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GC-MS STUDIES ON TRADITIONAL PLANT *ALLIUM FISTULOSUM L.* BULBS

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ABSTRACT

Plants are used medicinally in different countries and they are the source of many potent drugs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. The aim of this study was identification of bioactive compounds from the bulbs of ethanolic extract of *Allium fistulosum* by Gas chromatography and Mass spectrometry (GC-MS). GC-MS analysis of ethanolic extract was done by standard protocol using the equipment Perkin Elmer clarus 600 (EI) TurboMass ver 5.4.2. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra. The phytoconstituents present in the ethanolic extract were studied. The GC-MS analysis of the ethanolic extract revealed the presence of 31 compounds. The compounds present in this crude extract were found to be flavonoids, terpenoids, fatty acids, fatty alcohols and sulphur containing compounds. It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects.

KEYWORDS: *Allium fistulosum*, Thin layer chromatography (TLC), GC- MS analysis.

INTRODUCTION

For millennia, people around the world have healed the sick with herbal derived remedies and handed down through generations. Traditional medicine is the sum of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness.[1,2] Ayurveda stresses the use of plant-based medicines and treatments. It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life

style new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. Potentially valuable treasures in medicinal plants remain unexplored. By considering the scope of these medicinal plants we have to use more amounts of time and resources in developing medicines from medicinal plants. If we can come back to our nature, culture and tradition on use of medicinal plants it can bring up a bright and healthy new generation.[3] Most herbal medicines and their derivative products were often prepared from crude plant extracts, which comprises a complex mixture of different phytochemical constituents (plant secondary metabolites). Medicinal plants are at great interest to the researcher, as most of the drug industries depend in medicinal plants for the production of pharmaceutical compounds. They are used as pharmaceutical biochemicals, fragrances, food colours and flavours in different countries especially in India.

The chemical features of these constituents differ considerably among different species. Gas Chromatography Mass Spectrometry, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. It is used for the analysis of obtained extracts for determining the amount of active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry. The aim of this study was identification of bioactive compounds from the ethanolic bulb extract of *Allium fistulosum* by Gas chromatography and Mass spectrometry (GC-MS). GC-MS analysis of ethanolic extract was done by standard protocol using the equipment Perkin Elmer clarus 600 (EI) TurboMass ver 5.4.2. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.[4]

Allium fistulosum L. is a monocot perennial herb which was widely cultivated throughout the world from tropical Asia to Siberia, particularly in Japan, Korea and China. This species is known as Welsh onion derived from the German word '*welshche'* meaning 'foreign'. It is used as a Chinese folk medicine for treating febrile disease, headache, abdominal pain, diarrhoea, eye related disorders and habitual abortion. The bulb contains an essential oil that is rich in sulphur compounds. It is a promising source of bioactive moieties such as quercetin and flavonoids that exhibits various biological activities such as anticancer, antioxidant, anti-microbial, anti-platelet, anti-diabetic, anti-inflammatory, anti-asthmatic, anti-thrombotic, hypolipidaemic and anti-hypertensive.[5] In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species. To explore its medicinal

importance, the bulbs of *Allium fistulosum* .L were screened primarily for the phytochemicals present in it and was analyzed using GC-MS. The present study helps to predict the formula and structure of biomolecules present in ethanolic bulb extract of *Allium fistulosum*.

MATERIALS AND METHODS

I. Extraction

a. Collection and Authentication of Plants

The plant *Allium fistulosum L.* was collected from Chennai in the month of August 2017. The plant was identified and authenticated by DR. JAYARAMAN, Plant Anatomy Research Centre at Tambaram, Chennai.

b. Preparation Of Extracts:

The bulbs of the plant were cleaned, shade dried for about 2 weeks. The dried bulbs were pulverized to a coarse powder by grinding in mixer and stored in an air tight container.

Extraction is the preliminary step involved in the phytochemical studies. It is the separation of medicinally active portions of plant using selective solvents through standard procedures.

Hot Percolation Method

30g of the dried coarsely powdered plant material of *Allium fistulosum* L. was extracted with solvent of high polarity (Ethanol) using soxhlet apparatus at 60^0-70^0 for 18hrs, until the solvent become colourless in the siphon tube. The extracts were concentrated by rotary evaporator. The semisolid residue obtained was collected and stored in desiccators.

c. Chromatographic Studies[6-16]

Thin Layer Chromatography

Principle

Thin layer chromatography is based on adsorption principle in which separation depends on the selective adsorption of the components of a mixture on the surface of solid. The stationary phase was in the form of thin layer of silica gel adhering to a backing material. Through capillary action, mobile phase moves along the stationary phase. The compound with high affinity towards the stationary phase moves slower and the compound with less affinity towards stationary phase moves faster.

