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STUDY OF ANTHELMINTIC AND INSECTICIDAL ACTIVITIES OF DIFFERENT EXTRACTS OF *KAEMPFERIA GALANGA*

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ABSTRACT: *Kaempferia galanga* has profound implications in the treatment of nematocide, larvicide, abdominal discomfort, cholera, various inflammatory diseases and many more. It is a potential candidate in terms of evaluating its medicinal activities. Therefore, the present study was conducted to evaluate anthelmintic and insecticidal activities of different extracts of the rhizome of *Kaempferia galanga.* For anthelmintic activity, *Pheretima posthuma* was selected as test animal while 25, 50, 100 mg/ml concentrations of samples were tested in the bioassay, from which time of paralysis and time of death of worms were estimated. Evaluation of insecticidal activity was performed against *Sitophilus oryzae* to calculate the mortality rate. In anthelmintic study, extracts exhibited its activity in dose-dependent manner showing higher the concentration, higher the effect. Extracts of ACR, PEF, CHF and MEF in case of 100 mg/ml concentration exhibited its paralytic effect followed by death within a short period of time among which ACR extract gave the best result which only took approx. 20 mins to show paralytic effect and 35 min for death sentence. In insecticidal activity, all extracts showed potent activity with 100% mortality of rice insects *Sitophilus oryzae* at 80 mg/ml concentration in 24hrs, proving the activity also followed dose dependent pattern. 90% mortality rate observed in case of 80 mg/ml concentration of MEF extract in 12 hr whereas ACR and CHF extracts showed 70 % and PEF showed 50% mortality rate in treated rice insects. The present study shows that all the extracts of *Kaempferia galanga* are found to possess anthelmintic and insecticidal activities.

INTRODUCTION: Due to nutritive value plants can be a better alternative comparing to medicine as they possess number of pharmacological effects rather than side effects. To advance the efficacy and reduce toxicity, plants derived drugs serves as a prototype. Thus, medicinal plants are nowadays seek the attention because of its ability to possess potent antibacterial, anthelmintic and anti-inflammatory activities etc [1, 2].

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Nowadays the occurances of parasitic infections are increasing extensively. Despite of having numerous antibiotics available to encounter infections, antibiotics resistance has limited its use. As a result an alternative option can be phytotherapy to beat nematodes. At the same time, to invade different pathogenic organism's medicinal plants can be a potential choice to defeat infectious diseases.

An imperious medicinal plant entitled as *Kaempferia galanga*, a long-lived aromatic rhizomatous herb is belongs to Zingiberaceae family. The plant *Kaempferia galanga* is locally known as "Chandramalika". It is extensively concentrated in the tropic and sub-tropics especially in Africa and South East Asia.

Having numerous medicinal benefits, the rhizomes of the plant are widely used as an ailment such as carminative, expectorant, aromatic stomachic and diuretics. Moreover, the enrollment of the plant is dominant in the treatment of various skin disorders, diabetes mellitus, several inflammatory and lipid afflictions [3]. Apart from these, diversified mood of use of the plant is observed in different countries, i.e. potential redress for toothache, depuration for dandruff on the head in Bangladesh; counteract for rheumatism, hypertension, dyspepsia, pectoral and abdominal pains in China. It is also appointed to replenish fickleness, atrabilious, torment and anxiety due to its intense fragrance [4].

Some potential chemical constituents were successfully isolated from *Kaempferia galanga* rhizomes when treated with dichloromethane, hexane and methanol including ethyl-p-methylcinnamate (31.77%), methyl-cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%). The rhizome extract of *Kaempferia galanga* also confirmed the presence ofcineol, borneol, 3-carene, camphene, kaempferal, cinnamaldehyde, *p*-methoxycinnamic acid and ethyl cinnamate. Several studies had exposed that a potential constituent, named ethyl-p-methoxycinnamate liable to possess assorted pharmacological responses like anti-neoplastic. antimicrobial, mosquito repellent and nematicidal action. Another isolated vital compound possesses significant vesorelaxant activity [5].

Previous studies revealed that *Kaempferia galanga* leaf and rhizome extract; treated with acetone was found to be effective for sedative activity and its fraction showed antinociceptive property and again acetone and petroether fractions were prove to be operative in case of cytotoxic and antimicrobial activity. Thus, as a part of our continuing studies [3, 6, 7], we focused on the investigation of anthelmintic and insecticidal activities of different rhizome extract of *Kaempferia galanga.*

MATERIALS AND METHODS:

Collection of the plant: On December, 2011; the plant *Kaempferia galanga* was procured from Mauoa, the local area of Dhaka. All types of unwanted materials like dust, dirt, polen and residual were segregated cautiously. Then the plant was judged by Bushra Khan, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen has been deposited (DACB:36,064) for further reference.

