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Research Article **Botany**

IN SILICO DETERMINATION AND BIOLOGICAL EVALUATION OF METHANOL EXTRACT OF *Zingiber officinale*

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Abstract: In the present study *Zingiber officinale* was selected and investigated for the bioactive potential. The phytochemical screening aqueous extract of *Zingiber officinale* rhizome showed that the presence of tannin, flavonoids, alkaloids, saponins, phenolics, glycosides, anthriquinone and protein while terepenoids, triterpenoids, steroids, phlobatannins and carbohydrate were absent. Methanol extract of *Zingiber officinale* rhizome showed that the presence of alkaloids, steroids, saponins, flavonoids, tannin, terepenoids, phenolics, carbohydrate, anthriquinone, protein and glycosides while triterpenoids, and phlobatannins were absent. Significant quantity of flavonoids, terpenoids and phenol present in the extract.In the present study twenty one chemical components have been identified from extract of *Zingiber officinale* rhizome by Gas Chromatogram-Mass spectrometry (G C-MS) analysis. The prevailing compounds were 2-Octylcyclopropene-1-heptanol, Hexadecanoic acid ethyl ester, Timonacic, l-(+)- 9,12,15-Octadecatrienoic acid,, Phytol and 1,2-Benzenedicarboxylic acid Plant extract of *Zingiber officinale* was potential activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subti*lis species of bacteria. *In silico* molecules docking to analyze the binding properties of the mediator called JNK with Phytol compound reported from *Zingiber officinale* rhizome. The wet analysis carried out by us showed very good result with regard to anti-cancer property of this plant extract. So the present study may act as supportive evidence that substantiate property of this plant extract. Phytol has potential inhibiting ability was identified from this plant with JNK.

Keywords: *In silico*; Phytochemical screening; *Zingiber officinale* rhizome; Antibacteria.

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INTRODUCTION

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta *et al*., 2005; Sandhu , 2005).

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.

Computer aided method is an easy platform to search biologically active compounds with favorable ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and drug-likeness properties. The ultimate goal is to enrich set of molecules with good pharmacological properties and eliminate compounds with undesirable properties (inactive, reactive, toxic, poor, ADMET-PK etc.). In another words *in silico* modeling is used to significantly minimize time and resource requirements of chemical synthesis and biological testing. Thus computational modeling and simulations are needed to facilitate, expedite and streamline drug discovery and development, save time, money and resources. It is estimated that computer modeling and simulations account for 10% of pharmaceutical R&D expenditure and that they will rise to 20% by 2016 (Waterbeemd *et al.,* 2003). Recently, application and utility of this virtual screening approach in combination with activity-guided fractionation of medicinal plants was also demonstrated and coined “in combo screening”. While computational techniques have already provided significant benefits, they hold a great promise for future progress in drug discovery and development.Keeping in view, the present study to investigate the phytochemical and antibacterial properties of *Zingiber officinale* rhizome.

MATERIALS AND METHODS

Collection of Plant materials:

The *Zingiber officinale* rhizome were collected in January 2018 from, Thanjavur, Tamil Nadu, India.

Preparation of alcoholic extract:

The rhizome of *Zingiber officinale were* first washed well and dust was removed from the rhizome. *Zingiber officinale* rhizome were washed several times with distilled water to remove the traces of impurities from the rhizome. The rhizome were dried at room temperature and coarsely powdered. The powder was extracted with aqueous and methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

GC-MS Analysis

GC-MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32 mm, column length is 30 m, column thickness 0.50 µm), operating in electron impact mode at 70 eV; Helium gas (99.99 %) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 5 µI was employed (split ratio of 10:1), injector temperature 270 ºC; ion source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150 ºC, then 8 ºC/min to 250 ºC, ending with a 20 min isothermal at 280 ºC. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0 (Srinivasan and Ramarao, 2013).

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dukes, 2013).

Determination of antibacterial activity

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing *Staphylococcus aureus*, *Escherichia coli* specie and *Bacillus subtilis* of bacteria were spread on Nutrient agar plates for bacteria. Using sterile forceps, the sterile

filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37○C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antibacterial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

***In Silico* Docking Studies**

Protein and ligand structures were obtained from the protein data bank (PDB) database and Pubchem. Automated docking along with a graphical user interface, Auto Dock tools was utilized to generate grids, calculate dock score and evaluate the conformers of activators bound in the active site of protein as targets. A Lamarckian genetic algorithm method, implemented in the program Auto Dock 4.1, was employed. This software used for the estimation of energy during the interaction and identify the best flexible ligand pose with minimum energy. The scoring function is based on the inter-amolecular interaction of ligand and protein during docking. As per genetic algorithm all the torsions were allowed to rotate during docking. The grid map was centred at particular residues of the protein and was generated with Auto Grid. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters (Vidya et al., 2012; Shruthi et al., 2012). Complex structures were modeled using modeling software's Pymol (1.1 version, Delano Scientific LLC, San Carlos, CA, USA), Chimera (1.10.1 version UCSF Resources for biocomputing visualization and informatics, NIH, CA, USA) and Pose view (Trot and Olson, 2010) installed on a desktop equipped with Pentium (R) Dual-E6600 at 3.05 GHz 3.06 GHz processor (2 GB RAM Core CPU) running the Ubuntu 12.01 (LINUX) and Windows XP SP3 operating system.

