Analgesic, anxiolytic and sedative-like activities of leaves of *Alpinia calcarata* Roscoe in mice

Mahmuda Ferdous, A. F. M. Shahid Ud Daula, Shahnaz Naznin, Farjana Yeasmin and Mohammad Anwarul Basher*

Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

Received 22 January, 2020; Accepted 6 March, 2020

The objectives of the study were to evaluate the analgesic, anxiolytic and sedative-like activities of methanol extract of leaves of *Alpinia calcarata* Roscoe in mice model. Analgesic activity was investigated using the acetic acid-induced writhing test and formalin-induced paw licking test. *In vivo* neuropharmacological effects, including anxiolytic and sedative effects were examined by open field, light-dark, elevated plus maze, thiopeental sodium-induced sleeping time and hole cross tests behaviors in mice. The extract produced significant (p<0.001) reduction in writhing and licking response in acetic acid-induced writhing and formalin-induced paw licking tests, respectively. Administration at a dose of 400 mg/kg.bw of leaves extract significantly (p<0.001) attenuated anxiety-like behavior in mice by decreasing movement in open field, increasing the time spent and number of entries in the open arms of elevated plus maze, and a significant increase in the time spent in the illuminated compartment in the light box in the light-dark test. The extract significantly (p<0.01) potentiated thiopeental sodium-induced sleep and reduced the number of sectional crossings relative to the control group, indicating sedative effects. Based on the results obtained from *in vivo* activities, the leaves of *A. calcarata* was found to be a potential source of new analgesic, anxiolytic and sedative compounds.

**Key words:** *Alpinia calcarata*, Swiss albino mice, analgesic, anxiolytic, sedative

**INTRODUCTION**

The genus *Alpinia* belongs to the family Zingiberaceae, and has long been used for many decades for medicinal and non-medicinal purposes. Plants of this genus have extensively been reported by several research studies for their potential biological activities. For example, antioxidant, antibacterial, larvicidal, cytotoxic and vasodilator activities of *Alpinia purpurata* were reported (Chan and Wong, 2015); the principle phytoconstituents responsible for antibacterial effect are kumatakenin and two steroidal glycosides and were isolated from leaves extract (Villaflor et al., 2010). Fruit extract of *Alpinia oxyphylla* showed the presence of yakuchinone-A (Oonmetta-aree et al., 2006) and norcardinane (Muraoka et al., 2001) possessing cardiotonic effect; kernel of the plant contains protocatechuic acid having neuroprotective effect (An et al., 2006) and diarylheptanoids produce anti-inflammatory effect (Chun et al., 2002). The plant also showed antidiarrheal, antidiuretic, antineoplastic,*

*Corresponding author. E-mail: m.anwar.basher@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](http://creativecommons.org/licenses/by/4.0/).
antioxidant, anti-inflammatory, anti-allergic, viscera protective and anti-diabetic activities (Abubakar et al., 2018). Remarkable bioactivities such as anti-inflammatory, cytotoxicity, homeostasis, lipid regulation, antioxidant, antiviral, antimicrobial, antiosteoporosis of *Alpinia officinarum* were reported (Abubakar et al., 2018). *A. officinarum* showed the presence of two flavonoids, four diarylheptanoids, one sterol and one heptanone- all of these compounds possess antiemetic effect (Shin et al., 2002) and also cause inhibition of prostaglandin biosynthesis (Kiuchi et al., 1982). Alpinia zerumbet showed the presence of labdane-type diterpenes such as zerum A and zurumin B (Xu et al., 1996). These essential oils are believed to have antinociceptive effect (De Araújo Pinho et al., 2005); flavonoids with antihypertensive effect (de Moura et al., 2005; Mpalantinos et al., 1998) were also found in this species. Chemical constituents of aerial parts of *Alpinia katsumadai* include stilbenes, monoterpenes, diarylheptanoids, labdanes and chalcones (Ngo and Brown, 1998) and showed antioxidative activity (Lee et al., 2003). Rhizome of *Alpinia galanga* showed the presence of an antifungal compound named afetoxychavicol acetate (Janssen and Scheffer, 1985; Onnnetta-aree et al., 2006); the compound also exhibited antitumor activity (Itokawa et al., 1987). *Alpinia calcarata* Roscoe is widely distributed in different regions of Bangladesh. It is cultivated as ornamental plants in Bangladesh, India, Sri Lanka, and Malaysia. This rhizomatous plant is commonly used as systemic medicinal sources in Sri Lanka. Traditionally, *A. calcarata* has been used by various indigenous communities as a remedy for bronchitis, cough, respiratory ailments, diabetics, asthma and arthritis (Perera et al., 2014; Ramanayake, 1994). Anecdotal use of this plant against pain and inflammation was scientifically proven by the findings of potent antinociceptive effect in mice model; also, neurological effects were simultaneously performed and observed dose-dependent effect (Arumbewela et al., 2009a, b, 2004). Researchers also showed that rhizomes of *A. calcarata* possess antifungal, antibacterial, aphrodisiac, anesthetic, antioxidant, gastroprotective and anti diabetic activities (Arumbewela and Arawwawala, 2005; Arumbewela et al., 2010, 2009a, b; Ratnasooriya and Jayakody, 2006). Extensive chemical analyses of different parts of *A. calcarata* and diverse groups of phytochemicals were reported. Eighteen different volatile oils were found in rhizome, leaves and root; among these, rhizome and leaves mainly contain 1,8-cineole while α-fenchyl acetate is the principle essential oil in root (Rahman et al., 2013; Tewari et al., 1999). Kong et al. (2000) isolated four diterpenoids such as calcaratarians A-D, a sesquiterpenoid named shlyobunone and coumarins from rhizome extract of *A. calcarata* (Kong et al., 2000); in another study, flavonoids were reported in the same plant part (Hema and Nair, 2013).

