

ORIGINAL CONTRIBUTION

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Phytochemical investigation and in vitro and in vivo pharmacological activities of methanol extract of whole plant *Argyreia capitiiformis* (Poir.) Ooststr

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Abstract

Background *A. capitiiformis* (Poir.) Ooststr has a long history of usage as a medicinal cure for a wide variety of illnesses in many different cultures. Pharmacological properties and phytochemical characterization of the crude *A. capitiiformis* whole plant are evaluated, in this paper.

Methods Antioxidant activity was tested by the DPPH free radical scavenging method. In vitro anti-arthritis, anti-inflammatory, and cytotoxic effects were assessed using Bovine serum albumin (BSA), protein denaturation method, and brine shrimp mortality assays, respectively with antihelmintic activity through *Pheretima Posthuma* worms. Acetic acid-induced writhing, hot plate and tail immersion testing assessed in vivo analgesia. CNS activity was evaluated through elevated platform, open field, hole cross, and head dipping method.

Results Phytochemical investigation of *A. capitiiformis* showed the presence of alkaloid, saponin, terpenoids, steroid and flavonoids etc. with the % yield of crude 2.04%. With an IC_{50} of 45.35 $\mu\text{g/ml}$, the whole plant methanolic preparation has antioxidant activity equivalent to ascorbic acid. Anti-arthritis protein blocking dropped from $74.25 \pm 0.12\%$ to $12.18 \pm 0.12\%$. 1000 $\mu\text{g/ml}$ extract demonstrated $54.05 \pm 0.12\%$ anti-inflammatory activity with protein denaturation. In the cytotoxicity assay, the extract had 129.72 $\mu\text{g/ml}$ LC_{50} and the positive group 34.67 $\mu\text{g/ml}$. Unlike Albendazole, the methanol extract triggered mature earthworms at 50 mg/ml. The extract's analgesic efficacy at 200 and 400 mg/kg was statistically significant ($p < 0.001$) in the acetic acid writhing and tail immersion method. The hot plate technique yielded statistically significant results only at 400 mg/kg ($p < 0.001$). Only 400 mg/kg was statistically significant in the Elevated Platform and Hole Board Procedure ($p < 0.01$). The hole cross and open field methods yielded highly statistically significant outcomes at 200 and 400 mg/kg ($p < 0.001$).

Conclusion In this research, the whole crude methanol extract of *A. capitiiformis* revealed phytochemicals, antioxidants, in vitro anti-inflammatory and anti-arthritis properties, cytotoxicity, anti-helmintic, in vivo analgesic, and CNS inhibitory activities.

Keywords *A. Capitiiformis*, Phytochemicals, Antioxidant, Anti-arthritis, Cytotoxic, Analgesic

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Background

Plants continue to provide a rich supply of numerous phytochemicals employed in medication research, and a growing number of these compounds have been shown to have therapeutic benefit. The conventional medical system makes use of around 800 different flora, of which about 500 are listed in old texts as being useful [1]. Nowadays, humans are exploring different ways to treat, cure, relieve, and prevent medical issues and diseases [2]. Synthetic and semi-synthetic medications cause hospitalisations and fatalities. Adverse drug reactions cause 8% of US hospitalisations. Poisons kill 100,000 people annually [3]. These drugs are also pricey, making therapy challenging for patients. Plant-based herbal therapies are being investigated as a treatment for this condition. Thus, scientists are seeking new herbal therapies for pain, inflammation, diabetes, and other disorders. Plants contain phenols, glycosides, alkaloids, saponins, terpenoids, tannins, polysaccharides, flavonoids, plant lipids, resins, and essential oils [4, 5].

Oxidative stress is characterised by increased ROS and inadequate antioxidant systems. Reactive stress may damage cells and kill tissues [6]. SOD, epigallocatechin-3-O-gallate, CAT, lycopene, coenzyme Q10, ellagic acid, quercetin, indole-3-carbinol, genistein, vitamin E, and vitamin C have been widely explored as preventive and curative medicines [7]. 0.5–1.0% of Western adults have rheumatoid arthritis (RA), a chronic, debilitating, inflammatory, degenerative joint illness [8]. NSAIDs like aspirin have historically been the primary line of defence against rheumatoid arthritis pain [9]. Recently added therapies include corticosteroids and disease-modifying anti-rheumatic medications. The inflammatory reaction protects after removing harmful external effects, your body begins to restore itself. Inflammation may be acute or chronic [10–12]. As painkillers or anti-inflammatories, common medications include paracetamol, diclofenac, ketorolac, narcotics, etc. Aspirin, codeine, and morphine make up a basic painkiller routine, but they all come with their fair share of unwanted side effects, including those that can affect your stomach, heart, kidneys, brain, and immune system [3]. Cancer is a fatal illness characterized by cellular anomalies, uncontrolled cell division and growth, and eventually, cell mortality [13]. Current conventional treatments, such as surgery, radiotherapy, and chemotherapy, all have drawbacks. It's promising that natural cytotoxic compounds may help in cancer treatment by harming cancer cells. It is believed that close to half of the global population is afflicted with helminthiasis, which is increasing rapidly. Helminthic infection is a big problem because it affects both people and animals, can last for a long time, and makes it hard to move around. Benzimidazoles, macrocyclic lactones, and cholinergic

agonists, such as levamisole, are the three most popular anthelmintics in small ruminants. These drugs work by inhibiting the metabolism of the worms and causing paralysis or death [14]. Pain, also called algesia, is an unpleasant sensation brought on by tissue damage. Pain initially protects you from harm but can backfire later [15]. Analgesics are painkillers that target the peripheral or central nervous systems to reduce pain [16]. Traditional analgesic medicines include nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids [17]. Both anxiety and depression are prevalent mental diseases that often co-occur with one another and are collectively classified as internalizing disorders [18]. Anxiety disorders can be treated with medication, and common options include SSRIs, SNRIs, pregabalin, buspirone, tricyclic antidepressants, monoamine oxidase inhibitors and benzodiazepines [19]. These manufactured antidepressants and calming treatments are successful, but patients often don't stick with them because of side effects or a significant delay in efficacy.

The plant *Argyrea capitiformis* (Poir.) Ooststr belongs to the family of convolvulaceae, having various traditional uses. In the Chakma culture, bruising on the legs is treated by applying leaf paste to the afflicted regions. Leaf ash is used for ear discomfort, and leaves are used externally for healing trauma [20–22]. Traditional medicine also used *capitiformis* as a purgative to cure sexual debility and ear discomfort [23–25]. To treat sexual debility, seed powder is provided with milk [26]. The network pharmacology analysis indicated critical pathways involved in the anti-inflammatory activity caused by the chemical components detected in the methanolic extract of *A. capitiformis* stem [27]. GC-MS analysis of the methanolic stem extract of *A. capitiformis* revealed the presence of 49 different compounds with varying peak areas and retention times. The study found that chemicals like 3-furaldehyde, phenol, catechol, and hydroquinone found in the methanolic extract of *A. capitiformis* are very good at reducing inflammation. These compounds have a significant impact on the inflammation pathway by specifically targeting six proteins [27].

Numerous investigations have been described for the *Argyrea* species, including antioxidant, immunomodulatory, anti-inflammatory, and CNS properties with many bioactive chemicals [28, 29].

Based on traditional uses and other pharmacological activities of *Argyrea* species, we evaluated the in vitro and in vivo pharmacological actions of the methanolic extract of this plant. Additional research is needed to identify and isolate the novel compound responsible for these pharmacological activities.

Methods

Collection and identification of the plant

The developed whole plant was obtained in August 2022 with the assistance of a well-known local traditional healer. A famous taxonomist identified it as *A. capitiformis* with herbarium no MC 031022-16.

Preparation of crude extracts

Material from plants (leaves and stems) were cleaned, cut, and shade-dried for eight days. 700 g of ground *A. capitiformis* leaves, and stems were soaked in 3.5 L of methanol. The solution was passed through filters, and the resulting filtrate was concentrated using a rotary evaporator at reduced pressure (Stuart, UK). The total amount of *A. capitiformis* leaves and stems used to make the crude methanol extracts weighed 14.28 g. The following equation was used to determine the percentage (%) yield of the extract:

Percentage extract yield = (extract weight/powder weight) * 100. Crude methanol extracts of *A. capitiformis* whole plant yielded 2.04%.

Qualitative phytochemical screening

Using a standard procedure, preliminary phytochemical screening was performed to evaluate the qualitative detection of terpenoids, saponins, flavonoids, phenol, tannins, phlobatannins, steroids, glycosides, anthraquinones, alkaloids, resins, cardiac glycosides, carbohydrates, fat and oil, proteins, and coumarin [30]. The colour intensity or precipitate formation was utilized as analytic answers to these qualitative assessments.

