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CHEMICAL PROFILING AND ANTIOXIDANT POTENTIAL OF *DYSOXYLUM MALABARICUM* SEEDS

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ABSTRACT

Dysoxylum malabaricum is a constituent of ayurvedic thrigandha and its wood and fruits are being used in traditional medicine. The study aims at chemical profiling of the biologically active components present in the seeds of *Dysoxylum malabaricum* through GC-MS analysis in methanol and chloroform extracts. The analysis resulted in 28 phytochemicals and the major compounds identified includes, Methyl 4-O-methyl-d-arabinopyranoside, 1,2,4-Triazolo[4,3-a]pyridin-3(2H)-one,5-methyl-,2-Butenamide, 2-ethyl-3-methyl-N-phenyl-,Methylpalmitate,9-octadecenoicacid(z)-, methylester,1-Hexadecene, Heptadecane, Pseudolimonene, Cyclohexane, 1-methylene-4-(1-methylethenyl)-, Alpha, gamma-dipalmitin, 1-Tetradecene, 1-Octadecene with reported antioxidant and anti-microbial properties along with minor compounds of remarkable properties. The analysis further included an antioxidant assay which revealed the effective antioxidant property of the *Dysoxylum malabaricum* seeds.
Keywords : Antioxidant, *Dysoxylum malabaricum,* GC-MS, Phytoconstituents, Seeds

Introduction

The Western Ghats, India, is one of the 36 biological hotspots in tropics. The region is recorded with enormous number of medicinal plants with amazing properties. The medicinal plants contain an array of active components which can be used to treat chronic as well as infectious diseases. They are potent sources of antioxidants and presence of phytochemicals in medicinal plants represents a number of pharmacological actions (Nisha,Garima and Vivek, 2022).

Deep rooted knowledge on medicinal plants serves as a reservoir for drug designing (Kamalakar *et al*., 2022). Phytochemical analysis helps to identify crucial chemical classes present within a plant thus giving an idea on the properties they may exhibit. The Gas Chromatography-Mass Spectroscopy (GC-MS) help to profile the phytochemical constituents present in a plant and each of which serve as possible leads for drug identification.

Dysoxylum malabaricum belonging to the family Meliaceae, commonly known as white cedar. It is endemic to Southern Western Ghats and usually found in evergreen and semi-evergreen forests from 200 to1200 m above sea level. This canopy tree grows to a height of 30–40 m with 3–4 m girth (Sofia *et al*., 2013). The tree is considered to be both medicinally and industrially valued, and is one among ayurvedic thrigandha. Leaves of *D. malabaricum* possess strong larvicidal, pupicidal, and adulticidal activity against the malaria vector *Anopheles stephensi* (Senthil, Kalaivani and Sehoon, 2006). Triterpenes present in the *D. malabaricum* could be used as an active principle during the preparation of botanical insecticides (Govindachari, Suresh and Kumari, 1994). The wood and fruits are used in traditional medicine. A decoction of the wood is useful in the treatment of arthritis, anorexia, cardiac debility, expelling intestinal worms, inflammation, leprosy and rheumatism. The wood oil is used in treating ear and eye diseases in folklore medicine.

The present study focused profiling of the biologically active phytochemicals present in the seeds of *Dysoxylum malabaricum* through the GC-MS analysis further to evaluate the antioxidant potential in methanol and chloroform seed extracts.

Materials and Methods

Reagents

The methanol and chloroform for extraction of plant material were procured from the Merck and both the solvents are of analytical grade.

Collection of fruits/seeds

Ripened fruits of *Dysoxylum malabaricum* was collected from Kulamavu forest in Idukki district, Western Ghats Kerala located at latitude and longitude of 9°49'5.63", 76°53'25.06" respectively. The fruits were processed and seeds were dried, coarsely powdered and kept in an airtight container for further usage.

Preparation of extract

Soxhlet extraction

1 gm of dried seed powder of *D. malabaricum* was extracted with 250 ml of methanol and absolute chloroform using Soxhlet apparatus, separately for 8 hours until the

reflux becomes clear which is approximately 5 cycles of reflux at temperature below boiling point based on respective solvent. The extracts were filtered and evaporated with a rotary evaporator with 45°C of water bath temperature until concentrated extract was left behind. The extracts were filtered again to remove any solid particles and kept closed tightly in a micro centrifuge tube at 4°C for further use.

