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Phytochemical investigation of the hexane extract of stem bark of *Peltophorum pterocarpum* (DC.)

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ABSTRACT

The chemical investigation of the stem bark of Peltophorum pterocarpum (DC.) belonging to the family Leguminosae/Fabaceae led to the isolation of four phytoconstituents namely Stigmasterol, β-Sitosterol, Lupeol and Lupenone. The isolated compounds were characterized using various spectroscopic data as well as chemical studies. Out of the four compounds isolated, lupenone has been isolated for the first time from the plant.

Key words: *Peltophorum pterocarpum*, Leguminosae/Fabaceae, Hexane extract, Chemical constituents

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INTRODUCTION

Peltophorum pterocarpum (Copperpod, Golden Flamboyant, Yellow Flamboyant, Yellow Flame Tree, Yellow Poinciana and Radhachura in Bengali; Synonyms: *Peltophorum inermis* and *Peltophorum ferrugineum*) is a family of Leguminosae/Fabaceae native to tropical southeastern Asia and a popularly ornamental tree grown around the world. It is a deciduous tree growing to 15–25 m (rarely up to 50 m) tall, with a trunk diameter of up to 1 m. The leaves are bipinnate, 30-60 cm long, with 16-20 pinnae, each pinna with 20-40 oval leaflets 8-25 mm long and 4-10 mm broad. The flowers are yellow, 2.5-4 cm diameter, produced in large compound racemes up to 20 cm long. The fruit is a pod 5-10 cm long and 2.5 cm broad, red at first, ripening black, and containing one to four seeds. Trees begin to flower after about four years [1-2]. The plant is native to tropical southeastern Asia and northern Australasia, in Sri Lanka, Thailand, Vietnam, Indonesia, Malaysia, Papua New Guinea, Philippines and the islands of the coast of Northern Territory, Australia [1,3]. The plant is also found in different regions of India including Birbhum District, West Bengal. The wood of the plant is wide variety of uses, including cabinet-making [4] and the foliage is used as a fodder crop [1].

P. pterocarpum is a deciduous tree commonly used for ornamental purpose and as an avenue tree. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm and its flower extract is known to be a good sleep inducer and used in insomnia treatment [5-7]. Its bark is used as medicine for dysentery, as eye lotion, embrocation for pains and sores. The traditional healers use the leaves in the form of decoction for treating skin disorders. Stem infusion of the plant used in dysentery, for gargles, tooth powder and muscular pain [8]. Flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores [9]. Crude organic extracts of

different parts of this plant are reported to exhibit promising antimicrobial activities [5,8,10-15]. The aim of the present study is to isolate and characterize the phytoconstituents from the *n*-hexane extract of stem bark of *P. pterocarpum*.

MATERIALS AND METHODS

Phytochemical Investigation

General experimental procedures

All the melting points were recorded on Model No. Chemiline-715 melting point apparatus and were uncorrected. IR measurements were obtained on Perkin-Elmer (FT-IR) infrared spectrophotometer. TMS has been used as internal standard in recording ^{1}H-NMR spectra (Bruker DRX300; $CDCl_3$, 400 MHz), ^{13}C-NMR Spectrum was performed on 100 MHz instrument (Bruker DRX 300) using TMS as internal standard and EIMS Spectrum was carried out on JEOL-JMS 600 (70 eV). TLC was carried out using Silica-gel 60/ UV254 using precoated plates. Silica-gel (Marks 60-120 mesh) was used for Column Chromatography.

Plant material

The stem bark of *P. pterocarpum* was collected from Santiniketan, Birbhum District, West Bengal, India during July, 2012. It was authenticated by Dr. H.R. Choudhury, Department of Botany, Visva-Bharati, Santiniketan, West Bengal, India. A voucher specimen of the plant has been kept in the Department of Chemistry, Kulti College.

Preparation of the *n*-hexane extract

The stem bark of *P. pterocarpum* were collected and dried in shade. The dried bark was powdered (1kg) and exhaustively extracted by Soxhlet apparatus with *n*-hexane for 56 h. Then the *n*-hexane layer was decanted off. The solvent of the extract was distilled off by using rotary evaporator and the brown syrupy material thus obtained was allowed to evaporated to dryness and a brown mass (about 13.5 g) was obtained. The preliminary phytochemical studies were performed for testing the different phytoconstituents present in the n-hexane extract. The chemical tests revealed the presence of steroids and triterpenoids.

