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# Preliminary Phytochemical Screening and Determination of Anxiolytic, Analgesic, and Antibacterial Potentials of a Medicinal Plant - *Alpinia conchigera* Griff

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## Abstract

People in rural areas are using several traditional medicinal plants to make a inntial treatment against numerous diseases. Antibacterial activity of methanolic *Alpinia conchigera* Griff rhizomes extracts' was determined *in vitro* study through the disk diffusion method. The anxiolytic activity was tested on Swiss albino mice using both open field and hole cross methods, while analgesic activity was assessed via formalin-induced hind paw licking test and acetic acid-induced writhing test in mice. There was a wide spectrum of phytoconstituents found during the phytochemical screening process. The rhizome extract possesses moderate antimicrobial activity against gram-positive bacteria but is resistant to gram-negative bacteria. The rhizome extract significantly reduced locomotor activity of mice in an open field and hole cross methods in a dose-dependent manner (100, 200, and 400 mg/kg) compared to standard drugs diazepam (1 mg/kg). Acetic acid-induced writhing in mice was considerably ($p<0.05$) reduced by plant extracts at 29.11%, 35.44%, and 48.10% at 100, 200, and 400 mg/kg, respectively, compared to acetylsalicylic acid (standard drug) with 51.89%. 200 and 400 mg/kg of rhizomes extract provided positive results in formalin-induced hind paw licking test by 45.38% to 61.00% at an early phase and 62.50% to 69.70% at late phase, respectively, whereas aspirin (100 mg/kg) revealed 29.23% and 39.28% of inhibition at both phases sequentially. To recapitulate, the experimental can be used as traditional medicine against numerous diseases, but further studies are necessary to validate this plant extract as a drug.

## Keywords

*Alpinia conchigera* Griff, Antibacterial activity, Anxiolytic activity, Analgesic activity, Phytochemical screening

## Introduction

Every day, researchers look forward to novel medications that have special or better-quality therapeutic effects. Medicinal plants can be a potential source of lead compounds for developing noble drugs with fewer side effects and risks [1-3]. Such plants yield various bioactive natural chemicals used as starting materials for new medication development [4]. Due to the increasing consistency of traditional medicinal plants against a wide range of diseases and alleviating human suffering has had an important place in society since antiquity [5, 6]. A wide range of bioactive natural chemicals, alkaloid, glycoside, carbohydrate, protein, steroid, saponins, steroid, reducing sugar, and tannins were generated from

medicinal plants and used as a new drug development raw materials [7, 8]. These bioactive chemicals have therapeutic and pharmacological potential and are used to treat a variety of diseases. *A. conchigera* Griff is a slender herb with 0.6 - 1.5 m tall. This plants are available in Bangladesh, India, and Indonesia. The plant has been traditionally used for several purposes like antioxidant capacity, anti-inflammatory, antimicrobial activity, anthelmintic, antidiarrheal, anti-motility, and anti-cancer effects [9, 10]. But specific pharmacological activities of *A. conchigera* Griff rhizome is still under investigation.

Human pathogenic bacteria have developed resistance to commercial antimicrobial drugs due to widespread use in recent years [11]. Since certain antibiotics have unwanted side effects and previously rare infections are becoming prevalent, scientists have been pushed to explore new antimicrobial alternatives, such as medicinal plants. Antimicrobial activity screening of medicinal extract has revealed that the plant can be a novel source for anti-infective medicines [12]. So aimed to determine antimicrobial activities of *A. conchigera* Griff rhizome extract was against multidrug-resistant bacterial strain. The term "pain" refers to various unpleasant physical and emotional sensations that can range from mild to severe, depending on the severity of the underlying cause.

