



Original Research Paper

**“PHYTOCHEMICAL ANALYSIS AND ANTIDEPRESSANT
ACTIVITY OF ETHANOLIC EXTRACT OF *ZINGIBER
ZERUMBET*”**

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ABSTRACT

Zingiber zerumbet belongs to the family Zingiberaceae, are spread throughout the world growing under a wide range of climatic conditions. It has been widely used in Mexican traditional medicine for the treatment of different central nervous system disorders. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effect on the CNS. In this work, the effects of ethanolic root extract of *Zingiber zerumbet* were evaluated in mice using behavioral tests sensitive to clinically effective antidepressant compounds. The extract (100 and 200mg/kg), administered intraperitoneally, was able to decrease the immobility time of mice dose dependently when subjected to both tail suspension and forced swim tests and the effects are comparable to that of standard drugs fluoxetine (20mg/kg). Preliminary phytochemical analysis showed the presence of phenolic compounds, glycoside, steroids, alkaloids, flavonoids, saponins, tannins and amino acids in ethanolic extract of *Zingiber zerumbet*. It is concluded that *Z. zerumbet* possess antidepressant activity may be due to the presence of polyphenolic compounds and flavonoids. However further study is needed to understand mechanism of action and to identify active component responsible for antidepressant activity.

INTRODUCTION

According to the World Health report, approximately 450 million people suffer from a mental or behavioural disorder. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020. [1,2] Psychiatric illness is also often associated with suicide and there are between 10 and 20 million suicide attempts every year. Depression is one the most prevalent forms of psychiatric disorders and is a leading cause for mortality. [3] Lifetime prevalence of depression in the general population is 4.4-20% and suicides occur is up to 15% of individuals with severe major depression. [4] Therefore, major depression is a serious public health problem and causes a considerable heavy psychological and economic burden for families. [5] In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem. [6] Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. *Zingiber zerumbet* was investigation for antidepressant activity.

Zingiber zerumbet Linn, (Family-Zingiberaceae) are spread throughout the world, growing under a wide range of climatic conditions. This plant is characterized by the presence of stems approximately 1–2 m tall, which are erect, oblique, round and covered by sheaths of flat leaves. The leaves and inflorescences grow from a thick rhizome or underground stem. The rhizome is circular with epidermis, cortex, endodermis and closed, collateral vascular bundle. Transverse section of the rhizome showed outer single layered epidermis having rectangular and elongated cells, followed by thin walled cork cells of 6-10 layers, irregularly elongated. The methanolic extracts of rhizomes of *Zingiber zerumbet* (L) gave positive tests for alkaloids, saponin, tannin, amino acid, proteins, starch, flavonoids and reducing sugar. Starch, flavonoids and saponines were distributed throughout the rhizome except periderm. *Z. zerumbet* is a rich source of different classes of compounds that belong to a wide variety of chemical metabolites, such as polyphenols, alkaloids and terpene (Zerumbone). Zingiberaceae, which is a significant source of essential oil used in the perfume, cosmetics, and medical sectors 4,5. Other medicinal uses are as anti-allergic, immunomodulators 6. Traditional medicinal uses of *Zingiber zerumbet* include treating inflammatory condition fever, toothache, indigestion, constipation, diarrhea, severe sprain, and an antispasmodic and pain reliever and diuretic medication.

MATERIAL AND METHOD

Collection and Authentication of plant

Zingiber zerumbet Rhizome was collected from the Sanjeevani Gardan in the state of Madhya Pradesh District

Bhopal during the Month of May. The Rhizome have been identified and authenticated by Dr. Saba Naaz Head of the Department Botany at the Safia college of science, Bhopal (M.P.).

The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of *Zingiber zerumbet* Rhizome extract

Extraction of *Zingiber zerumbet* was done by maceration method. Maceration Extraction: Maceration method using 90% ethanol for extraction. Extract obtained was passed through the Whatman filter paper No.1 and the ethanol was evaporated (at 40°C) with the help of heating mantle and dried in a desiccator. Calculated percentage yield of the extract was 8.6%

Preliminary phytochemical Analysis

Preliminary phytochemical tests were done by the methods described by usual procedures. The crude extracts of plants obtained by solvent extraction was subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterols, saponins, tannin, flavonoids and protein as described by Kokate. [58,59]

Quantitative Phytochemical Estimation

TPC

The total phenolic content of *Zingiber zerumbet* extract was determined using the Folin-Ciocalteu Assay. The *Zingiber zerumbet* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic acid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically.

TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Zingiber zerumbet* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight. [60]

DPPH

The antioxidant activity of *Zingiber zerumbet* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared. Different concentration of *Zingiber zerumbet* extracts /standard (20 – 100µg/ml) were prepared from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using UV spectrophotometer (Shimadzu 1700) at 517 nm. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm.

Percentage antioxidant activity of sample/standard was calculated by using formula:

$$\% \text{ Inhibition} = \frac{[(\text{Ab of control} - \text{Ab of sample}) / \text{Ab of control} \times 100]}{}$$

Animals Male or female Swiss albino mice weighing between 20 – 25 g were used for the present study. The animals were maintained under standard environmental conditions (25 ± 2° C and relative humidity of 45 to 55%) and were fed with standard pellet diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee. CPCSEA guidelines were adhered during the maintenance and experiment.

Acute Oral Toxicity Study

Acute oral toxicity study was evaluated as per OECD guidelines (425) on albino mice. Before experimentation mice were fasted overnight with water ad libitum. Three animals were selected which receives dose of 2000 mg/kg. [18] All three animals were received dose of 2000 mg/kg body weight of ethanolic extract of plants extract + 1% aqueous CMC by gavage using oral cannula (limit test). Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, administered dose was found tolerable (as no death found). Therefore, two dose levels 100, and 200 mg/kg was selected for CNS depressant activity. [61]

In-vivo Assessment of Anti-Depressant Activity

Antidepressant activity of EEZZ was assessed using Forced swimming test and Tail Suspension Test.

FORCED SWIMMING TEST

Assessment of Antidepressant activity using forced swimming test in mice. Swiss Albino mice weighing around 20 to 25 gram were used for this study. They were divided into four groups each group consist of 6 animals (n=6). Each animal was placed individually in a 5 liter glass beakers, filled with water upto a height of 15 cm and were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test. A decrease in the

duration of immobility is indicative of an antidepressant like effect.

GROUP 1 RECEIVED - Normal saline (5ml/kg P.o)

GROUP 2 RECEIVED - Fluoxetine (20mg/kg P.o) (Standard)

GROUP 3 RECEIVED - EEZZ 100mg/kg, P.o

GROUP 4 RECEIVED - EEZZ 200mg/kg, P.o

TAIL SUSPENSION TEST

Assessment of Antidepressant activity using tail suspension test in mice. Swiss Albino mice weighing around 20 to 25 gram were used for this study. They were divided into four groups each group consist of 6 animals (n=6). The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless. A decrease in the duration of immobility is indicative of an antidepressant effect.

GROUP 1 RECEIVED – Normal saline (5ml/kg P.o)

GROUP 2 RECEIVED - Fluoxetine (20mg/kg P.o) (Standard)

GROUP 3 RECEIVED - EEZZ 100mg/kg (t1)

GROUP 4 RECEIVED - EEZZ 200mg/kg (t2)

Statistical Analysis

The collected data was subjected to appropriate statistical tests including one-way ANOVA (Analysis of Variance), followed by Dunnett's test. P values of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using Graph pad prism software of version 4.

RESULTS AND DISCUSSION

Depression is characterized by emotional symptoms such as hopelessness, apathy, and loss of self -confidence as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances and loss of appetite. Preliminary phytochemical analysis of Ethanolic Rhizome extract of *Zingiber zerumbet* showed the presence of Glycosides, alkaloids, Tannins, phenolic compounds, flavonoids, saponins, Protien and amino acids.

Table 01: Phytochemical screening of Ethanolic extract of *Zingiber zerumbet*

S. N	Identification Test	Test name	Result
1	Alkaloids	Mayer's test	+
		Dragendroff's test	-
		Wagner's test	+
2	Glycoside	Killer-killani test	+
3	Carbohydrate	Fehling test	-

4	Tannins & Phenols	Gelatine test	+
		Ferric chloride test	+
5	Flavonoids	Shinoda test	+
		Alkaline reagent test	+
6	Steroids	Liebermann-Burchard test	-
		Salkowski test	-
7	Saponins	Foam test	+
8	Proteins	Xanthoprotic	+

(+) = Present, (-) = Absent

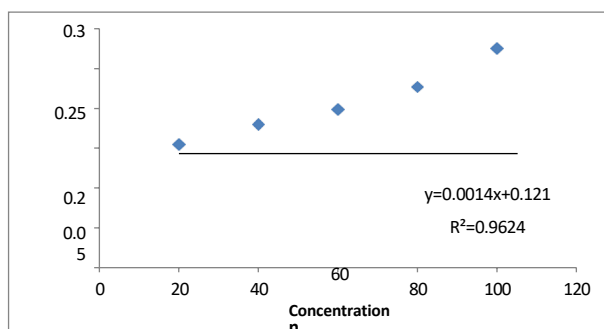
Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic content (TPC) estimation