Isolation of compounds from *n*-hexane soluble fraction

The *n*-hexane soluble fraction (12 g) was dissolved in minimum volume of *n*-hexane and adsorbed onto silica gel (60-120 mesh, 36 g). After evaporation of the solvent, the resulting mass was loaded on column packed with about 190 g of Silica-gel prepared in Petroleum ether (60-80 ^{0}C). The column was then eluted with different solvents with increasing polarity starting from *n*-hexane (100%) and ending with ethyl acetate (100%). The elutions were monitored by TLC (Silica gel-G; visualization by UV 254 nm, 366 nm and Vanillin-Sulphuric acid spraying reagent heated at 110 ^{0}C).

Elutions carried out with Hexane: Pet. Ether (60-80^{0}C) in 3:2 ratio resulted a single white amorphous solid compound and designated as **Compound I** (90 mg).

Elutions carried out with Hexane: Pet. Ether (60-80^{0}C) in 1:4 ratio resulted an amorphous white solid compound and designated as **Compound II** (85 mg).

Elutions carried out with Pet. ether (60-80^{0}C): Chloroform (3:1) resulted a single white amorphous solid compound and designated as **Compound III** (69 mg).

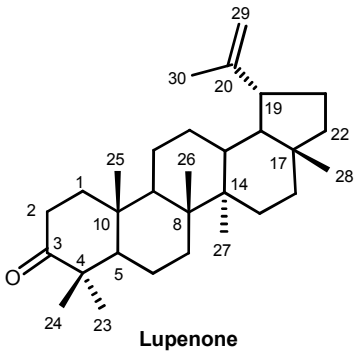
Elutions carried out with Pet. ether (60-80^{0}C): Chloroform (2:3) resulted another single white amorphous solid compound and designated as **Compound IV** (80 mg).

During elution with different solvents, compounds with very low quantity or mixtures of many compounds were collected occasionally but unable to process further analysis due to lack of infrastructures (unavailability of GC-Mass etc.) in our and nearby institutions.

CHARACTERIZATION

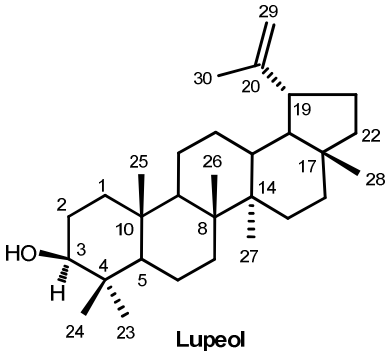
Lupenone (I): White amorphous solid; mp 167-168 ^{0}C. The compound **I** gave a positive response to Libermann-Burchard's test for triterpenoid. IR (KBr) ν_{max} 2918 cm^{-1} (aliphatic C-H stretching), 1706.4 cm^{-1} (C=O), 1641.7 cm^{-1} (C=C) and 882.4 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.76, 0.80, 0.90, 0.98, 1.08 and 1.35 (each 3H, s, Me-27, Me-25, Me-

26, Me-28, Me-23, Me-24, respectively), 1.65 (3H, br s, Me-30), 4.57 (1H, s, H-29a) & 4.69 (1H, s, H-29b), 2.32 (2H, m, H-2); ^{13}C NMR ($CDCl_3$): δ 217.9 (C-3), 151.2 (C-20), 110.0 (C-29), 55.4 (C-5), 49.8 (C-9), 48.3 (C-18), 47.9 (C-19), 47.1 (C-4), 42.9 (C-17, 14), 42.7 (C-8), 40.0 (C-22), 39.6 (C-1), 38.2 (C-13), 36.9 (C-10), 35.8 (C-16), 34.5 (C-2), 34.1 (C-7), 30.2 (C-21), 27.3 (C-15), 26.2 (C-23), 25.2 (C-12), 21.5 (C-11), 21.0 (C-24), 19.6 (C-6), 19.3 (C-30), 18.0 (C-28), 15.9 (C-26), 15.8 (C-25) and 14.4 (C-27); EIMS (70 ev): *m/z* 426 $[M+2H]^+$, 424 $[M]^+$, 409 $[M-CH_3]^+$, 408, 393 $[M-2CH_3-H]^+$, 383 $[M-C(Me)=CH_2]^+$, 313, 262, 218 $[M-C_{14}H_{22}O]^+$, 205 $[M-C_{16}H_{27}]^+$, 189 $[M-C_{16}H_{27}O]^+$, 161, 149, 135, 57 and 41.



