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IDENTIFICATION OF PHYTOCONSTITUENTS FROM THE METHANOLIC EXTRACTS OF *CISSUS ARNOTTIANA* USING GC-MS ANALYSIS

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ABSTRACTS

The present investigation deals with the identification of phytoconstituents of methanolic extracts from the stem part of *Cissus arnottiana* by GC - MS analysis. The plant materials were collected from foot hills of Kanjamalai hills located at 16 kilometers away from the city of Salem district in Tamil Nadu. *Cissus arnottiana* is an erect woody tree which belongs to Vitaceae family. The results of GC-MS analysis shows that twenty one compounds were identified and among them four compounds were consider as major (Eugenol, Phenol, 4-(ethoxymethyl)-2-methoxy, 3, 5-Cyclohexadiene-1, 2-dione, 3, 5-bis (1, 1-dimethylethyl), Diisooctyl phthalate) and rest of them consider as minor compounds which are denoted based on their percentage of peak values. The present investigation is needful for the identification of potential compound against microbial diseases.

Keywords: ***Cissus arnottiana, GC-MS analysis, Kanjamalai, Phytoconstituents.***

INTRODUCTION

Plants are rich source of the traditional medicine in several countries and produce a diverse array of bioactive molecules, the source of potential and powerful drugs (Vashist and Jindal, 2012). Medicinal plants have a global distribution although they are most abundant in the tropics (Shanmugapriya and Thayumanavan, 2013). The natural products from the medicinal plants still play a very important

role in pharmacological or biological activities (Bhore *et al.,* 2012). Plant extract has a potential application as natural medicine and to treat diseases of the human health (Subashkumar *et al.,* 2013). Herbal drugs are prescribed widely even when their biological active compounds are unknown, because of their effectiveness, lesser side effects, and relatively low cost (Yadav and Agarwala, 2011).

The plant parts are used in the form of extract, infusion and powder for the treatment of different diseases to humans and animals (Bharti *et al*., 2013). Medicinal plants are sources of important therapeutic aids for alleviating human ailments (Thenmozhi and Rajan, 2012). Many medicinal plants traditionally used for thousands of years are present in a group of herbal preparation of the Indian traditional health care system (Londonker and Tukappa, 2013). Medicinal plants are the backbone of traditional means, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Hunt, 2000).

Cissus arnottiana is an erect woody tree which belongs to Vitaceae family. *Cissus* is a genus of about 350 species of tropical and subtropical, chiefly woody vines of the grape family. This plant has erect shrubs, tendris simple. Leaves broadly ovate 9 x10 cm, margins glandular, membranous; petiole to 6 cm, stipules 5 mm, ovate, cauducous. Cymes axillary, leaf opposed, puberulus; bracts 5mm, ovate, pedicels 5 mm; calyx truncate, 4 – lobed, 1.5 mm; petals 4, stamens 4, the filaments 2 mm, disc prominent, 4 – lobed; ovary embedded, style 1 mm stout.

This plant flowering and fruiting time on April - June. They are often used as medicinal plants because they contain some bioactive compounds such as vitamins, proteins, carbohydrates and polyphenols among others. The bioactive compounds are contained in their leaves, stems and roots or bark, which makes these plants to be used medicinally in indigenous system of medicine (Singh *et al*., 1984).

MATERIALS AND METHODS

Plant Collection and Authentication

The stem of *Cissus arnottiana* was collected from foot hills of kanjamalai, Salem District, Tamil Nadu. The plant was authenticated (Ref No. BSI/SRC/5/23/2016/Tech/1071) by Botanical Survey of India (Southern Region), Tamil Nadu Agricultural University (TNAU), Coimbatore, India.

Preparation of Plant Extracts

Cissus arnottiana stem were collected and washed several times with distilled water and allowed to shade dry at room temperature for two weeks. The dried plant materials were made into a fine powder by mixer grinder. 10g of powdered material was dissolved in 100ml of methanol and the extracts were obtained by cold percolation method. The extract was stored at 4^0C until further use.

GC-MS analysis

The identification of phytochemical constituents of the stem samples was executed by Gas chromatography analysis (GC clarus 500 Perkin Elmer using Elite-5MS column (5% Diphenyl/ 95% Dimethyl poly siloxane with 30×0.25mn×0.25µm thickness). Helium was used as carrier gas at a flow of 1ml per minute. The injection port was maintained at 280ºC and the split ratio was 10:1. Oven temperature programming was done from 5 ºC to 280 ºC at 10 ºC per minute and it was kept at 280ºC for 9 minutes. Interface temperature was kept at 250ºC. The ionization mode was electron impact ionization and the scanning range from 45 to 450 (m/z). Mass spectra were obtained at 0-2 minute's interval. The spectra of the compounds were matched with NIST version 11 libraries (Srinivasan and Kumaravel, 2016).