Materials Required

Readymade TLC plates, Beaker, UV chamber, Iodine Chamber, capillary tube, watch glass.

Solvent System: Mobile phase: Hexane: Acetone (4:1)

Stationary phase: Silica gel

Procedure

The solvent system was prepared and kept aside for saturation. The readymade precoated TLC plates were cut into desired size. Spot the fraction above 1 cm from the bottom of the TLC plate using the capillary tube and allow the chromatogram to develop in solvent system tank (TLC chamber). The developed chromatogram was observed under UV at shorter and longer wavelength and it was photographed.

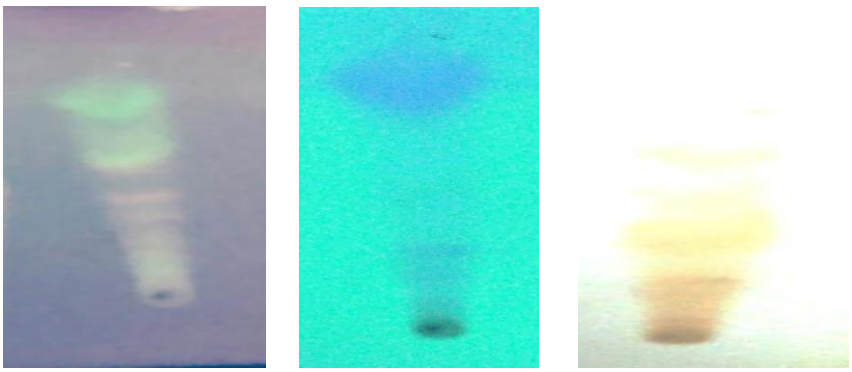
i. GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

RESULTS AND DISCUSSION

Thin layer chromatography of Ethanolic extract of *Allium fistulosum* (EEAF):

The TLC for EEAF was performed and its Rf value was mentioned in the Table 1.



visible light Long UV – 365nm Short UV – 254nm

Figure 1: TLC of EEAF.

Table 1: Thin layer chromatography of Ethanolic extract of *Allium fistulosum* (EEAF)

TLC of EEAF	R_f value
Long UV – 365nm	0.8, 0.72, 0.6, 0.5, 0.44, 0.36, 0.1
Short UV – 254nm	0.12, 0.24, 0.32, 0.5, 0.7
visible light	0.7, 0.56, 0.4, 0.24

GC-MS OF EEAF

The GC-MS analysis of ethanolic bulb extract of *Allium fistulosum L.* was performed using Perkin Elmer Elite - 5 capillary column and typical total ion chromatograms (TIC) of each sample were given in fig 2 respectively. The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in [Figure 2]. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments breaks into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The composition determined for this ethanolic extract corresponds to 100% of the entire GC-MS chromatogram. The comparison of the mass spectrums with the data base will give more than 90% match as well as confirmatory compound structure match.

The results pertaining to GC-MS of the EEAF lead to the identification of number of compounds. The various components present in bulbs of *Allium fistulosum L.* were detected by the GC-MS was shown in Table 2.

