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Antimicrobial and insecticidal activities of *Alpinia calcarata* rhizome

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Abstract
To explore antimicrobial and insecticidal effects of ethanolic extract of *Alpinia calcarata* rhizome and its different fractions. Antibacterial activity test was carried out by disc diffusion method against six different pathogenic bacteria whereas insecticidal activity was evaluated by surface film activity test against *Tribolium castaneum* (Herbst) insect. At a concentration of 800 µg/disc, the zones of inhibition produced by the crude ethanol extract and its petroleum ether and residual fractions were ranged from 6-13 mm, whereas water fraction showed no antibacterial activity. The crude ethanol extract and water soluble fraction showed a prominent insecticidal activity against *Tribolium castaneum* adults after 48 h of exposure with the LD_{50} values of 0.709 and 0.633 mg/cm^2, respectively. The residual and petroleum ether fraction did not exhibit any toxicity to *Tribolium castaneum*. It can be concluded that the ethanol extract of *Alpinia calcarata* is a potent resource to develop antimicrobial and insecticidal drugs.

Keywords: *Alpinia calcarata*, Antibacterial, Antimicrobial, Insecticidal activity, pathogenic bacteria, Disc diffusion method

1. Introduction
Despite the advancement in the production of a number of new antibiotics in the last few decades, infectious diseases and drug resistance remain a growing problem. Moreover, the toxic effect is limiting the use of synthetic antimicrobial drugs. Therefore, a great attention has been achieved in research on developing new effective antimicrobial drug from non-toxic and natural sources [1-2].

For many thousands of years, plants have been playing an important role in maintaining human health. According to WHO, about 80% of individuals from developed countries use traditional medicine derived from plants and these medicinal plants would be the best source to formulate a variety of drugs. The antimicrobial activity of various plant extracts have been reported by a number of researchers throughout the world [3]. *Tribolium castaneum* (Herbst) is a major insect in Bangladesh which is abundantly found in stored grain of different cereals. To control these insects synthetic insecticides and fumigants are used which lead to serious problems such as disturbances of the environment, increasing costs of application, pest resurgence, resistance to pesticides, lethal effects on non-target organism and direct toxicity to users [4]. Therefore, actions must be taken to develop a new pesticide of biological origin.

Alpinia calcarata Roscoe (Family: Zingiberaceae) is widely distributed in Srilanka, India, and Malaysia and traditionally used in medicinal systems in Sri Lanka [5]. Rhizome, the most important part of this plant, is used to treat cough, respiratory ailments, bronchitis asthma, arthritis and diabetes [6-9]. A Several studies have been conducted with ethanolic extract of this part and found to have antifungal, antihelminthic, anti-inflammatory, antinociceptive, aphrodisiac, gastroprotective, and antidiabetic activities [10-17]. Previously our group has reported the cytotoxic properties of ethanolic extract of *Alpinia calcarata* rhizome against Ehrlich ascites carcinoma (EAC) tumor bearing Swiss Albino mice [18]. Very limited number of research has reported the antibacterial activity of this rhizome extract while the insecticidal activity is yet to be investigated. Therefore, the present study was conducted to explore the antibacterial and insecticidal activity of ethanolic extract of *Alpinia calcarata* rhizome.

2. Materials and methods
2.1 Plant materials
Rhizome of *Alpinia calcarata* Rosc (Family: Zingiberaceae) were collected from Rajshahi University campus, Rajshahi, Bangladesh, in December, 2015 and were identified by a taxonomistat the Department of Botany, University of Rajshahi, where a voucher specimen (No. 2) of this collection was deposited.

2.2 Extraction and fractionation
Fresh rhizomes of *A. calcarata* were sun dried for 7 days and finally autoclaved in an electric oven below 40^0C for 23 hours. The dried powdered plant materials (800 g) of *A. calcarata* were extracted in room temperature with ethanol (4.0 L) in an aspirator bottle for a week and then filtered. The filtrate was then concentrated by using a rotary evaporator at 45^0C under reduced pressure. The crude ethanol extract (15.0 g) was fractionated by solvent-solvent partitioning with petroleum ether (40-60)^{0}C and distilled water, yielding petroleum ether fraction (11.97 gm) and water soluble fraction (6.17 gm) and residual semisolid fraction (6.26 gm).

2.3 Antimicrobial activity
The crude ethanol extract and their fractions were tested for their antibacterial activity against three Gram positive and three Gram negative bacteria by disc diffusion method [19]. Six pathogenic bacteria [*Bacillus cereus* QL 29, *Bacillus megaterium* QL 38, *Bacillus subtilis* QL 40, *Escherichia coli* ATCC 25922, *Salmonella typhi* AM 16406, *Shigella dysenteriae* ATCC 26131, were collected as pure culture from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample solution of the plant materials (extract and fractions) were prepared by dissolving

definite amounts of materials in appropriate solvent to attain the desired concentration and then applied to sterile disc (5 mm diameter, filter paper) followed by drying off the solvent in an aseptic hood. To compare the activity with standard antibiotics, Amphicillin discs (30 µg/disc) and blank discs impregnated with the respective solvents were used as positive control and negative control, respectively.

