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GC-MS ANALYSIS OF TERPENOIDS FROM *RADERMACHERA XYLOCARPA* (ROXB.) K. SCHUM

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ABSTRACT

The species under investigation is RET having medicinal attributes. In present context secondary metabolites mainly terpenoids are investigated. Proximate phytochemical analysis conducted as per standard protocols. Five different solvents systems were used viz. water, methanol, ethanol, chloroform and acetone. Additional compounds found are steroids, saponins, glycosides, alkaloids etc. Gas Chromatography Mass Spectroscopy (GCMS) analysis revealed presence of various biomolecules.

KEYWORDS: RET, Proximate Analysis, TLC, GCMS, *Radermachera xylocarpa*.

INTRODUCTION

Since long plants remained the principle source of medicine for most of organisms including man. Though he was not aware of the active compounds present in the plants but the effects of all those plants were well known to the fraternity of traditional health practitioners. India is the birth place of renowned system of indigenous medicine such as Siddha, Ayurveda and Unani. Traditional systems of medicine utilizes single plant or combinations of number of plants to prepare medicines **(Kalimuthu & Prabakaran, 2013)**. India is having number of tribal communities among which "Korkoo" community belongs to "Melghat" region of Maharashtra State. Korkoo's traditionally utilise seeds of *Radermachera xylocarpa* as antivenom, while bark-oil for combating joint pain **(Devarkar, 2002; Dhore, 2011)**. Such a prolong use of plants as a medicine by our ancestors laid the foundation of

"Phytochemistry", which encompasses extraction, isolation and identification of compounds obtained from plant source. The knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also for disclosing new sources of economic phyto-compounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies **(Panneerselvam et.al., 2013)**. Most important bioactive constituents of the plant are alkaloids, tannins, flavonoids and rest phenolic compounds **(Peter et.al., 2012)**. Plant derived natural products such as flavonoids, terpeonids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity **(Banskota et.al., 2000)**. Alkaloids have a bitter taste and may be toxic to other organisms **(Gupta et.al., 2010)**. Flavonoids are the group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent**(Frankel 1997)**. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity **(Reddy & Urooj, 2013; Salah et. al., 1995)**. Tannins are complex moieties produced by majority of plants as protective substances, they have wide pharmacological activities. They have been used since past as tanning agents and they possess astringent, anti-inflammatory, anti-diarrhoeal, antioxidant and antimicrobial activities **(Killedar & More, 2010)**.

Screening of active compounds from plants has laid the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer **(Turker & Usta, 2008; Sheeja & Kuttan, 2007)** and Alzheimer's disease **(Mukherjee, 2007)**.

The recent innovative technique like GCMS is providing the identification of compounds instantaneously. In recent years GC-MS studies have been increasingly applied for the analysis of Chinese medicinal plants as this technique has proved to be a valuable method for the analysis of nonpolar components and volatile essential oil, fatty acids, lipids**(Khare, 2004; Vaidya & Devasagayam, 2007)** and alkaloids **(Betz et.al., 1997)**.

The antivenom property of the species under investigation was the reason for its consideration in research. The species under investigation is medium sized tree, commonly known as Padri tree/Jaimangal/Khadshing. It is having dark green, bitter smelling, compound leaves; light grey bark; Long dehiscent fruit- Folicle is 2-3 feet nearly strait long and slightly

curved towards apex with numerous irregularly placed tubercles all over the kernel. Seeds are light, papery, creamish and winged. The species belongs to Family Bignoniaceae **(Khedkar & Oke, 2013)**.

MATERIAL AND METHOD

Rarely available and endangered plant specimen collected in the month of December and April, previous collection yielded verdant leaves while post yielded, fruits and flowers. The material identified at department of Botany, Shri Shivaji Science College, Amravati and authenticated and deposited at Botanical Survey of India (voucher no. AVORAX1). Shed-dried material pulverized to obtain coarse powders for individual organs. The secondary metabolites terpenoids **(Gadhvi et.al., 2013)** extracted using standard protocols. Every extract tested for the presence of respective group of compound and found positive, it was fractionated by Thin Layer Chromatography. Number of mobile phases tested for each group and one reflecting maximum, clear and sharp bands was taken for final development of chromatogram. After confirmation by using the same test for each compound the bands were eluted, dried and analysed on GCMS **(Harborne, 1998; Gadhvi et.al., 2013**).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanolic extract

GC-MS analysis were carried out on a GC-MS - 5975C AGILENT (GC-MS- QP 2010, SHIMADZU)) system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column DB-5ms Agilent (30m x 0.25mm 1D x µldf, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.9995%) was used as the carrier gas at constant flow rate 1.51 ml/min with a split ratio of 10:1. The oven temperature was operated according to the following oven temperature: 70°C held for 3 min, raising at the rate of 10°C min-1 up to 200°C with no Hel, raising at the rate of 5°C min-1 up to 300°C with 9 min held, injector temperature and volume 250°C and 1 µL, respectively. The total GC running time was about 36 min. The MS operating conditions were ionization voltage 70 eV, source temperature of 230°C, inlet line temperature of 240°C, mass scan (m/z)-40- 700, solvent delay: 5 min, total MS running time-34 min. **(Vinoth et.al., 2011)**. 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 chromatogram was a turbomass. The detection employed the NIST (2011) library.

