WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 1367-1376. Research Article ISSN 2277– 7105

PROFILING THE SECONDARY CHEMICAL CLASS OF IN VIVO MELIA COMPOSITA WILLD. LEAF USING ETHANOLIC FRACTION

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Article Received on
15 Oct 2015,

Revised on 05 Nov 2015,
Accepted on 25 Nov 2015,

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ABSTRACT

The present study was aimed to investigate the phytochemical components of Ethanolic in vivo leaf extract of *Melia composita* Willd. (Synonym: *Melia dubia Hiern* (not of Cav.)) member of Meliaceae by using GC-MS method. The chromatogram of in vivo leaf extract identified 33 phytochemicals as constituents with more than one peak area for 7 compounds. Quantitative analysis of the different compounds were performed on a GC Clarus 500 Perkin Elmer gas chromatograph equipped with Column: Elite-1 (100 % Dimethyl poly siloxane), 30m x 0.25 mm ID x 1.0 µ df at the oven temperature programme of 110°C -2 min hold up to 280°C at the rate of 5°C/min-9 min hold. 1µl of each sample was injected in triplicate splits and quantities represented relative area percentage as derived from the integrator. The major compounds identified from the in vivo leaf powder were Vitamin E with 35.46% peak area, followed by 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (18.13%), Phytol (11.28%), 1-Monolinoleoylglycerol trimethylsilyl ether (8.88%), Caryophyllene (4.50%), Tetradecanoic acid (2.94%), 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)- (2.62%) and 2-Pentanone, 4- hydroxy-4-methyl- (2.28%). The other compounds were represented by less than 2% peak area.

KEYWORDS: Gas chromatography-Mass Spectrometry, Phytochemical components, *Melia composita* Willd.

INTRODUCTION

Melia composita Willd.

Melia composita Willd. (syn. *Melia dubia* Hiern (not of Cav.)), a wild relative tree of *Azadirachta indica* Juss., is an important medicinal plant growing in the Eastern Gharts of Peninsular India. This tree, well known as Persian lilac, is native to India but is now grown in all the warmer parts of the world.

Nomenclature

Current Name:*Melia composita* Willd. Willdenow, Sp. Pl., ed. 4 2 (1): 559, 1799

Synonym:*Melia dubia* Hiern (not of Cav.) Hiern, Fl. Br. Ind., 1(1): 545, 1872

Family:Meliaceae

Common names:**Bengali** -Bakarjan, Ghora nim, Mahanim, Mahnim

English-Azedarach, Bead tree, China tree, Persian lilac, Pride of India, syringa

Hindi -Bakain, Bakarja, Betain, Deikna, Mallan nim

Kannada-Betta bevu; Heb-bevu

Sanskrit-Mahanimba

Malayalam-Aryaveppu; Malaveppu; Kattuveppu

Tamil-Malai vembu; puvembu

Trade name: Persian lilac

Currently, this species is treated as the synonym of *Melia azedarach* Linn. by some Taxonomists. However, these two species differ from each other. in vegetative and reproductive characters. *Melia composita* is a tall tree grows upto 25 m height with less number of branches; Leaves are much larger and bipinnately compound. Whereas, *M. azedarach* is a medium size tree with many lateral branches; and leaves are smaller than *M. composita*. In *M. composita*, flowers are white, anthers exceeding the laciniae of the white staminal tube and fruits ellipsoid, 1-1.5 in long; whereas in *M. azedarach*, flowers are lilac, anthers nearly equalling the laciniae of the purple staminal tube and fruits globose, 0.5-0.7 in long.[1] In several studies this species is treated as the synonym of *M. dubia* Hiern (wrongly quoted as *M. dubia* Cav., which is the synonym of *M. azedarach* Linn.).

Chemical constituents and properties

The plant parts are bitter tasting, refrigerant, toxic, vermifuge, analgesic and antiphlogistic. The bark contains margosine and tannic acid. The fruits contain azadine, resin, benzoic acid and meliotannic acid. The root cortex, the bark and the fruits contain toxic principles.[2] isolated two tetranortriterpenoids, compositin and compositolide from the seeds and leaves.