Analgesics are drugs that reduce pain without altering the patient's awareness through acting at the central nervous system (CNS) or peripheral pain mechanism [13]. As an example, Non-steroidal anti-inflammatory drugs, a class of painkillers, block the formation of prostaglandins or cyclooxygenase enzymes, respectively, in inflammatory pathways, thus decreasing pain and swelling [14]. Furthermore, opiates reduce pain by altering the CNS [15]. However, Non-steroidal anti-inflammatory drugs have serious side effects, such as stomach abrasions, whereas opiates can create tolerance or psychological dependence, making them unfeasible in many cases [16]. Our study speculated that the plant's rhizomes might contain chemicals with anti-hyperglycemic and analgesic properties. Psychiatric diseases such as anxiety and depression are ubiquitous, and they've been linked to disability and early death because they're the most common result of stress-related mood disorders [17]. More than 20% of people are experiencing one or more of these problems [18]. The most common drug used for anxiolytic therapies are benzodiazepines, which are also used for skeletal muscle relaxation, sedation-hypnosis, and anticonvulsant activity [19]. However, these substances have detrimental impacts on cognitive abilities, physical reliance, and endurance while also causing harm to the respiratory, digestive, and immune systems of the body [20]. Naturally occurring compounds have always been admired in search of potent anti-psychiatric agents due to their structural diversity. Therefore, we aimed to evaluate whether methanol extract from *A. conchigera* Griff rhizomes had any anxiolytic or antidepressant-like properties or not. Overall, in this study, we have screened phytochemicals and investigated analgesic, anxiolytic, and antibacterial activities of the methanol extract of *A. conchigera* Griff rhizomes.

# Materials and Methods

## Reagent (Drug and chemicals)

The acetic acid formalin was purchased from Sigma Chemicals (USA), while aspirin sedil (diazepam) and ciprocin (ciprofloxacin) were collected from square pharmaceuticals Ltd, Bangladesh. Analytical-grade reagents and materials were also utilized in this investigation.

## Plant materials collection and identification

Fresh and healthy rhizomes of *A. conchigera* Griff was collected in July 2020 from Sonapur, Noakhali and rinsed with distilled water. The Bangladesh National Herbarium (the national botanical garden) botanically identified and validated the plants (Accession number DACB: 55758). The plant material was exposed to a shadow background for ten days, dried in the sun, and turned into coarse powder through high capacity grinding machines.

## Plant extract preparation

To prepare methanol extract, 800 g of rhizome was emerged into 4.5-litre pure methanol in a desiccator with random pulsating and moving. This was then kept for 15 days in a dark room, and the mixer was filtered through autoclaved cotton and Whatman filter paper. Followed by rotatory evaporation was used to make the filtrate more concentrated and kept at room temperature to air to obtain brownish mass. The extract's percentage yield was considered based on the following formula:

$$\% \text{ of the yield of extract} = \frac{\text{Weight of the extracted materials}}{\text{Weight of the original plants materials used}} \times 100$$

## Phytochemical screening

Preliminary phytochemical screening evaluated several phytoconstituents in methanol rhizome extract of *A. conchigera* Griff [21, 22]. The available chemicals groups in each test were determined by using 10% (w/v) solution of extract in methanol [23] and characterized based on colour change using a standard procedure [24].

## Animal maintenance

Six weeks aged (both sex, 25 - 30g) healthy Swiss-albino mice were collected from purchased from the central animal house of the Department of Pharmacy, Jahangirnagar University, Savar Dhaka, Bangladesh. According to, Principle of Laboratory Animal Care (NIH publication no. 85 - 23, revised 1985), five mice in a group placed in a cage to adapted laboratory conditions including temperature (24 ± 3 °C), relative humidity (55 - 60%), light and dark cycle (12 h), and standard feed formulated diet and water before conduct final experiments. The experimental procedure in the mice model used in this research was performed according to the guideline of the Institutional Animal Ethics Committee.

## Experimental design

Experimental animals were sorted into three separate categories, including control, separate and experimental, and five

mice were used in each test group. Group I and Group II were considered control and standard, while Group-III, IV and V were marked for receiving experimental plant extract. Group-I was given the vehicle 1% tween 80 in water (at the dose of 10 ml/kg body weight), Group II received numerous standard drugs like acetylsalicylic acid, diazepam and ciprofloxacin at a different dose. In contrast, groups III, IV and V were received 100, 200 and 400 mg/kg of rhizome extract, respectively.

