International Journal of Ayurveda and Pharmaceutical Chemistry
www.ijapc.com

IJAPC

VOLUME 11 ISSUE 1 2019

E ISSN 2350-0204

GREENTREE GROUP PUBLISHERS

Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Anti-inflammatory Activity of a New Flavone Glycoside from the Seeds of *Tectona grandis* Linn.

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ABSTRACT

A new bio-active flavonol glycoside, m.f. $C_{35}H_{44}O_{22}$, m.p. 273-274 °C , $[M]^+$ 816 (FABMS) was isolated from ethanolic extract of seeds of *Tectona grandis* Linn. It was identified as 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavonol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside. Its structure was determined by various colour reactions, chemical degradation and spectral analysis. The new flavonol glycoside exhibited potent anti-inflammatory activity.

KEYWORDS

Tectona grandis Linn. , Verbenaceae, Flavonol glycoside, Seeds

Greentree Group Publishers

Received 13/04/19 Accepted 13/05/19 Published 10/07/19

INTRODUCTION

Tectona grandis Linn. belongs to Verbenaceae family and commonly known as "Sagun" in Hindi[1-2]. It is distributed in Konkan, W. Ghats of Bombay and Madras presidencies, Circars, Deccan, Carnatic, Central India, Burma, Malay peninsula-Sumatra Java. The plant has its utility in bronchitis, biliousness, piles, leucoderma, dysentery, anthelmintic, anti-inflammatory and diuretic activity. The present work deals with the isolation and structural elucidation of a new compound of flavonol glycoside "3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavonol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside" by various chemical degradation and spectral analysis.

MATERIAL AND METHODS

General Experimental Procedure

Melting points were determined on a melting point apparatus (JSGW, Model-3045). The IR spectra were taken on a Perkin-Elmer FT-IR spectrophtometer. ^{1}H-NMR spectra were recorded at 300 MHz using $CDCl_3$ as solvent. ^{13}C-NMR spectra were recorded at 90MHz using DMSO-d_6 as solvent.

Plant Material

The seeds of *Tectona grandis* Linn. were collected from Sagar region and identified by taxonomist, Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar, M.P. , India. A voucher specimen has been deposited in Natural Products Laboratory , Department of Chemistry of this university.