RESULT AND DISCUSSION

The GC-MS analysis of stem methanolic extracts of *Cissus arnottiana* showed twenty one compounds, among them most of the compounds play important role in antibacterial potential especially Eugenol, 1,2,4-Benzenetriol, Phenol, 4-(ethoxymethyl)-2-methoxy- and Ethanol, 2-(9-octadecenyloxy)-, (Z) etc., the crude extracts containing phenolic compounds, steroids and ketone compounds. In this present investigation the biological activity ketone groups were not assigned. High molecular weight of steroids compounds (481) were identified which act as antimicrobial, anti-inflammatory, antiarthritic, antiasthma, diuretic, hepatoprotective and antioxidant. The low molecular weight of the poly phenolic compound was identified which is also potential source for the antimicrobial, anti-inflammatory, analgesic and antioxidant. Compound name, molecular weight and biological activity presented in table 1.

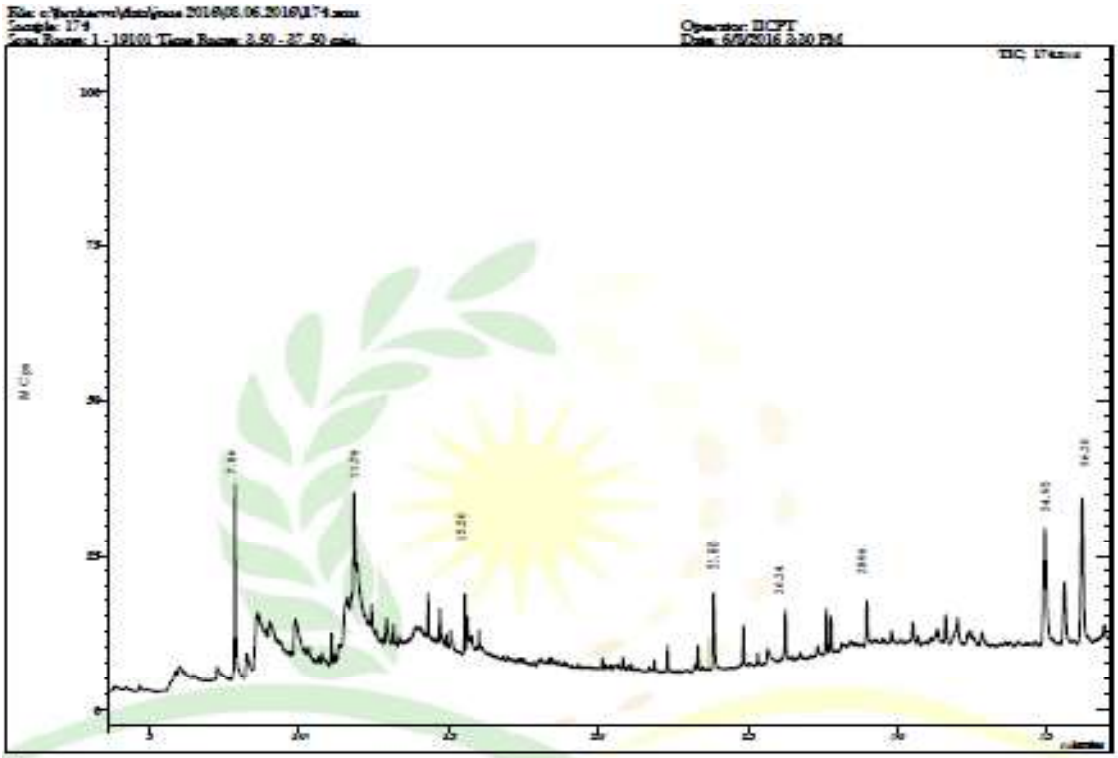

Figure.1: Chromatogram of methanolic extracts of *Cissus arnottiana* by GC-MS analysis

