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# Phytochemical screening and evaluation of pharmacological activity of leaf Methanolic extract of *Colocasia affinis* Schott

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## Abstract

**Background:** The purpose of the current study is to identify the phytochemicals as well as to determine the level of antioxidant, cytotoxic, antimicrobial and in vivo analgesic, anti-inflammatory and antidiarrheal activity of leaf methanolic extract of *Colocasia affinis* Schott (LMCA).

**Methods:** To appraise the antioxidant activities the extensively used total phenol, flavonoid, total antioxidant capacity, ferric reducing antioxidant power, cupric reducing antioxidant capacity and DPPH scavenging assay have been used. Cytotoxic potential was determined by brine shrimp lethality test whereas antimicrobial activity was assessed by disc diffusion method. Acetic acid induced writhing and formalin induced paw licking methods were employed to evaluate analgesic activity. To gauge anti-inflammatory activity, xylene-induced ear edema and carrageenan induced paw edema methods were used. Castor oil induced diarrhea and magnesium sulfate induced enteropooling methods were used to figure out antidiarrheal activity evaluation.

**Results:** Phytochemical screening affirmed the presence of alkaloids, flavonoids and tannins in the extract. LMCA contains marked amount of total phenol (3.89 ± 2.36 mg/g), total flavonoid (905.50 ± 2.12 mg/g) and total antioxidant (245.83 ± 2.36 mg/g). Remarkable cupric reducing power and ferric reducing power capability were observed. In DPPH radical scavenging assay, the extract showed moderate scavenging potential ($IC_{50}$ 395.97 μg/ml), whereas $IC_{50}$ value of standard ascorbic acid was 32.75 μg /ml. The extract showed a significant result in cytotoxicity test. In disk diffusion antimicrobial assay LMCA manifested broad spectrum of activity. In acetic acid induced writhing test, the highest dose (1000 mg/kg) showed 57.69% inhibition of abdominal constrictions compared to Diclofenac Sodium (74.62%). In case of formalin induced analgesic activity test the extract exhibited preeminent effect (LMCA 1000 mg/kg showed 58.64% inhibition). The extract inhibited 86.36% xylene induced ear edema (at dose 1000 mg/kg) where Aspirin inhibited 88.81%. In carrageenan induced test LMCA 1000 mg/kg manifested eloquent inhibition of inflammation at 6th hour (50.59%). The highest dose 1000 mg/kg of LMCA showed significant (80.95%) reduction in diarrhea and noticeable reduction of intestinal fluid secretion (24.29%).

**Conclusion:** To recapitulate, it is suggested that the leaf of *Colocasia affinis* Schott might be a potential source of useful bioactive molecules.

**Keywords:** *Colocasia affinis* Schott, Phytochemicals, Antioxidant, DPPH, Brine shrimp, Antimicrobial, Analgesic, Anti-inflammatory, Antidiarrheal

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## Introduction

From the very ancient time to till now plants have been the basis of many traditional medicine systems throughout the world and continued to furnish mankind with new remedies. A great variety of medicinal plants, their purified constituents and natural products from the medicinal plants provide unlimited opportunities for new drugs development because of the unparalleled accessibility of diverse chemical compounds [1] and also have been shown to have beneficial therapeutic potential. When our body cells use oxygen, they naturally produce free radicals which can cause damage to cell. Free radicals contribute to more than one hundred disorders in humans, including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, central nervous system injury, gastritis, and cancer [2]. Recent research has confirmed that antioxidants are the most effective tools to eliminate free radicals which cause oxidative stress and are possible protective agents that protect the cells from reactive oxygen spices and retard the progress of many diseases as well as lipid peroxidation [3–5]. Additionally, they also possess anti-inflammatory, anti-viral and anti-cancer properties [6]. The medicinal value of plant is related to their phytochemical component content and secondary metabolites, including: phenolic compounds, flavonoids, alkaloids, tannins, and other stress gene response products [7]. Recently, pathogenic microorganisms of human have exhibited multifarious drug resistance for frequent use of potent anti-microbial drugs in infectious diseases. In this situation, researchers are trying to seek of new anti-microbial lead compounds from medicinal plants, a good source of novel antimicrobial drugs [8]. The side effects of synthetic anti-inflammatory drugs including gastric injury, ulceration, bronchospasm, inhibition of platelet aggregation, liver and kidney toxicity have limited their use [9]. Therefore, it is requisite to investigate for new anti-inflammatory drugs with fewer side effects and plants may be our potential solution to combat this [10].

*Colocasia affinis* Schott (Family: **Araceae**), a perennial herb, height 46–91 cm. It is found all over in Bangladesh and locally known as Kochu (bengali). English name is **Dwarf elephant's ear.** Traditionally leaves are cooked as vegetables and are reported to be effective to treat cataract. The leaves are often boiled with coconut milk to make a soup which is rich in iron. To the best of our knowledge no attempts were taken before to evaluate in vitro antioxidant, anti-microbial, brine shrimp lethality bioassay, anti-inflammatory and antidiarrheal properties of methanol extract of leaf part of *C. affinis* Schott. So in this study we have investigated antioxidant, antimicrobial and brine shrimp lethality bioassay anti-nociceptive, anti-inflammatory and antidiarrheal activities of leaf methanolic extract of *C. affinis* Schott.