The leaves also contain essential oils monoterpenes and oxygenated monoterpenes, accompanied by a relatively much smaller amount of alkanes, sesquiterpene hydrocarbons and phenylpropanoids. The monoterpene camphene occurs as a major constituent of these leaf essential oils. The oxygenated monoterpenes are distinctly dominated by the presence of the bicyclic ketone camphor, while iso-borneol and borneol are detected in much smaller amounts.[3] Studies have yielded hydroxycoumarins (antifungal), alkaloids (antiinflammatory), monoterpenes (bacteriostatic) and limonoids (antifeedant), meliacarpin and meliacin (antiviral).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds.[4] The World Health Organization has reported that around 21,000 plants have been used for medicinal purpose in the world. About 500 higher species has been thoroughly investigated as potential source of new drugs. Nearly 119 pure chemicals were extracted from 90 plant species. Interest in phytomedicine has exploded in the last few years, and about 500 different plant species are used as key ingredients, and many are still being collected from the wild.[5]

Plants play a dominant role in the introduction of new therapeutic agents, and also drugs from the higher plants continue to occupy an important niche in modern medicine.[6] Phytochemical examination of a number of them has been carried out and active ingredients isolated and identified are being currently used as drugs. Chemical examination of hitherto unexplored medicinal plants will help in discovering new drugs.[7]

The active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components. The active compounds extracted from plants belong to a group

collectively known as secondary metabolites. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of pharmaceuticals.

Modern drugs or conventional medicine is often viewed as impersonal and expensive. It also brings some side effect, which are sometimes more dangerous than the disease itself. Scientists in many parts of the world have carried out extensive research and have proven to humanity the effective use of herbal medicine. The phytochemical screening of a medicinal plant is a prerequisite for the scientific verification of folklore claim of the inhabitants vis-à-vis their utility as a new source of herbal drug.

For the widespread acceptance of herbal medicines, standardization, quality control of the herbal materials, as well as evaluation of efficacy, safety and quality of the phytopharmaceutical are indispensable.[8] Identification of individual components of complex mixtures of phytochemicals requires the use of several techniques. One of the most popular methods of studying phytochemical composition is GC-MS, which allows the identification of the specific natural compounds found in a plant extracts by comparing their relative retention times and indices and their mass spectra.[9],[11]

The whole plant of *Melia composita* (=*M. dubia* / *M. azedarach*) possesses the active ingredients like caffeic acid, catechin, ferulic acid, fraxinellone, gedunin, glutamic acid, glycine, kaempferol, nimbin and quercetin. Leaf contains esculetin, quercitrin, rutin and scopoletin. Flower has astragalin and hyperin. Fruit has acetic acid, cinnamic acid, cycloeucalenol and vanillin. Seed possesses arginine, leucine, lysine, methionine, threonine, palmitic acid and stearic acid. The wood contains alpha-terpineol, aromadendrene, benzyl acetate, chavicol, creosol, eugenol, geranyl acetate and linalyl acetate. The root possesses benzoic acid and beta-sitosterol. Tannin and vanillic acid are found in the bark. An essential oil called carvacrol is found in the plant (IMPGC.com). This plant produces a limonoid toosendanin, which was first isolated from the bark of the tree *M. toosendan*.[11] Toosendanin is closely related to meliatoxins, the other group of anti-insect compounds produced in *M. azedarach*.[12] isolated[2] two tetranortriterpenoids, compositin and compositolide, from the seeds and leaves of *M. dubia* (syn. M. composita). This plant also contains the leaf essential oil chiefly of monoterpenes and oxygenated monoterpenes with small amount of alkanes, sesquiterpenes and phenylpropanoids.[3]

Medicinal uses

Fruits of this plant are used in folk medicine as an anthelmintic, astringent and in the treatment of colic. Paste made out of leaves/green fruits is used to treat scabies and maggot-infested sores. Root bark and trunk bark are used to kill intestinal parasites (anthelmintic). The recent studies of various parts of this plant also showed antifeedant[13] antimicrobial.[141516] and antidiabetic[17] activities.

