IJTA

International Journal of Therapeutic Applications

ISSN 2320-138X

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

Lincy Joseph*, Mathew George, Sreelakshmi.R

Pushpagiri College of Pharmacy, Tiruvalla, Kerala, India

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. Hydro alcoholic 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.

Result – Hydroalcoholic extract of Zingiber zerumbet shows significant anticonvulsant activity compard 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- Zinginer zerumbet, PTZ-Pentyene tetrazole,hydro-alcoholic extract

INTRODUCTION

Zingiberacaeae 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.[15] 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 Z*ingiberacea*e 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[15]

Fig.1: Zingiber zerumbet

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 s7oftened rhizome is pressed into the

Corresponding author:

Email: mathewlincg@yahoo.com

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[2], α-Caryophylleno,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* [4]has been documented by several workers [5]. 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 [10],analgesic[15](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 puthuppally, 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 powderd.

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 Laboratorylimited.), Pentylenetetrazol (Research Lab), α amylase enzyme (Ozone International).

Quantitative chemical analysis [1] (Table.2)

Determination of total flavonoids [11]

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 [11]

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 (Phenol–Sulfuric Acid method) [1]

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)[6], [7], [9]

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

Table1:Phytochemical analysis of Z.zerumbet

Phytochemical compound	Aq.ethnolic extract of *z.zerumbet*
Carbohydrates	+++
Flavonoids	++++
Tannins	+++
Triterpenoids	++
Steroids	-
Alkaloids	+

+ indicates presence - indicate absence

Table 2: Quantitative analysis of Phytochemicals

Phytochemical Compound	Percentage
Carbohydrate	8.5%
Flavonoids	91.6 µg QE/mg of extract
Tannins	86.9 µg GAE/mg of extract

RESULTS

Table.3: Evaluation of Anticonvulsant activity

GROUPS	TIME(min)
control	2.36±0.714
test	3.84±0.739
standard	6.56±0.694

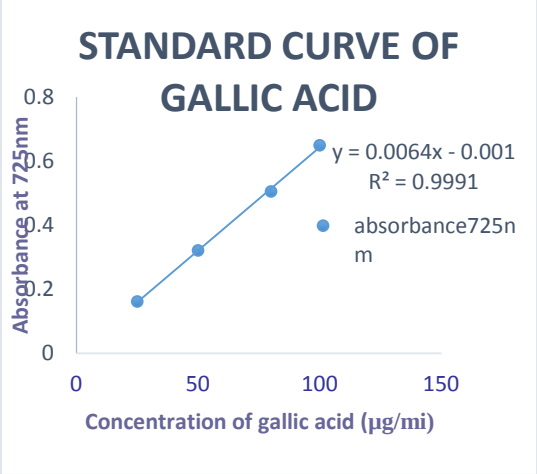

Fig.2: Standard Curve of Gallic Acid

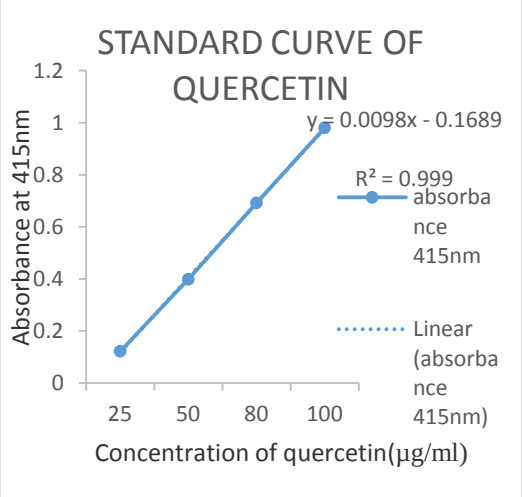

Fig.3: Standard Curve of Quercetin.

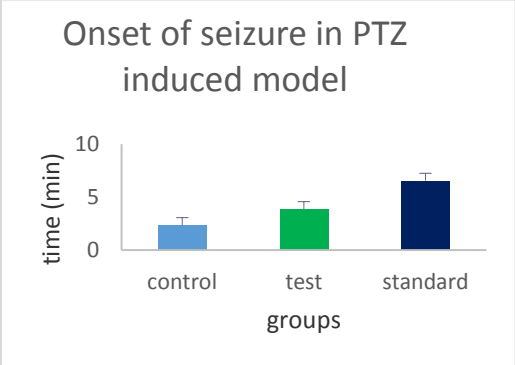

Fig.4: Effect of Hydro alcoholic Extract of Z. zerumbet (400mg/kg) and Standard (Diazepam 2mg/kg), compared with vehicle treated Control group (1% CMC).