## Materials and methods

### Plant material

Leaves of *Colocasia affinis* Schott were collected from the campus of Jahangirnagar University, Savar, Dhaka, Bangladesh and identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The voucher specimen of the plant had been deposited in the herbarium for further reference.

### Extraction process

Leaves of the plant were sun dried and then crushed through grinding machine. Then 100 g of dried leaf powder was taken in a separate container. To this 250 ml of methanol was added and kept for 24 h at room temperature with periodic shaking. Then filtered with Whatman No. 1 filter paper and the filtrate were collected. The procedure was repeated three times with fresh volume of methanol. The filtrates were pooled to ensure maximum extraction. Then the extract was dried and weighed. Yield was 41.50 g.

### Phytochemical screening

The freshly prepared crude extract was qualitatively tested to reveal the presence of phytochemical constituents such as alkaloid, carbohydrate, flavonoid, glycoside, tannin, steroid and saponin. These were identified by characteristic color changes using standard procedures [11].

### Tests for antioxidant activity

#### *Determination of total antioxidant capacity*

By the phosphomolybdenum method, the total antioxidant activity of the extract was evaluated according to Prieto et al, 1999 [12]. 0.3 ml extract solution was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were kept for incubation at 95 °C for 90 min. Then absorbance of the solution was evaluated at 695 nm using a spectrophotometer (UV visible spectrophotometer, Shimadzu, 1601) against blank after cooling at room temperature. Methanol (0.3 ml) is used as the blank. The antioxidant activity is expressed as the number of ascorbic acid equivalents.

#### *Determination of total phenolic contents*

To determine the total phenolic contents of LMCA, Folin–Ciocalteu reagent was used [13]. Plant extract solution (100 μl) was mixed with 500 μl of the Folin–Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and added distilled water up to 10 ml. The whole mixture was allowed to stand for 2 h. Then the absorbance was taken at 765 nm. These data were used to estimate total phenolic contents

using a standard curve obtained from various concentration of standard Gallic acid.

#### *Determination of total flavonoids content*

The content of total flavonoids in the extract was determined by the method by Chang et al., 2002 [14]. 1 ml of sample (LMCA) solution was mixed with methanol (3 ml), aluminum chloride (0.2 ml, 10%), potassium acetate (0.2 ml, 1 M) and distilled water (5.6 ml) and incubated the mixture for 30 min at room temperature. Then the absorbance was determined at 415 nm against blank. Methanol (1 ml) in the place of sample solution was used as the blank and Quercetin was used as the standard solution. The amount of flavonoids in plant extracts in Quercetin equivalents (QE) was calculated by the following formula: $X = (A \times m_0)/ (A_0 \times m)$, where X is the total flavonoid content, mg/mg plant extract in QE, A is the absorption of plant extract solution, $A_0$ is the absorption of standard quercetin solution, m is the weight of plant extract in mg and $m_0$ is the weight of quercetin in the solution in mg.

#### *Cupric reducing antioxidant capacity (CUPRAC)*

Cupric reducing antioxidant capacity of LMCA was determined according to Resat et al., 2004 [15]. Different concentrations of extract (5–200 μg) in 0.5 ml of distilled water were mixed with cupric chloride (1 ml, 0.01 M), ammonium acetate buffer (1 ml, pH 7.0), neocuproine (1 ml, 0.0075 M) and at last distilled water (0.6 ml). The mixture was incubated for 1 h at room temperature. Then the absorbance was measured at 450 nm against blank. Distilled water (0.5 ml) is used as the blank. From the slope of the calibration line concerned, the molar absorptivity of the CUPRAC method for each antioxidant was found. Ascorbic acid was used as the standard.

#### *Ferric reducing antioxidant power (FRAP)*

According to the method by Oyaizu, 1986 the ferric reducing antioxidant power was assessed [16]. In this method, the reduction of $Fe^{3+}$ to $Fe^{2+}$ is assessed by measuring the absorbance of Perl's Prussian blue complex. Different concentrations of extract solution (LMCA) (5–200 μg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C and waited for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) as well as $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference standard.

#### *DPPH free radical scavenging activity*

DPPH scavenging activity of the extract was determined by the method described by Braca et al., 2001 [17]. Extract solution (0.1 ml) of each concentrations were added to 3 ml of a 0.004% ethanol solution of DPPH. Absorbance at 517 nm was fixed to determined after 30 min and the percentage inhibition activity was estimated from $[(A_o\text{-}A_1)/A_o] \times 100$, where $A_o$ is the absorbance of the control (DPPH solution) and $A_1$ is the absorbance of the extract/standard. The DPPH free radical scavenging activity curves were prepared and $IC_{50}$ values were calculated.

#### *Cytotoxicity test*

Brine shrimp lethality bioassay method was applied to predict possible cytotoxic action [18, 19]. The eggs of brine shrimp (*Artemia salina* leach) were collected and hatched in a glass tank at a temperature around 37 °C with oxygen supply. Two days later the mature naupliies were allowed to assess for cytotoxic test. Stock solution of sample extract was prepared by dissolving required amount of extract in specific volume of dimethyl sulfoxide (DMSO). Ten alive nauplii were taken to each of the vial that contains different concentrations of test sample with Pasteur pipette. Then sample were transferred from the stock solution to the vials to get final sample concentration. In the control vials same volumes of DMSO (as in the sample vials) were taken. For the positive control, Vincristine sulphate was used. After 24 h the vials were observed and the number of nauplii was counted that were survived in each vial. From this, the percentage of mortality of brine shrimp nauplii was calculated for each concentration of the sample extract.

