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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS OF BIOACTIVE COMPOUNDS EXTRACTED FROM THE PLANT GLYCOSMIS PENTAPHYLLA RETZ. USING VARIOUS SOLVENTS

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ABSTRACT

Objective: The main motive behind this work is to study the individual phyto compounds present in *Glycosmis pentaphylla* Retz. commonly known as Orange berry through gas chromatography-mass spectroscopy (GC-MS) analysis. The plant contains secondary metabolites such as alkaloids, flavonoids, glycosides, terpenoids, phenolic compounds, and coumarins. However, there was a lack of proper information regarding the chemistry of these phytocompounds specifically.

Methods: The above objective was achieved through GC-MS method, both the ethanolic and hexane extract were prepared by maceration method. Then subjected to GC-MS analysis where the extract passed through fused silica column. Using the chromatogram the score, reverse match score and probability percentage were matched based on NIST and WILLEY library databases.

Results: The compounds in the ethanolic extract being Octadecyl 2-pentanyl sulfite, 4-amino-1-(4-fluorophenyl)pentan-1-one, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Ethyl hexadecanoate, Phytol, 11-Dodecyn-1-ol acetate, Acetyleugenol, and in the hexane extract were 1- nitro-2-propanol, Butyraldehyde semicarbazone, 4- hydrazino-5-hydroxyimino-4,5,6,7-tetrahydrobenzofuran, 3-cyclohexene-1-ethanol, 9-cyclohptadecen-1-one, N-methyl-2-nitropyridin-3-amine, 3-methyl-1,3-pentadiene, 4,7,8-trimethoxy-2,3-dihydrofuro[2,3-b]quinoline, 9,12-octadecadienoyl chloride, dihydromyrcene, and octadeca-9,12-dien-1-ol. These were noted along with their structure, molecular weight, chemical nature, etc., and displayed in the table. Furthermore, the theoretical action of the compounds was studied. Herewith, the presence of many potential compounds in the plant-*G. pentaphylla* was revealed.

Conclusion: From the observations, it is obvious that ethanolic extract has more compounds than the hexane extract of the plant *G. pentaphylla* Retz. having medicinal value. It supports the activity-based studies in near future. After thorough analysis, these plant derived medicaments were found to have promising therapeutic potential as reported in various databases. Hence it can lead to the discovery of newer phytocompounds.

Keywords: Chromatogram, Gas chromatography-mass spectroscopy, *Glycosmis pentaphylla*, Lead compounds, Phytocompounds.

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INTRODUCTION

Herbals are a major source of many medicines but these are on the verge of extinction; hence, the discovery of lead compounds in phytochemical extracts is an urgent need [1]. Moreover, there is a shred of evidence from ancient medicinal writings indicating herbals as a stock house of therapeutic agents that can pave the pathway toward new drug discovery. To be precise, many modern medicines available in the market today are derived from medicinal plants only.

The property of herbals depends on the biochemical compounds labeled as secondary metabolites [2]. Plant toxicity studies facilitate comprehending plant toxicity and thereby protecting humans and animals from natural poisons [3].

These bioactive molecules with their peculiar structure and properties can contribute to curing a lot of risky ailments such as snake bite, poisoning, and chemotherapy, gas chromatography-mass spectroscopy (GC-MS) is the most obvious technique for the structurally complex components in plant extracts [4,5]. The folklore remedies can be analyzed scientifically using techniques such as GC-MS which can analyze a wide range of compounds from alkaloids, flavonoids, fatty acids, lipids, essential oils, as well as nonpolar compounds [6].

Asian countries are recognizing traditional healthcare systems and are much more involved in framing quality control parameters for these drugs. The main drawback is the lack of scientific backbone which led to the underestimation of the effectiveness of these drugs [7].

The technique of GC-MS works two ways, that is, resolving the mixture into several individual components through gas chromatography and simultaneous analysis of each of the resolved ones through mass spectroscopy; further, interpretation can be done by matching with the available spectral data [8,9].

New drug development starts by searching for active principles from crude plant extracts. As per the current scenario, WHO is encouraging scientists to discover new lead compounds from herbals [10].

The phytochemical compounds derived from the plant under study here have proven anti-toxic properties. In this case, the first and foremost step is careful extraction without damaging the nature of the compounds. GC-MS technique is currently used to get information regarding the individual components in the crude extract [11].

