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Antidiarrheal and Analgesic Activities of *Bouea oppositifolia* (Roxb.) Adelb. in Experimental Animal Model

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Abstract

The current study was designed to evaluate the antidiarrheal and analgesic activities of the methanol extract of *Bouea oppositifolia* (Roxb.) Adelb. leaves through *iv vivo* studies in Swiss Albino mice. Oral administration of the extract at doses of 200- and 400-mg/kg of body weight demonstrated statistically significant ($p < 0.05$) antidiarrheal activities in castor oil-induced diarrheal mice. The extracts and standard loperamide alleviated diarrhea by margins of 31.82-, 45.45- and 68.17%, respectively. Potential of the plant extract to effectuate analgesia was ascertained both centrally and peripherally. In tail immersion method for the determination of central analgesic activity, the plant extract at both doses enhanced pain tolerance to maximum extents of 192.76% and 221.09%, respectively, compared to standard morphine (419.57%) after 90 minutes of sample administration. Comparable levels of central analgesia were also observed for both doses of the plant extracts at 30 and 60 minutes of pharmacological intervention and the p value of less than 0.05 illustrated statistical significance of the activity. On a similar note, potent peripheral analgesic activities were observed for both doses of the extract as evident by 45.45- and 54.55% inhibitions of acetic acid induced writhing responses, respectively. Compared to the standard acetylsalicylic acid characterized by 78.18% inhibition, the peripheral analgesic activity of the plant was found to be statistically significant ($p < 0.05$). The results of the study are indicative of the presence of potentially bioactive phytoconstituents with antidiarrheal and analgesic properties which might lead to newer drug candidates in future.

Key words: *Bouea oppositifolia*, antidiarrheal, central analgesic, peripheral analgesic.

Introduction

By virtue of their genetic ancestry and profound resilience within variant ecological systems, the plant kingdom have been illustrated with extensive mode of chemodiversity which have, in turn, established them as invaluable source of chemically complex bioactive molecules (McChesney, 1996). Furthermore, a long history of human utilization for any plant, imparts a relatively higher safety profile for the bioactive phytoconstituents available within the plant. Identification and characterization of such phytochemical entities generally commences with random biological assays of plant extracts involving *in vitro* simulations or experimental animal models. These assessments, in turn, enables us to ascertain the capacities of the plant components to demonstrate discernable biochemical interactions within the biological system (Fabricant and Farnsworth, 2001). Subsequently, plant extracts with promising pharmacological activities are subjected to bioactivity guided phytochemical analysis which may ultimately lead to either novel drug molecules or candidate lead compounds for future drug developments (Sasidharan *et al.*, 2011).

Belonging to the Anacardiaceae family, the genus *Bouea* is a relatively newer and smaller genus of the angiosperms. Among only the three accepted species of the genus, *Bouea oppositifolia* (Roxb.)

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Adelb. (Commonly known as Plum mango or Gandaria) is endemic to southeastern part of Asia including China, Myanmar, Thailand, Indonesia, Malaysia, Laos, Cambodia, Vietnam and the Andaman Islands. The plant is most popularly known by its synonym *B. microphylla*. Griff. (Lim, 2012; The Plant List, 2013). The plant is also located in the Khagrachari and Chittagong districts of Bangladesh where it is locally known as Uri Aam and considered as a critically endangered species (Rahman, 2018). The plant is mainly known for its fruits and also used in the household for ornamental purposes. Both the unripe and ripe fruits were illustrated with rich contents of carbohydrates, fats, amino acids, minerals including sodium, potassium, calcium, iron and phosphorus, as well as different vitamins like retinol, thiamine, riboflavin, niacin and carotenoids (Rajan and Bhat, 2020). Phytochemical analysis with the fruits revealed the presence of at least 82 and 121 volatile constituents in unripe and ripe fruits, respectively. Mono- and sesquiterpene hydrocarbons constituted the major extent of the volatile content (32.89% and 29.28%, respectively) along with variable extent of acid, ester, alcohol, aldehyde and ketone hydrocarbons (Rajan and Bhat, 2017). Another study investigating the antioxidative potential of the plant indicated the methanol extract of unripe fruit to be capable of scavenging DPPH and $ABTS^{+}$ free radical to an extent of 77.69% and 99.76%, respectively (Rajan and Bhat, 2016). Although, a number of variant medicinal properties has been associated occasionally with the plant, none of those activities were investigated scientifically. Hence, a systematic biological investigation of the plant was endeavored to evaluate potential pharmacological properties.