Free radical scavenging activity [DPPH]

Blois' technique [31] was used to quantitatively assess the radical scavenging capabilities of *A. capitiformis* whole crude methanol extracts. To summarize, a 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) in methanol was produced, and 1 ml of this solution was added to 3 ml of extracts at various concentrations (500–0.49 µg/ml). As a positive control, ascorbic acid was employed. After 30 min in the dark, the discolouration was detected at 517 nm. Measurements were obtained three times. Using the following equation, the ability to scavenge the DPPH free radical was computed and represented as an inhibition percentage:

% inhibition = (Absorbance of control – Absorbance of test/Absorbance of control) × 100

Using a regression equation developed from data collected at varying concentrations of methanol extracts, we were able to determine the IC₅₀ values (the concentration of sample necessary to scavenge 50% of free radicals).

In vitro anti-arthritis activity

Preparation of solutions

Each 0.5 mL of the test solutions was prepared by combining 0.45 mL of a 0.5%w/v aqueous solution of BSA with 0.05 mL of methanolic extract of whole crude methanol extract of six concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/mL). All test solutions were brought to a pH of 6.3 using 1 N HCl. The standard solution was made by combining 1000, 500, 250, 125, 62.5 31.25 µg/mL of diclofenac sodium with 0.05 mL of 0.5%w/v aqueous solution of BSA. Using 1 N HCl, the standard solutions were brought to a pH of 6.3. 0.45 mL of a 0.5% w/v aqueous solution of BSA were combined with 0.05 mL of distilled water to make 0.5 mL of the test control solution. Using 1 N HCl, the pH of the control solutions was brought down to 6.3 for the experiments. A product control solution of 0.5 mL was prepared by combining 0.05 mL of the test solution with 0.45 mL of distilled water. 1 N HCl was used to lower the pH of the product control solutions to 6.3. UV-Vis spectroscopy calculated the absorbance using distilled water as a blank.

Experimental design

Methanolic extract of whole crude methanol extract was tested for anti-arthritis effects in vitro utilizing the “inhibition of protein denaturation” technique [32–34]. All the solutions were first incubated at 37 degrees Celsius for 20 min, after which they were heated to 57 degrees Celsius and held there for 3 min. After that, we let the solutions cool off. The prior solutions were allowed to cool, and then 2.5 mL of newly produced phosphate buffer (pH 6.3) was added. A UV-Visible spectrophotometer was employed to determine the absorbance at 416 nm. Calculating the proportion of BSA denaturation inhibition required the following formula:

Percentage Inhibition = [100 - (optical density of test solution – optical density of product control) / (optical density of test control) × 100

Here, A(t)=Absorbance of test solution, A(pc)=Absorbance of product control, and A(tc)=Absorbance of test control.

In vitro protein denaturation method

Protein denaturation was performed in vitro by Sakat et al. [35]. The suppression of protein denaturation method was used to examine the anti-inflammatory potential of *A. capitiformis*. The reaction mixture (5 ml) contains 0.2 ml of fresh hen's egg albumin, 2.8 ml of phosphate-buffered saline (pH: 6.4), and 2 ml of different concentrations of plant extracts. As a control, a similar amount of double distilled water was used. The mixes were then incubated in an incubator at 37±2 °C for 15 min before being heated at 70 °C for 5 min. After cooling, their absorbance at 660 nm was measured using a vehicle

as a blank. Diclofenac at a final dosage of 1 mg/ml was employed as a reference medication and was handled identically for absorbance determination. The following percentage inhibition of protein denaturation was calculated:

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control} \times 100.$$

Evaluation of cytotoxic activity

Preparation of seawater

After dissolving 38 g of sea salt (iodine-free) in 1 L of distilled water, the solution was filtered to remove impurities. Using a 1 N NaOH solution, we could keep the seawater's pH stable between 8.0 and 8.5.

Brine shrimp eggs hatching

The brine shrimp eggs, or *Artemia salina* leach, were gathered from pet stores for the experiment. The little aquarium was filled with salt water and then stocked with shrimp eggs. The nauplii would develop from the eggs after two days. Throughout the hatching process, the oxygen pump ensured a steady supply. Shrimp nauplii were taken from the tank's lit area because the newly hatched shrimp were drawn to the light (phototaxis). A pipette was used to remove the nauplii from the aquarium. Framework for Research 10 nauplii was placed in 5 ml of seawater and treated with 1000, 800, 500, 250, 125, 62.5 and 31.25 µg/ml solutions of plant extract. After 24 h, the Petri plates were examined under a black light with a magnifying lens to tally the number of surviving nauplii. In this bioassay, death was defined as a lack of coordinated forward movement over the course of 30 s. The following equation was employed to calculate the percentage (%) of brine shrimp nauplii mortality at each concentration [36]:

$$\% \text{ Mortality} = \text{Nd} / \text{N} \times 100.$$

Here, Nd=Number of dead nauplii, N=Number of nauplii taken.

Determination of median lethal concentration (LC₅₀)

The LC₅₀ value was calculated using a linear regression model to estimate the extract concentration at which 50% of brine shrimp nauplii died after exposure to the compound for a certain period. A linear relationship was found when the concentration and the percentage of death were shown on a graph, and the concentration-response data were turned into a straight line using a trend line fit linear regression analysis. (Microsoft Excel 2007). Results for LC₅₀ were calculated by extrapolating the best-fit line.

In vitro evaluation of anthelmintic activity

The anthelmintic effectiveness of the plant extract was determined using the Ajaiyeoba procedure [37]. Adult

Pheretima Posthuma worms were used for this study. The flooded lands were the source for them. Veterinarians have determined that the length of the soil samples is 3–5 cm, the width is 0.1–0.2 cm, and the weight is 0.8–3.04 g. Salty water was used to clean them thoroughly. Plant extracts of varying strengths (10 mg/mL–50 mg/mL) were made. Each concentration was brought to a final volume of 10 mL before being put into individual Petri plates. Commercially available albendazole was the standard medication. Five earthworms were placed in each Petri dish upon delivery. Constant checks were done to see whether the earthworms showed any signs of paralysis or death.

In vivo studies of *A. capitiformis* extracts

Experimental animals

Male Swiss Albino mice weighing between 20 and 30 g were utilized in this investigation. The animals were kept in plastic cages and acclimated to controlled conditions for 14 days, including a temperature of 25±2 °C and a relative humidity of 60–70%. After acclimatization, we placed the animals on a 12-hour light/dark cycle and fed them pellets. Throughout the trial, the mice were provided a balanced diet and water to drink whenever they desired. Researchers employed chloroform anesthesia to end the mice's lives. At the end of each trial, the mice were killed under diethyl ether anesthesia. This publication strictly adheres to the ARRIVE Guidelines for reporting animal research. All studies were reviewed and approved by the ethics committee under permission number CC-307061.

Experimental design

For each analgesic activity assessment, four groups of mice were chosen, with five mice in each group. A control group was given 10 ml/kg of 1% tween-80; a standard group was given 50 mg/kg diclofenac sodium for the acetic acid writhing study and 10 mg/kg morphine sulfate for the tail immersion method and hot plate method; and the other groups were given 200 and 400 mg/kg doses of crude methanol extract of *A. capitiformis*. After conducting the acute toxicity test, we have chosen dosages of 200 mg/kg and 400 mg/kg for our research. Previous research on plant extracts with comparable pharmacological effects used identical dosages of 200 mg/kg and 400 mg/kg of plant extract [38]. To analyze the sedative and anxiolytic effects using two approaches, mice were chosen, weighed, and divided into four groups for individual testing. The control group got 1% tween-80 at a dose of 10 ml/kg, whereas the standard group received the usual medicine diazepam at a dose of 1 mg/kg, and the treatment group received 200 mg/kg and 400 mg/kg methanol extract. Each experimental period concluded

with the chloroform-anesthetized sacrifice of all treated animals.

Acute toxicity study

The investigation into the acute toxicity of the methanol extract of *A. capitiformis* was conducted to determine the appropriate dosage, following the recommendations set by the OECD [39]. The extract was orally supplied at escalating concentrations of 500, 1000, and 2000 mg/kg. The subjects were observed for 48 h to assess any changes in behaviour, toxicity, or death. No instances of fatalities or other acute adverse effects were observed. Hence, the doses used for the current study were 200 and 400 mg/kg body weight for the purpose of assessing analgesic and CNS depressant effects. The effective dose would be less than the median lethal dose (LD₅₀).

Evaluation of analgesic activity

Acetic acid writhing method

The painful stimulation in mice was proven using this analgesic behavioural observation evaluation approach. Dambisya and Lee's [40] adaptation of Koster's methodology was used for this investigation. All mice were injected with 0.7% glacial acetic acid (10 ml/kg) intraperitoneally (IP) 15 min after the standard administration and 30 min after the administration of the extract to cause discomfort manifested as abdominal constriction or writhing. After 5 min, 20 min of diligent observation were recorded for each mouse in all groups to tally the number of writhes. After each observation session, all treated mice were put to sleep with diethyl ether. Analgesia was measured by a percentage reduction in abdominal writhing, which was determined using the following formula:

$$\% \text{ of pain inhibition} = (N_c - N_t)/N_c.$$

Here, N_c=number of writhings in the control group, and N_t=number of writhings in the treatment group.

