

Evaluation of Phytoconstituents of *Syzygium arnottianum* Leaves

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ABSTRACT

Aim: The present study is carried out to explore the major phytoconstituents and functional groups present in the methanolic extract of the leaves of *Syzygium arnottianum* using FTIR and GC-MS. **Methods:** For the identification of the phytochemical constituents, Perkin – Elmer GC Clarus 500 system (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column is used. The molecular weight and structure of the compounds were determined by analysis of the mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST) library data. For the analysis of functional groups, ATR- FTIR spectroscope (Shimadzu IR Prestige-21) is used. **Results:** The GC-MS analysis of the methanolic extract of *S. arnottianum* revealed the presence of 11 bioactive compounds with valuable biological activities. The FTIR analysis indicated the presence of alcohol, alkane, alkene, alkyl halide, alkyne, amine, aromatic, carbonyl, ether, acid, aldehyde, anhydride and ester. **Conclusion:** The phytochemical profile of the plant *S. arnottianum* leaf extract indicates the presence of various bioactive compounds which can be utilised further for medicinal purposes.

Keywords: FT-IR, GC-MS, plant extract, Western Ghats and medicine.

INTRODUCTION

Phytochemical constituents are responsible for medicinal property of plant species. Plants are capable of producing a vast variety of secondary metabolites, which are low-molecular weight organic compounds, usually with unique and complex structures. Most of the herbal medicines and their derivatives were often prepared from crude plant extracts, which consist of a complex mixture of different phytochemical constituents. The chemical features of these constituents vary greatly among different species. The cell walls of different plant species have a variety of physically different polysaccharides and proteins. On the basis of the involvement in plant metabolism, phytochemicals are classified into two groups such as primary and secondary metabolites. Primary metabolites include carbohydrates, amino acids, proteins and chlorophylls whereas secondary metabolites include alkaloids, saponins, steroids, flavonoids, tannins and so on¹. The phytochemical constituents play a major part in the identification of crude drugs².

Syzygium arnottianum (Wight) Walp.) belongs to the family Myrtaceae (Fig.1) and the synonym is *Syzygium densiflorum*. It is endemic to southern Western Ghats and is coming under the category 'vulnerable species'. It is a tall tree with grey bark and simple and opposite leaves³. The tree has been used as food and source of wood. Essential oil of leaves of *Syzygium arnottianum* showed high anti-oxidant capacity⁴. The fruit has been used for the treatment of toothache whereas the leaves have been used for the treatment of diabetes mellitus from earlier time itself. Trace elements present in this tree make a good daily

supplement for people suffering from bone and anaemic disorders⁵. The potential phytochemical compounds such as tannins, saponins, flavonoids, alkaloids, quinine, cardiac glycosides, terpenoids, phenols and carbohydrates were present in the leaves of *S. arnottianum*⁶.

Identification and determination of bioactive compounds can be done with the help of FT-IR and GC-MS technologies^{7,8,9,10,11}. GC-MS is a unique method to recognise the bioactive components of long chain branched hydrocarbons, alcohols, acids, ester etc.¹². The functional groups in plant samples can be identified by FT-IR Spectroscopy. The present study evaluated the functional groups and phytochemicals present in methanol extract of *S. arnottianum* leaf with the help of FT-IR and GC-MS.

MATERIALS AND METHODS

Collection and processing of plant material

The leaves of *S. arnottianum* were collected from Pampadum Shola National Park, Kerala and identified with taxonomic keys¹³. Freshly fallen leaves of the plant were collected and thoroughly washed with distilled water. The leaves were then cut into small pieces and shade dried. The dried leaves were crushed into powder and preserved in air sealed polythene cover.

Preparation of plant extract

Soxhlet apparatus was employed to extract the dried leaves using methanol as a solvent. 20gm of plant material was extracted in 250ml of methanol, filtered and the extracts were concentrated using rotary evaporator at 100 rpm for



Figure 1: Plant of *Syzygium arnottianum*.

