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ORIGINAL ARTICLE

A Novel Compound Lup-20 (29)-ene-3α,6β-diol Identified in Petroleum Ether Extract of *Diospyros melanoxylon* Roxb. Leaves and to Reveal Its Antidiabetic Activity in Rats

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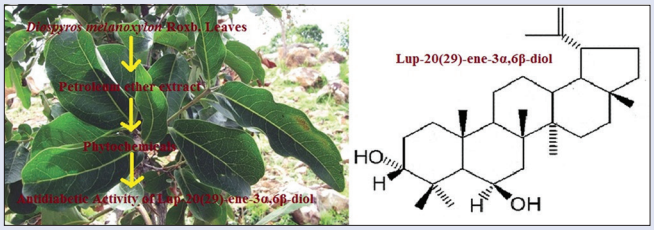
ABSTRACT

Background: *Diospyros melanoxylon* Roxb. belongs to the family *Ebenaceae* and its leaves show diuretic, carminative, laxative, and styptic bioactivities. **Objective:** The objective of this study was to give a direction for future investigators to carry out research on phytochemistry and develop a potent antidiabetic agent. **Materials and Methods:** Petroleum ether extract of *D. melanoxylon* Roxb. leaves was chromatographed over silica gel column. Isolated compounds were elucidated on the basis of different spectroscopic techniques (mass spectrometry, infrared, 1H, 13C nuclear magnetic resonance, and distortionless enhancement by polarization transfer). Antidiabetic activity of lup-20 (29)-ene-3α,6 β-diol was evaluated in streptozotocin diabetic rats. **Results:** A novel compound, lup-20 (29)-ene-3α,6 β-diol (6), was identified along with 11 known natural compounds, namely, lupeol (1), ceryl alcohol (2), octacosanol-1 (3), hentriacontanol-1 (4), β-sitosterol (5), diospyrin (7), 3α-methoxydiospyrin (8), betulin (9), ursolic acid (10), betulinic acid (11), and oleanolic acid (12). Further administration of lup-20 (29)-ene-3α,6 β-diol significantly improved the diabetes-induced oxidative stress. **Conclusion:** Investigation of this novel compound plays an important role in the field of drug development.

Key words: Antidiabetic, biological activities, *Diospyros,* Ebenaceae, phytochemicals, streptozotocin

SUMMARY

- A novel compound, lup-20(29)-ene-3α,6β-diol was identified with 11 other known natural compounds from petroleum ether extract of Diospyros melanoxylon Roxb. Leaves. Antidiabetic activity of lup-20(29)-ene-3α,6β-diol was evaluated in Streptozotocin diabetic rats. Administration of this natural compound significantly improved the diabetes induced oxidative stress.



Abbreviations used: STZ: (Streptozotocin); LPO: (Lipid Peroxidation); SOD: (Superoxide Dismutase); CAT: (Catalase); GSH: (Reduced Glutathione).

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INTRODUCTION

Plants of *Ebenaceae* are widespread chiefly in tropics and subtropics, occasionally extending into temperate areas. According to Hegnauer,[1] the family comprises of seven genera, namely *Diospyros*, *Euclea*, *Maba*, *Oncotheca*, *Rhaphidanthe*, *Royena,* and *Tetraclis*. The genus *Diospyros* is by far the largest with 500 species,[2] of which 41 species are found in India, mostly in the evergreen forests of Deccan, Assam, and Bengal. A few of them also occur in North India.[3] The genus *Diospyros* is also of great economic importance, with many species yielding edible fruits, ebony, and valuable timers.[4,5] A number of *Diospyros* species are reputed for their local herbo- medicinal uses.[6,7] Several parts of the plant have been used for a long time in the treatment of asthma, abdominal pain, dysentery, leprosy, whooping cough, menstrual troubles, and as antibiotics. *Diospyros melanoxylon* Roxb. (syn. *D. tupru*) belongs to the family *Ebenaceae* and commonly known as Tendu. It is a middle-sized deciduous tree reaching a height of 15 m. Different parts of this plant are used in various medicinal purposes. Its leaves are used for wrapping bidis. Their flavor, flexibility, and resistance to decay are the key factors in making their use valuable in the bidi industry. Its leaves also show diuretic, carminative, laxative, and styptic properties. Dried flowers of *D. melanoxylon* are recorded in Unani medicine for urinary discharges, inflammation of spleen, and enrichment of blood. Seeds of the plant find use in native medicine in dysentery. Bark of the plant is an astringent and its decoction is used in diarrhea and dyspepsia. A dilute extract of bark is used as an astringent lotion for eyes.[8]