Materials and Methods

Preparation of the plant material: Fresh leaves of *B. oppositifolia* were collected from the Botanical Garden, Dhaka, Bangladesh and voucher specimen was deposited in Bangladesh National Herbarium (BNH) for future reference (Accession no. DACB-56286). Properly cleaned leaves were shade-dried and ground into coarse powder. Then 650 g of this powder was completely immersed in 3000 ml methanol and kept in that condition for 15 days with occasional shaking and stirring. Afterwards, the content was filtered firstly through a cotton plug and then through Whatman No. 1 filter paper. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 40°C temperature to obtain crude methanol extract (MEBO, 73.5 gm).

Drugs and reagents: Methanol, Tween-80, high quality castor oil and acetic acid were procured from local market. Renowned pharmaceutical companies were contacted for acquiring different standard drugs including loperamide (Square Pharmaceuticals Ltd.), morphine (Gonoshasthaya Pharmaceuticals Ltd.), acetyl salicylic acid (Essential Drugs Company Ltd.) and normal saline (Opsonin Pharmaceuticals Ltd.). All other chemicals and solvents were of analytical grade.

Experimental animal: Swiss albino mice (25-35 g) of either sex, aged 4-5 weeks were obtained from the Department of Pharmacy, Jahangirnagar University, Bangladesh. The mice were maintained in appropriate conditions in the animal house of the State University of Bangladesh (SUB). Standard rodent feed were provided as required. Minimal physical exertion and pain induction was ensured for the experimental animals in accordance with the Federation of European Laboratory Animal Science Association's guidelines and recommendations. Sixteen Swiss albino mice were randomly divided into four groups of four animals in each group for each bioassay: positive control, negative control and two test groups receiving MEBO at doses of 200- and 400-mg/kg of body weight.

Determination of antidiarrheal activity: Antidiarrheal activity was assessed in castor oil induced diarrheal mice following the method developed by Agbor *et al.* (2014). Solution of 1% Tween-80 in normal saline was administered orally as the negative control at the dose of 10 ml/kg b.w. The positive control group was administered with loperamide at a dose of 50 mg/kg b.w. orally. Two test groups received the methanol extract of *B. oppositifolia* at doses of 200- and 400-mg/kg b.w.

Individual case was allocated for each mouse with regular change of the floor lining at the end of each hour. The number of diarrheal feces excreted by the animals was recorded for 5 hours after the intervention.

Determination of central analgesic activity: Tail immersion method estimates central analgesic activity of any sample by determining the extent of delaying pain response in mice (Dewey *et al*., 1970). Test samples and negative control were given orally by means of a feeding needle whereas positive control morphine was administered subcutaneously at the beginning. To determine the response of mice towards painful stimulus, 1-2 cm of the tail of each mouse was marked and immersed in warm water kept constant at 55°C. The time required by the mice to deflect their tails in response to that warm water was recorded as the pain response time. The first reading was discarded and the reaction time was recorded as a mean of the next three reading. A latency period of 20 seconds was defined as complete analgesia and the measurement was stopped to avoid injury to mice. The latent period of tail-flick response i.e. pain response time, was determined before the administration of drugs, followed by sample administration and a 30 minutes time period to ensure proper absorption. Then, the tail immersion times were measured at 30, 60 and 90 minutes of sample administration.

Determination of peripheral analgesic activity: Peripheral pain sensation was initiated by administration of 1% acetic acid through intra-peritoneal injection which, in turn, was physically manifested in the form biting or writhing of mice. Any treatment capable of producing peripheral analgesia will diminish the extent of such response (Saha and Ahmed, 2009). To start with, negative control, positive control and test sample were given orally by means of a feeding needle. A 30 minutes interval was allowed after oral administration to assure proper absorption, followed by the intra-peritoneal administration of acetic acid (0.1 ml/10 g b.w.). About 5 min after that, pain responses were recorded as number of squirms or writhing for 10 minutes.