Table 1: Preliminary phytochemical analysis of methanol extract of *S. arnottianum*.

Phytochemical constituents	Methanol Extract
Proteins	+
Alkaloids	+
Phytosterols	-
Diterpenes	+
Phenols	+
Flavonoids	+
Glycosides	-
Saponins	+
Carbohydrates	+
Phenols/Tannins	+

30 minutes. This residue was collected, dried and was kept in refrigerator at 4°C for further phytochemical analysis.

GC-MS Analysis

The GC – MS analysis was carried out using Perkin – Elmer GC Clarus 500 system (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column. The identification of components on the basis of comparing the relative retention time and mass spectra with NIST Library data¹⁴. The name, molecular weight and structure of the constituents of the sample material were also determined.

FT-IR Analysis

10 mg of the dried extract powder was condensed in 100 mg of KBr pellet. The powdered sample of each extract was loaded in ATR- FTIR spectroscope (Shimadzu IR Prestige-21 with ZnSe ATR crystal), with a scan range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹. All the analysis was repeated thrice. The functional groups of the active compounds present were identified on the basis of the peak values in the region of IR radiation¹⁵.

RESULTS AND DISCUSSION

The results showed that the leaves of *S. arnottianum* contains the foremost phytochemical constituents include alkaloids, proteins, phenols, tannins, carbohydrates, saponins, glycosides, flavonoids, diterpenes and phytosterols (Table 1).

The various phytochemical compounds detected are known to have beneficial importance in medical sciences. The secondary metabolites like phenolics and flavonoids from the plants are considered to be powerful free radical scavengers due to their inherent capacity to transform the body's response to allergies and virus; and they indicated their anti-allergic, anti-inflammatory, anti-microbial, anti-oxidant and anti-cancerous activities¹⁶. Flavonoids, a group of poly phenolic compounds, also can be used as an anti-inflammatory agent as it affects the radical scavenging, inhibition of hydrolytic and oxidative enzymes¹⁷.

Phenolic compounds perform as a cell supportive material; as they form an important part in the cell wall structure by polymeric phenolics¹⁸. Large quantity of phenolic content in any plant specifies its use in the treatment of inflammatory diseases and wound healing. Hence, pharmacologists are generally searching plants with high phenolic content. *S. arnottianum* leaves were already used indigenously for the treatment of inflammatory diseases.

Tannins are complex compounds of non-carbohydrate residue which have astringent, anti-inflammatory, antidiarrheal, antioxidant and antimicrobial activities like antiviral, antibacterial, antifungal and anti-tumorous activities¹⁹. It was also reported that certain tannins were able to prevent HIV replication selectively and was also used as diuretic. Hence, the leaves of *S. arnottianum* can also consider for such treatments as it exhibit these properties. The presence of saponins enhances the utilisation of *S. arnottianum* leaves for the treatment of hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss etc.

