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Analysis of Bioactive Compounds in Methanol Extract of *Globba Winitii* Rhizome Using GC-MS

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Abstract

The objective of this study was to determine the bioactive compounds from the methanolic extract of *Globba Winitii* rhizome and evaluate its biological activity by GC-MS analysis. The chemical constituents in this methanolic extract were subjected to the Perkin-Elmer Gas Chromatography - Mass Spectrometric analysis. Fifteen bioactive chemical compounds have been identified in the plant extract. This identification was based on the peak area, retention time, molecular weight and molecular formula. In this investigation the compounds like Cyclohexane, 1-Ethenyl – 1 – Methyl - 2, 4 – bis (1 – Methylethenyl) - 2, 4 - diisopropenyl -1- Methyl - 1 - Vinylcyclohexane, 5 – Oxatricyclo - [8.2.0.0 (4,6)] -Dodecane, KW3 Aus Epiglobulol, Bicyclo [5.3.0] nonane, 1,4 – Dimethyl - 3 - (2 - Methyl – 1- Propenyl 1- Cycloheptene, 10,12- Hexa decadien -1- ol, 9,12,15 - Octadecatrienoic acid, Androstan-17- one, 3 – ethyl - 3- hydroxyl - (5α) - 3- Ethyl - 3 - hydroxyandrostan - 17- one, n - Hexadecanoic acid, Doconexent cis - 4, 7, 10, 13, 16, 19 - Docosahexanoic acid and Azuleno [4,5 b] furan – 2 (3H) – one were predominantly found in the *Globba Winitii* rhizome extract.

Keywords: *Globba Winitii*, Bioactive compounds, GC-MS analysis

Full-length article **Corresponding author's* e-mail: madhiriramya2709@gmail.com

1. Introduction

Plants play a major potential role in the production of novel drugs and acts as a prominent natural resource for herbal medicine. In recent years a renewed interest flourished through the ‘’green medicine ‘with safe, non-toxic, less side effects and also available in affordable prices [11]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. [1]. The folk medicine or traditional medicine was now remembered as herbal medicine or phytotherapy treatment. It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problem and arterial hypertension [9].

In current, the herbal medicines were safer than synthetic medicine due to the presence of phytochemicals in the plant extract targeting the biochemical pathway [10]. It must be essential to know the chemical constituents of plant extract because of its biological activity carried in medicinal, poisonous and nutritive fields [4].

The chemical constituents in the plant extract were organized by primary and secondary metabolites which were based on their metabolic roles. The primary metabolism is an important for the growth and development of plants and includes of Sugars, amino acids, nucleic acids, fatty acids, chlorophylls etc., [8]. Secondary metabolites such as alkaloids, flavanoids, tannins, terpenoids, phenolics, steroids, saponiins were responsible for multiple biological effects including antimicrobial, antioxidant, aging, cancer, anti-proliferative, anti-inflammatory and immune modulatory activities. [2]. the separation, identification and structural elucidation of these phytochemicals were successfully carried out by analytical techniques like TLC, UV, NMR and GC-MS in these decades [14]. The aim of this study was to determine the bioactive compounds present in *Globba Winitii* rhizome extract with the aid of GC-MS technique which may provide an insight in its use in traditional medicine.

2. Material and Methods

The rhizome of *Globba Winitii* (Family: Zingiberaceae) were collected in the month of Janauary 2019 from the Koli hills, Tamil Nadu, India. The rhizome of the plant was identified and authenticated by Dr Madhava chetty SVU Tirupathi (0912)V no.

2.1. Preparation of extract

Globba Winitii rhizome was washed several times with distilled water to remove the traces of impurities from the rhizome. The rhizomes were dried at room temperature and finely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used.

2.2. Preliminary Phytochemical screening

The preliminary screening was carried out by using standard procedures described by Treese, Evans and Harborne.

2.3. GC-MS analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µ Mdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µ I was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9 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 36min. 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 Turbo Mass Ver 5.2.0.

2.4. Identification of components

Interpretation on Mass Spectrum 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.

