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RESEARCH ARTICLE

Phytoconstituents isolated from *Diospyros oocarpa* Thwatist

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ABSTRACT

The chemical examination of the roots of *D. oocarpa* afforded ten coumpounds on column chromatography and repeated crystallizations, Lupeol, 5-Hydroxy-4-methoxy-2-naphthaldehyde, 4-Hydroxy-5-methoxy-2-naphthaldehyde, 4-Hydroxy-3, 5-dimethoxy-2-naphthaldehyde, β-sitosterol, Plumbagin, Betulinaldehyde, Diospyrin, 8'hydroxyisodiospyrin, Umbelliferone. Isolation of umbelliferone belongs to coumarin is the first time record from this species and also from *Diospyros* genus. Occurrence of napthoquinones is very common in *Diospyros* species. The author could isolate these quinines from this species also and thus justified the species. Distribution of napthaldehydes in nature is very rare and this report of occurrence is new to the literature

Keywords: *Diospyros oocarpa,* Habibone, Diospyrin, umbelliferone, 8'hydroxyisodiospyrin, Betulinaldehyde

1. INTRODUCTION

The genus *Diospyros,* belongs to the family Ebenaceae, approximately consists of 450 species and is wide spread, chiefly in tropics and sub tropics[1-4]. About 90 species occur in India comprising mostly trees and rarely shrub. *Diospyros* species are known to elaborate a series of naphthoquinones and pentacyclic triterpenoid saponins. Phytochemical investigation of more than 130 *Diospyros* species led to the isolation of variety of compounds, the majority of which are triterpenoids naphthoquines and flavonoids. Then literature revealed that these plants also contain pentacyclic triterpenes and juglone based naphthoquinones[5-8]. This genus is so interesting that almost all plants were found to possess therapeutic properties[9]. Totally, *Diospyros* genus is economically and medicinally the most important genus of Ebenaceae. About 41 species are indigenous to India, grown mostly in Western Ghats of Karnataka, Goa and Maharashtra, forests of Deccan, Assam and Bengal, and a few are in North India. Medicinally, *Diospyros*species are used as anthelmentic, anti-inflammatory,antibacterial, antifungal, antioxidant, anticancer, antiviral, molluscicidal, piscicidal and termite resistant activities[10-13].

***D. oocarpa* Thwatist** (Syn: *D. marmorata*) is a moderate sized tree with shining leaves. Leaves are simple, flowers are unisexual and white in colour, and berry is oblong to ovoid, distributed throughout Amboli Ghat, Maharastra state, Western Ghats of India.

Ethnopharmacognosy: Our survey revealed that the traditional healers and Dhangars of Ramghat region of western ghat were using this plant to expelling worms, skin diseases, in the treatment of arthritis, dysentery and intestinal infections. From the other species of *Diospyros,* oxygenated-2-naphthaldehydes, naphthoic acids, naphthoates and naphthoquinone dimers have been earlier reported [14-16], with affinities to the African crocodile bark-tree, *D. quiloensis* which has been reported to produce several oxygenated 2-naphthaldehydes [17]. To the best of the author's knowledge there has been no report on the phytochemical constituents of this tree.

2. MATERIALS AND METHODS:

General Instrumentation: Melting points were recorded on a Cipla I-28, digital apparatus and were uncorrected. Silica gel (Acme) 60.120 mesh for column chromatography and silica gel (Acme) 100-200 mesh were used for

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preparative thin layer chromatography. Spots on chromatogram were detected under UV light and by spraying with 5% H2SO4 in methanol. UV spectra were recorded in methanol. Both 1-D and 2-D NMR spectra were run in CDCl3 (1H: 500 MHz; 13C: 125 MHz) on a Bruker AVANCE DRX-500 spectrometer. Accurate mass measurements were determined on a Kratos M525 RFA instrument. LC.MS (EI) was recorded using an Agilent 1100 series LC/MSD in the APCI mode.