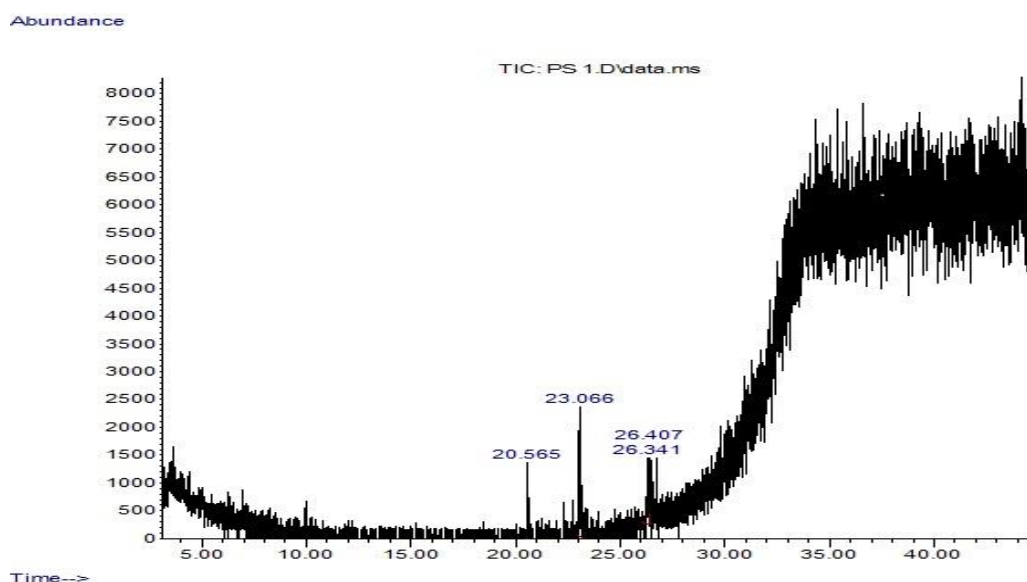
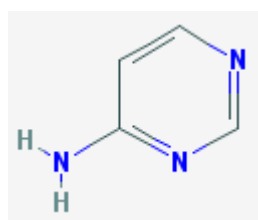
The GC-MS chromatogram was represented in fig. 2 and the constituents obtained from GC-MS analysis are given in table 2.

The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula (Table 2). Eleven phytochemical compounds are present in the leaves of *S. arnottianum*. These compounds are 4-Aminopyrimidine (RT=20.567), 5-methyl-1-Methyl-1H-1,2,4-triazole (RT=20.567), Oxazole (RT=23.068), 4,5-dihydro-2-methyl- Dichloro acetic acid (RT=23.068), hexyl ester Hydrazine (RT=23.068), [(1,2,3-eta.)-2-butenyl].eta.8-1,3,5,7-cyclooctatetraene)-Cyclopentane (RT=26.341), 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediy] bis-Propanedinitrile (RT=26.341), Cyclohexane (RT=26.410), 1,2,4,5-tetraethyl-2-Thiopheneacetic acid (RT=26.410), Oct-3-en-2-yl ester Cyclopentanone (RT=26.410 (1,2,3-eta.)-2-butenyl].eta.8-1,3,5,7-cyclooctatetraene)-Cyclopentane). The identified compounds have several biological properties²⁰.

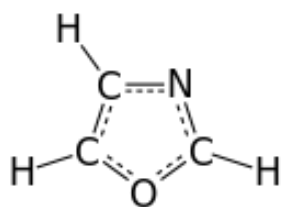
The results obtained from GC-MS analysis shows that most of the phytochemical groups in the leaves of *S. arnottianum* have pharmaceutical activities and some of are discussed here. 4-Aminopyrimidine has been used as a drug for curing multiple sclerosis²¹. This is used as a hair growth stimulant and potent convulsant²². Oxazole have anti-cancerous, anti-viral, anti-diabetic and antibiotic activity. Cyclopentanone, another compound obtained, has

Table 2: GC-MS spectral analysis of methanol extract of *Syzygium arnottianum*.

No.	RT (min)	Name of the compound	Library/ID	Ref#	CAS#	Peak Area %
1	20.567	4-Aminopyrimidine, 5-methyl-1-Methyl-1H-1,2,4-triazole	C:\Database\NIST11.L	2651 1287 1282	000591-54-8 005765-44-6 006086-21-1	14.32
2	23.068	Oxazole, 4,5-dihydro-2-methyl-Dichloroacetic acid, hexyl ester Hydrazine	C:\Database\NIST11.L	1564 71578 277	001120-64-5 037079-04-2 000057-14-7	41.34
3	26.341	[(1,2,3-.eta.)-2-butenyl].eta.8-1,3,5,7-cyclooctatetraene)-Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5pentanediyl]bis-Propanedinitrile,	C:\Database\NIST11.L	66801 148323 1015	039015-02-6 055255-85-1 000922-64-5	20.16
4	26.410	Cyclohexane, 1,2,4,5-tetraethyl-2-Thiopheneacetic acid, oct-3-en-2-yl ester Cyclopentanone	C:\Database\NIST11.L	58317 103739 3234	061142-00-5 1000299-38-6 001120-72-5	24.18

Figure 2: GC-MS Chromatogram of Methanol Extract of *syzygium arnottianum*.