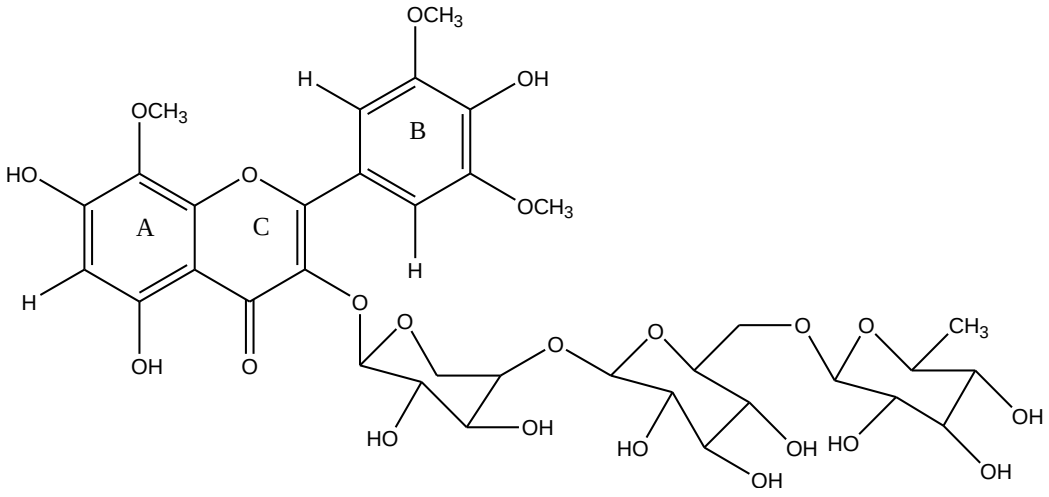
Extraction and Isolation

The powdered and air dried seeds (4 Kg) of the plant were extracted with ethanol using Soxhlet apparatus for consecutive seven days. The ethanolic extract so obtained was concentrated and dried by rotatory evaporator and further partitioned with Pet ether, $CHCl_3$, C_6H_6, EtOAc, Me_2CO and MeOH. The concentrated brown viscous mass of methanol soluble fraction obtained from ethanolic extract was examined through TLC using $CHCl_3$: MeOH:H_2O (6:4:2) in the form of solvent and Iodine vapours as indicator. The methanol soluble fraction was found to be a mixture of two compounds. The separation and purification of compound was done by column chromatography over silica gel using $CHCl_3$: MeOH as eluent. The two compounds were referred as A and B. Compound B was obtained in small amount hence rejected and further characterization of compound A was carried out. After removal of solvent and

crystallization from ether, compound A obtained with a yield of 1.95 g.

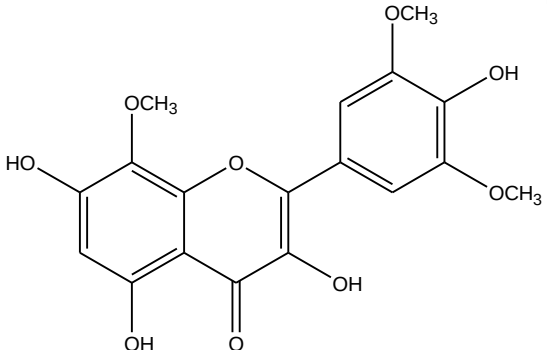
RESULTS AND DISCUSSION

Compound A obtained from methanol soluble fraction of seeds of the plant has m.f. $C_{35}H_{44}O_{22}$, m.p. 273-274°C , $[M]^+$ 816 (FABMS). The flavonoidal glycoside nature of compound A was confirmed by Molish and Shinoda test[3-4]. UV spectrum of compound A exhibits absorption bands at 366, 350, 252 nm indicating C-3-O substituted flavonol skeleton. IR spectra showed absorption band at 3480-3545 (-OH group), 1651 (-C=O), 2983 (C-H), 2872 (-OMe), 1642, 1545 (aromatic C=C), 1492-1021(O-gly), 830 cm^{-1}(Two adjacent C-atom in benzene ring). In ^{1}H-NMR spectrum of compound showed a singlet at δ 6.85 assigned for H-6 in ring A. Two doublets at δ 7.62 assigned for H-2′ and H-6′ in ring B. A singlet at δ 3.82 were assigned for 3H of –OCH_3 at C-8 position and two other singlet at δ 3.86 for methoxy protons at C-3′ and C-5′ of ring B. The signals for anomeric proton of sugars were observed at δ5.23 (1H, d, J 1.2 Hz), 5.48 (1H, d, J 7.4 Hz) and 5.68 (1H, d, J 7.2 Hz) were assigned to H-1′′′′, H-1′′′, H-1′′ of L-rhamnose, D-glucose and D-xylose, respectively. In the mass spectral details of compound A, showed characteristic fragments at m/z 816, 670, 508, and 376, were found by subsequent losses from molecular ion of each molecule of L-rhamnose, D-glucose, D-xylose linked at C-3 position. Compound A on acid hydrolysis (10% H_2SO_4) yielded aglycone A-1, m.f. $C_{18}H_{16}O_9$, m.p. 242-243 °C and $[M^+]$ 376 (FABMS) and was identified as 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxy flavone[5].



