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Research Article

Preliminary Phytochemical Analysis and Peripheral Analgesic Activity Studies of *Alpinia malaccensis* MeOH Extract

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Abstract

The analgesic effect of crude methanol extracts of *Alpinia malaccensis* (Burm.f.) Roscoe (Zingiberaceae) was evaluated in acetic acid-induced pain *model* experiment in Swiss albino mice. This study was designed to assess the nature of phytochemicals present and determine the peripheral pain relief properties of methanolic extract of aerial parts (MEAMA) and rhizome (MEAMR) of the plant. Both extracts contained alkaloids, flavonoids, steroids, saponins and tannins. In analgesic activity test, the methanol extract at doses of 50, 100, 200 and 400 mg/kg body weight dose-dependently reduced the number of writhing by 31.7, 46.3, 51.2 and 58.5% for MEAMA and 19.5, 41.5, 53.7 and 61.1% for MEAMR, respectively. The standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg/kg body weight reduced writhing by 41.5 and 63.4%, respectively. Compared to control, all the given doses except low dose (50 mg) of rhizome extract of *Alpinia malaccensis* significantly decreased the pain. Considering present data it can be suggested that the methanol extracts of *Alpinia malaccensis* have chemical compounds potentially responsible for alleviating pain and might pave the way towards development of a novel analgesic agent in future.

Keywords: *Analgesic; Alpinia malaccensis; Phytochemicals; Peripheral Pain; Zingiberaceae*

Introduction

Alpinia malaccensis (Burm.f.) Roscoe is a Zingiberaceae family plant. Originally native to Indonesia and Malaysia, it can be found both cultivated and in the wild from India to China, including neighboring countries like Bangladesh. The plant is known as 'Malacca ginger' in English and 'deotara' in Bangla. Not many studies have been published on the plant though it is considered a common member of the Zingiberaceae family.

Sahoo., *et al*. [1] reported the presence of α-phellandrene, β-cymene and β-pinene as major constituents in essential oil obtained from leaves of the plant. The authors also reported significant antioxidant activity in essential oil and methanol extract of leaves. It was further reported that the oil and extract showed inhibitory activity against four microbial strains. A compound present in rhizome extract of the plant, 1′-acetoxy chavicol acetate, present in rhizomes of the plant showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* [2].

Various Zingiberaceae family plants or plant parts and phytochemicals present within the plants like curcumin, gingerol and zingerone have been reported for their analgesic properties. Most pain (from different causes) can be alleviated with non-steroidal anti-inflammatory drugs (NSAIDs) and the analgesic phytochemicals of Zingiberaceae family reportedly have shown equal effectiveness as NSAIDs without the associated renal risks. However, the phytochemicals as well as NSAIDs are associated with bleeding risks [3], which necessitates fresh searches for better analgesic and anti-inflammatory agents.

Alpinia malaccensis is a common plant in Bangladesh. Since the analgesic properties of the plant, if any, are yet to be reported, the objective of the present study was to evaluate through acetic acid-induced writhing test in mice, the methanolic extract of rhizomes and aerial parts of the plant for any analgesic activity present. Preliminary phytochemical tests were also performed for various group of phytochemicals present in the plant.

Materials and Methods

Plant materials collection

Whole plants of *Alpinia malaccensis* including both aerial parts (leaves and stem) and rhizomes (root) were collected from Khulna city, Bangladesh (22.8456° N, 89.5403° E) and identified at the Bangladesh National Herbarium at Dhaka city. Voucher specimen was deposited with the Bangladesh National Herbarium. An accession number (43825) was given from there. The plant is readily available all over Bangladesh and is locally known as Deotara.

Preparation of methanol extracts

The aerial parts and rhizome of the plant were cut separately into small pieces and air-dried in the shade and grounded into a fine powder. Both aerial parts and rhizome powder (150g each) were then extracted with methanol at a ratio of 1:5 (w/v) for 48 hours at room temperature (30°C) with frequent stirring. The mixture was filtered; filtrate was collected and methanol was evaporated at 60°C and the extract stored at -20°C and used within 96 hours [4]. The final weight of the extract of aerial parts and rhizome was 14.36g and 9.49g, respectively.

