

## **Exploration of In vitro Phytochemical Screening, Cytotoxic, Anti-Arthritic and In vivo Analgesic Activities of Methanolic Extract of the Leaves of a Medicinal Plant**

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### **Abstract**

The plants have power over therapeutic activities or apply beneficial pharmacological outcomes on the human body are usually assigned as “Medicinal Plants”. After screening of leaves of *Leeaequata* under the family of Vitaceae, it has been found positive results for carbohydrates, Flavonoids, Phenols, Tannins, Phytosterols and Saponins which are good markers for further research with this plants. The plant *Leeaequata* has considerable Cytotoxic and Analgesic capacity which are very efficient. After investigation of antiarthritic activity of *Leeaequata* it shows moderate protein inhibition which may bring success of plant research. A method, utilizing brine shrimp is proposed as a simple bioassay for natural product research. In case of my plant *Leeaequata* it show mild cytotoxic effect. The significant pain reduction of *Leeaequata* plant extracts might be due to the presence of analgesic principle acting with the prostaglandin pathways.

**Keywords:** Phytochemical Screening; Cytotoxic; Anti-Arthritic; Analgesic, *Leeaequata*

### **Introduction**

Plant is any one of the vast number of organisms within the biological kingdom Plantae; in general, these species are considered of limited motility and generally manufacture their own food. They include a host of familiar organisms including trees, forbs, shrubs, grasses, vines, ferns, and mosses. Conventionally the term plant implies a taxon with characteristics of multicellularity, cell structure with walls containing cellulose, and organisms capable of photosynthesis [1]. Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important [2]. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties [3]. Basically phytochemical assay of the plant extract should be done for the ensuring the presence of bioactive components of the corresponding plant extract [4]. It is the primary stage of plant of work. After screening of the phytochemicals of any plants, we can assume the next steps what should to do with plant extract [5]. The selected plant is also known as Kakjanga in Bengali. The plant *Leeaequata* is traditionally used in the tribes areas for Astringent, Anthelmintic, Antipyretic, Alexiretic [6]. It is also useful in Bronchitis, Anaesthesia of skin, Dyspepsia, Bil-

ious fever, Leprosy, Itching, Tuberculous Ulcer [7]. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties [8]. The aim of the present project work was find out the In-vitro Phytochemical screening, Cytotoxic, Anti-Arthritis and In-vivo Analgesic activities of the methanolic extract of *Leeaequata*. Leaves of the plant *Leeaequata* were collected from KEPZ Hill tract area, KEPZ, Chittagong, Bangladesh in the month of January 2018.

### **Materials and Methods**

#### **Drugs and Chemicals**

Sea salt non ionized NaCl, Methanol, Distill H<sub>2</sub>O, Formalin, Acetic Acid, DMSO, Diclofenac Na, Bovin Serum Albumin (BSA).

#### **Animals**

Mice weighing about 28-32 gm aged about 1.5 month were purchased from animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. All the animals were acclimatized to new environment for a period of one week. During the experiment period the animals were kept in a well ventilated animal house at 25°C. They were supplied with standard pellets and fresh drinking water. All the mice were kept in cage and maintained with natural 12h light and dark cycle.

### In-vitro Anti-Arthritic Activity of Leeaeaquata [8]

0.05 ml various concentrations (800,400,200,100 µg/ml) of test dugs and standard drug diclofenac sodium (800,400,200,100 µg/ml) were taken respectively and 0.45 ml (0.5% w/v BSA) mixed.

The samples were incubated at 37°C for 20 minutes

The temperature was increased to keep the samples at 57°C for 3 minutes.

After cooling, add 2.5 ml of phosphate buffer to the above solutions.

The absorbance was measured using UV-Visible spectrophotometer at 416 nm.

The control represents 100% protein denaturation. The results were compared with diclofenac sodium. The percentage inhibition of protein denaturation can be calculated.

### In-vivo Analgesic activity[9]

#### Procedure for Acetic Acid Induced Test

Administered extract solution (200 & 400 mg/kg) all the groups of mice orally

Observed the condition of mice till 30minutes

Then 0.6% Acetic acid solution is injected in intraperitoneally

Then counts the number of writhing of first 20 minutes.

#### Procedure for Formalin Induced Paw Licking Test

Administered extract solution (200 & 400 mg/kg) all the groups of mice orally

Observed the condition of mice till 30minutes

2.5% Formalin solution is injected into the mice paw

In early phase, first 5 minute observation & count the no. of licking

Then break for 10 minute

In late phase, last 15 minute observation & count the no. of licking.

### In-vitro Cytotoxic Evaluation [10]

After 24 hours, the vials are observed using a magnifying glass and the number of survival nauplii in each vial were counted and recorded. From this data, the percentage of mortality of nauplii was calculated for each concentration of the sample. The median lethal concentration (LC50) of the test samples was calculated from straight line equation of graph. % of mortality can be calculated by using following equation:

$$\% \text{ Mortality} = \frac{N_o - N_1}{N_o} \times 100$$

Where,  $N_o$  = Number of nauplii taken.  
 $N_1$  = Number of nauplii dead.

### Results and Discussion Phytochemical Screening

The qualitative phytochemical screening was performed to ensure the presence or absence of secondary plant metabolites. In our investigation we have found positive results for carbohydrates, Alkaloids, Phytosterol, Phenol, Flavonoids, Tannins and Saponins. The result showed in (Table 1).

Table 1: Phytochemical Screening of Leeaeaquata.

