

**Evaluation of analgesic and antioxidant potential  
of the leaves of *Curcuma alismatifolia* Gagnep**

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**ABSTRACT**

In the present study, the antioxidant and analgesic potential of the 80% methanol extract of the leaves of *Curcuma alismatifolia* Gagnep was evaluated. The extract was investigated for its antioxidant activity using lipid peroxidation, total antioxidant capacity and reducing power assays. The extract showed significant antioxidant activities in lipid peroxidation assay compared to the standard antioxidant in a dose dependent manner. In lipid peroxidation assay, the IC<sub>50</sub> value was found to be 122.43µg/mL while the IC<sub>50</sub> value for the reference ascorbic acid was 147.87µg/ml. Moreover, *Curcuma alismatifolia* extract showed strong total antioxidant capacity and good reducing power. The analgesic activity was evaluated for its central and peripheral pharmacological actions using tail immersion method and acetic acid-induced writhing test in mice respectively. The extract, at the dose of 250 and 500 mg/kg, produced a significant ( $p < 0.05-0.001$ ) increase in pain threshold in tail immersion methods in a dose dependent manner. In acetic acid-induced writhing test the extract, at a dose of 500 mg/kg, showed a maximum of 60.5% inhibition ( $p < 0.001$ ) of writhing reaction compared to the reference drug diclofenac-sodium (75.0%). All experimental results suggest the use of this plant for the treatment of pain and inflammatory disorder.

**Key Words:** *Curcuma alismatifolia*, Antioxidant, Lipid peroxidation, Total antioxidant capacity, Reducing power, Analgesic, Tail immersion.

**INTRODUCTION**

Reactive oxygen species (ROS) are greatly reactive molecules, and include the hydroxyl radical (OH), the superoxide anion radical (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the peroxy (ROO), which consequently generate metabolic products that attack lipids in cell membranes or DNA. Lipid peroxidation occurring in cell membranes or DNA which involves a series of free radical chain reaction processes is associated with several types of biological damage, DNA damage, carcinogenesis, and cellular degeneration related to aging. Cells are protected by their endogenous scavenging systems or by other substances (Halliwell et al., 1990). ROS play an important role in the pathogenesis of clinical human diseases including neurodegenerative disorders, cardiovascular diseases, and mutagenesis (Kawanishi, 2001). Currently, the possible toxicity of synthetic antioxidants has been criticized. It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell et al., 1995). The interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakasha and Rao, 2002). Thus recent studies have investigated the potential of plant products to serve as antioxidants to protect against various diseases induced by free radicals. Plant products including phenolics, flavonoids, tannins, proanthocyanidins, and various plant or herbal extracts have been reported to be radical scavengers and inhibitors of lipid peroxidation (Xie et al., 1993; Formica and Regelson, 1995). Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause (Tripathi, 1999). Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency. *Curcuma alismatifolia* is a member of the family Zingiberaceae (ginger). Curcuma is a perennial herb having a fleshy corm with fibrous and fleshy ovoid storage roots. The plant has 6-8 lanceolate leaves with a pseudostem formed from leaf sheaths. The inflorescence is a splendid

compact spike with lotus flower-like bracts, and a long stalk of 60-70 cm. Previous phytochemical screenings on *Curcuma longa*, *Curcuma aromatica* etc. of the same family reveals the presence of curcumin, curcumol, resin, various essential oils including turmerone, alantone, zingiberone and other sesquiterpenes as the major constituents. *Curcuma aromatica* and *Curcuma longa* are applied to bruises and sprains, in snake bite, skin diseases and various inflammatory disorders. But to the best of our knowledge no biological activity of medicinal interest has so far been carried out on *Curcuma alismatifolia*.

As a part of our ongoing investigations on local medicinal plants of Bangladesh (Alam et al., 2008), in this paper, we reported antioxidant and analgesic activity of the leaves of *C. alismatifolia*.

## **MATERIALS AND METHODS**

### ***Chemicals and drugs***

Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India. Trichloroacetic acid and ferric chloride was obtained from Sigma Chemical Co. TBA (thiobarbituric acid) was obtained from Koch-Light Ltd, Suffolk, UK. Potassium ferricyanide from May and Backer, Dagenham, UK. Ammonium molybdate was purchased from Merck, Germany. Diclofenac-Na was collected from Square Pharmaceuticals Ltd., Bangladesh and Nalbuphine was from Incepta Pharmaceuticals Ltd., Bangladesh.

