Phytochemical Profiling and GC-MS analysis of methanolic extract of *Nymphaea caerulea* Savigny – Rhizome

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Abstract-
Background: Natural and distinctive medicinal plants serve dual purposes, both as remedies for a wide range of diseases and as a means of generating income. Ayurveda and other ancient Indian texts have extensively documented the utilization of plants in the treatment of diverse human health issues. Globally, medicinal plants hold significant importance as a valuable resource in the fight against severe diseases. *Nymphaea caerulea* belongs to family Nymphaeaceae and is well known for its medicinal properties. The present study was carried out to evaluate the phytochemicals and bioactive components present in the methanolic extract of *Nymphaea caerulea* rhizome using GCMS analysis.
Results: The phytochemical analysis of the methanolic extract of *Nymphaea caerulea* rhizome (NCRE) revealed the presence of carbohydrates and alkaloids. The chromatogram report showed mainly seven compounds with peculiar characteristics. The total content of extract is with Tetraborane (10)- 2.5%, N- Hexadecanoic acid - 25.3%, Propylidine furfurylamine – 55.3% (highest content),10-Dodecenol- 1.8% and the remaining contribute by other compounds.

Keywords: **Phytochemicals, Methanolic extract, *Nymphaea caeruleae*, Rhizome, GC-MS analysis.**

INTRODUCTION:

Plants play a crucial role in diverse cultural medicinal practices and serve as a rich reservoir of potent drugs, primarily owing to the presence of specific bioactive compounds relevant to the pharmaceutical sector [1]. Within plants, a variety of phytochemicals, also referred to as secondary metabolites, contribute significantly to their medicinal properties. These phytochemicals prove beneficial in addressing various disorders through their individual, additive, or synergistic actions, ultimately enhancing overall health [2, 3].

In the pharmaceutical industry, phytochemicals hold paramount importance for the development of novel drugs and the formulation of therapeutic agents [4]. The journey towards creating new pharmaceuticals commences with the identification of active principles sourced from nature. A contemporary approach involves the screening of plant extracts to uncover therapeutically active compounds within diverse plant species [1, 5].
Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids exhibit a spectrum of biological properties, encompassing antioxidant, anti-inflammatory, anti-diarrheal, anti-ulcer, and anticancer activities, among others [5]. This underscores the wide-ranging potential of plant-derived compounds in pharmaceutical development and the multifaceted biological roles played by various phytochemicals.

Nymphaea caerulea Savigny- familiar as Egyptian lotus, is a variety of Nymphaea nouchali. It is mainly cultivated at Eastern half of Africa and parts of Southern Arabia, which is now spread as Ornamental plant in many countries [6]. In Ayurveda and Siddha systems of medicines, the flower is widely used for diabetes, liver and urinary disorders, menstruation problems etc [7]. In ancient days, Rhizomes were taken as food and also used in making of perfumes [8]
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Gas chromatography-mass spectrometry (GC-MS) is a synergistic analytical method employed for the identification and quantification of compounds within a plant sample [9]. This technique holds a pivotal role in the analysis of phytochemicals and in conducting chemotaxonomic studies, particularly in medicinal plants containing biologically active constituents [10].

METHODS

Chemicals:

All the chemicals and reagents used for the research were of analytical grade.

Plant collection and authentication:

Fresh rhizomes of *Nymphaea caerulea* were collected from palakonda hills near palakondaraya temple, Kadapa District Fig.1. The rhizomes were identified and authenticated by Sri Venkateswara university of Herbarium. The plant material was deposited in the herbarium,
Department of Botany, S V university, Tirupati with the voucher number136

. Fig.1. *Nymphaea caerulea* rhizome

Preparation of plant material:

Nymphaea caerulea rhizomes were collected and washed thoroughly under tap water. It was cut into pieces and dried under the shade for 15- 20 days. These dried pieces are ground into moderately coarse powder and used for the extraction. The powder was macerated in 80% methanol and allowed to stand for 48h at room temperature. The mixture was filtered with Whatman No.1 filter paper and the filtrate was concentrated using a rotary evaporator to get a reddish-brown semisolid extract.

