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Research article

GC-MS profiling of *Magnolia griffithii* hook. f. & Thomson: an endangered plant species of Assam, North-east India

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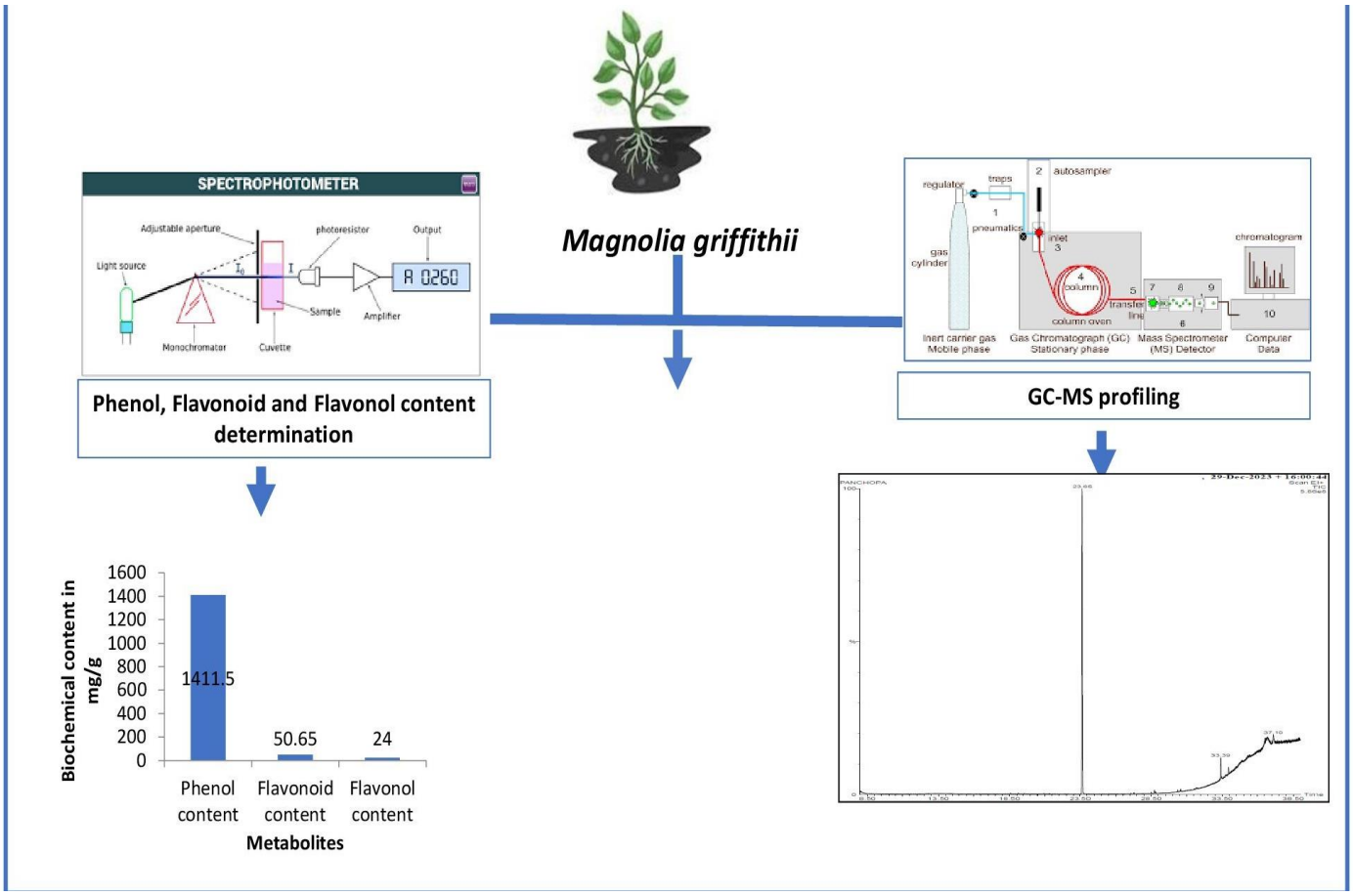
ABSTRACT



