GAS CHROMATOGRAPHY AND MASS SPECTROSCOPIC ANALYSIS OF PHYTO-COMPOUNDS IN *Dodonaea viscosa LEAF* EXTRACT

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ABSTRACT

The phytocomponents of Dodonaea viscosa Linn were analysed by Perkin-Elmer Gas Chromatography Mass Spectrometry. The WILEY8, NIST08s and FAME library were used for identification of compounds. The GC-MS analysis revealed the presence of various compounds via 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl, 4H-Pyran-4-One, 9,12,15-Octadecatrienoic Acid, N-Hexadecanoic Acid, Methyl Ester, 9,12-Octadecadienoic Acid, Phytol Isomer, 2-Hexadecen-1-Ol, 1,2Benzenedicarboxylic and N-Hexadecanoic Acid in the methanolic extract of Dodonaea viscosa . Findings of the present study support the use of Dodonaea viscosa for several disorders.

Keyword: Gas Chromatography Mass spectroscopy, *Dodonaea viscose*, Phytocompounds

INTRODUCTION

Plants area unit an upscale supply of secondary metabolites with fascinating biological activities. In general, these secondary metabolites measure a vital supply with a spread of structural arrangements and properties (De-Fátima *et al*., 2006). Different medicinal plants and their medicinal values are widely used for various ailments throughout the world. Various chemical compounds include phenols, flavonoids, phenolic glycosides, cyanogenic glycosides and saponins isolated and characterized from Boraginaceous plant species (Shahidi, 2000 and Shahidi, *et al*., 2008). Natural product from microorganism supplys are the first source of antibiotics, however with the increasing recognition of flavourer medication as another style of health care, the screening of healthful plants for active compounds has become terribly vital as a result of these might function proficient sources of book antibiotic prototypes (Meurer-Grimes *et al*., 1996; Koduru *et al*., 2006). It has been shown that *in vitro* screening ways might offer the required preliminary observations necessary to pick crude plant extracts with probably helpful properties for more chemical and medical specialty investigations (Mathekaga and Meyer, 1998).

Within a decade, there were a number of dramatic advances in analytical techniques including FTIR, UV, NMR and GC- MS that were powerful tools for separation, identification

and structural determination of phytochemicals. Gas Chromatography Mass Spectroscopy is the most commonly used technique for the identification of compounds. The organic compounds of unknown in a mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites, 1997). In the present study was to determine the bioactive compounds present in *Dodonaea viscose (*Family: Sapindaceae) leaf extract with the aid of GC-MS techniques.

MATERIALS AND METHODS

Plant materials:

The *Dodonaea viscosa leaves* were collected in March 2019 from Tamil University campus, Thanjavur, Tamil Nadu from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, Rapiant Herbarium and Center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts:

The collected *Dodonaea viscosa* leaves were dried at room temperature and coarsely powdered. The powder was extracted (Maceration) with methanol for 24 hours. The obtained solid extract was stored in desiccator until used. The extract contained both non-polar and polar phytocomponents of the plant material used.

GC –MS examination

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer followed by the method of Srinivasan et al., (2013).

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique, FAME and WILEY8 having more than 65 000 patterns. The spectrum of the unidentified component was associated with the spectrum of the recognized components stored in the WILEY8, NIST08s and FAME library. The name, molecular formula, molecular weight and structure of the component of the test material were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. GC MS solution ver. 2.53 Software used for mass spectra and chromatograms.

RESULTS AND DISCUSSION

GC-MS is an analytical method that associations the features of gas-chromatography and mass spectrometry to find different substances within a test sample. The use of GC-MS include fire investigation, drug detection, explosives investigation, environmental analysis, identification of unknown samples and inorganic, biochemistry. It can also identify trace materials that were

earlier thought to have degenerated beyond identification. GC-MS has been widely used as a "gold standard" for criminal substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system (Ronald Hites, 1997).