Pain is a common nonspecific manifestation in many diseases. Non-steroidal anti-inflammatory drugs and opiates are generally used in pain management, but many adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence (Pergolizzi Jr et al., 2016; Scheiman, 2016; Sevinsky et al., 2017). Everyone experiences pain at some points in life, but pain accompanied with depression and anxiety is hard to endure as well as treat. People affected with psychotic disorders generally tend to experience more severe and long-lasting pain than others (Ploghaus et al., 2001). Diseases such as fibromyalgia (Gracey et al., 2012), irritable bowel syndrome (Mudyanadzo et al., 2018), low back pain (Sagheer et al., 2013), headache (Beghi et al., 2010), nerve pain (Sieberg et al., 2018), etc. often display mixed symptoms by anxiety, depression and pain. In addition to this, there is a strong relationship between sedation and pain and procedural sedation is widely used in various painful medical conditions (Frolich et al., 2013). In patients with psychotic disorders as well as pain, various psychotherapies can be used on their own to treat pain or may be combined with drug treatment. Numerous scientific reports have been published on beneficial pharmacological effects of plant in pain and neurological disorders (Akhigbemen et al., 2019; Auhey et al., 2016; Benjumea et al., 2016; Huda et al., 2019; Khan et al., 2017; Roy et al., 2019); plants enriched with phytococonstituents effective again analgesia and neurological disorders would be boon for mankind. The therapeutic potentials of the rhizome of *A. calcarata* have been well established, and the medicinal effects of its leaves are yet to be explored. Thus, our present study aims to examine antinociceptive and neurological activities of methanolic extract of leaves of *A. calcarata*.

**MATERIALS AND METHODS**

**Experimental animals**

Swiss albino mice (20-25 g) of both sexes were obtained from International Centre for Diarrhoeal Disease Research and Jahangirnagar University, Bangladesh. Animals were housed in groups of six in cages (40 cm x 30 cm x 17 cm; made up of polypropylene base and stainless-steel net) under a standard 12 h light : 12 h dark cycle (light phase 7 a.m. – 7 p.m.) in a room maintained at 23-25°C and at approximately 50-55% relative humidity. Food and water were allowed *ad libitum* during the study period. In all experiments, mice were divided into four groups and each group consisted of six mice. All animal procedures and experimental protocols were approved by the Departmental Research Ethics Committee of the institution.

**Plant materials**

The leaves of *A. calcarata* were collected from botanical garden, Dhaka, Bangladesh and were authenticated by National Herbarium, Dhaka, Bangladesh. The leaves were sorted, cleaned, dried at room temperature and finally pulverized. About 400 g powdered plant material was taken separately in a clean, flat bottomed glass
container and soaked in 1500 ml of 80% methanol at room temperature for fifteen days with occasional shaking and stirring. Then the solution was filtered using filter cloth and Whatman filter paper No. 1 and concentrated with a rotary evaporator (RE-EV311-V, LabTeck S.R.L., Italy). It rendered a gummy concentrate of greenish black color. The gummy concentrate was designated as a crude methanolic extract.

### Chemicals and drugs

Methanol (Merck, Germany) was used as a solvent during extraction. 0.6% acetic acid (98% v/v) (Loba Chemie, India) aqueous solution was prepared and used to induce writhing on mice. Formaldehyde, 37% (Loba Chemie, India) was used for the preparation of 2.5% formalin. Aspirin (dose used: 100 mg/kg.bw; Albion Laboratories Limited, Bangladesh) was used as a positive control or standard during the study (acetic acid induced writhing and formalin-induced paw licking test); diazepam (1 mg/kg.bw) for neuropathological tests was also collected from the same source. Distilled water was used as control or vehicle. Distilled water, aspirin or diazepam and plant extracts were administered orally.