DISCUSSION

Plants and their phytoconstituents have important role in the development of a potent anti-convulsant agent. *Z.zerumbet*s one of the widely used species of the ginger family *Zingiberaceae*. Many members in this family shows potent anticonvusant 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) [16] 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)[8]. 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.

REFERENCES

[1] Geetha T.S.Geetha N.Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus* (DC) stapf. leaves from Kodaikanal hills, Tamilnadu:Int.J.PharmTech Res.2014,6(2), 521-529.

[2] Huang G-C, Chien T-Y, Chen L-G,Wang C-C. Antitumor effects of zerumbone from *Zingiber zerumbet* in P-388D1 cells in vitro and i*n vivo*. Planta Med 2005; 71(3):219-224.

[3] Huen M, Leung J, Ng W, Lui W, Chan M, Tze-Fei Wong J, Xue H. 5, 7- Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. J. Biochem. Pharmacol. 2003; 66:125–132.

[4] Jang DS, Han AR, Park G, Jhon GJ, Seo EK. Flavonoids and aromatic compounds from the rhizomes of *Zingiber zerumbet*. Arch Pharmacal Res 2004; 27(3):386-389.

[5] Jang DS, Seo EK. Potentially bioactive two new natural sesquiterpenoids from the rhizomes of *Zingiber zerumbet*. Arch Pharmacal Res 2005; 28(3):294-296.

[6] Kulkarni S. K., Dhir A.. An Overview of Curcumin in Neurological Disorders;Indian J Pharm Sci. 2010 Mar-Apr; 72(2): 149–154.

[7] Maggesissib R.S., Gardinob P.F., Guimarães-Souzaa E.M, Paes-de-Carvalhoa R., Silvaa, R.B. Calazaa K.C.Modulation of GABA release by nitric oxide in the chick retina: Different effects of nitric oxide depending on the cell population; Vision Research: 2009,49(20), 2494–2502

[8] Miliauskas G. V, Van Beek T. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. J. Food Chem. 2004; 85:231–237.

[9] Narapogu. Venkatanarayana, Md. Ghouse Basha, Naveen Pokala, T. Jayasree, S. Premendran John and Ch. Nagesh. Evaluation of anticonvulsant activity of ethanolic extract of *Zingiber officinale* in Swiss albino rat; Journal of Chemical and Pharmaceutical Research, 2013, 5(9):60-64.

[10] Nhareet S, Nur S.Antiinflammatory property of ethanol and water extracts of *Zingiber zerumbet*: Indian J Pharmacol 2003; 35(3):181.

[11] Rajeshwari Sahu, Jyoti Saxena.Screening of Total Phenolic and Flavonoid Content in Conventional and Non-Conventional Species of Curcuma;Journal of Pharmacognosy and Phytochemistry 2013 2(1):176-179

[12] Somchit M, Shukriyah M, Bustamam A, Zuraini A. Anti-pyretic and analgesic activity of Zingiber zerumbet. Int J Pharmacol 2005; 1(3):277-280.

[13] Sulaiman Mohammed, Fazilah Abd Manan.Analysis of total phenolics, tannins and flavonoids From Moringa oleifera seed extract;ournal of Chemical and Pharmaceutical Research, 2015, 7(1):132-135.

[14] Tewtrakul S, Subhadhirasakul S. Anti-allergic activity of some selected plants in the Zingiberaceae family. J Ethnopharmacol 2007; 109(3):535-538.

[15] Yob N, Jofrry SM, Affandi M, Teh L, Salleh M, Zakaria Z. *Zingiber zerumbet* (L.) Smith: a review of its ethnomedicinal, chemical, and pharmacological uses. Evid Based Complement Alternat Med 2011; 2011.

[16] Yu Dou; William D. Eldred. Gycine and GABA interact to regulate the nitric oxide/cGMP signaling pathway in the turtle retina: Vis Neurosci. 2005; 22(6): 825–838.

[17] Jang DS, Min HY, Kim MS, Han AR, Windono T, Jeohn GH, Kang SS, Lee SK, Seo EK. Humulene derivatives from *Zingiber zerumbet* with the inhibitory effects on lipopolysaccharide-induced nitric oxide production. Chem Pharm Bull (Tokyo). 2005; 53 (7):829-31.