Table 02- Standard table for Gallic acid

S. No.	Concentration($\mu\text{g/ml}$)	Absorbance
1.	20	0.155
2.	40	0.180
3.	60	0.198
4.	80	0.226
5.	100	0.275



Graph 01: Represent standard curve of Gallic acid

Total Phenolic Content in extract:

Table 03: Total Phenolic Content

S. No.	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.135	49.33mg/gm
2	0.182	
3	0.194	

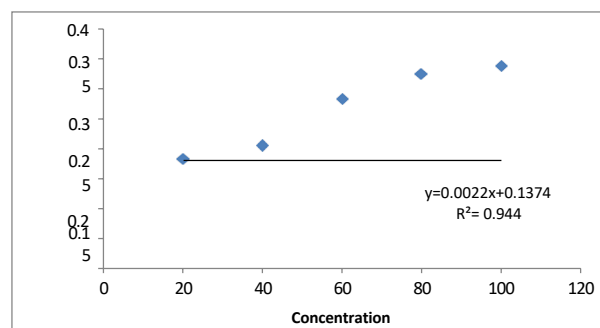
Table 04- Total Phenolic Content of extract *Zingiber zerumbet*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Ethanolic	49.33

Total Flavonoids content (TFC) estimation

Table 05: Standard Table for Rutin

S. No.	Concentration ($\mu\text{g/ml}$)	Absorbance
1.	20	0.182
2.	40	0.205
3.	60	0.283
4.	80	0.325
5.	100	0.337



Graph 02: Represent standard curve of Rutin**Total Flavonoid Content in extract****Table 06: Total Flavonoid Content**

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.155	18.16mg/gm
2	0.168	
3	0.197	

Table 07: Total Flavonoid Content of extract *Zingiber zerumbet*

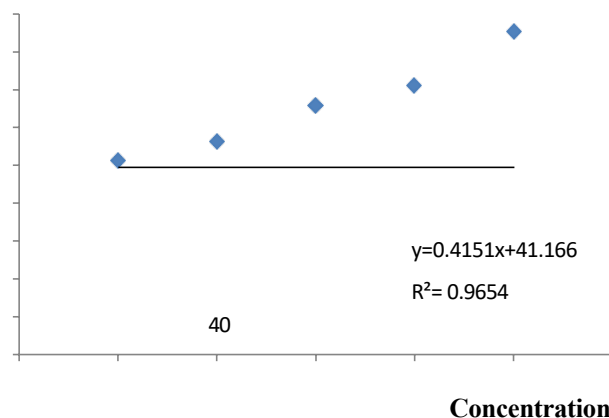
Extracts	Total Flavonoid content (mg/gm equivalent of Rutin)
Ethanolic	18.16

***In vitro* Antioxidant Assays**

In the present investigation, the *in vitro* antioxidant activity of extracts of *Zingiber zerumbet* was evaluated by DPPH radical scavenging activity. The results are summarized in Tables.

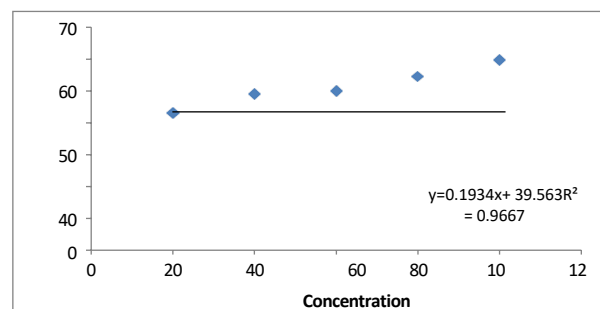
DPPH (1, 1- diphenyl-2-picryl hydrazyl Assay)**Table 08: DPPH Radical Scavenging activity of Std. Ascorbic acid**

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.483	51.457
40	0.435	56.281
60	0.339	65.929
80	0.287	71.155
100	0.144	85.527
Control		0.995
IC ₅₀		21.30

**Graph 03: DPPH radical scavenging activity of Std. Ascorbic acid****Table 09: DPPH radical scavenging activity of extract of *Zingiber zerumbet***

Concentration(µg/	Absorban	%
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ml)	ce	Inhibitio n
20	0.521	43.060
40	0.468	48.852
60	0.458	49.945
80	0.418	54.316
100	0.369	59.672
Control	0.915	
IC ₅₀ 54.09		

**Graph 04: Represents the Percentage Inhibition Vs Concentration of extract of *Zingiber zerumbet*****Acute Toxicity Studies (LD₅₀):**

In both phase I and Phase II procedures, none of the animal mortal or any signs of behavioral changes or show any toxicity upon the single administration of EEZZ (2000 mg/kg p.o.). Hence, the dose of 100mg/kg and 200mg/kg were chosen.