Tail immersion method

The central analgesic activity of the extracts under study was determined using a thermal technique. This study followed the procedure outlined by Di Stasi et al. [41]. Before the 30-minute treatment period, the length of time for each mice to extract its tail from water heated to 50±1 °C was recorded. Each mice tail was submerged for roughly 2–3 centimetres. Animals were chosen for the research based on their ability to respond with a flicking motion within three to five seconds. The tail protection cut-off time was set at 15 s. Mice given medication were evaluated 30, 60, 90, and 120 min after treatment began [42]. Animals were wrapped lightly to immobilize them for a short time so that measurements could be taken. All treated mice were killed under diethyl ether anaesthesia at the end of each observation period. The following

equation [43] was employed to determine the analgesic effect of the extract with (% MPE) was achieved.

$$\text{MPE (\%)} = (\text{Post drug latency} - \text{Pre drug latency}) / (\text{Cut off time} - \text{Pre drug latency}) \times 100.$$

The percentage of time elongation was calculated from the following equation [44]:

$$\text{Elongation (\%)} = (\text{Latency of Test} - \text{Latency of control}) / \text{Latency of test} \times 100.$$

Thermal stimulus-induced pain (hot plate test) in mice

The hot plate technique measures the time it takes for a person to respond to a continuous suprathreshold temperature, or "hot," on a hot plate. The animals were split into four groups of five to test the analgesic effects of whole crude methanol extract of the plant. Hot plate equipment was used for the experiment (UGO Basile of Italy). Group I got water, Group II administered ketorolac (2.5 mg/kg body weight animal), and Groups III and IV received crude extracts of the following plant at 200 and 400 mg/kg body weight, respectively. A metal surface was kept at a constant temperature of 55 °C, give or take 0.2 °C. The animals' ability to endure pain was measured by timing their reactions (paw licking, shaking, and leaping) on a hot plate before and after they were given the medicine. To prevent harm to the animals' paws, the time limit was set at 15 s. After the drugs were orally administered, the delay was measured at 15 min, 30 min, 60 min and 120 min [45]. A precise response to heat-induced discomfort was recorded. The mice were kept in conditions that ensured their comfort, including temperature, humidity, ventilation, noise level, and illumination requirements.

Evaluation of anxiolytic activity

Elevated plus maze method

An elevated plus maze is crucial for studying medicines' anxiolytic effects [46] and neuroprotective properties. Animals introduced to a new labyrinth avenue are likelier to engage in open-arm conflict than in closed-arm combat. As a result of their fear of heights and open areas, rodents prefer to spend more time in confined spaces such as a cage. When animals approach an open arm, they often get frightened, unable to move, or even defecate [47]. It has been shown that elevated plasma cortisol levels are an accurate depiction of anxiety. Benefits of this testing method include (a) its speed, ease, and short duration; (b) the absence of the need for unpleasant stimuli or training; and (c) the test's predictability and reliability in revealing the drug's anxiety-reducing [1, 48]. The elevated plus maze device is shaped like a plus sign 40 centimetres from the ground [49]. It has two open arms (25×5 cm, with a very little, 0.5 cm, wall) across from each other and perpendicular to two closed arms (25×5×16 cm). Each animal used in the experiment was

placed in the middle of the labyrinth with its head facing the open arm, and a timer was started. The following variables were tracked for a total of 5 min. First, we notice that mice like open arms over closed ones; second, we keep track of how many times each arm is opened and closed; and third, we label it an “arm entry” when a mouse fits all four paws inside. Animals were given saline as a placebo, the standard drug diazepam, and several test samples of *A. capitiformis* extract. After 30 min, the animals were kept alone in the maze’s centre. The average amount of time spent in the open arm entries number was compared across the two testing groups to determine whether animals favoured the open or closed arm.

Hole-board test method

The practice of head-dipping is performed in a confined space equipped with hole-board equipment [50]. The length and frequency of head dipping may be used as a proxy for neophilia or goal-oriented exploration. As a result, it may be concluded that the animal’s locomotor function is autonomous on the whole [51]. Generally speaking, neophilia is associated with a higher level of head dipping. In contrast, the lack of neophilia or an elevated anxiety-like condition in the testing animals is associated with decreased levels of head dipping [52]. As a result, head-dipping decreases in frequency, and frequency reduces anxiety, and vice versa [53]. Mice were placed singly on the hole board apparatus 30 min before being treated with control, standard, and test samples, and we recorded the number of times their heads dipped into the holes at the height of their eyes during the course of a 5-minute trial session.

Table 1 Phytochemical analysis of the methanolic extract of the plant

Phytochemicals	Result
Alkaloids	+++
Glycoside	++
Saponin	+++
Anthraquinone glycoside	++
Cardiac glycoside	++
Cyanogenetic glycoside	+
Phenol and tannin	+++
Flavonoid	+++
Protein and amino acid	+
Carbohydrate	++
Steroid	++
Fat and fixed oils	
Terpenoid	+++
Carboxylic acid	++
Triterpenoids	+++
phytosterols	++

Note: “+” represents the presence of phytochemicals and “–” represents the absence of phytochemicals. “++” and “+++” represents the intensity of presence of phytochemicals

Evaluation of sedative activity

Open-field test method

The floor size of the testing device was around 0.5 square meters, and the walls were 50 centimetres in height [54]. The floor comprises white and black squares that alternate in size. Once the control (saline), the standard (Diazepam), and the test samples (200 and 400 mg/kg) were orally administered, the number of squares visited by the mice was recorded every three minutes (using a tally counter) the first 0, 30, 60, 90, and 120 min.

Hole-crossed test apparatus

The experiment was conducted in a wooden box without a roof [55] with dimensions of 30×20×14 centimetres. A permanent wooden divider divides the room along the middle. The fixed wooden barrier included a circular aperture with a curvature of 3.5 cm in diameter and 7.5 cm in height. The number of times the mice crossed from one compartment to another was tallied using a tally counter every three minutes at 0 min, 30 min and 90 min.

Statistical analysis

The data are shown as the mean±SEM. One-way analysis of variance and Dunnett’s t-test were used for all statistical testing and a value of **P*<0.05 was considered significant. Furthermore, SPSS was used to do the statistical analysis. (Version: 20, IBM Corporation, New York, USA). Linear regression equations were used in Excel 2007 to estimate LC₅₀ values. (Microsoft, Redmond, Washing-ton, USA)

Results

The crude methanol extract contain alkaloid, glycoside, saponin, tannin, phenol flavonoid etc. which were showed in Table 1.

Anti-oxidant activity

Table 2 displays the percentage inhibition of free radical (DPPH) scavenging activity. The result showed that the methanolic extract has the higher antioxidant activity in 500 µg/ml is 87.55±0.98*, with an IC₅₀ value of 45.35 µg/ml, close to the standard ascorbic acid which is 90.01±0.02* (Fig. 1).

Anti-arthritic activity

Compared to the negative control, which exhibited no protein denaturation inhibition at any dose in the present in vitro anti-arthritic activity research, the positive control inhibited protein denaturation by 94.04±0.78to 15.29±0.25 (Table 3). Protein denaturation was successfully blocked by the whole crude methanol extract of the entire plant, with concentrations ranging from 74.25±0.12 to12.18±0.12 (Fig. 2).

Table 2 Antioxidant activity of the methanolic extract of the plant

Test samples	Concentration (µg/ml)	% inhibition	IC ₅₀ (µg/ml)
Standard(Ascorbic acid)	500	90.01 ± 0.02*	37.59
	250	87.06 ± 0.03*	
	125	76.19 ± 0.12*	
	62.5	70.12 ± 0.01*	
	31.25	64.09 ± 0.34*	
	15.63	60.01 ± 0.02	
	7.81	44.18 ± 0.78	
	3.91	26.19 ± 0.32	
	0.98	17.09 ± 0.90	
	0.49	10.12 ± 0.65	
MEAC	500	87.55 ± 0.98*	45.35
	250	82.01 ± 0.12*	
	125	72.03 ± 0.78*	
	62.5	70.01 ± 0.90*	
	31.25	54.50 ± 0.01	
	15.63	40.11 ± 0.34	
	7.81	38.10 ± 0.67	
	3.91	34.18 ± 0.23	
	0.98	30.09 ± 0.76	
	0.49	25.70 ± 0.12	

Note: Values are mean ± standard error of triplicate analysis. * shows significant differences ($p < 0.05$). MEAC=Methanol Extract of *A. capitiformis*

In vitro anti-inflammatory

Methanolic extract of the whole plant inhibited protein denaturation and reduced inflammation in vitro, as shown in Table 4. Maximum suppression $54.05 \pm 0.12^*$ was observed with 1000 µg/ml of *A. Capitoformis* extract.

Table 3 Anti-artritic activity of the methanolic extract of the plant

Test samples	Concentration (µg/ml)	% inhibition of protein denaturation	IC ₅₀ value(µg/ml)
Control	-	0	
Diclofenac-Na	1000	94.04 ± 0.78*	293.413
	500	79.09 ± 0.12*	
	250	65.14 ± 0.89*	
	125	37.28 ± 0.12	
	62.5	17.38 ± 0.34	
MEAC	31.25	15.29 ± 0.25	423.758
	1000	74.25 ± 0.12*	
	500	61.14 ± 0.54*	
	250	43.23 ± 0.12	
	125	33.67 ± 0.56	
	62.5	22.28 ± 0.78	
	31.25	12.18 ± 0.12	

Note: Values are mean ± standard error of triplicate analysis * shows significant differences ($p < 0.05$). MEAC=Methanol Extract of *A. capitiformis*

The lowest percentage of suppression followed with the extract was 20.06 ± 0.45 at a 25 µg/ml dose (Fig. 3).