SPECTRUM

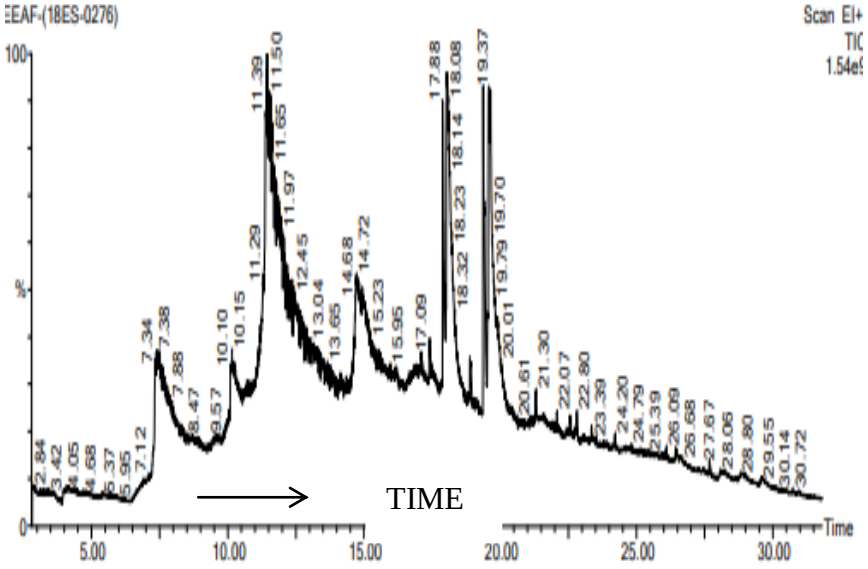
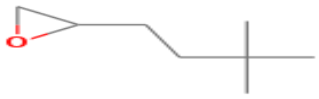
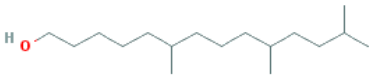
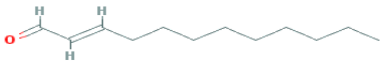
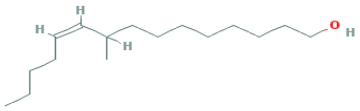
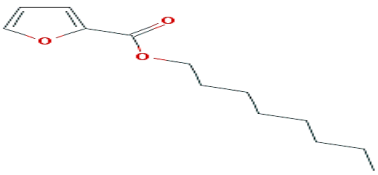
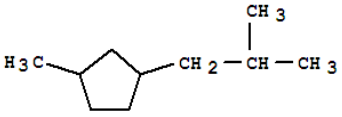
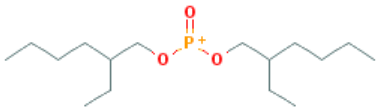
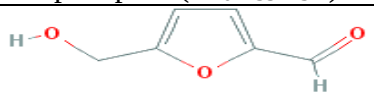
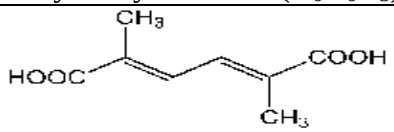
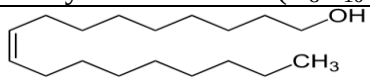
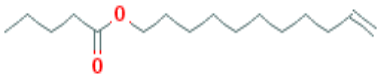
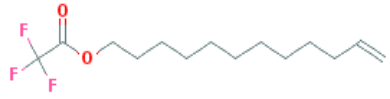
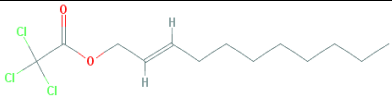
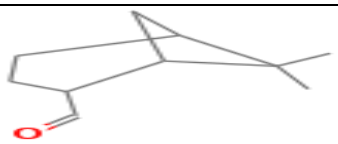
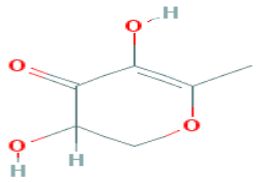
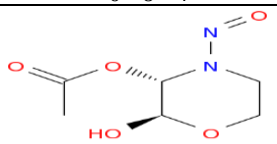
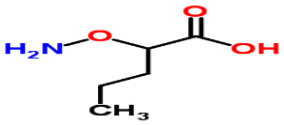
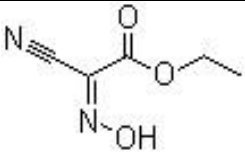
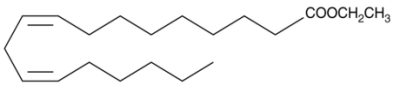
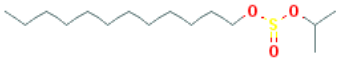
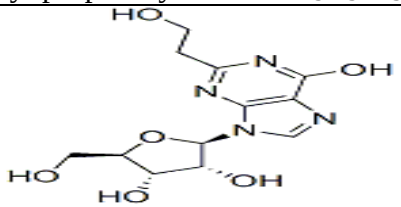
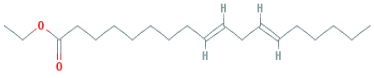


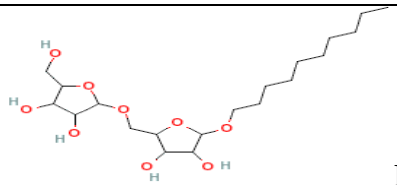
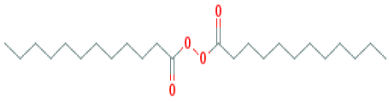
Figure 2: Total Ion Chromatogram of ethanolic extract of bulbs of *Allium fistulosum L.*

Table 2: Compounds identified in the EEAF in GC-MS.