Since plant-derived molecules are less toxic to human cells researchers are much oriented toward herbal medicaments. A wide array of secondary metabolites present in the plants *Glycosmis pentaphylla* exert their pharmacological action that is meant to be antitoxic [12,13]. The plant is already known for its anti-inflammatory, antimicrobial, antipyretic, antioxidant, and hepatoprotective activity [14].

G. pentaphylla family Rutaceae is a shrub indigenous to India, Srilanka, China, Thailand, Malaysia, Indonesia, and Vietnam. The plant is easily available as per its geographic distribution but fewer reports are there regarding the phytochemical profiling as well as evidence regarding the characterization of active compounds. Hence, the ethanolic extract of the plant was obtained and was subjected to GC-MS analysis [15,16].

METHODS

Collection of the plant material

G. pentaphylla Retz. was collected from the Iritty hills in Kannur district located in the North Malabar of Kerala. It was authenticated by certified botanist Dr. Ratheesh Narayanan M. K. H O D, Department of Botany, Payyanur College, Payyanur, Kannur District, Kerala. The voucher number is G16902022P/RIPSAR/PGPHY/2023-24/11. The herbarium was prepared and deposited in the same department. The fresh whole plants were washed, shade-dried, pulverized, and used for extraction through maceration.

Preparation of extracts

Initially, the plant material is defatted and then the solvents of varying polarity such as ethanol and hexane were chosen, then 500 g of the plant was comminuted and soaked in 750 mL of both solvents separately, kept closed for 2–3 days with intermittent stirring. The temperature to be maintained depends on the ability of the phytocompounds to withstand higher degrees of heat; hence, a normal temperature of about 10–15°C with occasional stirring was made for effective extraction. The above called method cold maceration followed. After which they were filtered first through cotton pieces and then using Whatman filter paper. The crude extracts thus obtained were kept in a desiccator.

Quantification of secondary metabolites

Alkaloids

Extract taken in separating funnel along with 20 mL NaOH and 20 mL chloroform, shake, and separate chloroform layer. Wash with water around 20 mL 2–3 times, add 20 mL 1N HCL, shake, again collect chloroform layer, add ammonia 15 mL again shake and collect chloroform layer, evaporate to dryness, calculate percentage weight.

Flavonoids

To 0.1 g of extract add 25 mL methanol and centrifuge for 20 min, take 0.5 mL add 1 mL methanol, 0.1 mL of aluminium chloride and sodium acetate, finally 2.8 mL water. Prepare standard solution using 1 mg of quercetin in same way. Incubate both for 30 min and read the absorbance at 415 nm.

$$\text{Flavonoid } \% = \frac{\text{Observed concentration} \times \text{Purity of standard}}{\text{Sample concentration}}$$

Phenols

To 0.5 g of extract add 25 mL water, sonicate and take 1 mL add 4 mL of sodium carbonate and 1 mL phenol, make up to 25 mL with water, sonicate after keeping in dark for 30 min. Repeat the same with 10 mg gallic acid as standard preparation. Read the absorbance at 760 nm.

GC-MS analysis of *G. pentaphylla* retz.

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×250 μm 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 1 μL 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 as follows: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70eV, a scan time 0.2 s and scan interval of 0.1 s. The mass fragments from 40 to 600 Da are found out. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library database [17].

Identification of components

The spectral data of the extracts were matched with the already available spectrum of known chemical compounds as depicted in the GC-MS NIST as well as the WILLEY library [18]. Thus, elucidations of unknown components were done based on molecular structure, molecular mass, and molecular weight and confirmed by comparison with their respective retention indices [19]. Moreover, quantification is also possible by calculating the average peak area. In this way, the compounds or class of secondary metabolites responsible for the therapeutic relevance of the plant can be found. On the whole, the traditional use of the plant in a particular aliment can be made evident with scientific reports [20].

RESULTS

Quantification of secondary metabolites

The amount of the secondary metabolites in the extracts was found out through conventional methods. It was found that the extract contains following metabolites.

