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Original article

Phytochemical, analgesic, antibacterial, and cytotoxic effects of *Alpinia nigra* (Gaertn.) Burtt leaf extract

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ABSTRACT

This research evaluated the phytochemical contents as well as the analgesic, cytotoxic, and antimicrobial effects of the methanolic extract of *Alpinia nigra* leaf. Phytochemical analysis was carried out using established methods. The analgesic effects of the extract were measured with the formalin test and tail immersion test. The antibacterial activity of the extract was evaluated using the disc diffusion technique. Cytotoxicity was assessed with the brine shrimp lethality bioassay. Data were analyzed with one-way analysis of variance using statistical software (SPSS, Version 19.0). The qualitative phytochemical screening of *A. nigra* leaf extract showed the presence of medicinally active secondary metabolites such as alkaloids, glycosides, cardiac glycosides, flavonoids, steroids, tannins, anthraquinone glycosides, and saponins. The extract at a dose of 200 mg/kg revealed a prevailed central nociception increasing the reaction time in response to thermal stimulation. The extract also showed a response to chemical nociceptors, causing pain inhibition in the late phase. The leaf extract (2 mg/disc) showed mild antibacterial activity compared to tetracycline (50 μg/disc). In the brine shrimp lethality bioassay, the LC_{50} (lethal concentration 50) value of the extract was found to be 57.12 μg/mL, implying a promising cytotoxic effect. The results evidenced the moderate analgesic and antibacterial effects with pronounced cytotoxic capability.

1. Introduction

Medicinal plants are the richest natural sources of medicinal products used in traditional and orthodox medicine.[1] The search for medicinal values of different plants has attracted increasing interest in the past couple of decades, presumably because of their potential as sources of potent pharmacological activities, convenience to users, economic viability, as well as low toxicity.[2] Plant-derived drugs proved to be relatively safer and dependable even in long-term use, where synthetic drugs are always feared in chronic cases.[3] Plants have also formed the basis of sophisticated traditional medicine systems in different therapeutic areas for thousands of years in many countries.[4] Therefore, continuous efforts in the search for newer sources of traditional medicines as well as screening of existing ones for newer therapeutic indications are inevitably urgent.

Alpinia nigra (Gaertn.) B.L. Burtt, which belongs to the Zingiberaceae family, is known as *Jongly Ada* or *Tara* in Bengali. This aromatic and rhizomatous herb is also referred to as Galangal, False galangal, Greater galangal, Black-Fruited, or Kala.[5,6] It is found in most parts of Bangladesh including Chittagong. It is widely distributed in Yunnan and Hainan provinces of China, and other Southeast Asian countries.[5] This plant is used as vegetable and hot culinary spice. Different parts of this herb are used as folk remedies to treat dyspepsia, gastric disease, and insect bites.[7] A previous phytochemical investigation revealed the presence of two flavone glycosides, astragalin and kaempferol-3-*O*-glucuronide,[8] which possess several biological activities, including antibacterial,[9,10] antioxidant,[11–13] antiprotozoal,[14] hepatoprotective,[15,16] and glycation inhibitory effects.[17] The crude shoot extract is reported to cause destruction of surface tegument leading to paralysis and death of intestinal parasites.[18] Recent studies have reported the antibacterial effect of seed extracts, the anti-inflammatory effects of rhizome

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extracts, and the anthelmintic effect of the shoot ethanol extract of this plant.[19–21] Although the different parts of this plant have been studied for several biological effects, the leaf extract is yet to be studied extensively. This research, therefore, was aimed to investigate the *in vivo* analgesic and *in vitro* antibacterial and cytotoxic effects of *A. nigra* leaf extract. Apart from these, other species of *Alpinia* were found to have analgesic, antimicrobial, and cytotoxic effects, which assisted us to formulate our hypothesis on *A. nigra*, presumably because of the interspecies similarities of the biological activities of different plant species.

2. Materials and methods

2.1. Drugs and chemicals

All chemicals and reagents used in this study were of analytical grade. Methanol (99.5%), which was used for extraction, was procured from Sigma-Aldrich (Humburg, Germany). Nalbuphine HCl was obtained from Square Pharmaceuticals Ltd. (Kaliakoir, Gazipur, Bangladesh). Formalin was purchased from CDH, New Delhi-110002, India. Diclofenac sodium (powder form) was a gift from GlaxoSmithKline Ltd. (Chittagong-4217, Bangladesh). Normal saline solution was purchased from Beximco Infusion Ltd (Dhaka).