Magnolia griffithii Hook. f. and Thompson, belongs to the family Magnoliaceae, is an endemic and threatened plant species of Assam, North-east India. However, its population has declined rapidly from its natural habitat due to overexploitation of the plant species. Additionally, very little information is still available on the taxonomy, distribution pattern or microbial ecology of the said species. There is also very less information about the diversity, bioprospection and commercial values of the species. In the current investigation, the phytochemical constituents of the methanolic extract of *M. griffithii* leaves were analysed qualitatively using gas chromatography-mass spectrometry (GC-MS). Qualitative estimation of the biochemical metabolites was done and the following compounds were found viz. alkaloids, tannins, flavonoids, lignin etc. Quantitative analysis revealed the existence of phenol content as 1411.5 mgg-1, flavonoid content as 50.65 mgg-1 and flavonol content as 24 mgg-1. Gas Chromatography - Mass Spectrometry analysis revealed the presence of 198 diverse compounds with varied biological activities, among which some significant compounds are reported as Safrole, Aciphyllene, Alloaromadendrene, Aminohippuric Acid, Androstan-17-One, 3,11-Bis [(Trimethylsilyl)Oxy]-,O-(Phenylmethyl) Oxim, Arsenous Acid, Tris(Trimethylsilyl) Ester Azulene, 1,2,3,3a,4,5,6,7-Octahydro-1,4-Dimethyl-7-(1-Methylethenyl), Caryophyllene, Curlone, Cyclobarbital, Cycloheptane, Bromo, Guaia-1(10),11-Dien, Isocaryophillene, Leucodrin, Linalool, Linalyl Acetate, Patchoulene , Andrographolide, Aromandendrene, Beta.-Bisabolene, Methane sulfonothioic Acid, S,S'-1,4-Butanediyl Ester, Neophytadiene, Pyrrolidin-2,5-Dione, Retinal, Santolina Triene, Silicic Acid, and Stigmasterol. Thus, the identification and characterisation of the phytochemicals in the extract favour the development of novel therapeutic agents.

Keywords: Phytochemicals, Alkaloids, Tannins, Flavonoids, Lignin, Phenolics.

INTRODUCTION

India is one of the richest megadiversity hotspot region, unique habitat for endemic and threatened species [1]. The country is rich in the diversity of *Magnolia* species, most of which are located in Himalayan region with many of them found locally [2].

Despite of the economic and pharmaceutical importance, the diversity of *Magnolia* species of the country is lessening at an alarming rate and are in the verge of extinction and, thus, can be classified as the key species for conservation needing prioritized attention [3].

One of the endemics and threatened plant species of Assam *Magnolia griffithii* Hook.f. And Thompson, belonging to Magnoliaceae is our target plant species and its population have declined rapidly from its natural habitat due to the over exploitation of the plant species. The plant species is distributed in the North eastern parts of India (mainly in Assam) to North Myanmar and possibly in Bangladesh. Found in an elevation of 1,500-2000 above msl (mean sea level) and also at 80-750 m above msl [4]. Till date, no information is available on the population of the said species. According to IUCN red list data availability, the conservation status of the plant is reported for declaring data deficient as no information is available on the population status. The plant species have a potential forest distribution of 148,687 km^2 but it is suspected that due to deforestation, the population have declined rapidly [5]. Very little information is available regarding the taxonomy, distribution pattern or microbial ecology of the said species. There is also very less information available on the diversity, bioprospection, phytochemical constituents and commercial value of the species. Moreover, the natural habitat of the plant species has been severely fragmented owing to industrialization and urbanization. Thus, this study was undertaken to enumerate the phytochemical constituents and secondary metabolites associated to *Magnolia griffithii*. Primary metabolites consist of small molecules such as sugars, amino acids, tricarboxylic acids or Krebs cycle intermediates, proteins, nucleic acids and polysaccharides.

Likewise, secondary metabolites are those compounds produced by plant cells through primary metabolic pathways. The secondary metabolites are known to possess various biological effects which imparts the various medicinal properties like antibiotic, antifungal and antiviral and, therefore, are known to protect the plants from phytopathogens. And into a bargain with it, it was observed that forage grasses such as clover or alfalfa can express estrogenic properties and interact with fertility of animals [6]. The secondary metabolites in plants are known to impart the biological and healing effects in plants which helps in plant protection.

Moreover, information from traditional medicine has resulted in determination of plant metabolites that display biological effects for their expected applications of being used as medicinal products and food supplements and biopesticides [7,8]. In intriguing of the above, the present investigation was undertaken to screen the leaves of the target plant for various phytochemicals and bring into light the bio-prospecting potential of the endangered plant species.

