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IDENTIFICATION OF PHYTOCOMPOUNDS IN *Lawsonia inermis* USING GC-MS TECHNIQUE

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Abstract — *GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from the whole plant methanolic extract of Lawsonia inermis by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like Caryophyllene, octodeconoic acids, hexadecanoic acid, ascorbic acid and squalene in the methanolic extract of Lawsonia inermis. Hence, the Lawsonia inermis may have antioxidant and antiinflammatory activity due to the presence of secondary metabolites in the methanolic extract. These findings support the traditional use of Lawsonia inermis in various disorders.*

Keywords- *Gas chromatography and Mass spectroscopy, Lawsonia inermis, Phytochemistry, GC—MS analysis.*

I. INTRODUCTION

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs [1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [2].

Plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function [3].

Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [4]. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [5]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals [6]. The aim of this paper is to determine the organic compounds present in the *Lawsonia inermis* leaves extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

II. MATERIAL AND METHODS

2.1 Plant materials:

The fully mature *Lawsonia inermis* leaves were collected from Tamil University campus, Thanjavur District, Tamil Nadu, India from a single tree.

2.2 Preparation of extracts:

The *Lawsonia inermis* Linn were first washed well and dust was removed from the plant. The plants were washed several times with water to remove the traces of impurities from the plant. Then the plants were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours using soxlet apparatus. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

2.3 GC –MS analysis

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 instrument employing the following conditions: column Elite-1

fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0

3. RESULTS AND DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions [7].

3.1 Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown 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. 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.

3.2 GC-MS ANALYSIS

Thirty compounds were identified in *Lawsonia inermis* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were Squalene (29.65), 2-Hydroxy-3-[(9e)-9-Octadecenoyl (29.55), Oleic acid (26.88), Octadecanoic acid (24.23), 1-(+)-Ascorbic acid 2,6-dihexadecanoate (22.02) and Caryophyllene (14.77).

Table 1 Shows the components identified in methanolic extract of Lawsonia inermis (GC MS study)

Peak	R.TIME	AREA%	NAME OF COMPOUND	MOLECULAR FORMULA	MOLECULR WEIGHT
1	5.847	0.28	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl	$C_{10}H_{16}$	136
2	13.852	0.27	Alfa.-copaene	$C_{15}H_{24}$	204
3	14.109	0.32	Cyclohexene, 1-methyl-4-(1methylethenyl)-, (r)	$C_{10}H_{16}$	136
4	14.770	4.37	Caryophyllene	$C_{15}H_{24}$	204
5	15.855	0.47	1,6-cyclodecadiene, 1-methyl-5-m	$C_{15}H_{24}$	204
6	17.596	1.00	(-)-5-oxatricyclo[8.2.0.0(4,6)]dodeca	$C_{15}H_{24}O$	220
7	18.285	0.32	1hcyclopropa[A]Naphthalene, 1a	$C_{15}H_{28}O_2$	240
8	18.806	0.46	Dotriacontane	$C_{32}H_{66}$	450
9	19.643	0.69	Tetradecanoic acid	$C_{24}H_{48}O_2$	368
10	20.612	0.48	2,6,10-trimethyl,14-ethylene-14-pe	$C_{20}H_{38}$	278
11	20.875	0.36	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
12	21.127	0.37	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
13	21.843	1.04	Oleic acid $$ 9-octadecenoic acid (z)	$C_{18}H_{34}O_2$	282
14	21.883	0.74	Nonanedioic acid, dibutyl ester	$C17H_{32}O_4$	300
15	22.025	17.32	L-(+)-Ascorbicacid2,6dihexadecanoate	$C_{38}H_{68}O_8$	562
16	23.089	0.82	Heptadecanoic acid	$C_{17}H_{34}O_2$	270
17	23.351	0.42	Behenic alcohol	$C_{22}H_{46}O$	326
18	23.717	1.78	Phytol isomer	$C_{20}H_{40}O$	296
19	23.792	0.48	Methyl stearate	$C_{19}H_{38}O_2$	298
20	24.000	25.39	Oleic acid	$C_{18}H_{34}O_2$	282
21	24.234	26.47	Octadecanoic acid	$C_{18}H_{36}O_2$	284

22	24.575	3.13	Cis-11,14-eicosadienoic acid, methyl ester	$C_{15}H_{26}O$	222
23	25.544	0.67	Nonadecanoic acid	$C_{19}H_{36}O_2$	296
24	26.110	0.64	Hexadecanoicacid,2hydroxy-1,3	$C_{37}H_{74}NO_8P$	691
25	26.800	0.75	Octadec-9-enoic acid	$C_{21}H_{42}O_4$	358
26	26.889	0.95	Oleic acid $$ 9-octadecenoic acid (z)	$C_{18}H_{34}O_2$	282
27	27.150	5.46	Icosanoic acid	$C_{20}H_{40}O_2$	312
28	29.558	1.04	2-Hydroxy-3-[(9e)-9-Octadecenoyl	$C_{39}H_{72}O_5$	620
29	29.657	2.05	Squalene	$C_{30}H_{50}$	410
30	30.072	1.43	Glycidol stearate	$C_{21}H_{40}O_3$	340

Table 2: Activity of phyto-components identified in the methanolic extracts of Lawsonia inermis.by GC-MS.

