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PHYTOCHEMICAL SCREENING AND GC–MS ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN ETHANOLIC LEAVES EXTRACT OF AN ENDEMIC PLANT *CHIONANTHUS MALA–ELENGI* (DENNST.) P.S.GREEN

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Abstract

Chionanthus mala-elengi (Dennst.) P.S. Green is an endemic tree species belongs to Oleaceae. It is one of the indigenous folk medicine in Western Ghats. It is used to treat giddiness, liver affection, epilepsy, brain related disease and wound healing. However, till date there are no reports available on its phytochemical constituents. The objective of the present study is to identify the phytochemicals present in ethanolic leaves extract of *Chionanthus mala-elengi* and to determine the bioactive compounds using GC-MS (Gas Chromatography-Mass Spectrometry). The phytochemical analysis revealed the presence of phenol, flavanoid, tannin, saponin, alkaloid, cardiac glycoside, terpenoid, volatile oil, sterol, anthraquinone, carbohydrate and balsam. The GC-MS results led to identification of seventeen phytoconstituents in ethanolic leaves extract of *C. mala-elengi*. Based on the peak area the major chemical constituents were Cuparophenol (19.26%), Ethyl p-methoxycinnamate (13.92%), Phytol (8.50%), Hexadecanoic acid (7.70%), 2H-Benzocyclohepten-2-one, 3, 4, 4A, 5, 6, 7, 8, 9 - octahydro-(7.45%), 2(3H)-Naphthalenone, 4, 4A, 5, 6, 7, 8-hexahydro-4A-methyl-(7.05%) and p-Vinylguaiacol (6.90%). The other corresponding constituents of *C. mala-elengi* were Phosphoric acid bis (trimethylsilyl) monomethyl ester (6.38%), Artumerone (5.43%), Beta-elemenone (4.23%), Beta-bisabolol (2.84%), B-asarone (1.98%), Curlone (1.91%), Methylterephthalaldehydate (1.76%), 2H-Benzocyclohepten-2-one, 3, 4, 4A, 5, 6, 7, 8, 9-octahydro- (1.62%), Methyl tetradecanoate (1.54%), 7, 9-Di-tert-butyl-1-Oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione (1.54%). The presence of different bioactive constituents confirms the pharmacological application of *C. mala-elengi* for various ailments by traditional folk practitioners. Therefore, the individual chemical constituent isolation may lead to discovery of new drugs.

Key words: *Chionanthus mala-elengi*, Endemic, GC-MS, Phytoconstituents.

Introduction

World Health Organization reported that 80% of world's population rely on the traditional remedy for their major health care system. Plants have been used for various incurable diseases (Alkhawalidy and Hossain, 2015). People have been exploring the medicinal flora for discovery of new drug (Savithramma *et al*., 2011). Herbal drug molecules are safer than the synthetic drug (Karthika *et al*., 2019). Medicinal plants discovery and screening of their extracts is important in medicinal sector, beneficial in establishment of new information towards economic and social benefits (Barbour *et al*., 2004; Umamaheswari *et al.,* 2007).

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Medicinal properties of plants present in their component phytochemicals such as alkaloid, tannin, flavanoid and other phenolic compounds produce a definite physiological action on the human body (Akinmoladun *et al.,* 2007). Secondary metabolites serve as potential sources of new compounds of therapeutic value and also as sources of lead compounds in the drug development. These constituents play a vital role in survival of the plants under harmful conditions and also in the protection from microbes. Therefore, phytochemical screening is necessary to find the bioactive components of plants of therapeutic significance (Singh and Bag, 2013; Priya *et al*., 2018).

In recent days, Although allopathic drugs are

extremely effective and easily available in treating various ailments, most of the people still wish to use traditional folk medicines due to their less harmful side effects (Iqbal *et al.*, 2015). Medicinal plants are the best source to obtain active compounds. They have various pharmacological potential that would allow them to play a vital role in terms of therapeutic action (Akeel *et al*., 2014; Obiang *et al*., 2019). Phytochemical constituents of the plants may exist in all parts of the plant such as roots, stems, leaves, bark, fruit, seeds, flower (Ye *et al*., 2015). Extraction is a vital step in the processing of phytochemicals for the detection of chemical constituents from medicinal plants (Dhanani *et al*., 2017).