MATERIALS AND METHODS

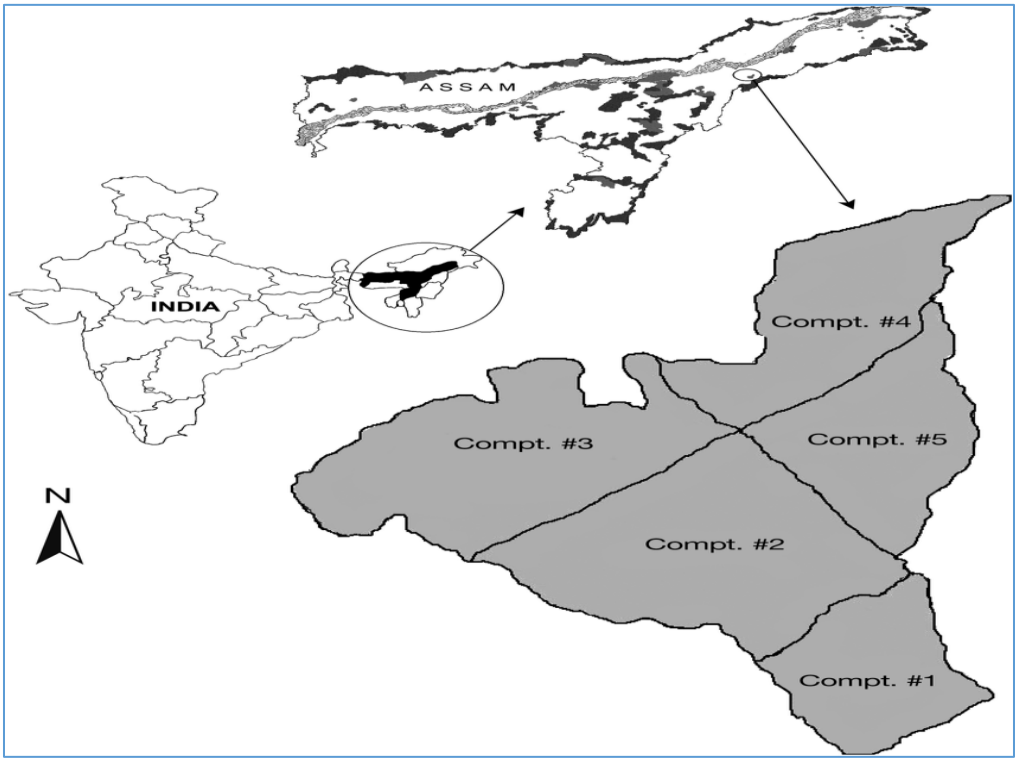
Collection site

The study site is selected at Hollongapara Gibbon Wild Life Sanctuary (HGWLS), Assam, and North-east India (Figure 1). The sanctuary is situated at 26° 40/ to 26° 45/ N and 94° 20/ to 94° 25/ E at an altitude of 100-120 m and has an area of 20.98 km^2, on the south of the mighty Brahmaputra in the district of Jorhat, Assam, India [9].

Preparation of Plant extract

To 0.5 mg of plant sample, 50 ml of 90% ethanol was added and the leaves were macerated in mortar and pestle. The macerated leaves are then kept in a hot air oven for a period of 15-20 minutes. Then the solution is filtered and extract was obtained.

Figure 1: Location map of the study area (Source: Das et al., 2014 [10])



Test for tannins ($FeCl_3$ test)

To the plant extract, 5% $FeCl_3$ was added and the solution turned deep blue black in colour indicating the presence of tannin.

Test for Flavonoids

Shinoda test

To the plant extract, a few fragments of Magnesium ribbons were added and then concentrated hydrochloric acid is added dropwise. The appearance of pink colour indicates the presence of flavonoids.

Zinc hydrochloride test

To the plant extract a mixture of zinc dust and concentrated HCl is added. The red colour indicates a positive reaction.

Test for lignin

Wiesner test

This test is performed by using a piece of softwood or of low-grade paper (phloroglucinol) dipped in alcohol and concentrated HCL. Development of a brilliant red color indicates the presence of aldehyde group in lignin.

Detection of alkaloids

The plant extracts were dissolved in diluted HCl and filtered. To detect the presence of alkaloids, Hager's test and Mayer's test were performed.

Mayer's test

To the filtrates, Mayer's reagent (potassium mercuric iodide) was added. Yellow coloured precipitate indicates the presence of alkaloids.

Hager's test

To the filtrate, Hager's reagent was added. Yellow ppt. indicates the presence of alkaloids.

Detection of carbohydrates

The plant extracts were dissolved individually in 5 ml SDW and filtered. The filtrate was used to test the presence of carbohydrates.

Fehling's Test

The filtrate was hydrolyzed with dil. HCl and neutralized with alkali. Then to the filtrate Fehling's solution A and B were added. The solution turned red, indicating the presence of reducing sugar.

Detection of glycosides

Sample extracts were hydrolyzed with dilute HCl and subjected to test for glycosides.

Detection of steroids and terpenoids

To 1.0 ml of plant extract, 1.0 ml chloroform was added followed by the addition of 2-3 ml acetic anhydride. To it, 1-2 drops of concentrated H_2SO_4 were added. The presence of dark green colour of the solution shows the presence of steroids while pink, red colour of solution indicates the presence of terpenoids.

Qualitative detection of phenols

Ferric Chloride test ($FeCl_3$)

To the crude extracts, 3-4 drops of $FeCl_3$ solution was added. The presence of bluish black colour indicated the presence of phenols.

Detection of proteins and amino acids

Xanthoproteic Test

The plant extracts were treated with a few drops of concentrated nitric acid (HNO_3). Formation of yellow colour indicated the presence of proteins.

Detection of flavonoids

To the plant extract, 10% NaOH was added followed by dilute HCl which resulted in change in colour from yellow to colourless indicating a positive reaction.