GC-MS profiling

The results of preliminary phytochemical screening plant showed secondary metabolites and there was a significant amount of amides and aromatic compounds like terpenoids. The phytocompounds present in the ethanolic and hexane whole plant extract of *G. pentaphylla* Retz. as revealed by the GC-MS technique showed the presence of much more compounds as indicated in chromatogram – Figs. 1 and 2. The chromatograms were compared with those of known databases from the NIST and WILLEY LIBRARY.

DISCUSSION

The quantification of secondary metabolites in both the extracts showed the presence of alkaloids, flavonoids, and phenols predominantly as shown in Table 1. These are known to have antioxidant, anti-inflammatory activities which have been found out.

The most prevalent ones in the ethanolic extract being octadecyl 2-pentanyl sulfite, 4-amino-1-(4-fluorophenyl)pentan-1-one, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, ethyl hexadecanoate, phytol, 11-dodecyn-1-ol acetate, and acetyleugenol, as shown in Table 2. The compound name, class, retention time, area %, molecular weight, and structure of those compounds are depicted in Tables 2 and 3 for both the extracts.

Among these many are having anti-inflammatory action [21]. Furthermore, one compound 4-amino-1-(4-fluorophenyl) pentan-

Table 1: Results of quantification of secondary metabolites using ethanolic and hexane extracts of *Glycosmis pentaphylla* Retz

Secondary metabolite	Relative abundance (mg/g)	
	Ethanolic extract	Hexane extract
Alkaloid	1.85±0.45	2.34±0.63
Flavonoid	0.07±0.24	0.04±0.02
Phenol	0.1±0.15	0.08±0.03

Values expressed as mean±SD

Table 2: GC-MS results of identified phytocompounds from the ethanolic extract of *Glycosmis pentaphylla* Retz

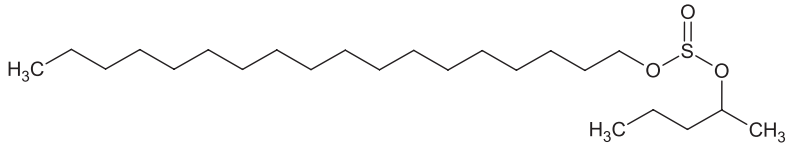
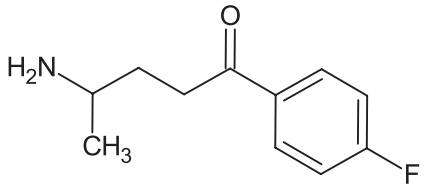
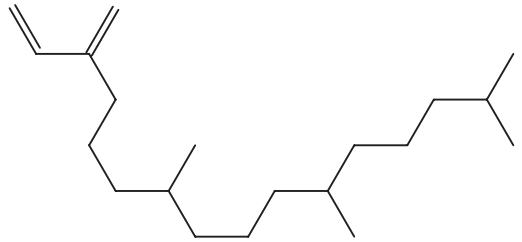
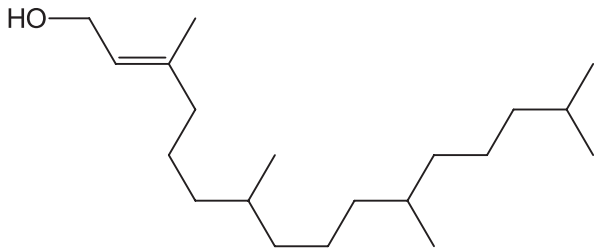
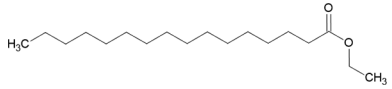
S. No.	Name of the compound	Class	Retention time	Area %	Therapeutic potential	Molecular formula	Molecular weight (g/mol)	2D molecular structure
1.	Octadecyl 2-pentanyl sulfite	Sulfite ester	17.103	1.31	Anti-oxidant, anti-inflammatory	$C_{23}H_{48}O_3S$	404.7	
2.	4-amino-1-(4-fluorophenyl) pentan-1-one	Cathinone	20.681	1.14	Analgesic, anti-inflammatory	$C_{12}H_{16}FNO$	209.26	
3.	Neophytadiene	Diterpene	22.263	13.75	Anti-inflammatory	$C_{20}H_{38}$	278.3	
4.	3,7,11,15-tetramethylhexadec-2-en-1-ol	Diterpenoid alcohol	23.295	5.85	Anti-cholinesterase	$C_{20}H_{40}O$	296.5	
5.	Ethyl hexadecanoate	Fatty acid ester	26.441	9.98	Anti-cancer	$C_{18}H_{36}O_2$	284.48	

