Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Chemica Sinica, 2013, 4(1):36-40

Der Chemica Sinica
Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Phytochemical characterization of *Melia dubia* for their biological properties

***S. Murugesan, N. Senthilkumar, C. Rajeshkannan and K. B. Vijayalakshmi**

Division of Bioprospecting, Institute of Forest Genetics & Tree Breeding, Coimbatore – 641 002, Tamil Nadu, India.

ABSTRACT

The phytochemical components of Melia dubia (Cav) leaf extract has been evaluated using GC-MS-MS. It represents the presence of unsaturated fatty acids, terpenoids (diterpenes and sesquiterpenes) antioxidants, phenolic derivatives and lipophylic organic compounds. Phytochemical compounds such as Linolenic acid, Palmitic acid, Caryophyllene, Humulene, Aromadendrene, Probucol, Germacrene-D, Phthalic acid 6-ethyl-3-octyl, Butylated hydroxy toluene. The bioactive constituents identified from M.dubia were found to possess insecticidal, insect antifeedant, anti tumor, anti inflammatory, antioxidant, antibacterial and fungicidal properties. The findings of the present study reveal the potential of M.dubia as an effective biopesticidal and pharmacological agents.

Key words: *Melia dubia*, GC-MS-MS, Terpenoids, Antioxidant, Antifeedant.

INTRODUCTION

Melia dubia (Cav) belonging to the family Meliaceae has shown great potential for the pest management in terms of secondary plant chemistry. It is a large deciduous and native tree species to India. It's timber is mainly used for furniture and agricultural implements (Amarashekara, 1995). Every part of the plant is being used as traditional herbal medicines, such as anthelmintics, treatment of leprosy, eczema, asthma, malaria, fevers and veneral diseases (Govindachari,1992), as well as cholelithiasis, acariasis and pain (Kokwaro, 1976). Fruits of *M.dubia* are considered to be important in colic and skin diseases and also as anthelmintic (Purushothaman, 1984). It is well known as a rich and valuable source of bioactive limonoids (Awang *et al*., 2007). Although hundreds of limonoids have been isolated from various plants but, their occurrence in the plant kingdom is more abundantly in Meliaceae and Rutaceae. Ongoing studies show that limonoids are highly oxygenated, modified terpenoids and have recently attracted attention because compounds belonging to this group have exhibited a range of biological activities like insecticidal, insect antifeedant especially on some of the forest insect pests and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans (Koul *et al*., 2004, Endo *et al*., 2002, Nakagawa *et al*., 2001). Several compounds present in plants are of great importance for their use in insect pest management and limonoids from meliaceae have potential to effectively control a variety of insect pests without harming the environment (Carpinella *et al*., 2002). Tetranortriterpenoids (Porushothaman *et al*., 1984) and monoterpenes (Nagalakshmi *et al*., 2001) are its major constituents and reported to be toxic to *Spodoptera litura* and *Helicoverpa armigera* (Opender *et al*., 2000). The extracts of *M.dubia* acts as a growth inhibitor, stomach poison, moulting disorders, morphogenetic defects and antifeedant to a number of insect pests (Bhuiyan *et al*., 2001). The environmental hazards posed by synthetic pesticides provide an impetus for investigation into some ecofriendly and biorational alternatives. In our preliminary

study on bioactivity of acetone extract of *M.dubia* against teak defoliator, *Hybleae puera* and Ailanthus defoliator *Atteva fabricella* and *Eligma narcissus* was found to be effective. Hence, the present study envisaged at identification and characterization of bioactive compounds from *M.dubia* that could be useful for the development of novel environmental compatible formulations for the management of pests.

MATERIALS AND METHODS

Collection of plant materials:
Fresh leaves of *M.dubia* were collected from Ikkarai Boluvampatti located between 10°58'18.72 "N latitude and 76°47'44.44"E longitude in the Kolli hills range in Western Ghats, Coimbatore district, Tamilnadu. Plant materials were collected by interaction with the hill tribes Irulas and Mudugas. The collected plant material was authenticated by a taxonomist at Institute of Forest Genetics and Tree Breeding, Coimbatore.