Chionanthus mala-elengi (*C. mala-elengi*) (Dennst.) P.S.Green is an endemic tree species of Peninsular India of Western Ghats belonging to Oleaceae. Local name is kallidala and mala-elengi. It is occasionally near threatened species. Flowering period is December to April. It is distributed in various countries like India, Burma, Thailand, Vietnam and Cambodia. In India it is recorded in evergreen forest area of Karnataka, Kerala and Tamilnadu (Deepa *et al*., 2016; Pius *et al*., 2015; Narayanan *et al*., 2018). It is an indigenous folk herbal medicine. Leaves, bark and kernel of the fruit is used for giddiness, liver affection, epilepsy and similar affection of the brain related ailments. Whole plant paste is used for wound healing (Manilal and Remesh, 2010; Kumar *et al*., 2016). Medicine preparation from natural compounds even by pharmaceutical companies may lead to major exposure of humans to natural products. In order to enhance the use of medicinal plants, it should be thoroughly investigated for the chemical compounds activity and thus validating the application (De *et al*., 2013). The literature search revealed that still no scientific work have been done on this plant. For this reason, the objective of this work was to identify the phytochemicals using standard methods and characterize the chemical constituents present in the ethanolic leaves extract of *C. mala-elengi* by using GC-MS analysis.

Materials and Methods

Collection and identification of the plant material

The fresh plant material (*C. mala-elengi*) were collected from Anaikatti, Coimbatore district, Tamilnadu, India. The plant material was identified and authenticated at the Botanical Survey of India, Coimbatore, Tamilnadu, India. Authentication number of the plant is BSI/SRC/5/23/2018/Tech/1850.

Plant material extraction

Leaves of plant material were washed with distilled water to remove the dirt. The plant material was shade dried and pulverized through mechanical grinder. 30g of powdered plant material was extracted with 150ml ethanolic solvent in Soxhlet extractor. After the extraction the solvent was evaporated and dried. Then, the extract was stored at -30°C until further use (Murugan and Parimelazhagan, 2014).

Phytochemical Screening

Preliminary phytochemical screening of secondary metabolites such as phenol, flavanoid, tannin, saponin, alkaloid, cardiac glycoside, terpenoid, volatile oil, sterol, anthraquinone, photobalamine, carbohydrate, protein and balsam was carried out according to standard phytochemical method (Iqbal *et al*., 2015; Prabhavathi *et al*., 2016; Carole *et al*., 2018).

The GC-MS analysis

GC–MS analysis of bioactive compounds in leaves ethanolic extract of *C. mala-elengi* was performed at Kerala Forest Research Institute, Thrissur District, Kerala (Casuga *et al.,* 2016).

Results and Discussion

Phytochemical Screening

The preliminary phytochemical screening of crude ethanolic extract of leaves of *C. mala-elengi* revealed the presence of some phytoconstituents such as phenol, flavanoid, tannin, saponin, alkaloid, cardiac glycoside, terpenoid, volatile oil, sterol, anthraquinone, carbohydrate and balsam. On the other hand protein and photobalamine were not detected in leaves extract of *C. mala-elengi* as shown in table 1. The presence of these phytochemicals are reported to have various biological and therapeutic properties (Senguttuvan *et al.,* 2014). This study also supports the utilization of *C. mala-elengi*

Table 1: Phytochemical analysis of ethanolic leaves extract of *C. mala-elengi.*

S. No.	Test	Ethanol
1	Phenol	+
2	Flavanoid	+
3	Tannin	+
4	Saponin	+
5	Alkaloid	+
6	Cardiac glycoside	+
7	Terpenoid	+
8	Volatile oil	+
9	Sterol	+
10	Anthraquinone	+
11	Photobalamine	-
12	Carbohydrate	+
13	Protein	-
14	Balsam	+

Table 2: Chemical constituents from ethanol extract of *Chionanthus mala-elengi* leaves.