### Animal sacrifice

After conducting the research experiments, an appropriate dose of chloroform was used to anaesthetize the testing animals. After sacrificing liver, heart, kidney, and stored fat were detached from the bodies and conserved in normal saline for evaluating further pharmacological activities.

### Antimicrobial activity

The antimicrobial activity of the rhizome extract was against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* were conducted according to the Bauer-Kirby disc diffusion method [25]. These strains were obtained from, Microbiology Department, Noakhali Science and Technology University (Accession no. Mi_NSTU: 062020) and their species authenticity was evaluated by polymerase chain reaction. The test organisms were inoculated and cultured overnight at 37 °C in the nutrient broth and finalized the 1 x $6^6$ cells/ml in Mueller-Hinton agar cultured media. The culture plate incorporated the autoclaved filter paper discs (6 mm diameter) with 400 µg/disc of rhizome extract disc. The plate was kept 24 h at low temperature for maximum dispersion of sample surrounding to media. The antibacterial activity of the rhizomes extract was determined by assessing the zone of inhibition. For quality control, regular antibiotic ciprofloxacin (5 g/disc) was a positive control, while blank discs were utilized as a negative control.

### Analgesic activity

#### Acetic acid-induced writhing test

Analgesic activity was evaluated according to Sen et al. [26]. Five groups with five mice were treated with 1% (w/v) tween-80 (10 ml/kg), aspirin (100 mg/kg), and rhizome extract (100, 200, and 400 mg/kg) of body weight. After 1 h, 0.7% acetic acid with the dose of 10 ml/kg body weight were injected intraperitoneally for all groups. The number of writhing or squirms responses achieved by individual animals was recorded at 5 min intervals for 30 min. Following equations calculated analgesic parentage:

$$\text{Inhibition rate \%} = \frac{\text{Number of writhes (control) - Number of writhes (treated)}}{\text{Number of writhes (control)}} \times 100$$

#### Formalin-induced analgesic assay

Like the acetic acid-induced writhing test, five groups with five mice were treated with 1% (w/v) tween-80 (10 ml/kg), acetylsalicylic acid (100 mg/kg) and rhizome extract (100, 200, and 400 mg/kg) of body weight. After 1 h, 20 µl of 1% formalin solution were injected subcutaneously beneath the plantar surface of the left hind paw of each mice to introduce pain. In a second, the time (in a second) spent in licking and biting response of the injected paw was an indicator of pain response. In this test, the determination of the anti-nociceptive effect involved two phases. The early phase (phase 1) was recorded during the first 5 min, while the late phase (phase 2) was counted during the last 20 - 30 min after formalin injection [26, 27].

$$\% \text{ inhibition} = \left[\left(R_c - R_t\right)/R_c \times 100\right]$$

Here, $R_c$ stands for the reaction time of the control group and $R_t$ stands for the reaction time of the test group.

### Anxiolytic activity

#### Open field test

The anxiolytic activity by open field test was carried out according to Adebesin et al. [28] with some modification. In an open field (100 cm x 100 cm x 40 cm) (white and black), a sequence of colored squares was used to build the open field apparatus. Five groups with five mice were treated by intraperitoneal with 1% (w/v) tween-80 in water (10 ml/kg), diazepam (1 mg/kg), and rhizome extract (100, 200, and 400 mg/kg) of body weight. After that, mice were spread on the floor of an open and the number of squares visited by individual animals were counted for 3 min at the time started at 0, 30, 60, 90, and 120 min later the administration of test substance.

#### Hole-cross test

According to Hussain et al., the hole-cross-methods was conducted using a cage (30 cm x 20 cm x 14 cm) and a steel barrier fixed in the middle. A hole of 3 cm diameter was made at the height of 7.5 cm in the middle cage [29]. Five groups with five mice were treated by intraperitoneal with 1% (w/v) tween-80 in water (10 ml/kg), diazepam (1 mg/kg), and rhizome extract (100, 200, and 400 mg/kg) of body weight. After 1 h of administration, each mouse was placed in a different part of the cage, where it preferred to move and dip its head into the holes. The numeral of head dips for a 5 min period was recorded for individual mice.