Cytotoxic assay

The cytotoxicity of a substance is measured using the brine shrimp assay. After 24 h, the LC₅₀ of each sample was determined by plotting the percentage of surviving shrimp against the logarithm of the sample concentration and then using regression analysis to get the best-fit line from the curve data. Compared to the negative control (seawater), the LC₅₀ value of the positive control was 34.67 µg/ml, which resulted in a statistically significant

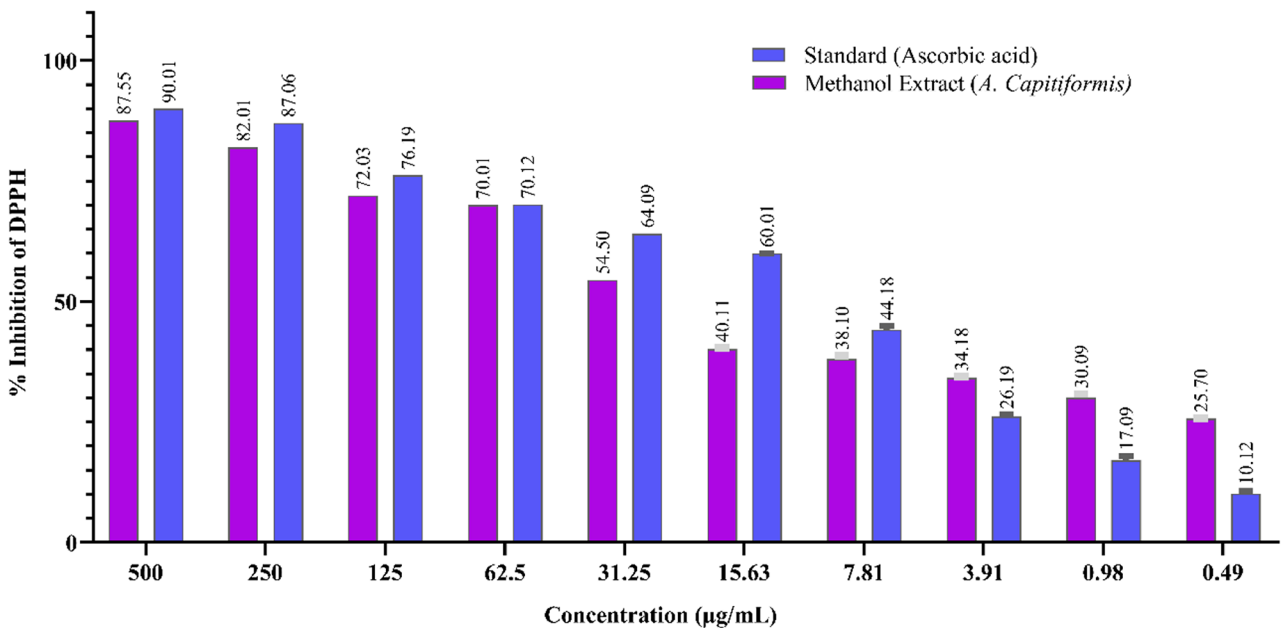


Fig. 1 Antioxidant activity of the methanolic extract of and standard

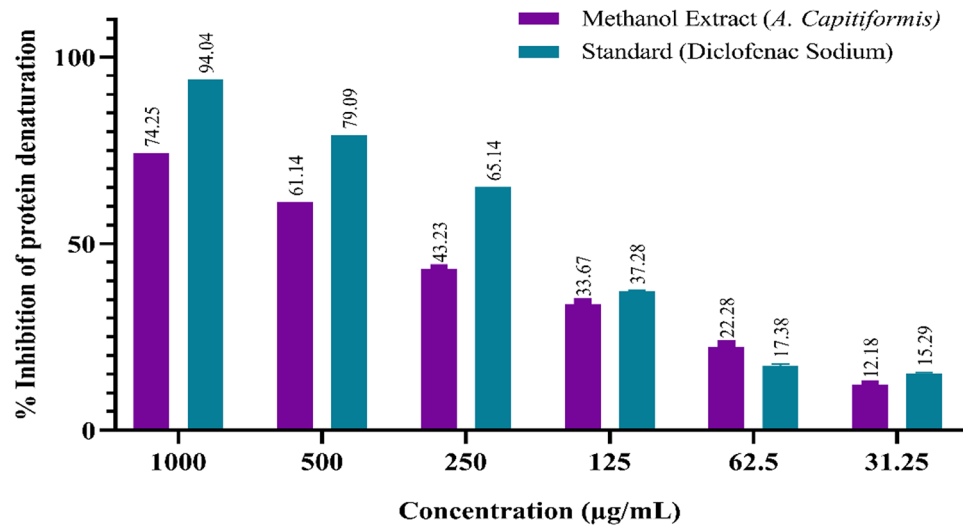


Fig. 2 Anti-arthritis activity of the methanolic extract of the plant and standard drug

Table 4 Anti-inflammatory activity of the methanolic extract of the plant

Test samples	Concentration(µg/ml)	% of protein denaturation	IC ₅₀ (µg/ml)
Control	-	0	26.03
Diclofenac-Na	1000	91.16 ± 0.56*	857.36
	600	86.34 ± 0.01*	
	400	80.01 ± 0.89*	
	200	75.33 ± 0.45*	
	100	65.14 ± 0.12*	
	50	53.81 ± 0.78	
	25	11.23 ± 0.12	
MEAC	1000	54.05 ± 0.12*	857.36
	600	44.08 ± 0.45	
	400	31.13 ± 0.34	
	200	27.36 ± 0.12	
	100	27.34 ± 0.67	
	50	25.11 ± 0.78	
	25	20.06 ± 0.45	

Note: Values are mean ± standard error of triplicate analysis * shows significant differences ($p < 0.05$). MEAC = Methanol Extract of *A. capitiformis*

increase in shrimp mortality (Table 5). *A. capitiformis* methanol extract LC_{50} values were determined to be $129.72 \mu\text{g/ml}$ Table 6.

In vitro evaluation of antihelmintic activity

Physical changes (such as paralysis and death) in the earthworms were monitored continuously. The whole crude methanol extract demonstrated considerable action on adult earthworms at the maximum dose (50 mg/ml), whereas the conventional medicine Albendazole exhibited significant effects at 20 mg/ml (Fig. 4).

Analgesic activity

Evaluation of peripheral analgesic effect

Acetic acid writhing method The crude methanolic extract showed highly statistically significant analgesic activity in both 200 mg/kg and 400 mg/kg body weight ($p < 0.001$) (Table 7) (Fig. 5).

Evaluation of central analgesic activity

Tail immersion test The methanolic extract caused a statistically significant ($p < 0.05$) rise in the percentage of delay time using the tail immersion technique (Table 8). This chart also displays the statistically meaningful response time and proportion of maximal potential effect in 30 and 60 min (Fig. 6) Table 9.

Hot plate method

Figure 7 demonstrates that the delayed reaction in the heated plate test was prolonged from 30 to 120 min after rodents were treated with morphine (10 mg/kg i.p.). However, *A. capitiformis* greatly affected the animals' response time to the heated plate at 400 mg/kg after 60 and 120 min of treatment ($P < 0.001$) following therapy (Table 10).

Elevated plus maze

Evaluation of anxiolytic activity

Compared to the control, methanolic extract of the whole plant of MEAC produced a significant ($p < 0.05$) increase in the time spent in the open arms and the number of entries in the open arm of the elevated plus maze, indicating anxiolytic activity (Table 11). But 200 mg/kg dose did not produce any statistically significant results (Fig. 8). When compared to the extract-treated groups,

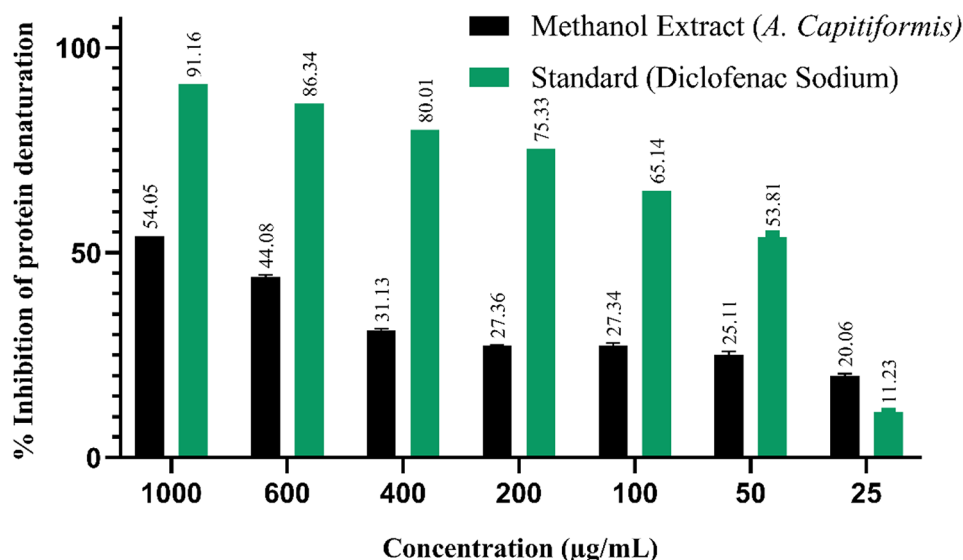


Fig. 3 Anti-inflammatory activity of the methanolic extract of the plant and standard