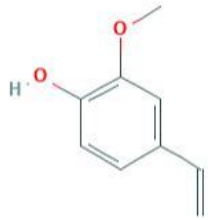
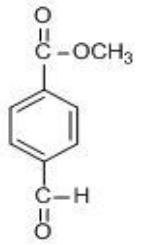
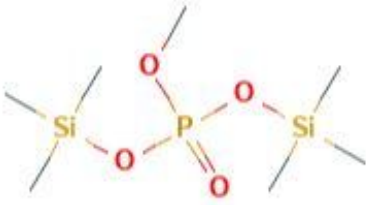
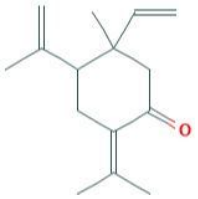
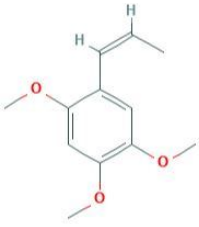
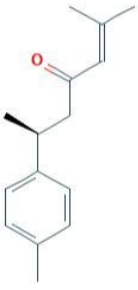
S. No.	Retention time (min)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area %	Compound Structure
1	10.291	p-Vinylguaiacol	C9H10O2	150.17	6.90	
2	11.133	Methylterephthalaldehydate	C9H8O3	164.16	1.76	
3	11.327	Phosphoric acid, bis (trimethylsilyl) mono methyl ester	C7H21O4PSi2	256.38	6.38	
4	12.333	Methyl tetradecanoate	C15H30O2	242	1.54	
5	13.845	Beta-elemenone	C15H12O	218.33	4.23	
6	13.917	B-asarone	C12H16O3	208.25	1.98	
7	14.577	Ar-tumerone	C15H20O	216.32	5.43	

Table 2 Continue ...

Continue Table 2 ...

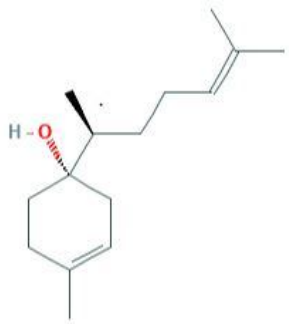
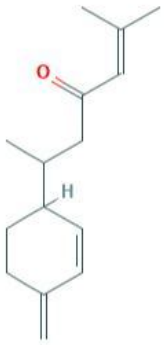
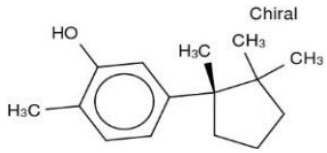
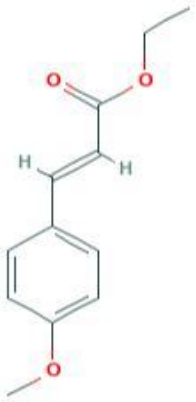
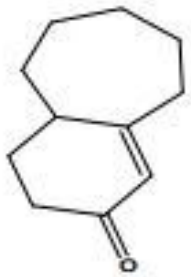
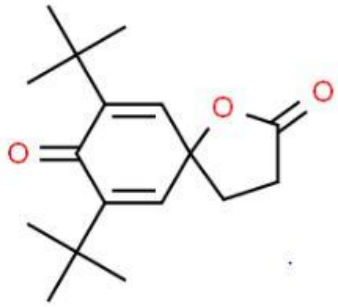
8	14.650	Beta-bisabolol	C15H26O	222.37	2.84	
9	15.023	Curlone	C15H22O	218.33	1.91	
10	15.589	Cuparophenol	C15H22O	202.34	19.26	
11	15.768	Ethyl p-methoxycinnamate	C12H14O3	206.24	13.92	
12	15.970	2H-Benzocyclohepten -2-one,3,4,4A,5,6,7, 8,9-octahydro-	C12H18O	178.27	7.45	
13	16.087	2(3H)-Naphthalenone, 4,4A,5,6,7,8-hexahydro -4A-methyl-	C11H16O	164.244	7.05	

Table 2 Continue ...