MATERIALS AND METHODS

General experimental procedures

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative and quantitative thin-layer chromatography was conducted on an aluminum sheet (Kieselgel 60 F254 [E. Merck]). Silica gel (E. Merck, 60–120 mesh, 550 g) was used for column chromatography (1.5 m × 4.0 cm). The infrared (IR) spectra were recorded on FTIR SHIMADZU 8400S spectrometer with

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KBr pellets. The 1H and 13C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ at 300 MHz and 75 MHz on a Brucker NMR instrument, respectively, using tetramethylsilane as internal standard. Fast atom bombardment (FAB) mass spectra were recorded on JEOL S × 102/DA-6000 mass spectrometer using argon/xenon as FAB gas.

Plant material

The plant material (leaves) of *Diospyros melanoxylon* Roxb. was collected from Jhalawar district of Rajasthan (India) and the authenticity was confirmed by the In-charge of Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen of the plant leaves was deposited at the Herbarium of the University (voucher no. RUBL-20111).

Extraction and isolation of the constituents

The shade-dried leaves (3.0 kg) were fine powdered and extracted with petroleum ether for 72 h on a water bath. The extract was filtered hot and the solvent was removed under reduced pressure where a semi-solid greenish mass (7.0 g) was obtained. The solvent-free extract was chromatographed over silica gel column. The column was eluted with different solvents in order of increasing polarity where 12 compounds were isolated, purified, and characterized with one novel compound, the details of which are as follows:

Lup-20 (29)-ene-3α,6 β-diol (6), $C_{30}H_{50}O_2$, colorless crystals, 180 mg, m. p. 190°C–191°C. Mass spectrometry (MS) (m/z) 443 [M + H] + ($C_{30}H_{50}O_2$), 425 [M-H_2O] +, 407 [425-H_2O] +, 392 [407-Me] +, 377 [392-Me] +, 362 [377-Me] +, 347 [362-Me] +; IR (KBr) ν_{max}, 3350-3310 (O-H str), 2950-2850 (C-H str.), 1640 (C = C str.), 1450, 1380, 1200, 1050 (C-O str.), 880, 550 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 4.64 (s, 1H, H-29a), 4.53 (s, 1H, H-29b), 4.40 (dd, 1H, H-6*α*, *J* = 5.3, 6.03 Hz), 3.60 (dd 1H, H-3 β, *J* = 4.9, 4.5 Hz), 2.35 (m, 1H, H-19), 1.61 (br, s, 3H, H-30), 1.2 (s, 3H, H-28), 0.97 (s, 3H, H-27), 0.95 (s, 3H, H-26), 0.85 (s, 3H, H-25), 0.81 (s, 3H, H-24), 0.76 (s, 3H, H-23), 1.30–1.55 (m, remaining 22 protons).

Animals

The present study was approved by Institutional Animal Ethics Committee, Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, India. The Indian National Science Academy (INSA) guidelines were maintained (CPCSEA 1678/Go/a/12) throughout the experiment for the use of animals (INSA, 2000). Colony-bred male albino rats of "Wistar strain" weighing 200 ± 10 g were divided into six groups with six rats in each group and allowed free access to food and water *ad libitum*.

Induction of experimental diabetes

Streptozotocin (STZ; Sigma, USA; 55 mg/kg body weight) was dissolved in 0.1 M sodium citrate buffer (pH 4.5) just prior to use and injected intraperitoneally to overnight-fasted rats. Control rats (Groups I, IIIa, and IVa) received an equivalent volume of citrate buffer. 72 h after STZ administration, blood was collected from tail vein and rats with serum glucose >250 mg/dl were randomized to three groups (Groups II, IIIb, and IVb).

Blood, serum, and tissue biochemical analysis

The lup-20(29)-ene-3*α*,6 β-diol was dissolved in 0.5 ml olive oil for oral dosage given to the rats for 21 consecutive days. Following the last dose administration, animals were overnight fasted, sacrificed, and blood was collected by cardiac puncture for serum separation. Serum was analyzed for glucose, protein,[9] albumin,[10] and insulin (Radioimmunoassay, Merck, Japan).

Pancreas was quickly removed and placed in cold saline solution, trimmed off adipose tissue, finely minced, homogenized and analyzed for protein,[9] lipid peroxidation (LPO),[11] and antioxidant defense system assays such as superoxide dismutase (SOD),[12] catalase (CAT),[13] and reduced glutathione (GSH).[14]

Statistical analysis

Results are expressed as mean ± standard error of mean and comparison among groups was performed using ANOVA (AnalystSoft StatPlus, Version 4.7.5.0, UK). The level of significance was set at $P < 0.01$ and $P < 0.001$ was regarded to be highly significant.