Identification of components

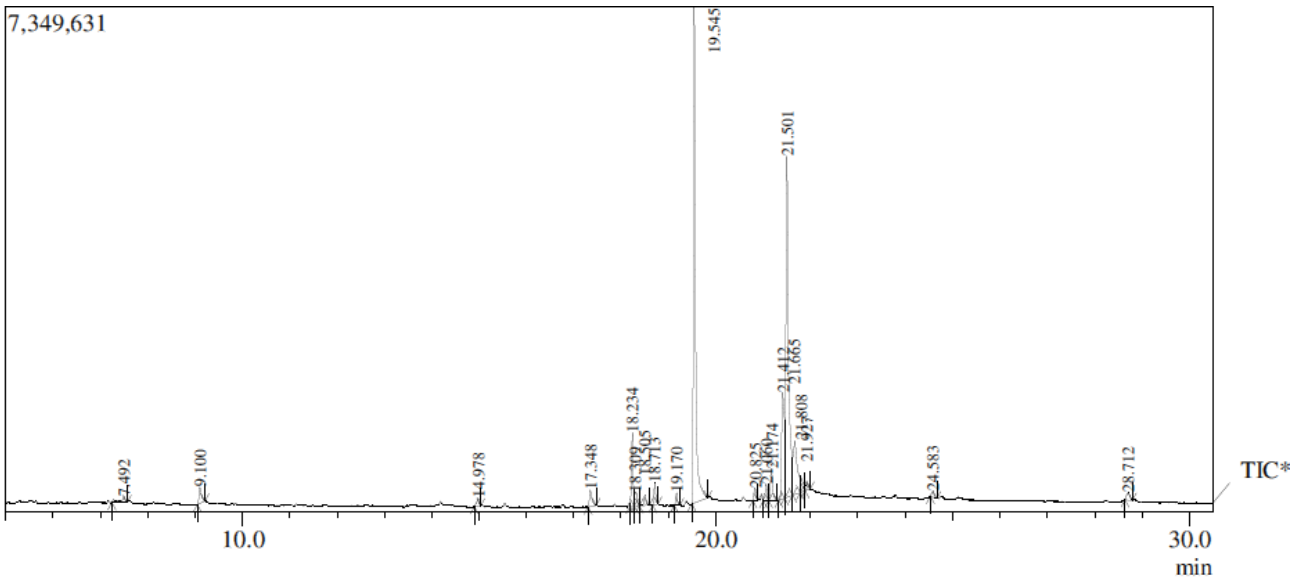
Table-1 shows the GC-MS analysis revealed the presence of phytochemical component in *Dodonaea viscosa* leaf. The biological activities listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA (Duke's, 2013). The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound can be identified from NIST library database.

GC-MS ANALYSIS

Twenty compounds were identified in *Dodonaea viscosa* by GC-MS analysis. The active principles with their Retention Time (RT), molecular weight (MW), molecular formula and concentration (%) are presented in (Table 1and Fig 1). The prevailing compounds were 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl, 4H-Pyran-4-One, 9,12,15-Octadecatrienoic Acid, N-Hexadecanoic Acid, Methyl Ester, 9,12-Octadecadienoic Acid, Phytol Isomer, 2-Hexadecen-1-Ol, 1,2Benzenedicarboxylic and N-Hexadecanoic Acid present in the extract. The blogical activity of *Dodonaea viscosa* is represented in table 2. This study explores the goodness of the leaf of the plant *Dodonaea viscosa which* has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

The investigation concluded that the stronger extraction capacity of methanol have produced number of active constituents responsible for many biological activities. So these might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer.

Fig 1: GC MS chromatogram of *Dodonaea viscosa* leaf extract



Phytol is reported to have antioxidant, antiallergic (Santos *et al*., 2013) antinociceptive and anti-inflammatory activities (Ryu *et al*., 2011). Recent studies have revealed that phytol is an excellent immunostimulant. It is superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity (Lim *et al*., 2006). Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Saikia *et al*., 2010). Similarly Maria Jancy Rani *et al.* (2011) observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (2011) in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* (Sathish kumar and Manimegalai, 2008). Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al.*, 2005). Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K_1. It is used along with simple sugar or corn syrup as a hardener in candies.

Hexadecanoic acid, ethyl ester is recommended to be a saturated fatty acid and it might act as an Antioxidant, anti-androgenic, hypocholesterolemic, hemolytic and alpha reductase inhibitor (Sermakkani, 2012). Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al.*, 2002) and *Melissa officinalis* (Sharafzadeh *et al.*, 2011). Parasuraman *et al.* (2009) identified 17 compounds with n-

Octadecanoic acid and Hexadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus*. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid (Siddig Ibrahim *et al.,* 2009). n-hexadecanoic acid, Hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid Phytol, 9, 12 - Octadecadienoic acid, and Squalene were Identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen kumar *et al.,* 2010). Squalene has earlier been reported as antimicrobial, antioxidant, anticancer , Neutralize different xenobiotics, anti-inflammatory, anti-anti-neoplastic and atherosclerotic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer (Ponnamma and Manjunath, 2012). Devi *et al.* (2009) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Das and Sudhakar Swamy (2016) determined the bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia* species from south India. Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid was the major compound identified. In *A. racemosa* also 27 compounds were identified and n- Hexadecanoic acid was the major compound.