Plant material: The roots of *D.oocarpa* were collected at Amboli ghat, of Maharastra state, Western ghats of India, The voucher specimen (SGDO-1) was deposited in the College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India

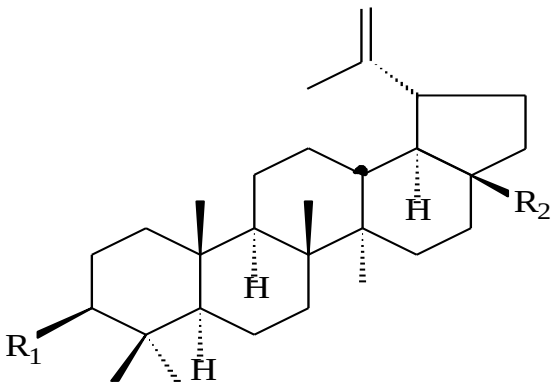
Extraction: Shade dried roots (1.6 kg) were powdered in a Wiley mill and then extracted over four days with chloroform (4 x 1.5 L) at room temperature. TLC examination of the residue showed a number of spots (solvent system: Benzene: Chloroform: Ethyl acetate. 1:3:1). The combined extracts were concentrated under reduced pressure to yield 28 g of reddish brown gum and hence a portion of the chloroform extract (20 g) was chromatographed over silica gel and eluted, in succession, with petroleum ether (b.p 40-60°C), petroleum ether-benzene mixtures containing increasing amounts of benzene, benzene and benzene- Chloroform mixtures containing increasing amounts of chloroform and 250 ml fractions were collected.

Chromatographic separation: TLC examination of the residue showed a number of spots (Benzene: Chloroform: Ethyl acetate. 1:3:1) on spraying with 5% alcoholic H2SO4 followed by heating. Column chromatography of the chloroform extract (12 g) over silica gel, on elution with petroleum ether containing increasing amounts of benzene and then Chloroform afforded three coloured bands which yielded pure crystalline material in the sequenceDNR-01 to DNR-10

3. RESULTS:

D0R-01 (0.120 g Lupeol)

Crystallized from pet. ether - benzene as colourless needles, mp 212-2130C. [ ] D30 + 240 (CHCl3 0.1) and analysed for the empirical formula C30H50O. It gave a pink colour in L.B reaction and a yellow colour with tetranitromethane. The IR showed absorption bands at 3540(-OH), 1380 and 1390 (gem dimethyl) and at 890 cm-1 (vinyl methylene). The 1H NMR exhibited signals (90 MHz, CDCl3,) at 0.78, 0.80, 0.83, 0.90 and 1.02 (18H, *s*, 6(CH3) 1.63 (3H, *s*, CH3-CH=CH2), 2.25 (1H, *d,* 19-H), 3.15 (1H, *m*, 3 -H), 4.51 (2H, *d*, CH2



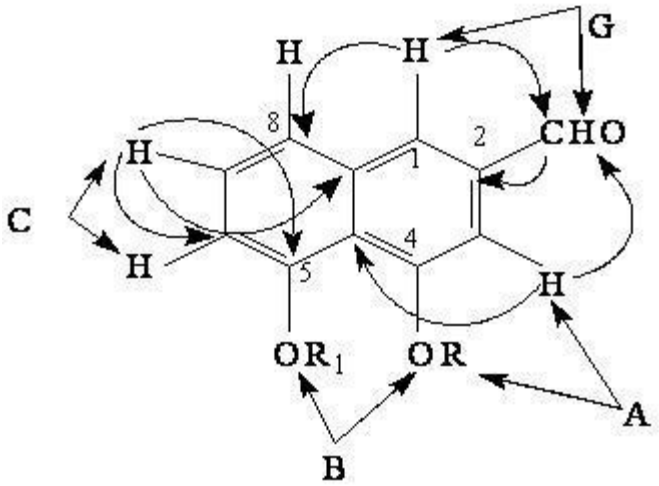
D0R-01 R_1: β-OH., R_2:CH_3

DOR-02 (0.025 g., 5-Hydroxy-4-methoxy-2-naphthaldehyde)

On repeated preparative silica gel TLC using petroleum ether-chloroform (50:50), the upper red band yielded orange-red needles from MeOH, mp 103-104°C.

^{1}H-NMR (δ $CDCl_3$, 500.13 MHZ)	: 7.92 *d* (1.3) H-1, 7.23 *d* (1.3) H-3, 7.08 *m* H-6, 7.48 *m* H-7/H-8, 10.10 *s* CHO, 9.30 *s* 5-OH, 4.14 *s* 4-OCH_3
^{13}CNMR (δ $CDCl_3$ 125.77 MHz)	: 130.7 (C-1), 134.6 (C-2), 98.9 (C-3), 157.5 (C-4), 117.8 (C-4a), 155.0 (C-5), 114.7 (C-6), 129.2 (C-7), 121.0 (C-8), 136.0 (C-8a), 191.9 (CHO), 56.7 (C-4OCH_3)
LC-MS (EIMS) (m/z) (%)	: $[M+H]^+$ 203 (100%)

Calculated for $C_{12}H_{10}O_3$ and found C. 71.28%, H. 4.98% and O. 23.74%.



