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7,4'-DIHYDROXY-3-METHYLFLAVONE-5-O-α-L-RHAMNOPYRANOSYL (1-4)-O-β-D-GLUCOPYRANOSIDE FROM THE SEEDS OF *LITSEA CHINENSIS* (LAM)

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ABSTRACT

The plant *Litsea chinensis*: Lam (NO. Lauraceae)[1], is known as Garbijour in hindi, is reported to be useful for curing diarrhoea and dysentery. Phyotochemical study of the ethyl acetate soluble part of the concentrated rectified spirit extract of the defatted, and powdered seeds of this plant, when worked up gave the flavones glycoside, which was identified as; 7,4'-dihydroxy-3-methylflavone-5-O-α-l-rhamnopyranosyl (1 → 4)-O-β-D-glucopyranoside by chemical analysis and UV, IR, ^{1}H NMR and Mass spectral studies.

Key words: *Litsea chinesis,* Flavone glycoside 7,4'-dihydroxy-3-methylflavone-5-O-α-l-rhamnopyranosyl (1 → 4)-O-β-D-glucopyranoside.

INTRODUCTION

The plant *Litsea chinesis* Lam is commonly known as Garbijour in hindi and belongs to natural order *Lauraceae*. Its bark is astringent and is useful in diarrhoea and dysentery. It also finds use as local antidote to bites of venomous animals and for dressing wounds.

It seeds on phytochemical examination revealed the presence of the flavonoidal glycoside; 7,4'-dihydroxy-3-methylflavones-5-O-α-L-rhamnopyranosyl (1 → 4) -O-β-D-glucopyranoside.

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EXPERIMENTAL

Air dried and powered seeds of the plant *Litses chinesis*: Lam was extracted with 95% ethanol. The concentrated ethanol extract was successively extracted with petroleum ether (60-80^{o}), benzene, chloroform, acetone, ethyl acetate and methanol, respectively.

The ethyl acetate solube fraction was subjected to concentration under reduced pressure and on TLC examination over (Butanol : Acetic acid : Water = 4 : 1 : 5 and iodine vapours and visualizing agent), showed a single spot. The above concentrated amorphous mass was crystallized from chloroform : methanol (1 : 1) gives brown colour product which had, mp. 206-207^{o}C molecular formula; $C_{28}H_{32}O_{14}$, IR ν (KBr) cm^{-1} 3478 (-OH), 2876 (C-Me), 1638 (α, β-unsated. > C = O), group 1614-1510 (C = C), 1064 (glycosidie C-group), UV λ_{max} (MeOH) nm: 266, 318, EMS [M^{+}] at m/z 592, 415, 445, 285, 283, 280, 120 and Retero-Diels-Alder fragment m/z, 152. 132. 93; $C_{28}H_{32}O_4$ found: C, 56.68%, H, 5.35%. It formed an acetyl derivative, m.p. 116-117^{o}C, molecular formula; $C_{44}H_{48}O_{22}$, [M^{+}] at m/z 928, found : C, 56.78%, H, 5.21%. ^{1}H NMR δ 6.75 (1H, d, J = 2Hz, H-5), δ 6.78 (1H, d J = 2 Hz, H-7), δ 6.72 (2H. d; J = 8.6 Hz.C-3', C-5'), δ 7.90 (2H, d, J = 8.0 Hz, C-2', C-6'), δ 1.80 (3H, 5, CH_3C-3), δ 2.45(3H,5,OAc-C-7), δ 2.40 (3H, 5, OAc-C-4'), δ 5.45 (1H, d, J = 7Hz.C-1" anomeric proton), δ 3.83-5.30 (10H, m, proton of glucose and rhamnose), δ 2.74-2.82 (18H, m acetoxyl protons of sugars), δ 5.38 (1H,d, J = 76 Hz); C-1"'anomeric protons), δ 0.88 (3H, d, J = 6.6 Hz-CH_3 of rhamnose protons).

The flavone glycoside was refluxed with 10% ethanolic H_2SO_4 for about 12 hours at room temperature and the mixture was then extracted with butanol. The butanol fraction, which contained the aglycon, was crystallized form $CHCl_3$: MeOH as brownish crystalline solid, m.p. 214-215^{o}C, molecular formula $C_{16}H_{12}O_5$ [M^{+}] at m/z 284 (EIMS), which was identified as 5,7,4'-trihydroxy-3-methylflavones (by Co-PC and Co-TLC with authentic sample).