#### *Antimicrobial activity by disc diffusion method*

The antimicrobial activity of the plant extract was figured out by disc diffusion method described by Bauer-Kirby [20, 21]. With the help of a sterile swab, the inoculums of microorganisms were spread over nutrient agar plates. One hundred milligram of the test sample was dissolved in 1 ml of methanol to obtain the concentration 100 μg/μl in an aseptic condition. Sterilized metrical filter paper discs (Whatman No. 1, 6 mm diameter) were soaked with different concentrations (30 μg/μl, 20 μg/μl and 10 μg/μl) of the test sample. Then the soaked discs were placed on the marked agar plate and dried. The extract was tested in triplicate and the plates were inoculated at 37 °C for 24 h. Amoxicillin was used as a positive control. Inhibition zones (diameters) were measured.

### In-vivo pharmacological activity evaluation

#### *Acute toxicity study*

Acute oral toxicity study for the test extract of the plant was carried out as per the guidelines set by Organization

for Economic Cooperation and Development (OECD). Overnight-fasted Swiss albino mice (30–45 g) and Wister rat (120–130 g) of either sex were used for the study. The animals were divided into seven groups of five animals each. Groups A to F received orally 250, 500, 1000, 2000, 3000, 4000 mg/kg of the extract respectively, while the control (group G), received distilled water (3 ml/kg) by the same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed for any signs of delayed toxicity for 2 weeks.

### Analgesic activity evaluation

#### *Acetic acid induced writhing test*

The method described by Koster et al., 1959 was employed for this test [22]. Four groups of five mice each were pretreated with extracts (500 and 1000 mg/kg), diclofenac (10 mg/kg) and distilled water (3 ml/kg). After forty five minutes, 0.7% acetic acid at a dose of 10 ml/kg body weight was injected intraperitoneally in each mouse. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 min of i.p. administration of acetic acid and the mean abdominal writhing for each group was obtained. The percentage inhibition of writhing was calculated by comparison with the control mice.

#### *Formalin induced paw licking test*

For formalin induced analgesic activity evaluation the method described by Hunskaar S et al. [23] was used. The control group received normal saline (0.1 ml/10 g) and standard group Aspirin (100 mg/kg). Extract solutions (500 mg/kg & 1000 mg/kg) were orally administered and after 30 min of treatment, 20 μl of 1% Formalin solution was injected subcutaneously in the right hind paw of the mice. The time spent in licking and biting of the affected paw was noted. The total paw licking response was measured as early phase (0–5 min) and late phase (15–20 min) after formalin injection [24, 25]. The percentage of pain inhibition was expressed by the given formula: Percentage inhibition = [(Control mean-Treated mean)/Control mean] × 100.

### Anti-inflammatory activity evaluation

#### *Xylene induced ear edema method*

Mice were divided into four groups of five animals each. Group A & B were treated orally with the LMCA solution 500 and 1000 mg/kg respectively while group C received diclofenac 10 mg/kg and group D was treated with distilled water with Tween 80 (10 ml/kg). Thirty minutes afterwards, edema was induced in each mouse group by applying a drop of xylene to the inner surface of the right ear. Fifteen minutes later, the animals were sacrificed under ether anesthesia and both ears cut off, sized and weighed. The anti-inflammatory activity was manifested as the percentage inhibition of edema in the treated mice in comparison with the control mice [26].

#### *Carrageenan induced rat paw edema method*

The anti-inflammatory activity of LMCA was investigated by carrageenan induced inflammation in rat paw by following the method of Winter et al. [27] with minor modifications. Rats were randomly divided into four groups, each consisting of five animals, of which group I was kept as control giving only distilled water. Group II was given indomethacin (5 mg/kg) as standard drug. Group III and group IV were given the test sample at the dose of 500 mg/kg and 1000 mg/kg body weight respectively. Half an hour after the oral administration of the test materials, 1% carrageenan in saline was injected to the left hind paw of each rat. The volume of paw edema was measured at 1,2,3,4 and 6 h using water plethysmometer after administration of carrageenan. The right hind paw served as a reference of non-inflamed paw for comparison.

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-.

% Inhibition of paw edema = $[(V_c - V_t) / V_c] \times 100$.

Where $V_c$ and $V_t$ represent average paw volume of control and treated animal respectively.

### Antidiarrheal activity evaluation

#### *Castor oil-induced diarrhea test*

The antidiarrheal activity of LMCA was studied according to the method described by Jebunnessa et al., 2009 [28]. Mice fasted for 24 h were divided into control (Group I), positive control (Group II: Loperamide) and test groups (Group III, IV) containing five animal in each group. Control group received distilled water at the dose of 10 ml/kg p.o. Positive control group was given Loperamide at the dose of 5 mg/kg p.o. Test groups III & IV were given LMCA at doses of 500, 1000 mg/kg respectively. After 1 h each group were treated with 0.5 ml of castor oil orally. Then each animal was placed in a separate cage with blotting paper lined floor. The blotting papers were changed every hour. The animals were observed for the next 4 h to record the time of onset of diarrhea, the total number of fecal output (frequency of defecation) and weight of feces excreted by the animals were recorded. The percent (%) inhibition of defecation was calculated using the formula: % Inhibition of defecation = [(A-B)/A] × 100 here, A = Mean number of defecation caused by castor oil and B = Mean number of defecation caused by drug or extract.