Test for phenol

The quantitative test of phenols was determined using the method by Singleton and Rossi (1965) [11] with slight modification. To 0.5 ml of plant extract, 0.5 ml of Folin Ciocalteu reagent is added (Previously diluted with 1:1 of distilled water). The mixture was then incubated at RT for 5 minutes, followed by addition of 1 ml of 2 % Na_2CO_3 solution and incubation at room temperature for 10 minutes. The absorbance was taken at 730 nm with a UV-VIS spectrophotometer.

Test for flavonoid

The amount of flavonoid was determined according to Zhishen et al (1999) [12] with minor modifications using quercetin as standard. To 0.25 ml of the plant extract, 1.25 ml of double distilled water were added followed by addition of 75 µL of 5 % $NaNO_2$. After incubating the solution at room temperature for 5 minutes, 0.15 ml of 10% $AlCl_3$ were added. After a further incubation of 6 minutes, the reaction mixture was treated with 0.5mL of 1mM NaOH solution. Finally, the reaction mixture was diluted with 275 µL of double distilled water. The absorbance was measured at 510 nm after incubating the mixture for 20 minutes at room temperature.

Test for Total Flavonols

The method developed by Kumaran and Karunakaran (2006) [14] was used to estimate the total flavonols using quercetin as a standard. To 2mL of plant extract, 2% $AlCl_3$ was added followed by the addition of 1 mL of 1% CH_3COONa and then incubated for 2.5 hours at room temperature. The absorbance was taken at 440 nm.

GC-MS analysis

The Perkin Elmer (USA) GCMS instrument, Model: Clarus 680 GC and Clarus 600C MS, which includes a liquid autosampler, was used to perform the GC-MS analysis of the sample extract. Turbo Mass Ver. 6.4.2 is the software utilized in the system. NIST-2014, a data analysis program, was used to examine the peaks. The stationary phase is 5% diphenyl 95% dimethyl polysiloxane and the capillary column used is called "Elite -5 MS," with dimensions of 60 m for length, 0.25 mm for ID and 0.25 µm for film thickness.

GC-Protocol

At a flow rate of 1 millilitre per minute, helium gas (99.99 %) was employed as the carrier gas, or mobile phase. In spitless mode, a 1 µl injection volume was used. The temperature of the ion source is 180°C and the injector is 280°C. The oven temperature was set at 60°C for one minute, then increased by 7°C per minute to 200°C (held for three minutes) and then by 10.C per minute to 300°C (held for 5 minutes). It turns for about 39 minutes in total. For eight minutes, the solvent delay was maintained.

MS Protocol

At 70 eV, mass spectra were recorded in electron impact positive (EI+) mode. For the MS scan, there was an 8-minute solvent delay. 50–600 amu is the mass range, or m/z range.

Identification of Peaks

By using the National Institute standard and Technology - 2014 (NIST-2014) database software to search the mass spectrum of corresponding peaks, the peaks that appeared in the GC chromatogram were interpretated. The compounds were identified using their names, molecular weights, empirical formulas and other characteristics after the mass spectra of the unknown and known components of the NIST library compared.

RESULT

The qualitative phytochemical screening of the plant extract revealed the presence of steroids, alkaloids, flavonoids, terpenes, phenols, glycosides, tannins and saponins, proteins and amino acids (Table 1). Similar results were previously reported [15] in *M. champaca* leaves. In the leaves of *M. nilagirica*, the presence of alkaloids, sterols, diterpenes, phenols, flavonoids, glycosides, saponins, carbohydrates ands tannins were reported [16]. Like-wise, the presence of alkaloids, flavonoids, terpenes, glycosides, phenols, steroids, tannins and saponins were reported in *Magnolia champaca* [17]. FTIR spectroscopic analysis revealed structurally related compounds. GC-MS/MS (Tandem mass spectrometry) analysis revealed the presence of 99 diverse compounds with varied biological activities, among which 1, 2, 4-butanetriol, n-hexadecanoic acid, cis vaccenic acid, phytol, trans longipinocarveol and caryophyllene oxide were found predominantly.

Table 1: Qualitative analysis of phytochemicals

Phytochemicals	Status
Phenol	+++
Flavonoid	+
Lignin	+
Alkaloid	+++
Steroids	++
Terpenoids	++
Carbohydrates	++
Glycosides	+
Saponin	+
Proteins	+
Aminoacids	+

+= Present, ++= Present in high amount.