### Statistical analysis

The result of the all experiment were expressed as mean ± SEM (n = 5). p-value was computed by one-way analysis of variance (ANOVA) using SPSS software, version 22 (IBM Corporation, New York, NY, USA). Dunnett's test was used to analyze the intergroup significance *p< 0.05, **p<0.01 and ***p< 0.001 to define weak, moderate and high significance, respectively.

## Results

Preliminary phytochemical screening of methanol rhizome's extract of *A. conchigera* Griff revealed a wide range of pharmacologically active compounds, including sugar reducing agent, alkaloid, tannin, glycosides, saponins, and steroids are tabulated in table 1.

The findings of the antibacterial activities of methanol extract of *A. conchigera* Griff rhizomes are summarized in table 2, where antibacterial activity against gram-positive bacteria was found to be only moderate but resistant for the gram-negative

**Table 1:** Preliminary phytochemical screening of *A. conchigera* Griff rhizomes

| Phytochemicals | Test | Result |
|---|---|---|
| Alkaloids | a) Wagner's test | + |
| | b) Dragendorff's reagents test | + |
| | c) Mayer's reagents test | - |
| Saponins | a) Froth test | + |
| Steroids | a) Lieberman-Burchard test | + |
| | b) Salkowski test | + |
| Reducing sugar | a) Fehling's solution test | + |
| | b) Benedict's test | + |
| Tannins | a) Potassium dichromate | - |
| | b) Mayer's test | + |
| | c) Dragendorff's reagent | + |

**Table 2:** Antibacterial activities of methanolic extracts of *A. conchigera* Griff rhizomes against both gram positive and gram negative bacteria.

| Test organisms | | Crude sample (400 µg/disc) | | |
|---|---|---|---|---|
| | | Zone of Inhibition | Relative % of inhibition | Ciprofloxacin (5 µg/disc) |
| **Gram-positive bacteria** | *Enterococcus faecalis*<br>*Staphylococcus aureus* | 12 mm<br>9 mm | 32%<br>26% | 38 mm<br>34 mm |
| **Gram-negative bacteria** | *Escherichia coli*<br>*Salmonella typhi*<br>*Klebsiella pneumoniae* | -<br>-<br>- | -<br>-<br>- | 30 mm<br>22 mm<br>28 mm |

**Table 3:** Effects of *A. conchigera* Griff rhizomes on acetic acid induced writhing in mice.

| Group | Dose (mg/kg) | Number of writhing (mean ± SEM) | % of inhibition of writhing |
|---|---|---|---|
| Control | 10 | 19.75 ± 0.25 | - |
| Standard | 100 | 9.50 ± 1.26* | 51.89 |
| ME | 100 | 14.00 ± 1.68* | 29.11 |
| ME | 200 | 12.75 ± 1.09** | 35.44 |
| ME | 400 | 10.25 ± 1.03*** | 48.10 |

Here, ME: Methanolic Extract, SEM: Standard Error Mean. Results are presented as mean values ± SEM (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 when compared to control group.

bacteria.

The analgesic properties of *A. conchigera* Griff rhizomes against acetic acid-induced writhing and formalin-induced hind paw licking were tested, and the result was tabulated in table 3 and table 4, respectively. From table 3, there was an average number of writhes in both experimental (Group III, IV, and V) and standard group (Group-II). Still, no writhes in the negative control group (Group I). Ethanoic extract at all testes doses 100, 200, and 400 mg/kg showed statistically significant analgesic activity 29.11%, 35.44%, and 48.10%, respectively, compared to standard drugs. The maximum dose considerably lowered the number of writhing than lower dose and moderate doses. Standard anti-nociceptive attributed 51.89% pain inhibition, which was also statistically significant ($p<0.05$) compared to control.

Table 4 reveals that 100, 200, and 400 mg/kg rhizomes extract could reduce the hind paw licking significantly with the rate of inhibition 34.61%, 45.38, and 61.00% and 51.80, 62.50%, and 69.70% throughout at early phase and late phase, respectively. Similarly, positive control drug aspirin reduced hind paw licking around 29.23% and 39.28% at the initial and late phases, respectively.

