Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (6): 39-47
Received: 01-01-2014
Accepted: 11-01-2014

Pravin P. Ekade
Research Scholar, Department of Botany, Sant Gadge Baba Amravati University, Amravati – 444 602, Maharashtra, India.
Mobile: +91-8308991653
E-mail: pravinekade005@gmail.com

Dr. S.R. Manik*
Professor and Head, Department of Botany, Sant Gadge Baba Amravati University, Amravati – 444 602, Maharashtra, India.
E-mail: Manik_bot@rediffemail.com

Correspondence:
Pravin P. Ekade
Research Scholar, Department of Botany, Sant Gadge Baba Amravati University, Amravati – 444 602, Maharashtra, India.
Mobile: +91-8308991653
E-mail: pravinekade005@gmail.com

Investigations on Secondary Metabolites in Different Parts of *Radermachera xylocarpa* Using GC-MS

Ekade P.P. & Manik S.R.

ABSTRACT

Objective: To explore the important secondary compounds contained in leaves, stem and root of *Radermachera xylocarpa*. **Methods**: All three parts are collected and shade dried. The preliminary phytochemical screening of leaves, stem and root reveals the presence of steroids, alkaloids and terpenoids in dichloromethane extract. These tests are performed using the standard methods of Harborne. The preliminary screening reveals the presence of steroids, alkaloids and terpenoids in all three parts in dichloromethane extract. Gas Chromatography – Mass Spectroscopy analysis of leaves, stem and root is performed on dichloromethane extract which reveals the presence of different secondary metabolites. **Results:** All samples, namely leaves, stem and root of *Radermachera xylocarpa* shows the presence of different steroidal, alkaloidal and terpenoidal compounds in dichloromethane extract. **Conclusion:** *Radermachera xylocarpa* is an excellent source of secondary metabolites especially steroidal compounds which may be used in drug formulations, medicines, pharmaceuticals etc. Further investigation on this plant may lead to important drug discoveries.

Keywords: *Radermachera xylocarpa*, Steroids, GC – MS, Phytochemical, Bignoniaceae, Secondary Metabolites

1. Introduction

Medicinal plants are being used for centuries as remedies for human health owing to its ability to synthesize biologically active chemical compounds as antimicrobial agent. Medicinal plants are richest source of traditional medicinal system of drugs, medicines of modern era, food supplements, nutraceuticals, folk medicines, pharmaceuticals, intermediates and chemicals used for synthetic drugs [1]. This importance which is gained by plants throughout the history of mankind is increasing day by day in this modern era also. The plants used by the ancient people are still in use today but the activities shown by these plants due to presence of their chemical constituents are yet to be identified and hence the exact mechanism of action of these medicinal plants is still not known for number of plant species. The medicinal and pharmacological properties of these medicinal plants is often related to the presence of bioactive compounds called secondary metabolites [2, 3]. Plants synthesize vast and different types of secondary metabolites for the protection needs against the biotic and abiotic environmental challenges [4]. Therefore these compounds give the plants more fitness as they possess the properties like antimicrobial, anti-herbivory, alleopathic etc. These chemical weapons are also used to avoid the attacks from viruses, bacteria, fungi, herbivores and/or minimize competition with other plants [5]. Phytosterols represent the diverse group of natural products and the knowledge about their occurrence in various plants and their chemical composition has gradually accumulated during the last 100 years. Much of the early research and utilization of phytosterols focused on their value as precursors in the synthetic synthesis of several steroid hormones. During the last 10 years most of the interest in phytosterols is due to their cholesterol lowering properties [6]. *Radermachera xylocarpa* is middle size deciduous tree belonging to family Bignoniaceae [7]. It is mainly distributed in areas of Deccan, Konkan, Khandesh and Western Ghats of India [8]. From phytochemical point of view, this plant has not been evaluated properly. The phytochemical, Dinatin-7-glucoronide is reported from leaves [9] and O-ocetylo-lenic acid, Stigmasterol, Radermachol are reported from roots [10, 11] Therefore very less is known about its chemical composition. The present study aims to find out the secondary metabolites in the leaves, stem and root of *Radermachera xylocarpa.*

2. Material and Methods

2.1 Collection of Plant Material

The leaves and stem of *Radermachera xylocarpa* were collected from Melghat forest of Amravati district, Maharashtra, India. The collected leaves were carefully examined for too old, etiolated, infected parts and were removed accordingly. Only fresh leaves were taken for the analysis. Only fresh stems which are light green in colour apart from tree trunk were collected. The leaves and stem are firstly washed by tap water to remove dust and other contaminants. For the purpose of collection of roots, seeds of *Radermachera xylocarpa* were grown in departmental garden. When the plantlets were reaching the height of 10-12 cm they were taken out and roots are separated from rest of the plant. These roots were washed with tap water to remove the soil particles. These plant parts were shade dried at room temperature. This dried material is converted to fine powder and used for further experimental analysis.

2.2 Extraction procedure

Five gram of powder leaves, stem and root of *Radermachera xylocarpa* were extracted using Soxhlet apparatus for 24 hours in dichloromethane solvent (250 ml for each part). These three extract were filtered through Whatman filter paper No. 42 to obtain free and clear extract. This extract then concentrated to 5 ml and stored at – 20 ^{0}C.

2.3 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The GC-MS analysis of three crude extract leaves, stem and root isolated from *Radermachera xylocarpa* was carried out using gas chromatography – high resolution mass spectrophotometer. 2 µl of each sample is employed for GC – MS analysis. The GC-MS analysis was carried out using Alegent Hp 7880 with column of 30 meter length, 0.25 mm ID, 0.32 thickness. Helium gas is used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at 100^0 C. The oven temperature were programmed from 50^0 C to 280^0 C at 10^0 C/ minute to 200^0 C then10^0 C/3 minutes to 250^0 C ending with a 5 minutes isothermal at 280^0 C. The sample was injected in split mode as 50:1.

2.4 Identification of compounds

Identification of the compounds was done by comparing the spectral data of sample compound with the reference spectra present in spectral libraries (NIST).

3. Results

3.1 Physical properties

The three crude extracts have different colour properties. Leaves extracted in dichloromethane are light green in colour, stem extracted in dichloromethane is yellow in colour and root extracted in dichloromethane is light yellow in colour.