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the extracted sample was analyzed using a Shimadzu QP-2010 GC–MS equipped with a Rxi-5Sil MS capillary column of 30m in length, 0.25mm in internal diameter, and 0.25µm in thickness. Helium (99.9995%) was used as the carrier gas, at a constant flow rate of 1mL/min. Injection port temperature was adjusted at 260°C and the injection was performed in split less mode with a split ratio of 100. A 1µl of the syringe filtered (0.22µm) sample was analyzed with the column held initially at 80°C for 2 min and then increased at the rate of 10°C/min to 260°C, which is held constant for 10 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 6 min. For GC– MS spectral detection, EI mode was adopted at 70 eV with 0.5 sec of scan time and fragments were recorded in the 50 to 500 m/z range. The ion source temperature was maintained at 200°C. The components of the sample were identified by comparing the retention time of chromatographic peaks with NIST and Wiley mass spectra libraries.

Identification of compounds

The chromatogram, retention times, fragmentation patterns along with m/z value base peak, mass peak, and peak intensities were obtained through GC-MS analysis. The identification of compounds was based on retention time, fragmentation patterns along with the m/z values. The mass spectra of the unknown compound obtained from sample extraction by GC-MS were matched with mass spectra of the known compounds stored in the database of the National Institute Standard and Technology (NIST) library. Their structures were defined by the percent similarity values. The name, molecular weight, molecular formula, and structure of the compounds were identified.

Antioxidant assay

The free radical scavenging activity is determined based on the stable free radical (DPPH)with antioxidant in organic/aqueous media resulting in bleaching of the DPPH due to its quenching by the interaction with the analytes. The decrease of absorbance of DPPH compared to blank measured spectrophotometrically at 516nm related to the concentration of antioxidants in the test solution.

Result and Discussion

The present study was aimed to explore the phytoconstituents and their bioactive properties of methanolic and chloroform extracts of *D. malabaricum* seeds by GC-MS analysis (Figure 1, 2) and to compare their antioxidant properties. A total of 18 compounds were found in methanolic extract (Table 1) and 10 compounds in chloroform extract (Table 2). Twelve compounds of methanolic extract and five compounds of Chloroform extracts showed immense biological activities.