4-Aminopyrimidine



Oxazole

Figure 3: The structure of various phytochemicals contribute to the medicinal activity.

been used as an intermediate for the production of medicines and perfumes. The structure of the various phytochemicals (Fig -3) which contribute to the medicinal activity of the plant methanol extract of *S. arnottianum*.

FTIR spectroscopy is used to be an essential and sensitive technique for find out the bio molecular composition²³. The results of FTIR spectroscopic analysis in the methanol extract of leaf litter of *Syzygium arnottianum* have revealed the presence of numerous chemical compounds (Fig. 4). The peak formation in the FTIR spectrum represents the functional groups (Table 3).

The absorption at 3363.86 cm^{-1} assigned to O-H of alcohols and phenol groups. The band at 3307.92 cm^{-1} , 3292.49 cm^{-1} , 3263.56 cm^{-1} , 3203.76 cm^{-1} , 1627.92 cm^{-1} , 1614.42 cm^{-1} is due to the N-H stretching that present in the extract. The band at 2918.30 cm^{-1} , 2850.79 cm^{-1} is due to C-H stretching of methylene asym. /sym. The IR finger prints of protein are featured by a set of absorption regions represented as the amide region and the C-H region. The band at 1379.10 cm^{-1} showed aromatic C-H in plane bend.

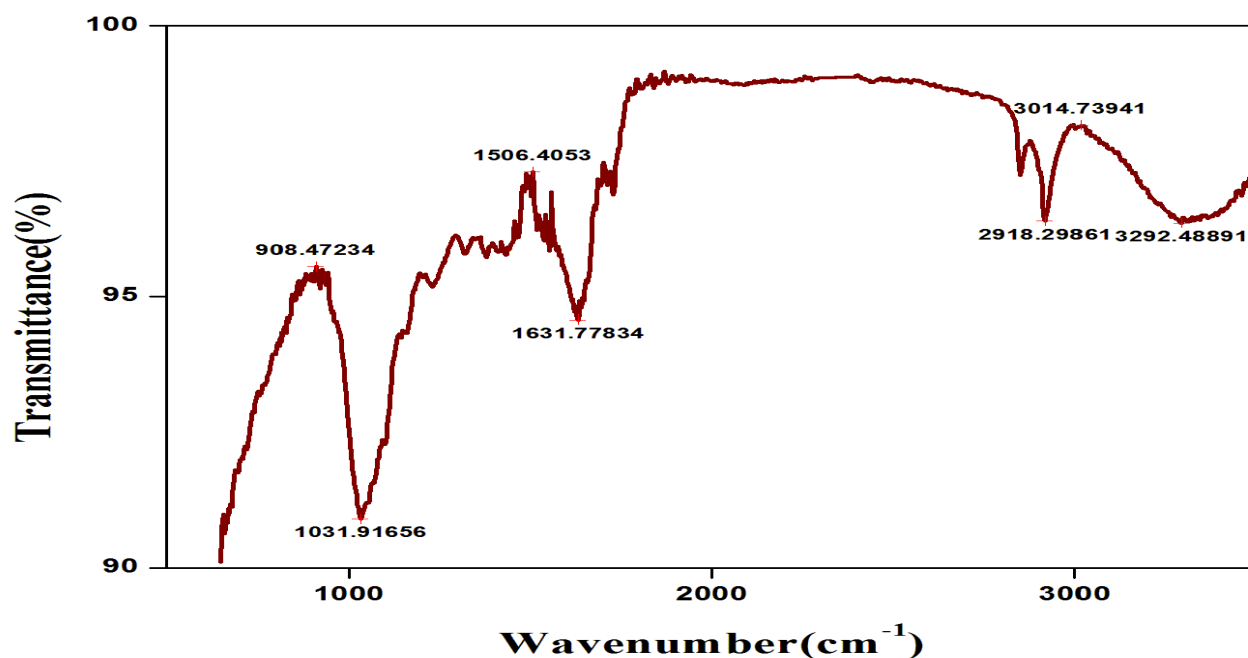


Figure 4: FT- IR spectrum of methanol extract of *Syzygium arnottianum*.