Preliminary Phytochemical Screening:

The phytochemical profiling of the crude extract and the aqueous methanol fraction obtained from *Nymphaea caerulea* rhizomes was conducted following established procedures outlined by Harborne [11], Trease and Evans [12], Harborne [13], and Soni and Sosa [14].

Gas chromatography-mass spectrometry (GC-MS) analysis:

Gas chromatography-mass spectrometry (GC-MS) analysis is a combined analytical technique used to identify and quantify compounds within a sample. This method involves separating and detecting individual components of a complex mixture, making it a powerful tool for chemical analysis. During GC-MS analysis, a sample is vaporized and introduced into a gas chromatograph, where it is separated into its individual components based on their physical and chemical properties. The separated compounds then enter a mass spectrometer, where they are ionized, and their mass-to-charge ratios are measured. The resulting mass spectrum provides information about the identity and abundance of the compounds present in the sample. GC-MS analysis is widely used in various fields, including Pharmacy, chemistry, biochemistry, environmental science, and forensic science for the characterization of organic compounds in different samples.

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 transferring 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.

Identification of Compounds:

The identification of components was accomplished by relying on their retention indices, and the interpretation of the mass spectrum was carried out utilizing the database of the National Institute of Standards and Technology (NIST). This extensive database encompasses over 62,000 patterns of known compounds. To elucidate the

composition of the unknown components within the obtained *Nymphaea caerulea* fraction, their spectra were systematically compared with the standard mass spectra of known components archived in the NIST library (NISTII). This comparative analysis facilitated the accurate identification of the constituents present in the sample.

Results:

Phytochemical screening of methanolic extract of *Nymphaea caerulea* rhizome revealed the presence of carbohydrates and alkaloids as shown in Table – I& fig. 2.

Table – I Phytoconstituents of methanolic extract of *Nymphaea caerulea*

Test for	**Methanolic extract**
Carbohydrates	+ve
Alkaloids (Mayer's test) (Wagner's test)	+ve +ve
Steroids & Triterpenoids	-ve
Flavonoids	-ve
Proteins	-ve
Saponins	-ve
Tannins	-ve