3.2 Chemical compound composition of different extract

The compounds found in leaves of dichloromethane extract are shown in table No. 1. The major identified compounds are Cyclopentanone, 2-(1-methylpropyl)- (0.31%), Naphthalene (2.21%), Dibutyl phthalate (19.93%), 1,2-Diydropyrido(3,2,1-kl) phenothiazin-3-one (10.23%), β-Sitosterol (57.55), Heptacosane, 1-chloro- (3.08%), Tetracosane, 12-decyl-12-nonyl (3.51%), 12-Hydroxyoctadecanethioic acid S-t-butyl ester (3.01%) and Cholesterol (0.13%). The compounds found in stem of dichloromethane extract are shown in table No. 2. The major identified compounds are Dibutyl phthalate (3.95%), Stigmast-4-en-one (4.52%), Stigmasterol (8.40%), Eicosane, 2-methyl- (2.35%), β-Sitosterol (9.37%), Heptacosane, 1-chloro- (4.51%), Cholest-4-en-one (60.55%), Ergosta-4,6,8(14),22-tetraen-3-one (3.15%), Heptacosane, 2,6,10,15-tetramethyl- (3.16%). The compounds found in root of dichloromethane extract are shown in table No. 3. The major identified compounds are Cyclopropane nonyl (1.47%), Dodecane, (1.43%), Tetradecane (2.00%), 7-Azaindole-3-carboxaldehyde (1.54%), 1-Hexadecane (2.19%), 1 Nonadecane (1.73%), Dibutyl phthalate (5.33%), n-Hexadecanoic acid (2.52%), Stigmast-4-en-3-one (28.28%), Heptacosane (5.40%), Heptacosane (7.82%), Heptacosane (12.03%), Squalene (12.97%) and Hentriacontane (13.36%).

Table No. 4 shows the secondary metabolites found in *Radermachera xylocarpa* leaves, stem and root, followed by mass spectrum of each secondary metabolite.

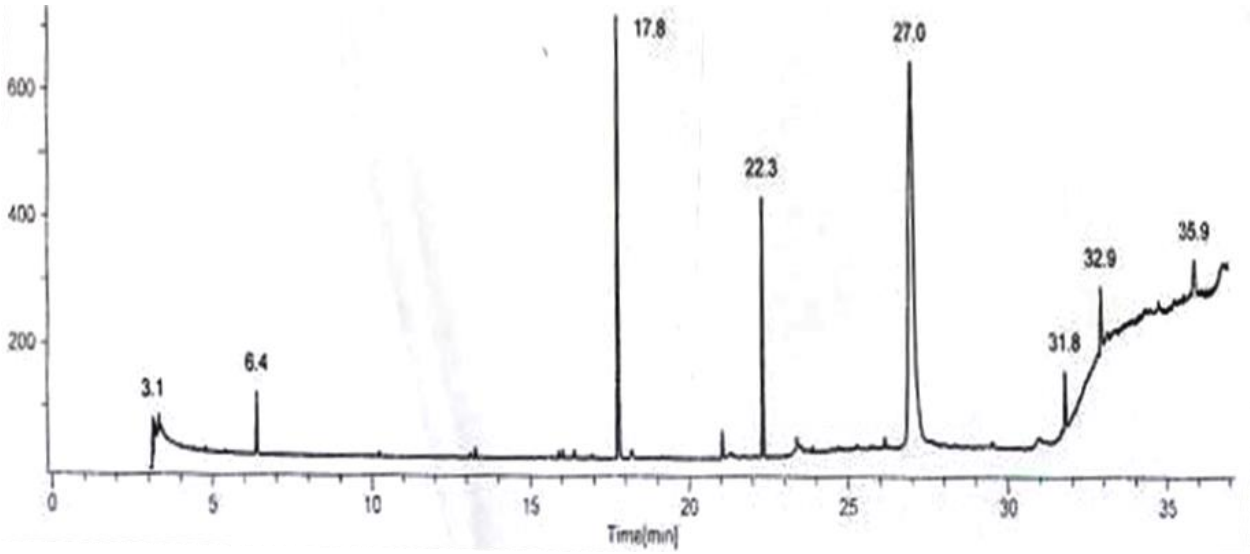

Fig 1: Chromatogram of dichloromethane extract of leaves

Table 1: Compounds Identified in leaves of dichloromethane extract

Sr. No.	RT	Name of Compound	Peak area %	MW	MF
1	3.1	Cyclopentanone, 2-(1-methylpropyl)	0.31	140	$C_9H_{16}O$
2	6.4	Naphthalene	2.21	128	$C_{10}H_8$
3	17.8	Dibutyl phthalate	19.93	278	$C_{16}H_{22}O_4$
4	22.3	1,2-Diydropyrido(3,2,1-kl) phenothiazin-3-one	10.23	253	$C_{15}H_{11}NOS$
5	27.0	β-Sitosterol	57.55	414	$C_{29}H_{50}O$
6	31.8	Heptacosane, 1-chloro-	3.08	415	$C_{27}H_{55}Cl$
7	32.9	Tetracosane,12-decyl-12-nonyl	3.51	605	$C_{43}H_{88}$
8	35.9	12-Hydroxyoctadecanethioic acid S-t-butyl ester	3.01	372	$C_{22}H_{44}O_2S$
9	36.0	Cholesterol	0.13	386	$C_{27}H_{46}O$

