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Analgesic activities of *Geodorum densiflorum*, *Diospyros blancoi*, *Baccaurea ramiflora* and *Trichosanthes dioica*

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Abstract

Geodorum densiflorum, *Diospyros blancoi*, *Baccaurea ramiflora* and *Trichosanthes dioica* are four important medicinal plants used traditionally in various diseases. Different parts of these plants have been used in different painful conditions. Therefore, the present study was designed to investigate the analgesic activities of methanol extract of pseudobulb of *G. densiflorum*, seeds of *D. blancoi*, Seeds of *B. ramiflora* and aerial parts of *T. dioica* using acetic acid-induced writhing and tail immersion test. The results demonstrated that aerial parts of *T. dioica* and seeds of *B. ramiflora* possess significant analgesic activity in both acetic acid-induced writhing as well as in tail immersion test. Pseudobulb of *G. densiflorum* and seeds of *D. blancoi* also possess moderate analgesic activity.

Keywords: Analgesic, Acetic acid induced writhing, Tail immersion, *Geodorum densiflorum*, *Diospyros blancoi*, *Baccaurea ramiflora*, *Trichosanthes dioica*.

Introduction

Nowadays, the use of medicinal plants for alleviating diseases is growing day by day around the world especially in Asia. Fewer side effects of medicinal plants play a big role for the popularity of the medicinal plants. Currently, many established drugs are not working against intended diseases due to drug resistance. In addition, new diseases are emerging which are threatening for human race^[1]. Thus, we have to have new arsenals against those threats. Moreover, Plants are the source of versatile chemical compounds. Herbal medicines are being increasingly used to treat clinical illnesses despite of having a little understanding of their way of action^[2]. Analgesic drugs has not been successful in all cases due to the adverse effects, therefore new drugs lacking those side effects are always a major area of interest^[3]. For developing new drugs, the traditional knowledge about medicinal plants has served as a basis undoubtedly^[4]. The costs of the drugs are also increasing significantly. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed^[5]. Painful diseases require safe, effective and potent drugs for treatment though of recent therapeutic advancement in pain therapies^[6]. Therapeutic regimens available for the management of peripheral and centrally acting pain often trigger potentially serious adverse effects^[7, 8]. It is the demand of time to stand with ethno pharmaceuticals as a therapeutic modality to treat diseases. Search for new pharmacologically active analgesics from plants in the near past has led to the discovery of some clinically useful drugs, such as aspirin and morphine^[9, 10].

Geodorum densiflorum (Lam.) Schltr. (Orchidaceae), locally known as 'Shankhamul', is an endangered terrestrial orchid found in Bangladesh and some areas of India. *G. densiflorum* is a medicinal plant that has been traditionally used for the treatment of various diseases. The root is used as an insecticide, to treat irregular menstrual cycle in women^[11] and in wound healing. The tuber and rhizome are used to treat impotency^[12, 13] and to increase sperm density^[14]. The pseudo bulb is used to treat diabetes^[15-16] and carbuncles^[17]. Pharmacological studies on various plant parts of *G. densiflorum* have reported its antimicrobial^[18-20], antioxidant^[20], cytotoxic^[20, 21], thrombolytic^[22], analgesic and sedative^[23] properties. Phytochemical investigation of the leaves and pseudobulb of orchid have reported presence of flavonoids, terpenoids, alkaloids and steroids.

Baccaurea ramiflora (Euphorbiaceae), locally known as 'Latkan', is a popular fruit in Bangladesh. It is a tall evergreen tree growing widely in the highland of Bangladesh, India, Burma, Thailand, Vietnam, Laos, Cambodia, Malaysia and China^[24]. It is utilized in Chinese Dai medicine as an antiphlogistic and anodyne against rheumatoid arthritis, cellulites, and abscesses^[25]. The fruit was reported to possess antiviral and antioxidant and the stem bark of

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the plant was reported to have diuretic activity [26]. The stems and the leaves evidently showed antioxidant activities [27]. Studies on *B. ramiflora* revealed the presence of phenols, flavonoids, flavonols, proanthocyanidins, 6'-*O*-vanilloylisotachioside, 6'-*O*-vanilloyltachioside, 4'-*O*-6-*O*-vanilloyl)- β -D-glucopyranosyl tachioside D, ramifloside, sapidolide A, 6'-*O*-vanilloyl picraquassioside D and 6'-*O*-vanilloylicaraside B5 [28, 29]. Pharmacological studies have reported its potential as antifungal [30] and anti-inflammatory [31] agents.

