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Determination of phytochemical and anthelmintic activity of rhizome of *Zingiber zerumbet*

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ABSTRACT: Background: Medicinal plants have many creative properties due to the presence of many complex chemical substances with different chemical composition which are found as secondary plant metabolites in many parts of these plants. So one of the medicinal plant Zingiber zerumbet is one of the important medicinal plants which show many pharmacologically as well as therapeutically effective for the different purposes for the human beings. **Aim:** The aim of the study is to extract Z. zerumbet powdered rhizomes, to carry out the phytochemical screening and to evaluate the anthelmintic activity. Methods: The rhizome of Z. zerumbet was extracted by Percolation method using the solvents hydroalcoholic and water. The anthelmintic activity of extract of Z. zerumbet was evaluated on the adult Indian earthworm Pheritima posthuma at different doses of 10, 20, 30, 40 and 50 mg/ml. The Albendazole was used as standard drug. The paralysis and death time of *P. postuma* were determined. **Results:** The phytochemial screening showed that the *Z*. zerumbet rhizome extract is containing phyto-constituents that are Glycosides, Carbohydrate, Phenolic compounds, Flavonoids and Saponins. The Z. zerumbet extract at all the doses exhibited the anthelmintic activity in dose dependent manner. The anthelmintic activity was well comparable with standard drug Albendazole. The water extract of Z. zerumbet rhizome showed greater activity than Albendazole. **Conclusion:** It could be concluded that the rhizome of Z. zerumbet exhibited significant anthelmintic study and research has to be progress to identify the chemical which is responsible for anthelmintic activity.

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INTRODUCTIONS:

Medicinal plants always have been the principal form of medicine in India. Medicinal plants have therapeutic property due to the presence of various complex chemical substances of the different composition which are found as the secondary plant metabolites is one or more parts of these plants^[1].

Zingiber zerumbet (Zingiberaceae) is also a very pharmacologically important plant. It is smooth, erect,

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herbaceous plant. This genus of plant is confined to the tropics of Asia, Malaysia, and the Pacific Islands ^[1,2]. The root stocks are tuberous and pale yellow. The leaf stem is 0.6 to 2.0 m high. These leafs are numerous and long narrow types. The flowering stem which directly grows from the root stock in late summer resembles pine cones ^[2,3]. This plant is found to contain many flavonoid and alkaloid. Some of the major chemicals isolated from Camphene, this plant are as camphor and monoterpenoids as gingerol, zingeberol, zingerone, sesquiterpenenoids zerumbone, zerumbone epoxide, oxalic acid, kaempferol derivative terpine and humulene ^[4]. It also contain many flavonoids like Afzelin 1,3, flavonoid glycosides, essential oils, chlorgenic acid and ferulic acid. The rhizome part possesses stimulating action, anti-hyper tensive action ^[1], carminative, flavouring ^[4]. It is also used to treat dyspepsia wound treatment, for hemorrhoids and flatulent colic for the cure of the stomach trouble and fever ^[1]. It is used for the treatment for leprosy^[4], peptic ulcer, mouth infection^[1], asthma^[4], rheumatism^[4], anticancer, anti-tumor effects ^[6], anti inflammatory effects ^[8], anti microbial ^[9], antifungal activity^[9], anti-bacterial^[8], anti-hyperglycemic activities ^[1]. Thus the aim of the study to evaluate the Z. zerumbet rhizome for anthelmintic activity.

MATERIALS AND METHODS:

The drug Albendazole was procured as gift sample from Bandy from Mankind company, Mumbai. The Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.



Fig 1. The Zingiber zerumbet plant.

Plants collection, Identification and processing:

The plant was collected from the Botanical garden of The Pharmaceutical College, Barpali, in the month of September 2017. The plant was identified by Prof. (Dr.) S.K. Dash, Retired Professor and H.O.D., PG Dept. of Biosciences, C.P.S., Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust, and then it was dried in sun light for 1 h and kept in shade dried. The dried plant was cut in to chips and powdered by means of wood grinder. The dried form of rhizome was stored in air tight container for further study.



Fig 2. The rhizome of the plant Zingiber zerumbet.

Preparation of extracts: *Preparation of hydroalcoholic extracts:*

The rhizome of *Z. zerumbet* powdered was extracted by using hydro-alcoholic and water solvents in the ratio 30: 70 (water: ethanol) respectively. The shade dried course powder of rhizome (75 g) was packed well in a percolator for 5 days in cold extraction method. After 5 days the extract was filtered and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccator to further experiment. The obtained extract was weighed and the percentage yield was calculated in terms of air-dried powdered crude material.

Preparation of water extract:

The aqueous extracts were prepared by dissolving 100 g of powdered plant material in 500 ml of distilled water in a glass percolator. It was allowed to macerate for 24 h at room temperature and the brew was filtered using Whatman number one filter paper. The process of percolation was repeated three times (500 ml). The combined filtrate was then concentrated in a water bath

to ensure the complete evaporation of the solvent. The final crude aqueous extract was transferred to a vial and kept air tight.