Preparation of plant extract:
The collected plant materials were air dried and ground into uniform powder. Dry powder of plant sample was extracted with acetone using soxhlet apparatus for 6 hours. The extract was filtered over anhydrous sodium sulphate followed by concentrated using rotary evaporator. The concentrated extract was subjected to freeze drying in a lyophilizer till dry powder was obtained. Finally the extracted powder was resuspended with methanol at the concentration of 100mg/ml (w/v) followed by filtration through Varian Bond Elute C18 solid phase extraction to remove impurities. 1µl of this solution was employed for GC-MS-MS analysis.

GC-MS-MS ANALYSIS:
The GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS with Fused silica 15m x 0.2 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

Identification of phytocompounds:
Interpretation on mass-spectrum GC-MS-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and molecular formula of the test materials were ascertained.

RESULTS AND DISCUSSION

Fourty two phytochemicals were identified by the GC-MS-MS analysis as constituents of *M.dubia* leaf extract. The compounds with their retention time, molecular formula, molecular weight and concentration (peak area %) are presented in Table1. GC-MS-MS chromatogram and the peaks of compounds detected were shown in figure1. In the present study, identified compounds are Octadecanoic acid (15.71%), Hexadecanoic acid (11.10%), Humelene (3.24%), Caryophyllene (6.07%), Aromadendrene (3.53%) and Germacrene-D (2.89%). Those molecules have been reported to possess pesticidal activity (Mansour *et al*., 2000, Duke, 1992, Abdolhamid *et al*., 2009, Murugesan *et al.*, 2012, Sathya *et al*., 2012, Shailesh, 2012). Nagalakshmi *et al*. (2001) reported that *M.dubia* leaf essential oil chiefly consists of monoterpene hydrocarbons and oxygenated monoterpenes accompanied by relatively much smaller amounts of alkanes sesquisterpene hydrocarbons and phenylpropanoids. Senthilkumar *et al.* (2012) reported that *M.dubia* leaf extract was effective against teak defoliator *H.puera*. Probucol identified from this study is a hydrophobic antioxidant drug, lowers plasma cholesterol in humans, rodents and may inhibit progression of atherosclerosis and postangioplasty retenosis (Nestle *et al*., 1981, Mclean *et al*., 1992, Kesaniemi *et al*., 1984, Hayek *et al*., 1991). Piperidine display important biological properties like antiviral activity (Finke *et al*., 2001), antidepressant effects (Trabaco *et al*., 2007) cytotoxic activity (Kobayashi *et al*., 1996) and antimalarial activity (Murata *et al*., 1998). Chrysanthemol is a trans-eudesmane type sesquiterpene possesses certain anti-inflammatory activity (Carmen *et al*., 2001). Valancene, Longifolene, Sinularene, Thujopsene, Widdrane, α-Longipinene, β – elemene are the terpenoid compounds which are present in trace amounts. Phytol is a diterpene which is used in the manufacture of Vitamin E and K. It is also used in regulating blood glucose level and reducing cholesterol levels in

blood. GC-MS-MS study of *M.dubia* leaf extract has shown many phytochemicals which contributes varied biological activity (Table 2).

Plant species such as *M.dubia, Azadirachta indica*, a related plant *Melia azadirachta* along with several other plant species belonging to Meliaceae are store-house of limonoids like azadirachtin and other related compounds that have feeding deterrent, insect-repellant, anti-hormone and other insect control properties against number of insect pests. Such reports provide an impetus to evaluate these compounds alone or in combination to identify their potential in commercial formulations that can be used as bio-pesticides in integrated pest management.

Table 1: Phytocomponents identified in the actone extract of *M.dubia* by GC-MS-MS.