RESULT AND DISCUSSION

Nine compounds are detected in root terpenoid, while twenty one in Bark terpenoids. Some of these are listed below with wide range of activities like Anti-oxident, Anti-biofilm, Anti-mutagenic, Anti-inflammatory etc. some are used in cosmetics while some are flavouring agent.

TRANS-NEROLIDOL-MF- $C_{15}H_{26}O$, MW 222.37

Nirolidol is a naturally occurring sesquiterpene alcohol that is present in various plants with a floral odor. It has been widely used in cosmetics (e.g., shampoos and perfumes) and in non-cosmetic products (e.g., detergents and cleansers). It is found in *Scutellaria abida* L., *Piper aleyreanum*, *Leonotis ocymifolia*, *Peperomia serpens* (Sw.), *Piper claussenianum*, *Strychnos spinosa* Lam, etc. In fact, U.S. Food and Drug Administration (FDA) has also permitted the use of nerolidol as a food flavoring agent **(Chan et.al., 2016)**. Nerolidol also has Antioxidant, Antibacterial, Anti-biofilm, Anti-fungal, Anti-trypanosomal, Anti-leishmanial, Anti-schistosomal, Anti-malarial, anti-parasite, Insecticidal, Antiulcer, Skin penetration enhancer, Anti-nociceptive and anti-inflammatory, Anti-cancer or anti-tumor properties **(Chan et.al., 2016)**.

FLORANTYRONE- MF- $C_{20}H_{14}O_3$, MW- 302.33

Florantyrone is the structural moiety formed with suitable substitution on benzene nucleus and side chain from γ-oxobenzenebutanoic acid, which is biologically active compound **(Srinivas et.al., 2004)**. Fluorantyrone is a drug used in the treatment of biliary dyskinesia. It is also known as a cholagogue and choleretic **(Fung, 1961)**.

CITRONELLOL –MF- $C_{10}H_{20}O$, MW- 156.269

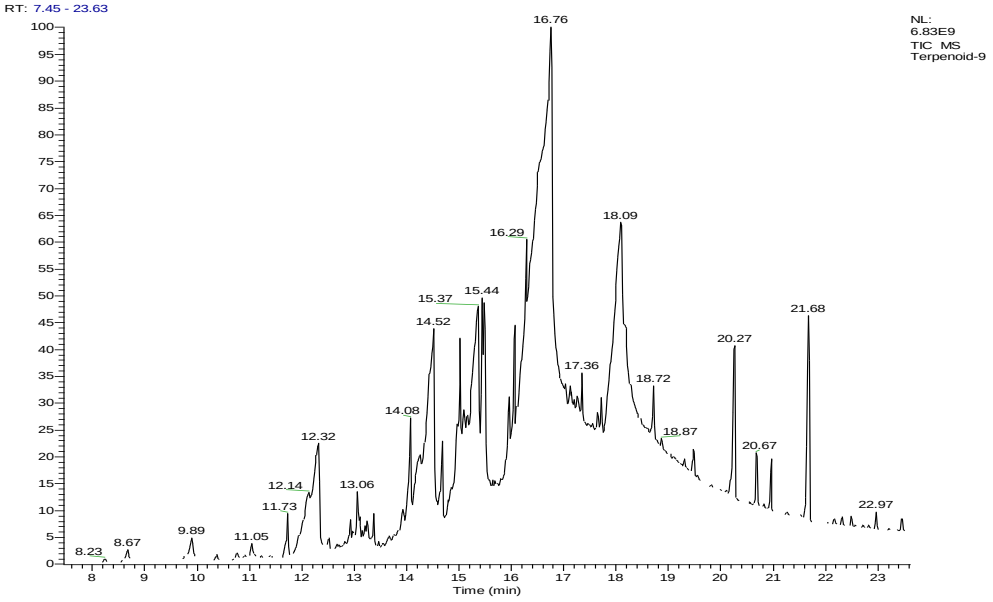
Citronellol is anticancer, anti-inflammatory and wound healing agent. The comparison of the clinical efficacy of flavored and non-flavored chewing gums in allergic rhinitis (pollenosis) showed that the peppermint gums enriched with l-menthol, 1,8-cineole, as well as geraniol or citronellol, more effectively attenuated rhinitis symptoms than the non-flavored gum and normal peppermint flavored gum **(De Cássia et.al., 2013)**.

