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Identification of Phytochemical Constituents of *Michelia nilagirica* Leaves

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Abstract

The present study is to find out the major phytochemical components and functional groups present in the leaves of *Michelia nilagirica* using FTIR and GC-MS. For the identification of the phytochemical constituents, TRACE 1300 Thermo Scientific gas chromatography and ISQ QD (Single Quadrapole) Thermo Scientific mass spectrometer equipped with TG-5MS capillary column (5% Diphenyl 95% dimethyl siloxane) (30 m x 0.25 mm x 0.25 µm) is used. The molecular weight and structure of the compounds were detected by investigation of the mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST) library data. For the exploration of functional groups, ATR- FTIR spectroscope (Shimadzu IR Prestige-21) is used. The GC-MS analysis of the leaves of *M. nilagirica* revealed the presence of twenty bioactive compounds with valuable biological activities. The FTIR analysis indicated the presence of amine, alkane, acetyl, alkene, alkyl fluoride, carbonyl and alkyl halides. The phytochemical profile of the *M. nilagirica* plant leaf extract specifies the occurrence of numerous bioactive compounds which can be further applied for pharmaceutical purposes.

Keywords: FT-IR; GC-MS; Medicinal property; Secondary metabolites; Western Ghats

Introduction

Phytochemicals are chemicals produced by plants through primary or secondary metabolism [1,2]. Phytochemical constituents are accountable for therapeutic activity of plant species. Plants are able to produce a wide range of secondary metabolites, normally with single and complex structures. Most of the herbal medicines and their by-products were often prepared from crude plant extracts, which consist of a complex mixture of diverse phytochemical components [3]. The chemical features of these components vary considerably among different species. The cell walls of different plant species have a collection of physically altered polysaccharides and proteins. Based on the role in plant metabolism, phytochemicals are characterized into two groups i.e. primary and secondary metabolites. Primary metabolites involve carbohydrates, amino acids, proteins and chlorophylls where, secondary metabolites comprise of alkaloids, saponins, steroids, flavonoids, tannins and so on [4]. The phytochemical constituents achieve a most essential part in the identification of crude drugs [5].

Michelia nilagirica Linn. belonging to the family Magnoliaceae is the scientific and binomial name of the commercially famous plant 'Kattu chempakam' (Figure 1). Several traditional experts used this plant parts for the herbal preparations for diabetes [6] and kidney diseases [7]. By practice, it is used for remediating fever, stomach pain, leprosy, post-partum protection [8] and eye sicknesses [9]. It has been informed to have antipyretic, anti-inflammatory [10], insecticidal [11], antimicrobial and leishmanicidal activities [12]. The active components stated in this plant are alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids. Identification and assessment of bioactive compounds by means of FT-IR and GC-MS technologies [13-17]; GC-MS is used to familiarize the bioactive constituents of long chain branched hydrocarbons, alcohols, acids, ester etc. [18]. The functional groups can be identified by FT-IR Spectroscopy. The present study estimated the phytocomponents and functional groups present in the methanol extract of leaves of *M. nilagirica* using GC-MS and FT-IR respectively.

Materials and Methods

Collection and processing of plant material

The leaves of *M. nilagirica* were collected from Pampadum Shola National Park, Kerala and identified with taxonomic keys [19]. Freshly fallen leaves of the plant were collected and thoroughly washed with distilled water. The leaves were then cut into slight pieces and shade dried. The dried leaves were crushed into powder form and conserved in airtight polythene cover.

Figure 1: *Michelia nilagirica*

Preparation of plant extract for phytochemical analysis

The leaves were shade dried, powdered and extracted using methanol. Extraction was done using Soxhlet apparatus and filtered. The extracts were concentrated by means of rotary evaporator at 100 rpm for 30 minutes. This residue was collected, dried and was preserved in refrigerator at 4°C for further phytochemical analysis.

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Thin layer chromatographic studies (TLC)

Pre-coated TLC plates with silica were used in which the supporting material is the aluminium foil. Thin layer chromatography was accomplished by put on the solute as a spot. The developed chromatogram was observed under short wavelength UV light (254 nm).

Column chromatography

The dried plant residue after rotary evaporation was dissolved in hexane for column chromatography [20]. The silica gel (60-120 mesh size) as slurry with hexane was used for column packing. Semisolid hexane extract was transferred through a funnel into the packed column. The solvent level was kept 2.5 cm above the extract. The solvents used were hexane, dichloromethane, ethyl acetate and methanol and the TLC plates were scanned using CAMAG TLC Scanner 4.