Extraction and fractionation of the plant material: The plant parts were extracted by a cold extraction method. The rhizome (900 g) was taken and soaked with 2700 ml of acetone for 3 consecutive days at 25ºC. The extract was filtered and evaporated on rotary evaporator under reduced pressure. Recovered solvent was again used for percolation for another 3 days. The process was repeated three times to obtain 58 g rhizome (yield 6.45%) extract of *Kaempferia galanga*. The rhizome extract was further partitioned using petroether, chloroform and methanol. The acetone extract of rhizome (ACR), as well as petroleum ether fraction (PEF), chloroform fraction (CHF) and methanol fraction (MEF) were examined for anthelmintic and insecticidal activity.

Drugs and chemicals: Albendazole was assorted from ACME Pharmaceuticals Ltd., Bangladesh; 0.9% sodium chloride solution (Normal saline) was purchased from Orion Infusion Ltd., Bangladesh and other reagents were of analytical grade.

Earthworms and Insects: Since the close anatomical and physiological resemblance to the human intestinal round worm parasite, *Pheretima posthuma (P. posthuma)*, adult earthworm were chosen to evaluate in vitro anthelmintic activity. Adult earthworms were collected easily from the moist soil of Mohakhali area of Dhaka, Bangladesh and for the purpose of eliminating all fecal matters normal saline water was used. According to all experimental protocol, the earthworms of 3-5 cm in lengths and 0.1-0.2 cm in width were selected for the evaluation of anthelmintic activity. *Sitophilus oryzae (S. oryzae)*, commonly known as rice weevil because of its habitat, was collected from old rice in local rice godown Bangladesh for detecting the insecticidal activity of *Kaempferia galanga*.

Anthelmintic activity: The anthelmintic activity was evaluated using adult Bangladeshi earthworm namely *Pheretima posthuma* having close anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Test samples of extract were prepared at 25, 50 and 100mg/ml concentration in normal saline water and

approximately equal size of six earthworms *(Pheretima posthuma)* were placed in each beaker containing 50ml of above test solutions of extract. Albendazole (10 mg/ml) was used as a reference standard and normal saline water as control. All the test and reference solutions were freshly prepared before initiating the experiment. The time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously [8]. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50ºC).

Insecticidal activity: To initiate the surface film activity test 60 mm petri dishes were taken for the extract and their replication. The sample solutions were prepared (2.5, 5, 10, 20, 40, 50, 60, 70, 80 mg/ml) by dissolving the extract into respective solvent. After that they were poured into the lower part of petri dish and allowed them to dry out. Then insects were released in each of the treated petri dish. A control experiment applying only the solvent into the petri dish was also set at the same environment. After performing the above steps, at room temperature treated petri dishes were placed in a secured place. The whole experiment was checked from time to time and mortality was observed first at 30 minutes and then after 12, 24 and 48 hrs of exposure and the data was recorded. A simple microscope was used to verify the movement of the insects to ascertain their death. Sometimes hot needle was taken closer to the bodies (without movement) to confirm death. Recovery of the insects if occurred should also be taken into consideration. The mortality rates of *Sitophilus oryzae*adults were corrected by the Abbott's formula [9].

$$\% \text{ of Mortality} = [C_a - T_a / C_a] \times 100$$

Where,
C_a = No. of live control insects after treatment
T_a = No. of live test insects after treatment

Statistical Analysis: All assays were performed in triplicate under strict aseptic conditions to ensure consistency of all findings. Data of all experiments were statistically analyzed and expressed as the mean ± SEM of three replicate experiments.

RESULTS AND DISCUSSION:

Anthelmintic activity: Despite of tremendous advancement in the sector of human medicine still infectious diseases caused by pathogens like bacteria, viruses, parasites, fungi, insects, rodents, helminthes are becoming an alarming threat to public health. Particularly, the developing countries are at risk for their survival, especially children are more vulnerable to infectious diseases. Due to the occurrence of relative anthelmintic drug resistance, the natural anthelmintic are gaining the demand to be the potential substitute for diseases management [10, 11]. In the present study, we analyzed anthelmintic activity of the plant extract of KG using adult Bangladeshi earthworms because of its easy accessibility and structural similarity with human intestinal roundworm parasites. When the earthworms (*Pheretima posthuma*) were being exposed to the *Kampferia galanga* crude extract, they started to loss their motility by giving stimulant effect initially.

Eventually, dose-dependent paralysis followed by death occur in each crude extract containing 25, 50 and 100mg/ml. **Table 1** represents the effects of *Kaempferia galanga* possessed anthelmintic activity in dose-dependent manner describing higher concentration of each crude extract exhibited paralytic effect much earlier and results in death within short period of time. As the **table 1** reflects it can be said that at 25mg/ml concentration in all extracts (ACR, PEF, CHF and MEF) showed paralytic effect approximately in 48 min and took more than 80 min for death sentence. However, for 50 and 100mg/ml concentration, within a very short time each extract successfully produced paralytic effect followed by death. The reference drug albendazole also showed strong anthelmintic action. As a whole, different extracts of *Kaempferia galanga* showed anthelmintic activity in a dose- dependent manner [11].

