PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON ZINGIBER ZERUMBET HYDRO-ALCOHOLIC EXTRACT FOR ANTICONVULSANT ACTIVITY

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ABSTRACT

Aim- The aim of this study is to evaluate anticonvulsant activity and to determine the Total flavonoid content (TFC), Total tannin, Total carbohydrates in hydroalcoholic extract of Z. zerumbet.

Materials and methods- Plant materials are collected from puthuppally. Hydroalcoholic extract was screened for different phytochemical constituents. Acute toxicity studies are done according to OECD guidelines. Hydroalcoholic extracts were screened for anticonvulsant activity (induced by PTZ) in Wistar Albino rats. Quantified total content of carbohydrates (phenol-sulfuric acid method), tannins and flavonoids were identified in the extract.

Result – Hydroalcoholic extract of Zingiber zerumbet shows significant anticonvulsant activity compared to control. Presence of carbohydrate, flavonoid, tannins and terpenoids were identified in the extract.

Conclusion- The result suggest that the hydro-alcoholic extract of zingiber zerumbet rhizome contains some active principles which may possess significant anticonvulsant activity.

Key words- Zingiber zerumbet, PTZ-Pentyene tetrazole, hydro-alcoholic extract

INTRODUCTION

Zingiberaceae family is a plant species endowed with anti-oxidative properties. It is the largest family of the order Zingiberales. It is widely distributed throughout the tropics particularly in Southeast Asia. In Southeast Asian region, several species of Zingiberaceae are used as spices, traditional medicines, flavoring agents and as the source of certain dyes. The common Zingiberaceae species are easily recognised because they are used as food flavour, mixtures in traditional medicine as well as an ornamental plant. Zingiber zerumbet (L) Smith (Fig.1) well known as shampoo ginger, is a wild ginger belonging to the Zingiberaceae family. Z. zerumbet grows to about 7 ft (2.1 m) tall with long narrow leaves arranged oppositely along the stem.

- Kingdom: Plantae – Plants;
- Subkingdom: Tracheobionta
- Division: Magnoliophyta
- Class: Liliopsida
- Subclass: Zingiberidae;
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: Zingiber
- Species: Zingiber zerumbet (L.)
- Common Names: Shampoo Ginger, Bitter Ginger, Pinecone Ginger, Wild Ginger

Z.zerumbet has been used as a traditional medicine for many years. Z. Zerumbet is used in local traditional medicine as a cure in swelling, sores and loss of appetite. The juice of the boiled rhizomes has also been used as medicine for worm infestation in children (Nik-Norulaini et al., 2009; Faizah et al., 2002). It is in fact used as a shampoo in Asia and Hawaii, and is one of the ingredients in several commercial shampoos. For toothache, the cooked and softened rhizome is pressed into the

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Fig.1: Zingiber zerumbet
cavity to reduce pain. In Thailand, they used the rhizomes to relieve stomach ache, macerated in alcohol is regarded as tonic and also as the spice ginger. The flowers are eaten as vegetable.

The Z. zerumbet oil contains several volatile compounds like zerumbone, α-Caryophyllene, and camphene. There are several previous researches reported about the high content of zerumbone in the oils. It is reported that volatile oils of Z. Zerumbet from Malaysia and southern India consist of high content of zerumbone in the oil (Baby et al., 2009). The Malaysian accession recorded the content of the zerumbone in the oil is about 68.9% while the southern Indian accession of Z. Zerumbet is 76.3% - 84.8% zerumbone content in their rhizomes oils. Other than oil from the rhizomes, the extraction of the oil from the leaves and flowers also can be commercialized. It is because, the oil from these two parts reported contain (E)-nerolidol, beta-caryophyllene and linalool. (Chane-Ming et al., 2003) E-nerolidol and Linalool has many application based on its pleasant scent in the manufacturing of fragrance or flavors.

The analysis and physico-chemical characterization of rhizome of Z. zerumbet has been documented by several workers. There are many research have been done in order to commercialize the value added extract from Z. zerumbet. These researches focused on the ability of Zerumbone to treat several diseases. The zerumbone has the potential to be used in the treatment of Alzheimer’s disease (Bustaman et al., 2008). Z. Zerumbet also showed potential to suppress tumor activity (Murakami et al., 2002). In addition, the extract shows other pharmacological activities such as anti-inflammatory (Chien et al., 2008; Mukarami et al., 2004) etc.

Epilepsy is a serious neurological disorder associated with recurrent episodes of seizures due to the abnormal electrical activity in the brain. Nearly 40 million people all over the world are affected by this disease. Prevalence rate of epilepsy is about 5.59 per 1000 population in India. Currently, many drugs are available for treating this disorder, but these drugs have drawbacks like teratogenicity and other dose-related side effects. In spite of daily treatment, nearly 30% of patients continue to have convulsions and fail to provide a complete cure. Wide range of medicinal plants have been identified by the ancient systems of medicines for treating these problems which are devoid of undesirable effects and are gaining popularity in most of the developing countries. The aim of this study is to evaluate anticonvulsant activity and to determine the Total flavonoid content (TFC), Total tannin, Total carbohydrates in hydroalcoholic extract of Z. zerumbet.

MATERIALS AND METHODS

Collection of plant materials

Rhizomes of the plant Z. Zerumbet were collected from putthupally, Kottayam district, Kerala during the month of September and authenticated by Botanist.