#### *Magnesium sulfate induced enteropooling*

To appraise the antidiarrheal activity through magnesium sulfate induced enteropooling method Wistar rats were fasted for 18 h and divided into four groups of five animals per group. Group I animals which received normal saline (2 ml, p.o.) served as the control group. Group II animals received loperamide (3 mg/kg, p.o.) and served as standard. Group III and IV received LMCA 500 mg/kg and 500 mg/kg respectively, p.o. Immediately after the treatment magnesium sulfate (10% *w*/*v*) was administered. After 30 min following administration of magnesium sulfate the rats were sacrificed, the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated [29, 30].

### Statistical analysis

The results were expressed as mean ± standard deviation (SD) from triplicate experiments and evaluated with the analysis of student's t-test. Differences were considered significant at a level of $P < 0.05$. $IC_{50}$ was calculated using Sigma Plot 11.0 software.

## Results

### Phytochemical screening

Preliminary phytochemical screening of LMCA revealed the presence of different kind of chemical groups that are summarized in Table 1.

### Determination of total antioxidant capacity

Total antioxidant capacity of the test sample was measured by the standard curve of ascorbic acid. The total antioxidant capacity of the extract was found 245.83 ± 2.36 mg/g AAE (Ascorbic acid equivalent) at 200 μg/ml extract concentration.

### Determination of total phenolic contents

The total phenolic contents of the test fraction were measured using the standard curve of gallic acid. Test fraction was found to contain a little amount of 3.89 ± 2.36 mg/gm Gallic acid equivalent (GAE).

### Determination of total flavonoids content

The total flavonoid content was calculated using the standard curve of quercetin and was expressed as Quercetin equivalents (QE) per gram of the plant extract. Flavonoid contents of the fraction were found as 905.5 ± 2.12 mg/g Quercetin equivalent (QAE) which might be supportive for its antioxidant activities.

### Cupric reducing antioxidant capacity (CUPRAC)

Preliminary phytochemical screening supported that polyphenolics and flavonoids are present in the LMCA. The plant extract showed dose dependent reducing capacity that is comparable to ascorbic acid (Fig. 1) in the current test. The standard ascorbic acid showed highest reducing capacity.

### Ferric reducing antioxidant power (FRAP)

The extract showed marked reducing power which was found to rise with increasing the concentrations of the extract (Fig. 2) whereas the standard ascorbic acid showed higher activity.

### DPPH radical scavenging activity

In DPPH radical scavenging assay, as shown in Fig. 3, by inhibiting DPPH radical, a concentration dependent anti-radical activity was exhibited by the extract. A well known antioxidant, ascorbic acid showed greater degree of free radical-scavenging activity than that of the plant extract at each concentration points. The $IC_{50}$ value of the leaf methanol extract was 560.931 μg/ml, whereas for standard ascorbic acid $IC_{50}$ value was 34.88 μg/ml.

### Cytotoxic activity test

LMCA showed good potentiality in Brine Shrimp Lethality Bioassay. Table 2 displays the result of the brine shrimp lethality assay of the samples and the positive control (Vincristine sulphate). In this study, LMCA was found to be good toxic to Brine Shrimp with $LC_{50}$ of 182.98 μg/ml whereas Vincristine sulphate showed $LC_{50}$ value 0.0699 μg/ml. In case of $LC_{90}$, the value for LMCA and Vincristine sulphate were 1099.13 μg/ml and 6.33 μg/ml respectively.

### Antimicrobial activity test

Plants are widely used as useful sources for the development of new chemotherapeutic agents. The in vitro antibacterial activity assay is the first step to achieve this goal [31]. The result of antimicrobial activities of LMCA is summarized in the Table 3. Though it is a preliminary test but the result reveals that tested fraction might have

**Table 1** Results of phytochemical screening of LMCA

| Extract | Alkaloid test | | | | Carbohydrate test | Flavonoid test | Glucoside test | Glycoside test | Saponin test | Steroid test | Tanin test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mayer's Test | Hager's Test | Wagner's Test | Dragendorff's Test | | | | | | | |
| LMCA | ++ | ++ | ++ | + | + | ++ | – | + | + | – | ++ |

*LMCA* Leaf methanol extract *of Colocasia affinis* Schott; [++ = Strong presence, + = Presence, – = Absence]