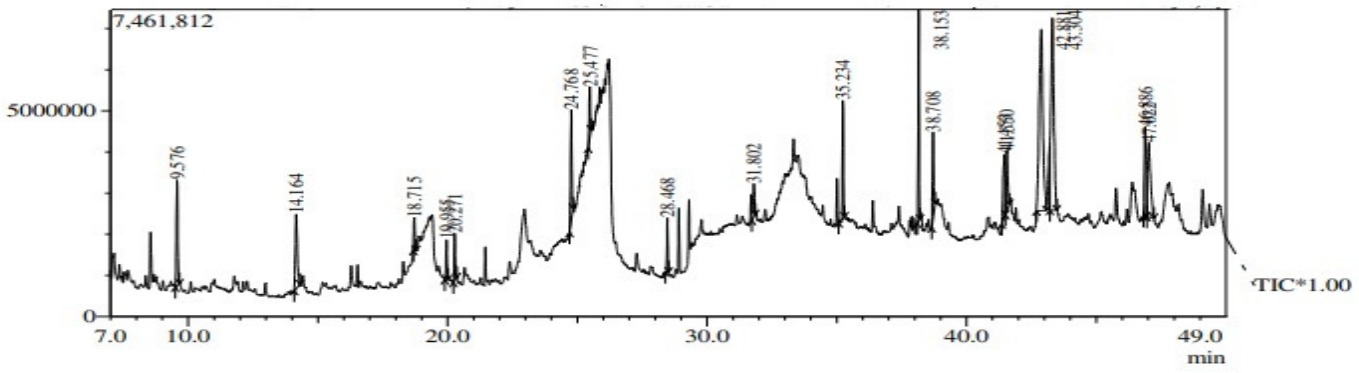


Fig. 1 : GC-MS spectrum of Methanol extract of *Dysoxulum malabaricum* seeds

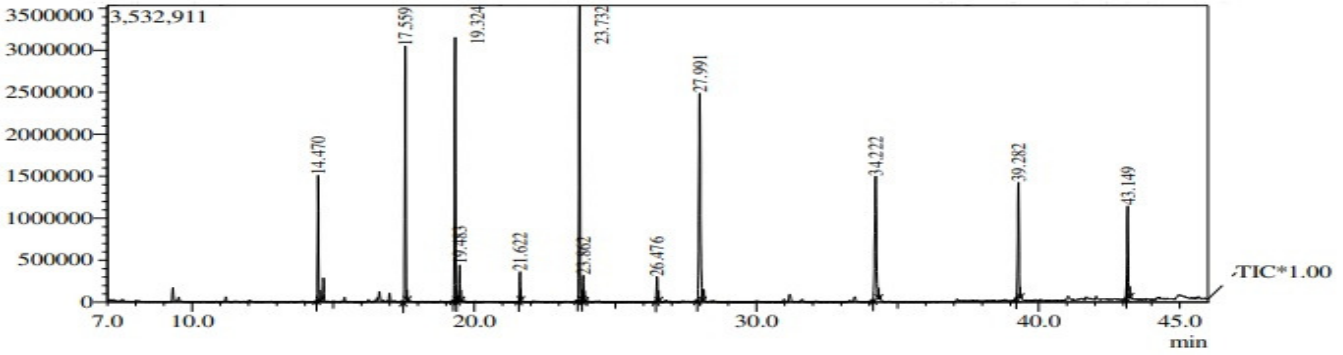
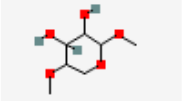
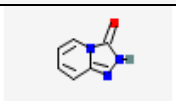
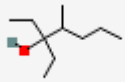
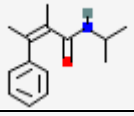
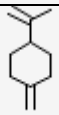
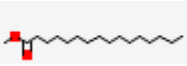
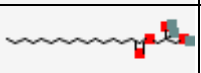
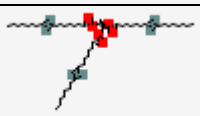
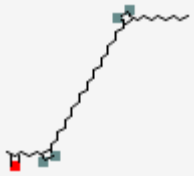


Fig. 2 : GC-MS spectrum of Chloroform extract of *Dysoxylum malabaricum* seeds

Table 1 : Biological activities of phytocomponents identified in the methanol extract of *Dysoxylum malabaricum* seeds.

No	Name of compound	Retention time	Molecular formula	Molecular weight	Peak area	Class compound	Compound structure	Activities
1.	Pyranone	9.576	$C_5H_4O_2$	96.08	4.65	Heterocyclic chemical compound		Cytotoxic Anticancerous Phototoxic (pubchem)
2.	Methyl 4-O-methyl-d-arabinopyranoside	14.164	$C_7H_{14}O_5$	178.18	5.03			Antibacterial, Antioxidant, Cytotoxic, Antineoplastic
3	1,2,4-Triazolo[4,3-a] pyridin-3(2H)-one, 5-methyl-	18.715	$C_6H_5N_3O$	135.12	1.25			Antioxidant
4	3-Ethyl-4-methyl-3-heptanol	19.955	$C_{10}H_{22}O$	158.28	1.52			No specific acitivity reported
5	2-Butenamide, 2-ethyl-3-methyl-N-phenyl-	20.271	$C_{14}H_{19}NO$	217.31	1.79			Antioxidant
6	Pseudolimonene	24.768	$C_{10}H_{16}$	136.23	4.57			Antiprotozoal Antibacterial
7	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	25.477	$C_{10}H_{16}$	136.23	2.05			Antimicrobial
8.	Methylpalmitate	28.468	$C_{17}H_{34}O_2$	270.5	1.66	Fatty acid methyl ester		Anti inflammatory Antioxidant, Antimicrobial Antifibrotic Vasodilator
9	9-octadecenoic acid (z)-, methyl ester	31.802	$C_{19}H_{36}O_2$	296.5	1.92	Fatty acid methyl ester		Antibacterial Antimicrobial Antioxidant Anticancerous Nematicide Anti arthritic Hepato protective
10	Alpha, gamma-dipalmitin	35.234	$C_{35}H_{68}O_5$	568.9	3.90			Antimicrobial
11	1,3-Dioleoylglycerol	38.153	$C_{39}H_{72}O_5$	621.0	6.51			No specific activity reported
12	Glycerol .beta.-palmitate	38.708	$C_{19}H_{38}O_4$	330.5	3.43			No specific activity reported
13	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	41.453	$C_{57}H_{104}O_6$	885.4	4.08			Antispasmodic and Immune modualtor
14	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	41.550	$C_{21}H_{32}O_4$	354.5	3.23			Hypocholesterolemic, Antieczemic, Nematicide, Hepatoprotective, Antioxidant, Antiacne, Haemolytic,
15.	Z,Z-6,28-Heptatriactontadien-2-one	42.881	$C_{37}H_{70}O$	530.9	22.73			Vasodialatory effect