R= CH_3, R_1=H

NOESY and HMBC correlations of DOR-02

DOR- 03 (0.030 g, 4-Hydroxy-5-methoxy-2-naphthalde)

On repeated preparative silica gel TLC using petroleum ether-chloroform (50:50), the lower band afforded yellow needles from MeOH, mp 85-90 °C.

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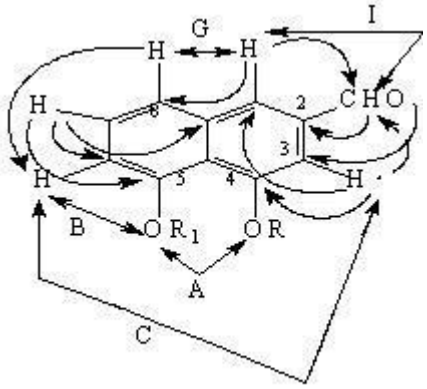
UV λ_{max} (log ε) : 221 (4.42), 254 (4.49) 379 (3.76)

^{1}H-NMR (δ $CDCl_3$, 500.13 MHZ) : 7.82 *d* (1.2) H-1, 7.31 *d* (1.2) H-3, 6.96 *d* (7.8) H-6, 7.44 *t* H-7 (7.9), 7.59 d (8.3) H-8, 10.07 *s* CHO, 9.44 *s* 4-OH, 4.11 *s* 5-OCH_3

^{13}CNMR (δ $CDCl_3$ 125.77 MHz) : 125.1 (C-1), 136.0 (C-2), 107.0 (C-3), 155.8 (C-4), 118.4 (C-4a), 156.4 (C-5), 107.4 (C-6), 127.2 (C-7), 123.6 (C-8), 136.3 (C-8a), 192.3 (CHO), 56.6 (C-4OCH_3),

HR-EIMS (EIMS) (m/z) (%) : 202^+ (100%)

$C_{12}H_{10}O_3$ was found 202.0627, requires 202.0630.



R=H, R_1=CH_3

NOESY and HMBC correlation DOR-03

DOR- 04 (0.023 g, 4-Hydroxy-3, 5-dimethoxy-2-naphthaldehyde)

On Fractional crystallization using methanol yielded yellow needles, mp 151-15

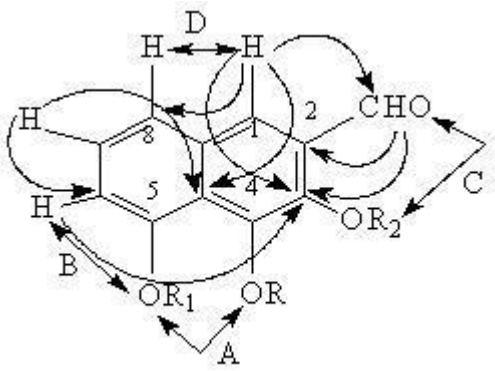
UV λ_{max} (log ε) : 231 (4.95), 260 (4.84), 291 (4.22), 390 (3.88)

^{1}H-NMR (δ $CDCl_3$, 500.13 MHZ) : 7.85 *s* H-1, 6.89 *d* (7.7) H-6, 7.32 *t* H-7 (7.9), 7.51 *d* (8.3) H-8, 10.53 *s* CHO, 9.46 *s* 4-OH, 4.07 *s* 3-OCH_3, 4.11 s 5-OCH_3

^{13}CNMR (δ $CDCl_3$ 125.77 MHz) : 119.7 (C-1), 129.7 (C-2), 144.1 (C-3), 147.1 (C-4), 119.2 (C-4a), 155.8 (C-5), 106.7 (C-6), 125.7 (C-7), 124.0 (C-8), 132.1 (C-8a), 191.0 (CHO), 62.1 (C-4OCH_3), 56.6 (C-5OCH_3),

HR-EIMS (EIMS) (m/z) (%) : 232^+ (100%)