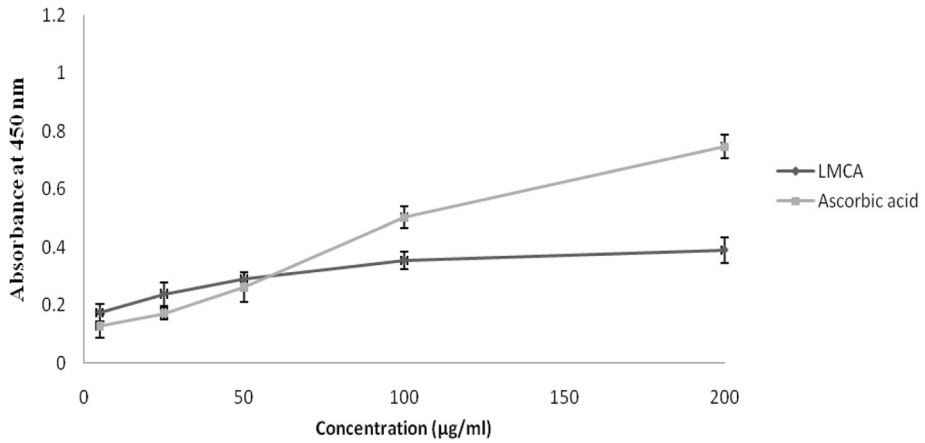


**Fig. 1** CUPRAC reducing antioxidant activity of leaf methanolic extract of *Colocasia affinis* Schott along with the standard Ascorbic acid. (Mean ± SD, n = 3)

active compounds that might be a source of antimicrobial agent.

## In-vivo pharmacological activity evaluation

### *Acute toxicity study*

In this study, it was observed that no mortality was found up to dose as high as 4000 mg/kg for the extract. So, the extract was considered to be safer with broader therapeutic range. Here, two comparative doses (500 and 1000 mg/kg) for the extract was taken for the in-vivo models.

## Analgesic activity evaluation

### *Acetic acid induced writhing test*

Table 4 shows the effect of LMCA in acetic acid induced writhing test. The % of inhibition of writhing by methanol extract at dose of 500 and 1000 mg/kg were 48.46% and 57.69% respectively whereas the standard Diclofenac showed 74.62% inhibition.

Values are presented as mean ± SEM ($n$ = 5). One way ANOVA followed by Dunnett's multiple comparisons was performed to analyze this data set. $^{*}p < 0.05$ was considered statistically significant when compared against control.

### *The formalin-induced paw licking test*

The current study showed a dose dependent inhibition of pain responses. The extract with dose 500 mg/kg produced significant ($P < 0.05$) inhibition (5.98%) in the early phase of the pain. The extract at both the doses (500 and 1000 mg/kg) produced significant ($P < 0.01$) inhibition of pain in the late phase, (Table 5) while the standard drug (Aspirin) produced significant reduction in pain response (78.07%) at dose 100 mg/kg.

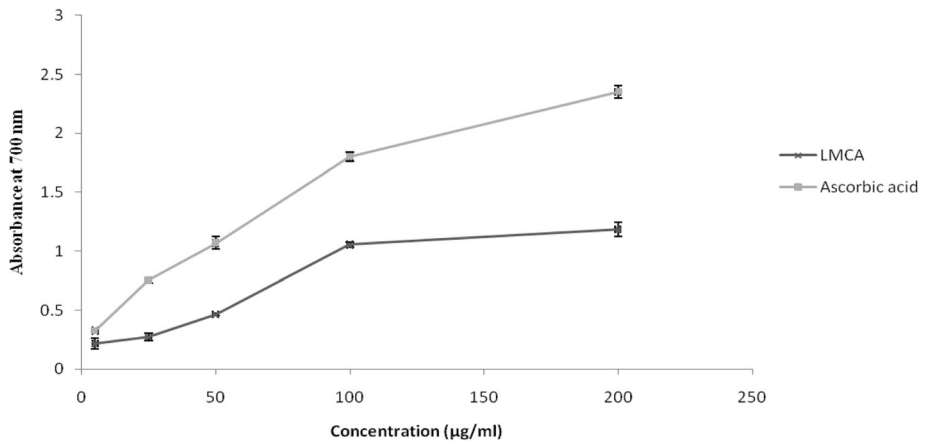


**Fig. 2** Ferric reducing antioxidant activity (FRAP) of leaf methanolic extract of *Colocasia affinis* Schott along with the standard Ascorbic acid. (Mean ± SD, $n$ = 3)

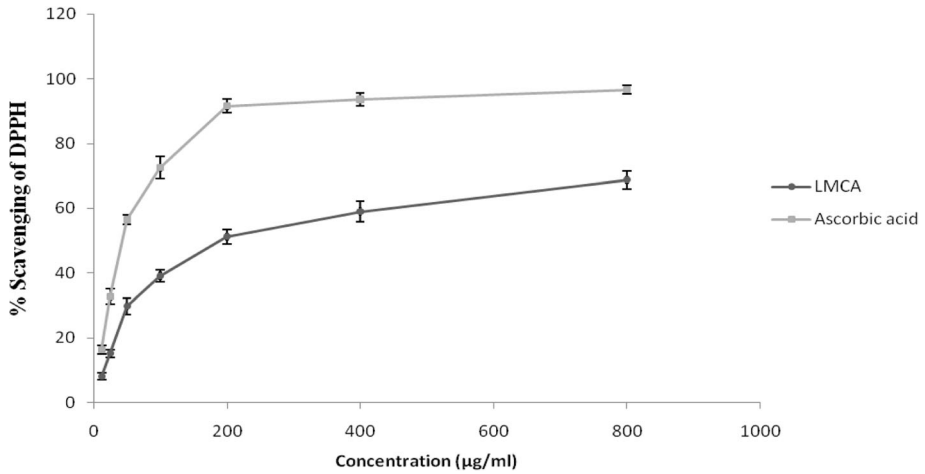


**Fig. 3** DPPH radical scavenging activity of leaf methanolic extract of *Colocasia affinis* Schott along with standard Ascorbic acid. (Mean ± SD, n = 3)

The animals were treated orally. Values are represented as mean ± SEM ($n = 6$ in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. *p < 0.05, **$p < 0.01$ were considered as significant.