### Analgesic test

#### Acetic acid induced writhing test

Acetic acid induced writhing test was performed as described in the literature (Hishe et al., 2018). The test samples and both controls were administered 30 min before induction of writhing by intraperitoneal injection of acetic acid (0.6%, 0.1 ml/10 g.bw). Number of writhing shown by each mouse was counted and recorded for 30 min. Contraction of the abdomen, elongation of the body, twisting of the trunk and/or pelvis ending with the extension of the limbs was considered as writhing. Results were expressed as mean percentage inhibition of writhing (PIW) (Hishe et al., 2018):

\[
\text{PIW} = \frac{\text{No. of Writhes (control) - No. of Writhes (sample)}}{\text{No. of Writhes (control)}} \times 100
\]

#### Formalin induced licking test

In the licking test, formalin (2.5%, 0.02 ml) was injected subcutaneously to plain surface of the left hind paw of mice after 1 h of administration of test samples. Licking and biting of the injected paw were considered as an indication of pain. The amount of time spent in licking was recorded in two phases: at first 0-5 min and last 20-30 min after formalin injection (Wheeler-Aceto et al., 1990). The results were expressed as percentage inhibition of licking response (PIL) (Hishe et al., 2018):

\[
\text{PIL} = \frac{\text{Time spent licking for Control - Time spent licking for Sample}}{\text{Time spent licking for Control}} \times 100
\]

### Behavioural assays

#### Open field test (OFT)

Open field test was performed in a box (72 cm x 72 cm x 36 cm) made of plywood and clear Plexiglas. The floor of the box was divided and marked into sixteen (18 cm x 18 cm) squares. Controls and extracts were administered orally 30 min prior to the test. Each mouse was then put into the apparatus and the number of square blocks visited by each mouse was calculated for 5 min on 0, 30, 60, 90 and 120 min intervals (Consolini et al., 2006).

#### Elevated plus maze test (EPM)

The apparatus used for elevated plus maze test was comprised of two open arms (35 cm x 5 cm) across from each other and perpendicular to two closed arms (35 cm x 5 cm x 15 cm) with a center platform (5 cm x 5 cm) with minor variations in the related literature (Hritcu et al., 2011). The open arms were exposed having no wall whereas the closed arms were enclosed by walls 15 cm high. Each mouse was placed in the center area of the maze with its head directed toward a closed arm after 1 h of treatment with samples and allowed to move freely about the maze for 5 min. After each trial, all arms and the center area were cleaned with 10% ethanol.

#### Light-Dark test (LDT)

The apparatus used for the light/dark transition test consisted of a cage (21 cm x 42 cm x 25 cm) divided into two sections by a partition with door (7 cm x 7 cm); the light compartment is 2/3 of the box, is brightly lit and open, the dark compartment is 1/3 of the total box and is covered and dark. Experimental animals were kept for an hour in a dark testing room to adapt with new environment before subjected to light-dark test. Then, each mouse was placed at the center of the light compartment with its back to the dark compartment, and then transition behavior over 5 min was observed with the following parameters: time spent in light compartment and number of transitions. Typically, mouse was expected to move around the periphery of the compartment until they find the door. All four paws must be placed into the opposite chamber to be considered an entry (Gong et al., 2006).

#### Thiopental Sodium induced sleeping time test

In this test, controls and extracts were administered to each mouse first. After 20 min, thiopental sodium (40 mg/kg.bw) was injected intraperitoneally. The animals were then observed for onset of sleep and duration of sleep (Moniruzzaman et al., 2015).

#### Hole cross test

Hole cross test was done in a steel cage having dimensions of 30 cm x 20 cm x 14 cm. A partition which has a hole of 3 cm diameter at a height of 7.5 cm was fixed in the middle of the cage. After administration of sample, each mouse was introduced into the cage and the number of passages from one chamber to other through the hole inside the cage was counted for 3 min on 0, 30, 60, 90 and 120 min intervals (Ali et al., 2014).

### Statistical analysis

Data were presented as mean ± SEM values. One-way ANOVA with Dunnett’s test was performed using GraphPad Prism (version 8.3). A probability level of 0.05 (adjusted P value according to GraphPad Prism) or less was accepted as significant; \( p < 0.05 \), \( p < 0.01 \), \( p < 0.001 \) vs. vehicle; \( p < 0.05 \), \( p < 0.01 \), \( p < 0.001 \) vs. either aspirin or diazepam.

### RESULTS

#### Writhing response in acetic-acid induced mice

As shown in Figure 1A, aspirin significantly (\( p<0.001 \))...
Figure 1. Effect of methanolic extract of leaves of A. calcarata on analgesic tests. 'Veh' stands for vehicle or control, 'Aspr' for aspirin and 'ME' for methanolic extract while 200 and 400 were the doses in mg/kg,bw. Values are mean ± SEM (n=6) and One-way ANOVA with Dunnett's test was performed.

a p<0.05, b p< 0.01, c p<0.001 vs. vehicle; α p<0.05, β p<0.01, γ p<0.001 vs. aspirin.

