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Analgesic and Sedative-hypnotic Potentiality of Crude Methanolic Extract of *Gomphandra tetrandra* Leaves

Gomphandra Tetrandra Yapraklarının Ham Metanolik Ekstraktının Analjezik ve Sedatif-hipnotik Potansiyeli

Short Title: Pharmacological investigation of *Gomphandra Tetrandra* Leaves
Gomphandra tetrandra Yapraklarının farmakolojik araştırması
Türkçe Kısa Başlık: Gomphandra tetrandra yapraklarının farmakolojik araştırması

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ABSTRACT
Objectives: *Gomphandra tetrandra* (leaves.) belonging to the family Icacinaceae has been investigated for preliminary phytochemical screening and evaluating their pharmacological activities in various pharmacological models.
Materials and methods: The crude methanolic extracts were then screened with different chemical reagents for the qualitative detection of different phytochemical groups. The peripheral analgesic function was determined using the acetic acid-induced writhing procedure and sedative-hypnotic behaviors were assessed using hole-board, open field, and hole-cross test using different doses of the extract (200 mg/kg and 400 mg/kg body weight).
Results: Phytochemical screening revealed that methanolic extract of *Gomphandra tetranda* laves contains steroids, gums, mucilages, phytosterol, carbohydrate, and flavonoids. The crude methanolic extract at 200 mg/kg and 400 mg/kg doses showed statistically significant activity in acetic acid-induced writhing inhibition test, with 60% ($p<0.01$) and 76.47% ($p<0.01$) percent inhibition, respectively, compared to control. The extract also had dose-dependent substantial

($p<0.01$) sedative-hypnotic activities compared to diazepam in the hole-board, open field, and hole-cross tests.
Conclusion: It may be assumed that the methanolic leaf extract of *G. tetrandra* possesses the strong possibility of having analgesic and sedative-hypnotic activity due to the presence of bioactive compounds in its leaves. Moreover, observed results have opened a new era of in-depth research to discover the possible mechanism of analgesic and sedative-hypnotic activity.
Keywords: *Gomphandra tetrandra,* methanolic extract, analgesics, sedative-hypnotic activity.
ÖZ
Amaç: Icacinaceae familyasına ait *Gomphandra tetrandra* (yapraklar.), Çeşitli farmakolojik modellerde ön fitokimyasal tarama ve farmakolojik aktivitelerinin değerlendirilmesi için araştırılmıştır.
Materyaller ve yöntemler: Ham metanolik ekstraktlar daha sonra farklı fitokimyasal grupların kalitatif tespiti için farklı kimyasal reaktiflerle tarandı. Periferik analjezik fonksiyon, asetik asit kaynaklı kıvranma prosedürü kullanılarak belirlendi ve sedatif-hipnotik davranışlar, ekstraktın farklı dozları (200 mg / kg ve 400 mg / kg) kullanılarak delikli tahta, açık alan ve delik çapraz testi kullanılarak değerlendirildi. vücut ağırlığı).
Sonuçlar: Fitokimyasal tarama, *Gomphandra tetranda* laves'in metanolik özütünün steroidler, sakızlar, müsilajlar, fitosterol, karbonhidrat ve flavonoidler içerdiğini ortaya koydu. 200 mg / kg ve 400 mg / kg dozlarında ham metanolik ekstrakt, sırasıyla% 60 ($p <0.01$) ve% 76.47 ($p <0.01$) inhibisyon ile asetik asit kaynaklı kıvranma inhibisyon testinde istatistiksel olarak anlamlı aktivite gösterdi. kontrol etmek. Ekstrakt ayrıca doza bağlı önemli ($p <0.01$) yatıştırıcı-hipnotik aktivitelere sahipti.
Sonuç: *G. tetrandra*'nın metanolik yaprak özütünün, yapraklarındaki biyoaktif bileşiklerin varlığı nedeniyle güçlü analjezik ve sedatif-hipnotik aktiviteye sahip olma olasılığına sahip olduğu varsayılabilir. Dahası, gözlemlenen sonuçlar, analjezik ve yatıştırıcı-hipnotik aktivitenin olası mekanizmasını keşfetmek için yeni bir derinlemesine araştırma dönemi açtı.
Anahtar Kelimeler: *Gomphandra tetrandra*, metanolik ekstrakt, analjezikler, sedatif-hipnotik aktivite.