Table 5 Cytotoxic activity of the methanolic extract of the plant

Sample	Concentration(µg/ml)	LogC	R2	LC ₅₀ (µg/ml)
Standard	1000	3	0.837256	34.67
	800	2.90		
	500	2.69		
	250	2.39		
	125	2.09		
	62.5	1.79		
	31.25	1.49		
MEAC	1000	3	0.769466945	129.72
	800	2.90		
	500	2.69		
	250	2.39		
	125	2.096		
	62.5	1.79		
	31.25	1.49		

Equation for standard, $y = 1.223x + 3.122$, Equation for crude, $y = 1.618x + 1.585$, MEAC=Methanol Extract of *A. capitiformis*

Table 6 Anthelmintic activity of methanolic extract of plant

Sample	Concentration(mg/ml)	Time taken for paralysis (mean ± SEM)	Time taken for death (mean ± SEM)
Control	20 ml of distilled water		
Standard (Albendazole)	20 mg/ml	13 ± 0.632	19.4 ± 0.509
MEAC100	10 mg/ml	40 ± 0.707	55.6 ± 0.678
MEAC 200	20 mg/ml	28.8 ± 0.374	32.2 ± 0.860
MEAC 300	30 mg/ml	21.4 ± 0.509	29 ± 0.707
MEAC 400	40 mg/ml	18.8 ± 0.374	21.6 ± 0.509
MEAC 500	50 mg/ml	16 ± 0.316	18.4 ± 0.509

MEAC=Methanol Extract of *A. capitiformis*

the delivery of diazepam (1.0 mg/kg body weight) considerably ($p < 0.05$) increased the number of entrances as well as the duration of remaining in the open arms.

Hole board method

The whole crude methanolic extract at 400 mg/kg body weight showed a significant rise ($p < 0.01$) in head dipping than the standard, indicating the extract's calming action (Table 12). The other dosage 200 mg/kg dose samples had no notable effect. Diazepam (1.0 mg/kg body weight) substantially enhanced the number of head dipping ($p < 0.05$) (Fig. 9).

Hole cross method

In the hole cross test, the number of times the rodents crossed between the compartments dropped steadily over 1.5 h (Table 13). In 90 min, the methanolic extract at 200 mg/kg dosage produced a highly significant outcome ($p < 0.001$). However, after 30 min of delivery, the result was significant ($p < 0.01$) for the 400 mg/kg dosage (Fig. 10).

Open field method

During the open field test, the number of areas visited by the rodents of the various test groups dropped over two hour period. (Table 14). At a dosage of 400 mg/kg, the methanolic extract of *A. capitiformis* revealed a reduced number of areas visited by the rodents over time compared to the control, suggesting that the plant extract may have calming properties. At 30 and 120 min, 200 mg/kg dosage yielded statistically significant results (Fig. 11).

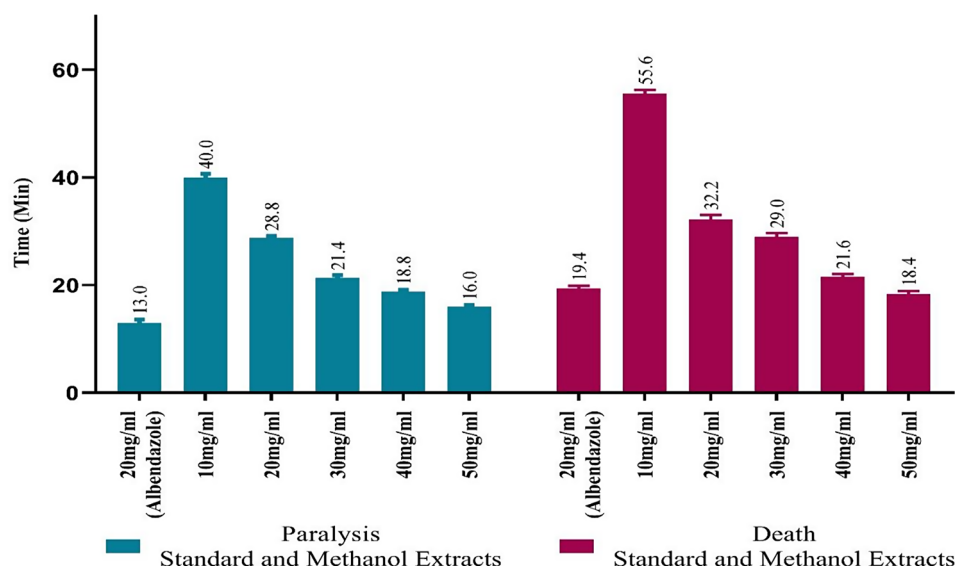


Fig. 4 Antihelmintic activity of methanolic extract of *A. capitiformis*

Table 7 Analgesic activity by acetic acid writhing method of the methanolic extract of the plant

Treatment	Writhing count Mean $\pm$ SEM	% of Inhibition
Control	44.4 $\pm$ 4.11	0
Standard (Diclofenac-Na)	5.4 $\pm$ 0.40***	86.73
MEAC 200 mg/kg	17.8 $\pm$ 0.86***	43.29
MEAC 400 mg/kg	10 $\pm$ 0.84***	75.48

The results were expressed in Mean $\pm$ SEM (standard mean error) * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MEAC=Methanol Extract of *A. capitiformis*. (* significant, ** more significant and *** highly significant)

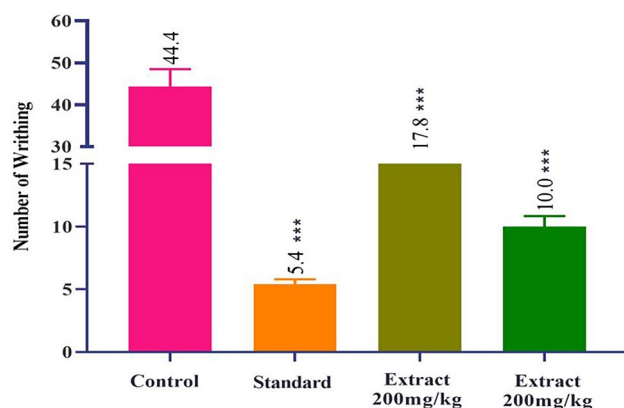


Fig. 5 Analgesic activity by acetic acid writhing method of methanolic extract of *A. capitiformis*

Discussion

Herbal remedies have been utilized to treat illnesses since prehistoric times. Here, we performed a phytochemical investigation and looked into the pharmacological effects of the whole crude extract of *A. Capitiformis*. Methanolic

preparation of the whole plant of *A. capitiformis* was analyzed for its phytochemical compounds, and the results exhibited the existence of alkaloids, carbohydrates, flavonoids, terpenoids, phenols, saponins, etc. Identifying potent phytochemicals, which could contribute to discovering and creating new medicines, may be aided by early phytochemical screening assays. Chemicals known as antioxidants (free radical scavengers) protect free radicals from damaging cells in the body by combining and neutralizing them [56]. As shown in Table 2, *A. capitiformis* showed powerful antioxidant ability against DPPH free radicals in the present research, with an IC_{50} value of 45.35 μ g/ml, which is very close to the IC_{50} value of ascorbic acid which have the IC_{50} value of 37.59 μ g/ml. So it can be said that methanolic extract of our experimental crude has potential antioxidant activity. Functional phytochemicals, including flavonoids, phenolic acids, and tannins, help plants scavenge free radicals and serve as antioxidants [57, 58]. Polyphenolic compounds may help the extract scavenge free radicals. Our results were in agreement with the findings of Arshad et al., 2017 [59].

Auto-antigens production is suggested to contribute to the progression of rheumatoid arthritis in several rheumatic diseases [60, 61]. Diclofenac sodium, a common anti-inflammatory drug, was used as a benchmark in this investigation of anti-arthritis efficacy. *A. capitiformis*'s anti-arthritis effects were significant and dose-dependent, with an IC_{50} of 423.76 μ g/mL compared to the corresponding values for the standard group Diclofenac-Na with an IC_{50} 293.413 μ g/mL which is lower than the crude extract. Less potential anti arthritic activity has been seen in the methanol extract of *A. capitiformis* when compared with the standard drug Diclofenac-Na.