Continue Table 2 ...

14	16.208	2H-Benzocyclohepten -2-one,3,4,4A,5,6,7,8, 9-octahydro-	C12H18O	178.27	1.62	
15	17.856	7,9-Di-tert-butyl- 1-Oxaspiro (4,5)deca -6,9- diene-2,8-dione	C17H24O3	276	1.54	
16	19.066	Hexadecanoic acid	C16H32O2	256.42	7.70	
17	22.309	Phytol	C20H40O	296.5	8.50	

in traditional medicine for treatment of various disease. So this species is expected to have many therapeutic uses. For example many alkaloid derived from medicinal plants show biological activities such as anti-inflammatory, antimalarial, antimicrobial, cytotoxicity, antispasmodic and pharmacological effects (Iqbal *et al*., 2015). According to research, tannins are known to have antibacterial, antitumor and antiviral activities (Senguttuvan *et al.,* 2014). Flavanoids play active role in antidiabetic, asthma, sclerosis, psoriasis, rheumatoid arthritis, antimicrobial, therapeutic activities against influenza virus, hepatitis virus (Wang *et al.,* 2018). Phenolics have been associated with the inhibition of atherosclerosis and cancer (Barros *et al*., 2007).

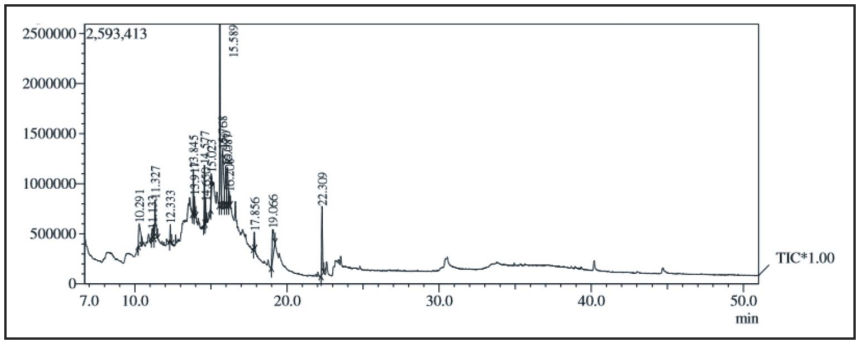


Fig. 1: GC-MS chromatogram of ethanolic leaves extract of *Chionanthus mala-elengi.*

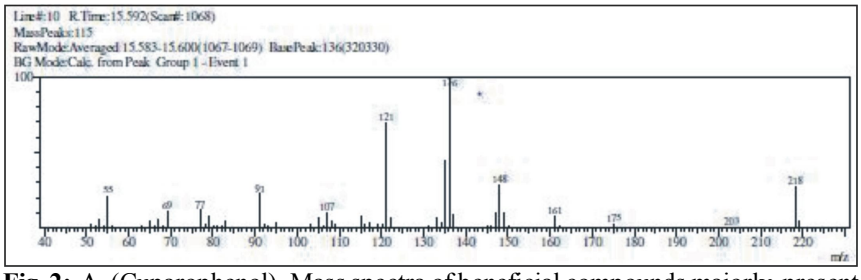


Fig. 2: A. (Cuparophenol). Mass spectra of beneficial compounds majorly present in leaves ethanolic extract of *C. mala-elengi.*

GC-MS analysis

GC-MS analysis of ethanolic leaves extract of *C. mala-elengi* confirmed the presence of 17 different chemical compounds are presented in (Table 2 and Fig. 1). Till date no reports are available on the GC-MS analysis of leaves extract of *C. mala-elengi*. In terms of percentage amount Cuparophenol (19.26%), Ethyl p-methoxycinnamate