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Table 2: ***(Continued).***

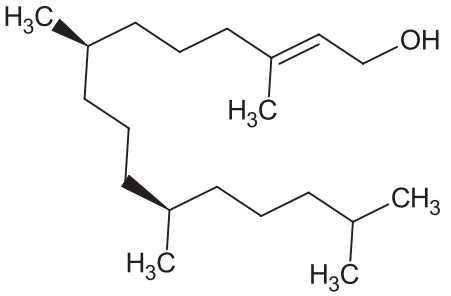
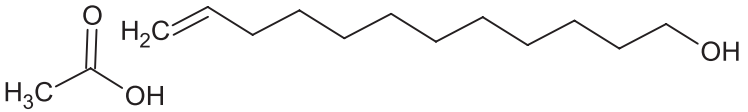
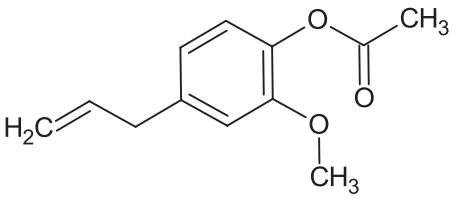
S. No.	Name of the compound	Class	Retention time	Area %	Therapeutic potential	Molecular formula	Molecular weight (g/mol)	2D molecular structure
6.	Phytol	Acyclic diterpenoid	29.273	59.16	Neuroprotective	$C_{20}H_{40}O$	296.5	
7.	11-Dodecyn-1-ol acetate	Carboxylic ester	30.486	7.40	Anti-inflammatory	$C_{14}H_{26}O_3$	226.35	
8.	Acetyleugenol	Phenyl propane	31.066	1.41	Anti-oxidant, anti-inflammatory	$C_{12}H_{14}O_3$	206.24	

Table 3: GC-MS results of identified phytocompounds from hexane extract of *Glycosmis pentaphylla* Retz

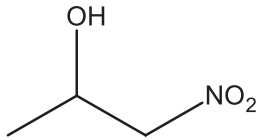
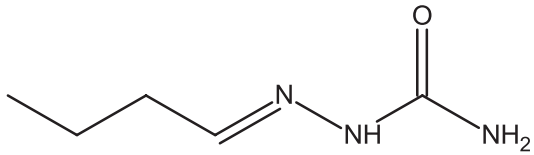
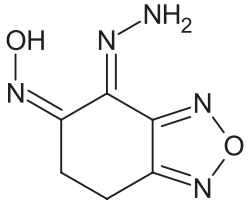
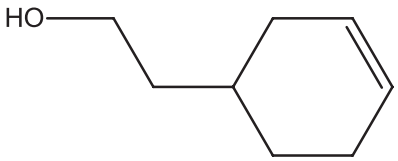
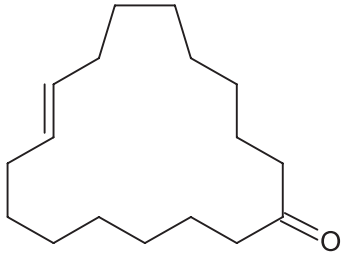
S. No.	Name of the compound	Class	Retention time	Area %	Therapeutic potential	Molecular formula	Molecular weight (g/mol)	2D Molecular structure
1.	1- nitro-2-propanol	Nitro alcohol	1.018	8.555	*Anti-microbial, anti-viral*	$C_3H_7NO_3$	105.09	
2.	Butyraldehyde semicarbazone	Nitro alcohol	13.193	24.033	Anti-oxidant, anti-inflammatory	$C_5H_{11}N_3O$	129.16	
3.	4- hydrazino-5-hydroxyimino-4,5,6,7-tetrahydrobenzofuran	Benzofuran	15.669	2.104	Antihypertensive	$C_6H_7N_5O_2$	181.18	
4.	3-cyclohexene-1-ethanol	Primary alcohol	16.710	1.666	Antifungal	$C_8H_{14}O$	126.2	
5.	9-cyclohptadecen-1-one	Cyclic ketone	16.765	1.713	Antioxidant	$C_{16}H_{28}O$	236.39	