RT= Retention Time, MW= Molecular Weight, MF= Molecular formula

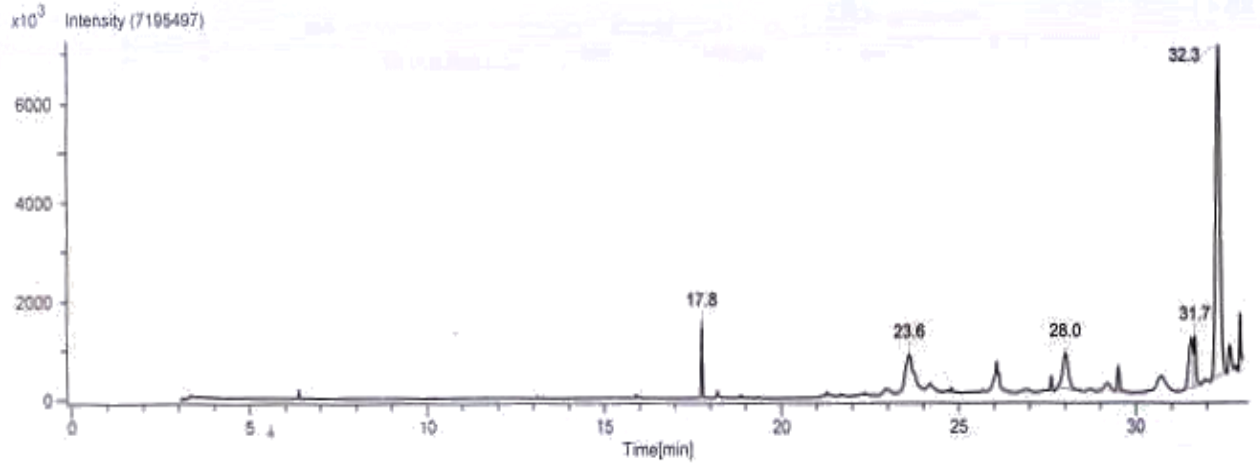

Fig 2: Chromatogram of dichloromethane extract of Stem

Table 2: Compounds Identified in stem of dichloromethane extract

Sr. No.	RT	Name of Compound	Peak area %	MW	MF
1	17.8	Dibutyl phthalate	3.95	278	$C_{16}H_{22}O_4$
2	23.6	Stigmast-4-en-3-one	4.52	412	$C_{29}H_{48}O$
3	28.0	Stigmasterol	8.40	412	$C_{29}H_{48}O$
4	29.5	Heptacosane	2.35	380	$C_{21}H_{44}$
5	31.6	β-Sitosterol	9.37	414	$C_{29}H_{50}O$
6	31.7	Heptacosane, 1-chloro-	4.51	415	$C_{27}H_{55}Cl$
7	32.3	Cholest-4-en-one	60.55	384	$C_{27}H_{44}O$
8	32.6	Ergosta-4,6,8(14),22-tetraen-3-one	3.15	392	$C_{28}H_{40}O$
9	32.9	Heptacosane, 2,6,10,15-tetramethyl	3.16	605	$C_{43}H_{88}$

RT= Retention Time, MW= Molecular Weight, MF= Molecular formula

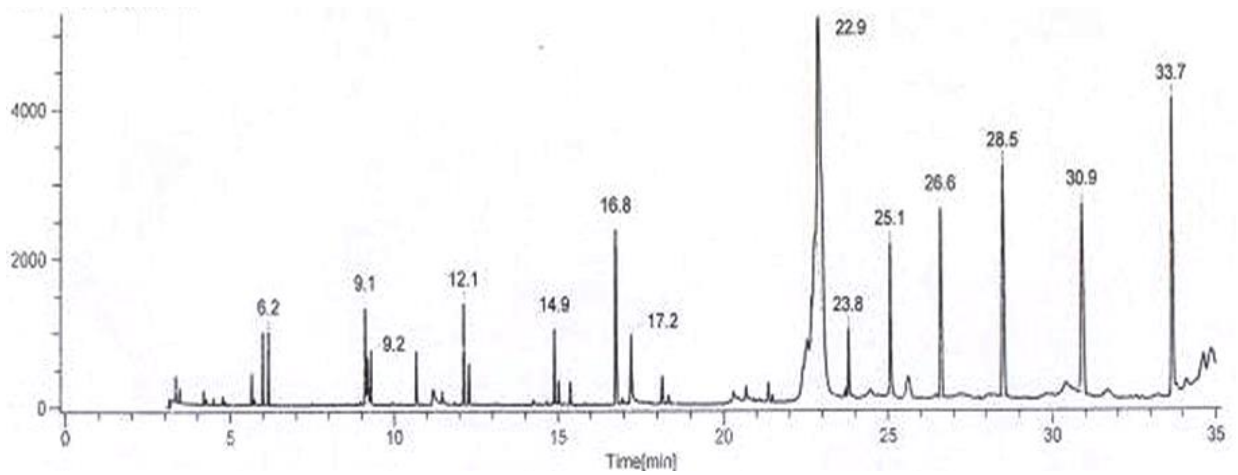

Fig 3: Chromatogram of dichloromethane extract of Root.

Table 3: Compounds Identified in roots of dichloromethane extract

Sr. No.	RT	Name of Compound	Peak area %	MW	MF
1	6.0	1-Decene	1.47	168	$C_{10}H_{20}$
2	6.2	Dodecane	1.43	170	$C_{12}H_{26}$
3	9.1	Tetradecane	2.00	198	$C_{14}H_{30}$
4	9.2	7-Azaindole-3-carboxaldehyde	1.54	152	$C_8H_6N_2O$
5	12.1	1-Hexadecane	2.19	224	$C_{18}H_{36}$
6	14.9	1-Nonadecane	1.73	266	$C_{19}H_{38}$
7	16.8	Dibutyl phthalate	5.33	278	$C_{16}H_{22}O_4$
8	17.2	n-Hexadecanoic acid	2.52	256	$C_{16}H_{32}O_2$
9	22.9	Stigmast-4-en-3-one	28.28	412	$C_{29}H_{48}O$
10	25.1	Tetracosane	5.40	338	$C_{24}H_{50}$
11	26.6	Octadecane, 2-methyl	7.82	268	$C_{19}H_{40}$
12	28.5	Heptacosane	12.03	380	$C_{27}H_{56}$
13	30.9	Squalene	12.97	410	$C_{30}H_{50}$
14	33.7	Hentriacontane	13.36	436	$C_{31}H_{64}$

RT= Retention Time, MW= Molecular Weight, MF= Molecular formula

Table 4: Secondary metabolites found in different parts of *Radermachera xylocarpa*.

