ANTIDIARRHOEAL AND ANTI-INFLAMMATORY ACTIVITIES OF *MURRAYA PANICULATA* (L.) JACK

Md. Atiqur Rahman[1]*, Md. Hasanuzzaman[1], Nazim Uddin[1], IZ Shahid[1]

[1]Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

*** Corresponding author:** Md. Atiqur Rahman
Present address: Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.
Phone: +8801717584231. E-mail: satikrahman@gmail.com

Summary

The ethanol extract of the dried leaves of *Murraya paniculata* (L.) Jack (Family - Rutaceae) was investigated for its possible antidiarrhoeal and anti-inflammatory activities in animal models. The extract showed antidiarrhoeal activity on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly ($P<0.001$, $P<0.01$) at the oral dose of 600 mg/kg of body weight which was comparable to the standard drug Loperamide at the dose of 50 mg/kg of body weight. Moreover, when given orally to rats at dose of 300 and 600 mg/kg of body weight, the extract showed a significant ($P<0.001$) anti-inflammatory activity against carrageenin induced paw edema in rats which was comparable to the standard drug aspirin at the dose of 150 mg/kg of body weight. The overall results tend to suggest the antidiarrhoeal and anti-inflammatory activities of the crude ethanolic extract of leaves of *Murraya paniculata* (L.) Jack. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: antidiarrhoeal activity, anti-inflammatory activity, *Murraya paniculata* (L.) Jack.

Introduction

Murraya paniculata (L.) Jack (English Name: Orange Jessamine, Family: Rutaceae, Synonym: *Chalcas paniculata* L. (basionym)) locally known as 'Kamini' in Bangladesh. It is also known as Kamini (Hindi), Kadu karibevu (Kannada), Kemuning (Malay), Maramulla (Malayalam), Kamini kusum (Manipuri), Kunti (Marathi), Kamuning (Tagalog), Nagagolungu (Telugu), Kattu Kariyilai or Vengarai (Tamil). It is a very variable evergreen shrubby plant with small white flowers, small oblong fruits and hard wood, commonly grows in plain areas throughout Bangladesh. It is also planted in gardens as an ornamental in many areas of the country. It is also distributed more or less throughout India, Srilanka, Myanmar, Philippines, Malaysia, China, Thailand, Cambodia, South Vietnam, East Africa and Australia[1-2].

Leaves of *Murraya paniculata* (L.) Jack are stimulant and astringent and are used in the treatment of diarrhoea, dysentery and diseases of teeth and gum; useful against rheumatism, coughs and hysteria[3-5]. Sawangjaroen et al. (2006) reported, it showed anti-amoebic activities[6].

The leaves and other tissues have both stimulant and astringent properties and are used to treat diarrhoea, dysentery, cuts, joint pain, body aches[7], venereal disease[8], and as an abortive[9]. In addition to essential oils, tissues of orange jasmine contain the indole alkaloid yuehchukene[9] and at least eight highly oxygenated flavones[8], leaves yield oil which contains sesquiterpenes (l-cadinene), a sesquiterpene alcohol and methyl anthranilate[3-4]. The principal constituents of the leaf oil were β-cyclocitral (22.9%), methylsalicylate (22.4%), trans-nerolidol (11.7%), α-cubebene (7.9%), (-)-cubenol (6.8%), β-cubebene (5.8%) and isogermacrene (5.7%) and among them the most prominent compounds were β-caryophyllene (24.1%), with lesser amounts of germacrene D (11.9%) and bicyclogermacrene (11.8)[10-12] . On the other hand, *Murraya Paniculata* contained abundance of caryophyllene oxide which has antifungal activities[13]. Paste of *Murraya paniculata* (L.) Jack bark is served as an expectorant. Its bark is also employed as a potent astringent[5].

From the existing information it is evident that the plant may possess some important biological activities. The main objective of this study was to evaluate the antidiarrhoeal and anti-inflammatory activities of the ethanol extract of dried leaves of *Murraya paniculata* (L.) Jack.

Materials and methods

Plant material

Leaves of *Murraya paniculata* (L.) Jack were collected from Khulna, Bangladesh in December 2007 and were authenticated by the experts at National Herbarium (Accession Number: 32092). After collection, leaves were sun dried for several days to remove moisture. After drying, the dried leaves were ground into course powder by 'Hammer' mill. About 400 gm of powdered leaves was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract. And this crude ethanolic extract was used for all phytochemical and pharmacological screening.

