

GC-MS Analysis of Bioactive Compounds in Methanolic Extract of Leaves of *Diospyros melanoxylon* Roxb.

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Abstract: *Diospyros melanoxylon* Roxb. belongs to the family Ebenaceae. The leaves are very well known for making beedi all over India but also well-known for its medicinal uses. Commonly called as 'Kendu' distributed in the forest all across Jharkhand state. The plant has been utilized extensively by tribal herbal practitioners to treat various ailments such as diarrhoea, cholera, dysentery, intermittent fevers, bleeding gums, bronchitis, carbuncles, cough, cramps, pneumonia, syphilis and tumors. In previous studies the antidiabetic, antioxidant, anticancer and antiulcerogenic activity of *Diospyros melanoxylon* has been reported. The medicinal value of a plant species depends upon its various phytochemical constituents. In the present study the chemical compositions of the methanolic extract of leaves of *Diospyros melanoxylon* were investigated using Gas chromatography Mass spectrometry. The investigation highlighted the presence of thirty-three bioactive phytochemical compounds. The major chemical constituents belonging to triterpenes are Alpha-amyrin(37.36%), Beta-amyrin(16.09%), Lanosterol(9.09%), Lup-20(29)-en-3-one(3.87%), Ursolic aldehyde(3.79%), Urs-12-en-28-al,3-(acetyloxy)-(3beta)-(3.47%), Lup-20(29)-en-3-one(2.45%), 2,2,4a,6a,8a,9,12b,14a-Octamethyl(2.81%), 2-Naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro-alpha-tetramethyl-(1.77%), Lupeol(1.61%), Neophytadiene(1.23%), 9,19-Cyclolanost-23-ene-3-O-beta(1.16%), Olean-12-en-28-al(1.09%), Squalene(0.99%) known for anti-inflammatory, antiviral, antimicrobial and antitumoral agents, followed by polyphenols 1,3,5-Benzenetriol(1.02%), 1,2,3-Benzenetriol(1.00%), and Vitamin E(0.98%) with high antioxidant property and sugar Mome inositol(3.41%) with anti-alopecic, anti-cirrhotic, anti-neuropathic and sweetening properties have been identified.

Key words- Bioactive compounds, *Diospyros melanoxylon*, GC-MS, Methanolic extract, Phytochemicals.

1. INTRODUCTION

Plants are the source of bioactive phytochemicals that contribute to health promotion and disease prevention. Macro and micronutrients in plants were thought to be one of the essential components for human health, phytochemicals have recently emerged as modulators of key cellular signalling pathway¹. Plants with proven ethnobotanical use can be the best option in developing natural products-based alternatives. Traditionally, a number of medicinal plants have shown their efficacy through ethno-pharmacological evaluation². Phytochemical extracts containing constituents such as plant-derived vitamins, flavonoids, alkaloids carotenoids, terpenoids, polyphenols, and phenolic compounds have been reported to exhibit antioxidant and anticancer activities³. *Diospyros melanoxylon* Roxb. Known as Coromandel Ebony or East Indian Ebony belongs to the family Ebenaceae, native to India and Sri Lanka. Widely distributed as dominant plant species in tropical moist deciduous and dry deciduous forest of Jharkhand. In Jharkhand commonly called as 'Kendu', whose leaves are dried and is used for wrapping bidis (a tobacco product know as Indian cheap smoke). *Diospyros melanoxylon* is one of the most valuable non-timber forest products found in Jharkhand⁴. Kendu leaf collection is being associated with rural livelihood, an economic resource and revenue generation for the Jharkhand state⁵. Traditionally, *Diospyros melanoxylon* is being used in the management of diverse diseases and treatment of

diabetes, anaemia, inflammation of spleen, dyspepsia, diarrhoea, scabies, hypotensive and used as carminative, laxative, diuretic and astringent⁶.