2.4 Collection of test insect

For insecticidal screening the insect *Tribolium castaneum* (Herbst) used in the experiment was provided from the stock cultures of the Crop Protection and Toxicology Laboratory, University of Rajshahi, Bangladesh.

2.5 Insecticidal screening

For the conduction of surface film activity test of the plant extract, 60 mm petridishes were taken. The plant extract (40 mg) was dissolved into 1 ml methanol. This was poured into the lower part of the Petridish. A control experiment applying only the solvent into the Petridish was also set at the same time under the same conditions [20]. After completing all the arrangements, treated petridishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed first after 12 hrs, 24 hrs, 36 hrs and finally after 48 hrs of exposure and data were recorded. A simple microscope was used to observe each and every beetle by tracing natural movements of each organism. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recover the insects if occurred.

3. Results

The results of antibacterial activities of crude ethanol extract and its petroleum ether and water, residual fractions of the rhizome of *A. calcarata* at doses of 400 µg/ disc and 800 µg/ disc respectively were presented in Table 1. The zones of inhibition produced by the crude ethanol extract and its petroleum ether and residual fractions were ranged from 6-13 mm, depending on dose, whereas water fraction showed no antibacterial activity.

The results of insecticidal activities of crude ethanol extract and its petroleum ether and water, residual fractions of *A. calcarata* rhizomes has been studied by testing it against the insect, *Tribolium castaneum* (Herbst) and the results are represented in Table 2. The water soluble fraction showed promising activity against *Tribolium castaneum* adults to offer LD_{50} values 1.178, 1.002, 0.745, and 0.633 mg/cm^2 after 12, 24, 36 and 48 h of exposure, respectively. The crude ethanol extract showed mortality against *Tribolium castaneum* adults where LD_{50} values were 1.806, 1.084, 0.821, and 0.709 mg/cm^2 after 12, 24, 36 and 48 h of exposure respectively. The residual and petroleum ether fraction did not exhibit any toxicity to *Tribolium castaneum*. It was found that the mortality rate of the *Tribolium castaneum* adults increased with the increase of the concentration of the sample and the increase of the exposure time as well.

Table 1: *In vitro* antimicrobial activity of crud ethanol extract and its different fractions

Test bacteria	Zone of inhibition (Diameter in mm)								
	Crude ethanol extract		petroleum ether fraction		water fraction		Residual fraction		Ampicillin
	400µg/ disc	800µg/disc	400µg/ disc	800µg/disc	400µg/disc	800µg/disc	400µg/disc	800µg/disc	30µg/disc
	Gram positive bacteria								
Bacillus cereus	7	8	-	8	-	-	7	13	28
Bacillus megaterium	6	8	-	6	-	-	7	10	24
Bacillus subtilis	-	6	-	6	-	-	-	7	28
	Gram negative bacteria								
Escherichia coli	6	8	-	-	-	-	7	11	31
Shigelladysenteriae	-	9	8	8	-	-	7	9	29
Salmonella typhi	-	7	7	9	-	-	7	9	28

Table 2: Dose mortality effect of crud ethanol extract and its different fractions against *T. castaneum* adults

Plant materials	Exposure time	LD_{50} (µg/cm^2)
Crud ethanol	12 hrs	1.806
	24 hrs	1.084
	36 hrs	0.821
	48 hrs	0.709
Water soluble fraction	12 hrs	1.178
	24 hrs	1.002
	36 hrs	0.745
	48 hrs	0.633

4. Discussion

Various medicinal plants used in the traditional medicine have attracted the attention of scientists due to their diverse biological activity. Plants secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids have been found to have antimicrobial activity and can be a safe alternatives to synthetic drugs to which many infectious microorganisms have become resistant [4]. The present study investigated the antibacterial activity of EEACR and found strong activity against species such as *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli*, *Shigella dysenteriae and Salmonella typhi*. This antibacterial activity could be attributed to different bioactive compounds present in the different fractionates. GC-MS analysis of *Alpinia calcarata* rhizome revealed the presence of different bioactive compounds such as 2-octanone, camphene, 1, 8-cineole, 2 hexanone and the antibacterial activity could be attributed to these compounds [21]. Since some active components can only be extracted by polar compounds, while some by less polar and yet some by non-polar compounds, therefore, different biological activities of plant extract largely depends on the type of solvent used in the extraction procedure. In the present study, residual fraction showed the highest

antibacterial activity which is probably due to the presence of active components in large amounts. Killing larvae of *Tribolium castaneum* heavily depends on the use of synthetic chemical insecticides whose repeated use creates environmental problems and develop drug resistance. Bioactive compounds present in the extract may offer an alternative source of insect-control agents with little or no harmful effect on non-target organisms and environment [4]. Different fractions of EEACR showed different toxic effects on *Tribolium castaneum*.

5. Conclusion

Crude ethanol extract and its petroleum ether and residual fractions of the rhizome showed a wide range of antimicrobial and insecticidal activities. Thus, the rhizome of this plant can be used for the remedy of infectious diseases caused by pathogenic bacteria as well as for the control of insect.

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7. References

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