Table 1. Effect of A. calcarata leaves extract on percentage inhibition in acetic acid induced writhing and hind paw licking.

| Group     | Acetic acid induced writhing (% inhibition) | Hind paw licking (% inhibition) |
|-----------|--------------------------------------------|---------------------------------|
|           |                                            | Early phase | Late phase |
| Vehicle   | -                                          | -           | -         |
| Aspirin   | 75.76                                      | 52.95       | 47.28     |
| ME200     | 41.10                                      | 36.50       | 28.38     |
| ME400     | 59.82                                      | 50.22       | 43.22     |

decreased the number of abdominal writhes (13.1) as compared to the control group (54.33). Similar to positive control (aspirin), leaves extract at both doses (ME200 and ME400) also exhibited significant reduction in the number of abdominal writhes against acetic acid induced pain. Moreover, percentage inhibition of writhing was observed 75.76, 41.10 and 59.82 for aspirin, ME200 and ME400 respectively (Table 1).

Paw licking in formalin-induced pain in mice

Figure 1B shows that animals administered leaves extract demonstrated significantly (p<0.001 for early phase and late phase) reduction of paw-licking time compared with the control group at both phases. Likewise, in both phases, aspirin also significantly (p<0.001) reduced the paw-licking time compared with the control group. However, no significant differences were observed among the leaves extract at the dose of 200 mg/kg (ME200). Calculating the percentage inhibition of licking, aspirin demonstrated highest percentage inhibition (early phase: 52.95% and late phase: 47.28%) followed by ME200 (early phase: 36.50% and late phase: 28.38%) and ME400 (early phase: 50.22% and late phase: 43.22%) of leaves extract, respectively (Table 1).

Anxiolytic effect in open field test (OF)

In open field test, number of square travelled was recorded after 0, 30, 60, 90 and 120 min. For all samples, square crossed was highest at 0 min that gradually decreased showing lowest number at 120 min (Table 2). Number of squares travelled at 0 min was 46.5 and 33.17 (p<0.001) for vehicle and diazepam respectively; these movements were slowly decreased demonstrating 33.0 and 13.17 at 120 (p<0.001) min. In case of methanolic extract, ME200 showed 43.0 and 24.00 (p<0.01) square movements at 0 and 120 min respectively while ME400 demonstrated nearly equal movements similar to diazepam at 0 and 120 min, producing 35.83 (p<0.01)
Table 2. Effect of A. calcarata leaves extract on anxiolytic responses in open field test.

| Group     | 0 min     | 30 min    | 60 min    | 90 min    | 120 min   |
|-----------|-----------|-----------|-----------|-----------|-----------|
| Vehicle   | 46.5±2.41  | 44.67±2.25 | 42.33±2.36 | 36.33±2.94 | 33.00±2.60 |
| Diazepam  | 33.17±1.14 | 30.67±0.99 | 26.00±1.39 | 19.5±1.96  | 13.17±1.52 |
| ME200     | 43.00±1.61 | 38.5±1.12  | 33.67±1.54 | 28.33±1.52 | 24.00±1.32  |
| ME400     | 35.83±2.52 | 30.67±2.42 | 24.17±2.06 | 20.00±1.63 | 16.33±1.63  |

Values are mean±SEM (n=6) and One-way ANOVA with Dunnett’s test was performed. a p<0.05, b p<0.01, c p<0.001 vs. vehicle; d p<0.05, e p<0.01, f p<0.001 vs. diazepam.

**Anxiolytic effect in elevated plus maze test (EPM)**

In the elevated plus maze test, the number of entry in the open arm of the apparatus for vehicle and diazepam were 9.33 and 16.17 (p<0.01) respectively; accordingly, time spent for the controls were 70.83 and 158.3 s (p<0.001) respectively (Figure 2A and 2B). Methanolic extracts produced a higher number of entry than the vehicle in the open arm showing 11.6 and 12.0 for ME200 and ME400 respectively. As a result, longer durations than vehicles were observed for both extracts showing 128.2 and 145.5 s (p<0.001) respectively.

**Anxiolytic effect in light dark test (LDT)**

In this experiment, both number of entry and time spent in the light box of the apparatus were increased after the administration of diazepam compared to vehicle (Figure 2C and 2D). For diazepam, the animals showed 21.5 appearances (p<0.01) staying 182.8 s (p<0.001) in the light chamber while the vehicle showed 10.0 entries (p<0.01) with total spent time of 43.5 s (p<0.001). ME200 and ME400 caused longer duration to stay of 142.5 s (p<0.001) and 173.8 s (p<0.001) respectively with 15.17 and 18.67 (p<0.05) entries.

