Research Article

GC-MS analysis for bioactive compounds from methanolic leaf and stem bark extracts of *Hildegardia populifolia* (Roxb.) Schott & Endl.

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ABSTRACT

The present study was aimed at to investigate the bioactive compounds from methanolic leaf and stem bark extracts of the endangered medicinal plant *Hildegardia populifolia* (Sterculiaceae) through GC-MS analysis. Four and twelve biochemical compounds in leaf and stem bark extracts have been identified respectively. Squalene (46.44 %) and 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester (43.87 %) were the major compounds present in leaf extract and Lup-20(29)-en-3-one (76.32 %) was the only major compound present in stem bark extract. Presence of these phytochemical constituents in plant extracts provides scientific evidence for the biological properties of this species.

Keywords: *Hildegardia populifolia,* endangered species, Sterculiaceae, GC-MS.

INTRODUCTION

Medicinal plants are used in the treatment and prevention of various health problems to mankind, thereby improving the quality of life. It is estimated that about 75% of useful bioactive plant derived pharmaceuticals used globally are discovered by systemic investigation of leads from traditional medicines[1]. However, the bioactive compounds are an important source with variety of structural arrangements and properties[2]. Therefore, the screening of medicinal plants for active compounds has become very significant because they may serve as endowed source of bulk antibiotic prototype.

Hildegardia populifolia, belongs to the family, Sterculiaceae is an endangered traditional medicinal tree species and mainly distributed in few tropical deciduous forests of Tamil Nadu and Andhra Pradesh. The bark of this plant is used for the treatment of dog bite and malaria in the traditional medical practice of Tamil Nadu and Andhra Pradesh[3]. Strong fibres obtained from the bark are used for making ropes. Various parts of this species are being prescribed by the local healers of southern India for various ailments. It is reported to have antimicrobial activity[4-6], antioxidant activity[7] and antiinflammatory activity[8]. However, no information is available on phytochemical constituents for this species. To address this lacuna, the present study was carried out to identify bioactive compounds from leaf and stem bark methanolic extracts of *H. populifolia.*

MATERIALS AND METHODS

Collection and identification of plant materials

The clean and healthy leaves and stem bark of the study species, *H. populifolia* were collected from the Forest Genetics Division, Bhavanisagar, Erode, Tamil Nadu and they were shade dried separately. The plant was identified and confirmed by a voucher specimen with authentic specimen (15211 (MH)) deposited in the herbarium at Botanical Survey of India, Southern Circle, Coimbatore, India.

Preparation of the plant extracts

The shade dried leaves and stem bark of the study species were made into fine powder of 40 mesh size using the pulvarizer separately. 100g of the powder was filled in the filter paper and successively extracted by using 500 mL methanol in soxhlet extractor for 8 to 10 hours [9]. Then the extracts were filtered through Whatman No. 1 filter paper separately to remove all undissolved matter, including cellular material and other constituents that are insoluble in the extraction solvent.

GC-MS analysis

Gas chromatographical analysis was made by following the method of Joachim and Hübschmann [10]. Five mL of leaf and stem bark methanol extracts were evaporated separately to dryness and reconstituted in 2 µL methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35 mr column (10 m × 0.5 mm, 0.25 µm film thickness). Heating programmes were executed at 100 - 250°C for 3 minutes by using helium as carrier gas with a flow rate of 1 mL/min in the split mode (1:50). An aliquot (2 µL) of oil was injected into the column with the injector heater at 250°C.

Analytical conditions

Injection temperature at 250°C, interface temperature at 200°C, quadruple temperature at 150°C and ion source temperature at 230°C were maintained. Injection was performed in split less mode.

Data analysis

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV, and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST [11,12].

RESULTS AND DISCUSSION

The methanol extracts of the leaf and stem bark of *H. populifolia* were subjected to GC-MS analysis and the results revealed the presence of rich variety of phytochemicals in this species. The phytochemical compounds with their retention time (RT/min.), molecular formula and molecular weight (*m/z*) for the two parts are presented in Tables 1 and 2. Derivatives and therapeutic activity of the compounds are given in the Tables 3 and 4. Four compounds in the leaf extract and twelve compounds in the stem bark extract have been identified. The major chemical constituents determined were Squalene (46.44%) and 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester (43.87%) in the leaf extract.

Table 1: Chemical components of the methanolic leaf extract of the *Hildegardia populifolia* using GC-MS analysis.

