Journey into Nature's Chemistry: Exploring the Chemical Riches of *Chrozophora rottleri* through GC-MS Analysis

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Abstract ***The study used gas chromatography and mass spectrometry (GCMS) to characterize the phyto constituents of the unknown plant Chrozophora rottleri. The entire plant was extracted with ethylene acetate at room temperature for 72 hours, producing a concentrated extract that was then subjected to GCMS analysis. Understanding the chemical composition of the plant has advanced significantly as a result of the results, which revealed a wide range of compounds within the extract. This initial investigation lays the groundwork for the isolation of the main active ingredients present in Chrozophora rottleri, opening the door for additional research into the pharmacological potential of the organism. Through the identification of these compounds, scientists hope to shed light on the plant's medicinal qualities while also possibly discovering entirely novel, pharmacologically active compounds. This work emphasizes the value of looking into lesser-known botanical sources for their phytochemical components, as these sources may contain compounds that could be useful in the development of novel therapeutics or natural products with pharmacological applications. In order to advance the medical and pharmaceutical sciences, more research can be done on the isolation, characterization, and biological activity testing of these compounds.***

Index Terms ***Gas chromatography, Mass spectrometry, Phyto constituents, Chrozophora rottleri, Pharmacological potential.***

I. INTRODUCTION

Chrozophora rottleri is one of the members of the 300 genus and 5000 7500 species strong Euphorbiaceae family, which is divided into seven genera. The natural behavior of Chrozophora plants increased awareness of the need to discover novel lead compounds for the treatment of various diseases [1]. Monoecious or under shrubs are present. The genus is widely distributed throughout Asia, Africa, and Europe. It is an annual herb with silvery hairs that thrives in open waste areas naturally throughout India [2]. A medical plant called Chrozophora rottleriis sometimes referred to as Croton rottleri Geiseler and Chrozophoraplicata var. rottleri (Geiseler). In Sanskrit, it is generally referred to as Suryavarti. It is an herbal plant with a variety of medicinal effects. In India, stem powder is used to cure jaundice in Sudan, while in Nepal, the leafy parts of the plant are used to treat colds and coughs. In order to cure skin conditions including sunburn and sunstroke, leaf powder is used. Leucoderma is treated using leaves. It is used in Ayurveda as a purgative, depurative, emetic, and cathartic. Concentrate of leaf exhibits phytotoxic effects on rice, wheat, and mustard as well as helmintic properties that are averse to Pheritimaposthuma (Indian Earthworm). Laxatives and purgatives are made from seeds. The strongest suppression of both Gram positive and Gram-negative bacteria is demonstrated by plant extraction with methanol [3]. Our ancestors have relied on the herbal remedy for at least 5000 years as a kind of treatment over the years[4]. In some ways, the rise of allopathic or modern medicine has reduced the importance of medicinal plants in favor of synthetic medications. Even today, a lot of newly discovered drugs have their roots in indigenous populations' use of medicinal plants[5]. The World Health Organization (WHO) estimates that due to their low risk of side effects and affordability, roughly 80% of the world's population relies on natural products for their health[6].

Fig 1. Chrozophora rottleri[7]

Ayurvedic medications saved countless lives before modern synthetic medicine reached the general public, and the use of products for therapeutic purposes has a long history in Indian medicine dating back to the Vedic Age. Additionally, there is a persistent market demand for "natural" and/or "preservative-free" cosmetics and food that are microbiologically safe[8]. More than 10,000 medicinal plants are employed in traditional medicine in India, of which 1800 are utilized in Ayurveda, 4700 in traditional medicine, 1100 in the Siddha medicinal system, 750 in ^{unani}medicine, 300 in homeopathy, 300 in Chinese medicine, and 100 in the allopathic system [9]. In poor nations like India, infectious diseases make up a large share of health issues. Antibiotic resistance among microorganisms has caused significant clinical issues in the management of infectious illnesses. Scientists are compelled to look for new antimicrobial compounds from a variety of sources, including medicinal plants, due to the limited availability and expensive cost of current generation antibiotics [10]. The bulk of the species in this family are found in tropical America and the Indo-Malayan region, where they are mostly found in the tropics. Tropical Africa has a wide range, but they are not as common or as diversified as in these two other tropical regions. There are numerous species of Euphorbia, though, in non-tropical regions such the Mediterranean Basin, the Middle East, South Africa, and the Southern United States [11]. The leaves have stipules and are alternating, rarely opposite. Most of them are simple, but when they are, they are always palmate and never pinnate. Stipules can occasionally be absent in succulent species or reduced to hairs, glands, or spines. Though occasionally an adrupe, the fruit is mostly a schizocarp. This family is home to a wide range of phytotoxins, primarily diterpeneesters, alkaloids, glycosides, and toxins of the ricin-type [12]. Many members of the Euphorbiaceae family of plants are planted for their decorative qualities, and certain species have shown promise in combating genital herpes (HSV-2) [13]. The Euphorbiacea family includes a variety of succulent and non-succulent plants, such as herbs, shrubs, trees, and several kinds of cacti. The milky juice that many of them contain is more or less hazardous, especially for cold-blooded creatures. The fruits are typically three-celled capsules with a single seed inside each cell. Some species of these fruits can provide poisonous, irritating, and vesicular seed oils. Croton, which has over 700 species, and spurge or Euphorbia, which has about 1600 species, are the two largest genera in the spurge family [14].