Histogram depicting the secondary metabolites content in *Magnolia griffithii* is shown in figure 2. The chromatogram obtained on phytochemical analysis of the extract through GC-MS is given in Figure 3. Till date, no reports have been made on the phytochemical constituent and gas chromatogram analysis of the said plant species. In our study, the quantitative analysis revealed that phenol content is 1411.5 mg/g, flavonoid content is 50.65 mg/g and flavonol content is 24 mg/g (Figure 2). GC-MS analysis revealed the presence of 198 diverse compounds with varied biological activities, among which some significant compounds are reported as Safrole, Aciphyllene, Alloaromadendrene, Aminohippuric Acid, Androstan-17-One, 3,11-Bis[(Trimethylsilyl)Oxy]-, O-(Phenylmethyl) Oxim, Arsenous Acid, Tris(Trimethylsilyl) Ester Azulene, 1,2,3,3a,4,5,6,7-Octahydro-1,4-Dimethyl-7-(1-Methylethenyl), Caryophyllene, Curlone,

Cyclobarbital, Cycloheptane, Bromo, Guaia-1(10), 11-Dien, Isocaryophillene, Leucodrin, Linalool, Linalyl Acetate, Patchoulene, Andrographolide, Aromandendrene, Beta.-Bisabolene, Methanesulfonothioic Acid, S,S'-1,4-Butanediyl Ester, Neophytadiene, Pyrrolidin-2,5-Dione, Retinal, Santolina Triene, Silicic Acid, and Stigmasterol (Figure 3 and Table 2). Similar results were reported by Cristea et al. (2024) [18], where they quantified different compounds: phenolic acids (6.259 to 27.883 mg/g dry weight), aglycone flavonoids (61.224 to 135.788 mg/g dw), glycosidic flavonoids (17.265 to 57.961 mg/g dw), and lignans (150.071 to 374.902 mg/g dw) and identified 76 volatile compounds, predominantly oxygenated monoterpenes and sesquiterpene hydrocarbons, which contribute to the antibacterial effectiveness of the extracts.

Regarding the GC MS analysis, it was reported that the ethanolic extract of *M. champaca* contained methyl β-d-galactopyranoside octadecatrienoic acid and phenol [19]. They also reported 9,12-octadecadienoic acid, methyl ester, (E, E), butanoic acid, 2-methyl-3-oxo-, ethyl ester and oleic acid in the methanolic extract of flowers of *M. champaca.* In another study, it was reported that, *M. officinalis* had the highest polyphenol content and displayed the most potential antioxidant activity and the flowers had lower polyphenol content compared to its bark [18].

In a similar study, the preliminary phytochemical screening of different leaf extracts of *Cassia alata* revealed the presence of various phytochemical compounds such as terpenoids, steroids, flavonoids, phenolic compounds, quinones, carbohydrates, tannins and alkaloids. Qualitative and quantitative determination of different biologically active compounds from the crude n-hexane extract using gas chromatography-mass spectrometry disclosed 20 compounds with varying amounts where main components were identified as palmitic acid (26.65%), stearic acid (14.27%), (E)-9-octadecadienoic acid (11.40%), erucylamide (8.34%), 1,19-palmitate (3.93%) and methyl 11-octadecenoate (3.32%) [20].

In our study, we found a wide variety of components with multiple usage. The plant species contain the metabolite linal, which also functions as an antibiotic, volatile oil component, and fragrance molecule. Home products, insecticides, soaps, fragrances, and flavourings for food are all made with linalool. Phytosterols are well known for their biological and medicinal properties. Our focus is on stigmasterol, a phytosterol that is widely recognized for its ability to control cholesterol levels as well as its anti-inflammatory and antioxidant characteristics. These biological traits could be linked to the functional elements of the tetracycline rings and the unsaturation of the anthracene group, which demonstrated the autoxidation property [21].

Santolina triene is used as an antifungal agent and possesses antioxidant properties [22]. Since retinal is almost exclusively coupled to form a light-sensitive complex (rhodopsin-like proteins), this rhodopsin-like protein may function as a blue light photoreceptor in higher plants [23]. Safrole belongs to the methylenedioxybenzene group, which includes a number of chemicals that are used in combination with insecticides. One such compound is piperonyl butoxide, which is made using safrole as a precursor. Another substance called ecstasy, or 3, 4-methylenedioxymethamphetamine, or MDMA, is derived from safrole. Because of its unique "candy-shop" aroma, safrole was used as a food flavor, until it was banned by the US FDA in 1960. It was put to soaps, chewing gum, toothpaste, root beer, and some prescription treatments. Safrole possesses both antibacterial and anti-angiogenic qualities [24, 25].

