



***Melia dubia* Fodder Phytochemicals: Non-Targeted Gas-Chromatography Mass-Spectrometry (GC-MS) Analysis and Corroboration for Biological Activities**

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Abstract: *Melia dubia* of Meliaceae family is an important agroforestry tree species indigenous to the Western Ghats region in India and is common in moist deciduous forests of south Gujarat. It has chemically diverse and structurally complex active phytochemicals with no allelopathic influence on understory crops. In present study, total 93 volatile phytochemical compounds were detected from *M. dubia* leaves from different locations of south Gujarat region in nontargeted gas-chromatography mass-spectrometry (GC-MS) analysis. The detected compounds are known to have antimicrobial, antifungal, antioxidant, anti-inflammatory, insecticidal, nematocidal, antibacterial, antiviral and other various biological properties which are beneficial to humans and animals as well.

Keywords: *Melia dubia*, GC-MS, Volatile phytochemical, Biological properties

The interest in natural products from plants and their use has increased tremendously in indigenous and unconventional plants to study bio compounds which may be beneficial to mankind (Achi et al 2017). *Melia dubia* of Meliaceae family is a large deciduous perennial tree growing from 6 to 30 meters in height. The species is indigenous to the Western Ghats region in India and is common in moist deciduous forests of the Indian states from Kerala to Gujarat (Saravanan et al 2013). *M. dubia* showed high potential as a raw material for pulp and paper industries (Sinha et al 2019) and is being planted under industrial agroforestry models. It is also reported to be an acceptable agroforestry species (Mohanty et al 2017, Thakur et al 2018) with no allelopathic influence on understory crops (Thakur et al 2017, Parmar et al 2019). Leaves and fruits of *M. dubia* possess a vast array of biologically active compounds which are chemically diverse and structurally complex (Paritala et al 2015, Parmar et al 2019, Sukhadiya et al 2021). Traditionally different plant part of species used as herbal medicines, such as anthelmintics, in treatment of leprosy, eczema, asthma, malaria, fevers and venereal diseases, as well as cholelithiasis, acariasis and pain (Murugesan et al 2013b). The study was conducted to unveil the various phytochemicals of *M. dubia* from south Gujarat region.

MATERIAL AND METHODS

The leaf samples of *M. dubia* were collected from distantly located eight different locations in Satpura and Shyadri ranges of Northern Western ghats in Gujarat. Various

phytochemicals were detected through GC-MS partly following Murugesan et al (2013b), and Sukhadiya et al (2021) as under:

One gm of the powdered sample was extracted using Hexene: Acetone (1:1) solvent in centrifuge tube and after 72 hrs of incubation the homogenate centrifuged for 20 min. at 3500 rpm and supernatant was collected. Pinch of activated charcoal was added to treat chlorophyll content. 2 ml of supernatant was collected in pre cleaned glass test tube and evaporated using cold nitrogen air drier. After drying 2 ml ACN solution was added to test tube, vortex for 2-3 min and sonicate for 2 min. The content was then filtered using injection and disk filter in to 2 ml glass sampling vile. Reading was taken in GC-MS (Thermo make trace GC ultra – ITQ 900). The GC-MS analysis was carried out on Thermo make Trace GC-ULTRA-ITQ 900 with fused silica capillary column (Rx-1-5MS) of 30 m length, 0.25 internal diameter and 0.25 μ m film thickness. The injection volume was 1 μ l and the total run time of the sample was 33.00 minutes. Three samples per location were injected for GC-MS analysis. Total of 93 different phytochemicals were detected from the *M. dubia* leaves and out of that 20 important phytochemicals are described in this paper and corroborated for their biological activities with other studies.

RESULTS AND DISCUSSION

Non targeted gas-chromatography mass-spectrometry (GC-MS) analysis of *M. dubia* leaves from different locations

of south Gujarat region revealed array of total 93 volatile phytochemical compounds (Fig. 1). Mudhafar et al (2020) reported 22 phytochemical compounds from *M. dubia* leaves using GC-MS analysis. Parmar et al (2019) reported 18 different types of phytochemicals in leaf litter of *M. dubia* through GC-MS analysis whereas; Sukhadiya et al (2022) mentioned 27 phytochemicals from fruit pulp. Murugesan et al (2013b) also reported 42 phytochemicals from the *M. dubia* leaves. Jahirhussain et al (2015) identified 33 phytochemicals as constituents with more than one peak area for 7 compounds from ethanolic leaf extract of *M. composita* using GC-MS method. Compound formula, molecular weight and peak area percentage (concentration) of the selected compounds are presented in Table 1.