RESULTS AND DISCUSSION

Identification of Lup-20 (29)-ene-3α,6 β-diol

Compound 6 was isolated as colorless crystals, m. p. 190°C–191°C. It was analyzed for its molecular formula as $C_{30}H_{50}O_2$. It answered Liebermann–Burchard and Noller tests for triterpenoids. Its IR spectrum displayed absorption bands for hydroxyl (3350–3310 cm^{-1}) and vinylidene groups (1640, 880 cm^{-1}). In its 1H NMR spectrum (300 MHz, $CDCl_3$), a pair of broad singlets at δ 4.64 and 4.53 integrated for each proton and a broad singlet δ 1.61 integrated for three protons of olefinic methyl group which indicated the presence of lup-20 (29)-ene system in the compound. A multiplet at δ 2.30 was ascribable to the H-19 proton of the cyclopentane ring. A doublet of doublets appeared at δ 3.60 (*J* = 4.9, 4.5 Hz) was assigned to the H-3 β oxymethine proton while another doublet of doublets at δ 4.40 (*J* = 5.3, 6.03 Hz) was attributed to the hydroxyl methane proton (H-6*α*). The orientation of hydroxyl groups at C-3 and C-6 position was ascertained by comparing chemical shift and coupling constant data of respective hydroxymethine protons with the literature values of pentacyclic triterpenes.[15] Singlets at δ 0.76 (3H), 0.81 (3H), 0.85 (3H), 0.95 (3H), 0.97 (3H), and 1.2 (3H) were exhibited by the six tertiary methyl groups. The sequence of protons was established by spin decoupling experiments. A detailed assignment of the various peaks in the 1H NMR spectrum is summarized in Table 1. The 13C NMR spectrum (75 MHz, $CDCl_3$) displayed thirty signals suggesting the presence of triterpene skeleton in the compound. The nature of various carbon atoms was determined by DEPT experiment

Table 1: 1H- and 13C-NMR data of compound 6 in $CDCl_3$ δ in ppm. *J* in Hz

Position	(δ) H	(δ) C
3	3.60 (*dd*, 1H, *J*=4.9, 4.5 Hz)	78.8 (d)
6	4.40 (*dd*, 1H, *J*=6.03, 5.3 Hz)	79.0 (d)
19	2.30 (*m*, 1H)	47.9 (d)
23	0.76 (*s*, 3H)	27.9 (q)
24	0.81 (*s*, 3H)	15.3 (q)
25	0.85 (*s*, 3H)	16.1 (q)
26	0.95 (*s*, 3H)	15.9 (q)
27	0.97 (*s*, 3H)	14.5 (q)
28	1.20 (*s*, 3H)	18.2 (q)
29	4.64 (*s*, 1H, H-29a) 4.53 (*s*, 1H, H-29b)	109.3 (t)
30	1.61 (br, *s*, 3H)	19.2 (q)
1, 2, 4, 5, 7-18, 20-22	1.30-1.50 (*m*, remaining hydrogen)	38.8 (t, C-1), 27.4 (t, C-2), 38.6 (s, C-4), 55.2 (d, C-5), 34.2 (t, C-7), 40.7 (s, C-8), 50.3 (d, C-9), 37.1 (s, C-10), 20.8 (t, C-11), 25.1 (t, C-12), 38.0 (d, C-13), 42.8 (s, C-14), 27.4 (t, C-15), 35.5 (t, C-16), 42.9 (s, C-17), 48.2 (d, C-18), 150.9 (s, C-20), 29.8 (t, C-21), 39.9 (t, C-22)

which shows the presence of $7CH_3$, $10CH_2$, 7CH, and 6 quaternary carbon atoms. The detailed 13C NMR values are summarized in Table 1. Its mass spectrum (ESIMS) exhibited reasonably strong molecular ion peak at m/z 443 [M + H] + corresponding to its molecular formula $C_{30}H_{50}O_2$. Other significant peaks were at 425 [M-H_2O] +, 407 [425-H_2O] +, 392 [407-Me] +, 377 [392-Me] +, 362 [377-Me] +, 347 [362-Me] +, etc.

