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PRELIMINARY ANALYSIS OF PHYTOCONSTITUENTS AND EVALUATION OF IN-VITRO ANTHELMINTIC, ANTI MICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF ZINGIBER ZERUMBET

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ABSTRACT

The total objective of our study must be to identify drugs to estimate human illness by a thorough analysis of plant Ayurveda and modern medicine techniques must be coupled in order to bring out high quality of herbal product with rapid onset of action and good bioavailability. **Aim**: The aim of the study is to investigate Phytochemical screening & Anthelmintic activity of Ethanolic Extract of *Zingiber zerumbet*. **Result & Conclusion**: The percentage yield of Ethanol and Aqueous extract were found to 7.88% and 4.52%w/w . The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, glycosides, saponins, protein, amino acids, flavonoids like compounds but do not shows the presence of phytosterol. The Powder analysis and fluorescence was observed in ultrashort, ultra long and visible. The results depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations with respect to drug Albendazole. The data revealed that the aqueous extract has a better wormicidal effect than Ethanolic extract with compared to the standard drug Albendazole.

KEYWORD: *Zingiber zerumbet*, Traditional use, Phytochemical screening, Albendazole, Anthelmintic activity.

INTRODUCTION

Herbal medicine refers to plant-based substances with nutritive, curative, or preventive properties. It is an interdisciplinary branch of herbal medicine, encompassing fields like botany, medicinal plant research, pharmacognosy, phytochemistry, phytotherapy, botanical medicines, Ayurveda, natural chemistry, agriculture science, Unani medicine, biotechnology, and biochemistry. An herbalist is someone who deals with herbs, especially medicinal herbs, and is known for their expertise. Herbal medicines are substances containing parts of plants or other plant materials as active ingredients, used for disease prevention and treatment, including herbs, herbal materials, preparations, and finished products.[1] Medicinal plants have been the primary form of medicine in India due to their therapeutic properties, which are derived from the presence of complex chemical substances found in secondary plant metabolites.[2] Herbal medicine is increasingit's popularity due to its potential to cause adverse events like cardio-, neuro-, nephro-toxicities, and cancers. The severity of these toxicity depends on the type of herb, preparation, and user, ranging from minor to severe and sometimes fatal. Generally herbal medicine is used for the treatment, mitigation, and prevention of diseases, particularly those endemic to the local environment of herbs.[3-5]

Plant Profile

This *Zingiber zerumbet* is better known as wild ginger or bitter ginger basically belongs to Zingiberaceae family which is most commonly found in some Asian countries like India, Indonesia, Malaysia, Bangladesh, Nepal, Sri Lanka, Thailand, etc. This plant was previously introduced by early Polynesians but they are native to the Asia (India) . All the parts of the pine cone ginger were spicy and fragrant. This wild ginger grows besting the lower part of the damp, open forest and can form continuous ground lower. It needs worm or wet and shade darkish region on wind words sides of islands where rain fall is plentiful.



Growing Plant *Z. zerumbet* Rhizome of *Z.zerumbet* Flower of *Z. zerumbet*

The stem is about 1.2 m tall in the upright position having oblique and round shape. It is smooth, erect, herbaceous plant .The root stocks are tuberous and pale yellow within. The leaf stem is 0.6 -2.0 m high .The leafs are numerous and long narrow types that they are distichous, lanceolate to oblong lanceolate, 15-30 cm long, 5-8 cm wide and leaves are oppositely arranged. The flowering heads are reddish green in color with 3-10cm long. Sometimes it is seen that yellowish-white color flower comes out from the fruit. The flowering stem which directly grows from the root stock in late summer resembles pine cones. The green cone turns red over a couple of weeks and then small creamy flowers appear on the cone. The inflorescences are spike, ovoid and ellipsoid in shape with greenish color, when it is young but it turns into red color when it becomes old with 6-12cm long.[2] The flower stems clothed with long ,appressed, obtuse sheath bearing at its apex an ovoid to oblong or cylindrical green or red spike 5-20 cm long The bract are numerous, imbricate or bicular, 2-3 cm long greenish merging in to red and holding water. The fruits are smooth, white and thin walled. The fruit is oblong red color and about 2.5 cm in length .The seeds are oblong and 4 mm in diameter. Typically this plant is a multi trunk or clumping stem. The rhizomes are thick, yellowish in color and are everlasting in nature. The stem is about 1.2 m tall in the upright position having oblique and round shape.[2,3]

Different names of the *Zingiber zerumbet*[2,6,7]

This *Zingiber zerumbet* plant has different names in the different countries whose synonym is *Amomum zerumbet* (L).The trivial names of the plant in different countries are as follow.