Diospyros blancoi A. DC (Ebenaceae), locally known as 'bilati gab', is a common fruit in Bangladesh. This plant is native to Philippine but also grows in Southeast Asia. Its traditional uses include wounds, diarrhea, dysentery, aphthous stomatitis, snakebites, heart problems, hypertension, spider bites, stomach aches, diabetes, and eczema [32, 33]. Studies on chemical constituents have reported the presence of lupeol, maslinic acid, betulin, ursolic acid, taraxerol, lanostane, plumbagin, diospyrin, isoquercetin, quercetin glucoside habibone, diospyrin 80-hydroxydiospyrin benzyl butyrate, butyl butyrate and (E)-cinnamyl butyrate 6 [34-36]. Pharmacological studies showed significant analgesic [32], antimicrobial [37], antioxidant [32], antidiarrhoeal [37] and anti-inflammatory [32] activities.

Trichosanthes dioica (Cucurbitaceae), locally known as 'potoi', is a common vegetable in Bangladesh. The herb is grown widely in Bangladesh, India, Pakistan, Burma, and Sri Lanka [38]. Besides the wide spread use as vegetable, this plant has significant medicinal values. The herb has been traditionally used in the treatment of constipation, fever, skin infection, wounds, appetite and digestion [39-41]. Leaves are used as febrifuge, tonic, oedema, alopecia and in liver diseases [42]. Fruits are used in fungal infection in nails [43]. Phytochemical screening has revealed the presence of vitamin A, vitamin C, tannins, saponin, phenol and flavonoids from *T. dioica* [44, 45]. Chemical constituents isolated from this plant include colocythin, trichosanthin, hentriacontane, cucurbita-

5,24-dienol [46], an amorphous saponin, a phyto sterol, a non-nitrogenous bitter glucosidic principle, essential oil, fixed oil and tannin [47], fatty acids like elaeostearic, linoleic, oleic and saturated acids [46]. Pharmacological studies have reported antidiabetic [48], antioxidant [49], antidiarrhoeal [49], antitumor [50], anti-inflammatory [51] and lipid lowering [52] activities of different parts of the plant.

To support the traditional uses of these precedent plants with scientific evidence, the present study was performed to evaluate the potential analgesic activity by acetic acid-induced writhing (chemical-induced) and tail immersion (heat-induced) method in swiss albino mice with the methanol extracts of the pseudo bulb of *G. densiflorum*, seeds of *B. ramiflora*, seeds of *D. blancoi* and aerial parts of *T. dioica*. Despite of a large number of pharmacological investigations carried out to explore the therapeutic potentials of the aforementioned four plants, to the best of our knowledge no combined study was performed yet with the plant parts that are used in the present study.

Materials and Methods

Plant materials and extract preparation

The selected plants were collected from different places of Bangladesh (Table 1) during the fruiting season of the plants and authenticated by the experts of National Herbarium Mirpur, Bangladesh where voucher specimens were deposited for future references (Table 1). The plants were then cleaned, dried and grounded. Maceration method has been followed to prepare the extracts using 500 ml of methanol for each sample. The supernatant liquid (extract) was then either decanted or filtered through a plug of cotton or glass wool. The process was repeated for complete extraction. The extracts were then poured in five Petri dishes and dried by evaporation until the extracts become concentrated and almost all the solvents have evaporated. Then the extract containing Petri dishes were labeled with specific information [53].

Table 1: Plant material collection and extract preparation.

Plant Name	Used Part	Place and Time of Collection	Used solvent	Weight of Dry powder
<i>Geodorum densiflorum</i>	Pseudobulb	Dhaka, 2013	Methanol	250g
<i>Diospyros blancoi</i>	Seed	Manikgong, 2013	Methanol	300 g
<i>Baccaurea ramiflora</i>	Seed	Norsingdhi, 2013	Methanol	300g
<i>Trichosanthes dioica</i>	Aerial parts	Rajshahi, 2013	Methanol	350g

Chemicals

Diclofenac sodium and 0.9% sodium chloride solution were generously gifted by Square Pharmaceuticals Ltd, Bangladesh and Orion Infusion Ltd, Bangladesh. Morphine sulphate (sigma, USA) and acetic acid (Merck, Germany) were purchased.

