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Scavenging the Bioactivities of Golden champak (*Ochna squarrosa*) of Bangladesh

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ABSTRACT: Background: *Ochna squarrosa* L. (Family: *Ochnaceae*) is a flowering plant of Bangladesh. Traditionally the plant extract was used by the tribal people for snake bites and menstrual disorders. **Aim:** This study aimed to screen *O. squarrosa* leaf extract for analgesic, anti-diarrheal, and hypoglycemic potentials along with sleep-inducing properties. **Method:** The sun-dried leaves were powdered and macerated in 1.5 L methanol for 7 days. After filtration, the filtrate was concentrated to yield the crude methanol extract. An acetic acid-induced writhing test was performed to determine peripheral analgesic activity using diclofenac sodium as standard. The tail flicking response to the thermal stimulus was assessed to determine central analgesic activity. Castor oil was used to induce diarrhea in the assay for anti-diarrheal activity. Phenobarbitone-sodium-induced sleeping time was determined to assess the extract's sleep-inducing potential. **Results:** The crude methanol extract (400 mg/kg body weight) demonstrated 81.53 % inhibition of writhing as compared to 70.37 % by diclofenac sodium. At the same dose, the extract reduced diarrheal feces by 84.40 %. In the 1st hour of observation, the extract lowered blood sugar levels by 34.62 % and 36.34 % at 200 and 400 mg/kg body weight doses, respectively. The total duration of phenobarbitone sodium–induced sleep was decreased by the extract in a dose-dependent manner. **Conclusion:** The phytocomponents responsible for the observed activity should be isolated and involved in further assessment.

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INTRODUCTION:

Ochna squarrosa L. (Synonyms: *O. obtusata*) is locally known as konok chapa (Fig 1). Other common names include golden champak, mickey mouse plant, and ramdhan champa. This small sub-deciduous flowering plant belongs to *Ochnaceae* family. In Bangladesh, the plant occurs in Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, and Mymensingh. The plant also grows in the Indian subcontinent and Pakistan. According to 'The Flora of Bangladesh database, the bark is stomachic while leaves are used in menstrual

disorders. Santals (a tribal community) use the root extract as an antidote to snake bites [1]. The leaf extract has not been intensively studied for useful phytocomponents. Other species of the same genus have been reported to possess Ochnaflavone- a derivative of isoflavone [2]. Some analgesic and anti-inflammatory compounds have also been reported from *O. squarrosa* root extract [3].

Medicinal plants have been used for centuries for the cure of human ailments. Many modern medicines were initially isolated from natural sources and later have been synthesized in the laboratory for large-scale production. Bangladesh is blessed with numerous flora and fauna with enormous chances to possess bioactive compounds [4, 5]. It is necessary to screen these medicinal plants for biological activities to identify suitable candidates for phytochemical screening. With this objective, some medicinal plants with a long history of traditional uses have been studied for bioactivities [6, 7]. The present study was also an attempt to evaluate *O. squarrosa* leaf extract for analgesic, anti-diarrheal, hypoglycemic, and sleep-inducing properties for the first time. Herein, the results of our investigation are reported.

Fig 1. *Ochna squarrosa* L leaf and flower.

MATERIALS AND METHODS:

Drugs and chemicals:

Morphine was procured from Gonoshastho Pharmaceuticals Ltd., Dhaka, Bangladesh. Diclofenac sodium BP, loperamide BP, and pure phenobarbitone sodium were obtained from Incepta Pharmaceuticals Ltd., Bangladesh. The rest of the reagents and solvents were analytical grade and were supplied by Sigma-Aldrich, Germany. All the reagents were used as received.

Collection of plant materials:

O. squarrosa leaf was collected from Chittagong, Bangladesh. This collection was identified in Salar Khan Herbarium, Department of Botany, University of Dhaka, and a voucher specimen have been preserved here for this collection.

Extraction and fractionation:

The collected leaves were cleaned, sun-dried, and ground into a powder. 1.5 L methanol was used for the maceration of the powdered plant material. The plant sample was macerated for 7 days at room temperature and occasionally shaken. After that, the macerated plant material was filtered. The filtrate was concentrated to yield the crude methanol extract

Animals:

International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) supplied healthy Swiss-albino mice (average weight 25 g). The animals were kept in small cages bedded with flake wood shavings with easy access to food and water. The animals were acclimatized to the standard laboratory environment (room temperature 25±2 °C; relative humidity 55 to 60 %; 12 h light-dark cycle) for 15 days before being involved in any experiments. The animals were kept fasting overnight before they were picked up randomly for experiments [8].