Processing of sample

The rhizome of the plant were collected, cleaned thoroughly with distilled water and chopped in to small pieces. Dried in oven for 2-3 days and powdered.

Preparation of extracts

The powdered plant material were subjected to extraction by soxhlet method using alcohol: water (hydro alcoholic) as solvent. Evaporation of solvent from the extract was done by rotary vacuum method. A sticky mass were obtained after evaporation of solvent. The samples were stored at 10ºC till further use. At the time of administration a suspension was prepared by using the extract in 1% w/v of sodium carboxy methyl cellulose (sodium CMC).

Phytochemical analysis

Freshly prepared extract of Z. zerumbet were qualitatively tested for presence of the various phytoconstituents and quantified total content of carbohydrates (phenol-sulfuric acid method), tannins and flavonoids (Table.1).

Experimental Animals

Wistar Albino rats of either sex 150-250 gm of body weight have been used. Animals were kept in standard animal housing condition. Rats were housed in groups of 6 per cage. All the animals were maintained under standard conditions, that is room temperature 26 ±1°C, relative humidity 45-55% and 12:12 h light-dark cycle. The cages were maintained clean and all experiments were conducted between 9am to 4pm.

Acute toxicity study

Wistar Albino Rats of either sex (150 - 250 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423 and animals were observed for
mortality and behavioral changes. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Drugs and Chemicals**

Ethanol (Spectrum Chemicals), Carboxy Methyl Cellulose (India Sea Foods), Inj. Diazepam (Abbott Laboratory Limited), Pentylenetetrazol (Research Lab), α amylase enzyme (Ozone International).

**Quantitative chemical analysis**

**Determination of total flavonoids**

Total flavonoids were determined by Aluminium Chloride Colorimetric method. 0.5ml of plant extract was mixed with 1.5ml of ethanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It was kept at room temperature for 30mins. The absorbance of the reaction mixture was measured at 415nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared with quercetin in different concentrations (12.5, 25, 50, 80 and 100 mg/L) (Fig.3).

**Determination of tannins**

The tannins were determined by Folin and Ciocalteu method. 0.1 ml of the sample extract was added with 7.5 ml of distilled water and add 0.5 ml of Folin Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with distilled water instead of the sample. A set of standard solutions of gallic acid is treated in the same manner as described earlier and read against a blank. The results of tannins are expressed in terms of Gallic acid mg/g of extract (Fig.2).

**Determination of total carbohydrate**

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20 min. The absorbance was read at 490 nm. The quantity calculated as follows

100 ml of sample solution contains ‘X’/0.1 ×100

**Pharmacological screening**

**Anticonvulsant screening method**

(Pentylenetetrazole (PTZ) induced convulsion)

The anticonvulsant activity was evaluated for pentylenetetrazole induced convulsion (PTZ) in rats. The convulsion is induced by administration of pentylenetetrazole (70 mg/kg, i.p.) to Wistar Albino rats. Rats those showing responses were divided into three groups of six animals each. The first group of animals were administered 1% CMC (1ml/100gm) orally which served as negative control. Group of II animals were treated with diazepam (2mg/kg, i.p.) which served as positive control. Group of III animals were treated with ethanolic extract of *Z. zerumbet* at a dose of (400 mg/kg p.o). Drug pretreatment was given 1 hr prior to the administration of pentylenetetrazole and Immediately after PTZ administration rats were observed for onset of convulsions (time from PTZ injection until convulsion occurred) (Fig.4).

**STATISTICAL ANALYSIS**

All data were represented as mean ±SEM values. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparison was made against the vehicle treated groups were performed using the Dunnett’s - tests. The level of statistical significance adopted was P < 0.001.
RESULTS

Plants and their phytoconstituents have important role in the development of a potent anti-convulsant agent. Z. zerumbet one of the widely used species of the ginger family Zingiberaceae. Many members in this family shows potent anticonvulsant activity. Pentylenetetrazol is a CNS stimulant epileptogenic properties have been used to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. As a non-competitive GABA antagonist, PTZ is specifically used in seizure assays as a method of assessing the excitability of the central nervous system and GABA activity. Moreover, experimental evidence demonstrated that PTZ increased the level of cGMP in many brain regions including cerebral cortex, hippocampus, striatum, and cerebellum. High antioxidant activity of Z. zerumbet and its compounds has been demonstrated in numerous reports. In addition to the above findings, NO also increases the level of cyclic guanosine monophosphate (cGMP) through the activation of soluble guanylyl cyclase (sGC), which influences a wide range of physiological functions including regulation of seizure threshold (Ni Dhi et al., 1999). Components of the NO/cGMP pathway modulate release of both excitatory and inhibitory amino acids in the central nervous system (Prast & Philippu, 2001; Yu & Eldred, 2005). Because imbalance between the excitatory and the inhibitory neurotransmission is the main reason of epileptic discharges, cGMP may affect convulsant activity in brain (Garthwaite & Boulton, 1995). Therefore, it seems that antioxidant property of Z. zerumbet and its nitric oxide synthase inhibitory effect can be, at least in part, responsible for such inhibiting response in this study. Flavonoids, an important class of naturally compounds, have demonstrated CNS activities such as affinity for GABA-A receptors and anticonvulsion effects. In conclusion hydroalcoholic extract of Z. zerumbet shows significant anticonvulsant activity compared to control, may be due to the presence of flavonoids.

DISCUSSION

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