Uraku (2016) examined the bioactive cconstituents of methanol fraction of *Spilanthes uliginosa* (Sw) Leaves. The major phytocompounds identified in the leaf extract are hepta-9, 10, 11-trienoic acid (19.36%), hexadecanoic acid (8.68%), hydroxylmethyl heptadecane (14.02%), octadecenoic acid (8.14%), 5- docosane aldehyde (41.72%) and 1-ethoxyoctadecane (8.08%).

Table-1: GC-MS analysis revealed the presence of phytochemical component in leaf of *Dodonaea viscosa*

Peak#	R.Time	Area %	Height %	Name of the compounds	Molecular Weight	Molecular formal
1	7.492	0.82	0.45	Benzene, 1,4-Diethyl-	134	$C_{10}H14$
2	9.100	1.38	1.26	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	144	$C_6H_8O_4$
3	14.978	0.64	0.64	2(4H)-Benzofuranone, 5,6,7,7a-Tetrahydro-4,4,7a-Trimethyl-, (R)-	180	$C_{11}H_{16}O_2$
4	17.348	1.39	1.30	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$

5	18.234	3.78	5.79	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	296	$C_{20}H_{40}O$
6	18.309	1.17	1.08	2-Isopropyl-5-Methyl-1-Heptanol	172	$C_{11}H_{24}O$
7	18.505	0.76	0.84	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	296	$C_{20}H_{40}O$
8	18.713	1.20	1.74	Oxirane, Tetradecyl	240	$C_{16}H_{32}O$
9	19.170	0.70	0.97	Tetracosanoic Acid, Methyl Ester	382	$C_{25}H_{50}O_2$
10	19.545	33.21	38.99	Hexadecanoic Acid	256	$C_{16}H_{32}O_2$
11	20.825	0.79	1.00	1-Hexadecanol, 2-Methyl-	256	$C_{17}H_{36}O$
12	21.060	1.08	1.32	9,12,15-Octadecatrienoic Acid, Methyl Ester	292	$C_{19}H_{32}O_2$
13	21.174	2.32	2.50	Phytol Isomer	296	$C_{20}H_{40}O$
14	21.412	8.92	8.35	9,12-Octadecadienoic Acid	280	$C_{18}H_{32}O_2$
15	21.501	31.68	26.81	9,12,15-Octadecatrienoic Acid, Methyl Ester	292	$C_{19}H_{32}O_2$
16	21.665	6.98	4.20	Octadecanoic Acid	284	$C_{18}H_{36}O_2$
17	21.808	1.34	1.09	Ethyl (9z,12z)-9,12-Octadecadienoate	308	$C_{20}H_{36}O_2$
18	21.927	0.65	0.65	N-Hexadecanoic Acid	256	$C_{16}H_{32}O_2$
19	24.583	0.45	0.46	4,7-Methano-5h-Inden-5-One, Octahydro-	150	$C_{10}H_{14}O$
20	28.712	0.75	0.56	1,2-Benzenedicarboxylic Acid, Dioctyl Ester	390	$C_{24}H_{38}O_4$
		100.00	100.00			

Table-2: GC-MS analysis revealed the presence of phytochemical component in leaf of *Dodonaea viscosa and* their biological activities

Peak	Area %	R.Time	Name of the compounds	Biological Activity**
1	1.38	9.100	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	Antimicrobial, Anti-inflammatory
2	1.55	18.375	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Antimicrobial, Anti-inflammatory, **Cancer - preventive**
3	33.21	19.545	N-Hexadecanoic Acid	Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor Antioxidant, Hypocholesterolemic
4	1.08	21.060	9,12,15-Octadecatrienoic Acid, Methyl Ester	Antiinflammatory,Hypocholesterolemic, **Cancer preventive**, Hepato protective, Nematicide,Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic,
5	2.32	21.174	Phytol Isomer	**Anticancer**, Anti-inflammatory Hypocholesterolemic, Nematicide, Anticoronary,
6	8.92	21.412	9,12-Octadecadienoic Acid	Hypocholesterolemic,5-Alpha reductase inhibitor, **Anticancer** Antihistaminic, Insectifuge, Antieczemic, Antiacne
7	0.79	21.204	2-Hexadecen-1-Ol	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents

8	0.65	21.927	N-Hexadecanoic Acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
9	0.75	28.712	1,2Benzenedicarboxylic Acid, Dioctyl Ester	Used as Softeners, Used in prepar ation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making.

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

Conclusion

The present study characterized the phytochemical profile of the *Dodonaea viscosa* leaf extract using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a fraction of retention time. The heights of the different peaks indicates the relative concentration of the compounds exist in the methanolic extract of *Dodonaea viscosa* leaf. The mass spectrometer analyses of the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the library. The identification of various bioactive compounds confirms the therapeutic application of *Dodonaea viscosa* leaf for a variety of diseases.

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