3. Results and Discussion

The preliminary screening test carried on different solvent extracts like methanol, water and 70% methanol. The results showed that the methanolic extract possess the highest contents of bioactive compounds. The healing properties of medicinal plants were depend on the presence of secondary metabolites such as alkaloids, saponins, flavonoids, phenolic compounds, tannins, phytosterol and terpenoids (Britto *et al* 2012) [3]. They were used in analgesic, and anti plasmodic and bacteriocidal activities [18]. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. The presence of phytochemicals is shown on the Table-1. In this present study we observed that phytochemicals like flavonoids, saponins, terpenoids, polysaccharides, and steroids were present in high concentration. Similarly, the following phytochemicals like alkaloids, tannins, proteins, glycosides were moderately found and anthroquinone was absent.

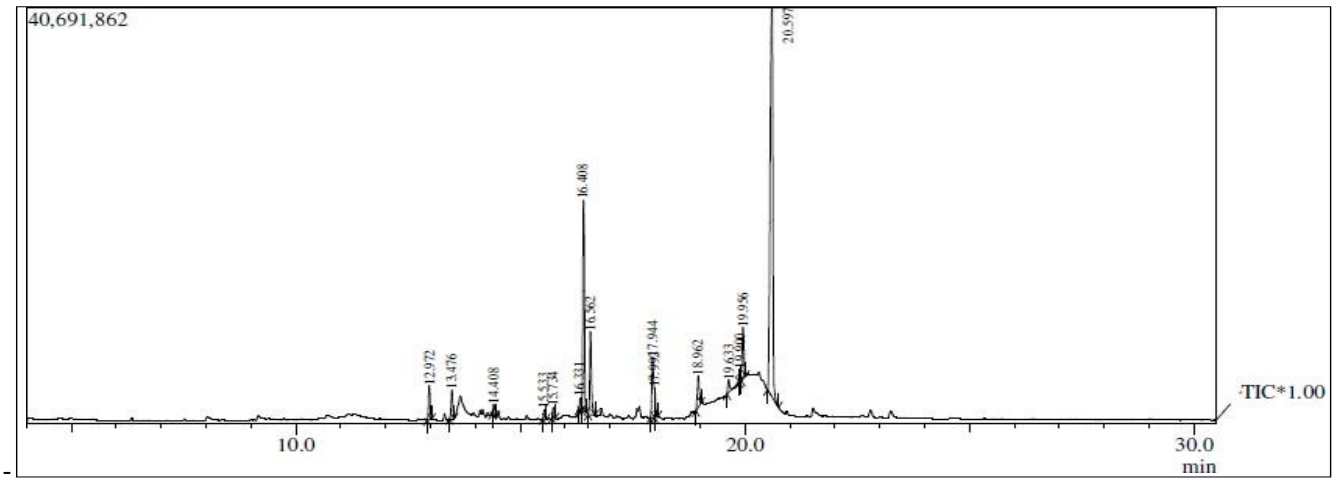
In figure-1 the chromatogram of GC-MS analysis confirmed the presence of fifteen bioactive compounds in the methanolic extract of *Globba Winitii* rhizome. The bioactive compounds were identified and characterized based on the mass spectra by comparing with the spectrum of the known components stored in the NIST and WILEY library. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. [16]. The active compounds in the rhizome extract of Globba Winitii with their Retention Time (RT), Molecular Formula (MF), Molecular Weight (MW), Structure and concentration (%) was given in Table -2. Among the fifteen the most prevailing compounds were KW3 Aus Epiglobulol, 9, 12, 15 - Octadecatrienoic acid and Azuleno [4,5-b] furan-2 (3H) – one. The other compounds like Cyclohexane,1-ethenyl-1-methyl - 2, 4-bis(1-methyl ethenyl) - 2,4-Diisopropenyl - 1- methyl -1-vinyl cyclohexane, Caryophyllene Bicyclo [7.2.0] undec - 4 - ene, Androstan -17-one -3-ethyl-3-hydroxy- (5- α) - 3-Ethyl-3-hydroxyandrostan-17-one, Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl-7-Isopropenyl-1-methyl – 4 - methylene decahydro azulene and Doconexent cis - 4, 7, 10, 13, 16, 19 - Docosahexanoic acid were moderately present. The compounds of 1,4 – Dimethyl –3 - (2 – Methyl – 1 – Propenyl) – 4 – Vinyl – 1 - Cycloheptene 1- Cycloheptene, 5 – Oxa tri cyclo [8.2.0.0 (4, 6)] Dodecane, 10, 12 - Hexadecadien – 1 - ol, n - Hexadecanoic acid and 6 - (Acetyloxy) - 5, 5, 8a – trimethyl – 2 methylenedecahydro -1-naphthalenyl] methyl acetate were present in lower concentration.