Sr. No.	Name of Compound	Category	Plant Part
1	1,2-Diydropyrido(3,2,1-kl) phenothiazin-3-one	Alkaloid	Leaves
2	7-Azaindole-3-carboxaldehyde	Alkaloid	Root
3	β-Sitosterol	Steroid	Leaves, Stem
4	Cholesterol	Steroid	Leaves
5	Stigmast-4-en-3-one	Steroid	Stem, Root
6	Stigmasterol	Steroid	Stem
7	Cholest-4-en-one	Steroid	Stem
8	Ergosta-4,6,8(14),22-tetraen-3-one	Steroid	Stem
9	Squalene	Terpenoid	Root

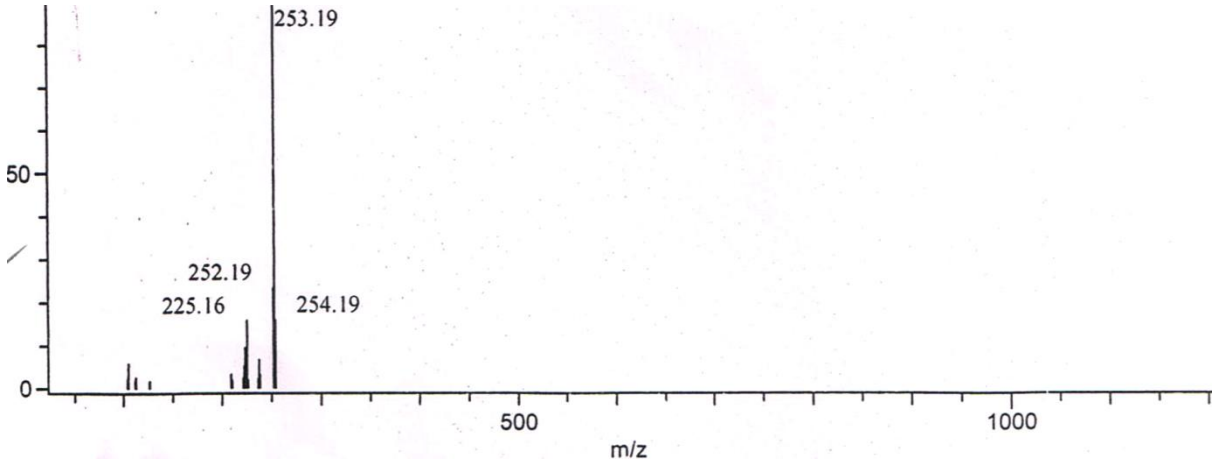

Fig 4: Mass spectrum of 1,2-Diydropyrido(3,2,1-kl) phenothiazin-3-one

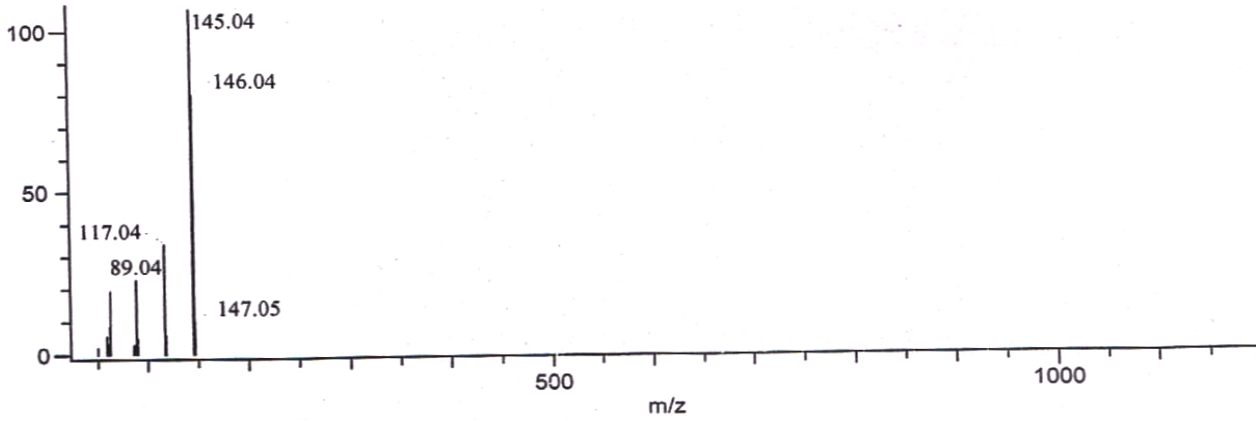

Fig 5: Mass spectrum of 7-Azaindole-3-carboxaldehyde

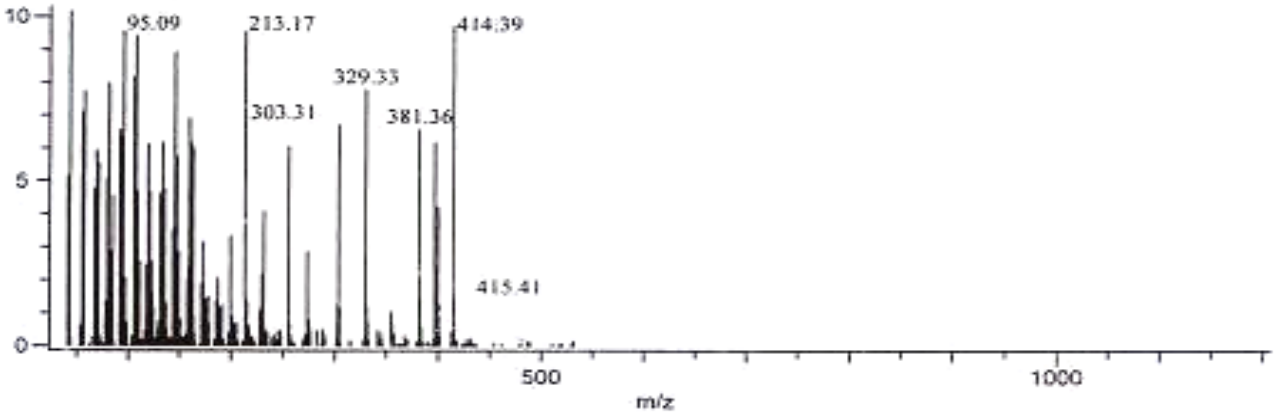

Fig 6: Mass spectrum of β-Sitosterol

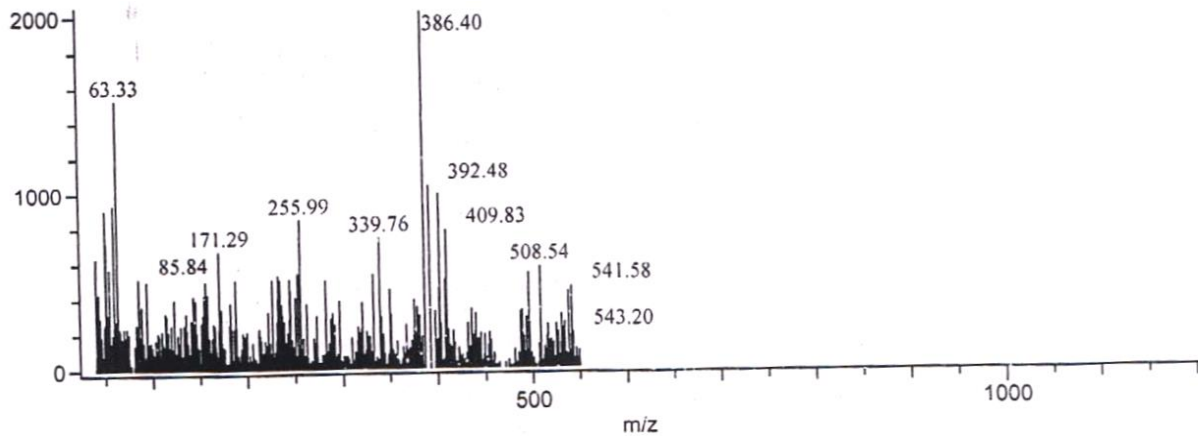

Fig 7: Mass spectrum of Cholesterol

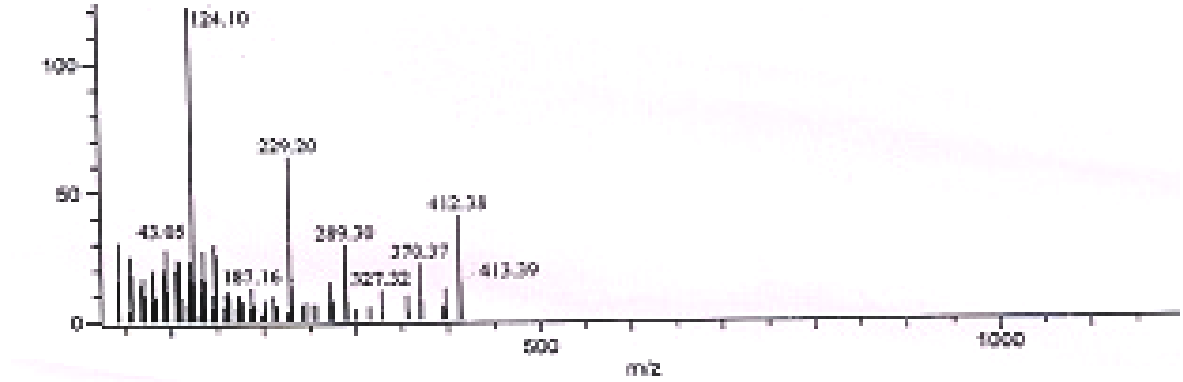

Fig 8: Mass spectrum of Stigmast-4-en-3-one

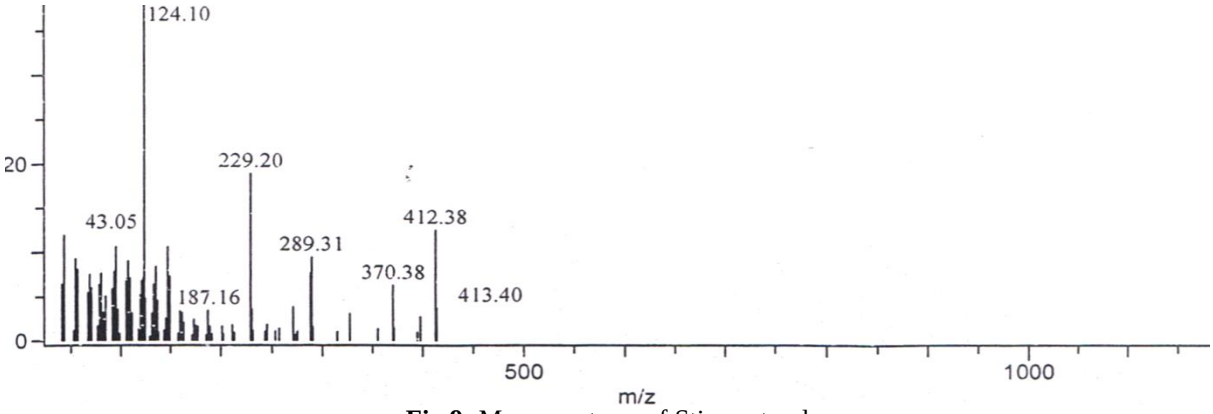

Fig 9: Mass spectrum of Stigmasterol

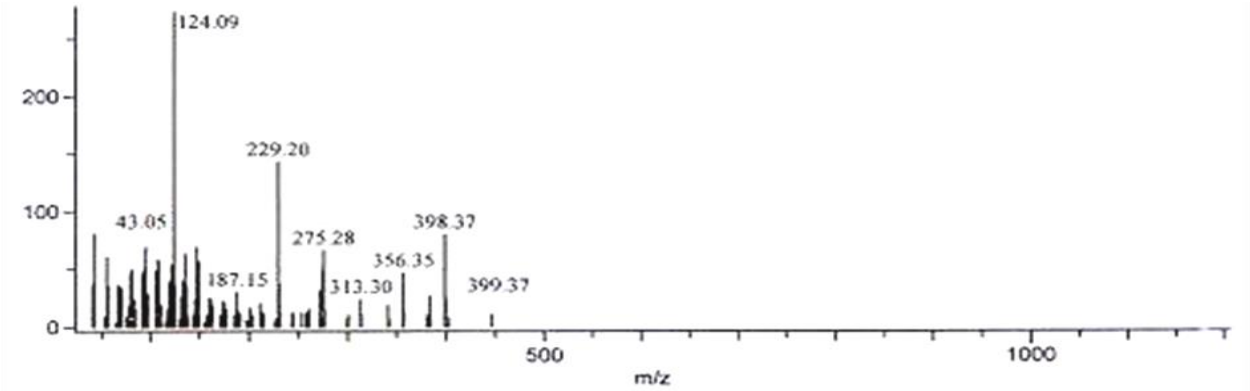

Fig 10: Mass spectrum of Cholest-4-en-one

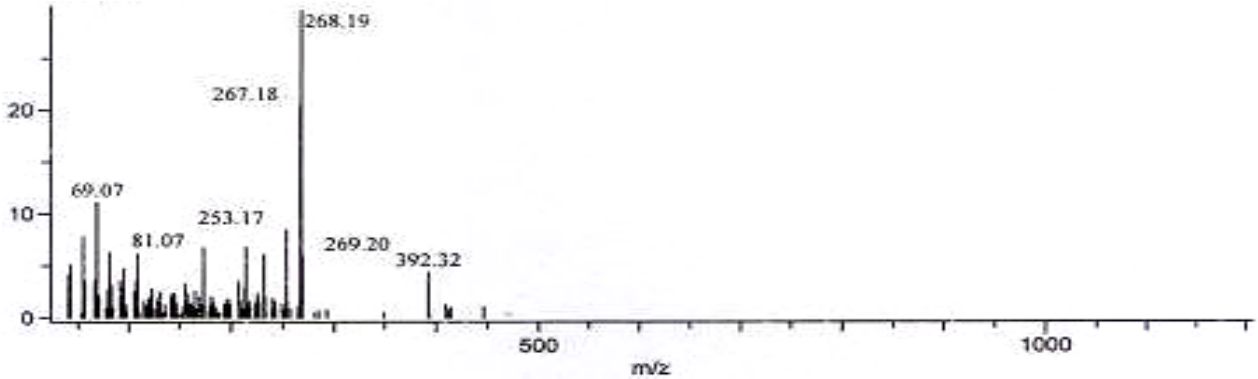

Fig 11: Mass Spectrum of Ergosta-4, 6, 8 (14), 22-tetraen-3-one

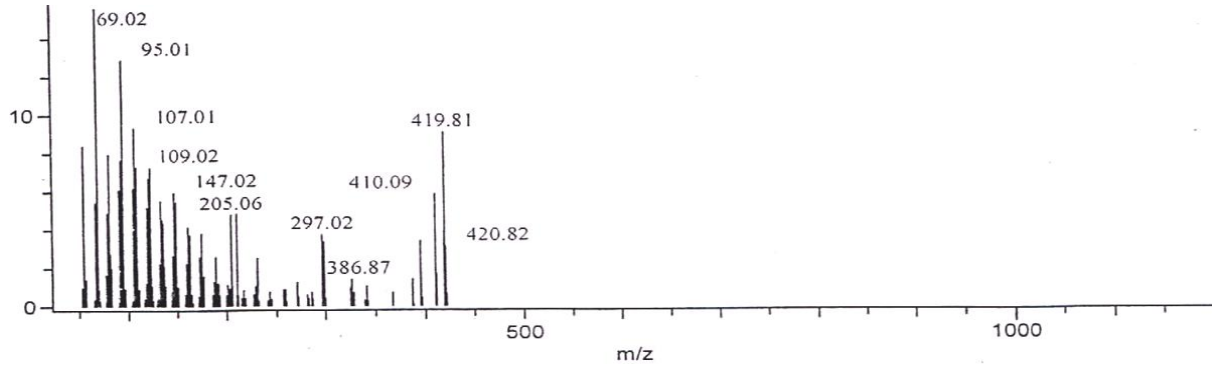

Fig 12: Mass spectrum of Squalene

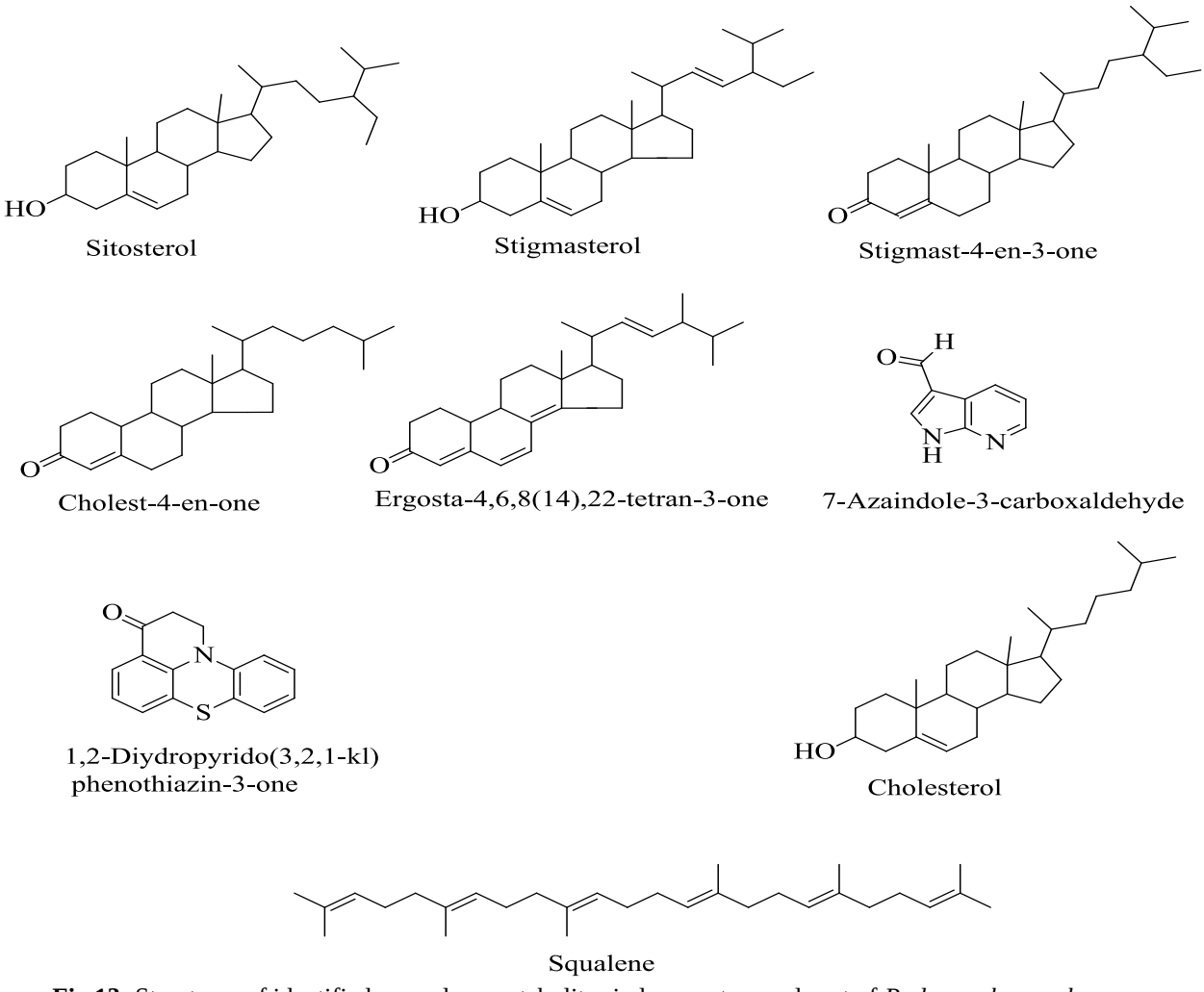

Fig 13: Structures of identified secondary metabolites in leaves stem and root of *Radermachera xylocarpa*.

4. Discussion

The preliminary phytochemical tests [12] have revealed the presence of important secondary groups in different parts of *Radermachera xylocarpa.