Phytochemistry

Plants are the potential source for the discovery of new products and fine chemicals for drug development. A variety of compounds are accumulated in plants accounting for their anti-inflammatory, hypotensive, hypoglycaemic, amoebicidal, antifertility, cytotoxic, antibiotic and anti-parkinsonism properties. The wealth of uninvestigated material available is illustrated by the fact that in 1985, it was reported that natural product research elicited some 3500 new chemical structures of which more than 2600 were from higher plants.[18]

With advances in experimental methods and new scientific tools, notably in the past three decades, numerous active principles from medicinal herbs have been isolated in pure form and these principles along with the synthetic compounds, are the drugs of present day now almost exclusively used in the management of many of the diseases. Rapid development in Phytochemistry and Pharmacology have enormously expanded the subject.

In the present study an attempt was made to investigate the phytochemical components in the in vivo methanolic leaf extracts of *Melia composita* through GC-MS analysis.

MATERIALS AND METHODS

Plant Materials

Leaf sample of in vivo plants of *M. composita* were collected freshly and subjected to air-drying for 3-5 days at room temperature. The dry leaves were powdered and extracted using ethanol to study the bioactive components in GC-MS.

Instrumentation

Quantitative analysis of the different compounds were performed on a GC Clarus 500 Perkin Elmer gas chromatograph equipped with Column: Elite-1 (100 % Dimethyl poly siloxane), 30m x 0.25 mm ID x 1.0 µ df at the oven temperature programme of 110 °C -2 min hold up to 280 °C at the rate of 5 °C/min-9 min hold. 1µl of each sample was injected in triplicate

splits and quantities represented relative area percentage as derived from the integrator. Injector temperature was 250ºC and the split ratio was 10 :1. Helium was used as a carrier gas with a constant flow at 1 ml/min and the detector was Mass detector-Turbo mass gold-Perkin Elmer. The inlet line temperature was 200 ºC and the source temperature was 200 ºC. The instrument was operated at 70 eV in electron impact mode. Full-scan analyses were performed in the mass range 45 – 400 m/z at 1 scan. Data were evaluated by Software-Turbo mass 5.1. The total GC time was 45 minutes and the MS time was 46 minutes.

Identification of Compounds

The relative amount of individual components of the total composition was expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds (NIST Ver.2.1) or by retention indices and mass spectra.[19]

RESULTS AND DISCUSSION

The phytochemical study was carried out by GC - MS analysis of *Melia composita* Willd. for in vivo ethanolic leaf extracts, (Fig.1; Table 1). In in vivo leaf extracts, the chromatogram showed multiple signal or peak areas for some compounds suggesting the presence of two or more stereoisomeric compounds.[20]

The chromatogram of in vivo leaf extract identified 33 phytochemicals as constituents with more than one peak area for 7 compounds (Fig.1; Table 1). The compound 2-Hexenoic acid (Mol. Wt. 114) showed 2 peak areas; Benzeneethanamine, 2,5-difluoro-á,3,4-trihydroxy-N-methyl- (Mol. Wt. 219) 3 peak areas; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol 3 peak areas (Mol. Wt. 296) 3 peak areas and 3-Trifluoroacetoxypentadecane (Mol. Wt. 324) 2 peak areas. The compound 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)- (Mol. Wt. 496) showed 5 peak areas and 1-Monolinoleoylglycerol trimethylsilyl ether (Mol. Wt. 498) showed 8 peak areas. The remaining compounds were represented by single peak area.

Table. 1. GC-MS analysis showing different phytochemical components identified from the *in vivo* ethanolic leaf extract of *Melia composita* Willd.