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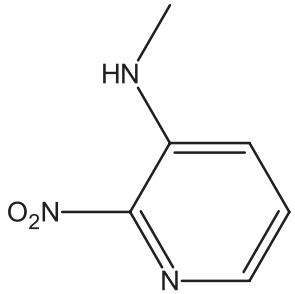
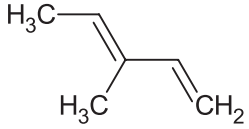
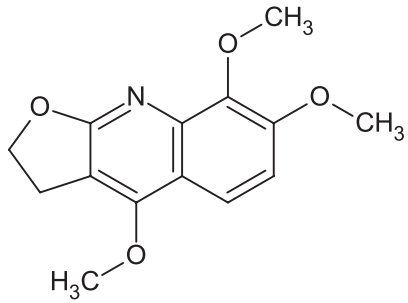
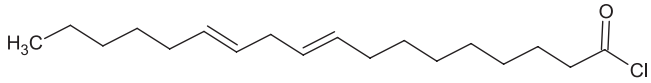
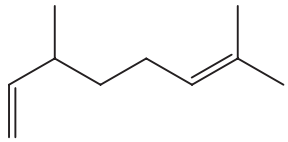
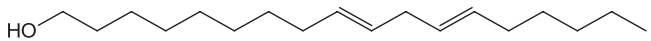
Table 3: ***(Continued).***

S. No.	Name of the compound	Class	Retention time	Area %	Therapeutic potential	Molecular formula	Molecular weight (g/mol)	2D Molecular structure
6.	N-methyl-2-nitropyridin-3-amine	Pyridine derivative	17.300	29.533	Antioxidant, anti-inflammatory	$C_6H_7N_3O_2$	153.14	
7.	3-Methyl-1, 3-pentadiene	Alkadienes	17.495	9.764	Anti-inflammatory	C_6H_{10}	82.14	
8.	4,7,8-Trimethoxy-2,3-dihydrofuro [2,3-b] quinoline	Furoquinoline	19.346	8.379	Neuroprotective, anti-inflammatory	$C_{14}H_{13}NO_4$	259.26	
9.	9,12-octadecadienoyl chloride	Acyl chloride	21.682	11.310	Anti-cholesterol, anti-oxidant, anti-inflammatory	$C_{18}H_{31}ClO$	298.9	
10.	Dihydromyrcene	Monoterpene	21.977	0.964	Anti-oxidant, anti-inflammatory	$C_{10}H_{18}$	138.25	
11.	Octadeca-9,12-dien-1-ol	Fatty alcohol	29.805	1.979	Anti-oxidant, anti-cancer	$C_{18}H_{34}O$	266.46	

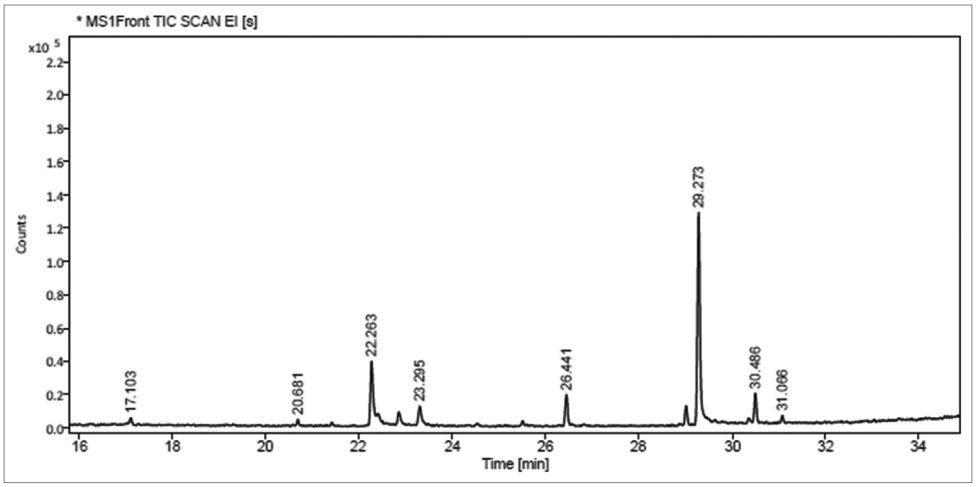


Fig. 1: Chromatographic report after the gas chromatography-mass spectroscopy study of ethanolic extract *Glycosmis pentaphylla* Retz