**Sedative effect in tiopental sodium induced sleeping time test**

The effect of extract on induction of sleep and its duration was shown in Figure 3A and 3B. Diazepam took far less time (11.5 min, p<0.001) to induce sleep in mice than vehicle (42.33 min, p<0.001). ME200 induced sleep in slightly less time (11 min, p<0.001) than diazepam; onset of sleep was further decreased when the dose of extract increased to 400 mg/kg, bw (ME400) (8.33 min, p<0.001). Consequently, duration of sleep followed similar trend (p<0.001) where the animals were asleep for longer period after the administration of diazepam and extract compared to the vehicle.

**Sedative effect in the hole cross test**

In this experiment, number of holes crossed at 0, 30, 60, 90 and 120 min were recorded and were continued to decline over time. Animals crossed around twenty holes (p<0.001) for diazepam and both doses of extracts while vehicle showed 31 passages (p<0.001) as the experiment commenced. The movements of mice were dropped as time elapsed; diazepam and ME400 produced nearly equal numbers at 120 min showing 5.17 (p<0.01) and 5.5 (p<0.001) respectively; ME200 caused moderately higher hole crossings (7.5, p<0.001) than diazepam at the same time span (Table 3).

**DISCUSSION**

In our research work, analgesic and neuropharmacological potentials of A. calcarata leaves were assessed. To determine antinociceptive effect, acetic acid induced writhing test and formalin-induced hind paw licking test were performed as peripheral models of pain. Both of these methods are commonly used for the assessment of the peripheral pathway of analgesic drugs (Jain et al., 2001). The plant extract demonstrated dose-dependent analgesic effect. In acetic acid induced writhing test, significant inhibition of nocifensive behaviors were observed at highest extract dose (59.82%) as compared to aspirin (75.76%). Acetic acid causes excitation of nociceptive nerve endings resulting to production of certain prostaglandins, activation of ion channels and increased capillary permeability (Pace et al., 2017; Ribeiro et al., 2000; Sutraddhar et al., 2007; Voilley, 2004). Production of prostaglandins in peripheral tissues involves the cyclooxygenase pathway, which is blocked by common NSAIDs. Reduced nocifensive behaviors by the methanolic extract of leaves in induced writhing experiment could be assumed by interference of any of these pains and inflammation induction pathways. In formalin licking test, the licking time was recorded in two phases. In both phases, nearly equivalent effects were demonstrated by extract at 400 mg/kg, bw and aspirin; extract produced 52.95% inhibition while for aspirin, it was 50.22% in early phase. Effects were slightly attenuated...
Figure 2. Effect of A. calcarata leaves extract on anxiolytic responses in (A and B) elevated plus maze and (C and D) light dark test. ‘Veh’ stands for vehicle or control, ‘Diaz’ for diazepam and ‘ME’ for methanolic extract while 200 and 400 were the doses in mg/kg.bw. Values are mean ± SEM (n=6) and One-way ANOVA with Dunnett’s test was performed. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle; αp<0.05, βp<0.01, γp<0.001 vs. diazepam.

Species from same genus such as A. zerumbet (De Araújo Pinho et al., 2005) and A. oxyphylla (Chun et al., 2002) showed antinociceptive and anti-inflammatory effects and these effects were believed to be produced by terpenoids. Phytochemical screening of A. calcarata showed the presence of alkaloids, phytosterols and flavonoids (Ferdous et al., 2018). Thus, any of these compounds or in combination could be responsible for the anti-nociceptive effect of extract of A. calcarata.

In case of neuropharmacological effects of the extract, the plant showed dose dependent activity in open field test. Standard drug- diazepam and plant extract at 400 mg/kg.bw demonstrated similar effect throughout the experimental period producing final locomotor activity of 13.17 and 16.33 respectively. Locomotion is believed to be mediated through dopaminergic pathway and other neural mechanisms (Raghav et al., 2018; Steidlet al., 2017).
It can be suggested that inhibitory effect of methanolic extract on locomotor activity could be mediated by interference in the GABA neurotransmission of central nervous system (Liu et al., 2015). Elevated plus maze test and light dark test also showed analogous results demonstrating increased entry and time spent in the open arm or illuminated areas which correspond with similar effects as diazepam and other anxiolytic drugs (Bourin and Hascoët, 2003; Kędzierska et al., 2018; Kosari-Nasab et al., 2018). Thus, our findings suggest that leaves of *A. calcarata* could have potential phytochemicals responsible for anxiolytic effect. The classic experimental methods for the evaluation of sedative effect include thiopental sodium induced sleeping test and hole cross test. The extract showed significant reduction in sleep latency and increased thiopental sodium induced sleeping time, indicating sedative effect. Thiopental is known to enhance the inhibitory action of the GABA receptor that decreases neuronal activity (Begum et al., 2019). On the other hand, in hole cross test, the results showed dose dependent activity corroborating with the findings of thiopental sodium induced sleeping test. The prolongation of thiopental sodium induced sleep and suppression of exploratory behavior indicates sedative effect of the plant extract. Neurological effects were reported by rhizome of *A. calcarata*, and presence of terpenoids and flavonoids could result to such effect (Hema and Nair, 2013; Kong et al., 2000). Another species of *Alpinia* genus- *A. oxyphylla* showed neuroprotective effect and contains protocatechuic acid (An et al., 2006). Presence of diverse types of phytochemicals in leaves extract of *A. calcarata* certainly contains compounds that impart neurological effects.

