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'The GC MS Analysis of Ethyl Acetate Extract of One Herbal Plant, '*Diospyrosmelanoxylon*'

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ABSTRACT

The present study deals with the GC MS pattern of the ethyl acetate leaf extract of one medicinal plant, '*Diospyrosmelanoxylon'***.** This plant was collected from nearby hills of Chengalpattu, Tamilnadu. The ethyl acetate extract of the leaves of the plant was subjected to GC MS study following standard protocols. It was observed that some very important molecules such as .beta.-Amyrin, Betulin, Lupeol werepresent in the extract which have various medicinal roles thereby indication the medicinal roles of this plant.

Keywords: GC MS, Ethyl acetate, .beta.-Amyrin, Betulin, Lupeol.,1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-,[S-(E,Z,E,E)]-

INTRODUCTION

'*Diospyrosmelanoxylon'* is also known as Kendu or Tendu in eastern parts of India. The fruits are edible and the leaves are used to make bidies which are sold commericially. Ethno-botanically this plant has various medicinal uses. It is known to have antibacterial andthe bark is also diuretic, carminative, laxative, and styptic and used as an astringent lotion for eyes (Rath*et al,* 2009; Mallavadhani*et al*., 1998). The seeds are prescribed for the treatment of mental disorders, palpitation of heart and nervous breakdown (Rathore, 1972). The anticancer role of *Diospyrosmelanoxylon*was studied by Rashid *et al*, 2017. Similarly the anticancer and antioxidant roles of the leaf extracts of this plant were reported by Rashid *et al*, 2018. Supriya*et al*, 2019 have reported the in vitro antioxidant and antibacterial activities of various extracts of this plant. Bodale and Shahare have done the phytochemical screening and GC MS study of a related species, *Diospyrosmontana.*This report is in continuation of our work to establish the efficacy of the herbal plants, Ayurvedic and Sidhha medicines. (Priyadarshini*et al,* 2017; Jayakumari*et al*, 2017; Rao*et al,* 2018; Vijayalakshmi and Rao, 2019; Yuvaraj*et al*, 2019; Mutteyi*et al,* 2019, Rao*et al*, 2019; Mutteyi*et al,* 2020; Vijayalakshmi and Rao, 2020; Janaki*et al*, 2021).

MATERIALS AND METHODS

The plant *Diospyrosmelanoxylon*was collected from the nearby hills at Chengalpattu, Tamil Nadu. The plant was identified by a qualified botanist at Chennai. The ethyl acetate extract of the shade dried leaves was collected after 48 h of soaking. The extract was evaporated and the dried powder was used for GC-MS analysis by standard procedures.

GC-MS Procedure

Instrument: GC (Agilent: GC: (G3440A) 7890A. MS/MS: 7000 Triple Quad GCMS) was equipped with MS detector.

Sample Preparation

About 100 ml sample was dissolved in 1 ml of suitable solvents. The solution was stirred vigorously using vortex stirrer for 10 s. The clear extract was determined using GC for analysis.

GC-MS Protocol

Column DB5 MS (30 mm × 0.25 mm ID ×0.25 µm, composed of 5% phenyl 95% methylpolysiloxane), electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min injector temperature 280°C; auxilary temperature: 290°C ion-source temperature 280°C.

The oven temperature was programmed from 50°C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10 min) fragments from 45 to 450 Da. Total GC running time is 32.02 min. The compounds are identified by GC-MS Library (NIST and WILEY).

RESULTS AND DISCUSSION

The results of the GC-MS analysis of the whole plant ethyl acetate extract, along with the possible medicinal role of each molecule of*Diospyrosmelanoxylon*extract are tabulated in Table 1. Figure 1 represents the GC-MS profile of ethyl acetate extract of the whole plant of *Diospyros melanoxylon.*

The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS along with the possible pharmaceutical roles of each bio molecule as per Dr. Duke's Phytochemical and ethnobotanical data base (National Agriculture Library, USA) and others as shown in Table 1. It is interesting to find that only three compounds are known for their medicinal role, namely, .beta.-Amyrin, Betulin, Lupeol. .beta.-Amyrin is known to have medicinal roles such as 17 beta hydroxysteroid dehydrogenase inhibitor, Antiamyloid beta, Anti TGF beta, Beta receptor agonist, Beta adrenergic receptor blocker, beta blocker, beta galactosidase inhibitor, beta glucuronidase inhibitor, ER beta binder. Betulinhas a role as a metabolite, an antiviral agent, an analgesic, an anti-inflammatory agent and an antineoplastic agent. Lupeol is known to be Anti-inflammatory,anti-arthritic, anti-mutagenic and anti-malarial. These aspects of thesethree compounds could contribute to the medicinal roles of *Diospyros melanoxylon, as claimed in ethnomedicine.* Themedicinal roles of most of the compounds such as Farnesylbromide,Androst-5-en-3-ol,4,4-dimethyl-,(3.beta.)-Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-,(4aR-trans)-, 1,3,6,10-Cyclotetradecatetraene,3,7,11-

trimethyl-14-(1-methylethyl)-,[S-(E,Z,E,E)]- are not known. Further work to identify the roles of these molecules will be useful in a better understanding of the medicinal aspects of this plant.