Chemicals and drugs

Glacial acetic acid, methanol and DMSO were obtained from Merck (Germany); aspirin was obtained from Reckitt Benckiser Bangladesh Ltd. All other chemicals were of analytical grade. Methanolic extracts of aerial parts and rhizomes of *Alpinia malaccensis* were designated as MEAMA and MEAMR, respectively.

Animals

Swiss albino mice comprising of both sexes and weighing between 15 - 18g were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were housed in cages (5 mice per cage) and acclimatized for 72 hours prior to experiment under conditions of 12 hour light and 12 hour darkness. During this time, the mice were fed with mice chow obtained from ICDDR,B and water *ad libitum*. The study was carried out in the Pharmaceutical Biotechnology Laboratory of Department of Biotechnology and Genetic Engineering and permission to carry out the experiment with mice was obtained from the Institutional Animal Ethical Committee of the University of Development Alternative. Care was taken that the animals did not suffer from unnecessary pain prior to or during the experiment.

Preliminary phytochemical analysis

The preliminary phytochemical screening of the methanol extract of both aerial parts and rhizome was carried out using standard protocols described by Kumar., *et al* [5].

Acetic acid-induced writhing test

Antinociceptive activity of methanol extract of aerial parts and rhizome of *Alpinia malaccensis* was examined using well recognized method conducted by Koster., *et al* [6]. In writhing test, visceral pain sensation was induced in the experimental mice through intra-peritoneal administration of 1% acetic acid at a dose of 10 mg/kg body weight. Mice were separated into eleven groups of five mice each. All groups received acetic acid with or without aspirin or extracts. Group I mice, serving as control were treated with 1% acetic acid only. The standard drug, aspirin was administered orally to Group II and Group III mice at doses of 200 and 400 mg/kg body weight, respectively. Groups IV to VII mice received MeOH extract of MEAMA, respectively at 50, 100, 200 and 400 mg/kg body weight orally 60 min before acetic acid injection. Similarly, Group VIII to XI mice received MeOH extract of MEAMR, respectively at 50, 100, 200 and 400 mg/kg body weight orally 60 min before acetic acid injection. Each animal was given 5 minutes to ensure bio-availability of acetic acid, followed by observing the number of writhing for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below:

Percent of Inhibition = $(1- W_e/W_c) X 100$

Where W_e and W_c represents, respectively, the number of writhings in Groups II to Group XI (Experimental) and Group I (Control).

Statistical analysis

Experimental values were expressed as the mean ± standard error of the mean (SEM). Statistical significance was determined by Student's *t*-test and $P < 0.05$ was considered to be statistically significant in all cases.

Results

Preliminary phytochemical analysis

Preliminary phytochemical screening for secondary metabolites from methanolic extracts of *Alpinia malaccensis* (both aerial parts and rhizome) confirmed the presence of alkaloids, flavonoids, steroids, saponins and tannins through qualitative color changes of test reagents as depicted in table 1.

Phytochemical	Reagent/Chemical	Observation	Result
Alkaloid	1% HCl + Wagner reagent	Brown precipitation	+
Flavonoid	Conc. HCl	Reddish coloration	+
Steroid	Chloroform + Conc. H_2SO_4	Red color	+
Saponin	Distilled water	Persistent frothing	+
Tannin	Distilled water + 1% ferric chloride solution	Blue-black precipitation	+

Table 1: *Phytochemical screening of methanol extracts of Alpinia malaccensis.*

'+': Present.