Table 10: Results of Acute oral toxicity study of EEZZ

Group Name	Animal Mark	Dose mg/kg	Body weight (gm)			Observation	Mortality (If any)
			1 day	7 days	14 days		
Control	H	Normal Saline (0.9 %)	153	148	146	No Sign of toxicity & all animals survived	No Mortality occurs
	B		155	150	152		
	T		138	135	132		
Test	HB	2000 mg/kg of EEAC (Once dosing at start of acute oral toxicity study)	200	204	186		
	BT		190	185	180		
	HT		175	165	168		

EVALUATION OF ANTI-DEPRESSANT ACTIVITY

FORCED SWIMMING TEST

Group I animals showed duration of immobility for 220.2±0.21 seconds on day 1 and on day 14, duration of immobility was slightly increased to 217.5±0.32 seconds. On day 1, Group II animals treated with fluoxetine showed lesser duration of immobility for about 56.3±0.31 seconds and on day 14, they showed further reduction in the duration of immobility for about 52.4±0.48 seconds. Group III and Group IV (100 mg/kg and 200mg/kg of extract) also showed reduction in the duration of immobility. A significant decrease in duration of immobility in mice was observed in test groups when compared to control group. The high dose of test compound (200mg/kg) exhibited a significant reduction in immobility compared to other test group of animals. The results were shown in the table no 11. The results coincide with the previous reported article.

Table 11: Effect of ethanolic extract of *Zingiber zerumbet* on Immobility Time in the Forced Swimming Test (FST) using mice

S. No	Treatment	Duration of immobility (seconds)		% Increase in immobility
		Day 1	Day 14	
1.	Group I (Normal saline-5ml/kg)	220.2±0.21	217.5±0.32	13 %
2.	Group II (Fluoxetine-20mg/kg)	56.3±0.31	52.4±0.48	71 %
3.	Group III (EEAC-100mg/kg)	63.6±0.26	60.9±0.60	47 %
4.	Group IV (EEAC-200mg/kg)	46.7±0.19	43.5±0.56	65 %

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. **P<0.01

FORCED SWIM TEST

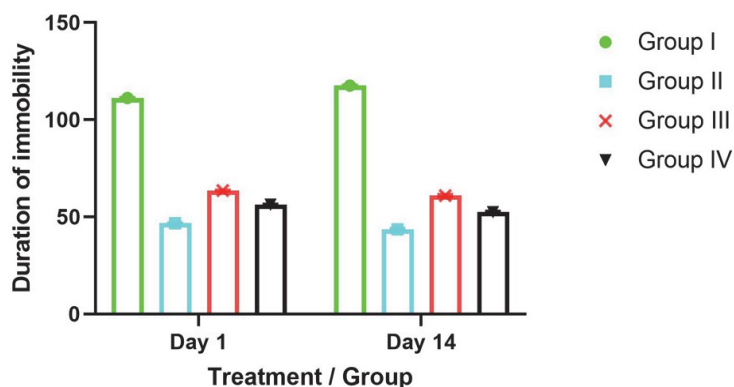


Figure 01: Graph of Effect of ethanolic extract of *Zingiber zerumbet* on Immobility Time in the Forced Swimming Test (FST) using mice

TAIL SUSPENSION TEST

Group I animals showed duration of immobility for 221.6±1.06 seconds on day 1 and on day 14, duration of immobility was found to be 218.4±0.96 seconds. On day 1, Group II animals treated with fluoxetine showed reduction in duration of immobility for about 147.2±0.83 seconds and on day 14, they showed further reduction in the duration of immobility for about 135.7±1.41. Group III and Group IV (100 mg/kg and 200mg/kg of extract) also showed reduction in the duration of immobility. A significant decrease in duration of immobility in mice was observed in test groups when compared to control group. The high dose

of test compound (200mg/kg) exhibited a significant reduction in immobility compared to other test group of animals. The results were shown in the table no.12.