Introduction:
The use of plants in all major systems of medicine, regardless of the underlying philosophical premise, illustrates their universal function in treating disease. [1] Plant species are being depleted at a rapid rate; they can't be botanically registered or chemically & pharmacologically studied. So, it is essential to create an increased effort toward conserving gene pools. [2]
Natural sources are credited for the excellent revolution in modern medicine, and since the beginning, medicinal plants have played a crucial role in the field of drug discovery. Bangladesh is a well-known source of medicinal plants. [3] Today, several medicines, such as morphine from *Papaver somniferum* and Atropine from *Atropa belladonna*, are extracted from medicinal plants. [4,5] Secondary metabolites (potential drug sources) are present in significant amounts in medicinal plants. Medicinal plants are in high demand in both developing and developed countries as a result of these factors. [6] In Bangladesh's rural and tribal communities, medicinal plants have an essential role in the socio-cultural, spiritual, and therapeutic spheres.
A survey conducted by World Health Organization (WHO) in 1993 depicts that traditional practitioners treat about 80% of patients in India, 85% in Burma, and 90% in Bangladesh. The use of plants for medicinal purposes dates back to 4000–5000 B.C., and the Chinese were the

first to use natural herbal preparations as medicines. Also, about 25% of all modern pharmacopeial drugs are plant-derived and many other synthetic analogues based on prototype compounds isolated from plants. [7]

The plant *Gomphandra tetrandra* is an herbaceous plant found in the forest which belongs to the family **Stemonuraceae** (Formerly Icacinaceae). This family has had a popular medicinal history for a long time in many countries globally, especially in the evergreen forest, tropical and subtropical regions. [8,9] Various pharmacological investigations have already been done in different plants of this family. For example, *Mappianthus iodoides* (Icacinaceae) are used for treating hepatitis, jaundice, rheumatalgia, and arthralgia. [10] Other studies revealed hepatoprotective and hypoglycemic activity of *Lasianthera africana* (Icacinacaeae/ Stemonuraceae) in animal models. [11,12] The plant (*L. africana*) leaves also showed potent *in vitro* antioxidant activities in DPPH free radical scavenging test. [13] In this study, we tried to explore the analgesic and neuropharmacological potentiality of the methanolic leaf extracts of *Gomphandra tetrandra*.

MATERIALS AND METHODS

Collection of the plant

Gomphandra tetrandra was collected from the Moulvibazar District, Bangladesh, for this study. One of the professional taxonomists at Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh, identified the plant, and a voucher was deposited there for future reference, and the plant identification number is 51332. The desired plant parts (leaves) were separated from unwanted materials, plants, or plant parts. They were allowed to dry for a week under sunlight. Using a suitable grinder, the sun-dried leaves were converted into coarse powder. An airtight container was used to store that coarse powder. The container was kept in a cool, dark, and dry place till analysis was initiated.

Preparation of Gomphandra tetrandra leaf extracts:

Approximately 400g of powdered plant material was soaked in 2100 ml of 80% methanol in a clean, flat-bottomed glass vessel. The glass vessel was properly sealed and stored for seven days, with periodic shaking and stirring. After that, the mixture was filtered twice, using a piece of clean cotton material and a cotton plug. The filtrate was evaporated until absolutely dry using a ceiling fan and a water bath. It produced a sticky material with a reddish black color that was identified as crude methanol extract.

Chemicals and solvents:

Different chemicals and solvents of analytical grade were used in this study, including methanol and acetic acid. Those were purchased from a local supplier and were used directly. Eskayef Pharmaceuticals Ltd., Bangladesh, and Orion Infusion Limited, Tejgaon, Dhaka, Bangladesh, supplied Diclofenac sodium and 0.9% NaCl solution, respectively. Incepta Pharmaceuticals Ltd provided the diazepam, which was used as standard in neuropharmacological activity tests.