HPTLC analysis

HPTLC (CAMAG LINOMAT-5, Switzerland) analysis was done mainly for the detection of major components present in the leaf extract [21]. This method was carried out on an aluminium foil backed silica gel GF 254 HPTLC plate (Merck) of size 6.0 x 11.0 cm, which was earlier saturated for 20 min with the chloroform: methanol (0.5:9.5) solvent system. The plate was then subjected to densitometric scanning using CAMAG TLC Scanner 4 at wavelength of 366 nm. The scanning procedure was realized using Win Cats software (version 1.4.4.6337)10. The detection of phytochemical components present in the fractioned plant samples was observed on the HPTLC plates in the form of bands.

Preliminary screening of phytochemical constituents

The following standard methods were used for the preliminary screening of leaves (Table 1) [22].

GC-MS analysis

GC-MS analysis were done using TRACE 1300 Thermo Scientific gas chromatography and ISQ QD Thermo Scientific mass spectrometer equipped with TG-5MS capillary column (5% Diphenyl 95% dimethyl siloxane) (30 m x 0.25 mm x 0.25 μm). ISQ QD Thermo Scientific mass detector was operated in EI mode. Helium was the carrier gas at a flow rate of 1 ml/min and the injector was operated at 250˚C. The identification of constituents was based on comparing the relative retention time and mass spectra with NIST Library data [18]. The name, molecular weight and structure of the components of the sample were also determined.

Major phytochemicals analysed	Tests done
Alkaloids	Mayer's Test, Wagner's Test, Dragendroff's Test, Hager's Test
Carbohydrates	Molisch's Test, Benedict's Test, Fehling's Test
Glycosides	Modified Borntrager's Test, Legal's Test
Saponins	Froth Test, Foam Test
Phytosterols	Salkowski's Test, Libermann Burchard's test
Phenols	Ferric Chloride Test
Tannins	Gelatin Test
Flavonoids	Alkaline Reagent Test, Lead acetate Test
Proteins and aminoacids	Xanthoproteic Test, Ninhydrin Test
Diterpenes	Copper acetate Test

Table 1: Tests used for phytochemical analysis

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}. The whole analysis was repeated thrice. The functional groups of the active compounds were identified based on the peak values in the region of IR radiation [23].

Results and Discussion

The leaves of *M. nilagirica* contain the foremost phytochemicals such as alkaloids, phenols, tannins, carbohydrates, saponins, glycosides, flavonoids, diterpenes and phytosterols (Table 2).

The phytochemical compounds obtained have significant value in medical sciences. The secondary metabolites like phenolics and flavonoids have anti-allergic, anti-inflammatory, anti-microbial, anti-oxidant and anti-cancerous activities [24]. Phenolic compounds also have wound healing, cardiovascular problems, antiageing [25], antiapoptosis, anti-inflammation and anti-artherosclerosis properties [26]. Traditionally, the leaves of *M. nilagirica* were used for the treatment of inflammatory diseases and it might be due to the presence of phenolics, flavonoids, terpenoids, alkaloids and saponins which is having biological activities such as antioxidant, cytotoxic and antimicrobial action [27].

Tannins have astringent, anti-inflammatory, antidiarrheal, antioxidant, antiviral, antibacterial, antifungal and anti-tumorous activities [28]. They also inhibit HIV replication and used as diuretic. Saponins present in the leaves which were used for remediating hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss, precipitating and coagulating red blood cells, cholesterol binding and haemolytic activity [29]. They are also used as detergents, molluscicides, foaming and surfactant [30].

Alkaloids have analgesic, antispasmodic and bactericidal activities [31]. Glycoside compounds are medically important due to their action on heart and are used in cardiac insufficiency [32]. Therefore, it can be used in the treatment of congestive heart failure and cardiac arrhythmia. Terpenoids encourages glutathione-S-transferase and cancer cell apoptosis [33].

The results shown that the leaves of *M. nilagirica* can be used as anti-allergic, anti-inflammatory, anti-microbial, anti-cancerous,

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

Table 2: Preliminary phytochemical analysis of methanol extract of *M. nilagirica*

hypercholesterolemia, hyperglycaemia, antioxidant, astringent, analgesic, antispasmodic, antiapoptosis, anti-ageing, anti-artherosclerosis, antidiarrheal etc.

Twenty fractions of components were obtained with chloroform: methanol in the ratio of 0.5:9.5 (Table 3). The fractions with same R_f value were isolated to give a single compound and the results showed some of the compounds have same R_f value.

HPTLC analysis of *M. nilagirica*

The bands formed in the TLC plates were observed under UV light at 254 nm. The major compounds were separated in the TLC plates and are seen as separable bands (Figure 2).

