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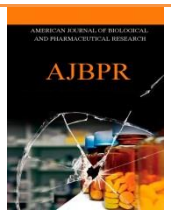

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF ETHANOL LEAVES EXTRACT OF CLERODENDRUM PHLOMIDIS (L.)

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ABSTRACT

Natural products are the source of synthetic and traditional herbal medicine.. *Clerodendrum phlomidis* L. is one of the medicinally elixir plant belonging to the family Verbenaceae. In present study the ethanolic leaves extract of *C. phlomidis* have been evaluated using Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extracts were matched with the National Institute of Standards and Technology (NIST) library. Gas chromatography mass spectrometry (GC-MS) analysis of ethanolic leaves extract of *C. phlomidis* revealed the presence of 18 bioactive compounds which showed the presence of Stigmasterol, Phytol, Squalene and n-Hexadecanoic acid. The study summarizes the information concerning the phytochemical constituents present in ethanolic leaves extract of *C. phlomidis*. These constituents can be responsible for pharmacological activities and they are very essential for the treatment of dangerous diseases and disorders.

INTRODUCTION

Plants are nature's gift to human beings for disease free healthy life. In India, different parts of several medicinal plants and their extracts are used for the treatment of various diseases. Nature is a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants have been used for centuries as remedies for human and animal diseases as they contain therapeutic value due to the presence of phytochemicals[1] .Over the last few decades, use of herbal drugs has been emphasized due to their easy availability, therapeutic potential, No side effects and minimum cost. At present nearly 80% of the world population rely on plant based drugs for their health care need.[2] The human civilization has been maintaining an intimate relationship with the plants from time immemorial. They depend on plants and other natural sources for their well-being and survival [3]. Various plants still available in the nature are yet to be explored for their medicinal potential.[4] Presently, phytoconstituents are playing pivotal role for development of novel compounds, which might be crucial for maintaining a healthy society.

The novel molecules from plant sources have been instrumental in development of structurally modified compounds, which assist a lot in the development of modern therapeutic system [5]. Phytochemicals are responsible for medicinal activity of plants these

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biochemicals are naturally occurring in the plants that have defence mechanism and protect from various diseases [6]. The phytochemical are very important in medicine and constitute most of the valuable drugs [7]. In this biochemicals are often referred to as Secondary metabolites which is useful to traditional medicine system and these bio chemicals are identify by using GC-MS technique [8]. In recent years Gas chromatography – Mass Spectrum (GC-MS) studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds [9].

The plant *Clerodendrum phlomidis* Linn., is one of the medicinally important plants belonging to the family verbenaceae locally known as 'Vathamudakki' a constituent of more than 50 indigenous medicinal formulations is used in the treatment of inflammation, diabetes, nervous disorders, asthma, rheumatism, digestive disorders and urinary disorders and also a bitter tonic. In clinical investigations have revealed anti-inflammatory, hypoglycemic, immune modulatory, antidiarrhoeal and antiplasmodial properties.[10] Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds. Hence the present study focused on Phytochemical Screening and analysis of Bioactive Compounds of Ethanolic Leaves Extract of *Clerodendrum phlomidis* (L.) using Gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Plant materials collection

The leaves of *Clerodendrum phlomidis* were collected from the field of Thanjavur region of Saliyamangalam. The leaves were washed with deionized water, shade dried till the weight become constant at room temperature and ground to fine powder. The species specimen was submitted in herbarium, SIMPRA Research Insitute, Thanjavur region of Tamil Nadu, India and got the voucher specimen No. SIMPRA32413.