+ve indicates positive & -ve indicates negative

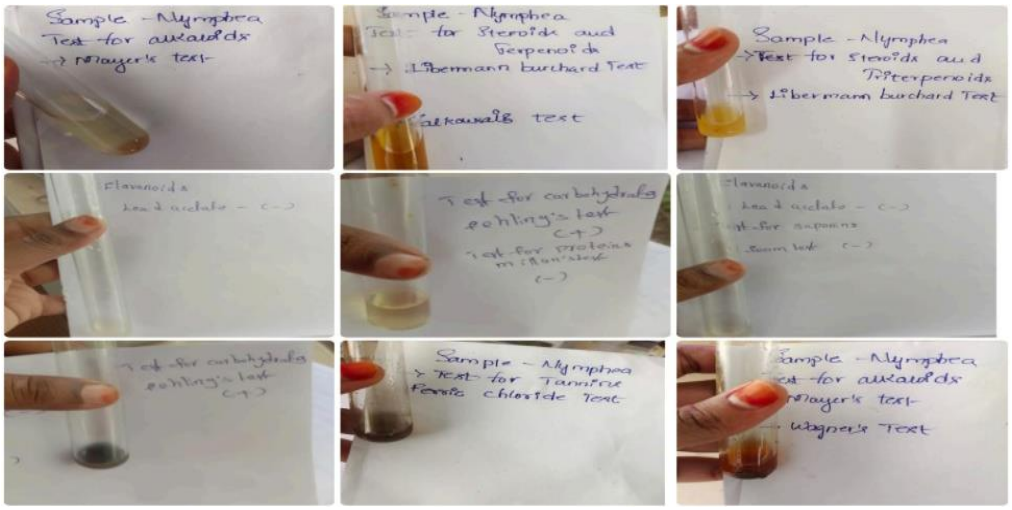


Fig .2 Phytochemical Screening of ***Nymphaea caerulea*** **rhizome**

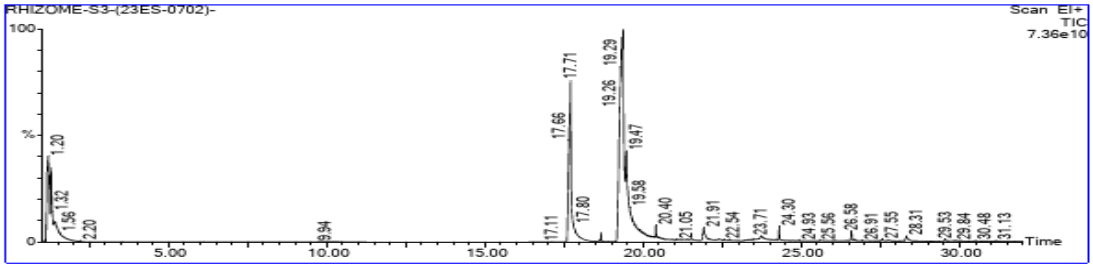
Gas chromatography-mass spectroscopy profiling of methanolic extract of *Nymphaea caerulea* rhizome:

A total of 5 compounds were identified from the GC-MS analysis of methanolic extract of *Nymphaea caerulea* rhizome. The chromatogram is presented in Fig.3, while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) in the methanolic extract are presented in Table. 2. The following bioactive compounds were present in the GC-MS analysis on methanolic extract of *Nymphaea caerulea* rhizome includes: Tetraborane(10), N-Hexadecanoic Acid, N-[3-[N-Aziridyl] Propylidene] Furfuryl amine, (Z)-9-Hydroxy-2,4-Dimethyl-Non-7-Enoic Acid Lactone, 10-Dodecenol, 7,11-Dimethyldodeca-2,6,10-Trien-1-Ol, 2,4-Dimethyl-7-Oxo-4,7-Dihydro-Triazolo (3,2-C)Triazine.

Fig.3: GC-MS chromatogram of methanolic extract of *Nymphaea caerulea* rhizome

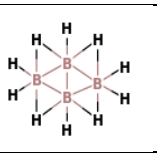
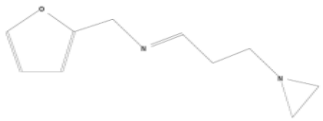
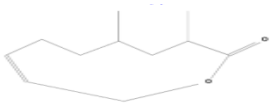
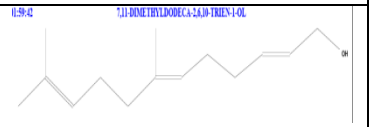
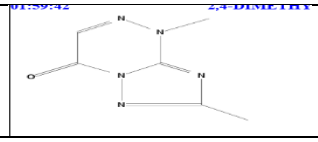
Qualitative Report

File: C:\TURBOMASS\2021.PRO\Data\RHIZOME-S3-(23ES-0702)-.raw
Acquired: 24-Aug-23 01:59:42 AM Printed: 25-Aug-23 10:31 AM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP Page 1 of 1
Sample ID: RHIZOME-S3-(23ES-0702)- Vial Number: 17



#	RT	Scan	Height	Area	Area %	Norm %
1	1.294	59	15,368,870,912	460,676,960.0	2.501	4.52
2	17.710	3341	55,021,846,528	4,666,432,512.0	25.333	45.83
3	19.371	3673	69,564,882,944	10,181,093,376.0	55.271	100.00
4	19.466	3692	26,947,661,824	2,784,580,608.0	15.117	27.35
5	21.912	4181	4,190,340,096	327,482,944.0	1.778	3.22