L-ASCORBIC ACID 2,6 DIHEXADECANOATE-MF-$C_{38}H_{68}O_8$, MW 652.954

The synonyms for this are 2,6-Di-O-palmitoyl-L-ascorbic Acid; L-Ascorbyl 2,6-dipalmitate. The Methanolic extract of *Evolvulus alsinoides* was analysed on GC-MS. The compound L-Ascorbic acid 2,6 dihexadecanoate was detected in plant extract. It possess anti-oxidant, anti-

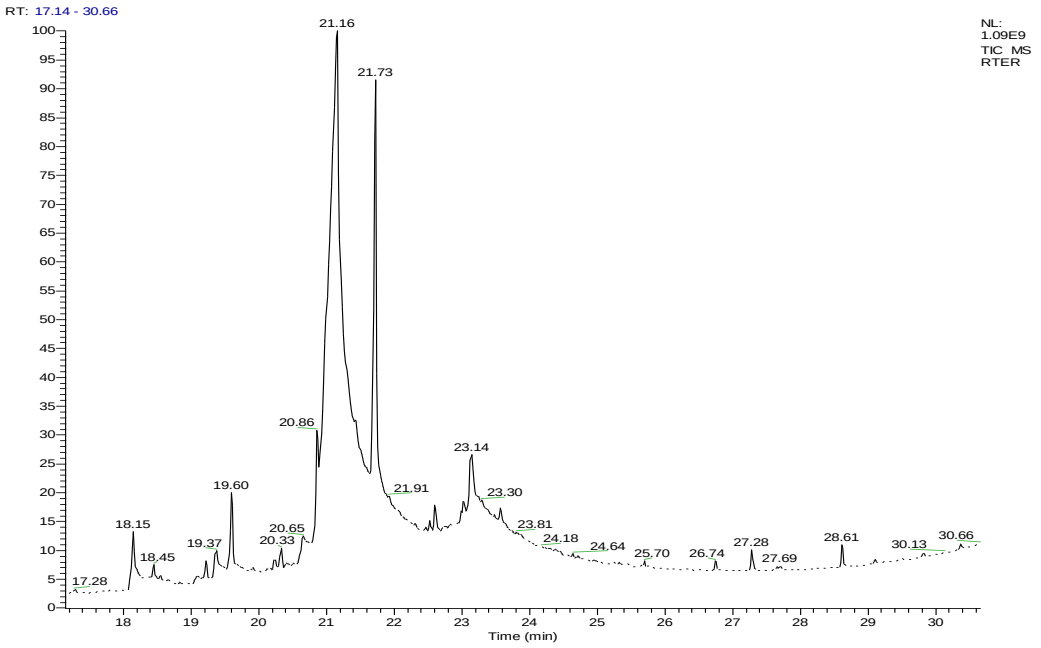
scorbutic, anti-inflammatory, anti-nociceptive, anti-mutagenic and wound healing activity **(Gomathi & Elango, 2015)**. It was also reported from *Nigella sativa* **(Hadi et.al., 2016).**

Bark Terpenoid]



SN	RT	**Identified Compounds** (Bark Terpenoid)	MW	Peak area
1	8.65	Nonanoic acid	158.24	634124558
2	9.88	Decanoic acid	172	1532178792
3	10.39	Trans- Geranyl acetone	194	245634158
4	10.74	9-Hexadeconoic acid	256	264509395
5	11.73	Trans-Nerolidol	222.37	1300307420
6	12.27	Dodecanoic acid	200	18006726990
7	13.06	Valeric acid 3 methyl phenyl ester	186	1328760124
8	14.08	Estran-2-one	260	2502619373
9	14.48	Myristic acid	228.37	63412415
10	14.67	Hexahydrofarnesyl acetone	268.485	2161280721
11	15.16	Heneicosane	296.57	2170283163
12	15.35	Pentadeconoic acid	222	8648958319
13	16.03	Cupressene	272	2474425483
14	16.75	Hexadecanoic acid	256	61874721228
15	18.09	Octadeconoic acid	NA	31084459727
16	18.72	Eicosane	282.55	1249191456
17	19.48	Tetracosane	338.65	916128207
18	20.27	Triacontane	422.81	5761244909
19	20.96	Eicosane	282.55	1838882784
20	21.68	Heptacosane	380.73	8294824130
21	22.15	Florantyrone	302.33	12061287

Root Terpenoid



SN	RT	**Identified compound** (Root Terpenoid)	MW	Peak area
1	17.01	Eicosane	282.55	39552264
2	18.11	Tetradecanoic acid	228.37	1192902847
3	18.44	Muscallure	322.62	2101663375
4	19.24	2-Methyl-5-Undecanone	184.323	227797370
5	21.15	L-Ascorbic acid 2,6 dihexadecanoate	562	11998834719
6	21.73	4 Hydroxydibenzo (b,Ejthiepin-11 (6H)-one	226.29	1513776718
7	22.60	Heptanolactone	128.17	81423722
8	23.14	Citronellol	156.269	592742822
9	26.72	3,6-Dimethoxyaurone	282.29	572253

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