RESULTS AND DISCUSSION

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Zingiber officinale* rhizome investigated and summarized in Table-1 and fig- 2 and 3. The phytochemical screening aqueous extract of *Zingiber officinale* rhizome showed that the presence of tannin, flavonoids, alkaloids, saponins, phenolics, glycosides, anthriquinone and protein while terepenoids, triterpenoids, steroids, phlobatannins and carbohydrate were absent. Methanol extract of *Zingiber officinale* rhizome showed that the presence of alkaloids, steroids, saponins, flavonoids, tannin, terepenoids, phenolics, carbohydrate, anthriquinone, protein and glycosides while triterpenoids, and phlobatannins were absent. Significant quantity of flavonoids, terpenoids and phenol present in the extract.

Table: 1 Phytochemical screening of *Zingiber officinale* rhizome

S.No	Phytochemical analysis	Aqueous extract	Methanol extract	Quantitative analysis (mg/gm)
1	Tannin	+	+	
2	Phlobatannins	---	---	
3	Saponin	+	+	
4	Flavonoids	+	+	130
5	Steroids	---	+	
6	Terepenoids	+	+	120
7	Triterpenoids	---	+	
8	Alkaloids	+	---	
9	Carbohydrate	---	+	
10	Protein	+	+	
11	Anthroquinone	+	+	
12	Polyphenol	+	+	180
13	Glycoside	+	+	

(+) Presence, (++) highly presence and (-) Absence

Falodun *et al*. (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *et al*. (2006) examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of phenols,

glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al*. (2007) extracted eight bioactive compounds from dry leaf of *Cnidoscolus aconitifolius* using water and ethanol. Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al*. (2007) for its phytochemical properties.

Onwukaeme *et al*. (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycanthus angolensis*. Uma Devi *et al*. (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated for phytochemical analysis and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins (Vaghasiya and Chanda, 2007). Ayoola *et al*. (2008) investigated the phytochemical components of four medicinal plants used for the treatment of malaria in Southwestern Nigeria. *Ichnocarpus frutescens* leaf, stem and root were investigated (Mishra *et al*., (2009) for its phytochemical and phytochemical properties.

GCMS Analysis

In the present study twenty one chemical constituents have been identified from extract of *Zingiber officinale* rhizome by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The prevailing compounds were 2-Octylcyclopropene-1-heptanol, Hexadecanoic acid ethyl ester, Timonacic, l-(+)-9,12,15-Octadecatrienoic acid,, Phytol and 1,2-Benzenedicarboxylic acid. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. (Fig 1, Table 2 and 3).

Fig.1: Chromatogram obtained from the GC-MS with the *Zingiber officinale* rhizome extract

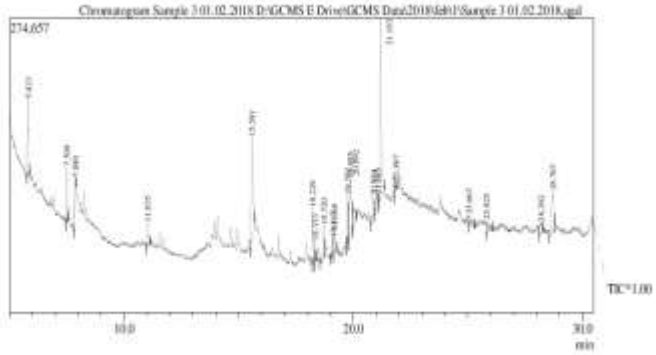


Table.2: Components identified in ethanolic extract of *Zingiber officinale* rhizome (GC- MS study)