2.2. Collection of plants

A. nigra leaves were collected from the Bangladesh Centre for Scientific and Industrial Research (Chittagong, Bangladesh) plantation area in April 2010 and authenticated by Dr Sheikh Bokhtear Uddin, a taxonomist (Department of Botany, University of Chittagong, Chittagong, Bangladesh). A taxonomical voucher specimen (No. BS-05/2010) is preserved at the Bangladesh Centre for Scientific and Industrial Research as an accession.

2.3. Preparation of extract

Fresh leaves of *A. nigra* were cleaned, sun-dried for about 5 days, and ground into coarse powder with a mechanical grinder (3 in 1 blender; Miyako, China). A total of 292 g powder was macerated into 800 mL of 98% methanol (Sigma Chemicals Co., St. Louis, MO, USA) for 5 days at room temperature (25 ± 1°C) with occasional stirring. The filtered extract was concentrated under reduced pressure below 50°C through a cyclone vacuum evaporator (RE200; Bibby Starling, Staffordshire, England). The concentrated extract was collected in a Petri dish and allowed to air-dry for the complete evaporation of methanol in the absence of sunlight. The whole process was repeated three times, and finally 15 g of blackish green, concentrated extract was obtained (yield 5.14%, w/w), which was kept in a refrigerator at 4°C.

$$\text{Yield } (\%) = \frac{\text{Weight of particular extract}}{\text{Total amount of coarse powder}} \times 100$$

2.4. Phytochemical screening of the extract

The freshly prepared crude extract was qualitatively tested for the presence of secondary metabolites especially saponins, flavonoids, steroids, anthroquinone, alkaloids, terpenoids, phlobatannins, tannins, and cardiac glycosides through established methods.[22]

2.5. Analgesic study

The analgesic effects of the extract were evaluated using two different animal pain models: the tail immersion test and formalin test.

2.6. Experimental animals

The experiments were carried out on 6-week-old Swiss albino mice of both sexes weighing 25–30 g. The animals were housed in standard polypropylene cages, at five mice per cage, under a 12-hour light/12-hour dark schedule at an ambient temperature of 25 ± 1°C. The mice were maintained on standard laboratory animal feed and water *ad libitum*. The animals were acclimatized for a week prior to commencing the experiment. They were deprived of food but not water 4 hours prior to the experiment. All animal experiments were maintained and carried out in compliance with Helsinki Animal Ethics guidelines.

2.7. Formalin test

The most predictive of the models for acute pain is the formalin test. The method used in our study was similar to that described previously by Shibata et al.[23] Briefly, 20 μL of 5% formalin was injected subcutaneously into the right hind paw of mice to produce a biphasic pain response. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured from 0 to 5 minutes (early phase, neurogenic) and 25–40 minutes after formalin injection (late phase, inflammatory).[24] The mice selected for this study were divided into three groups of five animals each. In the test group, the plant extract [200 mg/kg, intraperitonally (i.p.)] was administered 60 minutes prior to the formalin injection. Diclofenac sodium (10 mg/kg, i.p.) was administered 30 minutes prior to the formalin injection in the positive control group. The control group received the same volume of saline by oral administration.

2.8. Tail immersion test in mice

Analgesia was assessed according to the method described by Chandrashekar et al,[25] with slight modifications. The mice selected for this study were divided into three groups of five animals each and pretreated with 200 mg/kg (i.p.) of the extract for Group 1, whereas Group 2 received 10 mL/kg normal saline. As the reference standard, nalbuphine HCl (10 mg/kg) was administered to Group 3. After 30 minutes of drug administration, each mouse was held in position in a suitable restrainer with its tail extending out. About a 3 cm area of the tail was marked and immersed in the water bath thermostatistically maintained at 51°C. Within a few seconds, the mouse reacted by withdrawing the tail. The time it took for the mouse to remove its tail out of the water was recorded. The latency was evaluated at 0, 30, 60, and 90 minutes with 0 minute being the initial reading. The criterion for analgesia was postdrug latency, which was greater than two times the predrug average latency as reported by Janssen et al.[26] The mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs. To avoid injury to the tail, the maximum cutoff time for immersion was fixed at 15 seconds. The animals were allowed to adapt to the cages for 30 minutes prior to testing.

2.9. Assay of antibacterial activity

2.9.1. Bacterial strains used in the study

The bacterial strains selected for this research were *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*, *Vibrio cholerae*, and *Shigella sonnie*. All of these organisms were obtained from the Department of Microbiology, University of Chittagong.