The 1H NMR values of this compound were compared with that of isomeric known triterpene, i.e., 20 (29)-lupene-3 β,6 β-diol isolated from *Pleurostylia opposita*,[16] it was noticed that the chemical shift values of this dihydroxy triterpene were very close to that of compound 6 except the values of chemical shift of methane proton under oxygen function at position C-3. In all-known triterpenes of lupine series, where hydroxyl group at C-3 is *β*-oriented than the chemical shift of H-3*α* is always around δ 3.16. In case of *α*-oriented hydroxyl group, the position of H-3 β is always comparatively higher field with changed coupling constants. Hence, compound 6 has *α*-oriented hydroxyl group at C-3 position. From the above spectral data, the compound was characterized as lup-20(29)-ene-3*α*,6 β-diol [Figure 1]. This is the first report of the occurrence of this triterpene in nature.

Antidiabetic activity of Lup-20 (29)-ene-3α,6 β-diol

Serum glucose levels in control rats (Group I) and in lup-20 (29)-ene-3*α*,6 β-diol-treated control rats (Groups IIIa and IVa) were almost similar while Group II diabetic rats showed a highly significant increase. Following lup-20 (29)-ene-3*α*,6 β-diol administration, serum glucose level was markedly lowered. Serum total protein, albumin, and insulin levels of diabetic rats were found to be decreased while lup-20 (29)-ene-3*α*,6 β-diol administration brought about a significant increase in treated diabetic rats ($P < 0.001$). Serum total protein, albumin, and insulin levels of the Groups IIIa and IVa were also higher than that of Group II [Table 2].

Table 3 shows significant reduction in the pancreas protein, GSH, SOD, and CAT contents in diabetic rats (Group II). Treatment with lup-20(29)-ene-3*α*,6 β-diol dose dependently increased the pancreatic protein, GSH, SOD, and CAT contents. Control rats treated with lup-20(29)-ene-3*α*,6 β-diol also showed increase in pancreatic protein content as compared to Group I controls. Pancreas LPO level in diabetic rats was increased significantly as compared to Group I control rats,

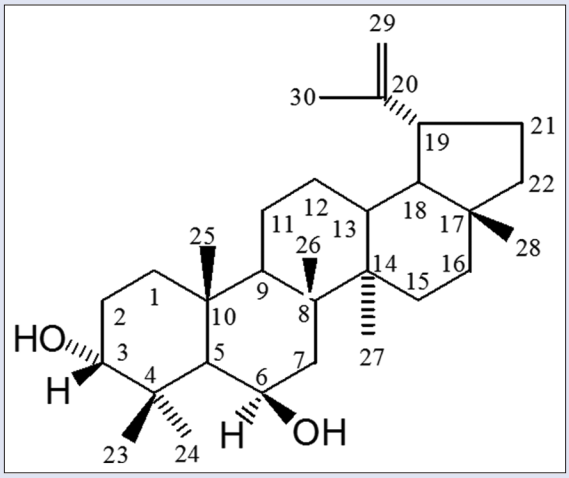

Figure 1: Structure of compound 6 isolated from *Diospyros melanoxylon*

Table 2: Effect of lup-20 (29)-ene-3*α*, 6*β*-diol on serum biochemical parameters in streptozotocin diabetic rat[a]

Group	Serum glucose (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)	Serum insulin (μU/ml)
Control (Group I)	86.78±6.35	10.23±0.66	5.47±0.21	17.86±0.56
Diabetic (Group II)	251.34±10.77**	5.87±0.21**	2.09±0.08**	6.11±0.21**
Lup-20 (29)-ene-3*α*, 6*β*-diol-treated control (15 mg/kg body weight/day) (Group IIIa)	81.27±11.63	12.28±0.32	5.81±0.24	18.85±0.24
Diabetic+lup-20 (29)-ene-3*α*, 6*β*-diol (15 mg/kg body weight/day) (Group IIIb)	227.21±18.31*, a+	7.23±0.12*,a+	2.78±0.09a+	09.37±0.21 (NS)a+
Lup-20 (29)-ene-3*α*, 6*β*-diol-treated control (15 mg/kg body weight/day) (Group IVa)	80.35±11.25	12.71±0.37	6.85±0.22	20.37±0.85
Diabetic+lup-20 (29)-ene-3*α*, 6*β*-diol (15 mg/kg body weight/day) (Group IVb)	152.63±19.53*, a+	8.83±0.26*,a+	3.34±0.08 (NS)a+	13.56±0.56 (NS)a+

[a]Values are given as mean±SEM from six rats in each group; diabetic group is compared with normal group; experimental groups are compared with normal and diabetic groups; values are statistically significant at *P<0.05; **P<0.001, as compared with the normal control; a+P<0.001, as compared with diabetic control. NS: Nonsignificant; SEM: Standard error of mean