Table 3: FTIR Frequency range and functional groups present in the leaf extracts of *Syzygium arnottianum*.

IR Frequency range	Functional Groups
3363.86	O-H
3307.92	N-H
3292.49	N-H
3263.56	N-H
3203.76	N-H
2918.30	C-H
2850.79	C-H
1728.22	C=O
1627.92	N-H
1614.42	N-H
1571.99	C=C
1537.27	C=C
1517.98	C=C
1433.11	C=C
1379.10	C-H
1317.38	C-F
1228.66	C-F
1220.94	C-F
1201.65	C-F
1153.43	C-F
1031.92	C-O
696.30	C-Cl

The band at 1571.99 cm^{-1} , 1537.27 cm^{-1} , 1517.98 cm^{-1} , 1433.11 cm^{-1} showed alkenyl C=C stretch. The band at 1728.22 cm^{-1} showed C=O stretching vibration of the peptide group means that some carbonyl compounds existed in the leaves of *S. arnottianum*. The band at 1317.38 cm^{-1} , 1228.66 cm^{-1} , 1220.94 cm^{-1} , 1201.65 cm^{-1} , 1153.43 cm^{-1} showed C-F stretching present in the extract. The band at 1031.92 cm^{-1} showed the stretching vibration of C-O. The band at 696.30 cm^{-1} showed the presence of carbohydrates present in the leaves. Bands in 1220 cm^{-1} represents C-O stretching of phenolics and asymmetric C-

C-O stretching of esters. The major components are identified based on the fingerprint characters of the peak positions, shapes and intensities²⁴. Based on the analysis of functional groups, it can be proved that carboxylic acids, aldehydes, aromatics, alkenes, phenols or tertiary alcohols, alkanes, aliphatic bromo compounds and alkynes might be accountable for numerous pharmaceutical properties of *S. arnottianum*^{25, 26}.

The FT-IR spectrum at 1101.28- 1152.10 cm^{-1} is because of the vibration stretching for (C-H) bond of aromatic compound which contains carboxylic acids, aldehydes, aromatics, alkenes, phenols, alkanes, aliphatic bromo compounds, alkynes, carbonyl and ether group²⁷. Alkenes are used as anaesthetics. Carboxylic acids help in maintaining the cell membrane and control nutrient use along with metabolism. Phenol is used as an antiseptic and is also used as a preservative in some vaccines. Alkynes have antifungal and antitumor activities. Phenol spray is used medically to help sore throat. The peak at 2923.95-2926.37 cm^{-1} is allocated to the C-H stretching means that some alkane compounds were present in rare medicinal plants²⁸. Hence, the FT-IR results showed that the leaves of *S. arnottianum* have antiseptic, anaesthetic, antimicrobial and antitumor activities. The functional groups appeared in FT-IR was related to qualitative phytochemical screening of methanol extracts and these studies pave a method for active separation of different phytochemical compounds with the help of GC-MS.

The plants species can be used for antifungal agents as it is confirmed that phenolic compounds from natural resources have antifungal activity²⁹. Carboxylic acids, present in various plant metabolites, were linked with numerous antimicrobial and antifungal activities³⁰. Hence the leaf extract of the studied species can be used as antibacterial agent.

Every organism is physiologically controlled by enzymes and hormones, which are basically proteins³¹. The leaves

of *S. arnottianum* contain rich content of proteins as observed in this study. Hence the present study substantiates the use of leaves as food supplement during the past.

CONCLUSION

The present study concluded that the leaves of *S. arnottianum* have numerous medicinal properties and isolation of individual phytochemical constituents may proceed to find out novel drugs.

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