Zhang et al. (2021) [26] have demonstrated the antibacterial activities of aciphyllene against a range of bacterial species. Alloaromadendrene oxide belongs to the phytochemical coumarin class, which is well-known for a variety of biological activities, such as anti-inflammatory, anti-oxidative, and anti-apoptotic qualities. In particular, the chemical's effects on photosensitization and its potential to treat breast cancer have been demonstrated. This demonstrates the material's potential usages in the treatment of numerous human ailments. Alloaromadendrene oxide is a viable choice for medical applications because of its many pharmacological properties [27]. B-Patchoulene (β-PAE), a tricyclic sesquiterpene, is found in Pogostemon cablin oil, commonly referred to as patchouli oil. It is widely used in traditional Chinese medicine to treat inflammatory diseases [28]

A diterpene lactone compound, called andrographolide is used to treat the ailments related to heart and brain as well as to shield the gallbladder and liver. It has immune-regulating, anti-inflammatory, antibacterial, antiviral, and anticancer qualities. There are strict production requirements for andrographolide due to its weak water solubility, bioavailability, and mild pharmacological effects [29]. Many naturally occurring derivatives of bisabolene have the ability to function as pheromones in a variety of insects, including fruit flies. Even though many fungi also produce them, it is yet unknown what role they play in fungus biology. β-Bisabolene has a balsamic scent and is approved as a food additive in Europe [30]. NPT-containing medicinal plants are used to treat rheumatism, headaches, and some skin disorders, despite the fact that NPT has showed analgesic, antipyretic, anti-inflammatory, and antioxidant characteristics [31].

Figure 2: Histogram depicting the secondary metabolites content in *Magnolia griffithii*