Previous phytochemical screening reported different extracts of *Kaempferia galanga* contain carbohydrates, tannins, flavonoids, proteins, steroids, alkaloids and resins [6]. Tannins which are polyphenolic compounds were known to have anthelmintic activities.

Reported anthelmintic effect of tannins is due to binding of tannins to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may be responsible for death [12]. Also some flavonoids and other secondary metabolites known to have anthelmintic activities but exact mechanisms are not clearly established [13]. It is possible that tannins, flavonoids, glycosides present in the rhizome extracts of *Kaempferia galanga* produced similar anthelmintic effects.

TABLE 1: EFFECT OF DIFFERENT EXTRACTS OF *KAEMPFERIA GALANGA* ON *PHERETIMA POSTHUMA*

Group	Dose	No. of worms	Time taken for paralysis	Time taken for death
Control	20 ml	6	--------	------
Standard	10	6	26±2	35±3
ACR	25	6	48±2	80±3
	50	6	40±3	60±3
	100	6	20±4	35±4
PEF	25	6	45±5	55±5
	50	6	33±2	46±3
	100	6	22±3	30±2
CHF	25	6	44±4	60±3
	50	6	35±2	55±2
	100	6	27±3	40±2
	25	6	55±3	70±4
MEF	50	6	30±4	50±9
	100	6	25± 3	43± 2

Control group received water 20 ml, standard group received Albendazole10 mg/ml, test groups ACR, PEF, CHF and MEF were treated with 25, 50 and 100 mg/ml of the extracts respectively. **ACR** = Acetone extract of rhizome, **PEF** = Petroleum ether fraction of rhizome, **CHF**=Chloroform fraction of rhizome, **MEF**=Methanol fraction of rhizome

Insecticidal activity: Resistance developed by insects is one of the greatest hinderance in case of using insecticides to fight against diseases due to its fewer efficacies. Hence, as a potent substitute plants are proved to be fruitful insecticides as they contain several bioactive chemicals [14]. The present study conducted on insecticidal activity against rice induced insect named *S. oryzae* by using different extracts of rhizome of *Kaempferia galanga*. The test samples were evaluated in different concentrations (2.5, 5, 10, 20, 40, 50, 60, 70 and 80 mg/ml) for 12 and 24hr. Among all the extracts, at the concentrations of 80mg/ml MEF showed the highest mortality (90%) in 12hr whereas ACR and CHF showed similar mortality rate (70%). Futhermore, PEF showed the lowest result (50%). However, at the concentration of 80 mg/ml in all the extracts 100% mortality was found in 24hr. Potent insecticidal activity of rhizome extracts of *Kaempferia galanga* were confirmed because of the traditional claims imposed on this herb. The mortality of the insect was found to be concentration dependent. It is observed that the carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins are having mosquito larvicidal activity [15]. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever [16]. Probably due to the presence of tannins, flavonoids, terpenoids and other secondary metabolites of plant may describe the toxic effects on the studied insects.

TABLE 2: INSECTICIDAL ACTIVITY OF *KAEMPFERIA GALANGA* ON *S. ORYZAE*

Sample Conc mg/ml	No. of insect taken	No. of insect dead								% of mortality							
		ACR		PEF		CHF		MEF		ACR		PEF		CHF		MEF	
		12hr	24hr	12hr	24hr	12hr	24hr	12hr	24hr	12hr	24hr	12hr	24hr	12hr	24hr	12hr	24hr
2.5	10	0	2	0	3	1	4	1	4	0	20	0	30	10	40	10	40
5	10	2	4	2	3	2	5	2	4	20	40	20	30	20	50	20	40

10	10	2	5	2	4	2	5	2	4	20	50	20	40	20	50	20	40
20	10	2	5	2	4	2	5	2	5	20	50	20	40	20	50	20	50
40	10	2	5	2	5	2	7	2	5	20	50	20	50	20	70	20	50
50	10	3	7	2	5	3	7	3	7	30	70	20	50	30	70	30	70
60	10	3	7	5	6	5	9	3	7	30	70	50	60	50	90	30	70
70	10	5	8	5	7	5	9	5	9	50	80	50	70	50	90	50	90
80	10	7	10	5	10	7	10	9	10	70	100	50	100	70	100	90	100

ACR = Acetone extract of rhizome, **PEF** = Petroleum ether fraction of rhizome, **CHF**=Chloroform fraction of rhizome, **MEF**=Methanol fraction of rhizome.

CONCLUSION: Researches on various plant extracts have been proved to be effective for diversified medicinal applications. The present study successfully established the anthelmintic and insecticidal properties of KG extract. As a result, the observed biological activities can play a great role for further investigation of certain activities of different plant extracts to signify the research on medicinal plants.

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