About nineteen compounds were obtained from the leaves of *M. nilagirica* (Table 4 and Figure 3). Many of these compounds have therapeutic activities also Octadecanoic acid is a fatty acid, which have antihelminthic and antibacterial activity. Methyl ester is having anti-inflammatory, antimicrobial and anti-cancerous activity. 1,2, 3,6-Tetrahydropyridine and (S)-(+)-5-Methyl-1-heptanol is having anti-inflammatory and antimicrobial activity respectively. 2-Dodecanol has been used for producing surfactants, lubricating oils, pharmaceuticals, development of monolithic polymers and also used as a flavour in food. In cosmetics, dodecanol has been used as an emollient. It is also the precursor to dodecanal, a vital fragrance. Myristic acid is having a positive impact on HDL (good cholesterol) to total cholesterol ratio [34]. It is also used as a drug for the treatment of cancer [35]. Hexadecanoic acid (Palmitic acid) has antipsychotic, used in the treatment of schizophrenia, an antioxidant and a source of vitamin A. Quinic acid is a better potent drug to combat prostate cancer [36] and a therapeutic agent in Alzheimer disease [37]. It is also used for the production of new drugs and medicine for the treatment of influenza A and B strains called Tamiflu. 9,12,15 octadecatrienal has been used as cancer preventive and have hypo cholesterolemic and anticoronary activity [38].

Sl. No.	Eluent solvent (100 ml)	Fractions	TLC Solvent System (Chloroform: Methanol)
			R_f: 0.5:9.5
1	Hexane	7	1.33, 1.21, 1.14, 1.19, 1.4, 1.23, 1.19
2	Ethyl acetate	7	1.14, 1.19, 1.4, 1.2, 1.1, 1.4, 1.6
3	Methanol	6	1.3, 1.2, 1.23, 1.3, 1.21, 1.24

Table 3: Column chromatography of methanolic extracts of *M. nilagirica* leaves

Lane 1 & 2: ***M. nilagirica***

Figure 2: TLC finger print of *M. nilagirica*

No.	RT (min)	Compounds identified	Molecular formula	Library/ID
1	6.840	1,2,3,6-Tetrahydropyridine, 1,1'-Bicyclopropyl 1-Propene	C_5H_9N	C:\ Xcalibur\ NIST11.L
2	10.445	(S)-(+)-5-Methyl-1-heptanol, 2,4,6-Trimethyl-1-nonene 1-Heptanol	$C_8H_{18}O$	C:\ Xcalibur\ NIST11.L
3	15.223	5-Octadecene, 1-Dodecene	$C_{18}H_{36}$ $C_{12}H_{24}$	C:\ Xcalibur\ NIST11.L
4	14.39	Linolenic acid, 9,12,15 octadecatrienal	$C_{18}H_{32}O_2$ $C_{18}H_{30}O$	C:\ Xcalibur\ NIST11.L
5	38.27	2-Hydroxyacetophenone	$C_8H_8O_2$	C:\ Xcalibur\ NIST11.L
6	7.55	3,5 dihydroxy-6-methyl-2,3-dihydro-4h-pyran-4-one	$C_6H_8O_4$	C:\ Xcalibur\ NIST11.L
7	10.951	Quinic acid, 2- Dodecanol	$C_7H_{12}O_6$ $C_{12}H_{26}O$	C:\ Xcalibur\ NIST11.L
8	16.828	Myristicacid, Hexadecanoic acid	$C_{14}H_{28}O_2$ $CH_3(CH_2)_{14}COOH$	C:\ Xcalibur\ NIST11.L
9	22.51	5-(Hydroxymethyl)dihydro-2(3H)-furanone	$C_5H_8O_3$	C:\ Xcalibur\ NIST11.L
10	11.09	N-Pentadecane	$C_{15}H_{32}$	C:\ Xcalibur\ NIST11.L
11	16.1	Methyl myristate	$C_{15}H_{30}O_2$	C:\ Xcalibur\ NIST11.L
12	14.680	Methyl, 19-octadecanoate	$C_{19}H_{38}O_2$	C:\ Xcalibur\ NIST11.L
13	14.928	Isopentyl isovalerate	$C_{10}H_{20}O_2$	C:\ Xcalibur\ NIST11.L