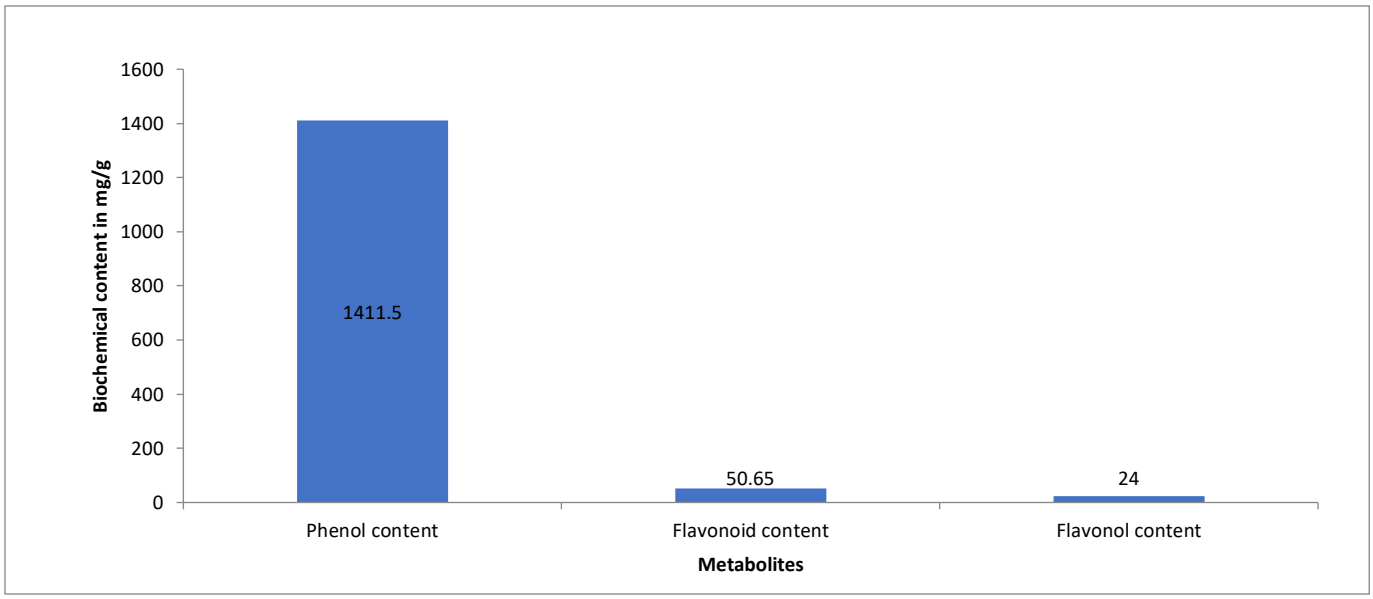


Figure 3: Gas chromatogram of *Magnolia griffithi*

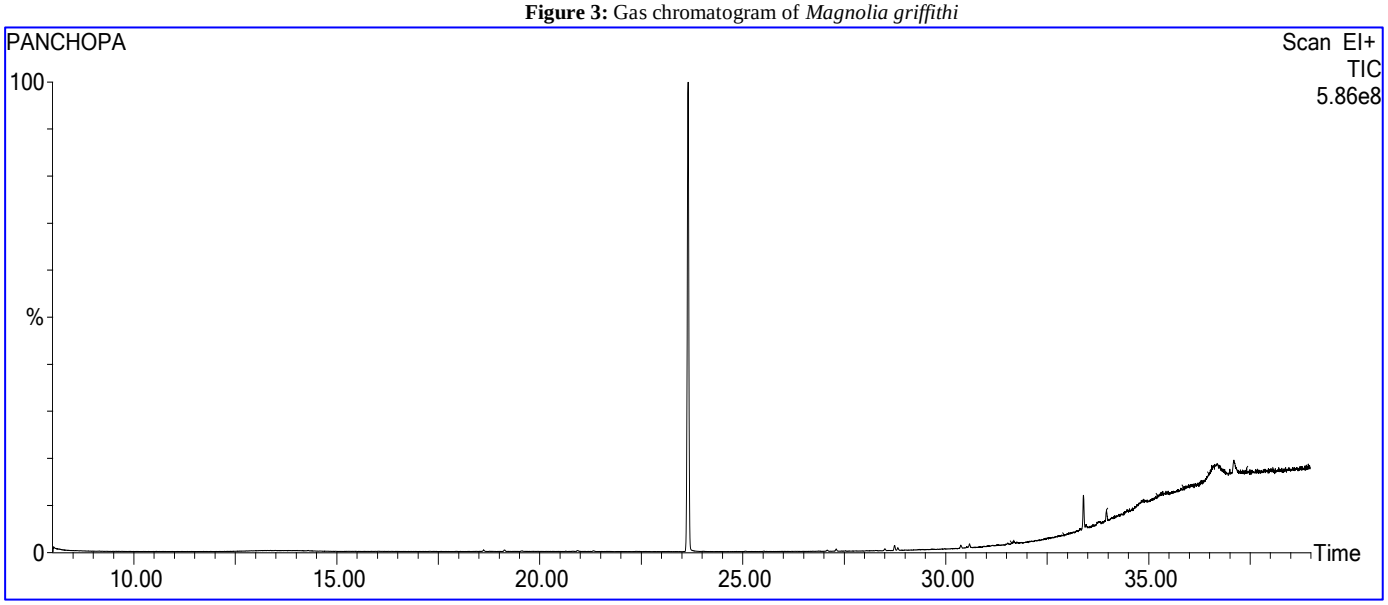
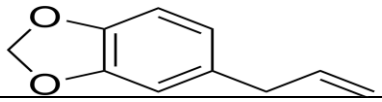
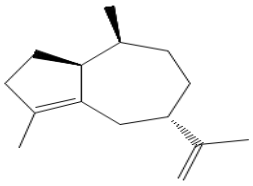
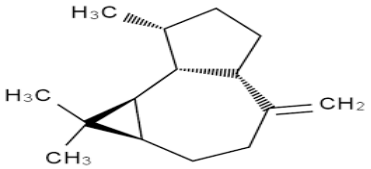
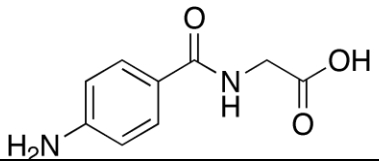
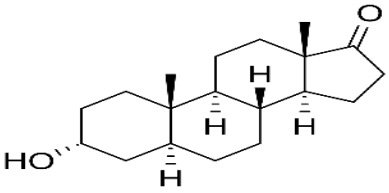
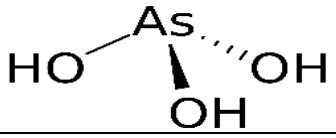
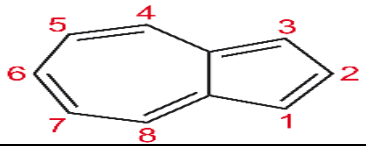
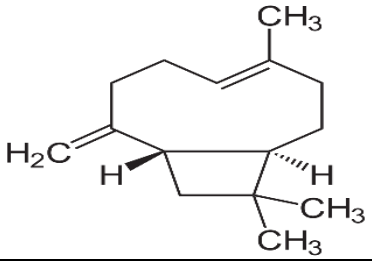
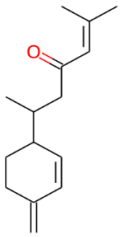
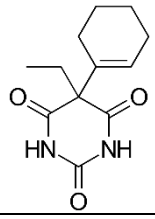
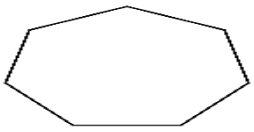
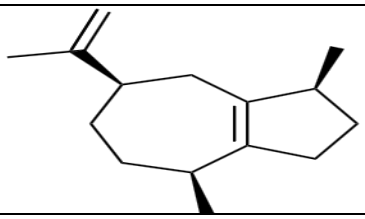
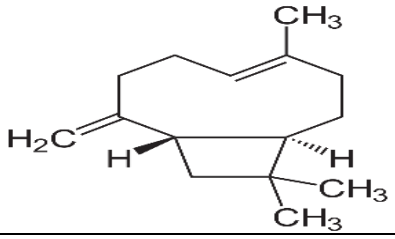
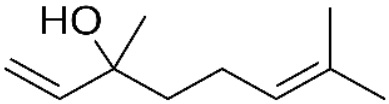
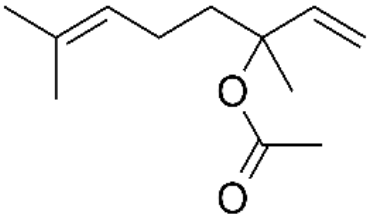
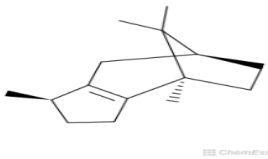
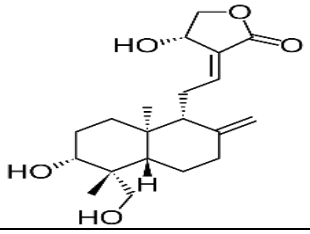
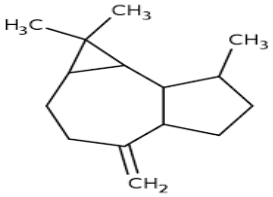
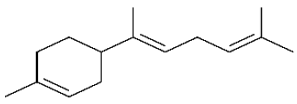
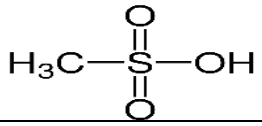
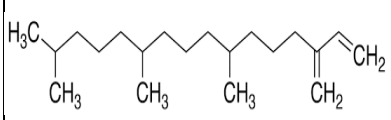
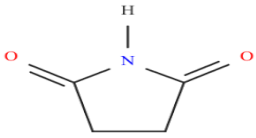
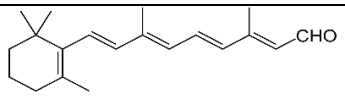
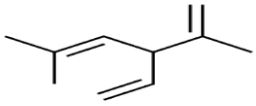
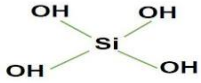
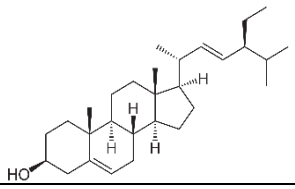