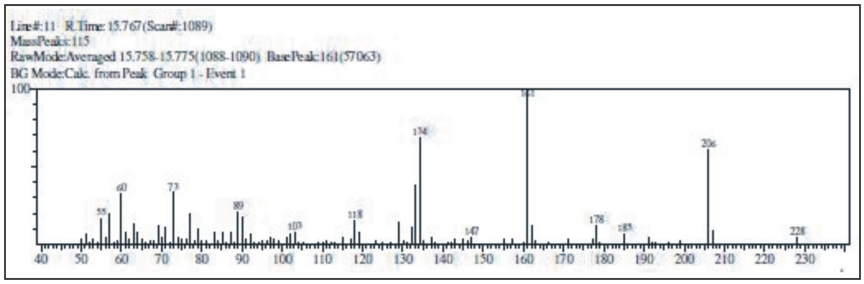


Fig. 2: B. (Ethyl p-methoxycinnamate). Mass spectra of beneficial compounds majorly present in leaves ethanolic extract of *C. mala-elengi.*

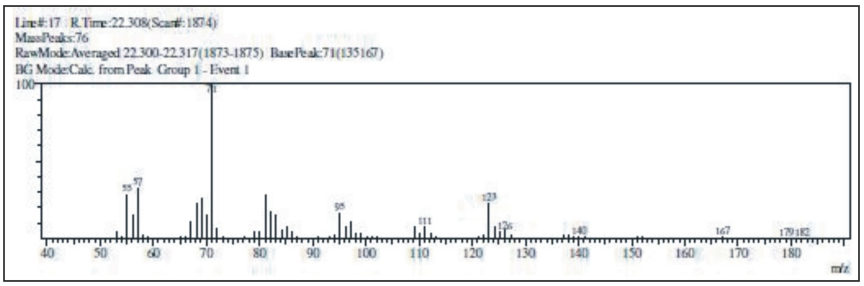


Fig. 2: C. (Phytol). Mass spectra of beneficial compounds majorly present in leaves ethanolic extract of *C. mala-elengi.*

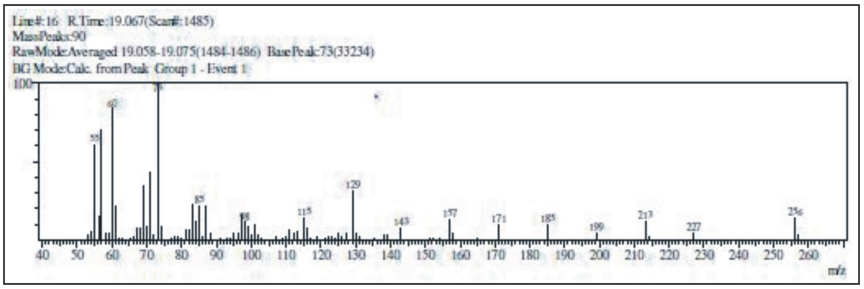


Fig. 2: D. (Hexadecanoic acid). Mass spectra of beneficial compounds majorly present in leaves ethanolic extract of *C. mala-elengi.*

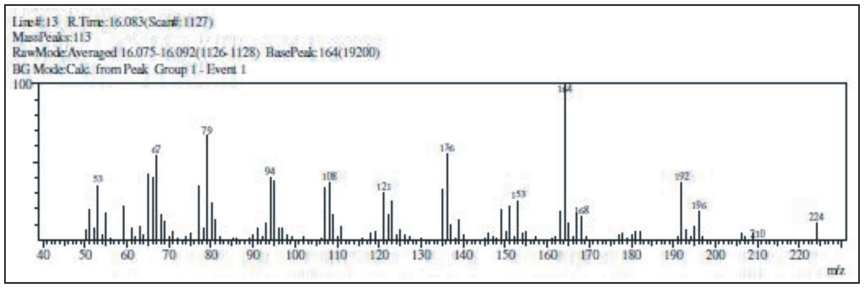


Fig. 2: E. (2(3H)-Naphthalenone 4,4A,5,6,7,8-hexahydro-4a methyl-). Mass spectra of beneficial compounds majorly present in leaves ethanolic extract of *C. mala-elengi.*

