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Phytochemical Studies on the Thorns and Leaves of *Canthium parviflorum* Lam.

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The isolation of taraxerol, d-mannitol, petunidin and long chain ester from the thorns, β-sitosterol, sakuranetin-4'-O-glycoside, long chain acids and alcohols from the leaves of *Canthium parviflorum* Lam. is being reported for the first time. The compounds were charecterized by spectral analysis and comparison with the authentic sample.

Key Words: *Canthium parviflorum* Lam., Rubiaceae, Taraxerol, Petunidin, Sakuranetin glycoside.

INTRODUCTION

Canthium parviflorum Lam. of Rubiaceae family is a rigid, thorny, deciduous shrub or small tree with stiff and spreading branches armed with stout, straight, nearly horizontal, paired spines above leaf axils. It is commonly called 'Kara' in Malayalam[1]. This plant occurs naturally on an elevation of 1000 m in western peninsular India from Gujarat and Maharashtra southwards, in Bihar, Orissa, Myanmar and in Sri Lanka[2]. The plant is sometimes used as an antispasmodic. The edible leaves and fruit constitute the Ayurvedic drug 'Kari', which is astringent and effective against cough and indigestion[1]. A decoction of the edible leaves, as well as the roots of this plant, is prescribed in certain stages of flux and the latter is supposed to have anthelmintic qualities[3,4].

Previous phytochemical screening of *C. parviflorum* leaves showed the presence of d-mannitol[5]. The chemical examination of the thorns of the plant was under taken for the first time.

EXPERIMENTAL

The thorns and leaves of *Canthium parviflorum* Lam. were collected from Trichur District of Kerala in May 2002 and was authenticated by Dr. A.K. Pradeep, Department of Botany, Calicut University.

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Column chromatographic separation of compounds from the crude extract was carried out using silica gel (60-120 mesh). IR spectra were recorded as KBr pellets using Shimadzu FTIR-8101-A spectrometer. UV spectra of the isolates were recorded using Shimadzu 1601 spectrometer. Proton NMR spectra were recorded using Bruker spectrometer at 400 MHz, 500 MHz and ^{13}C NMR at 125 MHz, in DMSO and $CDCl_3$ using TMS as internal standard. GC-MS was performed with Hewlett Packard HP 6890 series GC system with Hewlett Packard 5973 mass selective detector. DB-5 column (USA) of length 30 m with 0.32 mm internal diameter and film thickness of 0.25 mm was used. Helium was used as carrier gas at a flow rate of 2.5 mL/min. Temperature programme was 40 to 290 ºC with a heating rate of 5 ºC/min. For compound identifications Wiley 275. MS Library data was used.

Extraction and isolation of the compounds from the thorns of *Canthium parviflorum* Lam.: Shade dried and powdered thorns of *Canthium parviflorum* Lam. (2.8 kg) were successively extracted with petroleum ether (60-80 ºC, 3 × 7 L) and methanol (2 × 5 L). The solvents were removed by distillation under reduced pressure to obtain petroleum ether petroleum ether extract (110 g) and methanol extract (200 g), respectively. On standing a yellow powdery solid separated out from the petroleum ether extract. This yellow solid was subjected to column chromatography over silica gel. Elution of column with petroleum ether gave an impure solid which after recrystallization from chloroform yielded 150 mg of pure substance T_1, m.p. 78 ºC.

The crude petroleum ether extract, after the removal of yellow powdery solid, was adsorbed on 400 g of silica gel (60-120 mesh). Elution of the column with petroleum ether:ethyl acetate (4:1) gave the white crystalline solid, T_2 (1 g, 274 ºC).

The alcohol extract was suspended in water (*ca.* 300 mL) and exhaustively extracted with benzene and ethyl acetate (3 × 200 mL each). The benzene extract on column chromatography did not afford any compound. Ethyl acetate extract was chromatographed over silica gel. Fraction eluted out using 3:1 mixture of ethyl acetate-methanol on evaporation gave a pale yellow coloured compound, which upon recrystallization from hot ethanol yielded white fluffy solid T_3 (50 mg, m.p. 158 ºC).

The crude methanol extract of *Canthium parviflorum* Lam. thorns were refluxed with MeOH:conc. HCl (150 mL MeOH:40 mL conc. HCl) for 0.5 h. It was then cooled, filtered and the filtrate was twice washed with ethyl acetate to remove flavones[6]. The vine red coloured compound T_4 was then extracted with amyl alcohol and sufficiently concentrated for direct paper chromatography. It was chromatographed one dimensionally on paper (Whatmann no.1) in BAW (*n*-butanol:acetic acid:water, 4:1:5) and forestal

(HCl:acetic acid:water, 3:30:10) as eluents. The visible spectra of this compound showed bathochromic shift in the presence of 5 % alc. $AlCl_3$. The persistent vine red colour, even after heating with MeOH-conc. HCl suggested the presence of anthocyanidin.