CONCLUSION

From the above results it is clear that some of the molecules present in this plant do have medicinal values and further probe is warranted.

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REFERENCES

1. Rath, S. K., Mohapatra, N., Dubey, D., Panda, S. K., Thatoi, H. N., Dutta,S. K. (2009) Antimicrobial activity of *Diospyrosmelanoxylon* bark from Similipal Biosphere Reserve, Orissa, India. *African Journal of Biotechnology, 8 (9),*1924-1928,

2. Mallavadhani, U. V., Panda, A. K., Rao, Y. R. (1998). Pharmacology and chemotaxonomy of *Diospyros*.*Phytochem.49,* 901-951.

3. Rathore, J. S. (1972). Diospyrosmelanoxylon, a bread-winner tree of Indian.*Econ. Bot., 26*, 333-339

4. Rashid, M. H. A.,Mandal, V., Mandal, S. C., Thandavarayan, R. A.*Diospyrosmelanoxylon*Roxb in cancer prevention: pharmacological screening, pharmacokinetics and clinical studies. *Int J Pharmacognosy, 4(7),* 217-223

5. Rashid, Md., H., Bharadwaj, D. P. V. P, Majumder, S., Mandal, V., Pal, M., Mandal, S., Thandavarayan, R. A. (2018) Antioxidant and anticancer activity of extract and fractions obtained from *Diospyrosmelanoxylon*roxb. leaves and correlation with their polyphenolic profiles. *International Journal of Pharmacy and Pharmaceutical Sciences, 10(11)*, 6-16

6. Supriya, K. A., Growther, L .(2019) *In-vitro* antioxidant and antibacterial activity of different extracts of *Diospyrosmelanoxylon* roxb.*Int J Pharm Sci& Res, 10(4),* 1820-1827.

7. Bodele, S. K., Shahare, N. H.(2018) Phytochemical Screening and GC-MS Analysis of *Diospyrosmontana* (Roxb.) root. *Int. J. of Res. in Pharmacology &Pharmacotherapeutics, 7(2),*100-107

8. GomathiPriyadarshini, Arul Amutha Elizabeth, Jacintha Anthony, Mudiganti Ram Krishna Rao, Prabhu. K., Aiswarya Ramesh, Vani Krishna. (2017) The GC MS analysis of one medicinal plant, *Premnatomentosa. Journal of Pharmaceutical Sciences and Research,* ***9(9),*** 1595-1597

9. Jayakumari, S., Prabhu, K., Mudiganti Ram Krishna Rao, Bhupesh, G., Kumaran, D., Aishwariya Ramesh. (2017) The GC MS Analysis of a Rare Medicinal Plant *Aloe barbadensis. J. Pharm. Sci. & Res.9(7),* 1035-1037

10. Rao, M. R. K., Vijayalakshmi, N. (2018) Preliminary phytochemical and GC MS analysis of different extracts of *Sphaeranthusindicus* leaves*. Indo American J of Pharmaceuical Sciences, 5(3),* 1511-1520

11. Vijayalakshmi, N., Mudiganti Ram Krishna Rao. (2019) The antioxidant studies of two medicinal plants, *Sphaeranthusindicusand Psophocarpustetragonolobus*.*Asian J of pharmaceutical and Clinical Res, 12(1),* 321-327.

12. Yuvaraj, R., Mudiganti Ram Krishna Rao, Prabhu, K., Lakshmisundram, R., SampadShil, Sathish Kumar, M., Vijayalakshmi, N. (2019)The GC MS study of one medicinal plant, *Stachyterphetaindica*.*Drug Invention Today, 12(9),* 1665-1669

13. MutteviHyagreva Kumar, Prabhu, K., Mudiganti Ram Krishna Rao, Lakshmisundram, R., SampadShil, Sathish Kumar, M., Vijayalakshmi, N. (2019)The GC MS study of one medicinal plant, *Dodoneaangutifolia*. *Drug Invention Today, 12(9)*, 1661-1664

14. Mudiganti Ram Krishna Rao, Vijayalakshmi, N., Prabhu, K., Sathish Kumar, M. (2019)The gas chromatography–mass spectrometry study of *Moringaoleifera*seeds. *DIT, 12(10),* 2172-2175

15. MutteviHyagreva Kumar, Prabhu, K., Mudiganti Ram Krishna Rao, Lakshmisundram, R., SampadShil, Sathish Kumar, M., Vijayalakshmi, N.(2020)The GC MS study of one medicinal plant, *AristolochiaIndica .DIT, 12(12),* 2919-2923.