The summary of the hole cross test is tabulated in table 5. Overall, 9.50 ± 1.25*, 11.90 ± 1.21***, and 13.75 ± 1.75*** head dips were recorded by 100, 200, and 400 mg/kg, respectively. The extract significantly ($p<0.001$) alleviate the number of heads dipping at the dose of 200 mg/kg and 400 mg/kg. Similarly, mice treated with the control drug diazepam (1vmg/kg) showed no significant increases in the number of head dipping.

The open-field test took place for 120 min after the last dosage of the extract had been administered, and the result has been summarized in table 6. The number of the in the test animals was significantly ($p<0.001$) reduced in the experiment at the dose of 400 mg/kg (2.67 ± 0.33) and 200 mg/kg (9.33 ± 0.33) compared to 34.67 ± 2.60 and 24.00 ± 1.53 for the control group and standard groups, respectively. Moreover, the CNS depressant activity noticed for the extract was dose-dependent and noticeable result was found.

## Discussion

The use of medicinal plants as a raw material or as purified chemicals to treat various diseases, including from infectious [30, 31] to non-infectious disease [32] is a traditional approach.

**Table 4:** Effects of *A. conchigera* Griff rhizomes on formalin induced hind paw licking mice.

| Group | Dose (mg/kg) | Number of licking (0 - 5 min) (mean ± SEM) | % of inhibition (Early phase) (20 - 30 min) (Late phase) (mean ± SEM) | Number of licking | % of inhibition |
|---|---|---|---|---|---|
| Control | 10 | 32.50 ± 0.65 | - | 56 ± 0.49 | - |
| Standard | 100 | 23.00 ± 1.08* | 29.23 | 34 ± 0.97* | 39.28 |
| ME | 100 | 21.25 ± 0.85 | 34.61 | 27 ± 0.78 | 51.80 |
| ME | 200 | 17.75 ± 0.85** | 45.38 | 21 ± 1.01** | 62.50 |
| ME | 400 | 12.35 ± 1.22*** | 61.00 | 17 ± 0.88*** | 69.70 |

Here, ME: Methanolic Extract, SEM: Standard Error Mean. Results are presented as mean values ± SEM (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 when compared to control group.

**Table 5:** Effects of methanolic extract of *A. conchigera* Griff rhizomes on mice by hole cross method.

| Group | Dose (mg/kg) | Number of head dipping (mean ± SEM) |
|---|---|---|
| Control | 10 | 19.25 ± 0.47 |
| Standard | 1 | 6.75 ± 1.60 |
| ME 100 | 100 | 9.50 ± 1.25* |
| ME 200 | 200 | 11.90 ± 1.21*** |
| ME 400 | 400 | 13.75 ± 1.75*** |

Results are presented as mean values ± SEM (n = 5). *p < 0.05, ***p < 0.001 when compared to control group.

In our study, along with phytochemical screening, we attempted to assess the pharmacological quality of the methanol extract of *A. conchigera* Griff rhizomes *in vitro* and *in vivo* models. The early phytochemical screening revealed favourable results for alkaloid, carbohydrate, reducing sugar, flavonoid, terpenes, phenol, protein, amino acid, and tannins in the plant extract. These phytochemicals in a plant extract are responsible for numerous pharmacological activities, and our positive phytochemical screening set the foundation for further pharmacological investigation. Numerous studies indicate that alkaloids mediate therapeutic activities such as antihypertensive, anticancer, antimalarial, and antiarrhythmic [33]. Moreover, analgesic, antispasmodic, and bactericidal properties of pure alkaloids and their synthetic derivatives are also well documented [34]. It is impossible for the body to function without carbohydrates and reduce sugar, making the energy needed and supplying it to the brain, muscle, and blood [35]. Terpene has several therapeutic advantages, including antiulcer, anticancer, diuretic, and antimicrobial activities [34]. Phenols, flavonoids, and tannins are the primary groups responsible for antioxidant activity [36]. Studies in the past showed that saponins had antibacterial, anti-inflammatory, anticancer, and anti-diabetic properties [37]. Antibacterial agent meditates their activities against bacteria through numerous mechanism, including degrading cell wall [38], energy depletion [39], structural alteration [40].