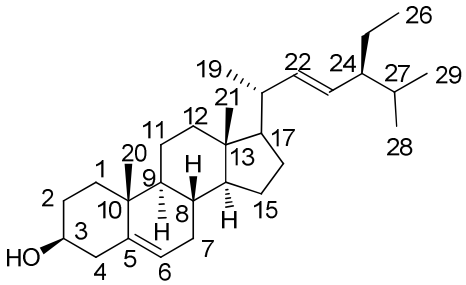
Lupenone

Lupeol (II): White amorphous solid; mp 211-213 ^{0}C. The compound **II** gave a positive response to Libermann-Burchard's test for triterpenoid. IR (KBr) ν_{max} cm^{-1}: 3327 (broad, O-H stretching), 3068, 2941, 2869, 1637 (C=C stretching), 1458, 1377, 1032, 881; ^{1}H NMR ($CDCl_3$): δ 0.76, 0.79, 0.83, 0.94, 0.97, 1.03 (each 3H, s, Me-23, Me-24, Me-25, Me-26, Me-27, Me-28), 1.68 (3H, br s, Me-30), 3.18 (1H, m, H-3), 4.57 (1H, br s, H_b-29), 4.68 (1H, br s, H_a-29); ^{13}C NMR ($CDCl_3$): δ 39.1 (C-1), 27.9 (C-2), 79.4 (C-3), 39.3 (C-4), 55.7(C-5), 18.7(C-6), 34.7(C-7), 41.2(C-8), 50.9(C-9), 37.6(C-10), 21.3(C-11), 25.6(C-12), 38.5(C-13), 43.2(C-14), 27.8(C-15), 36.0(C-16), 43.4(C-17), 48.4(C-18), 48.7(C-19), 151.4(C-20), 30.3(C-21), 40.4(C-22), 28.4(C-23), 15.8(C-24), 16.5(C-25), 16.4(C-26), 15.0(C-27), 18.4(C-28), 109.7(C-29), 19.7(C-30); EIMS (70 ev): *m/z* 426 ($[M]^+$), 425 $[M - H]^+$, 411 $[M-Me]^+$, 409 $[M - OH]^+$, 408 $[M - H_2O]^+$, 385 $[M -C(Me)=CH_2]^+$, 384, 366, 365, 219, 218, 207, 206, 189, 188, 178, 163, 135, 107, 105, 95, 79, 41.