Animals

Swiss albino mice (22–25 g) of either sex were obtained from the Animal Resources Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (icddr, b). The animals were maintained at constant room temperature (25.0 \pm 2.0 $^{\circ}$ C), humidity 55-65% and 12 h light: 12 h dark cycle. Pellets of mice food prepared by icddr, b were given to the mice with fresh water *ad libitum*.

Acetic acid-induced writhing test

Acetic acid-induced abdominal writhing reflex pain model was used to study the analgesic activity of the plants. Mice used in this study were randomly grouped (5 mice in each group) as Group 1 (negative control group) received 10 ml/kg body weight normal saline, group 2 (positive control group) received 10 mg/kg of diclofenac sodium; group 3 and 4 received 200 and 400 mg/kg of methanol extract of *G. densiflorum* pseudobulb (MPGD), group 5 and 6 received 200 and 400 mg/kg of methanol extract of *D. blancoi* seeds (MSDB), group 7 and 8 received 200 and 400 mg/kg of methanol extract of *B. ramiflora* seeds (MSBR) and group 9 and 10 received 200 and 400 mg/kg of methanol extract of *T. dioica* aerial parts (MATD) using oral gavage. 0.6% acetic

acid (10ml/kg) was administered intraperitoneally (I.P) to all the mice to induce abdominal contractions or writhings 15 and 30 minutes after drug and extract administration. Five minutes after the injection of acetic acid, the analgesic effect was assessed in each mouse for 10 minutes and recorded [54]. The contractions of the abdomen, elongation of the body, twisting of the trunk and/or pelvis ending with the extension of the limbs were considered as complete writhing.

Tail immersion test

Tail immersion test was performed to evaluate the central analgesic property. This procedure is based on the observation of prolonged tail withdrawal time from hot water in mice due to prior treatment with morphine like drugs [55]. One to two cm of tail of the mice pretreated with morphine or MPGD, MSDB, MSBR, MATD were immersed in warm water kept constant at $52 \pm 1^\circ\text{C}$. The latency between tail submersion and deflection of tail was recorded. Latency period between 1.5 and 3.5 s showed by the mice were selected for this experiment and the pre-treatment latency was recorded. A latency period of 20 s was maintained to avoid tail tissue damage in mice. The latency period of the tail-withdrawal response was taken as the

index of anti-nociception and was determined at 30, 60, 90, and 120 min after the administration of the drug and extracts.

Statistical analysis

The statistical analyses of the results were carried out by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test using SPSS 17.0. $p < 0.05-0.001$ were considered as statistically significant.

Results

Acetic acid-induced writhing

Table 2 shows various level of reduction of writhing response upon the administration of the extracts compared with the standard drug and the control group in mice. All of the extracts have given in two different doses to see any possible dose dependent increase of activity. This is clear from the present study that all of the extracts in larger doses gave high inhibitory effect. The standard drug, Diclofenac sodium (10 mg/kg), inhibited 81.55% writhing compared to the control group. Among the extracts MSBR in 400 mg/kg dose inhibited 69.02% writhing followed by MATD 66.07%. MATD has inhibited the writhing by 53.66% by the 200 mg/kg dose.

Table 2: Analgesic activities on acetic acid-induced writhing response by the extracts.

Treatment	Dose (mg/kg)	Number of writhing	Inhibition (%)
Vehicle	-	32.60±0.43	
Diclofenac Sodium	10	06.20±1.71*	81.55
MPGD	200	17.40±2.06	22.32
MPGD	400	09.40±1.50	58.03
MSDB	200	13.25±1.25*	42.39
MSDB	400	09.60±0.50*	57.14
MSBR	200	13.5±1.55	41.30
MSBR	400	07.10±0.43*	69.02
MATD	200	10.40±0.50*	53.60
MATD	400	07.60±0.50*	66.07

Each value is presented as the mean \pm SEM (n=5). MPGD =Methanol extract of *G. densiflorum* pseudobulb, MSDB= Methanol extract of *D. blancoi* seeds, MSBR= Methanol extract of *B. ramiflora* seeds and MATD= Methanol extract of *T. dioica* aerial parts. * $p < 0.001$ compared with the control group (Dunnett's test).