**Conclusion**

The results of the present study displayed significant analgesic activity of methanolic extract of leaves of *A. calcarata* thereby scientifically confirming the traditional usage of this species for arthritis. The findings of this
study also provide the first evidence of anxiolytic and sedative like effects of the A. calcarata leaves extract in the CNS in mice model. Further investigations are necessary for isolation and identification of the chemical compound(s) responsible for the observed biological effects of the extracts.

CONFLICT OF INTERESTS
The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT
The research work was partially funded by the institution from research allocations for Master’s students.

REFERENCES
Abubakar IB, Malami I, Yahaya Y, Manga SS (2018). A review on the ethnomedicinal uses, phytochemistry and pharmacology of Alpinia officinarum Hance. Journal of Ethnopharmacology 224:45-62.

Akhigbemen AM, Ozolua RI, Bator EE, Okwuofu EC (2019). Evaluation of some neuropharmacological effects of Caladium bicolor aiton (araceae) leaf extracts in mice. Metabolic Brain Disease 34(2):537-544.

Ali SA, Mamun MAA, Sayeed MA, Rahman MS, Rashid MA (2014). Sedative activity of methanolic extract of Glochidion multiloculare (Rottler ex Wild) Voigt leaves. Pakistan Journal of Biological Sciences 17(4):555-559.

An LJ, Guan S, Shi GF, Bao YM, Duan YL, Jiang B (2006). Protocatechuic acid from Alpinia oxyphylla against MPP+ induced neurotoxicity in PC12 cells. Food and Chemical Toxicology 44(3):436-443.

Aouey B, Samet AM, Fetoui H, Simmonds MS, Bouaziz M (2016). Antinociceptive activities of aqueous and ethanolic extracts of Alpinia calcarata rhizomes on ethanol induced gastric ulcers in rats. Pharmacognosy Magazine 5(19):226.

Arambewela L, Arawwawala L, Ratnasooriya W (2009a). Effect of Alpinia calcarata rhizomes on ethanol-induced gastric ulcers in rats. Pharmacognosy Magazine 5(20):412.

Arambewela LSR, Arawwawala LDAM, Ratnasooriya WD (2004). Antinociceptive activities of aqueous and ethanolic extracts of Alpinia calcarata rhizomes in rats. Journal of Ethnopharmacology 95:311-316.

Beghi E, Bussone G, D’Amico D, Cortelli P, Cevoli S, Manzoni GC, Torelli P, Tonini MC, Allais G, De Simone R, D’Onofrio F, Genco S, Moschiano F, Beghi M, Salvi S (2010). Headache, anxiety and depressive disorders: the HADAS study. Journal of Headache and Pain 11(2):141-150.

Begum A, Hossen A, Moly AA, Bhuiany MMR, Shahed-Al-Mahmud M (2019). In Vivo Sedative and Anxiolytic Activities of Thunbergia erecta (Acanthaceae) Leaves Activate Gamma-Aminobutyric Acid (GABA) Mediated Hyperpolarizationin Swiss Albino Mice. Pharmacology and Pharmacy 10:177-193.

Benjumea DM, Gómez-Betancur IC, Vásquez J, Alzate F, García-Silva A, Fontenla JA (2016). Neuropharmacological effects of the ethanolic extract of Sida acuta. Revista Brasileira de Farmacognosia 26(2):209-215.

Both FL, Kerber VA, Henriques AT, Elisabetsky E (2002). Analgesic properties of umbellatine from Psychotria umbellata. Pharmaceutical Biology 40(5):336-341.

Bourin M, Hascoët M (2003). The mouse light/dark box test. European Journal of Pharmacology 463(1-3):55-65.

Chan EWC, Wong SK (2015). Phytochemistry and pharmacology of ornamental ginger, Hedychium coronarium and Alpinia purpurata: a review. Journal of Integrative Medicine 13(6):368-379.

Chen J, Hang L, Zhang Y, Zhao X, Gao X, Liu Y (2016). Role of interleukin-1β activation of NMDA receptors in spinal dorsal horns of rats with neuro-pathic pain. Chinese Journal of Anesthesiology 36(11):1366-1370.

Chun K-S, Park K-K, Lee J, Kang M, Suh Y-J (2002). Inhibition of mouse skin tumor promotion by anti-inflammatory diarylheptanoids derived from Alpinia oxyphylla Miquel (Zingiberaceae). Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics 13(1):37-45.