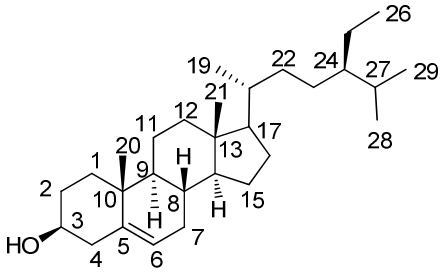
Lupeol

Stigmasterol (III): White amorphous solid; mp 169-170 ^{0}C. The compound **III** gave a red colour in Salkowski's test and a green colour in Libermann-Burchard's test specific for steroids. IR (KBr) ν_{max} 3375.4 cm^{-1} (broad, OH), 2937.8 cm^{-1} (C-H stretching in CH_3), 2837.6 cm^{-1} (C-H stretching in CH2), 1657.1 cm^{-1} (C=C stretching), 1454.5 cm^{-1} (C-H deformation in gem dimethyl groups), 1026.14 cm^{-1} (C-O stretching in secondary alcohol); ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, s, H-21), 0.87 to δ 0.91 (9H, m, H-26, H-27, H-29), 0.85 (3H, s, H-18), 0.854 (3H, s, H-19), 1.29 to 1.64 (18H, m, 9 x CH_2 and 7H, methine protons), 2.78 (1H, s, H-3), 2.01 to δ 2.39 (1H, m, OH), 5.37 (1H, s, vinylic protons), 5.31 to 5.64 (2H, m, H-22 and H-23); ^{13}C NMR ($CDCl_3$): δ 140.9 (C-5), 138.5 (C-22), 129.5 (C-23), 121.9 (C-6), 72.0 (C-3), 57.0 (C-14), 56.1 (C-17), 51.4 (C-24), 50.3 (C-9), 46.0 (C-25), 42.4 (C-13), 40.7 (C-20), 39.8 (C-12), 37.5 (C-4), 37.4 (C-1), 36.7 (C-10), 32.1 (C-8), 31.9 (C-7), 29.2 (C-16), 28.4 (C-2), 25.6 (C-28), 24.5 (C-15), 21.4 (C-21), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.1 (C-19), 12.2 (C-29), 12.1 (C-18); EIMS (70 ev): *m/z* 412 $[M]^+$, 351, 314, 300, 271, 229, 213, 55.



Stigmasterol

β-Sitosterol (IV): White amorphous solid; mp 136-139 ^{0}C. The compound **IV** gave a red colour in Salkowski's test and a green colour in Libermann-Burchard's test. IR (KBr) ν_{max} 3362.9 cm^{-1} (broad, OH)**,** 2940.8 cm^{-1} (C-H stretching in CH_3), 2833.5 cm^{-1} (C-H stretching in CH_2)**,** 1655.4 cm^{-1} (C=C stretching)**,** 1449.8 cm^{-1} (C-H deformation in gem dimethyl)**,** 1027.8 cm^{-1} (C-O stretching in secondary alcohol); ^{1}H NMR ($CDCl_3$): δ 0.85 to 1.02 (18H, m, 6 x CH_3, H-18, H-19, H-21, H-26, H-27, H-29), 1.25 to 1.5 (22H, d, 11 x CH_2), 1.60 to 2.32 (7H, m, methine protons)**,** 2.38 (1H, m, OH), 3.65, 3.68 (1H, m, C-3)**,** 5.35, 5.38 (1H, s, H-6, vinylic protons); ^{13}C NMR ($CDCl_3$): δ 140.9 (C-5), 34.2 (C-22), 26.3 (C-23), 121.9 (C-6), 72.0 (C-3), 56.9 (C-14), 56.3 (C-17), 46.1 (C-24), 50.3 (C-9), 23.3 (C-25), 42.6 (C-13), 19.0 (C-20), 39.9 (C-12), 42.5 (C-4), 37.5 (C-1), 36.7 (C-10), 32.1 (C-8), 32.0 (C-7), 28.5 (C-16), 31.9 (C-2), 34.2 (C-28), 26.3 (C-15), 12.0 (C-21), 21.3 (C-11), 29.4 (C-27), 12.2 (C-26), 19.2 (C-19), 19.6 (C-29), 36.3 (C-18); EIMS (70 ev): *m/z* 414 [M]$^+$, 351, 314, 300, 271, 229, 213, 55.



β-Sitosterol

RESULTS AND DISCUSSION

Chromatographic separation of the *n*-hexane extract of stem bark of *P. peltophorum* led to the isolation of four phytoconstituents belonging to the category of triterpenoids and steroids. They were characterized as Lupenone, Lupeol, Stigmasterol and β-Sitosterol using spectroscopic techniques like IR, ^{1}H NMR, ^{13}C NMR and EIMS as well as chemical studies. Lupenone has been isolated for the first time from this plant.

CONCLUSION

The phytochemical investigation of the *n*-Hexane extract of the stem bark of *P. peltophorum* belonging to the family Leguminosae/Fabaceae was successfully carried out. The chemical constituents isolated from this extract must account for the biological activities exhibited by the crude hexane extract of the plant. Therefore, it is now turn of the pharmacologists/biologists to explore the plant more systematically by carrying out individual bioactivity of the isolated chemical constituents. Therefore, the present work will boost the scientific communities to do more work on this important medicinal plant in near future.

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