Arabic	Zerunbah
English	Wild ginger
French	Amome sauvage
German	Wilder ginger. Zerumbet
Italians	Zenzerobianco, Zenzero salvatico
Portuguese	Gengibre amarg
Spanish	Jengibre, Jengibre amargo
Turkish	Yabani zencefil, Zerunbad, Zerunbat, Zernebat

Some vernacular names as follows[2,6,7]

Bengali	Narkachur
English	Wild ginger
Hindi	Narkachur
Malayalam	Kattingi
Oriya	Parsukedara
Sanskrit	Karpora haridra
Telugu	Karrallamu

Taxonomical Classification of the plant[2,6,7]

Synonym	*Amomum zerumbet*[2]
Family	*Zingibereaceae*
Kingdom	Plantae plant
Subkingdom	Tracheobionta
Super division	Spaermatophyta
Division	Magnoliophyta
Class	Liliopsida, Monocotyledons
Sub class	Zingiberidae
Order	Zingiberales
Genus	Zingiber
Species	zerumbet

Chemical constituents[8-12]

This plant is found to contain many Chemical constituents. Some of the major chemicals isolated from this plant are as follows.

Alkaloids[9]

Camphene, camphor and monoterpenoids as gingerol, zingeberol, zingerone, sesquiterpenenoids zerumbone, zerumbone epoxide, oxalic acid, kaempferol derivative terpine.[4] Humulene.[4]

Flavonoids[10-12]

Many flavonoids group are also found to be present in this plant which is as follows. Afzelin[8], flavonoid glycosides, essential oils, chlorgenic acid, ferulic acid etc.

Traditional use of the plant[13-15]

This drug has different used in the traditional as well as pharmaceutical purposed.Traditionally this can be used to treat fish poisoning, cough remedy, bacterial diseases, diabetes, skin diseases. The juice of the rhizomes is used for worm infection for children , for swelling, sores and for loss of appetites. The rhizome part also be used as for the stimulating action**.** It is used for anti-hypertensive action, for carminative purpose, for flavoring purpose , for perfume purpose. It is used as shampoo. It is also used to treat dyspepsia and fever. it is used for the treatment for leprosy, for peptic ulcer treatment and for other stomach problems and for mouth infection, for the treatment of asthma and for rheumatism purposes.

Pharmacological uses[16-18]

Some of the potent pharmacological activities of this plant Z. zerumbet as anti-cancer effects, anti-tumor effects, anti-inflammatory effects, anti-fungal activity, anti-microbial activity of zerumbone, anti-bacterial activity, anti-hyperglycemic activity has been reported from different literature survey studies.

MATERIALS AND METHODS

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, Wagner's, Dragendorff's, Fehling's A & B, α-naphthol, Ferric chloride,Conc. Sulphuric acid, Pyridine, Sodium nitropruside, Acetic anhydride, 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.

Plants collection, Identification and processing

The stems of *Zingiber zerumbet* were collected from adjoining area of Barpali ,Dist-Bargarh, Orissa in the month of October 2024. 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 on dated 02-10-2024. The plant was washed properly with water to remove the mud or dust, and then it was dried in sun light for one hour and the leaves of *Zingiber zerumbet* was dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of leaves was passed through sieve No. 16 to maintain uniformity and stored in cool and dry place for further study. The coarse powder have stored in air tight container for further studies.

Extraction

The dried powder (200 gm) plant material was extracted by soxhlet apparatus and was subjected to continuous cold as well as hot extraction with Ethanol and Aqueous in a ratio of 60:40 ratio until the completion of extraction. The powdered drug was extracted for 7 days with each solvent. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in a desiccator till experimentation. Obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material. The yield and % yield of both ethanolic and aqueous extracts of powdered root of *Zingiber zerumbet* were reported. The results thus obtained from the extraction of *Zingiber zerumbet* are shown in (Table 1).

Table 1: Extraction values of Hydro-alcoholic and aqueous extracts of *Zingiber zerumbet.*

Sl. No.	Extracts	% Yield (w/w)	Colour of extract
1.	Ethanol	7.88%	Dark reddish brown
2.	Aqueous	4.52%	LightYellowish Green

PRELIMINARY PHYTOCHEMICAL SCREENING[19-21]

The qualitative phytochemical analysis of Ethanolic and aqueous extract with a ratio of 60:40 extract of *Zingiber zerumbet* was taken And a preliminary identification of bioactive compounds such as alkaloids, glycosides, Cardiac Glycosides, flavonoids, tannins, carbohydrate, steroids and saponins and others was done as shown in Table. 2.

Table 2: Qualitative Phytochemical analysis of Ethanolic and Aqueous extracts of *Zingiber zerumbet.*

Phytochemical test	Ethanolic Extract	Aqueous Extract
Alkaloid test		
Mayer's test	Present	Present
Wagner's test	Present	Absent

Hager's test	Present	Absent
Dragendorff's test	Absent	Present
Carbohydrates		
Molish's test	Present	Present
Fehling's test	Absent	Absent
Barfoid's test	Absent	Absent
Benidict's test	Present	Absent
Borntrager's test	Present	Absent
Saponins		
Foam test	Absent	Present
Proteins & amino acid		
Millon's test	Present	Absent
Biuret's test	Present	Absent
Ninhydrin test	Absent	Present
Phenolic compounds & flavonoids		
Ferric chloride test	Absent	Absent
Lead acetate test	Present	Absent
Alkaline test	Absent	Present
Phytosterol :		
Libermann-Burchard's test	Absent	Absent

Table 3: Powder analysis with chemical reagent.