### ***Plant material***

For this present investigation the *C. alismatifolia* was collected from the district Comilla. The plant leaves and flowering top were collected for identification. Expert of Bangladesh National Herbarium Mirpur, Dhaka, identified the plant where the voucher specimen no. 32787 has been deposited. The flowering tops were discarded and the leaves were dried in hot air oven at 55°C for 3 days and at 40°C for the next 4 days.

### ***Extraction***

The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (8:2, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation.

### ***Animal***

For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature:  $24.0 \pm 1.0^\circ$ , relative humidity: 55-65% and 12hrs light/12 hrs dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experimentation (Chatterjee, 1993). All protocols for animal experiment were approved by the institutional animal research ethical committee.

## **PHYTOCHEMICAL SCREENING**

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann- Burchard reagent. Gum was tested using Molish reagent and concentrated sulfuric acid; reducing sugars with Benedict's reagent. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

## **TESTS FOR ANTIOXIDANT ACTIVITY**

### ***Lipid peroxidation assay***

The rat liver microsomal fraction was prepared by the method of Bouchet et al. (1998). The reaction mixture contained, in a final volume of 1.0mL, 500  $\mu$ L of liver microsomal fraction, 300  $\mu$ L buffer containing the plant extract (25–500  $\mu$ g), 100  $\mu$ L of  $\text{FeCl}_3$  (1 mM) and 100  $\mu$ L of vitamin C (1 mM) to start peroxidation. Samples were incubated at 37°C for 1 h before lipid peroxidation was measured using the reaction with thiobarbituric acid (TBA). Thiobarbituric acid reactive substances were determined as described in the literature Aruoma et al., (1989). The absorbance of the

organic layer was measured at 532 nm. All reactions were carried out in duplicate. Ascorbic acid was used as the positive control.

#### **Determination of total antioxidant capacity**

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml extract was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

#### **Reducing power**

The reducing power of *C. alismatifolia* was determined according to the method previously described by Oyaizu (Oyaizu, 1986). Different concentrations of *C. alismatifolia* extract (25 – 500 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 for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and  $FeCl_3$  (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

### **ANALGESIC SCREENING**

#### **Tail immersion test**

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma et al., 2003). The animals were treated as discussed above. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

#### **Acetic Acid-Induced Writhing Test**

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed et al., 2004).

#### **Statistical Analysis**

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. *p* values < 0.05 were considered to be statistically significant compared with the control.

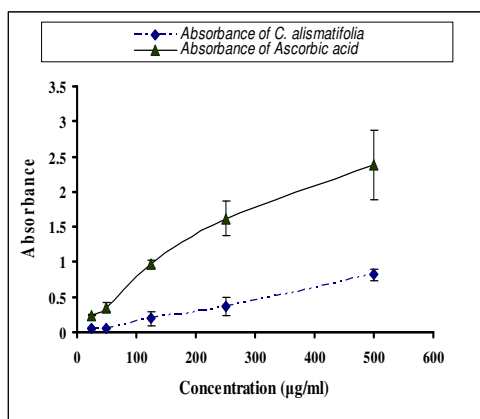
### **RESULTS AND DISCUSSION**

Phytochemical screening of the crude extract revealed the presence of flavonoids, alkaloid and gum, (see Table 1). Polyphenolic compounds, like flavonoids, tannins and phenolic acids commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998; Gil et al., 1999; Kähkönen et al., 1999; Vinson et al., 1995). Flavonoids present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action.

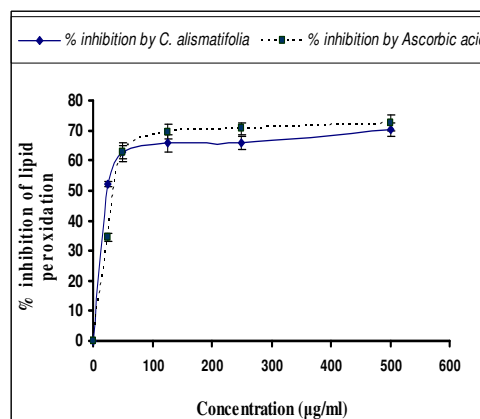
**Table 1: Result of chemical group test of the methanol extract of *C. alismatifolia***

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
ME of <i>C. alismatifolia</i>	-	+++	-	--	+	++	-

ME: Methanol extract; (+): Present; (-): Absent



**Figure 1: Reducing power of the methanol extract of *C. alismatifolia*. Values are the average of duplicate experiments and represented as mean± SD.**