After completion of each experiment, the animals were euthanized using carbon dioxide gas and the cervical dislocation method in order to minimize physical and mental suffering. The Swiss Academy of Medical Sciences formulated Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) were followed. All the experiments were performed under the approval of the Ethics Committee of the State University of Bangladesh.

Evaluation of peripheral analgesic potential by the acetic acid-induced writhing method:

The peripheral analgesic potential was determined using the acetic acid-induced writhing method [9]. After treating the test and control groups with 1% volume/volume acetic acid (10 ml/kg dose), the number of writhing exhibited by each animal was counted for 10 min.

Evaluation of central analgesic activity by tail flicking method:

In the central analgesic activity assay, a radiant heat source was used to observe the tail-flicking response [10,11]. The tail withdrawal time was recorded at the 30th, 60th, and 90th min of administration of test samples and standard.

Anti-diarrheal activity by castor oil challenge:

For the determination of anti-diarrheal activity, 1 ml of pure analytical-grade castor oil was fed to mice to induce diarrhea [12]. Here, loperamide (50 mg/kg) was used as the positive control.

Phenobarbitone- sodium-induced sleeping time test:

The assay was conducted according to the method of Williamson et al., (1996) [13]. In this study, phenobarbitone sodium (25 mg/kg body weight) was administered intraperitonially to induce sleep. The time of onset of sleep and total sleeping time was recorded.

Statistical analysis:

Three replicates of each sample were used for statistical analysis and all of the values are expressed as the mean ± standard deviation (SD). The results were evaluated by a two-tailed non-parametric paired student's t-test.

RESULTS:

This research was an effort to evaluate the crude methanol extract of *O. squarrosa* leaf for different bioactivities in an animal model.

Table 1. Effect of the crude methanol extract of *O. squarrosa* on acetic acid induced writhing in mice.

Groups	Number of writhing	Inhibition of writhing (%)
Control (10 ml/kg)	15.32±0.20	-
Diclofenac Na (Standard) (50 mg/kg)	4.54±0.21**	70.37
Methanolic crude extract (200 mg/kg)	4.11±0.13**	73.17
Methanolic crude extract (400 mg/kg)	2.83±0.24**	81.53

Values are expressed as mean ± Standard Deviation; **p<0.01.

The crude methanol extract of *O. squarrosa* leaf revealed statistically significant analgesic activity in the mice model. The mean number of writhing was significantly lower ($p < 0.01$) in mice receiving the test samples when compared to that of the negative control group. The crude methanol extract demonstrated 73.17 and 81.53 % inhibition of writhing at 200 and 400 mg/kg body weight doses, respectively in contrast to the standard, diclofenac sodium inhibiting writhing by 70.37 % (Table 1).

On the other hand, the test sample did not demonstrate a noteworthy central analgesic effect as compared with the standard used in the experiment (Table 2 and 3).

Table 2. Effect of morphine and *O. squarrosa* extract on tail flicking time of mice.

Groups	Latency period		
	30 min	60 min	90 min
Control (10 ml/kg)	6.40±0.34	6.40± 0.23	6.00± 0.14
Morphine (Standard) (2 mg/kg)	10.22± 0.04**	9.19± 0.33**	8.48± 0.29*
Methanolic crude extract (200 mg/kg)	4.67± 0.08	4.44± 0.24	5.73± 0.05
Methanolic crude extract (400 mg/kg)	4.84± 0.19	4.59± 0.31	4.33± 0.07

Values are expressed as mean ± Standard Deviation; *p<0.05, **p<0.01.

Table 3. Anti-nociceptive activity of the crude methanol extract of *O. squarrosa.*

Groups	% Pain inhibition		
	30 min	60 min	90 min
Morphine (standard) (2 mg/kg)	59.68	43.59	32.50
Methanolic crude extract (200 mg/kg)	27.03	30.63	4.50
Methanolic crude extract (400 mg/kg)	24.38	28.28	27.84

The crude methanol extract of *O. squarrosa* (400 mg/kg body weight) reduced diarrheal feces by 84.40 % which was found to be more prominent when compared to that of the standard Loperamide (70.67 %) (Table 4).

In the hypoglycemic activity assay, the extract lowered the blood sugar to a significant extent when compared with that of the control in the 1st hour of observation. However, the effect wearied down in the subsequent hours of observation (Table 5 and 6).

O. squarrosa extract decreased the total duration of phenobarbitone sodium–induced sleep in a dose-dependent manner (Table7).

