67.71% ($P < 0.001$) and 52.18% ($P < 0.001$) protection or writhing inhibition at orally doses of 500 mg/kg body weights of mice respectively in comparison with the standard chemical Diclofenac sodium having 75.78% of writhing inhibition at the dose of 25mg/kg body weights of mice. The results showed that Bark extract of *Aphanamixis polystachya* (wall.) Parker posseses much analgesic activity than that of Leaf extract. Besides these, methanolic extract of Leaves of *Aphanamixis polystachya* (wall.) Parker is a profound source of Alkaloid, Flavonoid, Tannin, Glycosides, and Saponinwhereas the Bark contains Flavonoid, Reducing Sugars, Tannin and Steroid. Both the extracts ensured the presence of plenty of Flavonoid which is reported to inhibit the release of autacoids and prostaglandins leading to the relief from pain sensation. Abundance of vital chemical groups in Bark and Leaf extracts with significant bioactivities has attracted *Aphanamixis polystachya* (wall.) Parker a overwhelming attention as a potential analgesic compound in the field of medicinally important natural products.

Conclusion

The experimental findings from the study showed that the methanolic extract of Leaves of *Aphanamixis polystachya* (wall.) Parkeris an immense source of Alkaloid, Flavonoid, Tannin, Glycosides, and Saponin whereas the Bark contains Flavonoid, Reducing Sugars, Tannin and Steroid.

The availability of various chemical groups in Bark and Leaf of this plant has brought *Aphanamixis polystachya* (wall.) Parker into light for pronounced pharmacological activity. The methanolic extract of Bark and Leaf of *Aphanamixis polystachya* (wall.) Parker produced 67.71% ($P < 0.001$) and 52.18% ($P < 0.001$)protection or writhing inhibition at orally doses of 500 mg/kg body weights of mice respectively in comparison with the standard chemical Diclofenac sodium having 75.78% of writhing inhibition at the dose of 25mg/kg body weights of mice(Table 2).The Bark extract showed much analgesic activity than that of Leaf extract. So the Bark of *Aphanamixis polystachya* (wall.) Parker is going to be a profound source of medicinally active compounds for analgesic activity for upcoming days and further study is required for development of mechanism and isolation of corresponding active compound from these extracts.

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