Qualitative phytochemical analysis:

Qualitative phytochemical studies of extract will be studied for the presence of different secondary metabolites responsible for the therapeutic values as per the standard procedures mentioned in Pharmacopoieae ^[9,10]. The solvent free extract of about 50 mg was stirred with few ml of dilute hydrochloric acid filtered. The filtrate was tested carefully for alkaloids (Mayer's, Wagner's, Hager's Dragendorff's and test), Carbohydrates and Glycosides (Molish's, Fehling, Barfoed and Benedicts test), Glycosides (Borntrager's and Legal's test), Saponins (Foam test), Proteins and Amino acids (Millon's, Biuret and Ninhydrine test), Phytosterols (Libermann-Burchard's test) and Phenolic and Flavonoids Compounds (Ferric chloride and Alkaline reagent test).

Determination of biological (Anthelmintic) activity:

The Anthelmintic activity was performed on adult Indian earthworm, *Pheretima posthuma* due too it's anatomical and physiological resemblance with human intestinal roundworm parasite ^{[11-14].} Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic activity ^[15-17]. Indian adult earthworms (*P. posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 3 to 5 cm in length and 0.1 to 0.2 cm in width were used for all the experimental protocol.

Earthworms were divided into 7 groups and each group consisting of 6 earthworms and were released into 20 ml of the desired formulation. Group I served as control and received only normal saline water, Group II served as standard and received standard drug Albendazole (10 mg/ml), Group III to VII, served as tests and recieved the *Z. zerumbet* rhizome (Water and hydro-alcoholic) extract of different doses of 10 to 50 mg/ml. The observations were done for the time taken for the paralysis and death of individual worms.

Paralysis was said to be occurred when there no movement of any sort could be observed except that the worms were shaking vigorously. Death was said to be occurred when the worms lost their motility followed with fading of their body color.

Statistical study:

All data thus obtained in study were verified by using statistical analysis like mean, standard deviation and standard error of mean ^[18].

RESULTS AND DISCUSSION:

The percentage yield of air-dried powdered crude material was found to be 1.03 %.

The finding of phytochemical detection was shown in Table 1. Qualitative Phytochemical analysis reports for presence of phytoconstituents in *Z. zerumbet* are Glycosides, Carbohydrate, Phenolic compounds, Flavonoids and Saponins.

The different extracts exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration. The Hydro-alcohlic extract of Z. zerumbet caused paralysis of 12.07 min and time of death of 12.05 min while water extract revealed paralysis of 21.25 min and death of 31.34 min against the earthworm P. posthuma. The reference drug Albendazole showed paralysis and death time of 10.15 and 12.25 min respectively. The anthelmintic activity was well comparable with standard drug Albendazole. The water extract of Z. zerumbet rhizome showed greater activity than Albendazole. The extent of activity shown by the extracts was found to be nearly equal than that of the standard drug Albendazole, which justifies its activity. The predominant effect of Albendazole on worm is to cause a flaccid paralysis which results in expulsion of the worm by peristalsis. The crude extracts of Z. zerumbet not only demonstrated paralysis but also caused death of worms especially at higher concentration of 100 mg/ml.

rnizome extract.			
Plant constituents	Hydro-alcohlic Extracts		
Alkaloids	-		
Saponins	+		
Glycosides	+		
Carbohydrates	+		
Phenolic Compound	+		
Phytosterols	-		
Flavonoids	+		
Steroids	-		
Proteins and Amino	-		
acids			

 Table 1. Phytochemical evaluation data of Z. zerumbet

 rhizome extract.

(-) – Absent and (+) – Present.

The results of the present study indicate that the hydroalcoholic extract of *Zingiber zerumbet* rhizome has significant anthelmintic activity property. The presence of various phytoconstituents may be responsible for exhibiting anthelmintic activity.

Drug	Dose	Paralysis	Death
	(mg/ml)	time (min)	time (min)
		(X±S.D.)	(X±S.D.)
WE	10	45±1.7	80.15±0.4
	20	41±0.56	65±0.17
	30	36±0.41	52.9±0.5
	40	26±0.12	45.5±0.54
	50	21.25±0.81	31.34±0.02
HAE	10	12.13±1.7	12.19±0.67
	20	12.10±0.56	12.15±0.96
	30	12.08±0.41	12.12±0.67
	40	12.06±0.12	12.10±0.87
	50	12.05 ± 0.88	12.07±0.54
NSW		231	331
ALB	10	10.15 ± 1.16	12.25±0.88

 Table 2. Anthelmintic activity of Z. zerumbet rhizome extract.

WE and HAE– Water and hydroalcoholic extract. NSW – Normal saline water. ALB – Albendazole. All data are represented as mean \pm standard deviation (n = 6).

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