S.no	Compound name	Biological activity**
1	Caryophyllene	Anti inflammatory, anti oxidant, anti tumor, antinociceptive, neuro protective, anxiolytic, anti depressent, anti alcoholism, anti pyritics, anti microbial, anti carcinogenic, anti dermatitic, allergenic, aldose- reductase inhibitor, anti acne, anti asthmatic, anti ulcer, anti proliferants, cyto protective, gastro protective, sedative, anti spasmodic, flavour.
2	Tetra decanoic acid	Anti oxidant, cancer preventive, hypercholesterolemic, nematicide, lubricant, cosmetic.
3	Penta decanoic acid	Antioxidant
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Anti microbial, anti inflammatory
5	Nonanedioic acid , dibutyl ester	Anti microbial, anti inflammatory, anti tumor, anti hyperpigmentative, anti proliferative, anti acne, cyto toxic, Anti leukemic, oxy radical scavenging activity.
6	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Anti oxidant, anti scorbutic, anti inflammatory, anti nociceptive, anti mutagenic, wound healing property.
7	Hepta decanoic acid	Anti oxidant, anti fungal, surfactant
8	Phytol isomer	Anti inflammatory, anti cancer, anti microbial, diuretic.
9	Oleic acid	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti inflammatory, **anti androgenic**, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D4, choleretic, dermatitigenic, hypocholestrolemic, insectifuge, perfumery, propecic, flavour.
10	Octadecanoic acid	5-α reductase inhibitor, hypo cholesterolemic, suppository, cosmetic, lubricant, surfactant & softening agent, perfumery, propecic, flavour.
11	Cis-11,14-Eicosadienoic acid, methyl ester	Anti inflammatory, anti oxidant, anti arthritic, anti coronary.
12	Hexa decanoic acid	Anti oxidant, hypocholesterolemic, nematicide, pesticide, lubricant, **anti androgenic**, flavour, hemolytic-5-α reductase inhibitor.
13	Squalene	Anti bacterial, anti oxidant, anti tumor, cancer preventive, Immuno stimulant, chemopreventive, lipoxygenase inhibitor, pesticide, diuretic.

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

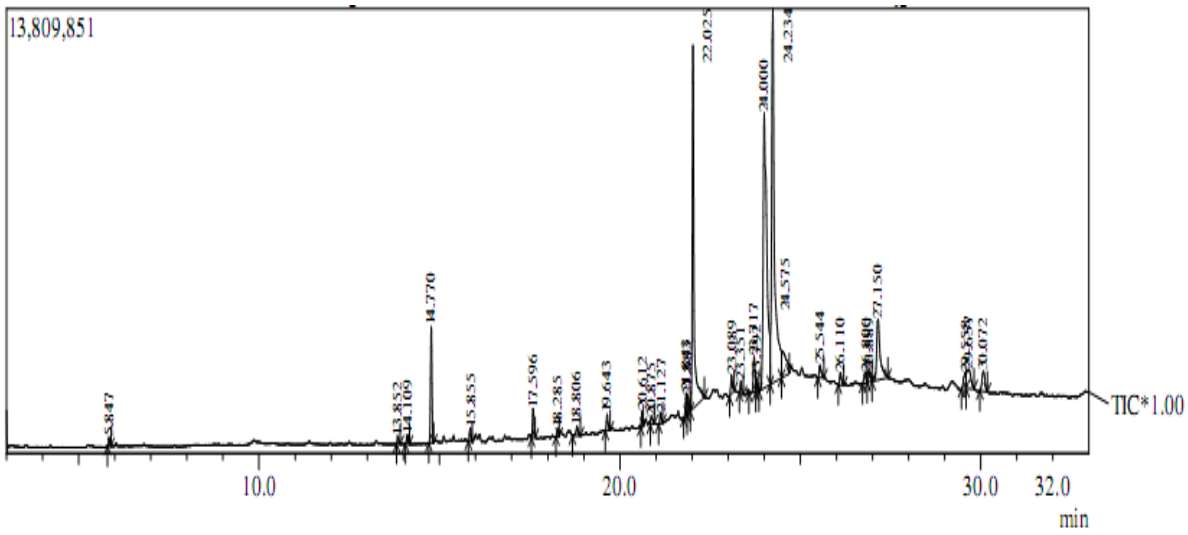

Figure 1: Chromatogram obtained from the GC/MS with the extract of Lawsonia inermis

Phytol is reported to have antioxidant, antiallergic [8] antinociceptive and anti-inflammatory activities. 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 [9]. Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* [10]. Similarly Maria Jancy Rani *et al.* (11) observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (12) in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* [13]. 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 [14]. 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.

Hexadecanoic acid, methyl ester is recommended to be a saturated fatty acid and it might as act as an Antioxidant, hypocholesterolemic, anti androgenic, hemolytic and alpha reductase inhibitor [15]. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* [16] and *Melissa officinalis* [17]. Parasuraman *et al.* [18] identified 17 compounds with n-Hexadecanoic acid and Octadecanoic 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 [19]. n-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15-Octadecatrienoic acid and Squalene were identified in the ethanol leaf extract of *Aloe vera* [20] and *Vitex negundo* [21]. Squalene has earlier been reported as antimicrobial, antioxidant, anticancer , Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer [22]. Devi *et al.* [23] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

III CONCLUSION

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those 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.

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