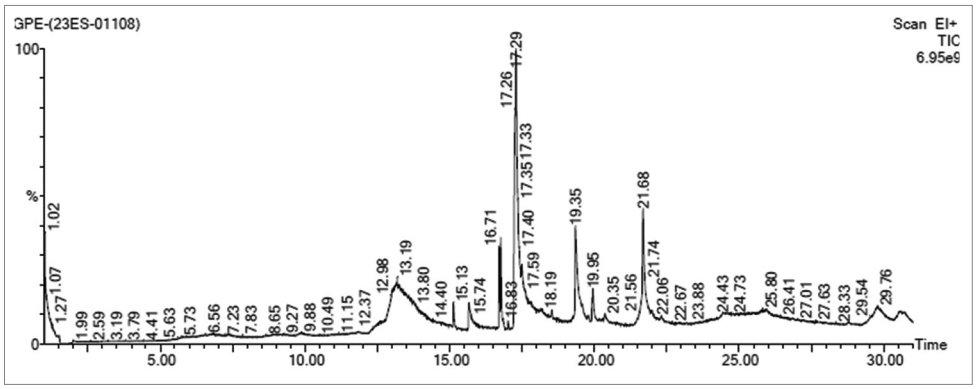


Fig. 2: Chromatographic report after the gas chromatography-mass spectroscopy study of hexane extract *Glycosmis pentaphylla* Retz

1-one having both analgesic and anti-inflammatory action contributing more to the pharmacological profile of the plant. One of the major findings 3,7,11,15-tetramethylhexadec-2-en-1-ol and phytol with anti-cholinesterase and neuroprotective action is the most significant [22].

The results due to the GC-MS analysis of the hexane extract of the has got the presence of many compounds out of which most prevalent ones were 1- nitro-2-propanol, butyraldehyde semicarbazone, 4- hydrazino-5-hydroxyimino-4,5,6,7-tetrahydrobenzofuran, 3-cyclohexene-1-ethanol, 9-cyclohptadecen-1-one, N-methyl-2-nitropyridin-3-amine, 3-methyl-1,3-pentadiene, 4,7,8-trimethoxy-2,3-dihydrofuro[2,3-b] quinoline, 9,12-octadecadienoyl chloride, dihydromyrcene, octadeca-9,12-dien-1-ol, as shown in Table 2. Most of the compounds in the hexane extract were found to have antioxidant action [23].

Similar to that of the ethanolic extract, the hexane extract also given compounds having neuroprotective and anticholinesterase action. However, the hexane extract shown much of the compounds having antioxidant and anti-inflammatory action whereas the ethanolic extract although got lesser number of peaks that they had prominent pharmacologic effect supporting the traditional use of the plant *G. pentaphylla* Retz. [24].

CONCLUSION

The study revealed that the ethanolic also hexane extract of the plant *G. pentaphylla* Retz. has got many potential compounds having medicinal value. This supports the use of plant for its medicinal value by traditional healers before ages. It will contribute to future activity-based studies of these compounds. Thus this study takes us to the pathway of lead compound discovery. Furthermore, pharmacological screening may be done to get evidence regarding the activity of the compounds.

The phytocompounds recognized in the both extracts of *G. pentaphylla* belong to terpenes, alkaloids, glycosides, and their derivatives using preliminary phytochemical screening. After thorough studies, these plant-derived medicaments were found to have promising therapeutic potential as reported in various scholarly articles. The most potent action among them is that many of these chemical derivatives were found to be having antitoxic action.

Further studies to be needed which should be going to pave way in the direction of compound isolation and *in vivo* analysis so that a confirmation about the action can be made through.

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AUTHORS CONTRIBUTIONS

Vyshnavy Devy D.K: Funding acquisition, data collection, and writing the original draft. S. Ruby: Supervision, analysis, manuscript review, and editing. M. Kumar: Conceptualization, project administration, data analysis, and manuscript review. Sajith Kumar P.N: Writing, original draft, methodology, and conceptualization. Vijayakumar B: Formal analysis, review and editing, visualization, and resources.

CONFLICTS OF INTEREST

There are no conflicts of interest for the author(s) with the article content.

AUTHOR FUNDING

None.

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