Furthermore, *D. melanoxylon* is reported to have anti-diabetic⁷, antioxidant, anti-cancer⁸, anti-ulcer⁹ and anti-bacterial activity¹⁰

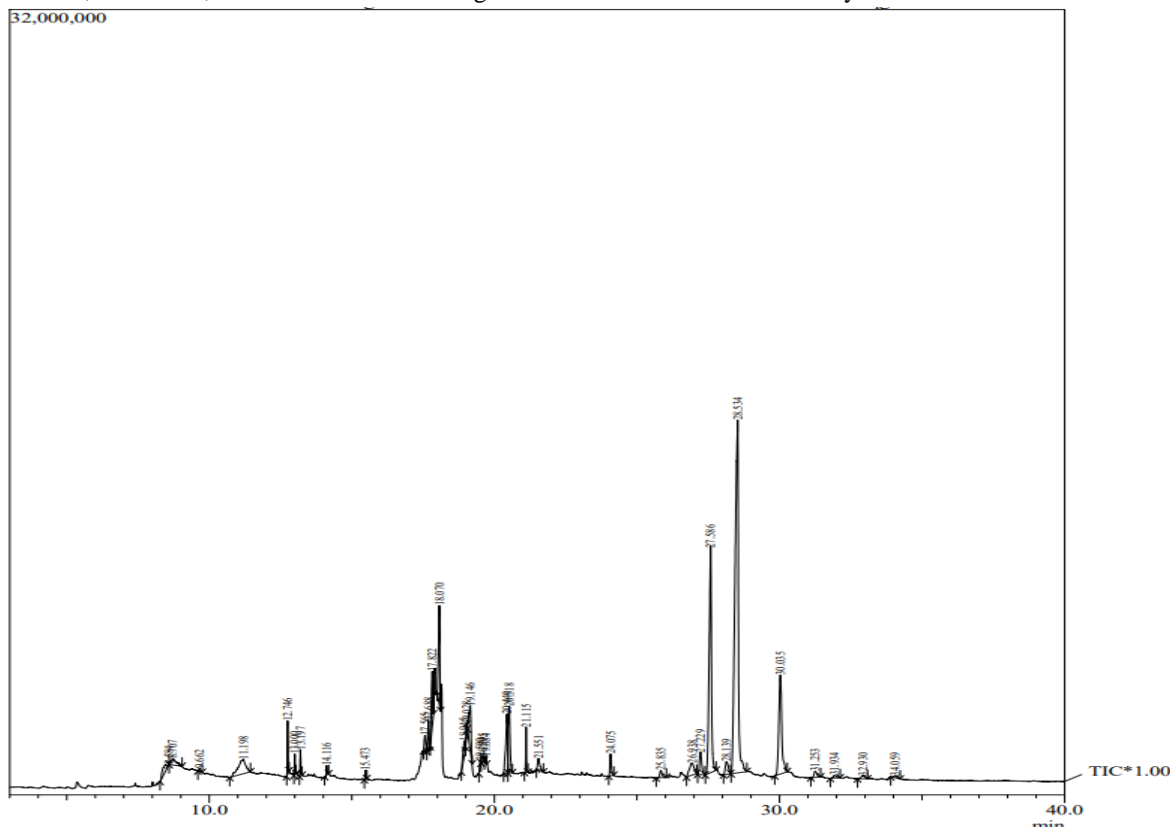


Fig. 1 GC-MS Chromatogram of methanolic extract of leaves of *Diospyros melanoxylon* Roxb.

2. MATERIALS AND METHODS

Preparation of extract- fresh and healthy leaves were collected from Garhkhatanga, Ranchi District, Jharkhand state. Collected fresh leaves were thoroughly washed and air dried in shade so as to prevent decomposition of active principle and fine powder was made. The leaves powder was soaked in 90% methanol in the ratio 1:10 and kept for 48-72 hours. The solution was filtered with Whatman filter paper (No.1). Filtrate evaporated at 37°C to obtain the extract and was kept at 20°C for further analysis by GC-MS.