Important volatile phytochemicals identified in *M. dubia* during the study have been corroborated with available literature. It is found that many of the compounds detected in *M. dubia*, in present study (Table 1), have been reported in other plant species and have one or the other beneficial biological activities (Table 2). The most common phytochemicals detected were l-(+)-Ascorbic acid 2,6-dihexadecanoate also known as vitamin C is beneficial in common cold, gum disease, acne and other skin infections,

bronchitis, stomach ulcers, tuberculosis, dysentery, boils and wounds, to prevent glaucoma, cataracts, gallbladder disease, dental cavities, constipation, hay fever, asthma, arthritis, back pain, diabetes, chronic fatigue syndrome, osteoporosis and boosting the immune system, antioxidant in the skin (Nazir et al 2021 and Okenwa and Okwunodulu 2014); Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) is alkylbenzene which act as antibacterial and is a moderate cytotoxic (Ren et al 2019, Elgorban et al 2019 and Alwar et al 2014); dl-a-Tocopherol commonly known as vitamin E is an antioxidant (Wojdylo et al 2021). Docosanal proved to be effective in treating recurrent herpes simplex labialis episodes (Shankar et al 2022) and 2,4-Di-tert-butylphenol have antioxidant, anti-inflammatory, insecticidal, nematocidal, antibacterial, antiviral and antifungal effect in various plants (Murugesan et al 2013b and Zhao et al 2020). The 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol also exhibits anti-inflammatory, anti-diuretic, antioxidant and hypocholesterolemic properties (Mujeeb et al 2014, Hamid et al 2016). The Phytol is a constituent of chlorophyll and after fermentation it converted to phytanic acid and stored in fats (Murugesan et al 2013b, Hamid et al 2016, Hossain et al 2013) (Table 2).

Table 1. Important phytochemicals detected in *M. dubia* leaf from south Gujarat, India

Compound	Formula (Molecular weight)	Peak area %
10-Heneicosene (c,t)	C ₂₁ H ₄₂ (294.55)	3.92
1-Hexadecanol	C ₁₆ H ₃₄ O (242.44)	4.96
2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O (206.32)	12.79
2-Pentadecanone, 6, 10, 14-trimethyl-	C ₁₈ H ₃₆ O (268.47)	3.96
3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O (296.53)	11.02
9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂ (278.42)	8.92
dl-a-Tocopherol (Vitamin E)	C ₂₉ H ₅₀ O ₂ (416.68)	13.45
Docosanal	C ₂₂ H ₄₄ O (326.61)	0.92
Eicosane	C ₂₀ H ₄₂ (282.54)	2.69
Ethanol, 2-(dodecyloxy)-	C ₁₄ H ₃₀ O ₂ (230.39)	2.50
Fumaric acid, cis-hex-3-enyl tetradecyl ester	C ₂₄ H ₄₂ O ₄ (280.36)	0.01
Hexadecanal	C ₁₆ H ₃₂ O (256.42)	0.53
l-(+)-Ascorbic acid 2,6-dihexadecanoate (vitamin C)	C ₃₈ H ₆₈ O ₈ (652.9)	18.14
Neophytadiene	C ₂₀ H ₃₈ (278.5)	9.87
Nonadecane, 2-methyl-	C ₂₀ H ₄₂ (282.5)	5.21
n-Tetracosanol-1	C ₂₄ H ₅₀ O (354.65)	2.87
n-Hexadecanoic acid (Palmitic acid)	C ₁₆ H ₃₂ O ₂ (256.42)	1.74
Octadecanoic acid (Stearic acid)	C ₁₈ H ₃₆ O ₂ (284.48)	4.23
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	C ₄₂ H ₆₃ O ₃ P (646.92)	13.83
Phthalic acid, hept-4-yl isobutyl ester	C ₁₉ H ₂₈ O ₄ (320.4)	1.41
Squalene	C ₃₀ H ₅₀ (422.8)	1.08