Plant sample Extraction

The powdered sample was successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus [11] for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The dried powder was subjected in to GC-MS analysis.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

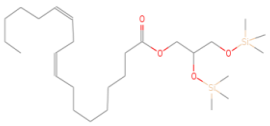
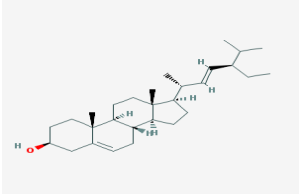
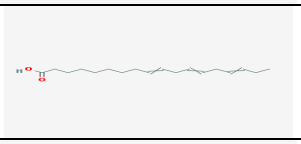
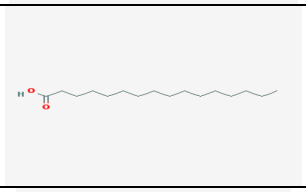
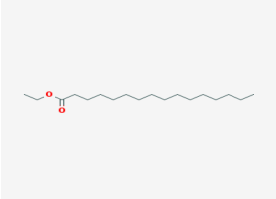
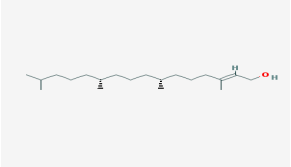
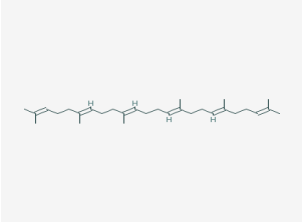
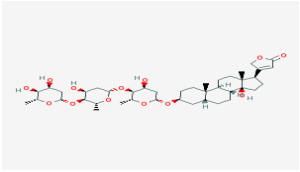
Identification of Compounds

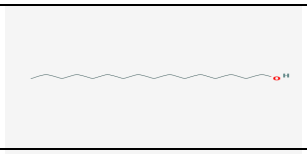
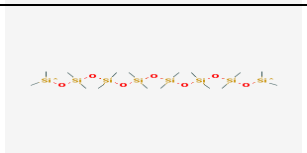
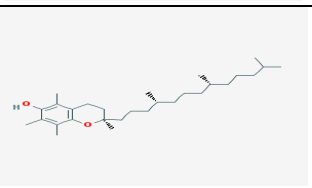
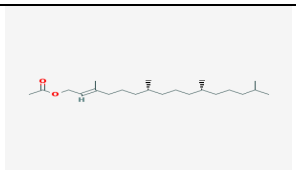
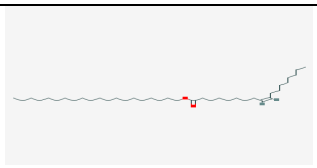
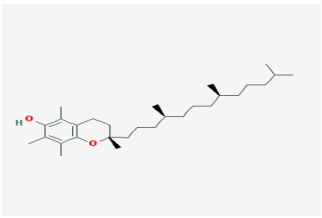
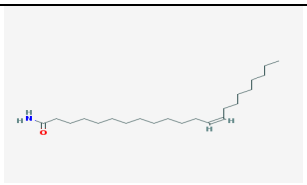
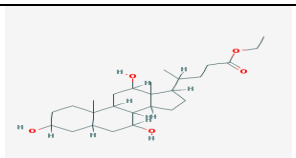
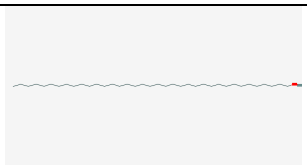
Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Table 1. Phytochemical Analysis of *Clerodendrum phlomidis* L

S.No	Test	Result
1	Alkaloids	+
2	Flavinoids	+
3	Saponins	+
4	Tannins	+
5	Terpinoids	+
6	Phenol	+
7	Cardiac glycosides	+
8	Anthraquinones	-