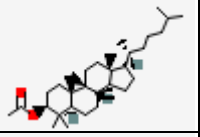
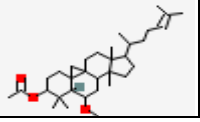
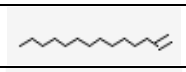
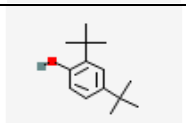
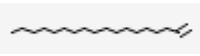
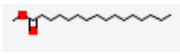
16	Cycloartenyl acetate	43.304	$C_{32}H_{54}O_2$	470.	18.62			No specific activity reported
17	3-O-Acetyl-cycloartenol	47.022	$C_{33}H_{54}O_3$	498.8	7.83			No specific activity reported

Table 2 : Biological activities of phytocomponents identified in the Chloroform extract of *Dysoxylum malabaricum* seeds.

No	Name of compound	Retention time	Molecular formula	Molecular weight	Class of compound	Compound structure	Peak area	Activities
1	1-Tetradecene	14.470	$C_{14}H_{28}$	196.37	Alkene		6.63	Antimicrobial Anticancerous
2	2,4-Di-T-butylphenol	17.559	$C_{14}H_{22}O$	206.32	Phenol		14.45	No specific acitivity reported
3	1-Hexadecene	19.324	$C_{16}H_{32}$	224.42	Alkene		14.22	Antibacterial Antifungal Antioxidant
4	Hexadecane	19.483	$C_{16}H_{34}$	226.44	Alkane		2.07	No specific activity reported
5	1-Tetradecanol, acrylate	21.622	$C_{14}H_{30}O$	214.39	Fatty Alcohol		1.54	No specific activity reported
6	1-Octadecene	23.732	$C_{18}H_{36}$	252.5			16.23	Antifungal, Antimicrobial, Antibacterial, Anti-diarrheal
7	Heptadecane	23.862	$C_{17}H_{36}$	240.5	Alkane		1.37	Antioxidant
8	Methylpalmitate	26.476	$C_{17}H_{34}O_2$	270.5	Fatty acid Methyl ester		1.47	No specific activity reported
9	n-Tetracosanol-	34.222	$C_{24}H_{50}O$	354.7	Fatty alcohol		11.45	Antimutagenic, Antibacterial activity, lowers cholesterol, enhancing immune functions, platelet aggregation and endothelial cell damage
10	1-Heptacosanol	39.282	$C_{27}H_{56}O$	396.7	Fatty alcohol		8.28	No specific activity reported