D-Xylose′′ **D-Glucose′′′** **L-rhamnose′′′′**

Permethylation was done for determining the position of sugar moiety in flavonol glycoside followed by acid hydrolysis, which yielded 2,3,4,-tri-O-methyl-L-rhamnose (R_G 1.01), 2,3,4,-tri-O-methyl-D-glucose (R_G 0.85), 2,3,-di-O-methyl-D-xylose (R_G 0.74) and 5,7,8,3′,4′,5′-hexamethoxy-3-hydroxy flavonol, showing that C-1‴′ of L-rhamnose is linked with C-6‴ of D-glucose, C-1‴ of D-glucose with C-4″ of D-xylose, and aglycone at C-3 was involved in glycosylation with C-1″ of D-xylose[5-6]. Periodate oxidation confirmed the presence of all three sugars in pyranose form[7]. Enzymatic hydrolysis of the compound A with Takadiastase enzyme liberated L-rhamnose indicating its α-linkage with proaglycone as 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavonol-3-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside. Proaglycone on further hydrolysis with enzyme almond emulsion liberated D-glucose first followed by D-xylose and aglycone as 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxy flavone[5,8]. From the above experimental evidences, the compound A is identified as,3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavonol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside.



Compound- A1

Study of Compound A

It was analysed for m.f. $C_{35}H_{44}O_{22}$, m.p. 273-274 °C , $[M]^+$ 816 (FABMS), found (%) C 51.49, H 5.91, O 43.12, Calcd. for m.f. $C_{35}H_{44}O_{22}$ (%) C 51.51, H 5.92, O 43.13. UV (MeOH) λ_{max} (nm) 366, 350, 252. IR (KBr) ν_{max} (cm^{-1})- 3480-3545 (-OH group), 1651 (-C=O), 2983 (C-H), 2872 (-OMe), 1642, 1545 (aromatic C=C), 1492-1021(O-gly), 830 cm^{-1}(Two adjacent carbon atom in benzene ring). ^{1}H-NMR (400 MHz, $CDCl_3$)- δ 12.25 (1H, s, 5-OH), 6.85 (1H, s, H-6), 7.62 (2H, d, J 2.7 Hz, H-2′,H-6′), 3.82 (3H, s, C-8 OMe), 3.86 (6H, s, C-3′-OMe, C-5′-OMe), 5.68 (1H, d, J 7.2 Hz, H-1″), 4.10-4.30 (3H, m, H-2″,H-3″, H-4″), 4.17 (2H, dd, J 6.10 Hz, 11.4 Hz, H-5″), 5.48 (1H, d, J 7.4 Hz, H-1‴), 4.45(1H, dd, J 8.2, 7.6 Hz, H-2‴), 4.28 (1H, dd, J 8.2 8.4 Hz, H-3‴), 3.93 (1H, m, H-4‴), 4.02 (1H, m, H-5‴), 4.21 (2H, dd, J 6.10, 11.2 Hz, H-6‴), 5.23 (1H, d, J 1.2 Hz H-1⁗), 4.19 (1H, dd, J 8.4 7.2 Hz, H-2⁗), 3.49 (1H, dd, J 8.3, 8.5 Hz, H-3⁗), 3.40 (1H, m, H-4⁗), 3.73(1H, m, H-5⁗), 1.21 (3H, d, J

6.1 Hz, H-6‴).^{13}C-NMR (90 MHz, DMSO-d_6)-56.6 (OMe-8), 56.3 (OMe-3′,5′), 157.6 (C-2), 138.2 (C-3),182.5 (C-4), 155.9 (C-5), 100.6 (C-6), 162.4 (C-7), 130.8 (C-8), 152.3(C-9), 106.0 (C-10), 127.5 (C-1′), 105.3 (C-2′), 150.1 (C-3′), 140. (C-4′),150.3 (C-5′), 105.2 (C-6′), 103.2 (C-1″), 76.4 (C-2″), 73.5 (C-3″), 71.3 (C-4″), 65.6 (C-5″), 101.2 (C-1‴), 80.2 (C-2‴), 75.8 (C-3‴), 70.1 (C-4‴), 74.1 (C-5‴),64.7 (C-6‴), 102.4 (C-1⁗), 72.4 (C-2⁗), 71.7 (C-3⁗), 75.3 (C-4⁗), 73.8 (C-5⁗), 17.9 (C-6⁗).