Table 2. GC-MS Analysis of *Clerodendrum Phlomidis* L

Name of the compound	Nature of the compound	Structure	Molecular Weight	Activity
1-Monolinoleoylglycerol trimethylsilyl ether	Steroid		498	Antimicrobial
Stigmasterol	Steroid		412	Antihepatotoxic, anti-oxidant, hypocholesterolemic, anti-inflammatory,Estrogenic, antiviral
9,12,15-octadecatrienoic acid	Linolenic acid		278	Anti-inflammatory, Anti-arthritic, Anti-coronar, Cancer preventive,Nematicide, Insectifuge
Hexadecanoic acid	Palmitic acid		258	Anti-oxidant, Nematicide
Hexadecanoic acid,ethyl ester	Ester compound		284	Anti-oxidant, Nematicide,Flavor, Pesticide
Phytol	Diterpene		296	Antimicrobial, Anticancer, Diuretic, Anti-inflammatory
Squalene	Triterpene		410	Antiageing, Analgesic, Antidiabetic, Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, . Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
Digitoxin	Steroid		764	Cardio tonic

1-Hexadecanol	Alcohol		242	Anti-oxidant
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15, -Hexadecamethyl	Volatile organic Compounds		578	Anti-microbial
DL-Alpha Tocopherol	Vitamin E compound		430	Anti-septic, preservative flavour stomach and Internal hemorrhoids
Phytol Acetate	Phytol compound		338	Anti-microbial, Antiinflammatory
Oleic acid, eicosyl ester	Ester		562	Antiinflammatory, Cancer preventive, Dermatitigenic
Vitamin E	Vitamin compound		430	Analgesic, Antidiabatic, Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Antispasmodic
13-Docosenamide,(Z)-	Amide group		337	Anti-microbial
Ethyl iso-allocholate	Steroid		436	Antimicrobial
1-Heptatriacotanol	Alcoholic		536	Antimicrobial

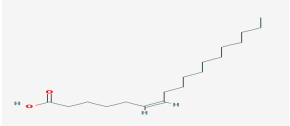
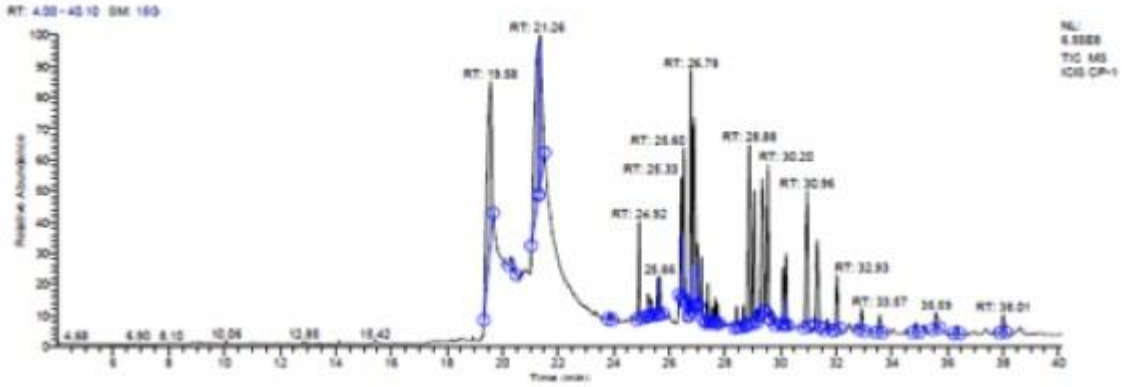
6-octadecenoic acid(z)-	Stearic acid		282	Cancer preventive insectifuge

Fig 1. GC-MS CHROMATOGRAM OF *Clerodendrum phlomidis* . L



RESULT AND DISCUSSION

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties.[12] The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [13]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [14]. In the present study reveals that ethanolic leaves extract *C.phlomidis* exhibited the presence of alkaloids, terpenoids, flavonoids, Cardiac glycosides and saponins. (Table-1.) GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The present study ,GC-MS analysis of ethanolic leaves extract *C.phlomidis* showed 18 major peaks (Table-2.) and have been identified after comparison of the mass spectra with NIST library (Table-2), indicating the presence of various phytocomponents(Fig.1). From the results, it was observed that presence of 1-Monolinoleoylglycerol trimethylsilyl ether, Stigmasterol, 9,12,15-octadecatrienoic acid, Hexadecanoic acid, Hexadecanoic acid-ethyl ester, Phytol, Squalene, Digitoxin,1 Hexadecanol, Octasiloxane,1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15,- Hexadecamethyl, DL-Alpha Tocopherol, Phytol Acetate, Oleic acid, eicosyl ester, Vitamin E, 13 Docosenamide,(Z)-, Ethyl iso-allocholate, 1-Heptatriacotanol, 6-octadecenoic acid(z).

Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies.It is also used to Antimicrobial, Antiinflammatory,[15] Anticancer and Diuretic activites. phytol usually have the insecticidal and antihelmintic or antiseptic activity. Hence *C.phlomidis* may be used as antiseptic drug.

Glycerin decreases intracranial pressure in numerous disease states, including Reye's syndrome, stroke, encephalitis, meningitis, pseudotumor cerebri, central nervous system tumor, and space occupying lesions. It is also effective in lowering intraocular pressure in glaucoma and shrinking the brain during neurosurgical procedures[16].

Squalene is a polyunsaturated triterpene widely found in plants. squalene protects the skin against UV-induced radiation damage due to its high secretion in Sebum. In addition, it has been reported that squalene, as a component of olive oil, is protective against breast cancer [17]and reduces serum cholesterol concentration [18]It is also used to Anti-cancer, Anti-inflammatory,and immunostimulating activities.

Stigmasterol is one of a group of plant sterols, or phytosterols. It is an unsaturated sterol occurring in the plant. It is used as a precursor in the manufacture of semi-synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogens effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids.[19] It is also used as the precursor of vitamin D_3. It is also useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers. It also possesses potent

antioxidant, hypoglycaemic and thyroid inhibiting properties.

Digitoxin is a cardiac glycoside. Digitoxin and related cardenolides display potent anticancer activity against a range of human cancer cell lines in vitro but the clinical use of digitoxin to treat cancer has been restricted by its narrow therapeutic index [20]. It is also used to treat heart failure, and also used to treat a certain type of irregular heartbeat (chronic atrial fibrillation.

Octadecanoic acid, Hexadecanoic acid and stigmasterol compounds have the property of antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory. Palmitic acid is reported to be an antioxidant, nematicide and a pesticide while Melitol and Phytol are said to be cancer preventive.

Vitamin E is synthesised only by plants. It is consists of two families of compounds, the tocopherols and tocotrienols. The major biological role of alpha-tocopherol is antioxidant activity contributing to the prevention of propagation of free radicals in various lipid structures within the organism. In addition to its antioxidant activity, alpha-tocopherol is also suggested to play a role in immune enhancement, inhibition of platelet aggregation and anti-inflammatory functions [21].

Tocotrienols provide valuable therapeutic and preventive options for the diseases. tocotrienols also used to promote new artery formation after a stroke, lower homocysteine levels, improve insulin sensitivity, protect vital brain circuitry, and even prevent bone loss.

In addition, tocotrienols have powerful lipid-lowering, anticancer, and neuroprotective properties. Due to the presence of above mentioned compounds in the ethanolic leaves extract of *C. phlomidis*, which can be utilized for various pharmaceutical and industrial applications in future research activities.

CONCLUSION

In the present study 18 compounds from the ethanol leaves extract of *C. phlomidis* were identified by Gas-chromatography– Mass spectrometry (GC-MS) analysis. The biological activities of each of the identified phytocomponents used for antimicrobial, antioxidant, nematicide and pesticide activities. The research findings have shown that the leaves extract of. *C. phlomidis* is extensively rich in secondary metabolites. The plant leaves has a high potential for a vast number of bioactive compounds which justified its use for various ailments by traditional practitioners.

These findings have provided scientific basis to the ethnomedical usage of the plant. However, isolation of the individual phytochemical constituents, subjecting it to biological activity and toxicity profile will give fruitful results.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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