$C_{13}H_{12}O_4$ was calculated and found 232.0733 and requires 232.0736



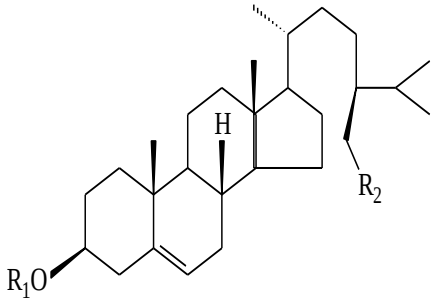
R=H., R_1= R_2 =CH_3

NOESY and HMBC correlations of DOR-04

DOR-05 (0.033g, β-sitosterol)

Crystallized from pet. ether-benzene as colourless needless, mp 134-136 °C, $[\alpha]^{30}_D$ - 36 ° ($CHCl_3$, *c*, 1.01) and had an empirical formulae C_{29} H_{50} O and was found to be C, 83.7; H, 12.7 % and requires C, 84.0; H, 12.2 %. The IR spectrum showed absorption bands IR ν^{KBR}_{max} 3440, 2970, 2910, 2880, 1470, 1385, 1380, 1055 cm^{-1}.

The above data coincided well with that of β-sitosterol and the identity was confirmed by comparison with an authentic sample through m.mp and Co-TLC.



R_1:H., R_2:CH_3

DOR-06 (0.023g, Plumbagin)

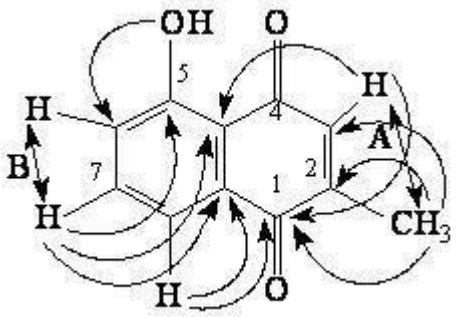
On crystallization from methanol, orange needles were obtained. It had an m.p. 78-79° C

IR ν^{KBR}_{max} (cm^{-1}) **:1**665, 1645 cm^{-1}

^{1}H-NMR (δ $CDCl_3$, 500.13 MHZ) **:** 2.20 *d* (1.5) 2-CH_3, 6.81 *d* (1.5) H-3, 11.97 *s* 5-OH, 7.25 *dd* (8.1, 1.4) H-6, 7.60 *br. t* (7.6) H-7, 7.64 *dd* (7.5, 1.4) H-8

^{13}CNMR (δ $CDCl_3$ 125.77 MHz) **:**185.0 (C-1), 149.8 (C-2), 16.7 (2-CH3), 135.7 (C-3), 190.5 (C-4), 161.4 (C-5OH), 124.4 (C6), 136.3 (C-7), 119.5 (C-8), 115.4 (C-4a), 132.3 (C-8a),

Calculated for $C_{11}H_8O_3$ and found C. 70.2%, H. 4.28% and O. 25.51%.



Selected NOESY and HMBC correlations of DOR-06

DOR-07 (0.035 g, Betulinaldehyde)

Betulinaldehyde (lupane type) was crystallized from methanol as colourless needles, mp. 185-187· Molecular formula $C_{30}H_{48}O_2$.

IR ν^{KBR}_{max} 3380, 3060, 2950, 2880, 1730, 1470 cm^{-1}.

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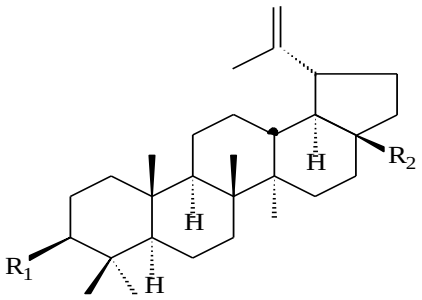
Specific rotation $[\alpha]_D^{20}$ + 27 ° ($CHCl_3$, *c*, 1.01) indicating it to be a dextrorotatory compound.

^{1}H NMR exhibited signals (300 MHz, $CHCl_3$, δ) at 0.73 (3H, *s*, 27(Me), 0.80 (3H, *s*, 25-Me), 0.90 (3H, *s*, 26-Me), 0.95 (6H, *s*, 23-Me, 24-Me), 1.68 (3H, *s, (br)*, 29-Me), 2.80 (1H, *m,* H-19), 3.15 (1H, *dd, J* = 11 Hz and 6 Hz, H-3), 4.60, 4.72 (2H, 2 x *s (br)* H-30), 9.65 (1H, *s*, H-28). From the above data the compound DAR-07 was identified and confirmed by comparison with an authentic sample through m.m.p. and Co- TLC [18, 19]