S.NO	Retention Time	Name of the Compound	Structure, Synonym and molecular formula	M.W	Peak area %
1.	7.450	Oxirane, (3,3- dimethyl butyl	 3,3-dimethyl-1,2-epoxybutane($C_8H_{16}O$)	128	17.32
2.	7.450	6,10,13- Trimethyl tetradecanol	 6,10,13- trimethyl1-tetradecanol($C_{17}H_{36}O$)	256	17.32
3.	7.450	Trans-2-dodecen-1-ol	 Pentyl2-methyl butyrate, dodecenol($C_{12}H_{24}O$)	184	17.32
4.	7.450	2(3H)-Furanone, 3-(15-Hexadecynylidene)dihydro-4-hydroxy-5-methyl-	(3Z)-3-(hexadec-15-yn-1-ylidene)-4-hydroxy-5-methyloxolan2-one($C_{21}H_{34}O_3$)	334	17.32
5.	7.450	3-methyl silacyclopent-3-ene,	 1-Methyl-2,5- dihydro-1H-Silole($C_5H_{10}Si$)	98	17.32
6.	7.450	9-methyl-Z-10-pentadecen-1-ol	 $C_{16}H_{32}0$	240	17.32
7.	7.450	2-ethylhexyl -2-Furancarboxylic acid, ester	 Octyl furan2-carboxylate($C_{13}H_{20}O_3$)	224	17.32
8.	7.450	1-Methyl-3-(2-methyl propyl)-cyclopentane	 1-methyl -3-isobutyl cyclopentane($C_{10}H_{20}$)	140	17.32

9.	7.450	Bis(2-ethylhexyl) hydrogen phosphite	 Di-2-ethylhexyl hydrogen phosphite($C_{16}H_{35}O_3P$)	306	17.32
10.	11.567	5-Hydroxymethyl 2-furan carboxaldehyde	 5-oxymethylfurfurole ($C_6H_6O_3$)	126	15.50
11.	11.567	2,5 Dimethyl-2,4-hexadienedioic acid	 Dimethyl muconic acid ($C_8H_{10}O_4$)	170	15.50
12.	19.64	Oleyl alcohol	 Octadecenol($C_{18}H_{36}O$)	268	10.96
13.	19.64	1,19, Eicosadiene	 1,19- Icosadiene($C_{20}H_{38}$)	278	10.96
14.	19.64	17-octadecynoic acid	 Alkynyl stearic acid($C_{18}H_{32}O_2$)	280	10.96
15.	19.64	10-Undecenylpentanoic acid	 Undec-10-enyl pentanoate ($C_{16}H_{30}O_2$)	254	10.96
16.	19.64	1-Eicosyne	 Icos-1-yne ($C_{20}H_{38}$)	278	10.96
17.	19.64	Cis -9- eicose-1-ol	 Gadoleyl alcohol($C_{20}H_{40}O$)	296	10.96
18.	19.64	11-Dodecen-1-ol Trifluoroacetate	 $C_{14}H_{23}O_2F_3$	280	10.96
19.	19.64	1,16-Hexadecanediol	 Hexadecamethylene glycol$C_{16}H_{34}O_2$	258	10.96
20.	19.64	Undec-10-enyl trichloroacetic acid	 10, undecenyl trichloroacetate - $C_{13}H_{21}O_2Cl_3$	314	10.96

21.	7.45	Bicyclo[3.1.1]heptane-2-carboxaldehyde, 6,6-dimethyl-	 Myrtanal - $C_{10}H_{16}O$	152	17.32
22.	10.151	4H –Pyran—4-one 2,3-dihydro 3,5-dihydroxy-6-methyl,	 $C_6H_8O_4$	144	4.88
23.	10.151	trans-4-Nitroso-2-3-morpholindiol-3-acetate	 $C_6H_{10}N_2O_5$	190	4.88
24	10.15	Pentanoic acid, 2-(aminoxy)	 2- aminoxy valeric acid- ($C_5H_{11}NO_3$)	133	4.88
25	11.47	Ethyl cyanoglyoxylate 2-oxime	 Ethyl 2-cyano 2-hydroxyamino acetate - $C_5H_6N_2O_3$	142	4.88
26	19.36	Linoleic acid ethyl	 Telfairic acid ethyl ester-$C_{20}H_{36}O_2$	308	9.87
27	14.93	Sulfurous acid, dodecyl 2-propyl ester	 dod ecyl propan2-yl sulfite-$C_{15}H_{32}O_3S$	292	4.09
28	14.93	2-[2-Hydroxyethyl]-9-[β -d-ribofuranosyl]hypoxanthine	 $C_{12}H_{16}N_4O_6$	212	. 0.4.09
29	14.93	9,12, Octadecadienoic acid, ethyl	 Linolelaidic acid ethyl ester - $C_{20}H_{36}O_2$	308	4.09