The biological activities of compounds listed in Table - 2 were based on Dr. Dukes Phytochemical and Ethnobotanical Data bases by Dr. Jim Duke of the Agricultural Research Service / USDA Similarly, various phytochemicals which contribute their biological activity were presented in Table-3. Similarly the work was done by GC-MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae). Thirteen compounds were identified. 1,2-Benzenedicarboxylic 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%). The presence of various bioactive compounds justifies the use of the leaf for various ailments by traditional practitioners. So, it is recommended as a plant of phytopharmaceutical importance [13]. The GC-MS analysis of Caesalpinia italica leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties.

Table 1: Preliminary Phytochemical screening of *Globba Winitii* rhizome

S. No	Phytochemical	*Globba Winitii* Extract		
		Methanol	**Aqueous**	**70% Methanol**
01	Alkaloids	+	+	+ +
02	Flavonoids	+ +	+ +	+ +
03	Saponins	+ +	+ +	+ +
04	Tannins	+ +	+	+
05	Terpenoids	+	+ +	+ +
06	Proteins	+ +	+	+
07	Polysaccharides	+ +	+ +	+ +
08	Steroids	+ +	+ +	+ +
09	Glycosides	+ +	+ +	+
10	Phlobatanins	+ +	+ +	+ +
11	Triterpenoids	+ +	+ +	+
12	Polyphenols	+	+	+ +
13	Anthroquinone	-	-	-

- Absent, + Present, ++ High concentration



- **Fig 1:** GC-MS Analysis of *Globba Winitii* rhizome extract

Table 2: Phyto components in the *Globba Winitii* rhizome extract

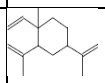
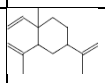
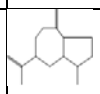
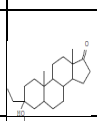
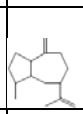
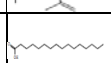
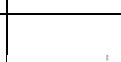
Peak	RT (mins)	Area (%	Name of the Compound	Molecular Formula	Molecular Weight	Chemical Structure
1	12.972	2.50	xane,1-ethenyl - 1-methyl - 2, 4-bis(1-methyl ethenyl) -2,4-Diisopropenyl -1- methyl -1-vinyl cyclohexane	C15H24	204	
2	13.476	2.25	Caryophyllene Bicyclo [7.2.0] undec - 4 -ene	C15H24	204	
3	14.408	0.73	yl-1-Propenyl) – 4 – Vinyl - 1-Cycloheptene 1- Cycloheptene	C15H24	204	
4	15.533	0.63	clohexane, 1-Ethenyl-1-Methyl -2, 4-Bis (1-Methylethenyl) -, [1s-(1α,2β, 4β)] 2, 4-Diisopropenyl-1-Methyl-1-Vinylcyclohexane)	C15H24	204	
5	15.734	0.76	5 - Oxatricyclo [8.2.0.0 (4, 6)] Dodecane,	C15H24O	220	
6	16.331	0.67	10,12-Hexadecadien-1-ol	C16H30O	238	
7	16.408	15.58	9, 12, 15-Octadecatrienoic acid,	C18H30O2	278	
8	16.562	7.19	KW3 Aus Epiglobulol Azulene	C15H24	204	
9	17.944	5.95	7-one -3-ethyl-3-hydroxy- (5-α) - 3-Ethyl-3- hydroxyandrostan-17-or	C21H34 O2	318	
10	17.992	1.54	3, 4, 4a, 5, 6, 7 - octahydro-naphthalen-2-yl)- prop-2-en-1-ol	C15H24O	220	
11	18.962	3.10	o [5.3.0] decane, 2-methylene -5-(1-methylvinyl)-8-methyl- 7-Isopropenyl-1-methyl-4-methylenedecahydroazulene	C15H24	204	
12	19.633	1.29	n-Hexadecanoic acid	C16H32 O2	256	
13	19.900	0.85	6- (Acetyloxy)-5, 5, 8a-trimethyl-2-methylenedecahydro -1-naphthalenyl] methyl acetate	C19H30 O4	322	
14	19.956	4.41	Doconexent cis-4, 7, 10, 13, 16, 19-Docosahexanoic acid	C22H32 O2	328	
15	20.597	52.57	Azuleno [4,5-b] furan – 2 (3H) - one	C15H18 O2	230	