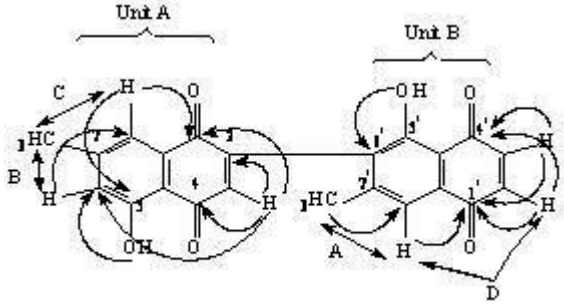
(DOR-07) Betulinaldehyde., R_1: β-OH., R_2: CHO

DOR-08 (0.040g, Diospyrin)

On silica gel eluting with $CHCl_3$-MeOH (99:1) afforded the less polar orange red prisms, m.p 258 °C. It had an R_f 0.27 in benzene.

UV λ_{max} nm (log ε)	: 252 (4.34), 432 (3.9).
^{1}HNMR (δ $CDCl_3$, 500.13 MHz)	: 6.91 *s* H-3, 11.90 *s* 5-OH, 7.14 *s* H-6, 2.47 s 7-CH_3, 7.51 *s* H-8, 6.96 *s* H-2'/H-3', 12.10 *s* 5'-OH, 2.32 *s* 7'-CH_3, 7.57 *s* H-8',
^{13}CNMR (δ $CDCl_3$ 125.77 MHz)	: 182.8 (C-1), 146.0 (C-2), 139.1 (C-3), 189.1 (C-4), 162.0 (C-OH), 124.5 (C-6), 148.9 (C-7), 22.5 (7-CH_3), 121.5(C-8), 131.9 (C-9), 113.4 (C-10), 184.3 (C-1'), 139.7 (C-2'), 139.0 (C-3'), 190.0 (C-4'), 159.4 (C-OH), 129.1 (C-6'), 146.7 (C-7'), 21.3 (7-CH3), 121.0 (C-8'), 131.6 (C-9'), 113.2 (C-10'),
HR-MS (EIMS) (m/z) (%)	: 374 ($M^{+\cdot}$ 100%), 359 (20), 328 (14), 187 (16), 163(12), 134 (18), 106 (54) and was found to be a dimer.

It was calculated for $C_{22}H_{14}O_6$ and found C.70.59%, H. 3.77% and O. 25.64%.



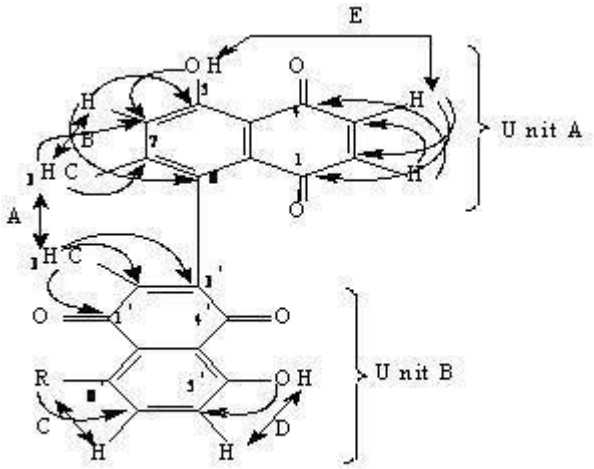
NOESY and HMBC correlations of DOR-08

DOR- 09 (0.070 g, 8'hydroxyisodiospyrin)

On silica gel eluting with $CHCl_3$-MeOH (99:1) afforded a more polar eluate that on crystallization using benzene yielded 0.070 g of red crystals, m.p 275-277 °C with R_f 0.12 (benzene).