Extraction, fractionation and isolation of compounds from the leaves of *C. parviflorum* Lam.: Shade dried finely powdered leaves of *C. parviflorum* (3.1 kg) were successively extracted with petroleum ether (60-80 ºC, 3 × 7 L) and methanol (3 × 5 L). The petroleum ether extract on column chromatography did not yield any compound.

On standing a white crystalline solid separated out from the crude methanol extract. It was recrystallized from hot methanol as white fluffy substance L_1 (1.5 g, m.p. 158 ºC). The concentrated methanol extract was filtered and the residue in the filter paper was chromatographed over silica gel. The elution of the column with petroleum ether:ethyl acetate (6:1) gave an impure solid, which after recrystallization from methanol furnished colourless needles L_2. The methanol extract was then fractionated into benzene and ethyl acetate extracts as explained earlier.

The benzene extract was chromatographed over silica gel. Fractions eluted using 8:1 mixture of petroleum ether-ethyl acetate, yielded deep orange red coloured oily mass which was dissolved in petroleum ether and a wet column was prepared. Elution of the column with 10:1 mixture of petroleum ether-ethyl acetate, gave an orange red viscous oily liquid (L_3) and was analyzed by GC-MS.

The ethyl acetate extract was subjected to chromatography over silica gel. Elution of the column with ethyl acetate furnished yellow flakes. Further, purification by preparative TLC on silica gel G plates, using 7:1 mixture of ethyl acetate-methanol as eluents yielded a yellow powdery solid L_4 (600 mg, m.p. 232 ºC).

RESULTS AND DISCUSSION

Characterization of T_1: The compound T_1 melted at 78 ºC. It gave a brown spot on spraying with H_2SO_4. These was no response to Liebermann Burchard reaction, indicating that it was not a triterpenoid or a sterol. Mass spectrum of this compound showed M^+ at m/z 522. Fragmentation pattern with a regular difference of 14 mass units showed its straight chain hydrocarbon nature. The IR spectrum gave characteristic absorption at 1736 cm^{-1} indicating the presence of ester carbonyl group and also other absorption bands at 734 and 719 cm^{-1} were indicative of a long hydrocarbon chain. The mass spectral fragmentation pattern and IR data suggested that it was a long chain ester with the molecular formula $C_{35}H_{70}O_2$.

Characterization of T_2 (Taraxerol): This compound was obtained as colourless needles (1 g), m.p. 274 ºC and give positive Liebermann-Burchard

reaction showing a persistent pink colour typical for triterpenes. Mass spectrum of T_2 showed M^+ ion at m/z 426 corresponding to a molecular formula $C_{30}H_{50}O$. The IR spectrum showed an –OH band at 3487 cm^{-1}.

The compound was identified as taraxerol by direct comparison with an authentic sample (m.m.p., TLC, ^1H NMR, ^{13}C NMR). The spectral data of the compound were found to be quite identical with those of taraxerol reported in the literature[7].

Characterization of T_3 (d-mannitol): Compound T_3 was isolated as a dull yellow coloured fluffy substance (500 mg). It was recrystallized from hot EtOH and had a m.p. of 158 ºC. It was soluble in water and failed to give positive Molisch's test. It gave brown colour with alkaline $AgNO_3$. The compound was identified as d-mannitol by m.m.p. and co-TLC with an authentic sample.

Characterization of T_4 (Petunidin): The persistent vine red colour of crude methanol extract, when heated with 2 M HCl for 0.5 h at 100 ºC indicated the presence of anthocyanidin. This on alkalification changed to blue-green and the colour slowly faded. The visible absorption in MeOH-HCl showed absorption at 544 nm and the observed bathochromic shift by the addition of 2 drops of 5 % alc. $AlCl_3$ and the R_f values ($R_f \times 100$) were in good agreement with that of the anthocyanidin, petunidin present in black grapes[8].

Characterization of L_1 (d-mannitol): The compound L_1 was identified as d-mannitol by direct comparison with the authentic sample (m.m.p., co-TLC, *etc.*)

Characterization of L_2 (β-Sitosterol): This compound was obtained as colourless needles (1.6 g) m.p. 139 ºC. It gave bluish-green colour with Liebermann-Burchard reagent. IR spectrum exibited absorption bands at 3441 (–OH), 2930, 2816, 1470, 1464, 1385 and 1383 cm^{-1}. Proton NMR specturm was identical with that of β-sitosterol. A direct comparison of R_f (0.33 in benzene) and m.m.p. with an authentic sample established its identity.