Table 8 Analgesic activity by tail immersion method of the methanolic extract of the plant

Test Samples	Dose (mg/kg)	Pretreatment	Reaction times in seconds (Mean ± SEM) and %MPE			
			30 min	60 min	90 min	120 min
Control	10 ml/kg	2.42 ± 0.07%	2.64 ± 0.05	2.82 ± 0.06	2.49 ± 0.11	2.55 ± 0.13
	body weight		1.86%	1.67%	2.6%	1.9%
Standard (Morphine sulfate)	50	2.55 ± 0.13%	6.96 ± 0.41***	6.64 ± 0.32***	4.26 ± 0.87	3.77 ± 0.69
			23%	21.4%	14.78%	9.01%
MEAC	Dose 200 mg/kg	2.37 ± 0.03%	7.22 ± 0.23***	6.51 ± 0.37***	2.56 ± 0.15	2.47 ± 0.15
			9.2%	7.23%	5.12%	3.19%
MEAC	Dose 400 mg/kg	2.40 ± 0.04%	8.5 ± 0.24***	8.32 ± 0.26***	2.5 ± 0.14	2.31 ± 0.14
			21.01%	16.9%	12.67%	10.71%

The results were expressed in Mean±SEM (standard mean error) **p*<0.05, ***p*<0.01, and ****p*<0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett’s test) compared to the control. MEAC=Methanol Extract of *A. capitiformis*. (* significant, ** more significant and *** highly significant)

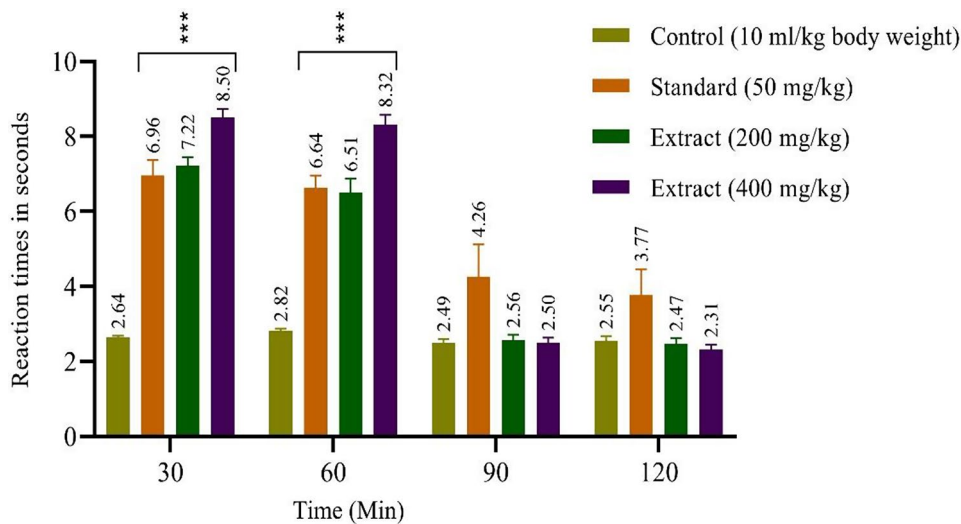


Fig. 6 Analgesic activity by tail immersion method of methanolic extractof *A. Capitiformis*

Table 9 Percent elongation of latency time after administrationof all test samples

Test Samples	Dose (mg/kg)	% elongation of latency time			
		30 min	60 min	90 min	120 min
Standard (Morphine sulfate)	50	73.2%	67.81%	59.32%	47.67%
MEAC	Dose 200 mg/kg	40.12%	32.45%	25.12%	16.89%
MEAC	Dose 400 mg/kg	58.12%	45.19%	39.91%	34.12%

The results were expressed in Mean±SEM (standard mean error) **p*<0.05, ***p*<0.01, and ****p*<0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett’s test) compared to the control. MEAC=Methanol Extract of *A. capitiformis*

Some plants have rich alkaloids, and polyphenols have anti-arthritic and anti-inflammatory properties which were supported by the previous studies done on plant extract [62–64]. According to qualitative analyses, *A. capitiformis* has higher levels of alkaloids, phenols, and flavonoids.

Inflammation-a complex biochemical response of vascular tissue to harmful stimuli, pathogens, and allergens—causes redness, warmth, oedema, and pain [65].

Salicylic acid and phenylbutazone inhibit heat-induced protein denaturation in a dose-dependent manner [66]. Here, we compare the IC₅₀ values of the standard drug Diclofenac-Na (26.03 µg/ml) with those of the methanolic extract of the whole plant (857.37 µg/ml). The IC₅₀ value of our methanolic extract has very higher value when compared with the standard drug Diclofenac-Na. So we can conclude that, the plant extract has lower anti-inflammatory activity when compared with standard drug. According to research, 3-furaldehyde, phenol, catechol, and hydroquinone are potent anti-inflammatory chemicals discovered in the methanolic extract of *A. capitiformis* that play a significant role in the inflammation process by targeting proteins [27]. Foyet et al.’s (2014) research on the effects of *Vitellaria paradoxa* stem bark extract on inflammation and arthritis further corroborated the anti-inflammatory and anti-arthritic properties of the herbal extract in our study [67]. Chandel et al. conducted a study in 2013 and found a notable anti-arthritic effect in the fruit extract of plants [68].

Brine shrimp lethality is a general test that shows cytotoxicity and a wide range of pharmaceutical actions of

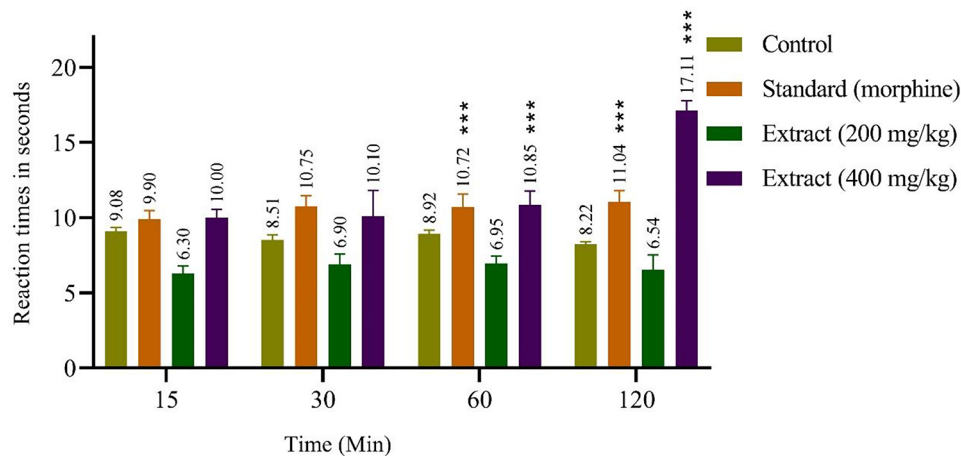


Fig. 7 Analgesic activity by hot plate method of methanolic extractof *A. Capitiiformis*

Table 10 Analgesic activity by hot plate method of the methanolic extract of the plant

Groups	Reaction time in seconds			
	15 min	30 min	60 min	120 min
Control	9.08±0.28	8.51±0.36	8.92±0.26	8.22±0.18
Standard (Morphine sulfate)	9.90±0.58	10.75±0.72	10.72±0.85***	11.04±0.76***
MEAC 200 mg/kg	6.30±0.49	6.90±0.68	6.95±0.51	6.54±0.99
MEAC 400 mg/kg	10±0.55	10.10±1.71	10.85±0.93***	17.11±0.67***

The results were expressed in Mean±SEM (standard mean error) **p*<0.05, ***p*<0.01, and ****p*<0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MEAC=Methanol Extract of *A. capitiformis*. (*significant, ** more significant and *** highly significant)

Table 11 Anxiolytic activity by elevated plus maze method of the methanolic extract of the plant

Test Samples	Dose (mg/kg)	Open Arm (OA)		Closed Arm (CA)	
		Time Spent (sec)	No. of Entries	Time Spent (sec)	No. of Entries
Control	10 ml/kg	104±25.62	10.2±2.37	196±25.61	7.20±2.08
Standard (Diazepam)	1	213±3.74**	16.8±0.58*	87±3.74*	5±0.71
MEAC	200	118±21.54	8.8±1.20	182±21.54	8.80±2.35
MEAC	400	204±20.15*	18.4±0.51**	96±20.14	4.6±0.68

The results were expressed in Mean±SEM (standard mean error) **p*<0.05, ***p*<0.01, and ****p*<0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MEAC=Methanol Extract of *A. capitiformis*. (*significant, **more significant and ***highly significant)

chemicals and plant preparations, such as antimicrobial, pesticidal, antiviral, anticancer, and so on [69]. The LC₅₀ value (129.72 µg/ml) for methanol extract was very high compared to the standard drug which had the LC₅₀ value of 34.67 µg/ml, indicating that the plant extract is safe

at therapeutic levels. Secondary plant metabolites were linked to cell death [70]. A preliminary qualitative phytochemical examination of *A. capitiformis* revealed cancer-fighting alkaloids, flavonoids, and tannins [71, 72]. This relationship suits our present research. A number of bioactive substances, including quercetin, trans cinnamic acid, o-coumaric acid, apigenin-7-o-glucoside, luteolin, and protocatechuic acid, have been shown to help fight cancer which were also justified by the previous studies [73]. Our findings indicate that *A. capitiformis* methanol extract exhibits significant cytotoxicity, necessitating further exploration of its effects on these specific cell lines.