Table 4: GC-MS spectral analysis of methanol extract of *M. nilagiric*

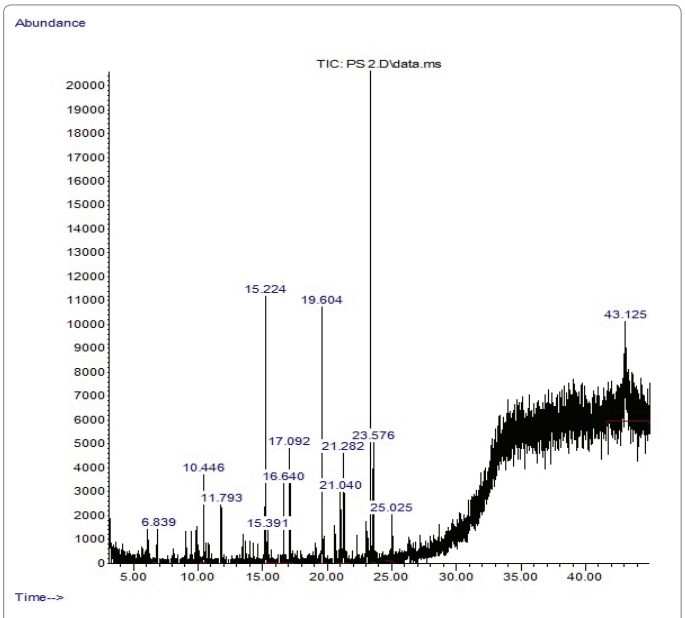

Figure 3: Mass spectra of identified compounds from *Michelia nilagirica*.

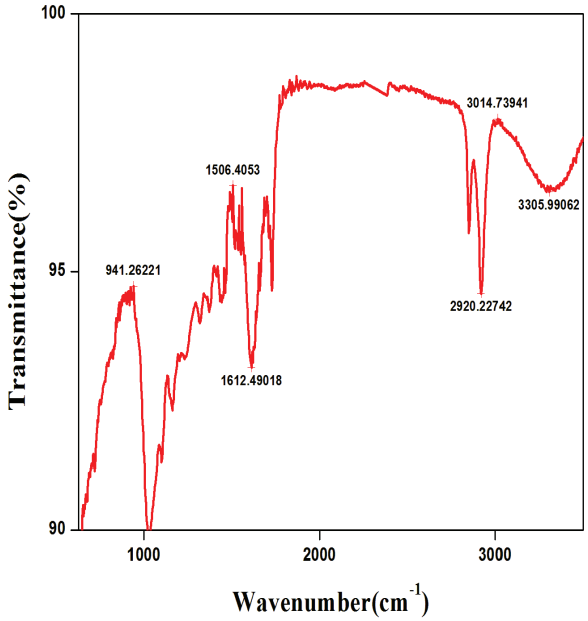

Figure 4: FT- IR spectrum of *Michelia nilagirica*.

Linolenic acid is used for treating diseases of the heart and blood vessels. It is used to prevent heart attacks, reduce high blood pressure, cholesterol and reverse atherosclerosis. It is also used to treat rheumatoid arthritis (RA), multiple sclerosis (MS), lupus, diabetes, renal disease, ulcerative colitis and Crohn's disease. Other uses include treatment of chronic obstructive pulmonary disease (COPD), migraine headache, skin cancer, depression, allergic and inflammatory situations such as psoriasis and eczema. Hexadecanoic acid is a fatty acid ester and it may be used as an antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicide [39,40].

The results of FTIR spectroscopic analysis in the methanol extract of leaves of *M. nilagirica* have revealed the presence of several chemical compounds (Figure 4). The peak formation in the FTIR spectrum denotes the functional groups (Table 5).

Based on FTIR spectra, the methanol extract of leaves of *M. nilagirica* contains seven major functional groups like N-H, C-H, C=O, C=C, C-F, C-O, C-Cl etc. The band at 3305.99 cm^{-1} is due to the N-H stretching present in the extract. The band at 2920.22 cm^{-1} is due to C-H stretching of methylene asym. /sym. The band at 1612.49 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 *M. nilagirica*. The band at 1506.40 cm^{-1}, showed C-F stretching present in the extract. The band at 1141.86 cm^{-1} showed the stretching vibration of C-O. The band width at 941.26 cm^{-1} showed C-Cl stretching of alkyl halide group. The major components are identified on the basis of the fingerprint characters of the peak positions, shapes and intensities [41]. Based on the identification of functional groups, it can be proved that common groups like amine, alkane, acetyl, alkene, alkyl fluoride, carbonyl and alkyl halides were present.

Alkenes, alkanes, amines and alkyl halides are used as anaesthetics. Alkanes have antifungal and antitumor activities. Acetyl group have anti-inflammatant property. Hence, the FT-IR results showed that the leaves of *M. nilagirica* have antiseptic, anaesthetic, antimicrobial and antitumor activities. Hence the leaf extract of the *M. nilagirica* can be used for developing drugs for numerous diseases.

IR Band Width	Functional Groups
3305.99	N-H
2920.22	C-H
1728.22	C=O
1612.49	C=C
1506.40	C-F
1141.86	C-O
941.26	C-Cl

Table 5: FTIR band width and functional groups present in the leaves of *Michaelia nilagirica*

Conclusion

The medicinal properties of the plants have commercial importance in both research institutes and pharmaceutical companies for the preparation of the novel drugs for curing various diseases. Thus the present study concluded that the important therapeutic properties of M. nilagirica will be helpful in the treatment of various diseases.

Conflict of Interest

The authors declared no conflict of interest with anyone.

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