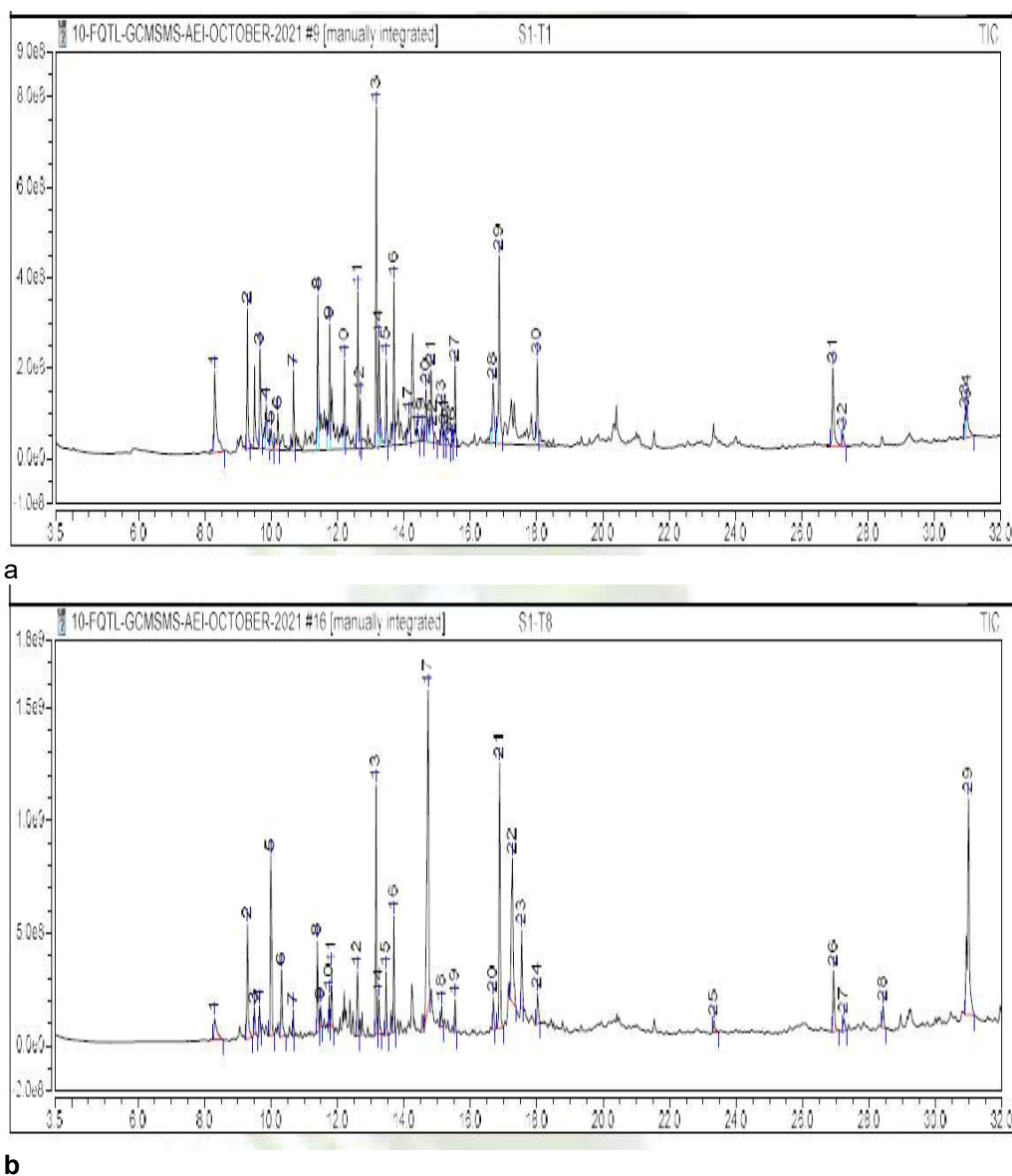
Table 2. Corroboration of *M. dubia* phytochemicals detected in present study for their beneficial biological activity