Table 4. Effect of the crude methanol extract of *O. squarrosa* on castor oil (1 ml/mice) induced diarrhea in mice.

Groups	Number of diarrheal feces	% Reduction of diarrhea
Control (10 ml/kg)	16.80±0.19	---
Loperamide (Standard) (50 mg/kg)	4.81±0.33**	70.67
Methanolic crude extract (200 mg/kg)	4.40±0.11**	75.61
Methanolic crude extract (400 mg/kg)	2.62±0.23**	84.40

Values are expressed as mean ± Standard Deviation (n = 3); **p<0.01.

Table 5. Effect of *O. squarrosa* flower extract on blood glucose of Swiss albino mice.

Groups	Blood Glucose Level (mmol/L)			
	0 min	1st hour	2nd hour	3rd hour
Control (10 ml/kg)	6.98± 0.12	10.40± 0.27	7.46± 0.11	7.08± 0.21
Standard Group (5 mg/kg)	6.26± 0.26	3.76± 0.11**	3.66± 0.40**	3.44± 0.34**
Methanolic crude extract (200 mg/kg)	6.80± 0.22	6.80± 0.41*	6.67± 0.23	6.45± 0.30
Methanolic crude extract (400 mg/kg)	6.80± 0.31	6.61± 0.19*	6.21± 0.17	6.17± 0.19

Values are expressed as mean ± Standard deviation (n = 3); **p<0.01, *p<0.05.

Table 6. Percentage of blood glucose level reduction in Swiss albino mice.

Groups	% Reduction of blood glucose level		
	1st hour	2nd hour	3rd hour
Standard Group (5 mg/kg)	63.85	50.94	51.41
Methanolic crude extract (200 mg/kg)	34.62	10.58	8.89
Methanolic crude extract (400 mg/kg)	36.44	16.75	12.85

DISCUSSION:

Two new furano-flavonoids, 4′-hydroxy-3′-methoxyfurano flavone, and 3′, 4′dihydroxyfurano flavone, two new chalcone dimers, lophirone A and lophirone H were isolated from *O. squarrosa* root and bark. All these compounds demonstrated notable analgesic and anti-inflammatory properties [3]. Squarrosin, an isoflavone extracted from the root extract of the species, has been reported to possess strong analgesic and anti-inflammatory effects [14]. The observed peripheral analgesic activity might be due to the presence of these compounds in the leaf extract. An aqueous extract of *O. schweinfurthiana*, another species of the same genus also showed prominent analgesic and anti-inflammatory activity [15].

Table 7. Effect of the crude methanol extract of *O. squarrosa* on phenobarbitone sodium–induced sleeping time.

Groups	Time of onset of sleep (min)	Total sleeping time (min)
Control (10 ml/kg)	16.34±0.50	123.32±0.35
Crude methanol extract (200 mg/kg)	24.11±0.06	78.41±0.21
Crude methanol extract (400 mg/kg)	27.05±0.19	59.33±0.34

Values are expressed as mean ± Standard deviation (n = 3).

Plant extracts rich in flavonoids have been reported to possess antidiarrheal activity [16-18]. Flavonoids inhibit intestinal motility and hydro electrolytic secretions [19]. *Ochna* species are known to be rich in bioflavonoids and isoflavonoids [20-22]. The flavonoids found to be present in the root and bark extract might also be present in the leaves and demonstrate the observed anti-diarrheal effect [3].

Another species of the same genus, *O. obtusata* was reported to possess antidiabetic activity [23]. It can be assumed that some common phytocomponents between the two species might contribute to the observed hypoglycemic effect.

CONCLUSION:

Traditional medicines have been used for centuries for the treatment of many human diseases. Mother nature has given the world not only the disease but also its cure within her natural resources. Herbal medicines are gaining popularity because of their long history of use and being available in ‘close to the natural state’ formulations. More often, people find modern medicines

costly and associated with side effects. All these factors contribute to the increasing popularity of traditional medicines. In this study, the crude methanol extract of *O. squarrosa* leaf was screened for peripheral and central analgesic, anti-diarrheal, hypoglycemic activities, and sleep induction properties. *O. squarrosa* leaf extract demonstrated highly significant peripheral analgesic and anti-diarrheal activities. It is therefore necessary to screen the extract for possible compounds that might be useful for pain management, arthritis, and diarrheal diseases. The previously reported high flavonoid content in this species might suggest that some flavonoid-type compounds might contribute to the observed activity. Thus, *O. squarrosa* is a very suitable candidate for the identification of useful phytocomponents that can be further studied and structurally modified for large-scale commercial production of the active moiety.

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