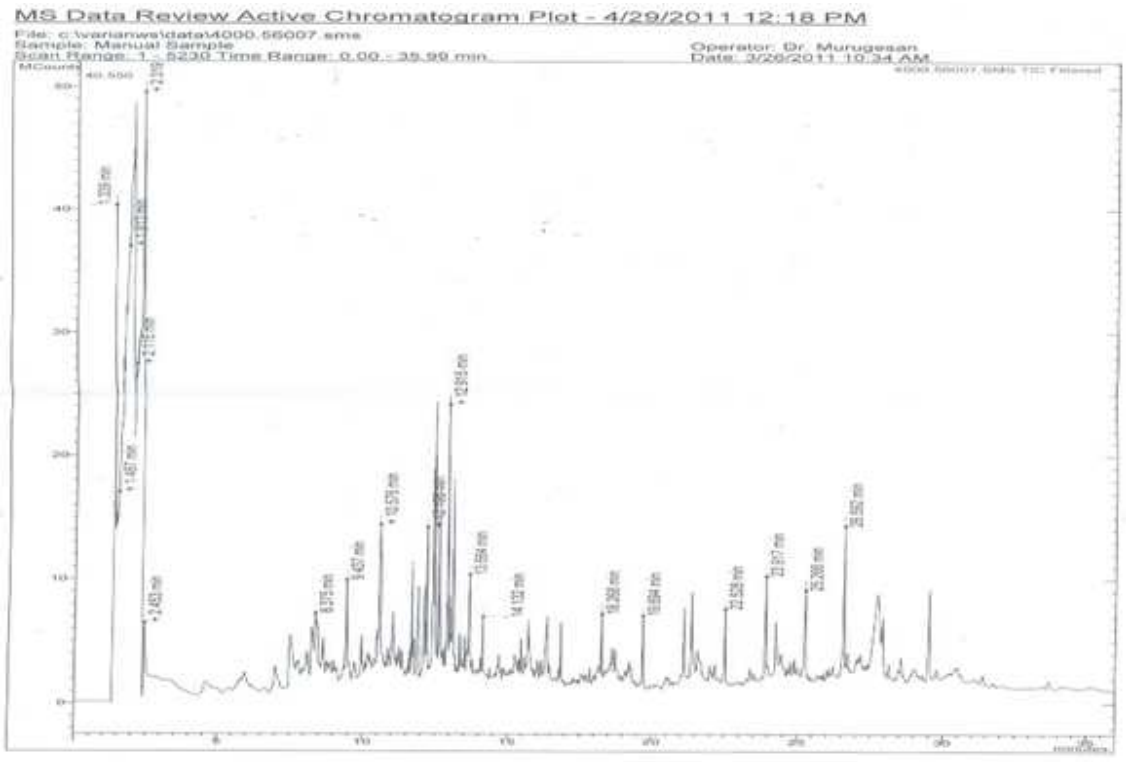
S.No	Retention Time (min)	Name of the Compound	Peak Area	Molecular Weight	Molecular formula
1	1.278	Guanosine	1.23	283	$C_{10}H_{13}N_5O_5$
2	1.278	Dodecanoic acid	1.64	229	$C_{13}H_{27}No_2$
3	1.978	Morpholine	2.43	191	$C_{12}H_{17}NO$
4	2.184	Piperidine hydrochloride	4.16	121	$C_5H_{12}C_1N$
5	2.326	Cyclopropane methanol	3.23	100	$C_6H_{12}O$
6	3.920	Limonen-6-ol, Piralate	1.53	236	$C_{15}H_{24}O_2$
7	5.184	Octadecane,1-(ethenyl oxy)	1.02	296	$C_{20}H_{40}O$
8	5.391	Silane, trichloroeicosyl	0.85	414	$C_{20}H_{41}C_{13}S$
9	5.728	Isotridecanol	1.11	200	$C_{13}H_{28}O$
10	5.947	Heptaflurobutyric acid	0.27	282	$C_{19}H_{38}O$
11	7.000	Bromodocosane	0.44	388	$C_{22}H_xBr$
12	7.508	Caryophyllene	6.07	204	$C_{15}H_{24}$
13	7.508	Humulene	3.24	204	$C_{15}H_{24}$
14	7.508	Aromadendrene	3.53	204	$C_{15}H_{24}$
15	7.508	Valancene	0.19	204	$C_{15}H_{24}$
16	7.508	Longifolene	0.16	204	$C_{15}H_{24}$
17	7.508	Sinularene	0.07	204	$C_{15}H_{24}$
18	7.508	Thujopsene	0.07	204	$C_{15}H_{24}$
19	7.508	Widdrane	0.07	204	$C_{15}H_{24}$
20	7.508	α-Longipinene	0.07	204	$C_{15}H_{24}$
21	7.508	β-elemene	0.05	204	$C_{15}H_{24}$
22	7.508	Aristolen	0.04	204	$C_{15}H_{24}$
23	7.508	Sativen	0.05	204	$C_{15}H_{24}$
24	7.508	Germacrene-D	2.89	204	$C_{15}H_{24}$
25	8.375	Phenol,2,4-bis(1,1-dimethyl)	2.67	206	$C_{14}H_{22}O$
26	11.891	Trans-Chrysanthemol	2.88	152	$C_{10}H_{16}O$
27	13.110	Butylated Hydroxytoluene	2.59	220	$C_{15}H_{24}O$
28	13.336	Benzenepropanoic acid	8.36	292	$C_{18}H_{28}O_3$
29	13.664	1,2- Benzenedicarboxylic acid	0.96	362	$C_{22}H_{34}O_4$
30	7.508	Phthalic acid, 6-ethyl-3-octyl	2.85	404	$C_{25}H_{40}O_4$
31	16.047	9,10- Anthracenedione, 2- methyl	7.68	222	$C_{15}H_{10}O_2$
32	16.156	Phytol	1.52	296	$C_{20}H_{40}O$
33	16.354	Octadecanoic acid	15.71	284	$C_{18}H_{36}O_2$
34	21.377	Hexadeconic acid	11.10	330	$C_{19}H_{38}O_4$
35	21.377	Palmitic anhydride	0.2	494	$C_{32}H_{62}O_3$
36	26.866	Serverogenin acetate	0.11	544	$C_{29}H_{36}O_{10}$
37	26.866	Betulin	0.18	168	$C_{10}H_{16}O_2$
38	27.733	Probucol	3.67	516	$C_{31}H_{48}O_2S_2$
39	27.733	Musk ketone	1.7	294	$C_{20}H_{22}O_2$
40	27.733	Yohimbane	0.67	280	$C_{19}H_{24}N_2$
41	29.011	Sarain-C	0.15	536	$C_{34}H_{52}N_2O_3$
42	29.298	Porphyrine	0.26	534	$C_{28}H_{46}O_6S$