Tail immersion test

The analgesic activity of the extracts and morphine demonstrated in tail immersion test are given in (Table 3). In tail immersion method, Morphine showed highest increase in the latency period to hot-water induced thermal stimuli ($p < 0.001$). MATD and MSBR in both doses have also

significantly increased the latency period compared to the other two extracts. The responses given by the mice have followed a dose dependent increase in case of all of the extracts (Table 3). MPGD and MSDB showed moderate increase in the latency period.

Table 3: Analgesic activities given by the extracts using tail immersion test.

Treatment	Dose (mg/kg)	Response Times (in seconds)				
		Pretreatment	30 min	60 min	90 min	120 min
Control	0.1ml/mice	2.83±0.48	2.62±0.37	2.68±0.46	2.66±0.43	2.59±0.48
Morphine	5	2.66±0.56	5.42±0.47*	6.21±0.41*	4.64±0.31*	3.57±0.40
MPGD	200	2.31±0.37	3.25±0.47	3.98±0.17	3.14±0.12	2.62±0.24
MPGD	400	2.39±0.22	3.46±0.17	4.26±0.46	5.19±0.29	4.06±0.13
MSDB	200	2.96±0.10	3.46±0.16	4.22±0.34	4.38±0.24	3.94±0.51
MSDB	400	2.65±0.15	3.38±0.16	4.85±0.29*	4.94±0.30	3.62±0.44
MSBR	200	2.66±0.12	3.66±0.35	4.52±0.32	3.88±0.34	2.97±0.17
MSBR	400	2.26±0.16	3.40±0.06	5.73±0.32*	5.93±0.32*	4.46±0.16
MATD	200	2.46±0.31	3.46±0.17	5.47±0.23	3.33±0.18	3.13±0.23
MATD	400	2.54±0.49	3.87±0.35*	5.44±0.36*	4.16±0.34*	3.14±0.24

Each value is presented as the mean \pm SEM (n=5). MPGD =Methanol extract of *G. densiflorum* pseudobulb, MSDB= Methanol extract of *D. blancoi* seeds, MSBR= Methanol extract of *B. ramiflora* seeds and MATD= Methanol extract of *T. dioica* aerial parts. * $p < 0.001$ compared with the control group (Dunnett's test).

Discussion

Assessment of analgesia by natural products can be done by various pharmacological models of pain [56]. For peripherally acting drugs, the acetic acid-induced abdominal constriction test is usually used. Endogenous mediators as well as arachidonic acid, prostaglandins are liberated for the induction of pain [57]. Writhing can be defined as the constriction of the abdominal muscles along with the extension of forelimbs and elongation of the body [56]. Present study demonstrated the peripheral and central antinociceptive effects of the extracts assessed by chemical (writhing test) and thermal (tail-immersion) models of nociception. The results of the current study showed that MATD and MSBR have significantly reduced the abdominal constriction induced by acetic acid in a dose dependent manner. MSDB and MPGD have also demonstrated dose dependent reduction of writhing. Therefore, it could be suggested that MATD and MSBR might contain some pharmacologically active molecules that are responsible for eliciting analgesic activity. Tail immersion model is an acute pain model where tail withdrawal response is considered to be discriminating for centrally acting analgesics [58, 59]. Peripherally acting analgesics are known to be inactive in these types of stimuli [60]. In this study, the increased reaction times of the animals treated with the extracts to the thermal induced pain simply imply the analgesic potentials of the extracts. However, thermal induced hyperalgesia attenuated by the extracts are not as significant as chemical induced hyperalgesia. The results of the study suggest that *T. dioica* and *B. ramiflora* have significant analgesic potentials and they must contain pharmacologically active phytoconstituents that may act centrally and peripherally. However, to understand the exact mechanism of action, future study should be designed to isolate the active constituents responsible for the specified effect.

Conclusion

In conclusion, the results of this study demonstrate that *T. dioica* and *B. ramiflora* possess significant analgesic activity both by peripheral and central mechanisms. Moreover, this study also provides a pharmacological rationale for the folk uses of these plants in painful conditions.

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