Sl. No	Name of the Compound	Mol. Formula	Mol. Wt.	R T
1.	2-Pentanone, 4-hydroxy-4-methyl-	C6H12O2	116	2.47
2.	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	C11H17NO2	195	3.55
3.	Tetraacetyl-d-xylonic nitrile	C14H17NO9	343	3.92
4.	Cyclohexanone, 3-hydroxy-	C6H10O2	114	4.18
5.	2-Hexenoic acid	C6H10O2	114	4.53
6.	2-Hexenoic acid	C6H10O2	114	4.64
7.	1-Octadecanamine, N-methyl-	C19H41N	283	5.45
8.	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	C11H17NO2	195	10.06
9.	Pterin-6-carboxylic acid	C7H5N5O3	207	10.53
10.	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl Caryophyllene Pterin-6-carboxylic acid	C6H12O2	116	2.47
11.	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	C13H18O2	206	10.95
12.	Lactose	C15H24	204	11.43
13.	ç-Elemene	C7H5N5O3	207	11.92
14.	Benzeneethanamine, 2,5-difluoro-	C12H20O	180	12.19
15.	á,3,4-trihydroxy-N-methyl-	C12H22O11	342	12.31
16	Dodecanoic acid Imidazole, 2-amino-5-[(2-carboxy)vinyl]-Benzeneethanamine, 2,5-difluoro-	C15H24	204	12.44
17.	á,3,4 -trihydroxy-N-methyl-	C9H11F2NO3	219	12.76
18.	Benzeneethanamine, 2,5-difluoro-	C12H24O2	200	12.99
19.	á,3,4- trihydroxy-N-methyl-9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)	C6H7N3O2	153	13.20
20.	oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-Imidazole, 2-amino-5-[(2-carboxy)vinyl]- 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)	C9H11F2NO3	219	13.29
21.	oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)- 3-Trifluoroacetoxypentadecane	C9H11F2NO3	219	14.28
22.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C27H52O4Si2	496	14.71
23	3- Trifluoroacetoxypentadecane 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C6H7N3O2	153	14.96
24.	1,2-Benzenedicarboxylic acid, butyl octyl ester	C27H52O4Si2	496	15.36
25.	Phytol	C17H31F3O2	324	16.10
26.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)	C20H40O	296	16.17
27.	oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	C17H31F3O2	324	16.22
28.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)	C20H40O	296	16.43
29.	oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	C20H40O	296	16.63
30.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)	C20H30O4	334	17.55
31.	oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	C20H40O	296	18.96
32.	1-Monolinoleoylglycerol trimethylsilyl ether	C27H52O4Si2	496	22.25
33.	Heptacosane(reference gas)	C27H52O4Si2	496	22.57
34.	1-Monolinoleoylglycerol trimethylsilyl ether	C27H52O4Si2	496	23.04
35.	Heptacosane(reference gas)	C27H56	380	23.85
36.	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498	24.76
37.	Vitamin E	C27H54O4Si2	498	25.02