Table 3: Pharmacological activity of components in the *Globba Winitii* rhizome extract

S.No	Name of the Compound	Pharmacological activity
01	Cyclohexane,1-ethenyl - 1- meth - 2, 4-bis(1-methyl ethenyl) -2,4-Diisopropenyl -1-methyl -1-vinyl cyclohexane	se-Inhibitor, A Methyl-Donor, A Methyl-Guanidine- Inhibitor.
02	ricyclo [8.2.0.0 (4, 6)] Dodecane,	Smart-Drug, (+)-Inotropic, (-)-Chronotropic, (-)-Inotropic, 11B-HSD-Inhibitor, 1 Lipoxygenase-Inhibitor, 17-beta-hydroxysteroid dehydrogenase-Inhibitor, 5-Alph Reductase-Inhibitor, 5-HETE-Inhibitor, 5-HT-Inhibitor, 5-Lipoxygenase-Inhibitor, 8-HETE-Inhibitor, Anti Allergenic, ABeta-Inhibitor,, Acantholytic, Acaricide,
03	10,12-Hexadecadien-1-ol	Oligosaccharide Provider.
04	9, 12, 15-Octadecatrienoic acid,	bitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric acid.
05	Androstan-17-one -3-ethyl-3-ydroxy-(5.alpha.)- 3-Ethyl-3-hydroxyandrostan-17-one	steroid dehydrogenase-Inhibitor, Aryl-Hydrocarbon-Hydroxylase- Inhibitor, Testosterone Hydroxylase-Inducer.
06	Bicyclo [5.3.0] decane, 2-methylene -5-(1-methylvinyl)- 8-methyl- 7-Isopropenyl-1- methyl-4- methylenedecahydroazulene	Anticancer (Mammary & Mouth), Antidote (Heavy Metals, Manganese, Mercury, Morphine, Muscarine, Mushroom & Methtrexate), Antitumor (Mammary & Mouth), Catechol -Methyltransferase –Inhibitor, Improve Microcirculation, Increase Alpha - Mannosidase Activity, Magnesium & Manganese Bioavailability, Inhibit Malonyl Co - A Interleukin – 6 – mRNA - Inhibitor, Maltase -Promoter, Mannosidase - Inhibitor, Inhibitor (MAO, MAO - A, MAO - B & MAPK), Marrow -Suppresant, Mast - Cell, Mastogenic, Inhibitor MDR&MDR – Efflux Pump), Melaninogenic, Melatoninergicel atoninogenic Membrano lytic, Membranomo dulator,Memorigenic Memorilytic, Memor - Enhancer, Memranolytic, Metal -Chelator (Copper), Metalloproteinase - Inhibitor, Metastatic, Methyl – Donor,Methyl – Guanidine -Inhibitor, Microphago cytogenic, Microva sculokinetic, Mineral- corticoid, Miotic, Mitochondriaprotective, Mitochondria stimulant, Mitotic, Inhibitor - (MMP - 2, MMP - 7 & MMP - 9), Moisturizing, Molluscicide, Molluscifuge, Mollusciphile, Monoamine - Precursor, Monoaminergic, Monooxygenase - Inhibitor, Mosquitocide, Mosquitofuge, Motor - Depressant, Motor - Stimulant, Mucogenic, Mucoirritant, Mucokinetic, Mucolytic, Mucoregulator, Mucotropic, Muscarinic -Agonist, Musculotropic, Mutagenic, Anti Mutagenic, Mycardio depressant, Myco bactericide, Mycolytic, Mycoplasmicide, Mycoplasmistat, Mycorelaxant, Mydriatic, Myelinogenic, Myelosuppressant, Myo-neuro-stimulant, Myocardiocontractant, Myocardiostimulant, Myocardiotonic, Myocontractant, Myodepressant, Myolytic, Myoneuro - stimulant, Myoparalytic.