II. GC - MS INTRODUCTION

There are numerous applications for gas chromatography. However, its primary and initial application is in the separation and analysis of multi-component mixtures such solvents, hydrocarbons, and essential oils [15.17]. Gas chromatography has the inherent ability to quantitatively identify compounds present at very low concentrations by using the flame ionization detector and the electron capture detector, both of which have extremely high sensitivities. It follows that studies of pollution, forensic work, and general trace analysis are the second-most significant application areas. One of the most crucial instruments in chemistry is gas chromatography, which separates components of mixtures with ease, sensitivity, and efficiency. It is frequently used to analyze mixtures

quantitatively and qualitatively, to purify substances, and to figure out thermo chemical constants like vapor pressure, activity coefficients, and temperatures of solution and vaporization [18–23]. The discovery of therapeutic agents is desirable, but it is also important to understand the chemical makeup of plants because this knowledge may be crucial for identifying new sources of valuable phytocompounds for the synthesis of complex chemicals and for understanding the true value of folk remedies. Higher plants continue to be important sources of bioactive chemicals for maintaining human health. Green plant studies offer a source of potent chemotherapeutics that are non-phytotoxic, more systemic, and readily biodegradable [24–26]. Because many phytochemicals have complimentary and overlapping mechanisms of action, a thorough validation of herbal medications has evolved as a new discipline of study that emphasizes and prioritizes the standardization of natural drugs and goods. Since this approach has proven to be a useful tool for the examination of non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids, GC-MS investigations have been used more frequently in recent years for the research of medicinal plants [27–30].

III. PLANT COLLECTION AND AUTHENTICATION

The plant Chrozophora rottleri (Geiseler) Spreng were collected fromlocality: Ramanathapuram(dt), Tamil nadu and was authenticated by Fischer Herbarium(Acronym: FRC) of the ICFRE-Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore. The herbarium of this specimen was kept in the department for further reference.

IV. MATERIALS AND METHODS

A. PREPARATION OF PLANT EXTRACT

The leaves of these plants were washed thoroughly under running tap water and then dry at Room Temperature for 2 hours and then dried in an oven at 60°C for 8 hours. The dried plant material was pulverized to fine powder in a grinder, stored in air tight bottle, labeled and kept in a dark room[31].

B. Maceration

Extraction of leaves of respective plants was carried out by maceration technique. The solubility of sample checked with different solvents, the leaf powder was freely soluble in the ethanol, methanol and ethyl acetate. 20 gm of dried powder was macerated separately in 250 ml of ethanol, methanol and ethyl acetate in test tube. The flasks were covered with aluminum foil and allowed to stand in a dark for 72 hrs for extraction. These extracts were filtered and the filtrate was evaporated to dryness in heating plate [32].

Fig 2. Extraction of Ethyl Acetate Chrozophora rottleriis.

C. GC – MS METHODOLOGY

The ethyl acetate extract of endophytic fungi was identified into HP -5 column (30 m x 0.25 mm with 0.25 μm film thickness) Agilent technologies 6890 N JEOL GC Mate II GC-MS Model. Following Chromatographic conditions were used, Helium as carrier gas, flow rate of 100 C / min; and column oven temperature was programmed as 500 - 2500 C at a rate of 100 C / min injection mode. Following MS conditions were used to screen all the fungal endophytecrude using computer searches on a National Institute Standard and Technology (NIST Ver 2.1) MS Data library.

V. RESULTS AND DISCUSSION

An effective method for determining the secondary metabolites contained in Chrozophora rottleriis is to examine the crude ethyl acetate extract using gas chromatography-mass spectrometry (GCMS). With the use of this technique, different chemicals present in the extract may be precisely identified and characterized. Researchers can identify the molecules in the extract by comparing the mass spectra of the compounds they have found with those in the NIST library. This procedure aids in deciphering the plant's

chemical makeup and locating putative bioactive substances with potential therapeutic or other advantageous uses [33]. In Figure 3, we provide an extensive list of compounds that have been identified using this method, all of which are found within the extract, Figure 4, on the other hand, visually represents the peaks corresponding to each individual compound. These peaks offer a graphical representation of the abundance and distribution of the identified compounds within the chromatogram, allowing for a deeper understanding of their relative concentrations and chromatographic behavior.