Compound	Phytochemical reported in other plants	Reported beneficial biological activity	Reference
10-Heneicosene (c,t)	<i>Luisia tenuifolia</i>	Pheromone, Antifungal	Sethuraman et al (2022)
1-Hexadecanol	<i>Memecylon umbellatum</i> , <i>Enhalusa coroides</i>	Antioxidant	Murugesan and Panneerselvam (2013a) Amudha et al (2018)
2,4-Di-tert-butylphenol	<i>M. dubia</i> , <i>Carica papaya</i> <i>Terminalia travancorensis</i> <i>Juglans regia</i> , <i>Albizia julibrissin</i> , <i>Eucalyptus globulus</i>	Antioxidant, Anti-inflammatory, insecticidal, nematicidal, antibacterial, antiviral and antifungal	Murugesan et al (2013b) Zhao et al (2020)
2-Pentadecanone, 6,10,14-trimethyl-	<i>Azadirachta indica</i> , <i>E. acoroides</i>	Hypocholesterolemic, antioxidant and lubrication	Kumar et al (2018) Amudha et al (2018)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	<i>Grewia pubescens</i> , <i>Aegle marmelos</i>	Anti-inflammatory, anti-diuretic, Antioxidant and hypo-cholesterolemic A constituent of chlorophyll, after fermentation converted to phytanic acid and stored in fats.	Hamid et al (2016) Mujeeb et al (2014) Murugesan et al (2013b) Hossain et al (2013)
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	<i>A. indica</i> , <i>A. occidentale</i> , <i>P. guajava</i> , <i>T. catappa</i> , <i>Salvadora persica</i> , <i>Silybum marianum</i>	Anticancer, anti-inflammatory, antibacterial, antioxidant	Balasubramanian et al (2014) Chikezie et al (2020), Bratty et al (2020), Padma et al (2019)
dl- α -Tocopherol (Vitamin E)	<i>Prunus armeniaca</i> <i>P. persica</i> , <i>P. domestica</i> <i>Malus domestica</i>	Antioxidant	Wojdyto et al (2021)
Docosanal	<i>Rhus chinensis</i>	Recurrent herpes simplex labialis episodes	Zhu et al (2007) Shankar et al (2022)
Eicosane	<i>Trichilia connaroides</i> , <i>Gymnema sylvestre</i>	Bronchodilators, Drug for throat disorder	Senthilkumar et al (2012) Subramanian et al (2020)
Ethanol, 2-(dodecyloxy)-	<i>T. connaroides</i>	Sclerosing agent for the treatment of esophageal and gastric varices and varicose veins	Senthilkumar et al (2012)
Fumaric acid, cis-hex-3-enyl tetradecyl ester	<i>Uncaria sessilifructus</i>	Food additive, to treat the autoimmune condition psoriasis and multiple sclerosis, feed additive to lower methane production	Wang and Huang (2012)
Hexadecanal	<i>Abutilon pannosum</i> , <i>Grewia tenax</i>	Reduce aggression, anthelmintic	Aadesariya et al (2017) Mishor et al (2021)
l-(+)-Ascorbic acid 2,6-dihexadecanoate (vitamin C)	<i>Elaeagnus umbellate</i> , <i>Phyllanthu samarus</i>	Preventing and treating common cold, gum disease, acne and other skin infections, bronchitis, stomach ulcers, tuberculosis, dysentery, boils and wounds. To prevent glaucoma, cataracts, gallbladder disease, dental cavities, constipation, hay fever, asthma, arthritis, back pain, diabetes, chronic fatigue syndrome, osteoporosis and boosting the immune system.	Nazir et al (2021) Okenwa and Okwunodulu (2014)
Neophytadiene	<i>A. pannosum</i> , <i>G. tenax</i> , <i>Plectranthus amboinicus</i> , <i>Eupatorium odoratum</i>	Sesquiterpenoids, an anti-inflammatory agent, a plant metabolite and an algal metabolite. analgesic, antipyretic, antimicrobial, and antioxidant	Aadesariya et al (2017) Swamy et al (2017) Raman et al (2012)
Nonadecane, 2-methyl-	<i>A. pannosum</i> , <i>G. tenax</i> <i>T. connaroides</i>	Alkanes, antioxidant	Aadesariya et al (2017) Senthilkumar et al (2012) Bratty et al (2020)
n-Tetracosanol-1	<i>Combretum microphyllum</i> , <i>E. acoroides</i>	Antioxidant	Makhafola et al (2017) Amudha et al (2018)
n-Hexadecanoic acid (Palmitic acid)	<i>Entada rheedii</i> , <i>A. indica</i>	Saturated fatty acid, anti-Inflammatory	Ruthisha et al (2017) Balasubramanian et al (2014) Apama et al (2012)