GC-MS analysis of bioactive compounds- Sample was sent for GC-MS analysis at Advanced Instrumentation Research Facility, JNU, New Delhi. The results pertaining to GC-MS analysis leads to the identification of number of chemical constituents from the GC fractions of methanolic extract of leaves of *Diospyros melanoxylon*. Thirty-three bioactive compounds were identified. Chromatogram is displayed in figure 1 and the retention time (RT), % of peak area, molecular formula, molecular weight and biological activities of the major compounds are presented in Table 1.

Table 1. Bioactive compounds from methanolic extract of leaves of *Diospyros melanoxylon*

Retention Time	% of Peak Area	Molecular Name	Molecular formula	Molecular weight(g/mol)
8.584	1.00	1,2,3-Benzenetriol	C7H10O6S	222.22
8.707	1.02	1,3,5-Benzenetriol	C13H12O5	248.23
9.662	0.10	2-Buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)	C14H24O	208.34
11.198	3.41	Mome Inositol	C6H12O6	180.16
12.746	1.23	Neophytadiene	C20H38	278.5

13.000	0.47	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296.5
13.197	0.61	Neophytadiene	C20H38	278.5
14.116	0.23	n-Hexadecanoic acid	C16H32O2	256.42
15.473	0.24	Phytol	C20H40O	296.5
17.565	0.69	Lup-20(29)-en-3-one	C30H48O	424.7
17.688	1.09	Olean-12-en-28-al	C32H52S2	500.9
17.822	2.45	Lup-20(29)-en-3-one	C30H48O	424.7
18.070	3.87	Lup-20(29)-en-3-one	C30H48O	424.7
18.945	0.82	2,2,4a,6a,8a,9,12b,14a-Octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14a,14b-eicosahydricene	C30H50	410.7
19.028	0.38	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	C32H54O2	470.8
19.146	2.81	2,2,4a,6a,8a,9,12b,14a-Octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14a,14b-eicosahydricene	C30H50	410.7
19.595	0.35	2,2-dimethyl-3-[3,7,12-trimethyl-14-[3-methyl-3-(4-methyl-3-pentenyl)-2-oxiranyl]-(3E,7E,11E)-3,7,11-tetradecatrienyl]oxirane	C30H50O2	442.7
19.684	0.12	Humulane-1,6-dien-3-ol	C15H26O	222.37
20.440	3.47	Urs-12-en-3-ol, acetate, (3.beta.)-	C32H52O2	468.8
20.518	3.79	Ursolic aldehyde	C30H48O2	440.7
21.115	0.99	Squalene	C30H50	410.7
21.551	0.81	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-	C20H28O6	364.4
24.075	0.98	Vitamin E	C29H50O2	430.7
25.835	0.46	Stigmasterol	C29H48O	412.7
26.938	1.77	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-alpha, alpha,4a,8-tetramethyl	C15H26O	222.37
27.229	1.61	Lupeol	C30H50O	426.7
27.586	16.09	beta-Amyrin	C30H50O	426.7
28.139	1.16	9,19-Cyclolanost-24-en-3-ol, acetate, (3beta.)-	C32H52O2	468.8
28.534	37.36	alpha-Amyrin	C30H50O	426.7
30.035	9.09	Lanosterol	C30H50O	426.7
31.253	0.46	2,2-dimethyl-3-[3,7,12-trimethyl-14-[3-methyl-3-(4-methyl-3-pentenyl)-2-oxiranyl]-(3E,7E,11E)-3,7,11-tetradecatrienyl]oxirane	C30H50O2	442.7
31.934	0.30	3-(1,5-Dimethyl-hexyl)-3a,10,10,12b-tetramethyl-1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradecahydro-benzo[4,5]cyclohepta[1,2-E]indene	C30H50	410.7
32.930	0.38	Urs-12-en-28-al	C30H48O	424.7