$[\alpha]_D^{23}$ (dioxan, *c* 0.1)	: -72 ° (laevorotatory)
UV λ_{max} nm (log ε)	: 253 (4.24), 432 (3.84), 460 (3.92), 4.84 (3.91) 554 (3.66)
^{1}H-NMR ($\delta CDCl_3$, 500.13 MHZ)	: 6.78 *d* (15.7) H-2, 6.95 *d* (15.7), 12.35 *s* 5-OH, 7.30 *m* H-5, 2.20 *s* 7-CH_3, 1.87 *s* 2-CH_3, 12.32 *s* 5'-OH, 7.29 *m* H-6', 7.29 *m* H-7', 12.67 *s* 8'-OH,
^{13}CNMR (δ $CDCl_3$ 125.77 MHz)	: 184.9 (C-1), 139.9 (C-2), 138.2 (C-3), 190.1 (C-4), 162.3 (C-5OH), 125.9 (C-6), 146.8 (C-7), 20.8 (7-CH_3), 127.2 (C-8), 129.3 (C-9), 114.2 (C-10), 186.7 (C-1'), 147.3 (C-2'), 13.6 (2'-CH_3), 143.0 (C-3'), 158.9 (C-5'OH), 129.9 (C-6'), 129.9 (C-7'), 158.9 (C-8'OH)), 112.3 (C-9').
LC-EIMS, m/z (%)	: 390 (M^+, 100%), 359 (23), 127 (14), 121 (18), 115 (43), 97 (15), 95 (14), 94 (28), 92 (60), 92 (78), 77 (22), 76 (21), 69 (23)

Calculated for $C_{22}H_{14}O_7$ and found C. 70.59%, H. 3.77% and O. 30.81%.



R=OH

NOESY and HMBC correlation of DOR-09

DOR- 10 (0.124 g, Umbelliferone)

On silica gel eluting with $CHCl_3$-MeOH (99:1) afforded a more polar eluate that on crystallization using ethylacetate yielded 0.124 g of colourless prisms, m.p 229-231 °C.

Analysed for the Molecular formula $C_9H_6O_3$.

UV λ_{max}	: 206, 325 nm.

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IR ν $^{cm-1}$	: 3159, 1681,1620, 1568,1322,1235,1134and 835 cm^{-1}.
Mass spectrum at EIMS (70 ev) m/z (%)	: 162 (100), 134 (81), 51 (21).
NMR spectrum (400 MHz, in DMSO-$_{d6}$)	: **δ** 6.19 (d,*j*= 9.4 Hz, 1H), 7.93 (d,*j*= 9.4 Hz, **δ** 7.52 (1H, d,*j* = 8.4 Hz, H-5), **δ** 6.87(1H, dd, *j* = 0.2, 8.4 Hz, H-6) and **δ** 6.70 (1H, d, *j* = 0.16 Hz, H-8)
^{13}CNMR spectra (100MHz.DMSO)	: **δ**161.3, 144.5, 111.4, 129.7, 113, 1, 160.4, 102.1, 155.5.

4. DISCUSSIONS:

The chemical examination of the roots of *D. oocarpa* afforded ten coumpounds on column chromatography and repeated crystallizations, Lupeol (**DOR-01**), 5-Hydroxy-4-methoxy-2-naphthaldehyde (**DOR-02**), 4-Hydroxy-5-methoxy-2-naphthaldehyde (**DOR- 03**), 4-Hydroxy-3, 5-dimethoxy-2-naphthaldehyde (**DOR- 04**), β-sitosterol, (**DOR-05**), Plumbagin (**DOR- 06**), Betulinaldehyde (**DOR-07**), Diospyrin (**DOR-08**), 8'hydroxyisodiospyrin (**DOR- 09**), Umbelliferone (**DOR-10**). All these compounds are characterized by conventional chemical tests, physical properties and spectroscopic methods like UV, IR, NMR, ^{13}C NMR and MASS. Out of these compounds, **DOR-1, DOR-4** and **DOR-7** belong to triterpene groups and **DOR-2**, **DOR3, DOR-4, DOR-6, DOR-8** and **DOR -9** are napthoquinones. The compound **DOR-10** named as umbelliferone, interestingly is a coumarin class. Occurrence of napthoquinones is very common in *Diospyros* species. The author could isolate these quinines from this species also and thus justified the species. The compound diospyrin is also frequent in the genus *Diospyros* and thus serving as chemotaxonomic marker of the genus. It is also interesting to note that three napthaldehydes were also isolated from this species. All these three aldehydes, 5-Hydroxy-4-methoxy-2-naphthaldehyde (DOR-02), 4-Hydroxy-5-methoxy-2-naphthaldehyde (**DOR- 03**), 4-Hydroxy-3, 5-dimethoxy-2-$naphthaldehyde (**DOR- 04**) are the first time report from this species. Distribution of napthaldehydes in nature is very rare and this report of occurrence is new to the literature. The compound (**DOR-07**) betulinaldehyde was isolated in very good quantity i.e upto 5% yield.

5. ACKNOWLEDGEMENTS:

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Conflict of Interest: None Declared

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