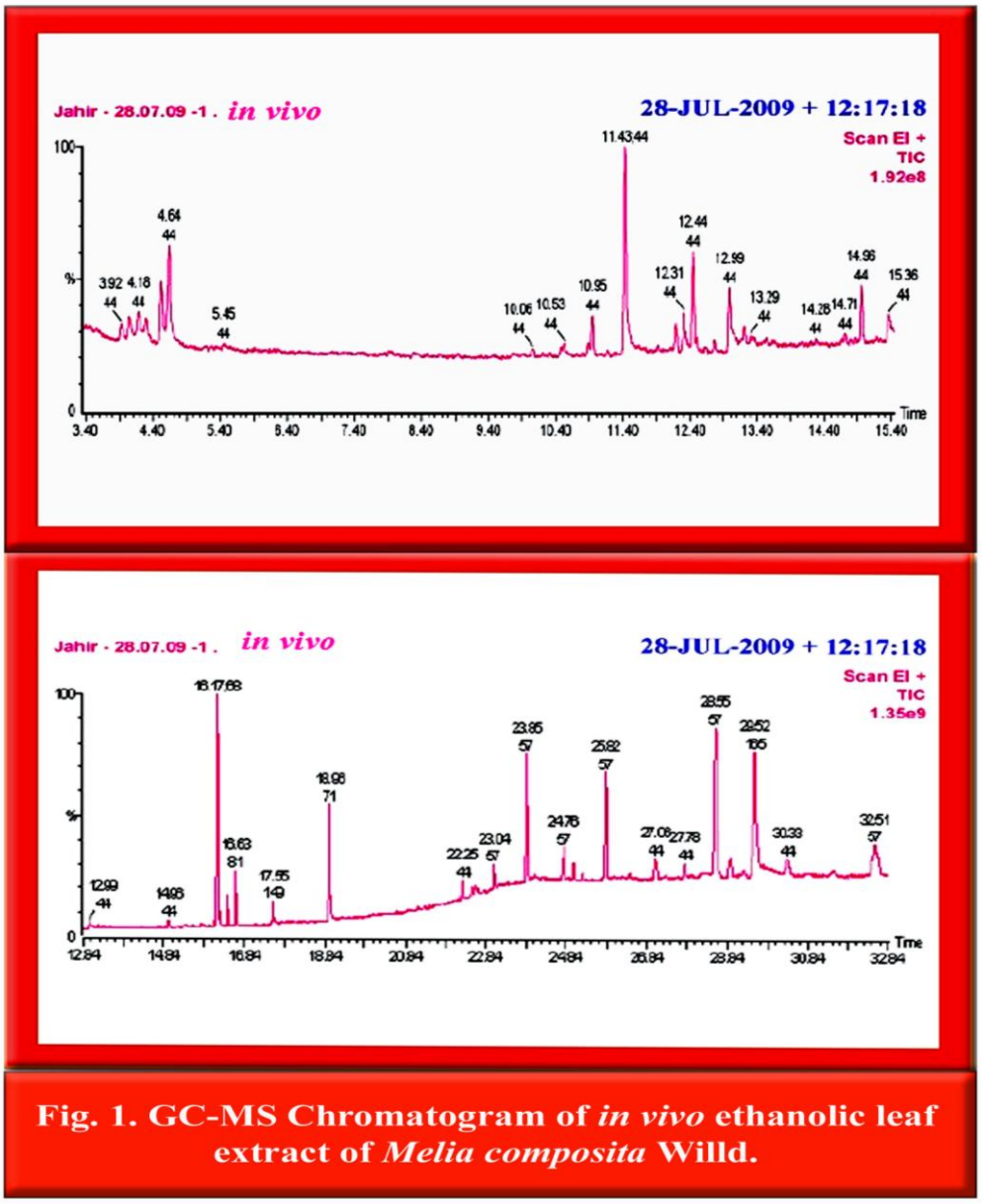

Fig. 1. GC-MS Chromatogram of *in vivo* ethanolic leaf extract of *Melia composita* Willd.

CONCLUSION

Phytochemical study was carried out by using GC-MS analysis of *Melia composita* Willd. in vivo leaf extracts. The chromatogram of in vivo leaf extract identified 33 phytochemicals with Vitamin E (35.46%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (18.13%), Phytol (11.28%), 1-Monolinoleoylglycerol trimethylsilyl ether (8.88%) and Caryophyllene (4.50%) as major compounds.

ACKNOWLEDGEMENT

Author express indebtedness to Dr. K. Kala Regional Joint Director of Collegiate Education, Trichy for guidance, valuable suggestions, continuous encouragement, and help. The author

gratefully acknowledge to Dr. R. Pari, Principal, Govt. Arts College (Autonomous), Karur, for their continuous encouragement and support in carrying out this research work. The author heartfelt thank to Mr. V. Tamilselvan, Mr. V. Muniappan, Mr. P. Rajkumar research scholars, P.G. and Research department of botany Govt. Arts College (Autonomous), Karur for their support and timely help to carry out this research work.