## Anti-inflammatory activity evaluation

### *Xylene induced ear edema method*

In this study, the extract reduced the ear edema in dose dependent manner (Table 6). In case of extract, the value of % of inhibition was 63.29% at lower doses (500 mg/kg) whereas at higher doses (1000 mg/kg) it was 86.36%. The standard drug aspirin showed significant inhibition (88.81%).

**Table 2** Mortality % of Brine shrimp produced by LMCA along with the standard Vincristine sulphate

| Test sample | Concentration (µg/ml) | Log concentration | Mortality% | Corrected mortality % | $LC_{50}$ (µg/ml) | $LC_{90}$ (µg/ml) |
|---|---|---|---|---|---|---|
| LMCA | 12.5 | 1.09691 | 10 | 0 | 182.98 | 1099.13 |
| | 25 | 1.39794 | 20 | 11.11 | | |
| | 50 | 1.69897 | 20 | 11.11 | | |
| | 100 | 2 | 30 | 22.22 | | |
| | 200 | 2.30103 | 50 | 44.44 | | |
| | 400 | 2.60206 | 80 | 77.78 | | |
| | 800 | 2.90309 | 90 | 88.89 | | |
| VS | 0.06 | −1.22185 | 10 | 0 | 0.0699 | 6.33 |
| | 0.125 | −0.90309 | 20 | 11.11 | | |
| | 0.25 | −0.60206 | 30 | 22.22 | | |
| | 0.5 | −0.30103 | 40 | 33.33 | | |
| | 1 | 0 | 50 | 44.44 | | |
| | 5 | 0.69897 | 90 | 88.88 | | |
| | 10 | 1 | 100 | 100 | | |

*LMCA* Leaf Methanolic extract of *C. affinis* Schott, *VS* Vincristine sulphate

### *Carrageenan induced paw edema method*

In carrageenan induced rat paw edema method, anti-inflammatory activity of tested plant extract was presented in Table 7. Current study revealed that LMCA (1000 mg/kg dose) exhibited significant reduction in edema volume (17.98%, 50.59%) after one hour and 5 h respectively. Standard anti-inflammatory drug (Indomethacin 5 mg/kg dose) revealed effective inhibition (64.65%) at five hour.

## Antidiarrheal activity evaluation

### *Castor oil-induced diarrhea test*

LMCA was found to be effective in a dose dependent manner against castor oil induced diarrhea on experimental mice at all tested doses. At all the doses (500, 1000 mg/kg bodyweight), the extract produced a significant decrease in the severity of diarrhea in terms of reduction in the rate of defecation and consistency of feces in albino mice. The highest dose (1000 mg/kg) of extract showed 75.61% reduction in defecation and 80.95% inhibition in diarrhea that is compare with that of the standard drug Loperamide that showed 88.89% inhibition of diarrhea as well as 81.71% reduction in defecation (Table 8).

### *Magnesium sulfate induced enteropooling*

Magnesium Sulfate induced enteropooling test result was displayed in Table 9 where extract indicated dose dependent lessening in the intestinal fluid secretion. Plant extract (LMCA 500 mg/kg b.w) treatment reduced the intestinal fluid secretion significantly (24.29%) while the standard drug, Loperamide (3 mg/kg, p.o.), produced a more marked and significantly greater ($p < 0.05$) inhibitory effects on magnesium sulfate induced fluid accumulation (49.53%).

**Table 3** Zone of Inhibition produced by LMCA against some Gram positive and Gram negative bacteria

| Test microorganisms | Zone of inhibition in mm | | | |
|---|---|---|---|---|
| | Extract Conc. (μl/disc) | | | Standard Amoxicillin (10 μl/disc) |
| | 10 | 20 | 30 | |
| *Bacillus subtilis* | 6 | 6.2 | 7.5 | 28.5 |
| *Bacillus cereus* | 7.5 | 8.5 | 10.5 | 22.2 |
| *Staphylococcus aureus* | 7 | 7 | 7.3 | 12.5 |
| *Proteus mirabilis* | 8.5 | 8.5 | 9.5 | 15 |
| *Escherichia coli* | 7.5 | 8 | 8 | 9 |
| *Salmonella typhi* | 7.5 | 8 | 8.5 | 12 |
| *Pseudomonas spp.* | 7.5 | 8 | 12 | 20.5 |

## Discussion

The findings of the preliminary phytochemical screening laid the foundation for further works as it showed positive result for alkaloid, tannin, flavonoid, carbohydrate etc.