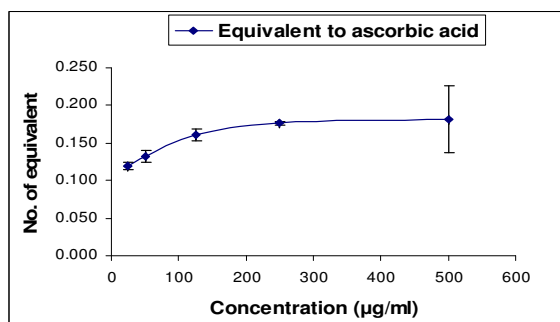


**Figure 2: Lipid peroxidation of the methanol extract of *C. alismatifolia*. Values are the average of duplicate experiments and represented as mean± SD.**

During lipid peroxidation, low molecular weight end products, generally malonaldehyde, are formed by oxidation of polyunsaturated fatty acids that may react with two molecules of thiobarbituric acid to give a pinkish red chromogen. The extract showed inhibition of non-enzymatic lipid peroxidation in rat liver microsomes at all concentrations and the IC<sub>50</sub> value was found to be 122.43µg/mL while the IC<sub>50</sub> value for the reference ascorbic acid was 147.87µg/ml.

A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported (Tanaka et al., 1988). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh et al., 1999; Gordon, 1990). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares et al., 1997).

The tail withdrawal reflex time following administration of the extract of *C. alismatifolia* was found to increase with increasing dose of the sample. The result was statistically significant ( $p < 0.05-0.001$ ) and was comparable to the reference drug Nalbuphine. The result is summarized in the Table- 2. Table- 3 shows the effects of the extract on acetic acid-induced writhing in mice.



**Figure 3: Total antioxidant capacity of the methanol extract of *C. alismatifolia*. Values are the average of duplicate experiments and represented as mean± SD.**

**Table 2: Effects of the methanol extract of *C. alismatifolia* on tail withdrawal reflex of mice induced by tail immersion method.**

Groups	Dose (mg/kg)	Mean reaction time (s) before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Group-I	Vehicle	1.98±0.174	2.44±0.144	2.44±0.219	2.32±0.116	-	-	-
Group-II	10	1.96±0.217	4.28±0.317**	10.50±1.337**	11.53±1.317**	42.9	76.8	79.9
Group-III	250	2.15±0.334	3.62±0.661*	5.67±1.174*	4.93±0.779*	32.6	57.0	52.9
Group-IV	500	2.22±0.190	3.73±0.506*	5.76±1.209*	5.36±0.875*	34.5	57.7	56.7

Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg/kg body weight, Group III and Group IV were treated with 250 and 500 mg/kg body weight (p.o.) of the crude extract of *C. alismatifolia*. Values are mean ±SEM, (n = 5); \*  $p < 0.05$ , \*\*  $p < 0.001$ , Dunnet test as compared to control

The oral administration of both doses of *C. alismatifolia* extract significantly ( $p < 0.001$ ) inhibited writhing response induced by acetic acid in a dose dependent manner. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid (Ahmed et al., 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ronaldo et al., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Vogel and Vogel, 1997).

Preliminary phytochemical screening showed the presence of flavonoids, alkaloid and gum in the plant extract. So, the observed analgesic activity may be attributed to these compounds. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Parke and Sapota, 1996). There are also reports on the role of flavonoid, a powerful antioxidant (Brown and Rice-Evans, 1998; Vinson et al., 1995), in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al., 2001; Rao et al., 1998). Again the plant extract demonstrated good antioxidant action in the tested models. So it can be assumed that Cyclooxygenase (COX) inhibitory activity together with antioxidant activity may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relieve pain-sensation. Tail immersion test are considered to be selective to examine compounds acting through opioid receptor; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain (Elisabetsky et al., 1995; Pal et al., 1999).

**Table 3: Effects of the methanol extract of *C. alismatifolia* on acetic acid-induced writhing in mice.**

Groups	Dose (mg/kg)	No. of Writhing	% Protection
Group-I	Vehicle	40±0.520**	-
Group-II	10	10±0.323**	75.0
Group-III	250	20.6±0.528**	48.5
Group-IV	500	15.8±0.552**	60.5

Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg/kg body weight, Group III and Group IV were treated with 250 and 500 mg/kg body weight (p.o.) of the crude extract of *C. alismatifolia*. Values are mean ±SEM, (n = 5); \*\*  $p < 0.001$ , Dunnet' t test as compared to control.

## CONCLUSION

Based on the results of the present study, it can be concluded that the plant extract possesses remarkable antioxidant and analgesic potential. However, further studies are needed to understand the exact mechanisms of antioxidant and analgesic action and to isolate the compound (s) responsible for such activity.

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