* The GC-MS analysis supports the same and shows the presence of important bioactive compounds. The relative concentrations of various compounds were calculated by the use of gas chromatogram which gives the many peaks. The height of the peak corresponds to the relative concentration of compound. The compounds which are eluted at different timings through gas chromatogram are picked up by the mass analyzer and produce particular fragmentation pattern. This fragmentation pattern is compared to the compounds present in reference library (NIST) on which the structure of compounds is determined. This provides the unique chemical fingerprint that shows the importance of plant under study. In the present investigation leaves, stem and root of *Radermachera xylocarpa* were extracted in dichloromethane solvent and analyzed by gas chromatography-mass spectroscopy. It is important to note that steroids are found in large number with very high concentration as compared to other chemical constituents. Phytosterols, which cannot be synthesized by human and therefore all plant sterols and stanols in the human body are of diet origin [13]. These phytosterols are known to have many bioactive qualities and possible implications on human health [14].
Leaves contain the steroids, β-Sitosterol with 57.55% concentration and cholesterol with 0.13% concentration. Stem shows the presence of five steroidal compounds namely, Stigmast-4-en-one, Stigmasterol, β-Sitosterol, Ergosta-4, 6, 8(14), 22-tetraen-3-one and Cholest-4-en-one with 4.52%, 8.40%, 9.37%, 3.15% and 60.55% concentration respectively. Whereas root shows the presence of Stigmast-4-en-3-one with 28.28% concentration.