Consolani AE, Ragone MI, Migliori GN, Conforti P, Volonte MG (2006). Cardiotonic and sedative effects of Cecropia pachystachya Mart. (ambay) on isolated rat hearts and conscious mice. Journal of Ethnopharmacology 106(1):90-96.

De Araújo Pinho F, Coelho-de-Souza A, Moraes S, Santos CF, Leal-Cardoso J (2005). Antinociceptive effects of the essential oil of Alpinia zerumbet on mice. Phytomedicine 12(6-7):482-486.

de Moura RS, Emiliano A, de Carvalho LCM, Souza MA, Guedes D, Tano T, Resende A (2005). Antihypertensive and endothelium-dependent vasodilator effects of Alpinia zerumbet, a medicinal plant. Journal of Cardiovascular Pharmacology 46(3):288-294.

Demirovic R, Rashid K, Ciganovic O, Vladic-Kresevic S, Skoda M, Visnic A (2015). Myricitrin exhibits antioxidant, anti-inflammatory and antifibrotic activity in carbon tetrachloride-intoxicated mice. Chemico-Biological Interactions 230:21-29.

Ferdous M, Bashker MA, Khan I, Ahmed F, Sobuz MSI, Daula ASU (2018). Evaluation of Phytochemicals, antioxidant and antibacterial potentials of Alpinia calcarata. Journal of Medicinal Plants Studies 6(2):152-158.

Frolich MA, Zhang K, Ness TJ (2013). Effect of sedation on pain perception. Anesthesiology 118(3):611-21.

Gong ZH, Li YF, Zhao N, Yang HJ, Su RB, Luo ZP, Li J (2006). Anxiolytic effect of agmatine in rats and mice. European Journal of Pharmacology 550(1-3):112-116.

Gracely RH, Ceko M, Bushnell MC (2012). Fibromyalgia and depression. Pain Research and Treatment 2012:486590. 10.1155/2012/486590.

Hegazi NM, Sobeh M, Rezq S, El-Ramey MA, Mahmoud MF, Wink M (2019). Characterization of phenolic compounds from Eugenia supra-axillaris leaf extract using HPLC-PDA-MS/MS and its antioxidant, anti-inflammatory, antipyretic and pain killing activities in vivo. Scientific Reports 9(1):11122.

Hema PS, Nair MS (2013). Flavonoids and other constituents from the rhizomes of Alpinia calcarata. Biochemical Systematics and Ecology 37:52-54.

Hishe HZ, Ambach TA, Hiben MG, Fanta BS (2018). Anti-nociceptive effect of methanol extract of leaves of Senna singueana in mice. Journal of Ethnopharmacology 217:49-53.

Hritcu L, Foyet HS, Stefan M, Mihasan A, Asongalem AE, Kamchatou P (2011). Neuroprotective effect of the methanolic extract of Hibiscus asper leaves in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Journal of Ethnopharmacology 137(1):585-591.

Huda N, Daula AFMSU, Barek MA, Bashker MA (2019). In vivo antidepressant and anxiolytic effects of methanol extract of leaves of Zingiber rubens Roxb. in mice model. EC Pharmacology and Toxicology 7(3):188-194.

Itokawa H, Morita H, Sumitomo T, Totsuka N, Takeya K (1987). Antitumour principles from Alpinia galanga. Planta Medica 53(01):32-33.

Jain NK, Patil C, Singh A, Kulkarni SK (2001). Sildenafil and Hyperpolarization in Swiss Albino Mice. Anti-inflammatory and analgesic activities of umbellatine from Psychotria umbellata. Pharmaceutical Biology 40(5):336-341.

Begum A, Hossen A, Moly AA, Bhuiany MMR, Shahed-Al-Mahmud M (2019). In Vivo Sedative and Anxiolytic Activities of Thunbergia erecta (Acanthaceae) Leaves Activate Gamma-Aminobutyric Acid (GABA) Mediated Hyperpolarization in Swiss Albino Mice.
pathway. Brain Research 909(1-2):170-178.

Janss AM, Scheffer JJ (1985). Acetoxychavicol acetate, an antifungal component of Alpinia galanga. Planta Medica 51(6):507-11.

Kędzierska E, Dąbkowska L, Górska-Bryła M, Polakowska M, Poleszak E, Wiaz P, Szewczyk K, Kotlińska J (2018). Synergistic Action of Sodium Selenite with some Antidepressants and Diazepam in Mice. Pharmaceutics 10(4):270.

Khan MJ, Saraf S, Saraf S (2017). Anti-inflammatory and associated analgesic activities of HPLC standardized alcoholic extract of known ayurvedic plant Schleichera oleosa. Journal of Ethnopharmacology 197:257-265.

Kiuchi F, Shibuya M, Sankawa U (1982). Inhibitors of prostaglandin biosynthesis from Alpinia officinarum. Chemical and pharmaceutical bulletin 30(6):2279-2282.