Phytochemical screening of methanol extracts of G. tetrandra leaf:

The primary phytochemical studies include testing of various chemical groups present in the extract. Different chemical tests were accomplished to ensure the presence of carbohydrates (using Molisch reagent), tannins (using bromine water), alkaloids (using Wagner's reagent), saponins (foam formation by olive oil), flavonoids (using NaOH), phenols (using $FeCl_3$ solution), anthraquinone glycosides, cardiac glycosides, proteins, and gums & mucilage (swelling properties). [14]

Test animals:

Swiss-albino mice were obtained from the Jahangirnagar University's animal house which were of both sex, 4-5 weeks old and 20-30 gm weight. They were held in a controlled environment and served ICDDR,B developed rodent food as well as water (ad-libitum). During the expeiments, the mice were chosen randomly and irrespective of sex and divided into different groups following the standard procedures [15]. Since these mice are susceptible to habitat changes, they are held in the experiment's condition for at least 3-4 days prior to the test. This study strictly adheres to globally recognized principles for standard use of experimental animal models developed by the International Council for Laboratory Animal Science (ICLAS) and National Institutes of Health (NIH) [16,17] and finally approved by the local Ethics Committee.

Evaluation of analgesic activity by acetic acid-induced writhing Test:

The writhing test is an experimental procedure for inducing peripheral pain in mice by administering irritants such as acetic acid. The test compound's analgesic efficacy is determined by a reduction in the number of writhings. [18] Twenty Swiss albino mice (weighing 20-30 gm) were randomly taken and divided into four groups, each with five mice. Group I mice were given normal saline (10 ml/kg per body weight, orally), group II mice were given Diclofenac Sodium (50 mg/kg per body weight, orally) as a standard drug. In contrast, groups III and IV received methanolic extract (200 and 400 mg/kg per body weight, orally) as the sample. One hour after this treatment, 0.1 ml of 0.6% (v/v) acetic acid was administered intraperitoneally. The animal's abdominal muscle writhing characterized by stretching with a twitch at the back limb was taken as writhing movements which is an indicator of pain response. The number of writhing motions in individual mice was counted for 15 minutes after the acetic acid administration. The % inhibition of writhing was counted from the following equation:

$$Inhibition\ (\%) = \frac{Average\ writhing\ of\ control - Average\ writhing\ of\ sample}{Average\ writhing\ of\ control} \times 100\%$$

Evaluation of Sedative-hypnotic activity

Healthy Swiss albino mice of both sex, 4-5 weeks old and 20-30 gm weight, were used for the test. A positive control group was treated with Diazepam (1 mg/kg i.p), and the negative control group was administered with vehicle (10 ml/kg, normal saline orally). The test groups (groups II & III) received two different doses (200 and 400 mg/kg, respectively) of *Gomphandra tetrandra* leaf extract by oral administration. The following three methods evaluated the sedative-hypnotic activity:

i) Hole-Board Test

By studying the exploratory activities of mice, this test is widely recognized as a way to assay potential sedative and anxiolytic effects of any compound. The equipment contained 16 equal size and evenly spaced holes. Normal saline (0.1 ml/mice, p.o.) and diazepam (1 mg/kg, i.p.) were given to the control and standard groups, respectively. It was established that the animals' head-dipping activity is directly relevant to their emotional situation. [19] The mice were put on the perforated flat platform after a period of 30 minutes in the case of the control and crude extract, and 15 minutes in the case of the standard, and the head dips' number in a period of five minutes was registered.

ii) Open field test

It is performed to evaluate exploratory behavior and anxiety along with to evaluate anxiolytic, anxiogenic, and non-pharmacological treatment. The test was performed to assess the anxiolytic activity of the test compound on mice CNS as described by Gupta *et al.* [20] The open field apparatus consisted of a half-square-meter square shaped wooden field with a series of squares

painted in black and white alternately. The apparatus also contained a 30 cm high wall and was kept in a dimly lit place. The experimental animals were treated with vehicle, extract, or diazepam and were held in the open field's center. After the treatment, the number of squares crossed by the animals was counted for 3 minutes at 30, 60, 90, and 120 minutes.

iii) Hole cross test:

In this experiment, a cage with a dimension of 30×20×14 cm was used, with a fixed partition in the middle with a hole of 3 cm diameter. [21] Mice received either a negative control, standard, or an extract before being allowed to pass the hole from one chamber to the next. The animals were observed for 3 minutes, and the number of passages was counted at 30, 60, 90, and 120 minutes after the treatments.

In all three methods, the percent inhibition (%) value was calculated as follows:

$$Inhibition\ (\%) = \frac{Reaction\ time\ (control) - Reaction\ time\ (sample)}{Reaction\ time\ (control)} \times 100\%$$

Statistical analysis:

One-way ANOVA followed by Dunnett's t-test was used to determine statistically significant differences between means. Results were considered significant at $P<0.05$. All the data analysis and statistical analysis were done using Microsoft Excel Version 13.0 and SPSS (StatisticalPackage for Social Sciences) version 22.0.