The hydrolysate was filtered off. The filtrate was concentrated. Hydrolysate was found to contain glucose and rhamnose (confirmed by Co-PC and Co- TLC with authentic sample).

25 mg of the flavones glycoside was treated with CH_3 I (2 mL) and Ag_2O (25 mg) in dimethyl formamide (6 mL) at room temperature for two days. The reaction mixture was concentrated under reduced pressure and hydrolysed with 25% ethanolic H_2SO_4 when it yeilld 5-hydroxyl-3-methylflavone-4', 7-dimethyl ether, mp 199-200^{o}C, m.f. $C_{18}H_{16}O_5$, [M^{+}] at m/z 312 found : C, 69.30% ; H, 5.14% ; calc. C, 69.26, H, 5.16% and methylated sugars,

which were identified as; 2,3,4-tri-O-methyl rhamnose and 2,3,6-tri-O-methyl glucose, which confirmed that rhamnose was attached to glucose by (1 → 4) linkage and C-1 of the glucose was attached at C-5 of the aglycone.

The flavone glycoside was dissolved in methyl alcohol and treated with sodium metaperiodate, when it was found to consume 3.02 mole of periodate and liberated 1.05 moles of formic acid. This indicated that the sugars were in an equimolecular ratio of 1 : 1 and both the sugars were present in the pyranose from in the glycoside.

The glycoside in methyl alcohol was treated with volume of almond emulsion solution for 36 hours thereby confirming β-linkage between D-glucose and aglycone. It was then further subjected to hydrolysis with enzyme takadiastase, which confirmed α-linkage between L-rhamnose and D-glucose.

RESULTS AND DISCUSSION

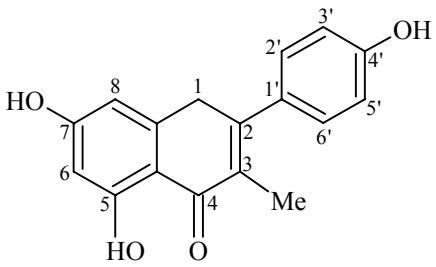
The air dried powdered and defatted seeds of *Litsea chinensis* Lam were extracted with rectified spirit and the rectifided spirit extract was concentrated under reduced pressure. Then it was fractionated with various solvents of increasing polarity by subjecting the concentrated rectified spirit extract to column chromatography. The eluates from ethyl acetate on TLC examination showed a single spot. It was thereafter crystillsed as light yellow coloured crystalline compound havine m.p. 206-207^{o}C, molecular formula $C_{28}H_{32}O_{14}$, M^{+}, m/z = 592. It respoaded to positive test of flavonoidal glyciside[2,3].

It displayed strong absorption bands at 3468 (OH), 1657 (α, β. Unsaturated > C = O group) 2876 (C-CH_3 group) and 1058 cm^{-1} (glycosidic C-O group).

The wavelength of maximum absorbance as observed in the UV spectrum of the glycoside were at 266 nm and 310 nm (MeOH), Characteristic bathochromic shift of 46 nm and 18 nm in band I & II on addition of $NaOCH_3$ showed the prersence of free-OH groups at C-4' and C-7 position in the flavonoidal glycoside[4].

On acid hydrolysis with 7% H_2SO_4, the glycoside gave L-rhamnsode and D-glusoce (identified[5] by Co-PC and Co-TLC with authentic samples) as sugar moieties along with the aglycone m.p. 214-215^{o}C, molecular formula $C_{16}H_{12}O_5$, M^{+} = 284 (found C 67.54% H. 4.24%, calculated C = 67.64%, H = 4.27%).

The aglycone was identified as; 5,7,4'-trihydroxy-3-methylflavones by comparison of its spectral data as well as superimposable spectral analysis with authentic sample.