Table 2: List of some of the isolated phytochemicals

Compound	Molecular structure	Molecular formula	Molecular Weight (gmol-1)
Safrole		$C_{10}H_{10}O_2$	162.188 gmol-1
Aciphyllene		$C_{15}H_{24}$	204.35 gmol-1
Alloaromadendrene		$C_{15}H_{24}$	204.35 gmol-1

Aminohippuric Acid		$C_9H_{10}N_2O_3$	194.190 gmol-1
Androstan-17-One, 3,11-Bis[(Trimethylsilyl)Oxy]-, O-(Phenylmethyl)Oxim		$C_{19}H_{30}O_2$	290.447 gmol-1
Arsenous Acid		H_3AsO_3	125.94 gmol-1
Tris (Trimethylsilyl) Ester Azulene, 1,2,3,3a,4,5,6,7-Octahydro-1,4-Dimethyl-7-(1-Methylethenyl)		$C_{10}H_8$	128.174 gmol-1
Caryophyllene		$C_{15}H_{24}$	204.357 gmol-1
Curlone		$C_{15}H_{22}O$	218.33 gmol-1
Cyclobarbital		$C_{12}H_{16}N_2O_3$	236.271 gmol-1
Cycloheptane		C_7H_{14}	98.189 gmol-1
Bromo, Guaia-1(10),11-Dien		$C_{15}H_{24}$	204.357 gmol-1

Isocaryophillene		$C_{15}H_{24}$	204.357 gmol-1
Linalool		$C_{10}H_{18}O$	154.253 gmol-1
Linalyl Acetate		$C_{12}H_{20}O_2$	196.290 gmol-1
Patchoulene		$C_{15}H_{24}$	204.357 gmol-1
Andrographolide		$C_{20}H_{30}O_5$	350.455 gmol-1
Aromandendrene		$C_{15}H_{12}O_6$	288.25 gmol-1
Beta-Bisabolene		$C_{15}H_{24}$	204.357 gmol-1
Methanesulfonothioic Acid S,S'-1,4-Butanediyl Ester		CH_4O_3S	96.10 gmol-1
Neophytadiene		$C_{20}H_{38}$	278.5 gmol-1
Pyrrolidin-2,5-Dione		$C_{10}H_{10}N_2O_2$	190.20 gmol-1

Retinal		$C_{20}H_{28}O$	284.443 gmol-1
Santolina Triene		$C_{10}H_{16}$	136.23 gmol-1
Silicic Acid		H_4O_4Si	96.113 gmol-1
Stigmasterol.		$C_{29}H_{48}O$	412.69 gmol-1

CONCLUSION

The results of the current investigation shows that leaves of *Magnolia griffithii* contains a variety of phytoconstituents, including the alkaloids, sugars, flavonoids, glycosides, phenolics, tannins, saponins, steroids, tannins, and terpenoids. The therapeutic value of this plant is probably due to these chemical constituents. As a result, these plant parts can be utilized to create novel or complementary medications for various illnesses. In-depth analyses of the subject matter being studied are crucial for this.

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Conflict of Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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