Compounds	Peak area %	Retention time (min)	Molecular weight (*m/z*)	Molecular formula
1a,2b,5a-2,6,6-Trimethylbicyclo(3.1.1)heptane	4.61	16.506	136.24	$C_{10}H_{18}$
Squalene	46.44	24.871	410.72	$C_{30}H_{50}$
tert-Butyl(5-isopropyl-2-methyl phenyl)dimethylsilane	5.08	27.064	264.478394	$C_{16}H_{28}OSi$
1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	43.87	29.824	265.347992	$C_{15}H_{23}NO_3$

Table 2: Chemical components of the methanolic stem bark extract of the *Hildegardia populifolia* using GC-MS analysis.

compounds	Peak area %	Retention time (min)	Molecular weight (*m/z*)	Molecular formula
Undecane	0.26	6.892	156.31	$C_{11}H_{24}$
Adenosine, N6-phenylacetic acid	2.36	12.149	401.37336	$C_{18}H_{19}N_5O_6$
Hexadecanoic acid, methyl ester	0.23	17.378	270.4507	$C_{17}H_{34}O_2$
Phenazine, 2-methoxy-, 5 oxide	0.61	18.133	226.2307	$C_{13}H_{10}N_2O_2$
9,12-Octadecadienoic acid, methyl ester	0.15	19.004	280.4455	$C_{19}H_{34}O_2$
3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	0.10	23.898	0	$C_{12}H_9F_2NO_3$
2-Heptadecanone	0.69	25.452	254.45718000	$C_{17}H_{34}O$
9H-Fluorene-4-carboxylic acid, 9-oxo-, (2,6-dimethyl phenyl)amide	0.07	26.440	327.375885	$C_{22}H_{17}NO_2$
Octadecane	2.13	26.658	254.50	$C_{18}H_{38}$
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	1.11	29.040	424.701508	$C_{30}H_{48}O$
Lup-20(29)-en-3-one	76.32	29.606	424.7015	$C_{30}H_{48}O$
1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	11.68	29.838	265.347992	$C_{15}H_{23}NO_3$

Table 3: Derivatives and therapeutic activity of the compounds identified in methanolic leaf extracts of *Hildegardia populifolia.*

Compound	Derivatives	Therapeutic activity
1a,2b,5a-2,6,6- Trimethylbicyclo(3.1.1)heptane	Terpene	Mainly it is used for the fragrance and flavour purpose, as well as in the pharmaceutical and chemical industries.
Squalene	Tritepene	It acts as a natural moisturizer to human skin and it has good antioxidant property.
tert-Butyl(5-isopropyl-2-methyl phenyl)dimethylsilane	alkaloid	No activity reported
1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	Fatty acid	No activity reported

Table 4: Derivatives and therapeutic activity of the compounds identified in methanolic stem bark extracts of *Hildegardia populifolia.*

Compound	Derivatives	Therapeutic activity
Undecane	Fatty acid	It is used as a mild sex attractant for various types of moths and cockroaches, and an alert signal for a variety of ants.
Adenosine, N6-phenylacetic acid	Phenol	Antioxidant activity.
Hexadecanoic acid, methyl ester	Fatty acid	Mainly used to produce soaps, cosmetics, and release agents.
Phenazine, 2-methoxy-, 5 oxide	Aromatic alkaloids	Antimicrobial activity.
9,12-Octadecadienoic acid, methyl ester	Fatty acid	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, antioxidant and anticancer properties.
3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	alkaloid	No activity reported
2-Heptadecanone	Essential oil	No activity reported
9H-Fluorene-4-carboxylic acid, 9-oxo-, (2,6-dimethyl phenyl) amide	alkaloid	No activity reported
Octadecane	Paraffin wax	No activity reported
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	Triterpene	No activity reported
Lup-20(29)-en-3-one	Triterpene	Antimalarial, antiinflammatory activity.
1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	Fatty acid	No activity reported