People and animals are plagued by parasitic helminths. Traditional medicines, which include anthelmintic herbs, are a good source of affordable, effective anthelmintics for impoverished nations [74, 75]. In this research, the time required for immobility and mortality of individual worms is compared to the pure extract and the standard medication Albendazole. The current research finds that the plant under study possesses substantial anthelmintic action in a dose-dependent manner compared to the conventional medication Albendazole. The 50 mg/mL group exhibited potent anthelmintic action compared to other groups, with a paralysis time of 16±0.316 min and a death time of 18.4±0.509 min. The group treated with the conventional medicine Albendazole 20 mg/ml showed a strong anthelmintic effect compared to the other groups. The paralysis time was 13±0.632 min and the death time was 19.4±0.509 min, which is similar to the findings obtained with a 50 mg/kg dosage of methanolic extract of *A. capitiformis*. This is justified and exemplified by the discovery of several beneficial phytochemicals, such as alkaloids [76], tannins [77], flavonoids [78], and phenolics [78], during the qualitative phytochemical analysis of *A. capitiformis*. Tannins, a type of polyphenolic compound, can exhibit anthelmintic activity by either binding to free proteins in the gastrointestinal tract of the host animal

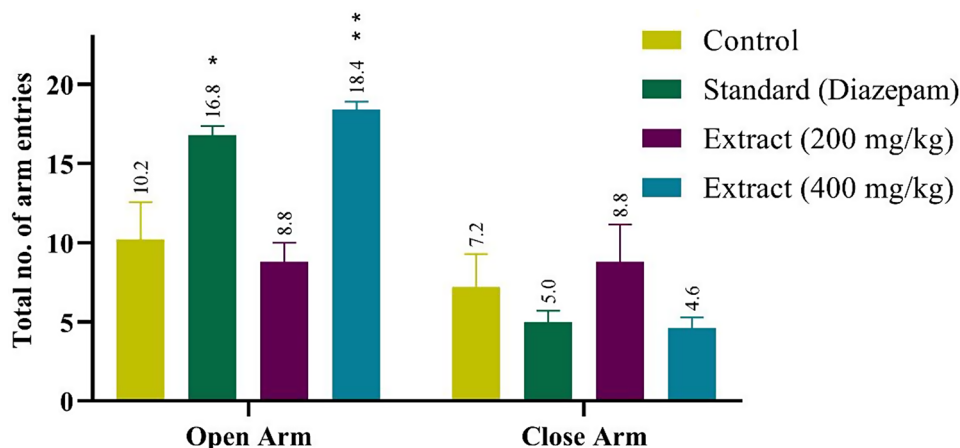


Fig. 8 Anxiolytic activity by elevated plus maze method of methanolic extract of *A. capitiformis*

Table 12 Anxiolytic activity by hole board method of the methanolic extract of the plant

Groups	Dose (mg/kg)	Number of head dipping (Mean $\pm$ SEM)
Control	10 ml/kg	28 $\pm$ 0.71
Standard (Diazepam)	1	41 $\pm$ 1.08
MEAC	200	33.4 $\pm$ 3.39
MEAC	400	53 $\pm$ 7.52**

The results were expressed in Mean $\pm$ SEM (standard mean error) * p < 0.05, ** p < 0.01, and *** p < 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MEAC = Methanol Extract of *A. capitiformis*. (* significant, ** more significant and *** highly significant)

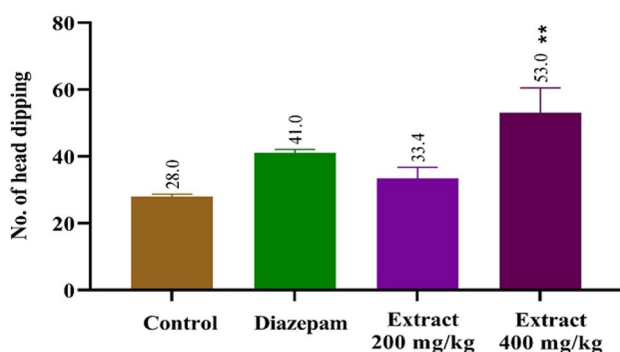


Fig. 9 Anxiolytic activity by hole board method of methanolic extract of *A. Capitiformis*

Table 13 Anxiolytic activity by hole cross method of the methanolic extract of the plant

Test Samples	Dose (mg/kg)	Number of movements			
		0 min	30 min	60 min	90 min
Control	10 ml/kg	9.4 $\pm$ 0.51	7.6 $\pm$ 0.51	4.8 $\pm$ 0.66	4.2 $\pm$ 0.58
Standard (Diazepam)	1	17 $\pm$ 0.44***	5.8 $\pm$ 0.66	6 $\pm$ 0.71	3.2 $\pm$ 0.37
MEAC	200	10 $\pm$ 0.71	8.2 $\pm$ 0.37	7.4 $\pm$ 0.51	9 $\pm$ 0.71***
MEAC	400	9 $\pm$ 0.11	4.2 $\pm$ 0.58**	2.8 $\pm$ 0.37	3.8 $\pm$ 0.36

The results were expressed in Mean $\pm$ SEM (standard mean error) * p < 0.05, ** p < 0.01, and *** p < 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MEAC = Methanol Extract of *A. capitiformis*. (* significant, ** more significant and *** highly significant)

or disrupting the energy generation process of worms by uncoupling oxidative phosphorylation. They can also bind to glycoproteins on the parasite's cuticle, causing worm death [79].

Pain is the body's natural response to noxious stimulation, acting as a warning mechanism and eliciting a defensive response. Although useful, it can produce a great deal of discomfort and negative reactions [80]. In mice, the acetic acid-induced pain response manifests as belly contraction (writhing) and hindlimb stretching. Such pain stimulation causes a localised inflammatory response by releasing free arachidonic acid from tissue phospholipids [81, 82]. In the acetic acid-induced writhing technique, a lower number of writhings and a larger percentage of writhing inhibition are associated with a stronger peripheral analgesic effect in mice. This research uses acetic-acid writhing to assess *A. capitiformis*' analgesic effects. Pain signals from the brain cause abdominal tightness once digestive tract inflammation enters the circulation and the dorsal horn of the central nervous system [83]. Methanolic extract of the whole plant of *A. capitiformis* decreased the number of abdominal constrictions caused by acetic acid in rodents by a statistically significant amount (p < 0.001) at both the 200 mg/kg and 400 mg/kg doses (Fig. 5). Between the two dose 200 and 400 mg/kg, 400 mg/kg showed higher percent inhibition which is 75.48% near to the standard drug diclofenac

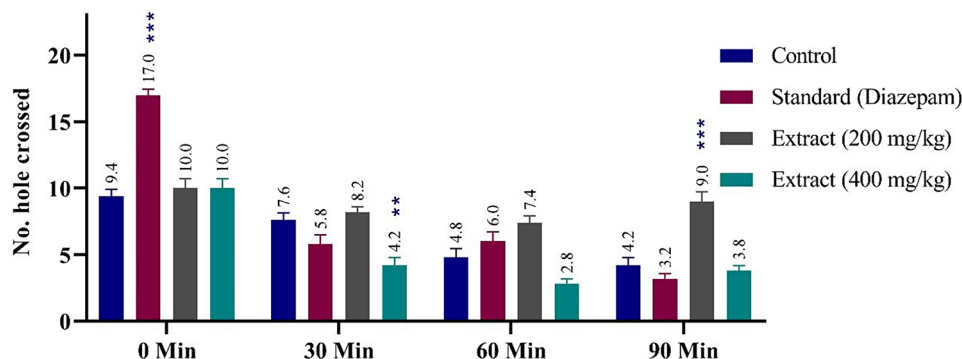


Fig. 10 Anxiolytic activity by hole cross method of methanolic extract of *A. Capitifformis*

Table 14 Anxiolytic activity by open field method of the methanolic extract of the plant

Test sample	Dose mg/kg	Number of passes squares(mean±SEM)				
		0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg	45±1.86	43.8±1.77	40.2±1.59	35.6±1.75	32.2±1.16
Standard (Diazepam)	1	34±2.35	28±2.57***	22.40±0.93***	16±0.45	8±0.84***
MEAC	200	47±1.16	27±2.4***	33±2.88	48±3.09	51±2.12**
MEAC	400	49±6.11	22±0.71***	29±1.32**	47±5.38	43±5.38

The results were expressed in Mean±SEM (standard mean error) * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control. MEAC=Methanol Extract of *A. capitiformis*. (* significant, ** more significant and *** highly significant)

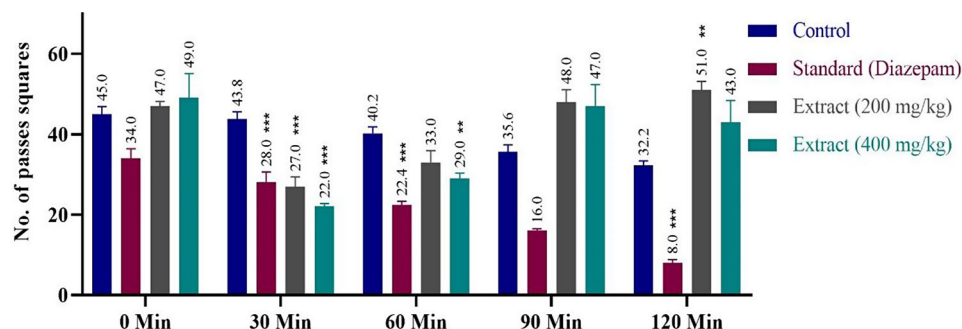


Fig. 11 Anxiolytic activity by open field method of methanolic extract of *A. Capitifformis*

Na having percent inhibition of 86.73%. Research shows that plants' flavonoids, phenols, terpenoids, and polyphenols relax and relieve pain which were justified by the previous studies done on different plant extract [83, 84]. *A. capitiformis* possesses high levels of phenolics, carbohydrates, flavonoids, alkaloids, and tannins.