The present investigation were reported that the potential biological compounds were identified using GC-MS analysis from the various species of Vitaceae family especially in *Cissus quadraguaris* determined thirty eight compounds among them n-hexadecanoic acid, ethan-1,1-diethoxy,9,12,15-octadecatrienoic acid-methyl ester(Z,Z,Z) as major constituents (Sathish Kumar, *et al*., 2012). Similarly, Rosy and Rosakutty, (2012) were documented of methanol extracts from various *Cissus* species like *Cissus xavierensis* revealed that presence of sixteen active compounds with highest peak level in 35.09%. *Cissus quandraguaris* twenty three bioactive compounds were identified with the highest area peak in 12.39%. *Cissus vitiginea* was determined twenty three bioactive compounds with the highest peak level 16.18%. *Cissus sicyoides* reported significantly reduced the levels of blood glucose, urinary glucose and urinary urea, as well as both the food and fluid intake and the volume of urine excreted, in streptozotocin-diabetic rats (Pepato *et al.,* 2003). The *Cissus quadrangularis* exhibit anti-diabetic property in alloxan induced diabetic rats by Stimulation of Surviving ß-cell to release more Insulin (Anuj e*t al.,* 2011).

Chellaperumal *et al.*, (2014) identified the twenty bioactive compounds from ethanolic extract of *Cayratia trifolia.* A peak level in the chromatogram graph indicates the maximum amount of phytol (40%) present in the extract was showed bioactive compounds posses many biological activities such as anti-cancer, anti- inflammatory, anti-microbial, anti-diabetic *etc*., (Nakashima, 2013; Yang, 2011; Mahendran, 2012; Ajila, 2010; Sasidharan, 2011). Chipiti *et al*., 2015 were analyzed the GC-MS of the aqueous and ethanol extracts of the roots indicated the presence of the common aromatic phenolic compounds, pyrogallol, resorcinol and catechol, a fatty acid, n-hexadecanoic acid and an aldehyde, vanillin.

Table: 1 GCMS analysis of methanolic extracts of *Cissus arnottiana*

No.	RT	Name of the compound	Molecular Formulae	M W	Peak Area %	Compound Nature	**Activity
1.	7.86	Eugenol	$C_{10}H_{12}O_2$	164	17.98	Phenolic compound	Antimicrobial Anti-inflammatory Antioxidant Fragrance Analgesic Antiseptic Anesthetic
2.	8.69	1,2,4-Benzenetriol	$C_6H_6O_3$	126	2.72	Poly Phenolic compound	Antimicrobial Anti-inflammatory Analgesic Antioxidant
3.	9.87	D-Glucose, 6-O-α-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	5.99	Sugar moiety	Preservative CNS depressant Decalcifier Alcohol Detoxicant DNA Protective Diuretic Food dye Anticancer
4.	11.79	Phenol, 4-(ethoxymethyl)-2-methoxy-	$C_{10}H_{14}O_3$	182	18.25	Phenolic compound	Antimicrobial Anti-inflammatory Analgesic
5.	14.31	1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl	$C_{13}H_{20}O$	192	8.05	Aromatic compound	Hepatoprotective Herbicide HIV-RT-inhibitor Histamine inhibitor Hypercholesterolemic
6.	15.50	3,5-Cyclohexadiene-1,2-dione, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	220	16.96	Ketone compound	No activity reported
7.	22.36	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	0.67	Alcoholic compound	Antimicrobial
8.	23.38	Octadecanoic	$C_{19}H_{38}O_3$	314	1.51	Ester compound	No activity reported

		acid, 4-hydroxy-, methyl ester					
9.	23.80	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	13.10	Plasticizer compound	Antimicrobial Anti-fouling
10.	24.81	Heptacosane	$C_{27}H_{56}$	380	2.17	Alkane compound	No activity reported
11.	25.65	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	1.44	Steroid	Antimicrobial Anti-inflammatory Antiarthritic Antiasthma Diuretic Hepatoprotective Antioxidant
12.	26.24	4-Methyldocosane	$C_{23}H_{48}$	324	2.95	Alkane compound	No activity reported
13.	27.60	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	2.56	Alkane compound	No activity reported
14.	28.96	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	$C_{27}H_{40}O_4$	428	2.05	Ketone compound	No activity reported
15.	30.54	Androstane-11,17-dione, 3-((trimethylsilyl)oxy)-, 17-(O-(phenylmethyl)oxime), (3α,5α)-	$C_{29}H_{43}NO_3Si$	481	0.31	Steroid	Antimicrobial Anti-inflammatory Antiarthritic Antiasthma Diuretic Hepatoprotective Antioxidant
16.	31.38	3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo(f)chromen-7-one	$C_{26}H_{36}O_3$	396	0.49	Color pigment	Antimicrobial Anti-inflammatory
17.	31.65	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	$C_{31}H_{50}O_2$	454	0.45	Steroid	Antimicrobial Anti-inflammatory Antiarthritic Antiasthma Diuretic Hepatoprotective Antioxidant
18.	32.03	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-	$C_{30}H_{48}O_2$	440	0.76	Steroid	Antimicrobial Anti-inflammatory Antiarthritic Antiasthma Diuretic Hepatoprotective Antioxidant
19.	34.95	Campesterol	$C_{28}H_{48}O$	400	0.32	Steroid	Antimicrobial Anti-inflammatory Antiarthritic Antiasthma Diuretic Hepatoprotective Antioxidant
20.	35.64	Stigmasterol	$C_{29}H_{48}O$	412	0.23	Steroid	Antioxidant Anti-inflammatory Sedative