Characterization of L_3: An orange red viscous oily liquid exhibiting strong absorption at 3451 cm^{-1} in the IR spectrum indicating the presence of –OH group. It also gave absorptions at 1713 cm^{-1} (carbonyl group) and 1641 cm^{-1} (unsaturation). GC-MS analysis of the compound resulted in the identification of 13 compounds, of which 9, 12, 15-octadecatrien-1-ol (43.8 %), hexadecanoic acid (25.7 %), octadecanoic acid (6.17 %) and dodecanoic acid (4.99 %) were the major constituents.

Characterization of L_4 (Sakuranetin-4'-O-glycoside): The compoud L_4 was isolated as pale yellow powdery solid (600 mg) which melted at 232 ºC. This compound gave a yellow spot on tlc and the colour being intensified by fuming with NH_3. With Mg-conc. HCl (Shinoda test), it gave intense cherry-red colour, which indicated the flavonoid nature of the compound[9].

The compound exhibited characteristic UV absorption characteristic of flavanones at 229, 267 and 305 nm in EtOH and showed bathochromic shift with 2 N NaOH, indicating the presence of phenolic group[10]. The compound was identified as sakuranetin-4'-O-glycoside by comparison with reported spectral data[11].

IR (KBr, ν_{max}, cm^{-1}): 3466 (-OH), 2926, 1653 (due to H-bonded carbonyl group), 1491, 1363, 1091, 895. ^{1}H NMR (DMSO, δ ppm): 12.55 (1H, s, H-5), 3.66 (2H, s, -OCH_3); 8.06 (2H, d, *J* = 15 Hz, H-2' & H-6'); 6.85 (2H, d, *J* = 15Hz, H-3', H-5'); 6.34 (1H, d, *J* = 2.5 Hz, H-8); 6.11 (1H, d, *J* = 2.5 Hz, H-6); 5.2 (1H, dd, J_{trans} = 12 Hz, J_{cis} = 3 Hz, H-2); 2.7 (2H, *J* = 17 Hz, 3Hz, H-3), 5.36 (H"-1 proton of the sugar moiety); 3.54, 3.46, 3.38, 3.34, 3.23 (sugar protons); 1.24 (rhamnosyl -CH_3). ^{13}C NMR (DMSO, δ ppm): 76.2 (C-2), 41.6 (C-3), 197 (C-4), 161.06 (C-5), 99.59 (C-6), 156.3 (C-9), 102.2 (C-10), 121.1 (C-1), 131.4 (C-2', C-6'), 115.3 (C-3', C-5'), 160.2 (C-4'), 68.2 (-OMe). EIMS (70 eV): m/z 286 (aglycone), 285 (aglycone-1)$^+$, 269 (aglycone-OH)$^+$, 258 (aglycone-CO)$^+$, 241 (aglycone-CO-OH)$^+$, 229, 213, 161, 121 (retro-Diels-Alder cleavage with H. transfer), 105 and 93.

Based on above chemical and spectral evidences, L_4 was identified as sakuranetin-4'-O-glycoside.

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REFERENCES

1. J.A. Parrotta, Healing Plants of Peninsular India, CABI Publishing, New York, p. 615 (2001).
2. Anonymous, The Wealth of India-Raw Materials, PID, CSIR, New Delhi, Vol. 2, p. 66 (1950).
3. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, PID, CSIR, New Delhi, p. 49 (1956).
4. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, Bischensingh Mahendrapalsingh, Dehra Dun, Vol. 2, p. 1284 (1984).
5. H.K. Dutta, S.N. Ganguly and A.K. Bhattacharya, *J. Indian Chem. Soc.*, **43**, 380 (1966).
6. J.B. Harborne, Phytochemical Methods, Chapman and Hall Ltd, London, p. 61 (1973).
7. N. Sakurai, Y. Yaguchi and T. Inoue, *Phytochemistry*, **26**, 217 (1987).
8. J.B. Harborne, Phytochemical Methods, Chapman and Hall Ltd., London, p. 64 (1973).
9. P.M. Dey and J.B. Harborne, Methods in Plant Biochemistry, Academic Press, New York, Vol. 1, p. 292 (1989).
10. J.B. Harborne, T.J. Mabry and M. Helga, The Flavonoids, Chapman and Hall, London, p. 46 (1975).
11. A. Haq, A. Malik and S.B. Khan, *Polish J. Chem.*, **78**, 1851 (2004).

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