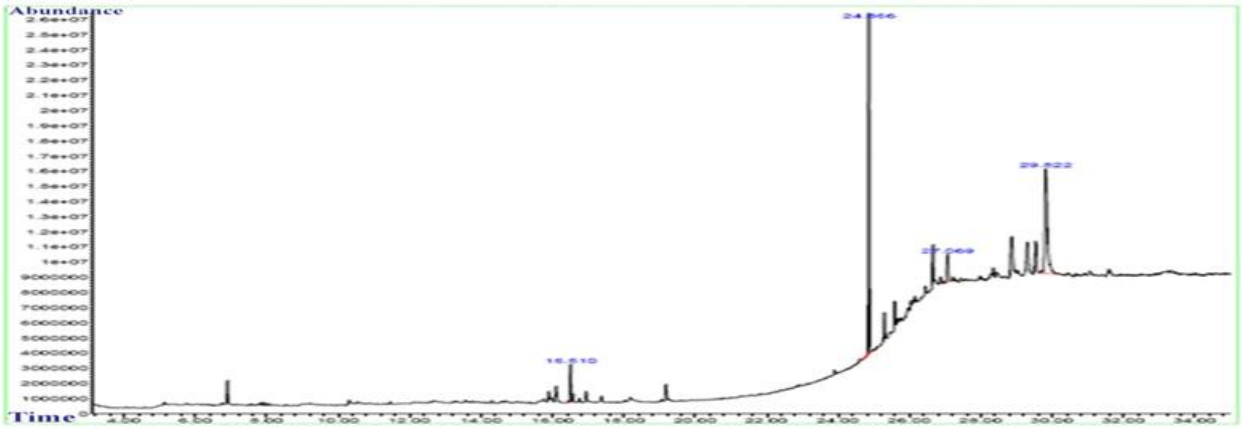

Figure 1: Gas chromatogram of methanolic leaf extract of *Hildegardia populifolia.*

Figure 2: Gas chromatogram for methanolic stem bark extract of *Hildegardia populifolia.*

Squalene a triterpenoid compound, has been reported for many medicinal properties in plants. It acts as a natural moisturizer to human skin and it has good antioxidant property also[13]. Abirami and Rajendran[14] reported that this compound present in *Vernonia cinerea* added medicinal properties to this species despite the presence of eight other chemical compounds. The other major compound reported in the study species, *Hildegardia populifolia,* 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] erhydro-methyl ester is a fatty acid compound. Kale *et al.*[15] also identified major fatty acid compounds in Sterculiaceae member, *Sterculia foetida* through GC-MS analysis.

Among the twelve compounds identified in the stem bark of *H. populifolia,* four are fatty acid group (Undecane; Hexadecanoic acid, methyl ester and 9,12-Octadecadienoic acid, methyl ester; 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester), one is phenolic group (Adenosine, N6-phenylacetic acid); three are alkaloid group (Phenazine, 2-methoxy-, 5 oxide; 3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester and 9H-Fluorene-4-carboxylic acid, 9-oxo-, (2,6-dimethyl phenyl) amide), one is essential oil group (2-Heptadecanone), one is compound of paraffin wax group (Octadecane), two are triterpenoid group, (4,4,6a,6b,8a,11,11,14b-Octamethyl-1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro-2H-picen-3- one and Lup-20(29)-en-3-one).

Among the identified chemical compounds in stem bark, triterpene derivatives of Lup-20(29)-en-3-one (76.32%) is the predominent compound. Gallo and Sarachine [16] reported that this compound has a complex pharmacology in humans, displaying antiprotozoal, antimicrobial, antiinflammatory, antitumor and chemopreventive properties. Sampathkumar and Ramakrishnan [17] identified this compound in the plant species, *Naringi crenulata* as major constituent by GC-MS analysis and investigated the medicinal uses of Lup-20(29)-en-3-one.

Both the leaf and stem bark of *Hildegardia populifolia* contained the triterpenoids as major bioactive compounds. Generally, terpenoids are important volatile part of plants and play vital role in traditional herbal remedies. They are used as antibacterial, antineoplastic, anticarcinogenic, antimalarial and antiulcer agents and for hepaticidal, diuretic and other pharmaceutical functions[18,19]. Most of the thousands of terpenoids produced by plants have no discernible role in growth and development. Few of the terpenoid compounds have been investigated in depth and they are thought to serve primarily in ecological roles, providing defense against or acting as attractants for animals that disperse pollen or seeds or as inhibitors of germination and growth of neighboring plants [20].

CONCLUSION

In conclusion the presence of various bioactive compounds justifies the use of the leaf and stem bark parts of *H. populifolia* for various ailments by traditional practitioners. Isolation of these compounds was supportive to identify new drugs to treat various diseases. Therefore, it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to ascertain fully for its bioactivity, toxicity profile and effect on ecosystem and agricultural products.

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