							Antihepatotoxic Caner-preventive Antiviral Ovulant Hypocholesterolemic Estrogenic Artemicide
21.	**36.20**	**Lanosterol**	**$C_{30}H_{50}O$**	**426**	**1.04**	**Steroid**	**Antimicrobial Anti-inflammatory Antiarthritic Antiasthma Diuretic Hepatoprotective Antioxidant**

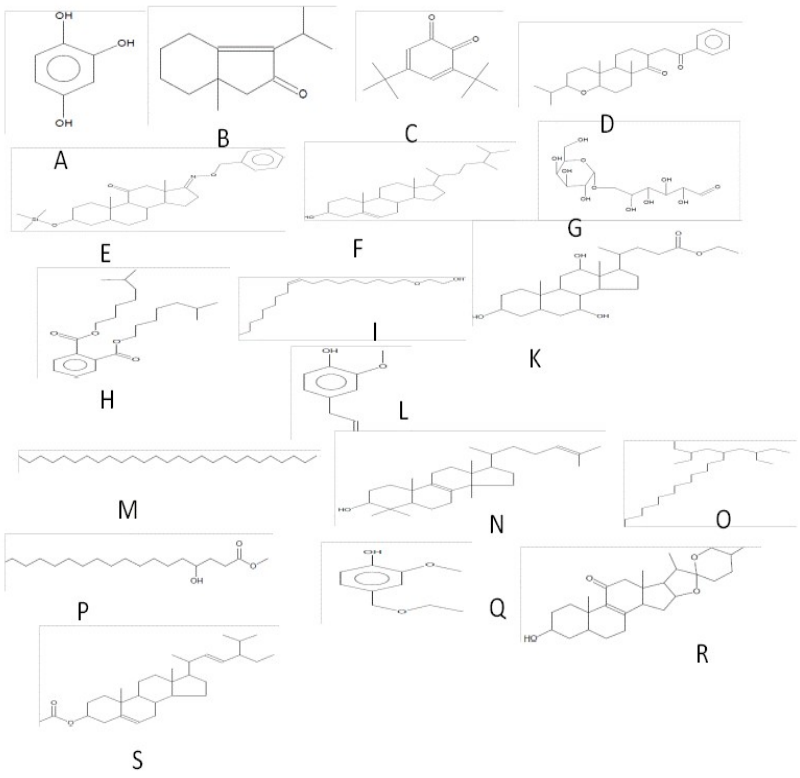

Figure 2: Chemical Structures of *Cissus arnottiana* by GC-MS analysis

A - (mainlib) 1,2,4-Benzenetriol, B - 1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl, C - 3,5-Cyclohexadiene-1,2-dione, 3,5-bis(1,1-dimethylethyl), D - 3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one, E- Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α), F - Campesterol, G - D-Glucose, 6-O-α-D-galactopyranosyl, H - Diisooctyl phthalate, I - Ethanol, 2-(9-octadecenyloxy)-, (Z), K - Ethyl iso-allocholate, L – Eugenol, M – Heptacosane, N – Lanosterol, O - Octadecane, 3-ethyl-5-(2-ethylbutyl), P - Octadecanoic acid, 4-hydroxy-, methyl ester, Q - Phenol, 4-(ethoxymethyl)-2-methoxy, R - Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R), S - Stigmasta-5,22-dien-3-ol, acetate, (3β).

CONCLUSION

In the present study was concluded that more than twenty chemical constituents have been identified from methanolic extract of stem parts of *Cissus arnottiana* by GC-MS analysis. The analysis reveals major components with the high peak values which are possess more number of biological activities. The presence of various bioactive compounds justifies the use of stem various ailments by traditional practitioners.

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