A NOVEL ACYLATED FLAVONOIDIC GLYCOSIDE FROM THE WOOD OF CULTIVATED *ACACIA NILOTICA* (L.) WILLD. EX. DELILE.

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Acacia is a fast-growing plant that has high potential in commercial plantations in tropical areas. It is already being grown as a plantation crop for building and industrial raw materials, as well as for reforestation of difficult sites. Extensive cultivation of this promising tree would enrich the natural resources besides being useful for industrial raw material, waste land management, and afforestation. In addition, *Acacia* exhibits extensive medicinal values. In view of the medicinal importance of *Acacia nilotica* and the therapeutic utility of flavonoids, an attempt has been made to isolate novel flavonoids from the wood of cultivated *A. nilotica*. The extraction of crude ethanol extract from the *A. nilotica* wood was followed by fractionation with chloroform, ethyl acetate, and methanol in increasing order of polarity of the solvent. The mixed ethyl acetate and methanol extract afforded three pure compounds through column chromtomatography and fractional crystallization. Among the isolated phenolic compounds, a new acylated flavonoidic glycoside, tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (**1**) was isolated from the wood of *A. nilotica* together with two known compounds, gallic acid and apigenin. Their structures were established by chemical evidence, spectroscopic techniques (FT-IR, ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, and ESI-MS), and by comparison with already existing spectroscopic data. The yield of novel tricin glucoside showed that it make up to 0.0786% of mixed ethyl acetate and acetone extract.

Keywords: Acacia nilotica; Flavonoids; Gallic acid; Apigenin; Tricin-4'-O-β-(6''-hydroxycinnamic)-glucoside

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INTRODUCTION

Wood is a versatile natural resource based material used for engineering and structural applications (Bhat et al. 2010). The fibrous nature of *Acacia* wood has made it one of the most suitable materials for a variety of applications (Abdul Khalil et al. 2011). *Acacia* is favored as a fast growing, medium-sized wood species used in plantation forestry programs in Asian countries, due to its rapid growth, reduced susceptability to disease, and tolerance of poor soils (Abdul Khalil et al. 2010). The timber from *Acacia spp.* can supply the raw materials required for the production of lumber, plywood, pulp and paper, particleboard, and other composite products used for heavy construction. In

addition to its versatility in applied sciences, the *Acacia* plant is rich in other natural compounds of biological importance.

Acacia nilotica (Linn.) belongs to the Leguminosae family and sub-family Mimosaceae. It is a tree 5 to 20 m height with a dense spherical crown, stems and branches usually dark or black colored, fissured bark, grey-pinkish slash, exuding a reddish low quality gum. It is a common Nigerian and West African plant reportedly native to Egypt but presently spread to the Arabian Peninsula, the Indian subcontinent, and most of Africa (Fagg 1990). It has been subjected to long term clinical trials in folk medicine (EI-Tahir et al. 1999). The bark, root, gum, leaves, and flowers have found use for skin diseases, diarrhoea, dysentery, cough, diabetes, eczema, wound healing, burning sensation, and as an astringent, demulcent, and anti-asthmatic etc. The tender twigs are used as toothbrushes. In Northern Nigeria, it is used in the treatment of malaria fever, gall bladder disease, indurations of the liver and spleen, hemorrhoids, etc. It has been used by traditional healers of different regions of India mainly in the treatment of mouth, bone, and skin cancers. In West Africa, the bark or gum is used for cancers and tumors of ear, eye, or testicles. Recently the plant was found to exhibit antioxidant (Singh et al. 2010), antimalarial (Jigam et al. 2010), and anticancer activites (Singh et al. 2009). Extensive chemical studies of the phytoconstituents of *Acacia* have been reported due to its medicinal properties. These medicinal activities have been attributed to the presence of secondary metabolites. The various parts of *A. nilotica* are rich in diverse class of compounds such as flavonoids, terpenoids, coumarins, tannins, stearic acid, ascorbic acid, carotene, crude protein, crude fiber, arabin, calcium, magnesium, and selenium (Basu et al.1947; Swain 1965; Gupta and Bokadia 1975; New 1984; Chakraborti et al. 1988; Tybirk 1989; Marohasy 1995; Sotohy et al. 1997; Seigler 2003; Singh et al. 2010; Eldeen et al 2010). Among the secondary metabolites of the plants, flavonoid represents a promising and expanding platform for biologically active natural compounds whose potential is applicable to human health care.