Certain phytoconstituents extracted using methanol as solvent like Methyl 4-O-methyl-d-arabinopyranoside, 1,2,4-Triazolo[4,3-a]pyridin-3(2H)-one, 5-methyl-, 2-Butenamide, 2-ethyl-3-methyl-N-phenyl-, Methylpalmitate, 9-Octadecenoicacid (Z)-, Methylester, Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-, are highly antioxidant in nature (Vengadesh *et al*., 2019; Thoraya *et al*., 2011; Suraj *et al*., 2020; Zhu, Zhu and Tian, 2012; Alwin, Reginald and Irene, 2021), whereas, Methyl 4-O-methyl-d-arabinopyranoside,Pseudolimonene,Cyclohexane,1-methylene-4-(1-methylethenyl)-, Methylpalmitate, 9-octadecenoic acid (Z)-, Methyl ester, Alpha, Gamma-Dipalmitin, possess antimicrobial properties (Morenike *et al*., 2018; Mary *et al*., 2021; Sonia, Salima and Abdel, 2015; Obidi *et al*., 2013; Krishnaveni, Ravi and Nagaraj, 2014). While certain compounds possess some unique properties like, Antispasmodic and immune modulator-9-Octadecenoic acid, 1, 2, 3-propanetriyl ester, (E, E, E)-) ([Duke], 2010). Vasodilatory effect - Z,Z-6, 28-Heptatriactontadien-2-one (Ali, Muhammed and Imad, 2016), cytotoxic, anticancer and phototoxic (Pyranone) (Manjari *et al*., 2014). Similarly compounds from chloroform extract also contain strong antimicrobial and antioxidant properties. For example, Heptadecane, 1-octadecene, 1-Hexadecene, 1-Tetradecene, are having both antioxidant and antimicrobial properties (NCBI, 2022; Shyamala and Manikandan, 2019; Madhavan, Priyadarshini and Sripriya, 2021; Belakhdar, Benjouad and Abdennebi, 2015; Nargani *et al*., 2016).

Antioxidant assay resulted that both the methanolic and chloroform extracts possess antioxidant properties (Figure3, 4). On comparing both, it is found that the IC_{50} value of Chloroform extract is found to be less than (965.21 mg/Kg) that of methanolic extract (1355.67mg/Kg). Hence, chloroform extract has more potential than the methanolic extract. It may be due to the presence of alkene and alkane groups of compounds that present in the chloroform extract.

However further studies are needed to explore its bioactivity and toxicity profile.

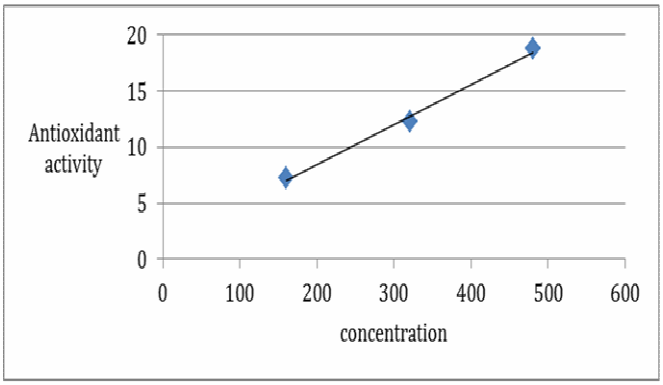


Fig. 3 : Graph showing Antioxidant activity in Methanol extract of *D. malabaricum* seeds

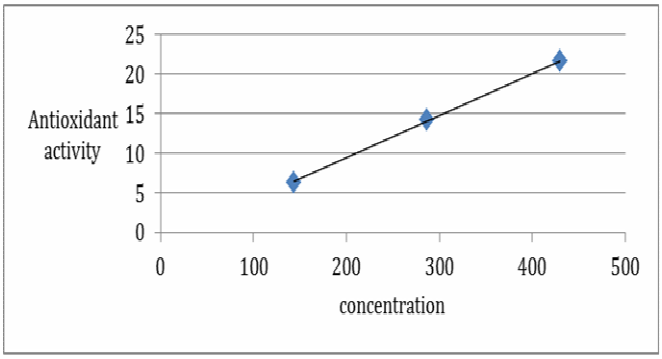


Fig. 4 : Graph showing Antioxidant activity of Chloroform extract of *D. malabaricum* seeds

Conclusion

The GC-MS analysis of *Dysoxylum malabaricum* seeds in both methanol and chloroform extracts showed presence of 28 phytochemicals with wide biological properties. Most of the compounds were with strong antioxidant and antimicrobial properties. On comparing the solvents for extraction it is found that chloroform extracts resulted in more compounds with antioxidant properties and all of them with high retention time. The present study revealed that seeds of *D.malabaricum* is a good source of biologically active components, and further study may lead to discovery of novel drugs.

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