β-sitosterol is a waxy substance which is white in colour. It has been already well known from wheat gram oil, cotton seed oil, corn oil and soybean oil in abundant quantity [15]. β-sitosterol present in soybean oil is reported to lower down the cholesterol level in blood [16], this is due to the property of β-sitosterol to inhibit the absorption of cholesterol in the body [17]. Stigmasterol, also known as stigmasterin is an unsaturated plant sterol present in various medicinal plants. Stigmasterol is utilized in number of chemical processes which are designed to yield synthetic and semi-synthetic compounds for pharmaceutical industry. It acts as a precursor in the synthesis progesterone and acts as an intermediate in the biosynthesis of androgens, estrogens, corticoids and in the synthesis of Vitamin D_3 [18, 19]. Stigmasterol has many pharmacological properties like anti-osteoarthritic activity [20], anti-hypercholestrolemic activity [21], cyto-toxicity activity [22], anti-tumor activity [23], the synergism between the stigmasterol and sitosterol is found to have hypoglycemic activity [24], anti-oxidant activity [25], anti-mutagenic activity [26], anti-inflammatory activity [27] etc. It has been reported that stigmasterol and spermidine have influence on the chemical composition of chamomile plant [28]. The derivative of stigmasterol, stigmast-4-en-one is known to have hypoglycemic activity [29]. Ergost-4,6,8(14), 22-tetraen-3-one is an bioactive steroid that has been isolated from fungus, *P. umbellatus* [30]. This compound is reported to possess cytotoxic activity, diuretic activity, and immunosuppressive