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area%	Height%	A/H	Mark	Name
1	5.823	5.758	5.867	5.87	9.08	2.96		Butane, 1,1-diethoxy-2-methyl-
2	7.509	7.467	7.567	3.13	6.49	2.21		Decane, 3,7-dimethyl-
3	7.880	7.800	7.917	3.07	2.74	5.13		Propane, 1,1,3-triethoxy-
4	11.035	10.992	11.117	1.97	2.78	3.26		Decane, 3,7-dimethyl-
5	15.581	15.525	15.717	13.85	12.43	5.11		Phthalic acid, di-(1-hexen-5-yl) ester
6	18.229	18.183	18.292	4.60	6.49	3.25	V	Pentadecanal-
7	18.333	18.292	18.408	2.16	2.50	3.96	V	1,3-Dimethylcyclopentanol
8	18.720	18.542	18.758	2.60	3.94	3.03	V	2-Octylcyclopropene-1-heptanol
9	19.117	19.033	19.133	1.77	1.96	4.13		Methylphosphonic acid, fluoroanhydride, 3-cy
10	19.168	19.133	19.308	3.74	2.82	6.10	V	Cyclohexanol, 1-ethyl-
11	19.757	19.700	19.783	4.75	5.66	3.85	V	(Z)-2-(Hex-3-enyloxycarbonyl)benzoic acid
12	19.885	19.783	19.958	10.33	6.70	7.06	V	HEXADECANOIC ACID, ETHYL ESTER
13	20.092	19.983	20.117	2.71	1.56	7.95	V	Timonacic
14	20.884	20.792	20.958	3.45	2.33	6.78		Pentadecanal-
15	21.085	20.958	21.117	2.04	1.22	7.64	V	9,12,15-Octadecatrienoic acid, 2-[(trimethylsil
16	21.183	21.117	21.342	19.85	21.60	4.21		Phytol
17	21.867	21.775	21.883	1.77	1.09	7.40	V	1H-IMIDAZOLE-1-ACETAMIDE, 2-METH
18	25.067	25.033	25.308	1.66	1.07	7.12		3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-tetrame
19	25.825	25.800	26.067	1.61	1.27	5.82		1,2-Bis(trimethylsilyl)benzene
20	28.202	28.133	28.317	1.71	1.24	6.33	V	TETRASILOXANE, DECAMETHYL-
21	28.707	28.575	28.767	7.37	5.04	6.69		1,2-BENZENEDICARBOXYLIC ACID, DIN
				100.00	100.00			

Table 3 Biological Activity of phyto-components identified in the ethanolic extracts of the *Zingiber officinale* rhizome by GC-MS.

S.NO.	R.Time	Name of the compound	Biological activity **
1.	18.720	2-Octylcyclopropene-1-heptanol,	Antibacterial activity
2.	19.885	Hexadecanoic acid ethyl ester,	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor
3.	20.092	Timonacic	Antineoplastic and antioxidant activities
4.	21.183	Phytol	Antibacterial, **Anticancer**, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic
5.	21.085	9,12-Octadecadienoic Acid	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic,Antiacne
6.	28.707	1,2-Benzenedicarboxylic acid,	Antibacterial, Antifouling

****Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database.**

Karpagasundari and Kulothungan, (2014) screened the bioactive components of *Physalisminima* leaves have been evaluated using GC-MS. GC-MS analysis of extract of *Physalisminima* leaves revealed the existence of heneicosanoic acid (25.22), bicyclo [4.1.0] hepta-2, 4-dien (27.41) octadecanoic acid (CAS), stearic acid (31.19) and octadeca-9, 12-dienoic acid (32.02).

Similarly the work was done by GC-MS analysis of bioactive components of *Hugonia mystax L*. (Linaceae). Thirteen compounds were identified. 1,2-benzene dicarboxylic acid, diisooctyl ester (48.75 %) was found to be major component followed by n-hexadecanoic acid (13.52 %), phytol (9.25 %), squalene (6.41 %), vitamin E (4.09 %), dianhydromannitol (3.56 %), 9,12–octadecadienoic acid (Z,Z)–(3.20%) and 3,7,11,15 – tetramethyl -2- hexadecen -1-ol (2.85 %) .

Antibacterial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antibacterial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003).

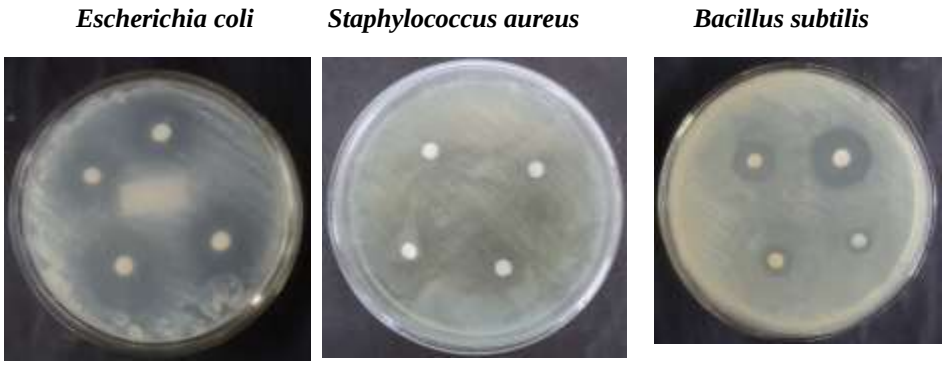
This situation forced scientists to search for new antibacterial substances with plant origin. Plant extract of *Zingiber officinale* was screened against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* species of bacteria were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antibacterial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antibacterial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antibacterial activity of the *Zingiber officinale* rhizome extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic plate 4. The inhibitory activities in culture media of the *Candida albicans* reported in Table 4 were comparable with standard antimicrobiotic viz. chloramphenicol.