Kong LG, Qin MJ, Niwa M (2000). Diterpenoids from the rhizomes of Alpinia calcarata. Journal of Natural Products 63(7):939-942.

Kosari A, Naderi M, Nourani MR, Soares de Moura R, Parente J, Kuster R (1998). Antioxidant activity of sedative and hypnotic action of Alpinia officinarum. Journal of Biologically Active Products from Natural Products 12(6):1123-1131.

Lee SE, Shin HT, Hwang HJ, Kim JH (2003). Antioxidant activity of extracts from Alpinia katsumadai seed. Phytotherapy Research 17(9):1041-1047.

Li X-H, Miao H-H, Zhuo M (2019). NMDA receptor dependent long-term potentiation in chronic pain. Neurochemical Research 44(3):531-538.

Liu J, Zhai W, Chi H, Wang S, Jiang X, Fan L, Guo J-Y (2015). GABA and 5-HT systems are implicated in the anxiolytic-like effect of spinosin in mice. Pharmacology, Biochemistry and Behavior 128:41-49.

Moniruzzaman M, Rahman MA, Ferdous A (2015). Evaluation of sedative and hypnotic activity of ethanolic extract of Scoparia dulcis linn. Journal of Evidence-Based Complementary and Alternative Medicine 10.1155/2015/873954.

Mpalantinos M, Soares de Moura R, Parente J, Kuster R (1998). Biochemically active flavonoids and kava pyrones from the aqueous extract of Alpinia zerumbet. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 12(6):442-444.

Mudyanatdo TA, Hauzaree C, Yerokhina O, Architha NN, Ashgar HM (2018). Entabale Bowell Syndrome and Malaria: a mouse model of mild traumatic brain injury. Toxicology and Applied Pharmacology 338:159-173.

Lee SE, Shin HT, Hwang HJ, Kim JH (2003). Antioxidant activity of extracts from Alpinia kutsumadai seed. Phytotherapy Research 17(9):1041-1047.

Li X-H, Miao H-H, Zhuo M (2019). NMDA receptor dependent long-term potentiation in chronic pain. Neurochemical Research 44(3):531-538.

Liu J, Zhai W, Chi H, Wang S, Jiang X, Fan L, Guo J-Y (2015). GABA and 5-HT systems are implicated in the anxiolytic-like effect of spinosin in mice. Pharmacology, Biochemistry and Behavior 128:41-49.

Onasanwo SA, Eleigbe RA (2006). Antinociceptive and antiinflammatory properties of the leaf extract of Hedranthera barteri in rats and mice. African Journal of Biomedical Research 9:109-117.

Oommetta-aree J, Suzuki T, Gasaluck P, Eumkeb G (2006). Antimicrobial properties and action of galangal (Alpinia galanga Linn.) on Staphylococcus aureus. LWT-Food Science and Technology 39(10):1214-1220.

Pace S, Rossi A, Krauth V, Dehm F, Troisi F, Bilancia R, Weinigel C, Krauth V, Dehm F, Troisi F, Bilancia R, Weinigel C (2018). Anxiolytic-like effect of spinosin in mice. Pharmacology, Biochemistry and Behavior 128:41-49.

Ngo K-S, Brown GD (1998). Slibbens, monoterpenes, diarylheptanoids labdanes and chalcones from Alpinia katsumadai. Phytochemistry 47(6):1117-1123.

Onasanwo SA, Eleigbe RA (2006). Antinociceptive and antiinflammatory properties of the leaf extracts of Hedranthera barteri in rats and mice. African Journal of Biomedical Research 9:109-117.

Oommetta-aree J, Suzuki T, Gasaluck P, Eumkeb G (2006). Antimicrobial properties and action of galangal (Alpinia galanga Linn.) on Staphylococcus aureus. LWT-Food Science and Technology 39(10):1214-1220.

Pace S, Rossi A, Krauth V, Dehm F, Troisi F, Bilancia R, Weinigel C, Rummiller S, Werz O, Saubelin L (2017). **Sex differences in prostaglandin biosynthesis in neutrophils during acute inflammation.** Scientific Reports 7:3759.

Perera PK, Perera M, Kumarasinghe N (2014). Effect of Sri Lankan traditional medicine and Ayurveda on Sandhigata Vata (ostearthritids of knee joint). Ayu (An International Quarterly Journal of Research in Ayurveda) 35(4):411-415. 10.4103/0974-8520.159007.

Pergolizzi Jr JV, Rafia RB, Nalamachu S, Taylor Jr R (2016). Evolution to low-dose NSAID therapy. Pain Management 6(2):175-189.

Ploegh J, Spilarska C, Beckmann CF, Bantick S, Wise R, Matthews PM, Rawlins JNP, Tracey I (2001). Excititation of pain by anxiety is associated with activity in a hippocampal network. Journal of Neuroscience 21(24):9896-9903.