Acid hydrolysis of Compound A

Compound A (150 mg) was dissolved in ethanol (20 ml) and refluxed with 10% H_2SO_4 (15 ml) on water bath for 7-8 h. The contents of reaction mixture were concentrated, cooled and further extracted with Et_2O. The residue obtained on washing the etheral layer with water was chromatographed over silica-gel using $CHCl_3$: MeOH (7:3) that gave aglycone A-1. $BaCO_3$ was utilized for treating the aqueous hydrolysate obtained on acid hydrolysis and $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography using nBuOH-AcOH-H_2O (4:1:5) as solvent and Ninhydrin as spraying reagent which showed the presence of L-rhamnose (0.37), D-xylose (0.28) and D-glucose (0.18). Aglycone A-1 was identified as 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxy flavone, m.f. $C_{18}H_{16}O_9$, m.p. 242-243 °C and [M^+] 376 (FABMS).

Permethylation of Compound A

Compound A was refluxed for 48 h with MeI (5 ml), Ag_2O (35 mg), in DMF (7 ml) and then filtered. The filtrate was dried and again hydrolysed with 10% alcoholic H_2SO_4 for 10 h, that yielded methylated aglycone which was identified as, 5,7,8,3′,4′,5′-hexamethoxy-3-hydroxy flavonol and methylated sugars as 2,3,4,-tri-O-methyl-L-rhamnose(R_G1.01), 2,3,4,-tri-O-methyl-D-glucose (R_G 0.85), 2,3,-di-O-methyl-D-xylose (R_G 0.74).

Enzymatic hydrolysis of Compound A

Compound A (45mg) was dissolved in methanol (20 ml) and on hydrolysis with Takadiastase yielded L-rhamnose showing α-linkage proaglycone and on hydrolysis with almond emulsion yielded D-glucose and D-xylose showing β-linkage with aglycone.

In-Vitro Anti-inflammatory activity of Compound A

The anti-inflammatory activity of Compound A was determined by albumin denaturation technique[9]. The reaction mixture was prepared using 2 ml of 1% albumin fraction, 1 ml of phosphate buffer saline (pH 6.4) , 5 ml each of compound A and standard drug Diclofenac sodium of different concentration of solutions (50,

100, 200, 300, 400, 500 µg/ml) were prepared separately. Diclofenac sodium was taken as a standard drug. Double distilled water was taken as control. All the reaction mixtures were incubated for 20 minutes at 37°C and then heated at 51°C for 15 minute. After cooling, absorbance of all reaction mixtures of compound A, standard drug and control were measured at 660 nm by Systronics-2201 UV/Vis Double Beam spectrophotometer. The results of % inhibition of protein denaturation have been shown in **Table 1 & 2.**

The % inhibition of protein denaturation was calculated using following formula:-

% Inhibition = 100 × [Vt / Vc – 1] (**As per reference no. 9**)

Vt = Absorbance of test sample

Vc = Absorbance of control.

Absorbace of Control =0.320

Table 1 Effect of Diclofenac Sodium on protein denaturation

Concentration (µg/mL)	Absorbance	% Inhibition
50	0.355	10.93
100	0.395	23.43
200	0.435	35.93
300	0.452	41.25
400	0.512	60
500	0.695	117.18

Table 2 Effect of Compound A on protein denaturation

Concentration (µg/mL)	Absorbance	% Inhibition
50	0.385	20.31
100	0.412	28.75
200	0.477	49.06
300	0.572	78.75
400	0.697	117.81
500	0.710	121.87

CONCLUSION

According to our investigation, the structure of compound (A) was established as 3,5,7,4′-tetrahydroxy-8,3′,5′-trimethoxyflavonol-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside.Compound A showed significant anti-inflammatory activity, and may be used as a potent anti-inflammatory agent.

Acknowledgement

Authors are thankful to Department of Chemistry, Dr. H. S. Gour Vishwavidyalaya Sagar (M.P.) for providing necessary laboratory facilities, Department of Botany, Dr. H. S. Gour Vishwavidyalaya Sagar (M.P.) for plant identification. One of the author is grateful to UGC for financial assistance.

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