Reagents	Colour of powder
Powder as such	Light Brown
Powder + conc. HCI	Light yellow
Powder + conc. HNO_3	Yellowish
Powder + conc. H_2SO_4	Deep brown
Powder + glacial acetic acid	Light Brown
Powder + dil. HCI	Brown
Powder + NaOH sol.	Light brown
Powder + $FeCl_3$	Yellowish
Powder + picric acid	Yellow
Powder + ammonia	Light Green
Powder +Iodine	Light Brown

Table 4: Fluorescence analysis of powder drug.

Reagent	Colour observed (naked eye)	Colour observed (U.V short wave length)	Colour observed (U.V long wave length)
Powder as such	**Brown**	**Light Brown**	**brown**
Powder +1N NaOH in methanol	Yellowish	green	Dark brown
Powder + NaOH in water	Light brown	Light brown	Dark brown
Powder + 50% HCl	yellowish	green	Black
Powder +50% H_2SO_4	brown	green	Black
Powder +50% HNO_3	Light brown	green	brown
Powder + petroleum ether	brown	Light brown	Dark brown
Powder + chloroform	brown	green	green
Powder + picric acid	yellow	Dark brown	green
Powder + 5% $FeCl_3$	Light Yellow	Black	Black
Powder + 5% iodine solution	Dark	Dark green	Light Black
Powder + methanol	Light Green	brown	Dark brown
Powder + (HNO_3 + NH_3)	brown	green	Light brown

DETERMINATION OF ANTHELMINTIC ACTIVITY[22-24]

The anthelmintic study was done by using one in-vitro species adult earthworms Pheretima posthuma. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm; average size of the earthworm was 8-9 cm. These earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms. The suspensions of various extracts were prepared in 2%

gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard drug albendazole were also prepared in distilled water. Two ml of each concentration of various extracts of *Zingiber zerumbet* and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm Pheritima posthuma due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 5: Anthelmintic effect of *Zingiber zerumbet* extracts.

Group	Concentration of Extract (%)	Time in minutes (Mean ± SEM)	
		Paralysis time(Min)	Death time(Min)
Albendazole (standard)	10 mg/ml	18min,18 sec ±17	14min,20 sec ±42
	30 mg/ml	14min,21 sec ±12	15 min, 20sec ±10
	50 mg/ml	12 min,12 sec ±14	14min,40 sec ±11
Ethanolic extract	15 mg/ml	20min,16 sec ±17	24min,15 sec ±48
	30 mg/ml	17min,26 sec ±12	25 min,26 sec ±12
	50 mg/ml	14min,48 sec ±14	20 min,48 sec ±14
Aqueous extract	15 mg/ml	12min,19 sec ±17	17min,15 sec ±48
	30 mg/ml	10min,26 sec ±12	15min,26 sec ±12
	50 mg/ml	08 min,48 sec ±14	10min,14 sec ±10
Control	-	-	-

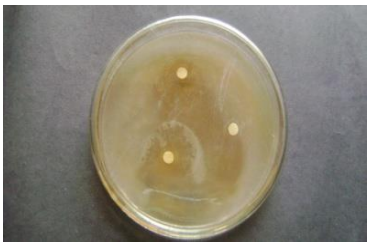
Results are expressed as mean ± SEM from six observations, *Control worms were alive upto 24 hrs. of observation,* N/A= No Activity shown within 24 hours.

ANTIMICROBIAL ASSAY OF ZINGIBER ZERUMBET[25-26]

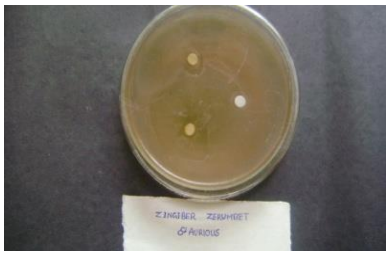
The term microbiological assay designedated as a type of biological assay, performed with microorganisms like bacteria, yeast &moulds. This involves the measurement of the relative potency of activity of compounds by determining the amount required to produce a stipulated effect on a suitable organism under standard conditions.

Table 6: In-Vitro evaluation of antimicrobial activity of Ethanolic extract (Agar Plate Method).

SL No	Microorganism	Zone of inhibition (at 1000 µg)
1	Staphylococcus aureus	1.33
2	Escherichia coli	1.6



S. Aureus.



E. Coli.

RESULTS AND DISCUSSION

The percentage yield of Ethanol and Aqueous extract were found to 7.88% and 4.52%w/w (Table-1). The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, glycosides, saponins, Protein, Amino acids,Flavonoids like compounds (Table-2) but do not shows the presence of Phytosterol. The Powder analysis and fluorescence was observed in ultra short, ultra long and visible. The results were shown in (table-3 and 4) .The results (Table-5) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Ethanolic extract with compared to the standard drug Albendazole. The Zone of inhibition for Anti-Microbial

assay of Staphylococcus aureus at 1000 µg is 1.33 and that of Escherichia coli is 1.6.

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