30	14.93	β -d-lyxofuranoside, 5-o-(β-d-lyxofuranosyl)-decyl	 Dec yl 5-o- pentofuranosyl pentofuranoside - $C_{20}H_{38}O_9$	422	4.09
31	14.93	Lauroyl peroxide	 Laurydol- $C_{24}H_{46}O_4$	398	4.09

The GC-MS analysis revealed the presence of various compounds like (3,3- dimethyl butyl) oxirane, 6,10,13-Trimethyl tetradecanol, Trans-2-dodecen-1-ol, 3-(15-Hexadecynylidene)dihydro-4-hydroxy-5-methyl-2(3H)-Furanone, 3-methyl silacyclopent-3-ene, 9-methyl-Z-10-pentadecen-1-ol 2-ethylhexyl -2-Furancarboxylic acid(ester), 1-Methyl-3-(2-methyl propyl)-cyclopentane, 1,16-Hexadecanediol, Undec-10-enyl trichloroacetic acid, 6,6-dimethyl-bicyclo[3.1.1]heptane-2-carboxaldehyde, 5-Hydroxymethyl 2-furan carboxaldehyde, Bis(2-ethylhexyl) hydrogen phosphite, 2,5 Dimethyl-2,4-hexadienedioic acid, Oleyl alcohol, 1,19, Eicosadiene, 17-octadecynoic acid, 10-Undecenylpentanoic acid, 1-Eicosyne, Cis -9- eicose-1-ol, 11-Dodecen-1-ol Trifluoroacetate, 2,3-dihydro 3,5-dihydroxy-6-methyl 4H-Pyran-4-one, trans-4-Nitroso-2-3-morpholindiol-3-acetate, 2-(aminoxy) Pentanoic acid, Ethyl cyanoglyoxylate 2-oxime, ethyl Linoleic acid, dodecyl 2-propyl Sulfurous acid (ester), 2-[2-Hydroxyethyl]-9-[beta-d-ribofuranosyl]hypoxanthine, 9,12, Octadecadienoic acid, 5-O-(β-d-lyxofuranosyl)-decyl ethyl β-d-lyxofuranoside, Lauroyl peroxide in the ethanolic extract of *Allium fistulosum*. The GC-MS analysis of the concentrated ethanol extract resulted many compounds which have diverse use. Hence, the *Allium fistulosum* may have chemopreventive, anticancer, anti-microbial activity, antioxidant and antidiabetic activity, anti-inflammatory, antibacterial, antifungal, skin conditioning properties due to the presence of secondary metabolites in the ethanolic extract. The compound 2,3-dihydro 3,5-dihydroxy-6-methyl 4H –Pyran-4-one is having antibacterial, antifungal properties and it inhibits melanin production. Anti-inflammatory compounds like Hexadecanoic acid, fragrance and flavouring agents such as 2-octenoic acid, pentadecanoic acid etc. were identified. Due to the presence of esters, it can be used as a flavouring agent in food industries. The concentrated ethanol extract contains a variety of fatty acids.

Terpenoids are an important part of volatiles from plants which were well known as preservative in food, drugs and cosmetics, has been tested *invitro* for antibacterial, antifungal

activity. It is also suggested as potential anti-carcinogenic agent exhibit cytotoxic activity against several solid tumour cell lines.[9-10] The compounds identified possess many biological properties for instance, 9,12-Octadecadienoic acid (Z,Z) – Linolenic acid and 9-Octadecenoic acid (Z)-,methyl ester, a fatty acid ester possess anti-inflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, antiarthritic, antieczemic, 5-alpha reductase inhibitor, antiandrogenic, anticoronary properties. n-hexadecanoic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic, 5-Alpha reductase inhibitors. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata*[17] and *Melissa officinalis*.[18] Parasuraman *et al*.,[19] identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* The ethanol extract contains hexadecanoic acid which has anti-inflammatory activity, flavouring agents like pentadecanoic acid, linoleic acid, laureayl peroxide which is a skin conditioning agent.

These findings support the traditional use of *Allium fistulosum* and further investigation may lead to isolation and their structural elucidation of active principle are needed to elucidate their exact mechanism of action and screening of pharmacological activity will be helpful for further drug development in various disorders. This study explores the goodness of the bulb of the plant *Allium fistulosum* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

CONCLUSION

The presence of various bio-active compounds detected after GC-MS analysis using the ethanolic extract of *Allium fistulosum* justifies the use of plant for various ailments by traditional practitioner. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. It will open a new area of investigation of individual components and their pharmacological potency. From the results, it can be recommended as a plant of phyto-pharmaceutical importance and as a potential source for new drugs.

CONFLICT OF INTEREST: None

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