Table -2 Bioactive compounds found in methanolic extract of *Nymphaea caerulea* rhizome

S.No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak Area	Structure of compounds
1.	1.294	Tetraborane (10)	$CHO_2C_{13}S$	54	4.52	
2.	17.710	N-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256	45.83	
3.	19.371	N-[3-[N-Aziridyl]Propylidene]Furfurylamine	$C_{10}H_{14}ON_2$	178	100.00	
4.	19.466	(Z)-9-Hydroxy-2,4-Dimethyl-Non-7-Enoic Acid Lactone	$C_{11}H_{18}O_2$	182	27.35	
5.	21.912	10-Dodecenol	$C_{12}H_{24}O$	184	3.22	
6.	24.298	7,11-Dimethyldodeca-2,6,10-Trien-1-Ol	$C_{14}H_{24}O$	208		
7.	28.554	2,4-Dimethyl-7-Oxo-4,7-Dihydro-Triazolo(3,2-C) Triazine	$C_6H_7ON_5$	165		

Discussion:

Phytochemical screening of methanolic extract of *Nymphaea caerulea* rhizome revealed the presence of the Phyto-compounds, carbohydrates and Alkaloids only. Alkaloids are also present in the aqueous extract of leaf and flower of this plant [15]. These are the derivatives of amino acids which noted for therapeutic and recreational purposes since ancient times [16

The chromatogram report of the rhizome showed mainly seven compounds with peculiar characteristics. The total content of extract is with Tetraborane (10)- 2.5%, N- Hexadecanoic acid -25.3%, Propylidine furfurylamine – 55.3% (highest content), 10-Dodecenol- 1.8% and the remaining contribute by other compounds. Generally, the flower of *Nymphaea caerulea* contains 33 bioactive compounds, mainly Benzyl acetate, Pentadecane, 6,9-Heptadecadiene, 8-Heptadecane which are key for fragrance and maturation process [17]. Whereas the compounds present in the rhizome are mainly noted for the industrial uses. Propylidine furfurylamine is an aromatic amine currently treated as important bio-based molecule in the manufacturing of plastics, lubricants, resins, food additives, perfumes etc. [18]. But it acts as irritant for skin, mucous membrane and respiratory track due to its high volatilic nature [19]. N-Hexadecanoic acid which is called as palmitic acid also present in essential oil of fenugreek seeds, Centaurea species [20] used as surfactant, Food stuffs, Natural additive in organic products etc. As it is a saturated fatty acid, high usage in food raises LDL and total cholesterol content that leads to heart problems. 10-dodecenol is commonly known as Lauryl alcohol present in palm kernal and coconut oil with floral odor, used as surfactant, lubricant, flavour in food additives and emollient in cosmetics [21]. It is mainly used in Shampoo manufacturing. 7,11-dimethyl dodeca-2,6,10-trien-1-ol is also known as Farnesol, is one of the compounds of soaps, bath lotions, skin care lotions and sunscreen lotions. (Z)-9-Hydroxy-2, 4 dimethyl -non-7-enoic acid lactone is unsaturated fatty acid used as flavour agent which odor as milky. Tetraborane (10) is used widely in the synthesis of various chemical compounds industrially. It has foul smelling and toxic to central nervous system. It reacts with oxygen and nitric acid in the air and causes ignition [22].

Conclusion:

In the present study, *Nymphaea caerulea* rhizome have shown to have various secondary metabolites which possess many pharmacological properties of which antioxidant activity is one. The GC-MS analysis showed the presence of 5 major phytochemical constituents which contribute the activities. Though these compounds have a wide industrial usage but not been reported for any pharmacological activity and are found in certain amount in *Nymphaea caerulea* rhizome extract for easy isolation. Further investigations are required for possible development of novel drugs using these bioactive compounds found in *Nymphaea caerulea* rhizome.

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