Table: 4 Antibacterial activities of *Zingiber officinale* rhizome

Microbial Organism	50µl	100 µl	150 µl	Standard
Escherichia coli (mm)	7.50±0.52	8.50±0.59	9.25±0.64	8.50±0.59
Staphylococcus aureus (mm)	9.75±0.68	10.23±0.71	13.10±0.91	11.00±0.77
Bacillus subtilis (mm)	9.75±0.68	10.75±0.75	11.75±0.82	10.75±0.75

Values were expressed as Mean ± SD.
Bacterial standard - Chloramphenicol
Fungal standard - Fluconazole

Plate.4: Antibacterial activity of *Zingiber officinale* rhizome



IN SILICO DOCKING ANALYSIS OF PHYTOL AGAINST C-JUN NH2-TERMINAL KINASES (JNKs)

Molecular docking and modeling studies improve the reliability, accuracy of biological test and show possible interactions between molecules and their target receptors. So in present study Ascorbic acid, Phytol, Caryophyllene and Tetradecanoic acid identified by GC-MS study were subjected to molecular docking for better recognition of their interaction with c-Jun NH2-terminal kinases (JNKs).

Identification and screening of bioactive molecules and their binding sites in the protein are challenging for drug development. Therefore, we planned to find out the binding ability of Phytol with JNK using computational biology tools. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 4. Hydrogen bond was indicated by dashed lines in green between atoms involved and rest of the interactions were hydrophobic. Phytol was found be -7.48 Kcal/mol. From the *in silico* docking results, it is quite evident that plant-derived compounds have the great potential against anti-cancer activity of cancer mediator protein JNK.

Molecular docking and modeling studies improve the reliability, accuracy of biological test and show possible interactions between molecules and their target receptors. So in present study Phytol identified by GC-MS study were subjected to molecular docking for better recognition of their interaction with c-Jun NH2-terminal kinases (JNKs).

Table 5. Docking results of Plant derived compounds against Jun NH2-terminal kinases Enzyme

Sl.No.	Ligands	Moleccular formula	Molecular Weight [g/mol]	Hydrogen donor	Hydrogen acceptor	Docking Energy Level (*kcal/mol*)
2.	Phytol	$C_{20}H_{40}O$	296.53	01	01	-8.48

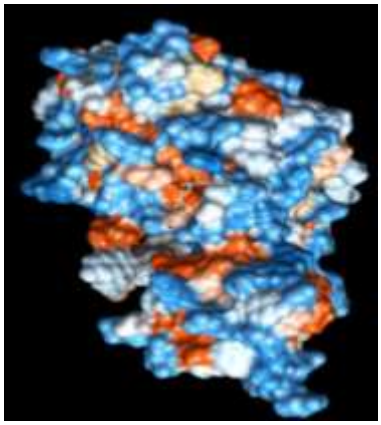


Fig.3: Electrostatic surface of JNK alongside of the amino acids motif with Ascorbic acid and Phytol

In silico molecular docking in one of the most powerful techniques to discover novel ligand for proteins of known structure and thus play key role in structure based drug. Investigators often use docking computer programs to find the binding affinity for molecules that fit a binding site on the protein. Hence in this present work we have carried out *in silico* molecules docking to analyze the binding properties of the mediator called JNK with Phytol compound reported from *Furcraea foetida* leaf. The wet analysis carried out by us showed very good result with regard to anti-cancer property of this plant extract. So the present study may act as supportive evidence that substantiate property of this plant extract. Phytol has potential inhibiting ability was identified from this plant with JNK.

CONCLUSION

Results obtained from this study, indicated that, the *Zingiber officinale* rhizome extracts showed the strongest antibacterial activity. Overall, the *Zingiber officinale* rhizome are a rich source of phytochemicals and potential antibacterial activity that can be important in infectious disease prevention and health preservation. The *in silico* studies confirmed the anticancer activity of *Zingiber officinale* rhizome extract.

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