Heat stimuli evaluated tail immersion's central analgesic activity [85, 86]. Sensory neurons sensitised nociceptors, reducing prostaglandin participation [87] and raising pain limits [88]. In this investigation, the analgesic impact of *A. capitiformis* was assessed by a rise in either the %MPE, PRT, or latency time at 200 and 400 mg/kg doses. PRT, MPE, and latency period increased dose-dependently until 120 min. Highest reaction time were seen in after the 30 and 60 min of extract administration at both the 200 and 400 mg/kg dose. The 400 mg/kg dosage had a greater impact than the 200 mg/kg dose, with response times of $8.5\pm0.24^{***}$ seconds for 30 min and

$8.32\pm0.26^{***}$ seconds for 60 min, respectively, which is highly statistically significant ($p<0.001$). The administration of the standard medication, Morphine sulfate, resulted in a very significant ($p<0.001$) consequence at both 30 and 60 min, as shown by response times of $6.96\pm0.41^{***}$ seconds and $6.64\pm0.32^{***}$ seconds, respectively. Therefore, it may be inferred that our extract has potent central analgesic properties. This extract may have affected the spinal and supraspinal analgesic pathways like morphine sulphate and these results were also supported by prior tests. [88]. Thus, the extract's analgesic properties may be due to the presence of saponins [89].

High-intensity phasic stimulation is used in the hot plate technique [90]. *A. capitiformis* showed an anti-nociceptive effect at 400 mg/kg after 60 and 120 min in a hot plate test, with highly significant findings ($p<0.001$). The reaction times for 60 and 120 min were 10.85 ± 0.93 min and 17.11 ± 0.67 min, respectively, for a dose of 400 mg/

kg, which is highly statistically significant ($p < 0.001$). At 200 mg/kg, the extract had no significant impact. At both 60 and 120 min, the standard medication, Morphine sulfate, had a highly significant ($p < 0.001$) impact, with reaction times of $10.72 \pm 0.85^{***}$ and $11.04 \pm 0.76^{***}$ seconds, respectively. When compared to the control, the same effects were observed with a dose of 400 mg/kg of methanolic extract of *A. capitiformis*. *A. capitiformis* extract may produce opioid peptides or act on central opioid receptors [91]. Therefore, the presence of saponins in the extract may contribute to its analgesic activities, considering the pharmacological background of these substances in analgesic and antispasmodic effects which was also justified by previous studies done [92]. Together with the results of the acetic acid-induced writhing technique, tail immersion method, and hot plate method, we may conclude that the extract exerted its analgesic effect through both central and peripheral mechanisms.

Anxiety disorders and severe symptoms typically need many treatments. Psychotherapy and anxiolytics treat worry [93]. Benzodiazepines have been the go-to anxiety drug for 40 years. They cause sleepiness, amnesia, muscular relaxation, and drug dependency [94, 95]. The hole board test may simulate animal anxiety by increasing head-dipping [52, 96]. To evaluate anxiolytic effects, the hole-board test provides a simple tool for assessing the pattern of mouse responses to an unfamiliar environment. This strategy makes it simple to monitor and measure the behavioral reaction; nonetheless, the animal's head-dipping behavior is closely connected to its emotional state [97]. The frequency and duration of head-dipping are considered indicators of neophilia (directed exploration) in mice, which is independent of overall locomotor activity. Strong levels of head-dipping are usually interpreted as an indicator of neophilia, whereas low levels imply a lack of neophilia or a strong anxiety-like condition in the animal [98]. The findings of our study demonstrated that a 400 mg/kg dose of *A. capitiformis* significantly increased head lowering which was $53 \pm 7.52^{**}$ ($p < 0.01$), that corroborated the anxiolytic-like impact earlier shown in the light-dark test, but the extract failed to demonstrate statistically significant effects in the instance of a 200 mg/kg dosage when compared with the control.

The EPM test presupposes that the approach-avoidance conflict elicited by an EPM is significantly more intense than that generated by a confined arm [99]. The open arm behaviors of mice in EPM indicate a conflict between the animal's inherent method to maintain itself in a safe zone (e.g., closed arms) and the motivation to explore in a novel environment, where the anxiolytic components promote their exploratory actions in the open arm [47]. In this technique, when comparing our methanolic extract of the whole plant to the standard medication,

the 400 mg/kg dosage revealed statistically significant ($p < 0.01$) findings, while the 200 mg/kg dose did not. At a dosage of 400 mg/kg, the duration of time spent in the open arm was $204 \pm 20.15^*$ seconds, and the number of entries into the open arm was $18.4 \pm 0.51^{**}$. The duration spent in the close arm for the same dose was 96 ± 20.14 s, and the number of entries was 4.6 ± 0.68 . The conventional medicine, Diazepam, has a statistically significant action in comparison to the control group.

The open-field test and hole-cross test were used to investigate the effects of methanol extract on locomotor activity. Sedative medicines reduce motility, which may be measured as a lack of exploratory behavior when a mouse is exposed to a novel environment [100]. Locomotor activity is thought to reflect a mental state as an indication of consciousness and awareness, and decreased movement acts as a signal of drowsiness and a general lack of agitation, which can be read as lower CNS excitability [101]. Our methanolic extract demonstrated statistically significant outcomes at a 400 mg/kg dosage after 30 and 60 min in an open field test and number of squares passed were $22 \pm 0.71^{***}$ and $29 \pm 1.32^{**}$ respectively compared to the gold standard medication, diazepam. The number of squares passed was $27 \pm 2.4^{***}$ and $51 \pm 2.12^{**}$ after 30 and 120 min, respectively, of administering the extract at a dosage of 200 mg/kg, which yields statistically significant results in 0, 30, 60, 90, and 120 min time courses when compared with the control group. The conventional medicine, Diazepam, has highly statistically significant action at 30, 60, and 120 min compared to the control group.

Both extract dosages, 200 mg/kg and 400 mg/kg, demonstrated calming action in the hole cross-test by reducing random motor activity. The findings were statistically significant just 30 min after the 400 mg/kg dose of extract was administered, with a number of hole crosses of $4.2 \pm 0.58^{**}$ ($p < 0.05$) in a 0, 30, 60, and 90 min time course. Nonetheless, the number of hole crosses was $9 \pm 0.71^{***}$, and the findings were highly statistically significant ($p < 0.001$) when administered at a dosage of 200 mg/kg when compared with the control group. The standard drug also showed statistically significant results when compared with the control group. Chemical and pharmacological studies have shown that saponins have a calming action [102]. Previous research has shown that plants containing alkaloids, flavonoids, terpenes, and saponins have sedative, anxiolytic, and antiepileptic activities because of the GABAergic system's affinity for the benzodiazepine [103]. These phytoconstituents may function as ligands for neurotransmitter receptors, imitating neuroactive hormones like GABA. Our research proved that our plant has terpenes, glycosides, alkaloids, flavonoids, phenols, and saponins.

Conclusion

In this study, methanolic extracts of the whole crude methanol extract of *A. capitiformis* showed a mixture of phytochemicals and antioxidants, in vitro anti-inflammatory and anti-arthritic effects, cytotoxicity, anti-helminthic, in vivo analgesic, and CNS inhibitory action. It may have contributed to the study of herbal medicine, but more advanced research on this medical plant is needed to determine its exact biological impact.

Abbreviations

DPPH	Diphenylpicrylhydrazyl
BSA	Bovine Serum Albumin
ROS	Reactive Oxygen Species
MPE	Maximal Possible Effect
UGO, PRT	Pain Reaction Time
EPM	Elevated Plus Maze
MEAC	Methanol Extract of <i>A. capitiformis</i>

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Author contributions

RAS designed the experiments, conception, and supervised the research work. MMC, MIS and AI collected the plant material, performed the pharmacological assays, statistical analysis, and drafted the manuscript. BC contributed to perform pharmacological analysis. RAS and MMC helped in the statistical analysis and write-up of the manuscript, critically revised the manuscript, provided punctual assistance, and gave the final approval for the submission of a revised version. Finally, all authors gave their final consent for the submission. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the present study are available upon reasonable request from the relevant author, and they were also discovered online by searching Google Scholar, PubMed, websites, book chapters, etc.

Declarations

Ethics approval and consent to participate

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) were followed, as well as specific national laws where applicable. Ethical approval has been taken from Animal Ethics Review Board, Faculty of Biological Sciences, University of Chittagong, Chittagong, Bangladesh.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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