Animals

For antidiarrhoeal activity study, Swiss-albino mice of either sex, weighing 20-25 g, bred in the animal house of the Department of Pharmacy, Jahangirnagar University, were used. On the other hand, Wistar rats of either sex, weighing 180-200 g, purchased from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for anti-inflammatory activity study. All the animals were acclimatized one week prior to the experiments.The animals were kept under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12h light-dark cycle) and fed with standard diets and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol. All the experiments were conducted on an isolated and noiseless condition.

Drugs
Loperamide (Square Pharmaceuticals Ltd., Bangladesh), Carrageenin (Sigma Chemicals, USA), Aspirin (Square Pharmaceuticals Ltd, Bangladesh).

Preliminary phytochemical analysis
The ethanol extract of leaves of *Murraya paniculata* (L.) Jack was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% (w/v) solution of the extract in ethanol was used unless otherwise specified in individual test[14-15].

Tests for reducing sugar
Benedict's Test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

Tests for tannins
Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

Test for flavonoids
A few drops of concentrated hydrochloric acid were added to 5 ml of the extract.

Test for saponins
1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

Test for gums
5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

Tests for steroids
Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

Tests for alkaloids
Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Tests for Glycosides
A small amount of extract was taken in 1 ml water. Then few drops of aqueous sodium hydroxide were added. Yellow precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was taken in 1 ml water and boiled with 5 ml Fehling's solution in a boiling water bath. Brick-red precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with 5 ml Fehling's solution in a boiling water bath. Brick red precipitate is considered as an indication for the presence of glycosides.

Pharmacological Studies

Antidiarrhoeal activity
Antidiarrhoeal activity of the ethanolic extract of leaves of *Murraya paniculata* (L.) Jack was tested using the model of castor oil-induced diarrhoea in mice[16]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug loperamide at a dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at a dose of 600 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour old papers were replaced by the new ones.

Anti-inflammatory activity
Anti-inflammatory activity of ethanolic extract of the dried leaves of *Murraya paniculata* (L.) Jack was tested using the carrageenin-induced rat paw edema model as described by Winter *et al*. (1962)[17]. Experimental animals (Wistar rats) were randomly divided into four groups with six animals in each group. Control group received vehicle (1% Tween 80 in water) at the dose of 10 ml/kg of body weight. Positive control group received aspirin (standard drug) at the dose of 150 mg/kg of body weight and the test groups were treated with ethanolic extract of the dried leaves of *Murraya paniculata* (L.) Jack at the doses of 300 and 600 mg/kg of body weight. The drugs were administered orally 1h prior to the injection of 0.1 ml of 1% freshly prepared suspension of

carrageenin into the left hind paw of each rat. The paw volume was measured by using a plethysmometer (Ugo Basile 7140, Italy) every hour for 5 hours after the carrageenin injection.

Statistical analysis:
Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

Results

Chemical group test
Results of different chemical tests on the ethanolic extract of leaves of *Murraya paniculata* (L.) Jack showed the presence of alkaloids, tannins, saponins, gums, reducing sugar, flavonoids and glycosides (Table 1).

Table 1: Results of different chemical group tests of the extract of leaves of *Murraya paniculata* (L.) Jack

Extract	Reducing Sugar	Steroids	Alkaloids	Tannins	Gums	Flavonoids	Glycosides	Saponins
Ethanolic extract of leaves of *Murraya paniculata* (L.) Jack	+	-	+	+	+	+	+	+

Key: + = Presence, - = Absence

Antidiarrhoeal activity
Antidiarrhoeal activity of the ethanol extract of leaves of *Murraya paniculata* (L.) Jack was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (1.82 h) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg of body weight significantly ($P<0.001$) which was comparable to the standard drug loperamide at the dose of 50 mg/kg of body weight where the value was 1.98 h ($P<0.001$) (Table 2a). The extract also decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd, 3rd, 4th and 5th h of study were 1.4, 1.0, 1.2, 1.2 and 1.4 respectively and in standard drug the values were 1.2, 1.0, 1.0, 1.2 and 1.4 respectively (Table 2b).

Table 2a. Effect of the extract of leaves of *Murraya paniculata* (L.) Jack on castor oil induced diarrhoea in mice (Latent period)

Animal Group / Treatment	Dose(/kg, p.o.)	Latent Period (h)
Group-I (control) 1% tween-80	10 ml	0.86 ± 0.140
Group-II (positive control) Loperamide	50 mg	1.98 ± 0.114*
Group - III Ethanolic extract	500 mg	1.82 ± 0.119*

Values are expressed as Mean±S.E.M (n=5), **P*<0.001, p.o. = per oral.