Table 3: Effect of lup-20 (29)-ene-3*α*, 6*β*-diol on biochemical parameters of pancreas in streptozotocin diabetic rat[a]

Group	Protein (mg/g)	SOD (μM/mg protein)	GSH (nM/g tissue)	CAT (μM H2O2 consumed/min/mg protein)	LPO (nM MDA/mg protein)
Control (Group I)	65.47±5.14	7.45±0.21	4.65±0.24	108.89±8.21	4.63±0.18
Diabetic (Group II)	24.97±3.37**	2.34±0.34**	1.09±0.06**	47.52±6.47**	14.77±0.36**
Lup-20 (29)-ene-3*α*, 6*β*-diol-treated control (15 mg/kg body weight/day) (Group IIIa)	68.97±6.31	8.47±0.23	5.35±0.21	113.56±6.32	4.19±0.26
Diabetic+lup-20 (29)-ene-3*α*, 6*β*-diol (15 mg/kg body weight/day) (Group IIIb)	33.78±3.66a++	3.18±0.25 (NS)a++	1.86±0.14 (NS)a+	52.78±5.54 (NS)a++	10.47±0.45 (NS)a+
Lup-20 (29)-ene-3*α*, 6*β*-diol-treated control (15 mg/kg body weight/day) (Group IVa)	71.34±6.63	8.56±0.11	6.56±0.25	117.62±6.47	3.54±0.12
Diabetic+lup-20 (29)-ene-3*α*, 6*β*-diol (15 mg/kg body weight/day) (Group IVb)	38.47±3.58*,a++	4.33±0.31 (NS)a++	2.98±0.16 (NS)a+	58.36±5.47a++	8.06±0.21 (NS)a+

[a]Values are given as mean±SEM from six rats in each group; diabetic group is compared with normal group; experimental groups are compared with normal and diabetic groups; values are statistically significant at *P<0.05; **P<0.001, as compared with the normal control; a+P<0.05; a++P<0.001, as compared with diabetic control. NS: Nonsignificant; SEM: Standard error of mean; SOD: Superoxide dismutase; GSH: Glutathione; CAT: Catalase; LPO: Lipid peroxidation

whereas administration of lup-20 (29)-ene-3*α*,6 β-diol dose dependently decreased ($P < 0.001$) the pancreatic level.

CONCLUSION

Using chromatography techniques, we isolated 12 phytoconstituents and they are identified and characterized on the basis of different spectroscopic techniques. These compounds have very useful medicinal activities. This study also suggests lup-20(29)-ene-3*α*,6 β-diol as prominent antihyperglycemic agent. Hence, the present work gives a direction for future investigators to carry out research on phytochemistry so that they could get some medicinally important drugs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hegnauer R. Chemotaxonomy of plant. Vol. 4: Birkhauser Basel Publisher, Switzerland; 1966. p. 45-54.
2. Willis JC, Airy Shaw HK. A Dictionary of the Flowering Plants and Ferns. 8th ed. London: Cambridge University Press; 1973. p. 360-97.
3. Sastri BN. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Vol. 3. New Delhi: CSIR; 1952. p. 76.
4. Irvine FR. Woody Plants of Ghana. Oxford: Oxford University Press; 1961. p. 576.
5. Yan XZ, Kuo YH, Lee TJ, Shih TS, Chen CH, Mcphail DR, et al. Cytotoxic components of Diospyros morrisiana. Phytochemistry 1989;28:1541.
6. Watt JM, Breyer-Brandwijk MG. Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd ed. Edinburgh, London: E and S Livingstone Ltd.; 1962. p. 369.
7. Kokwaro JO. Medicinal Plants of East Africa. Kampala, Nairobi and Dar es Salaam: East African Literature Bureau; 1976.
8. Sastri BN. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Vol. 3. New Delhi: CSIR; 1952. p. 33.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
10. Corcoran RM, Durnan SM. Albumin determination by a modified bromcresol green method. Clin Chem 1977;23:765-6.
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
12. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
13. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
14. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
15. Rizvi SA, Sultana T. Chemical studies of a fixed oil, terpenoids and other compounds from the pods of Heterophragma adenophyllum. Planta Med 1973;23:125-31.
16. Dantanarayana AP, Kumar NS, Sultanbawa MU, Balasubramaniam S. Structure of four oxygenated lupanes from *Pleurostylia oppsita* (celastraceae). J Chem Soc Perkin Trans I 1981;10:2717-23.