Compound Table

RT	Name	Area	Height	Mass	Formula	Area %
3.15	Propanamide, 2-hydroxy-	205334	27377	89	C3H7NO2	2.73
3.57	Tetrachloroethylene	141330	82142	163.9	C2Cl4	1.88
17.54	2-Decanol	34136	11117	158.2	C10H22O	0.45
17.91	Ethanamine, 1-(2-benzodioxanyl)-N-(2-benzodioxanylmethyl)-	35273	17469	327.1	C19H21NO4	0.48
18.56	Oxirane, 2-butyl-3-methyl-, cis-	33594	14009	114.1	C7H14O	0.45
20.68	2-Nonanol	47493	20679	144.2	C9H20O	0.63
20.80	2-Tetradecanol	73402	30016	214.2	C14H30O	0.98
21.02	4-Ethoxy-3-anisaldehyde	101207	24099	180.1	C10H12O3	1.35
23.64	N-[Dimethylaminomethyl]aziridine	59759	28333	100.1	C5H12N2	0.80
23.77	Dodecane, 1,1-dimethoxy-	439629	172922	230.2	C14H30O2	5.85
24.62	Phthalic acid, dodecyl 2-(2-methoxyethyl)hexyl ester	39757	13179	476.4	C29H48O5	0.53
25.09	Methoxyacetic acid, dodecyl ester	67826	25922	258.2	C15H30O3	0.90
25.85	2-Undecanol	30195	11836	172.2	C11H24O	0.40
26.11	5-(1-Iodo-1-methyl-ethyl)-3,3-dimethyl-dihydro-furan-2-one	438330	151346	282	C9H15IO2	5.84
26.39	4-Octadecenal	1739159	832133	266.3	C18H34O	23.15
26.52	cis-1,2-Cyclododecanediol	1160515	434395	200.2	C12H24O2	15.45
26.63	2-Octene, 1-(methoxymethoxy)-, (E)-	39919	14966	172.1	C10H20O2	0.53
27.38	Decanoic anhydride	605381	254306	326.3	C20H38O3	8.06
27.54	Fumaric acid, butyl pent-4-en-2-yl ester	1451006	562220	240.1	C13H20O4	19.32
27.81	Vinyl decanoate	71691	27979	198.2	C12H22O2	0.95
27.96	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	47960	13432	210.1	C11H18N2O2	0.64
28.86	2-Pentadecanol	28013	13593	228.2	C15H32O	0.37
28.99	2-Tridecanol	61153	13680	200.2	C13H28O	0.81
31.36	2-Hexanol	50447	17640	102.1	C6H14O	0.67
32.72	4-Nitrobenzoic acid, 2-butyl ester	147480	63105	223.1	C11H13NO4	1.96
34.19	N-cyclohexyl-3,4-methylenedioxyamphetamine	368689	101761	261.2	C16H23NO2	4.80

Fig 3. GC-MS Analysis Table.

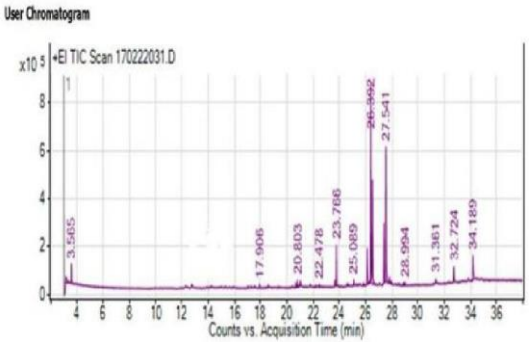

Fig 4. GC-MS Analysis Chromatogram.

CONCLUSION

In conclusion, Chrozophora rottleri plant was successfully identified and subjected to extraction using ethyl acetate. Further analysis and exploration may be warranted to uncover the potential therapeutic or chemical significance of these compounds. Then we send extracted sample for GCMS activities in that we provided a list of chemical compounds identified through GCMS analysis, including propanamide, 2-hydroxy, tetrachloroethylene, 2-Decanol, ethamine, 1-(2-benzodioxanyl)-n-(2 benzodioxanylmethyi)-, oxirane, 2-butyi-3-methyi-, cis-, 2-nonanol, 2-tetradecanol, 4-ehoxy-3-anisaldehyde, n-dimethyiamiomethyi aziridine, dodecane, 1,1-dimethoxy, phthalic acid, dodecyl ester, 2-undecanol, 5-1-iodo1-methyi-ethyi 3,3-dimethyi-dihydro-furan2-one, 4-octadecenal, cis 1,2-cyclododecanediol, 2-octene, 1-methoxymethoxy,(E), decanoic anhydride, fumaric acid, pyrrolo, 2-pentadecanol, 2-tridecanol, 2-hexanol, 4-nitrobenzoic acid, and n-cyclohexyl. This is the preliminary study to identify the phyto constituents from the plant Chrozophora rottleri. Based on these report future study want to be design to isolate the major constituents from the Chrozophora rottleri to develop therapeutic herbal medicine.

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