Table 2b. Effect of the ethanolic extract of leaves of *Murraya paniculata* (L.) Jack on castor oil induced diarrhoea in mice (Number of stools)

Animal Group/Treatment	Dose (/kg, p.o.)	Period of study (h)	Total number of stool
Group-I (control) 1% tween-80 solution in water	10 ml	1 2 3 4 5	3.0 ± 0.304 3.2 ± 0.357 3.0 ± 0.295 3.4 ± 0.364 3.2± 0.390
Group-II (positive control) Loperamide	50 mg	1 2 3 4 5	1.2 ± 0.219* 1.0 ± 0.320* 1.0 ± 0.332* 1.2 ± 0.287* 1.4 ± 0.224*
Group-III Ethanolic extract	500 mg	1 2 3 4 5	1.4 ± 0.357* 1.0 ± 0.418* 1.2 ± 0.370* 1.2 ± 0.327* 1.4 ± 0.312*

Values are expressed as Mean±S.E.M (n=5), **P*<0.01, p.o. = per oral.

Anti-inflammatory activity

In the carrageenin induced rat paw edema model of anti-inflammatory activity, the ethanolic extract of dried leaves of *Murraya paniculata* (L.) Jack showed a significant inhibitory effect on the edema formation from the first hour to fifth hour. The highest inhibitory effect was found during the third hour where the inhibition was 26.43% (*P*<0.001) and 42.71% (*P*<0.001) at the dose of 300 and 600 mg/kg of body weight respectively. These findings were comparable to standard drug aspirin where the inhibition was 51.38% (*P*<0.001) at the dose of 150 mg/kg of body weight (Table 3).

Table 3. Effect of ethanolic extract of leaves of *Murraya paniculata* (L.) Jack on carrageenin induced rat paw edema

Animal group / Treatment	Time after carrageenin injection				
	1 hr	2 hr	3 hr	4 hr	5 hr
	Edema volume × 1000 (ml) (Percent inhibition)				
Control (1% Tween 80) 10 ml/kg; p.o.	16.65±0.57	167.80±2.15	249.10±2.32	285.30±2.62	232.70±2.47
Positive control Aspirin 150 mg/kg; p.o.	11.20±0.73* (32.73)	101.20±2.13* (39.69)	121.10±1.90* (51.38)	183.40±2.31* (35.72)	155.15±1.98* (33.32)
Test group-1 Ethanol extract 300 mg/kg; p.o.	13.70±0.44* (17.72)	131.20±2.52* (21.81)	183.25±2.64* (26.43)	229.20±2.80* (19.66)	191.45±2.71* (17.73)
Test group-2 Ethanol extract 600 mg/kg; p.o.	12.15±0.91* (27.03)	112.10±2.86* (33.19)	142.70±2.97* (42.71)	202.10±3.05* (29.16)	168.50±2.95* (27.59)

Values are expressed as Mean±S.E.M (n=6); **P* <0.001; p.o. = per oral

Discussion

Antidiarrhoeal activity of the ethanol extract of leaves of *Murraya paniculata* (L.) Jack was tested using the model of castor oil induced diarrhoea in mice[16]. Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase[18] or release prostaglandin[19]. The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanol extract of leaves of *Murraya paniculata* (L.) Jack might possess antidiarrhoeal activity.

Carrageenin induced rat paw edema model is one of the most widely used primary test for the screening of new anti-inflammatory agents[17]. The edema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and

serotonin[20] and the delayed edema is due to the release of bradykinin and prostaglandins[21-22]. It has been reported that the second phase of edema is sensitive to steroidal and non-steroidal anti-inflammatory agents[21]. The extract reduced the paw volume significantly from 1h to 5h in which the highest effects were found at the third hour. These results tend to suggest the probable anti-inflammatory activity of the extract. The inhibitory activity of the extracts observed in the first phase of carrageenin induced inflammation may be due to inhibition of early mediators, such as histamine and serotonin and the action on the second phase be due to the inhibition of bradykinin and prostaglandins.

In conclusion, it could be suggested that the crude ethanolic extract of leaves of *Murraya paniculata* (L.) Jack might possess antidiarrhoeal and anti-inflammatory activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential to confirm its pharmacological properties.

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