On the basis of several reports, it has been vested that phenolic content is closely related with antioxidative activity of the fruits and vegetables. It is also evident that in various diseases like cardiovascular disease, aging and cancer, Phenolic compounds (natural antioxidants) exhibit their therapeutic activity [32]. In addition, phenolic compounds exhibit their antioxidant activity for their redox properties [33]. It has been also revealed that flavonoids posses antioxidant activities. Due to having the ability of scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation, flavonoids show the antioxidative properties [34]. The reactive hydroxyl groups of polyphenolics, oligomeric flavonoids are oxidized to the corresponding quinines with CUPRAC reagent [15, 35]. The presence of phenolic compound in the extract purports it to be a potential antioxidant. In reducing power assays, the presence of antioxidants can reduce the oxidized form of iron ($Fe^{3+}$) to its reduced form ($Fe^{2+}$) by donating an electron. Thus, it might be assumed that the presence of reductants (i.e. antioxidants) in LMCA causes the reduction of the $Fe^{3+}$/ferricyanide complex to the ferrous form which reveals the antioxidative nature of the extract [36]. The DPPH antioxidant assay was done on the basis of the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, which is decolorized in the presence of antioxidants [37]. The findings of DPPH scavenging test impose a status that the plant might have active principles which showed antioxidant activity due to their redox properties, play a vital role in absorbing and neutralizing free radicals.

**Table 4** Effect of LMCA in Acetic Acid Induced Writhing test

| Group | Dose | No of Writhing | Inhibition (%) |
|---|---|---|---|
| Control | – | 21.67 ± 1.11555 | – |
| Standard (Diclofenac) | 100 | 5.507 ± 0.99163* | 74.62 |
| LMCA | 500 | 11.17 ± 1.10805 | 48.46 |
| LMCA | 1000 | 9.17 ± 1.74005* | 57.69 |

**Table 5** Effect of LMCA in Formalin induced paw licking test

| Treatment | Dose (mg/kg) | Licking of hind paw (sec) | | | |
|---|---|---|---|---|---|
| | | (0–5 min) | % Inhibition | (15–20 min) | % Inhibition |
| Control | 10 | 95.14 ± 2.19 | – | 98.32 ± 2.18 | – |
| Aspirin | 100 | 91.73 ± 1.67 | – | 22.23 ± 2.43** | 78.07 |
| LMCA | 500 | 89.45 ± 2.10 | 5.98 | 65.31 ± 1.31* | 33.87 |
| LMCA | 1000 | 81.83 ± 2.18* | 13.99 | 41.16 ± 1.54** | 58.64 |

There is a correlation between the brine shrimp assay and in vitro growth inhibition of human solid tumor cell lines demonstrated by the National Cancer Institute (NCI, USA). The significant toxicity of plants is principally contributed by the presence of alkaloids, glycosides, steroids, tannins and flavonoids which were showed in preliminary phytochemical screening [38]. Our findings supported that the extract might have active constituents which is responsible for anti-tumor potential of the extract.

In anti microbial assay by disc diffusion assay the extract showed dose dependent inhibition. It is evident that alkaloids have potentials to show antimicrobial activity [39]. In phytochemical screening we found alkaloid in the extract which may be correlated in this context. Presence of tannin in LMCA may also be responsible for antimicrobial activity [40].

For evaluation of analgesic activity two established method were employed. Acetic acid induced writhing **test is** a chemical method used to induce pain of peripheral origin by injection. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [41]. It is a sensitive procedure used for the

**Table 6** Effect of LMCA in Xyline induced ear edema test

| Group | Doses (mg/kg) | Ear weight Difference (mg) | Inhibition (%) |
|---|---|---|---|
| Control | 1% Tween 80 in Water (10 ml/kg) | 2.86 ± 0.206 | – |
| Standard (Aspirin) | 100 | 0.32 ± 0.0663*** | 88.81 |
| LMCA | 500 | 1.05 ± 0.09** | 63.29 |
| LMCA | 1000 | 0.39 ± 0.11*** | 86.36 |

Values are presented as mean ± SEM ($n = 5$). A) One way ANOVA followed by Dunnet's multiple comparisons was performed to analyze this data set when compared against control

B) One way ANOVA followed by Tukey multiple comparison was performed to analyze inter-comparison among different groups and standard in this data set. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ were considered statistically significant

**Table 7** Anti-inflammatory activity of LMCA by carrageenan induced paw edema method

| Treatment group | 0 Hour (mm) | 1st Hour (mm) | 2nd Hour (mm) | 3rd Hour (mm) | 4th Hour (mm) | 6th Hour (mm) |
|---|---|---|---|---|---|---|
| Control | 0.81 ± 0.025 | 0.89 ± 0.035 | 1.21 ± 0.193 | 2.73 ± 0.012 | 3.46 ± 0.014 | 5.12 ± 1.02 |
| Diclofenac Sodium | 0.71 ± 0.089 | 0.69 ± 0.046 (22.47%) | 0.87 ± 0.013 (28.10%) | 1.32 ± 0.052 (51.65%) | 1.47 ± 0.063 (57.51%) | 1.81 ± 0.032** (64.65%) |
| LMCA (500 mg/kg) | 0.68 ± 0.041 | 0.76 ± 0.021 (14.61%) | 0.96 ± 0.016 (20.67%) | 1.83 ± 0.029 (32.97%) | 2.22 ± 0.057 (35.84%) | 2.98 ± 0.017** (41.80%) |
| LMCA (1000 mg/kg) | 0.63 ± 0.023 | 0.73 ± 0.026 (17.98%) | 0.89 ± 0.073 (26.45%) | 1.69 ± 0.029 (38.10%) | 1.89 ± 0.031 (45.38%) | 3.13 ± 0.72*** (50.59%) |

Values in the table are expressed as mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significantly different in comparison with control, *SEM* Standard error of Mean

evaluation of peripherally acting analgesics. Peritoneal mast cells, acid sensing ion channels and the prostaglandin are responsible for mediating the response [42, 43]. In this test the extract produced significant inhibition of pain which indicates the analgesic potential of the extract.