In our present study, *A. conchigera* Griff rhizomes attributed growth inhibition to some gram-positive bacteria. In the extract, this antimicrobial activity is might due to the existence of secondary metabolites, including tannins, flavonoids, glycosides, carbohydrates, organic acids, etc. The CNS depressive activity of *A. conchigera* Griff rhizomes was investigated using two different neuro-pharmacological models: the open field and hole-cross test. It was shown that using the plant extracts resulted in considerable ($p<0.001$) sedative-hypnotic action in the test animals, proving their CNS depressive properties. Methanol rhizome extract may work by directly activating the GABA receptors or causing the CNS's GABAergic inhibition to be induced by membrane hyperpolarization, which reduces the firing rate of essential brain neurons [41]. Furthermore, plants rich in flavonoids, alkaloids, and tannins can help treat various CNS illnesses by reducing the CNS's locomotor activity [42]. These phytochemicals were screened from *A. conchigera* Griff rhizomes in the earlier section of the study.

The purpose of the acetic acid-induced writhing experiment was to assess the peripheral analgesic properties of the plant extract [43, 44]. It is well known that acetic acid stimulates the neurons responsible for pain feeling, which are sensitive to anti-inflammatory medicines, by causing them to secrete endogenous pain mediators. In this study, *A. conchigera* Griff rhizomes were found to have a statistically significant analgesic effect compared to the control drug in mice's acetic acid-induced writhing test. This indicates that the plant extract's active principle(s) has peripheral analgesic action via lowering the writhing number. This mechanism might be inhibited the synthesis of prostaglandin [45]. For further information about analgesic activities, the persistent-pain model of formalin-induced hind paw licking was conducted. The early phase (0 - 5 min) is characterized by neurogenic pain generated by C-fibre activation due to formalin stimulation of peripheral nociceptors [46]. After 15 to 30 min, the second burst of licking behaviour occurs. The second phase is triggered by tissue damage, resulting in histamine, serotonin, prostaglandin, and excitatory amino acids [47, 48].

## Conclusion

Our study reported that methanolic extract of *A. conchigera* Griff rhizomes exhibited significant antibacterial activities against gram-positive bacteria, including *E. faecalis* and *S. aureus*. The present study also showed the extract has significant CNS depressant and analgesic activities in various models at different doses. These pharmacological activities of the extract may be due to the existence of different types of secondary metabolites like sugar reducing agents, alkaloids, tannin, glycosides, saponins, and steroids. So our research potentiates the plant's use against bacterial, neurological, and pain-related diseases. However, further advantage research on HPLC fingerprints for chemical compounds characterization of the

**Table 6:** Effects of the methanolic extract of *A. conchigera* Griff rhizomes on mice stay in the open field.

| Groups | Number of Movements (Mean ± SEM) | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | - | - | - | - | - |
| Standard | 57.50 ± 2.50 | 47.50 ± 2.50 | 46.67 ± 1.76 | 45.33 ± 5.33 | 34.67 ± 2.60 |
| ME 100 | 45.00 ± 2.00 | 33.00 ± 2.00* | 31.33 ± 2.03** | 15.33 ± 0.88** | 24.00 ± 1.53** |
| ME 200 | 38.50 ±1.50** | 32.50 ± 2.50 | 32.00 ± 2.00** | 23.33 ±1.45* | 9.33 ± 0.33*** |
| ME 400 | 36.50 ± 0.50 | 29.50 ± 0.50 | 20.67 ± 3.04** | 4.33 ± 0.33** | 2.67 ± 0.33*** |

Results are presented as mean values ± SEM (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 when compared to control group.

extract and *in vitro* and *in vivo* evaluations is required to find out pharmacokinetic properties, accurate therapeutics dose, possible routes of administration, and established convenient routes nanoformulation strategies.

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## Conflict of Interest

The authors declare that they do not have any conflict of interest.

## Credit Author Statement

All Authors contributed equally to the work.

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