Table 2: Biological activity of phytochemicals identified from *M.dubia*.

S.No	Name of the Compound	Nature of compound	Biological activity
1	Hexadecanoic acid	Palmitic acid	Antioxidant, hypercholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, Alpha reductase inhibitor.
2	Octadecanoic acid	Linolenic acid	Antiinflammatory,hypercholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, diuretic.
3	Caryophyllene	Sesquiterpene	Anti-tumor, analgesic, antibacterial, antiinflammatory, sedative, fungicide.
4	Aromadendrene	Sesquiterpene	Anti-tumor, analgesic, antibacterial, antiinflammatory, sedative, fungicide.
5	Phytol	Diterpene	Antimicrobial, anticancer, antiinflammatory, diuretic.
6	Probucol	Lipophylic organic compound	Antihyperlipidemic drug, Powerful antioxidant
7	Morpholine	Heterocyclic organic compound	Fungicide,ergosterol biosynthesis inhibitors,
8	Butylated hydroxy toluene	Lipophylic organic compound	Antioxidant
9	Longifolene	Sesquiterpene	Antiproliferative
10	Piperidine	Heterocyclic organic compound	Antiviral, antidepressant, cytotoxic and antimalarial activity.

(Source: Dr.Duke's Phytochemical and Ethnobotanical databases).

Figure 1: GC-MS-MS spectrum of *Melia dubia* Acetone extract.



Acknowledgement
Authors are grateful to The Director, IFGTB, Coimbatore, for providing the facilities during the course of study.

REFERENCES

[1] B. Abdolhamid., M. Asma, *Digest J Nanomat Biostruct.*, **2009**, 4 (2), 323-328.
[2] H. S. Amarasekara., Alternative time species-A review of their properties and uses. In: Forestry and Environment Symposium, **1995.**