Cont...

Table 2. Corroboration of *M. dubia* phytochemicals detected in present study for their beneficial biological activity

Compound	Phytochemical reported in other plants	Reported beneficial biological activity	Reference
Octadecanoic acid (Stearic acid)	<i>M. dubia</i>	lowered LDL cholesterol	Murugesan et al (2013b) Hunter et al (2009)
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	<i>Pseudomonas fluorescens</i> , <i>Alternaria</i> spp., <i>Gracilaria gracilis</i>	Alkylbenzene, anti-bacteria, antibacterial moderate cytotoxic	Ren et al (2019), Elgorban et al (2019), Alwar et al (2014)
Phthalic acid, hept-4-yl isobutyl ester	<i>Spondias mombin</i>	Allelopathic, antimicrobial, insecticidal	Osuntokun and Cristina (2019) Huang et al (2021)
Squalene	<i>Persea americana</i> , <i>M. domestica</i>	Triterpene, biochemical intermediate to sterol biosynthesis	Lewis (1972)

**Fig. 1.** Chromatograms showing retention time and relative abundance of phytochemical compounds detected in *M. dubia* leaf fodder in location 1 (a) and 8 (b)

Neophytadiene is sesquiterpenoid which act as an anti-inflammatory agent, a plant metabolite and an algal metabolite (Table 2). It has analgesic, antipyretic, antimicrobial, and antioxidant properties (Aadesariya, et al 2017, Swamy et al 2017 and Raman et al 2012). The 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- is reported to have anticancer, anti-inflammatory, antibacterial, antioxidant properties (Balasubramanian et al 2014, Bratty et al 2020, Padma et al 2019). Nonadecane, 2-methyl- have antioxidant properties (Senthilkumar et al 2012 Aadesariya et al 2017, Bratty et al 2020); 1-Hexadecanol had Antioxidant properties (Murugesan 2013a and Amudha et al 2018). Octadecanoic acid reported to lower LDL cholesterol (Murugesan et al 2013b and Hunter et al 2009). Eicosane can be used as a bronchodilators, drug for throat disorder (Senthilkumar et al 2012, Subramanian et al 2020). The Ethanol, 2-(dodecyloxy)- act as a sclerosing agent for the treatment of esophageal and gastric varices and varicose veins (Senthilkumar et al 2012) and Fumaric acid, cis-hex-3-enyl tetradecyl ester is a food additive and used to treat the autoimmune condition psoriasis, multiple sclerosis and lower methane production (Wang and Huang 2012, Gold et al 2012 and Bayaru et al 2000). The 10-Heneicosene (c,t) is a pheromone and has antifungal properties (Sethuraman et al 2022), 2-Pentadecanone, 6,10,14-trimethyl- is a hypocholesterolemic, antioxidant and used in lubrication (Amudha et al 2018). Phthalic acid, hept-4-yl isobutyl ester also known to have allelopathic, antimicrobial, insecticidal properties (Osuntokun and Cristina 2019 and Huang et al 2021) and Squalene which is a triterpene act as a biochemical intermediate to sterol biosynthesis (Lewis 1972) (Table 2).

CONCLUSION

Total 93 of biological active compound were reported from *M. dubia* leaves collected from various locations from South Gujarat. The non-volatile compounds findings through GCMS analysis inferred that *M. dubia* leaves has beneficial biological active phytochemicals which may be beneficial to human and animals.

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