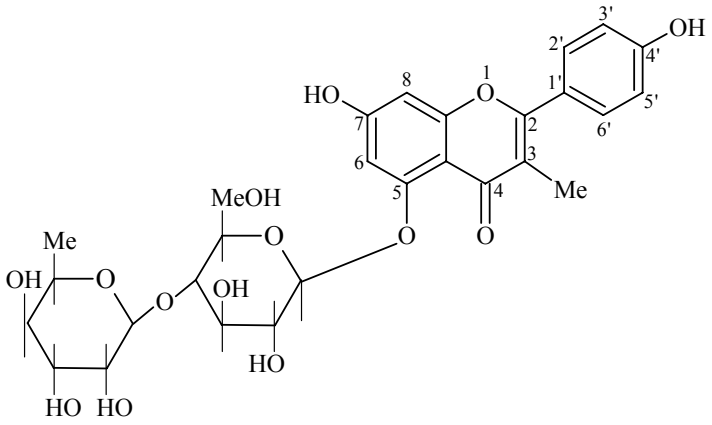
The aglycone also displayed a bathochromic shift of 45 nm in band I on addition of $AlCl_3$ (with respect to MeOH), which did not change on addition of HCl suggesting free OH group at C-5 position in it, where as it was not free in the glycoside; so it must be involved in glycosilation in the flavone glycoside. The flavone glycoside on acetylation with acetic anhydride and pyridine gave a crystalline octaacetate, mp 114°-115°C, molecular formula $C_{44}H_{48}O_{22}$, [M^+] at 928. It's ^1H NMR spectrum showed the presence of doublets at δ 6.70 (2H, d, J = 7.8 Hz) and δ 7.89 (2H, d, J = 8.6 Hz) suggesting ortho coupled portions at C-3', C-5' and C-2', C-6' respectively in the ring B. The ^1H NMR spectrum gave singlet at δ 1.80 (3H, s) of three proton intensity for one methyl group at C-3 position. Two doublets at δ 6.64 (1H, d, J = 2Hz) and 6.76 (1H, d, J = 2 Hz) were due to presence of to H-6 and H-8 portions. Other two singlets each of three proton intensity were recorded at δ 2.45 and δ 2.40 which were due to phenolic acetoxyls at C-7 and C-4' position. The multiples of 18 proton intensity at δ 2.70-2.90 integrated for sugar acetoxyls were also observed. The signals for anomeric proton was observed at δ 5.45 (J = 7 Hz) and δ 4.35 (J = 7.6 Hz) corresponding for glucose and rhamnsoe respectively and a doublet at δ 0.80 (J = 6.6 Hz) integrating for methyl of rhamnosyl proton.

The peaks in the mass spectrum of, the flavone glycoside [M^+] at m/z 592 and other peaks were in agreement with structure assigned to it. The disaccharide unit from the molecular ion showed fragment ion peak at m/z 284, 283, 282, 121 and the Retro-Diels-Alder fragmentation pattern was due to formation of ion peaks at m/z 152, 132, 93. These fragmentation pattern supported the presence of methyl group at C-3 and one hydroxyl group in ring A and one hydroxyl group in ring B. Permethylation[6] of the glycoside with 25% H_2SO_4 gave 5, hydroxyl 3-methylflavones 4', 7'-dimethyl ether m.p. 199-200°C molecular formula $C_{18}H_{16}O_5$, [M^+ m/z 312]. It's identity was confirmed by comparison of the spectral data with those of the known sample. The methylated sugars were identified as 2,3,4-tri-O-methyl rhamnose and 2,3,6-tri-O-methyl glusoce (Co-PC and Co-TLC)', which confirmed that C-1 of the glucose was attached to C-5 of the aglycone and there was (1 → 4) linkage between L-rhamnone and D-glucose[7].

Periodate oxidation of the glycoside indicated the pyranose fomm of both the sugars[8]. Enzymatic hydrolysis of the glycoside with alomond emulsion liberated L-rhamnose and D-

glucose indicating that L-rhamnose was attached to D-glucose through β-linkage and hydrolysis with takadiastase indicated the presence of α-linkage between D-glucose and the aglycone. Quantitative estimation of sugar indicated that both the sugars were in an equimolecular ration of 1 : 1 per mole of the aglycone[9].

All the nabove evidences, led to the identification of the glycoside as; 7,4'-dihydroxy-3-methyl flavone-5-O-α-L-rhamnopyranosyl (1 → 4)-O-β-D-glucopyranoside.



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