Table 12: Effect of ethanolic extract of *Zingiber zerumbet* on Immobility Time in the Tail Suspension Test (TST) using mice

S. No	Treat ment	Duration of immobility (seconds)		% Increase in immobility
		Day 1	Day 14	
1.	Group I (Normal saline-5ml/kg)	221.6±1.06	218.4±0.96	13%
2.	Group II (Fluoxetine-20mg/kg)	147.2±0.83	135.7±1.41	88%
3.	Group III (EEAC-100mg/kg)	122.2±0.83	115.7±1.41	69%
4.	Group IV (EEAC-200mg/kg)	123.3±1.45	114.2±0.71	78%

All values are expressed as mean ± S.E.M.; (n=6) animals in each group.
**P<0.01

Tail suspension test

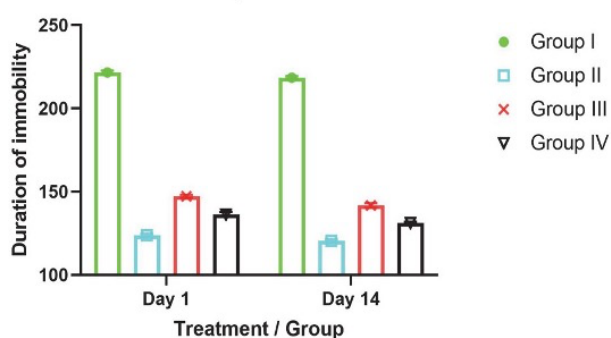


Figure 02: Graph of Effect of ethanolic extract of *Zingiber zerumbet* on Immobility Time in the Tail Suspension Test (TST) using mice

DISCUSSION

The results of phytochemical analysis showed the presence of phytoconstituents other than Flavonoids like Alkaloids, Triterpenes, Tannins, saponins, glycosides etc., in EEZZ. Previous research reports reveal that the presence of saponins and flavonoids attributed to the antidepressant activity of several plants.

The total phenolic content (TPC) of the ethanolic

extract of *Zingiber zerumbet* Rhizome was determined using the Folin–Ciocalteu colorimetric method, with gallic acid as the reference standard. The ethanolic extract exhibited a total phenolic content of 49.33 mg GAE/g of extract. The total flavonoid content (TFC) of the methanolic extract of *Zingiber zerumbet* Rhizome was determined using the aluminium chloride colorimetric method, with rutin as the reference standard. The ethanolic extract exhibited a total flavonoid content of 18.16 mg RE/g of extract. Although the antioxidant activity of the methanolic extract was slightly lower than that of standard ascorbic acid, the difference in IC₅₀ values was relatively small (54.09 µg/mL for extract compared with 21.30 µg/mL for ascorbic acid), demonstrating that the extract possesses strong radical scavenging ability.

The present study showed the effect of ethanolic Rhizome extracts of *Zingiber zerumbet* Antidepressant activity was Forced Swim Test in mice. The immobility period of mice in water is the parameter measured. The results show that Test Group III (100mg/kg) produced less immobility period while Test Group IV (200mg/kg) showed a significant immobility time compared to standard group (Fluoxetine – 20mg/kg). The % increase of immobility time in Group III, Group IV & standard groups are 47%, 65% and 71%. The showed the effect of ethanolic extracts of *Zingiber zerumbet* Rhizome Antidepressant activity was Tail Suspension Test in mice. The immobility period of mice in water is the parameter measured. The results show that Test Group III (100mg/kg) produced less immobility period while Test Group IV (200mg/kg) showed a significant immobility time compared to standard group (Fluoxetine – 20mg/kg). The % increase of immobility time in Group III, Group IV & standard groups are 69%, 78% and 88%. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

CONCLUSION

The preliminary Phytochemical screening of the extract of *Zingiber zerumbet* Rhizome showed the presence of carbohydrates, glycosides, sterols, flavonoids, saponins, tannins, terpenoids and phenolic compounds. The present study was designed to evaluate the anti-depressant activity of selected EEZZ using Forced Swimming Test and Tail Suspension Test using the standard drug Fluoxetine (20 mg/kg). The present study demonstrates that ethanolic extracts of *Zingiber zerumbet* Rhizome (100 & 200mg/kg) has antidepressant activity. This action of *Zingiber zerumbet* is functionally similar to Fluoxetine which are widely used as antidepressant. In conclusion, EEZZ showed significant antidepressant activity without producing motor in coordination in mice. Hence EEZZ may become potential resource for natural psychotherapeutic agent against various

anxiety related disorders and behavioral depression with fewer side effects compared to current available therapies.

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