07	n-Hexadecanoic acid	Arachidonic acid-Inhibitor Arachidonic – Acid - Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid,Anaphylactic (antidote = Neostigmine), Antitumor (Na sopharynx), Arylamine – N – Acetyl transferase - Inhibitor Decrease Norepinephrine Production, Down regulation of nuclear and cytosol androgen, GABA-adrenergic, Increase natural killer cell activity, Inhibit Production of Tumor-Necrosis-Factor, Myo-neuro-stimulant.
08	KW3 Aus Epiglobulol Azulene	Anti Allergenic, Alpha-Agonist, Alpha - Amylase-Inhibitor, Alpha - Glucosidase-Inhibitor, Alpha – Reductase -Inhibitor.
09	Azuleno [4,5-b] furan – 2 (3H) - one	Alpha – Reductase - Inhibitor, Anti Allergenic, Beta – Inhibitor, ACE-Inhibitor, Acetyl choline - antagonist, Acetyl-Co**A** – Carboxylase - Inhibitor, Acetylcholinergic, AChE – Inhibtor, Activities similar to Sanguinarine, Adaptogen, Adaptogenic, Addictive, Additiv Adenosine - Antagonist, Adenylate – Cyclase - Activator, Anti Adenylate –Cyclase - Activator, Adenylate – Cyclase - Inhibitor, Adjuvant, Adrenal - Support, Adrenalin - Pressor, Adrenalytic, Adrenergic, Adrenergic - Blocker, Adrenocorticotrophic, Adrenolitic, AHH - Inhibitor, Akt – Inhibitor, Alcohol – Dehydrogenase - Inhibitor, Aldehyde – Oxidase - Inhibitor, Aldose – Reductase –Inhibitor, Anti Allergenic, Allergic Alpha - Agonist, Alpha – Amylase - Inhibitor, Alpha – Glucosidase – Inhibitor, Anti Amphiglycemic, Amphitensive, Anabolic, Analeptic, Analgesic, Analgesic - Synergist, Anaphrodisiac, Anaphylactic, Anaphylactic (antidote = Neostigmine), Ancylostomicide, Anesthetic, Anesthetic - potentiator, Angiogenic, Angiotensin – Receptor - Blocker, Anhidrotic, Anhydrotic, Anti Anorectic, Anti- Repellent.

For instance, 9,12,15- Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid possesses anti- inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n- Hexadecanoic acid - palmitic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol- Diterpene is an antimicrobial, anticancer, antiinflammatory and diuretic agent (Praveen kumar *et al*., 2010). 9, 12, 15- Octadecatrienoic acid, methyl ester, (Z, Z, Z)-, n- Hexadecanoic acid, 1, 2-Benzenedicarboxylic acid and di-isooctyl ester were present in Caesalpinia sappan ethanol extract [15]. Similar types of compounds were identified among the fifteen compounds of this present study.

4. Conclusions

The results obtained in this study thus suggest that the identified phytochemicals are pharmacologically active compounds. Therefore the data generated from this experiment provide the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. This study offers a platform for using *Globba Winitii* rhizome as herbal alternative for emerging diseases like diabetes, cancer, microbial infection, cardiovascular and inflammatory etc.

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