16. Vijayalakshmi, N., Mudiganti Ram Krishna Rao. (2020) 'Preliminary phytochemical and antioxidant studies of leaf extracts of one medicinal plant, *Vitexnegundo*".*RJPT, 13(5),* 2167-2173

17. Janaki C. S.', Prabhu K., Mudiganti Ram Krishna Rao,VenkatRamaiah, ShrutiDinkar, Vijayalakshmi, N., Kalaivannan. J. (2021) The GC MS analysis of Ethyl acetate extract of *Merremiaemerginata'.Ind J of Natural Sciences, 12(67),* 33638-33646

18. Dr.Duke's Phytochemcial and Ehnobotanical Databases.U.S. Department of Agriculture, Agricultural Research Service.1992-2016. Dr. Duke's Phytochemical and Ethnobotanical Databases. Home Page, http://phytochem.nal.usda.gov/ http://dx.doi.org/10.15482/USDA.ADC/1239279

Figure 1. Shows the GC MS profile graph of ethyl acetate extract of *Diospyrosmelanoxylon*

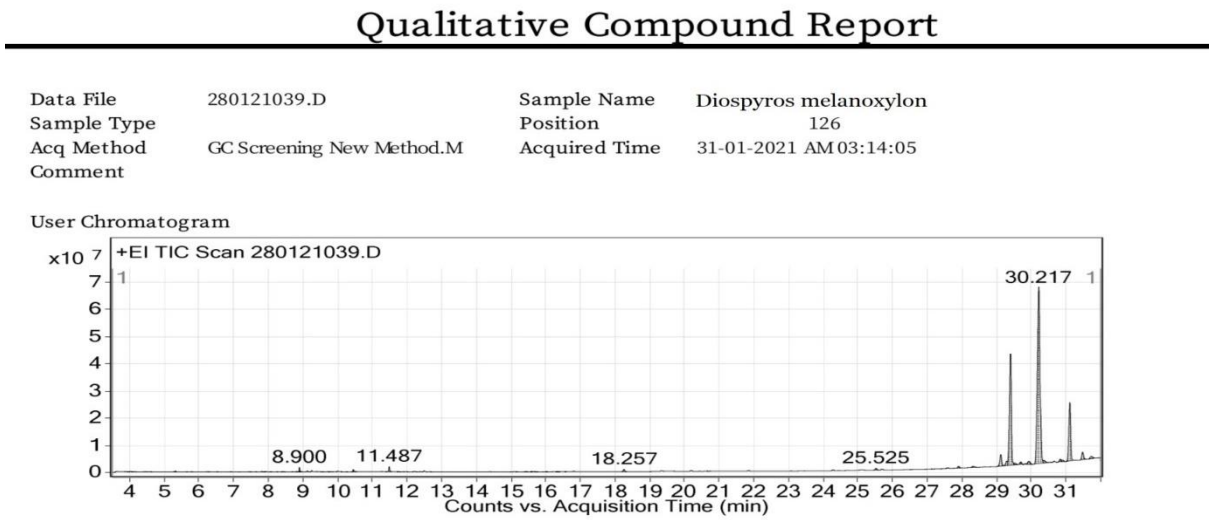

Table1. Indicates the retentions time, types of possible compound, molecular formula, molecular mass, Percentage peak area and the possible medicinal roles of each compound as shown in the GC MS profile of *Diospyrosmelanoxylon.*

Ret. Time	Compound	Mol. Formula	Mol. Mass	% Peak Area	Possible Medicinal Role

29.12	Farnesylbromide	C15H25Br	284.1	3.15	Not Known
29.93	Androst-5-en-3-ol,4,4-dimethyl-,(3.beta.)-	C21H34O	302.3	1.27	Not Known
30.22	.beta.-Amyrin	C30H50O	426.4	70.44	17 beta hydroxysteroid dehydrogenase inhibitor, Antiamyloid beta, Anti TGF beta, Beta receptor agonist, Beta adrenergic receptor blocker, beta blocker, beta galactosidase inhibitor, beta glucuronidase inhibitor, ER beta binder
30.37	Betulin	C30H50O2	442.4	0.89	It has a role as a metabolite, an antiviral agent, an analgesic, an anti-inflammatory agent and an antineoplastic agent
30.83	Fenretinide	C26H33NO2	391.3	0.59	Not Known
31.11	1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-,[S-(E,Z,E,E)]-	C20H32	272.3	16.42	Not Known
31.48	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-,(4aR-trans)-	C15H24	204.2	2.01	Not Known
31.73	Lupeol	C30H50O	426.4	0.77	Anti-inflammatory, anti-arthritic, anti-mutagenic and anti-malarial