Analgesic activity through acetic acid-induced writhing test

All the given doses of MEAMA and MEAMR dose-dependently reduced the number of writhing produced by acetic acid in mice, when compared with untreated control group and with the exception of MEAMR (Group VIII) demonstrated statistically significant peripheral

analgesic activity. The percentage inhibition of writhing on administration of test extracts was found to be increased up to 58.5 with MEAMA at 400 mg/kg and 61.1 with MEAMR at 400 mg/kg. The results shown in table 2 demonstrate that MEAMR at the highest dose was comparatively better than that of MEAMA, signifying MEAMR was more analgesic. The reference standard drug, aspirin at the dose of 400 mg/kg showed maximum inhibition in experimental mice (63.4%), however; the inhibition was only marginally larger than inhibition obtained with MEAMR at 400 mg per kg body weight at 61.1%.

Treatment	Dose (mg/kg body weight)	Mean number of writhing(s)	% inhibition
Control	10 ml	8.2 ± 0.58	-
Aspirin (Standard I)	200 mg	4.8 ± 0.37	41.5*
Aspirin (Standard II)	400 mg	3.0 ± 0.32	63.4*
MEAMA (Group IV)	50 mg	5.6 ± 0.51	31.7*
MEAMA (Group V)	100 mg	4.4 ± 0.51	46.3*
MEAMA (Group VI)	200 mg	4 ± 0.55	51.2*
MEAMA (Group VII)	400 mg	3.4 ± 0.4	58.5*
MEAMR (Group VIII)	50 mg	6.6 ± 0.68	19.5
MEAMR (Group IX)	100 mg	4.8 ± 0.37	41.5*
MEAMR (Group X)	200 mg	3.8 ± 0.37	53.7*
MEAMR (Group XI)	400 mg	3.2 ± 0.2	61.1*

Table 2: *Analgesic effect of crude methanol extract of A. malaccensis aerial parts (MEAMA) and rhizome (MEAMR) in acetic acid-induced pain model mice.*

*Values represented as mean ± SEM, (n = 5); *P < 0.05; significant compared to control.*

Discussion and Conclusion

Plants have been a unique source of a number of secondary metabolites such as flavonoids, saponins, tannins, alkaloids etc., which reportedly exhibited considerable anti-inflammatory, anti-oxidant, analgesic and anti-diabetic activities, leaving a profound contribution in folk medicine from time immemorial [7]. Our current research presented acetic acid induced writhing in experimental mice and further provided some scientific insights about the analgesic potential of *Alpinia malaccensis* methanol extract of aerial parts and rhizome, which might be attributed to the plant derived active compounds confirmed in the extracts. The peripheral pain response produced by acetic acid is an effective but non-selective model, which involves the release of prostaglandins (prostaglandins E_2 and $F_2\alpha$) through the activation of cyclooxygenase enzyme in arachidonic acid pathways [8,9]. Analgesic drugs like aspirin lowers the number of writhing due to their ability to inhibit prostaglandin synthesis and are most commonly used to treat pain and pain associated diseases [10,11].

A number of studies have earlier reported the analgesic and anti-inflammatory potential of phytochemicals especially flavonoids, saponins and tannins by suppressing pain sensation through inactivating the enzymes involved in the arachidonic acid metabolic pathway and inhibiting nitric oxide synthesis [12-18]. A recent study indicated a similar result that aqueous extract of *Varronia multispicata* leaves rich in flavonoids caused inhibition of NO and prostaglandin synthesis, thus alleviating inflammation and pain [19]. Therefore, the presence of flavonoids, saponins, tannins and alkaloids in the extracts of aerial parts and rhizome may contribute significantly to the analgesic as well as anti-inflammatory characteristics of *Alpinia malaccensis*.

Since the mechanism of exact active constituent responsible for this analgesic property is still unknown, further investigation on the characterization of these phytochemicals need to be conducted at molecular level in order to validate the role of *Alpinia malaccensis* extracts in traditional medicine. Interestingly, rhizomes of the plant are boiled in water and taken for relief of abdominal pain by indigenous people of northeast India [20]. As such, the present study is supportive of the ethnomedicinal use reported for the plant.

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Conflict of Interest

The authors declare that they have no conflict of interests.

Informed Consent

Manuscript is not reporting any study with humans.

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