RESULTS

Phytochemical screening:

A series of chemical reactions were performed for the qualitative assessment of phytochemicals present in the crude methanolic extract of *Gomphandra tetrandra* leaves. Table 1 summarizes the results of different chemical tests used to detect and identify chemical constituents:

Table 1: Phytochemical screening of methanolic extract of *Gomphandra tetrandra* leaves.

Phytochemicals	**Outcomes**
Phytosterol	++
Steroids	+++
Carbohydrates	+++
Saponins	-
Gums & Mucillages	+++
Soluble Starch	-
Cardiac Glycosides	-
Anthraquinone Glycosides	-
Tannins	-
Flavonoids	++
Proteins	-
Terpenoids	-

(+) means detected; (-) means not detected

Acetic acid-induced analgesic activity

According to the statistical analysis, the extract in both doses exerted a dose-dependant analgesic activity in mice (Table 2). In this experiment, the reference drug (Diclofenac sodium) 50 mg/kg and *Gomphandra tetrandra* leaf extract at 200 and 400 mg/kg significantly reduced the mean number of abdominal constrictions or writhes.

Table 2: Analgesic activity of methanolic extract of *Gomphandra tetrandra* leaf in the acetic acid-induced writhing test.

Group	No. of writhing	% of inhibition writhing
Control	21.25 ±2.87	-
Standard	3.75±0.96	82.35***
Extract (200 mg/g)	8.5±1.00	60.00**
Extract (400 mg/kg)	5±0.82	76.47***

The number of writhing is expressed as a mean + SEM (n=5), and significance of the percent of inhibition writhing is determined comparing to the control group, where ** P<0.01, *** P<0.001

Sedative-hypnotic activity

(i) Hole board test

The effect of the plant extract as a sedative on mice using the hole board test is summarized in Table 3. The observed result suggests that the leaf extract of *Gomphandra tetrandra* possesses the significant potentiality of having sedative-hypnotic activity compared to standard.

Table 3: Effect of the *Gomphandra tetrandra* leaf extract as sedative-hypnotics on hole board test.

Treatment	Head Dips Mean ± S.D.	% inhibition
Control	20.5±0.58	-
Standard	4±0.82	80.49***
Extract (200mg/kg)	7.25±0.96	64.63***
Extract (400mg/kg)	2.75±0.96	86.59***

Values are expressed in Mean ± S.D. (n=5), where, *** P<0.001

(ii) Open field test

The observed result reveals that the extracts substantially ($p<0.05$) decreased the mice's locomotion in the open field examination. The suppressive effect began after 30 minutes and lasted until 120 minutes after the extract was administered. The impact of the plant extract on the animal model using open field test is summarized in Table 4. It suggested that the extract at both doses has significant activity.

Table 4: Effect of the *Gomphandra tetrandra* leaf extract as antidepressants on the open field test.

Time interval (minute)	Mean ±SD value of four groups (number of square crossing)				% inhibition		
	Control (10ml/kg)	Standard (100mg/kg)	Extract (200mg/kg)	Extract (400mg/kg)	Standard (100mg/kg)	Extract (200mg/kg)	Extract (400mg/kg)
30	54±1.83	17.75±0.96	33.25±2.75	22.25±0.50	67.13***	38.43***	58.80***
60	56.75±2.06	10.25±1.26	31±2.16	21±1.83	81.94***	45.37***	63.11***
90	59.5±1.29	7.5±1.29	26.25±2.50	16.25±0.50	87.39***	55.88***	72.69***
120	56.5±1.29	4.75±0.50	31.5±4.20	20.25±0.96	91.59***	44.25***	64.16***

*** P<0.001.

(iii) Hole cross test

The effect of the plant extract as a sedative-hypnotic using the hole cross test is summarized in Table 5. It suggested that the extract at both doses has significant activity compared to the standard.

Table 5: Effect of the *Gomphandra tetrandra* leaf extract as antidepressants on hole cross test.