3. RESULTS

The prevailing compounds identified by GC-MS were Alpha-amyrin(37.36%), Beta-amyrin(16.09%), Lanosterol(9.09%), Lup-20(29)-en-3-one(3.87%), Ursolic aldehyde(3.79%), Mome inositol(3.41%), Urs-12-en-28-al,3-(acetyloxy)-,(3beta)-(3.47%), Lup-20(29)-en-3-one(2.45%), 2,2,4a,6a,8a,9,12b,14a-Octamethyl(2.81%), 2-Naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro-alpha-tetramethyl-(1.77%), Lupeol(1.61%), Neophytadiene(1.23%), 9,19-Cyclolanost-23-ene-3-O-beta(1.16%), Olean-12-en-28-al(1.09%), Squalene(0.99%), 1,3,5-Benzenetriol(1.02%),

1,2,3-Benzenetriol(1.00%), Vitamin E(0.98%), Octamethyl eicosahydricene(0.82%), 1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-(0.81%), Stigmasterol(0.46%).

4. DISCUSSION

The GC-MS analysis of methanolic extract of leaves of *Diospyros melanoxylon* Roxb. showed the presence of thirty-three different phytochemical compounds. Most of the compounds identified from extract consists of hydrocarbons of pentacyclic

triterpenes and triterpenes: Alpha-amyrin(37.36%), Beta-amyrin(16.09%), Lanosterol(9.09%), Lup-20(29)-en-3-one(3.87%), Ursolic aldehyde(3.79%), Urs-12-en-28-al,3-(acetyloxy)-,(3beta)-(3.47%), 2,2,4a,6a,8a,9,12b,14a-Octamethyl(2.81%), 2-Naphthalenemethanol(1.77%), Lupeol(1.61%), Neophytadiene(1.23%), Olean-12-en-28-al(1.09%), Squalene(0.99%), phenols: 1,3,5-Benzenetriol(1.02%), 1,2,3-Benzenetriol(1.00%), Vitamin E(0.98%) and polysaccharide: Mome inositol(3.41%) were identified as active phytochemicals with the highest peak area. Various *in-vitro* and *in-vivo* studies have been conducted for chemoprevention and therapy of breast cancer, and pancreatic cancer using triterpenoids as these phytochemicals exert their chemo-preventive and anti-cancer activities via enhancing apoptosis¹¹. The pentacyclic triterpenes have pharmacologically importance in activities like anti-cancer, anti-HIV and anti-inflammatory¹². Vitamin E is a fat-soluble antioxidant leads to stops the production of ROS¹³. Plant phenols are a vital human dietary component and exhibit a tremendous antioxidant activity. Phenols are well documented for health protective effects like antimicrobial, anticancer, anti-inflammatory, anti-mutagenic¹⁴. Squalene has been reported a highly effective oxygen-scavenging agent possess antioxidant properties. Subsequent to oxidative stress such as sunlight exposure, squalene functions as an efficient quencher of singlet oxygen and prevents the corresponding lipid peroxidation at the human skin surface¹⁵. Neophytadiene found to have role as anti-inflammatory agent, an antimicrobial agent¹⁶⁻¹⁸. Mome inositol have role in anti-neuropathic, anti-cirrhotic and sweetening properties of the plant¹⁹.

5. CONCLUSION

The crude extracts were analyzed qualitatively using GC-MS to determine the chemical composition of methanol extract of leaves of *Diospyros melanoxylon* Roxb. A total of thirty-three compounds were identified. The compounds were chosen based on the higher percentage peak area. The major compounds present belonging to pentacyclic triterpenes proven to have pharmacologically importance and presence of other chemical components responsible for its potent medicinal activity promising antioxidant, anti-cancer,

anti-inflammatory and anti-microbial properties. Overall, the GC-MS results of *Diospyros melanoxylon* Roxb. obtained provide a biochemical rationale for further studies with chemotherapeutic focus.

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