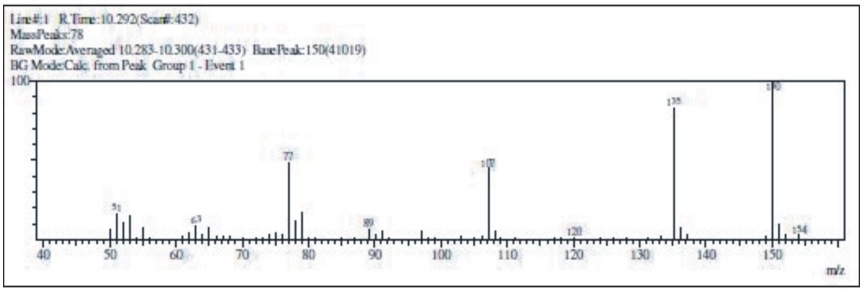


Fig. 2: F. (p-Vinylguaiacol). Mass spectra of beneficial compounds majorly present in leaves ethanolic extract of *C. mala-elengi.*

(13.92%), Phy-tol (8.50%), Hexadecanoic acid (7.70%), 2(3H)-Naphthalenone, 4,4A,5,6,7,8-hexahydro-4A-methyl- (7.05%) and p-Vinylguaiacol (6.90%) are predominant in the extract. These six major compounds have some vital medicinal activity in upcoming drug discovery system such as Cuparophenol (Sesquiterpene) shows antimicrobial activity (Agger *et al*., 2009). On the other hand Ethyl p-methoxycinnamate (Aromatic ester) having anti-inflammatory effect, analgesic effect and anti-angiogenic effect (Umar *et al*., 2014). Phytol (diterpene alcohol), a precursor of synthetic vitamin E and vitamin K was proven to be cytotoxic against breast cancer cell lines (MCF7). It has antimicrobial, anti-inflammatory, anticancer, diuretic, resistant gonorrhoea, joint dislocation, headache, hernia and antimalarial (Tyagi and Agarwal, 2017). It is also used as anticonvulsant, antispasmodic, antinociceptive and antioxidant (Costa *et al*., 2012; Pongprayoon *et al*., 1992; Santos *et al.,* 2013).

Hexadecanoic acid (fatty acid) shows antioxidant, hypocholestrolemic, antiandrogenic and hemolytic effect. It has various activities such as nematicide, pesticide, lubricant and flavoring agent. It also act as alpha reductase inhibitor and hemolytic-5-alpha reductase inhibitor. 2(3H)-Naphthalenone, 4, 4A, 5, 6, 7, 8-hexahydro-4a-methyl- (ketone) has anti-inflammatory activity (Muthusamy *et al*., 2015; Kavitha *et al*., 2012). p-Vinylguaiacol (phenolic compound) revealed antioxidant, antimicrobial and anti-inflammatory activity (Ravikumar *et al*., 2012). The individual fragmentation for few of the major beneficial components is illustrated in Fig. 2A-2F. The Retention time (min), Name of the compound, Molecular formula, Molecular weight (g/mol), Peak area (%) and Compound structure of test material were determined. The

percentage of relative amount of each component was confirmed by comparing its average peak area to the total areas.

Conclusion

The present study revealed that the various phytochemicals and bioactive constituents present in ethanolic leaves extract of *C. mala-elengi*. This study confirmed the use of leaves of *C. mala-elengi* for various disease by traditional practitioners. These beneficial compounds have antioxidant, anticancer, antimicrobial and anti-inflammatory activity. Further detailed *in vitro* and *in vivo* correlation studies together with isolation of active chemical constituents are needed on *C. mala-elengi* to find a novel drug.

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