Time interval (minute)	Hole crossing Mean ±SD value of four group				% inhibition		
	Control (10ml/kg)	Standard (100mg/kg)	Extract (200mg/kg)	Extract (400mg/kg)	Standard (100mg/kg)	Extract (200mg/kg)	Extract (400mg/kg)
30	10.75±1.26	4.25±0.96	7.75±0.96	4.5±1.29	60.47***	27.91*	58.14***
60	12.75±0.50	3.5±0.58	8±0.82	4.5±0.58	72.55***	37.25***	64.71***
90	12±0.82	3.25±0.50	7±0.82	4±0.82	72.92***	41.67***	66.67***
120	12.5±1.73	2.75±0.50	6.5±0.58	3.75±0.50	78***	48***	70***

Values are expressed in Mean ± SD (n=5). Here, *P<0.05, ** P<0.01 and *** P<0.001

DISCUSSION

The findings of this study demonstrated that the extract of *Gomphandra tetrandra* leaf possessed analgesic activity evident in the model, which is suggestive of the presence of peripherally mediated mechanisms. The acetic acid-induced writhing response is a commonly used method for determining the peripheral analgesic function of any plant component. In an animal model, acetic acid is a primary pain inducer. [22] Several studies reveal that pain response induced by acetic acid involves peritoneal mast cells and prostaglandin pathways. [23,24] The intraperitoneal administration of acetic acid has enriched the release of some inflammatory mediators, including histamine, serotonin, substance P, prostaglandins, and bradykinin. The release of these inflammatory mediators produces further abdominal constriction or discomfort. [25] Deraedt *et al.* reported an accumulation of prostaglandins PGE2e PGF2 within 30 minutes of acetic acid injection. [26] Jiang *et al.* also found an elevated level of lipoxygenase enzyme in peritoneal fluid following intraperitoneal acetic acid injection. [27] Various flavonoids, including rutin, quercetin, pectolinarin, and gossypin have been shown to induce considerable analgesic activity in various pain tests in previous studies. [28] Therefore, the flavonoids' involvement in the extract could play a vital role in the observed analgesic activity as they may inhibit the release of these inflammatory mediators. These findings also provide scientific credence for the traditional use of the leaf of *Gomphandra tetrandra* as an analgesic.

The obtained results suggest that the methanolic leaf extract of *Gomphandra tetrandra* possesses significant neuropharmacological activity in the animal model. The sedative-hypnotic activity was assessed carefully based on motor activity and exploratory behavior in the hole board, open field, and hole cross test. The open field is the most popular observational approach for general motor function. The most significant advantage of assessing motor movement in the open field is that the trend and qualitative profile of action can be detected explicitly. [29] Moreover, the Hole board test has also been achieved wide acceptance to assess the sedative-hypnotic activity of any drugs in the rodent model. Hole-poking (also known as head-dipping) is a standard prepotent action that has been proven to be highly responsive to drug effects. [30] There are many

physiologic conditions that can potentiate insomnia and other sleep disorders. Nervousness, tension, and indecision, both of which are accompanied by physiological arousal, may exacerbate sleep disruptions. [31] Synaptic transmission inhibition is required in this case, and agonists of the γ-aminobutyric acid receptor type A ($GABA_A$) are commonly used to achieve this (e.g., benzodiazepines). [32]

Furthermore, muscle fatigue, reduced ambulatory function, and sedation are well-known results of CNS depressant medications like benzodiazepines. [33,34] Plants containing flavonoids and tannins are well-known for their ability to treat various CNS disorders. [35] As a consequence, the extract's sedative-hypnotic properties are most likely attributed to the flavonoids coupling to the $GABA_A$-Benzodiazepine complex.

CONCLUSION

Phytochemical screening revealed that the methanolic extract of Gomphandra tetrandra contains Steroids, Carbohydrates, Phytosterols, Gums, flavonoids, and mucilages. The leaf extract of *Gomphandra tetrandra is* endowed with the significant potentiality of having analgesic and sedative-hypnotic activity. It reduced the pain of mice where acetic acid was injected intraperitoneally. The results of these in-vivo experiments inspire us to investigate the animals' motor performance further in order to determine the potentiality of antinociceptive involvement in the central and peripheral nervous systems. However, the findings demand a more of-depth investigation in animal models to uncover their molecular mechanisms of action in analgesic and sedative-hypnotic activity.

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CONFLICTS OF INTEREST STATEMENT:

The authors declare no conflict of interest.

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