[3] K.Awang, C. S. Lim, K.Mohamad, H.Morita , Y.Hirasawa, K.Takeya, O.Thoison, A. H. A.Hadi, A.Ceramicine, A. Walsogyne, *Bioorg. Med. Chem*. **2007**, 15, 5997.
[4] K. R.Bhuiyan, E. Hassan, M. B. Z. Isman, *Pflanzenkr*, *Pflanzenschutz* ., **2001**, 108, 82–88.
[5] C.Carmen, H.T. Gloria, U.Antonio, C.Francoise, *J. Asian Nat Prod Res*., **2001**, 3(2), 5517-5520.
[6] C. Carpinella, C.Ferrayoli, G.Valladares, M.Defago, S.Palacios, *Biosci. Biotechnol. Biochem*., **2002**, 66, 1731 - 1736.
[7] J.A.Duke, *Handbook of phytochemical constituents of GRAS herbs and other economical plants* (CRC Press Inc, Boca Ratan FL **1992**) 654.
[8] T.Endo, M.Kita, T.Shimada, T.Moriguchi, T.Hidaka, R.Matsumoto, S.Hasegawa, M.Omura, *Plant Biotechnol*., **2002**, 19, 397- 403.
[9] P.E.Finke, B.Oates, S.G.Mills, M.MacCoss, L.Malkowitz, M.S.Springer, S.L.Gould, J.A.Demartino, A.Carella, G.Carver, K.Holmes, R.Danzeisen, D.Hazuda, J.Kessler, J.Lineberger, M.Miller, W. A.Schleif, E. A.Emini, , *Bioorg. Med. Chem. Lett* ., **2001**, 11, 2475.
[10] T. R.Govindachari, *Current Science*., **1992**, 63, 117- 122.
[11] T.Hayek, T.Chajek-Shaul, A.Walsh, N.Azrolan, J.L. Breslow, *Arterioscler Thromb*., **1991**, 11, 1295–1302.
[12] Y.A.Kesaniemi, S.M.Grundy, *J. Lipid Res*., **1984**, 25, 780 –790.
[13] J.Kobayashi, M.Ishibashi, *Heterocycles*., **1996**, *42*, 943-970.
[14] J. O.Kokwaro, Medicinal Plants of East Africa (East African Literature Bureau, Nairobi, **1976**) 157.
[15] O.Koul, G.Singh, R.Singh, J.Singh, W. M.Daniewski, S.Berlozecki, *J. Biosci*., **2004**, 29, 409–416.
[16] S.A.Mansour, S.S.Messeha, S.E.Gengaihi, *J .Nat Toxins*., **2000**, 9, 49-62.
[17] L.R. McLean, C.E. Thomas, B.Weintraub, K.A.Hagaman, *J. Biol Chem*., **1992**, 267, 12291–12298.
[18] K.Murata, F.Takano, S.Fushiya, Y.Oshima, *J. Nat. Prod*., **1998**, 61**,** 729-733.
[19] S.Murugesan, C.Rajeshkannan, D.Sureshbabu, R.Sumathi, P.Manivachakam, *Advances in Applied Science Research*., **2012**, 3(5), 2754-2759.
[20] M.A.H.Nagalakshmi, D.Thangadurai, T.Anuradha, T.Pullaiah, *Flav and Fragrance Journal*.,**2001**,16, 241-244.
[21] H.Nakagawa, H.Duan, Y.Takaishi, *Chem. Pharm. Bull.*, **2001**, 49, 649 - 651.
[22] P.J.Nestel, T. Billington, *Atherosclerosis*., **1981**, 38: 203–209.
[23] K.Opender, M. P.Jain, V. K.Sharma, *Indian J. Exp. Biol*., **2000**, 38, 63-68.
[24] K. K.Porushothaman, D.Kalyani, C. D. Joseph, *Phytochemistry*.,**1984**,23,135-138.
[25] K. K.Purushothaman, K.Duraiswamy, and J. D.Conolly, (). *Phytochemistry*., **1984**, 23 (1), 135 – 137.
[26] N.Senthilkumar, S.Murugesan, K.B.Vijayalakshmi, M.Monisha, D.Sureshbabu, R.Lakshmidevi, P.Manivachakam, *European Journal of Experimental Biology*., **2012**, 2(3), 513-519.
[27] A. A.Trabaco, N.Aerts, R. M.Alvarez, J. I.Andres, I.Boeckx, J.Fernandez, A.Gomez, F.E.Janssens, J.E.Leenaerts, A.I.D.Lucas, E.Matesanz, T.Steckler, S.Pullan, *Bioorg. Med. Chem. Lett* ., **2007**, 17, 3860.
[28] A.Sathya, V.Ambikapathy, *Asian J. Plant Sci. Res*., **2012**, 2(4), 486-489.
[29] M.K.Shailesh, *Asian J. Plant Sci. Res*., **2012**, 2(4), 421-427