Research found that the early phase pain sensation (immediately after injection) might be caused by C-fiber activation as a result of peripheral stimulus where as the late phase (starting approximately 15 min after formalin injection) appears to be dependent on the integration of an inflammatory reflection, NMDA exhilaration and non-NMDA receptors and NO cascade in the peripheral tissue and the functional changes in the dorsal horn of the spinal cord. The result of the test suggests that LMCA causes partial inactivation of NMDA and non-NMDA [44, 45].

Xylene produces severe vasodilation, edematous changes of skin and of inflammatory cellular infiltration when applied topically [46]. As the extract reduced inflammatory symptoms it would be the reflective sign of the anti inflammatory potential of LMCA. The findings of the most classical acute anti-inflammatory activity evaluation method, carrageenan induced paw edema test [47] suggest that LMCA might have potential which is responsible for potent anti-inflammatory action possibly due to the inhibition the mediators of inflammation, principally prostaglandin [48].

Research has been made to show the anti-diarrheal activity may be due to the presence of tannins, saponin and terpenoids [49, 50]. It is withal evident that flavonoids and polyphenols were exposed to have anti-diarrheal activities [51]. As in case of castor oil-induced diarrhea test and magnesium induced enteropooling test the extract produced marked reduction in defecation and intestinal fluid secretion respectively, it might be concluded that the extract posses denoting antidiarrheal activity.

## Conclusion

In the current investigation, LMCA was evaluated for in vitro antioxidant, brine shrimp lethality, antimicrobial activity and in vivo analgesic activity, anti-inflammatory activity, antidiarrheal activity. The outcomes of this evaluation have exhibited that methanolic leaf extract is rich in antioxidant properties and has significant cytotoxic potentials also. Both Gram positive and Gram negative bacterial strains growth were controlled effectively by the extract. This study also showed that the LMCA have significant analgesic and anti-inflammatory effects at different doses. At different doses the plant extract showed significant dose dependent delayed onset of diarrhea induced by castor oil and magnesium when compared with the control. Different types of phytochemical constituents like alkaloids, flavonoids, glycosides, saponins and tannins present in the plant, which may be responsible for the observed activities. However well-structured in vitro and in vivo evaluations would be required to identify the bioactive compounds responsible for these activities as well as to examine the underlying mechanism action to find out novel lead molecules.

**Table 8** Effect of leaf methanolic extract of LMCA in castor oil induced diarrhea in mice

| Group | Doses (mg/kg) | Total feces | % inhibition of defection | Total diarrhea feces | % inhibition of diarrhea |
|---|---|---|---|---|---|
| Control | – | 82 | 0 | 63 | 0 |
| Standard (Loperamide) | 5 | 15* | 81.71 | 6* | 90.48 |
| LMCA | 500 | 40 | 51.22 | 20 | 68.25 |
| LMCA | 1000 | 20* | 75.61 | 12** | 80.95 |

Values are presented as mean ± SEM ($n = 5$). One way ANOVA followed by Dunnett's multiple comparisons was performed to analyze this dataset. *$p < 0.05$, **$p < 0.01$ significantly different in comparison with control

**Table 9** Effect of LMCA in magnesium sulphate induced enteropooling

| Treatment | Dose (mg/kg) | Volume of fluid (ml) | Weight of intestinal content (gm) | % inhibition |
|---|---|---|---|---|
| Control | – | 7.25 ± 0.45 | 13.71 ± 0.56 | – |
| Loperamide | 3 | 3.25 ± 0.61 | 6.92 ± 0.32 * | 49.53 |
| LMCA | 500 | 6.12 ± 0.24 | 11.57 ± 0.67 | 15.61 |
| LMCA | 1000 | 5.13 ± 0.18 | 10.38 ± 0.71 ** | 24.29 |

Results are expressed as mean ± SEM; $n = 5$ in each group. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparisons test. *$p < 0.05$, **$p < 0.01$ significantly different in comparison with control. *SEM* Standard error of Mean

**Abbreviations**
ANOVA: Analysis of variance; DPPH: 1,1-diphenyl-2-picrylhydrazyl; $IC_{50}$: Half maximal inhibitory concentration; LMCA: Leaf methanolic extract of *Colocasia affinis* Schott; SEM: Standard error of mean; VS: Vincristine sulphate

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**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**
MM, ND and TI, APS participated in the conduction of the experiments. MM, MSH and SKK contributed in designing concept and conduction of research. MM, MSH, ABR, SZS analyzed and interpreted data. MM, MSH, TI and SB participated in drafting the manuscript while MM, ABR and SKK revised the manuscript critically for important intellectual content. MSH made corrections and gave final approval for the submission of revised version. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**
The animal experiment performed in this study has been approved by the Biosafety, Biosecurity and Ethical Committee of Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. Ref. no. BBEC, JU/M2018(1)3.

**Consent for publication**
Not applicable.

**Competing interests**
All authors declared that they have no competing interests.

## Publisher's Note


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