activity [31-33] In present study cholesterol found in leaves with very low concentration, about which there is widespread belief among the people and even in chemists that plants do not contain cholesterol, this false assumption made due to the reasons that plants generally contain very small amount of cholesterol and analytical techniques for detection of it has been recently developed [34]. Cholesterol occurs as a component of plant membrane and as a part of surface lipids of leaves where it is sometimes the major sterol. Sources of plant cholesterol are palm oil, coconut oil soybean oil, olive oil etc [35].
Squalene is a triterpenoid and widely distributed in nature, with reasonable amount found in olive oil, palm oil, wheat germ oil etc. It is an important intermediate in the biosynthesis of sterols in the plants and animals [36, 37]. The alkaloids namely, 1,2-Diydropyrido (3,2,1-kl) phenothiazin-3-one from leaves and 7-Azaindole-3-carboxaldehyde from root were also observed.
The present study helps to understand the chemical composition contained in *Radermachera xylocarpa* which shows that the plant is highly a rich source of steroidal compounds with higher amount of concentrations. The compounds show the great prospects in pharmaceutical, medicine and in drug formulations. Therefore to explore the full significance of the plant, it is essential to carry out extensive chemical research on the same plant.

5. Acknowledgment

The authors express sincere thanks to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Mumbai for supporting instrumental analysis for this research work.