Result and Discussion

In vivo evaluation of antidiarrheal activity in castor oil induced diarrheal mice (Table 1) demonstrated a statistically significant ($p < 0.01$) 68.17% inhibition of diarrheal response as exerted by the standard drug, loperamide. Compared to this, moderate degree of statistically significant ($p < 0.05$) antidiarrheal potential was illustrated by the methanol extract of leaves of *B. oppositifolia* characterized by 31.82 and 45.45% reductions of diarrhea at doses of 200 and 400 mg/kg, respectively.

Table 1. Antidiarrheal activity of methanol extract of *B. oppositifolia* in castor oil induced diarrhea in Swiss Albino mice.

Test groups	Dose (mg/ kg of body weight)	Number of diarrheal feces (Mean ± SEM)	Inhibition of diarrhea (%)
Negative control (Normal saline)	10 ml	7.33	-
Positive control (Loperamide)	50	2.33	68.17**
Methanol extract of *B. oppositifolia*	200	5.00	31.79*
	400	4.00	45.43*

All values are expressed as mean ± SEM (n = 3), *indicates statistical significance of $p < 0.05$ whereas **indicates statistical significance of $p < 0.01$.

In the tail immersion test for the determination of central analgesic activity, the methanol extract of *B. oppositifolia* at doses of 200 and 400 mg/kg b.w., enhanced pain tolerance by 192.76- and 221.09%, respectively, after 90 minutes of sample administration, compared to that of the standard morphine (419.57% after 90 minutes). Similar patterns of analgesic activities were also observed at

30 and 60 minutes of sample administrations. All the responses were found to be statistically significant ($p < 0.05$) indicating potent central analgesic potential for the plant (Table 2).

The peripheral analgesic activity of the methanol extract of *B. oppositifolia* assessed through acetic acid induced writhing test (Table 3) revealed significant activity ($p < 0.05$) for the standard acetylsalicylic acid as indicated by 78.18% inhibition of writhing or pain response. Similarly, the methanol extract of the plant at doses of 200- and 400-mg/kg b.w. exhibited comparable analgesic activity to the standard as evident by 45.45% and 54.55% inhibition, respectively.

Table 2. Central analgesic activity of methanol extract of *B. oppositifolia* in tail immersion method.

Test groups	Dose (mg/kg)	Time of tail immersion (Mean ± SEM)			Elongation of pain inhibition (%)		
		30 min	60 min	90 min	30 min	60 min	90 min
Negative control	10 ml	2.03 ± 0.10	2.36 ± 0.11	2.62 ± 0.33	-	_	_
Positive control (Morphine)	2	5.88 ± 0.12	9.72 ± 0.09	13.60 ± 0.06	189.02	311.14	419.57
MEBO	200	3.74 ± 0.08	5.81 ± 0.07	7.68 ± 0.26	84.10	145.70	192.76
MEBO	400	3.89 ± .18	6.57 ± 0.09	8.42 ± 0.47	91.31	177.86	221.09

All values are expressed as mean ± SEM (n = 3), all elongation values were found to be statistically significant ($p < 0.05$).

Table 3. Peripheral analgesic activity of *B. oppositifolia* in acetic acid induced writhing test of Swiss Albino mice.

Test groups	Dose (mg/ kg of body weight)	Average number of writhing (Mean ± SEM)	Writhing/ pain response (%)	Inhibition of pain response (%)
Negative Control (Normal saline)	10 ml	18.33 ± 0.33	100	-
Positive control (Acetylsalicylic acid)	50	4.00 ± 0.58	21.82	78.18
Methanol extract of *B. oppositifolia*	200	8.33 ± 1.33	54.55	45.45
	400	10.00 ± 0.58	45.45	54.55

All values are expressed as mean ± SEM (n = 3), all elongation values were found to be statistically significant ($p < 0.05$).

Conclusion

Our preliminary studies with the methanol extract of the leaves of *B. oppositifolia* has demonstrated prominent central and peripheral analgesia as well as antidiarrheal activity within the biological system of Swiss Albino mice. This, in turn, may provide scientific basis to warrant future in-depth investigations into the plant material to identify possible bioactive constituents with aforementioned pharmacological activities.

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