REFERENCES

1. Gamble, J. S. and Fisher, C. E. C. The Flora of the Presidency of Madras. Parts I-II. Aldard and Son and West New Man Ltd., London, 1935; 1: 175-176.
2. Purushothaman, K.P. Kalyani, D. and Connely, J.D. 1984. Tetranortriterpenoids from *Melia dubia*. Phytochemistry, 1984, 23:135–137.
3. Nagalakshmi, M.A.H., Thangadurai, D., Anuradha, T. and Pullaiah, T. Essential oil constituents of *Melia dubia*, a wild relative of *Azadirachta indica* growing in the eastern ghats of peninsular India. Flavour and Fragrance Journal, 2001; 16(4): 241-244.
4. Hill AF (1952). Economic Botany. A textbook of useful plants and plant products. 2nd edn. McGarw-Hill Book Company Inc, New York.
5. Mendelsohnm, R. and Balick, M. The value of undiscovered pharmaceuticals in tropical forests. Econ. Bot., 1994; 49(2):223-228.
6. Dev, S. Ethno therapeutics and modern drug development: The potential of Ayurvedha. Curr. Sci., 1997; 73(11): 909-928.
7. Dayal, R. Chemical investigation of medicinal plants-Chemistry Division Forest - Res Inst Dehradun India. Indian Forester, 1997; 123(3): 249-255.
8. Huie, C.W. A review of modern sample preparation techniques for the extraction and analysis of medicinal plant. Anal Bioanal. Chem., 2002; 373: 23-30.
9. Yani, V. V., Oyedeji, O. A., Grierson, D. S. and Afolayan, A. J. Chemical analysis and antimicrobial activity of essential oil extracted from *Helichrysum aureonitens*. S. A. J. Bot., 2005; 71(2): 239 - 241.
10. Adams, R. P. 2007. Identification of essential oil components by Gas Chromatography/Mass Spectrometry. Allured publishing corporation, Illinois, USA.
11. Shu and Liang, 1980Shu, G. and Liang, X., Acta Chim. Sin., 1980; 38: 196–198.
12. Macleod, J. K., Moeller, P. D. R., Molinski, T. F. and Koul, O., J. Chem. Ecol., 1990; 16: 2511–2518.
13. Koul, O., Jain, M.P. and Verma, V.K. Growth inhibitory and antifeedant activity of extracts from *Melia dubia* to *Spodoptera litura* and *Helicoverpa armigera* larva. Indian Jour. Exp. Biol., 2000; 38: 63-68.

14. Nagalakshmi, M.A,, Thangadurai, D, and Pullaiah, T. In vitro antimicrobial efficacy of leaf essential oils of *Chukrasia tabularis* Adr Juss and *Melia dubia* Cav (Meliaceae). Phytother Res., 2003; 17(4): 414-416.
15. Vijayan, P., Raghu, C., Ashok, G., Dhanraj, S.A. and Suresh, B. Antiviral activity of medicinal plants of Nilgiris. Indian J. Med.Res., 2004; 120: 24–29.
16. Gerige, S.J.R. Antimicrobial activity of *Melia dubia* leaf volatile oil and camphene compound against skin pathogens. Int. Jour. Pl. Sci., 2007; 2(2), 166-8.
17. Susheela, T., Padma, B., Jane, T., Narender Reddy, T. and Reddy, P.U.M. Evaluation of hypoglycaemic and antidiabetic effect of *Melia dubia* CAV fruits in mice Curr. Sci., 2008; 94(9): 1191-1195.
18. Evans, W.C., Trease and Evans' Pharmacognosy. 14th edn. W.B. Saunders Company Limited, London, 1996; 612.
19. Mondello, L., Dugo, P., Basile, A., Dugo, G. and Bartle, K. D. Interactive use of linear retention indices, on polar and apolar columns, with MS library for reliable identification of complex mixtures. J. Microcol. Sep., 1995; 7: 581-591.
20. Pizzolattia, M.G., Venson, A.F. , Artur Smaˆnia Ju´ niorb, Elza de F. A. Smaˆniab and Braz-Filho, R. Two Epimeric Flavalignans from *Trichilia catigua* (Meliaceae) with Antimicrobial Activity. Z. Naturforsch., 2002; 57: 483-488.
21. Hassan, N.A., Yavari, I., Chalabian, F., Kiarostami, V., Ghaffarzadeh, F. and Nasirian, A. Chemical constituents and antimicrobial activities of the essential oil of Acroptilon repens (L.) DC. Flavour and Fragrance Journal, 2005; 21(2): 247-249.
22. Yu, J., Lei, J., Yu, H., Cai, X and Zou, G. Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. Phytochemistry, 2004; 65(7): 881-884.