(+)= Present ,(++)= Rapidly present  
 (-) = Absent

S. no.	Test Name	Observation	
1	Carbohydrate Test	Molish Test	++
		Benedict Test	+
		Fehling A&B	-
2	Alkaloid Test	Mayer Test	+
		Wagners Test	+
3	Glycosides Test	-	
4	Phytosterol Test	+	
5	Phenolic Test	++	
6	Flavoniods Test	++	
7	Protein Test	-	
8	Saponins Test	+	
9	Tannins test	+	

### In-vitro Cytotoxic Activity

Test samples showed different mortality rate at different concentration. The mortality rate of brine shrimp nauplii was found to be increased with the increase with the concentration of the sample. The median lethal concentration (LC50) was calculated. The LC50 values of methanol extract of Leea Aequata was 87.82µg/ml (Table 2). So, it is evident that the methanol extract of Leeaeaquata have high cytotoxic activity.

Table 2: Effect of a.

Conc. (µg/ml)	No. of nauplii taken	No. of nauplii dead	% mortality	LC <sub>50</sub> (µg/ml)
25	10	2	20	
50	10	3	30	
100	10	6	60	87.82(µg/ml)
150	10	8	80	
200	10	10	100	

### In-vitro Anti-arthritic Activity

The plant extract inhibited the protein denaturation by from a lowest 5% when conc. was 125µg/ml. To the highest % of inhibition was 35% when concentration was 1000 µg/ml as compared to standard drug diclofenac Na inhibited by 82% at 1000 µg/ml which is shown in (Table 3).

### Acetic Acid Induced Writhing Test

In acetic acid induced writhing test, the methanol extract of leaves of Leeaeaquata significantly and dose dependently suppressed the frequency of acetic acid-induced writhing in mice after oral administration. At 200 mg/kg body weight, the extract showed 34.26 writhing inhibition, at 400 mg/kg body weight, the extract showed 68.48 writhing inhibition which is shown in (Table 4). So, the plant extract showed analgesic activity at the dose of 400 mg/kg body weight was comparable to the standard drug Diclofenac sodium that inhibited 70.79 writhing at the dose of 10 mg/kg body weight.

Table-3: Anti-arthritic activity of *Leeaeaquata*. In-Vivo Analgesic Activity:

No	Concentration	Test	Test	Product	% of	% of
	µg/ml	Control	Solution	Control	Protein Inhibition	Protein Inhibition
		Abs.	Abs.	Abs.	By Extract	By Diclofenac Na
0.1	125	0.04	0.002	0.053	5%	38%
0.2	250	0.04	0.005	0.078	12.50%	47.50%
0.3	500	0.04	0.009	0.116	22.50%	58.50%
0.4	1000	0.04	0.014	0.192	35%	82%

Table 4: Effect of *Leeaeaquata* extract on acetic acid induced writhing response in mice.

Values are expressed as mean ± S.E.M. (n = 3).

Group	Treatments	Dose, Route	No. of writhing	% inhibition
Control	1% Tween 80 in water	10ml/kg, p.o	59.33±1.84	-----
Standard	Diclofenac Na	10mg/kg, p.o	17.33±0.47	70.79
Test	MELA	200mg/kg, p.o	39±1.73	34.26
	MELA	400mg/kg, p.o	18.66±1.20	68.54

MELA=Methanol extract of *Leeaeaquata*

### Formalin Induced Paw Licking Test

In formalin induced licking test, the methanol extract of *Leeaeaquata* significantly and dose dependently suppressed the frequency of formalin-induced licking in mice after oral administration. At 200 mg/kg body weight, the extract showed 67.51±4.09% licking time inhibition in early phase which is 50.59±2.68% in late phase. At 400 mg/kg body weight, the extract showed 50.74±1.71% licking time inhibition in early phase which 46.56±2.84% the late phase. (Table 5) So, the plant extract showed analgesic activity at the dose of 400 mg/kg body weight was comparable to the standard drug diclofenac sodium that inhibited 73.91±1.05% and 68.22±2.26% early and late phase respectively licking time at the dose of 10 mg/kg body weight.

Table 5: Effect of the methanol extract of *Leeaeaquata* hind paw licking in the mice.

Formalin test in mice. Values are expressed as mean ± S.E.M., (n = 3)

Groups	Treatments	Dose, Route	Early phase(sec)	% inhibition	Late phase(sec)	% inhibition
Control	1% tween 80 in water	10ml/kg, p.o	57.31±1.06		41.74±0.60	
Standard	Diclofenac Na	10mg/kg, i.p	14.95±0.60	73.91±1.05	13.26±0.94	68.22±2.26
	MELA	200mg/kg, p.o	18.62±2.34	67.51±4.09	22.62±1.12	50.59±2.68
Test	MELA	400mg/kg, p.o	28.23±0.98	50.74±1.71	18.13±1.19	46.56±2.84

MELA=Methanol extract of *Leeaeaquata*

### Conclusion

In conclusion, this study evaluated significant phytochemical

screening in-vitro Cytotoxic and Anti-Arthritis evaluation and in vivo Analgesic properties of the leaves of methanolic extract of *Leeaeaquata* at different doses. The plant contains a lot of major groups of organic compounds. The study showed significant result in case of Anti-Arthritic study & Analgesic activity against both acetic acid & formalin induced pain in mice. According to study of the results and discussions of this plant extract it is proved that the plant might be a source of effective herbal drug & pain reducing agents. Although, All pharmacological profile were not performed for a single plant further improvements on such techniques and advancements in the procedure could be acquired through careful and systemic methods and hence requires advance new supplementary approaches. More precise studies are needed to elucidate their mechanism of actions for individual photochemical.

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