6. References

1. Hammer KA, Carson CF and Riley TV. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbial 1999; 86 (6): 985.
2. Bruneton, J. Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept. Ltd. England, U.K.; 1999.
3. Heinrich, M, Barnes, J, Gibbons S. and Williamson EM. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK. 2004.
4. Dixon RA. Natural products and disease resistance. Nature (2001); 411: 843-847.
5. Rohloff J and Bones A. Volatile profiling of Arabidopsis thaliana in plant communication. Phytochemistry (2005); 66: 1941-9155.
6. Moreau RA, Whitekar BD, Hicks KB. Phytosterols, phytostenols and their conjugates in foods: structural diversity, quantitative analysis, and health promoting uses. Progress in Lipid Research 2002; 41: 475-500.
7. Chopra, RN, Nayar SL, and Chopra RC. Glossary of Indian medicinal plants; CSIR, New Delhi; 1956; 208.
8. Shetgiri NP, Kokitkar SV and Sawant SN. Radermachera xylocarpa; the highly efficient source of lapachol and synthesis of its derivatives. Acta poloniac pharmaceutia – Drug research 2001; 58 (2): 133-135.
9. Subramanian SS, Nagarajan S, Sulochana S. Phytochemisrty. 1972; 11: 1499.
10. Desai HK, Gawad DG, Joshi BS, Parthsarathy PC, Ravindranath KR, Saindane MT, Sidhaye AR, Vishvanathan N. Chemical investigation of Indian plants. part X. Ind J Chem 1977; 14B:291-292.
11. Joshi BS, Gawad DH, Pelletier SW, Kartha G, Bhandary K. Tetrahydrone Latters; 1984; 25:5847.
12. Herborne J. Phytochemical methods; a guide to modern techniques of plant analysis. Springer, Nature; 1998; 302.
13. Jong A, Plat J, and Mensink RP. Metabolic effect of plant sterols and stanols. J Nurt Biochem 2003; 14:362-369.
14. Norman L, Bryngelsson S, Johnsson M, Evheden P, Ellegard L, Brants H, Andersson H. and Dutts P. The phytosterols content of some cereal foods commonly consumed in Sweden and in the Netherlands. J. Food Comp. Anal.2002; 15: 693-704.

15. Chen RR. Plant oil. Biomedicine, Taipai Publishers, Taiwan. 1991; 283-287.
16. Cicero AF, Fiorito A, Panourgia MP, Sangiorgi Z and Gaddi A. Effect of new beta-sitosterol supplement to plasma lipid in moderately hypercholesterolemic subjects. J. Am. Diet Assoc.2002; 102: 1807-1811.
17. Tatu A, Miettinen A, Helena G. Ineffective decrese of serum chlolesterol by simvastatin in a subgroup of hypercholesterolemic coronary patients. Atherosclerosis; 2002; 164:147-152.
18. Sundararaman P and Djerassi C. A convenient synthesis of progesterone from stigmasterol. J Org Chem 1977; 42 (22): 3633-3634.
19. Kametani T, Furuyama H. Synthesis of vitamin D3 and related compounds. Med Res Rev 1987; 7 (2): 147-171.
20. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, et al. Stigmasterol: a phytosterols with potential anti-osteoarthitic properties. Osteoarthritis Cartilage 2010; 18(1): 106-116.
21. Chandler RF, Hooper SN, Ismail HA. Antihypercholesterolemic studies with sterols; beta-sitosterol and stigmasterol. J Pharma Sci 1979; 68(2): 245-247.
22. Huang JG, Zhou LJ, Xu HH, Li WO. Insecticidal and cytotoxic activities of extracts of *Cacalia tangutica* and its two active ingredients against *Musca domestica* and *Aedes albapictus*. Journal of Economic Entomology 2009; 102 (4): 1444-1447.
23. Kasahara Y, Kumaki K, Katagiri S, Yasukawa K, Yamanouchi S, Takido M. *Carthami flos* extract and its component, stigmasterol, inhibit tumor promotion in mouse skin two stage carcinogenesis. Phytotherapy Research 1994; 8 (6): 327-331.
24. Jamaluddin F, Mohamed S, Ligis MN. Hypoglycaemic effect of Parkia speciosa seeds due to the synergistic action of β-sitosterol and stigmasterol. Food Chemistry1994; 49 (4): 339-345.
25. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, anti-peroxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia 2009; 80 (2):13-126.
26. Jae-Chul L, Jong HP, Malos B, Alexander K, Yeong-Hwan H, Byung-Soo K. Anti-mutagenic constituents from the thorns of *Gleditsia sinesis*. Chem. Pharm. Bull 2005; 53(5):561-564.
27. Garcia MD, Saenz MT, Gomez MA, Fernandez MA. Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. Phytotherapy Research 1999; 13 (1):78-80.
28. Abd El-Wahed MSA, Krima MG. Stimulation effect of spermidine and stigmasterol on growth, flowering, biochemical constituents and essential oil of chamomile plant (*Chamomilla recutita* L., Rausch). Bulg J Plant Physiol 2004; 30(3-4):48-60.
29. Alexander-Lindo RL, Morrison EY, Nair MG. Hypoglycemic effect of stigmast-4-en-3-one and its corresponding alcohol from the bark of *Anacardium occidentale* (cashew). Phytother Res 2004; 18(5):403-407.
30. Zhao YY, Zhao Y, Zhang YM, Lin RC, Sun WJ. Qualitative and quantitative analysis of the diuretic component ergone in *Polypororus umbellatus* by HPLC with fluorescence detection and HPLC-APCII-MS/MS. Pharmazie 2009; 64:366-370.
31. Lee WY, Park Y, Ahn JK, Lee HJ. Cytotoxic activity of Ergost-4,6,8(14), 22-tetraen-3-one from the sclerotia of *Polyporous umbellatus*, Bull Korean Chem Soc 2005; 26:1464-1466.
32. Youn D, Mori J, Komatsu KI, Makino T, Kano Y. An anti-aldosteronic diuretic component (drain dampness) in *Polyporous sclerotium*. Biol Pharm Bull 2004; 27:867-870.
33. Fujimoto H, Nakamura E, Okuyama E, Ishibashi M. Six immunosuppressive features from an ascomycete, *Zopfiella longicaudata*, found in a screening study monitored by immunomodulatory activity. Chem. Pharma. Bull. 2004; 52:1005-1008.
34. Rossell JB. In analysis of oil seeds, fats and fatty foods; Rossell JB and Pritchard JLR. Chapter 7, Table 7.11; Eds.; Elseveir; London: 1991.
35. Mudd JB. The Biochemistry of Plants. Stumpf, PK.; Conn, E.E. Eds.; Acedamic press, New York; 4; 1980: 514-515.
36. Psomiadou E and Tsimidou M. On the role of squalene in olive oil stability. J Agric Food Chem 1999; 47:4025-4032.
37. Bargossi AM, Battino M, Gaddi A, et al. Exogenous CoQ10 preserves plasma ubiquinone levels in patients treated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Int J Clin Lab Res; 1994; 24; 171-176.