Keeping these considerations in view, it was considered significant to carry out a phytochemical investigation on the cultivated *A. nilotica* wood. As a part of the continuing effort to discover flavonoids, a novel acylated flavonoidic glycoside has been encountered along with gallic acid (Ebada et al; 2008) and apigenin (Harborne 1967; Duarte-Almeida et al. 2011). The present study deals with the isolation and characterization of novel acylated falvonoidic glycoside from *A. nilotica*.

EXPERIMENTAL

Plant Material and Reagents

The cultivated *A. nilotica* were collected from the Botanical Garden, Aligarh Muslim University, Aligarh, India. It was identified by Dr. Athar Ali, Taxonomist, Department of Botany, Aligarh Muslim University, Aligarh, India. The wood of *A. nilotica* (2 Kg) was collected and dried under shade at room temperature. The wood was cut into small cubes of approximately 5 cm^3. Silica gel of 60 to 120 mesh was used for different chromatographic purposes. The reagents and solvents used were mostly of LR grade. Authentic samples purchased were used as checks.

FT-IR and UV-Vis Characterization of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1) .

FTIR spectra were taken on a Shimadzu IR-408 Perkin Elmer 1800 (FTIR). Approximately 1 mg of samples were dispersed in a matrix of KBr (100 mg) and ground thoroughly in an agate mortar. The mixture was compressed by means of a hydraulic compressor to form a transparent pellet. The pellets were observed under FTIR spectrophotometer, and bands were recorded from 4000 to 400 cm^{-1}. Electronic spectra were recorded on UV-1700 PharmaSpec UV- vis spectrophotometer (Shimadzu). Data were reported as λ_{max} in nm.

NMR and Mass Spectral Characterization of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1).

^{1}H, ^{13}C and 2D NMR (HMBC, HSQC) spectra were recorded on a Bruker DRX-300 spectrometer. The required quantity of samples was dissolved in DMSO-d_6. Tetramethyl silane (TMS) was taken as internal standard. The spectra were run on δ scale (0 to 14 ppm). The recording is presented as a series of peaks. Peak areas were measured by electronic integrator, which is proportional to the number of protons. For ^{13}C NMR spectra were simply a series of singlets corresponding to each variety of carbon atoms present, and the spectra were recorded on δ scale (0 to 200 ppm).

The MS spectra were recorded on a JEOL MSD-300 spectrometer. About 1µg of sample was required for the analysis. The spectrum recorded in the form of the graph, ratio of mass to charge (m/z) versus relative abundance. The peak corresponding to each m/z value usually represents fragment of the molecule. Furthermore, Microanalysis (C,H,N) were obtained on a Carlo Erba Analyzer Model 1108.

Extraction and Isolation of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The pieces of wood (2 Kg) were kept in a round bottom flask (5 litres), which was attached with a jointed distillation tube. The wood was defatted with light petroleum ether (60–80 ^{o}C) by refluxing over water bath at 100 ^{o}C. The ether-soluble part obtained from wood was discarded, as it mainly contained chlorophyll and waxes. The wood was air dried to remove traces of petroleum ether and then extracted thoroughly with 95% ethanol in a soxhlet apparatus. The ethanol extract was evaporated to dryness under reduced pressure at 60 ^{o}C. The dark brown viscous mass (350 g) left behind was extracted successively with chloroform, ethyl acetate and methanol over water bath, in a round bottom flask (2 litres) with jointed distillation tube at 100 ^{o}C.

Thin layer chromatography (TLC) examination of ethyl acetate and methanol extracts revealed similar behavior on the TLC plates, and hence they were mixed together (210 g). Further, TLC examination of the combined extract in different solvent systems for flavonoids i.e. benzene-pyridine-formic acid (36: 9: 5 v/v), toluene-ethyl formate-formic acid (5:4:1 v/v), ethyl acetate-ethyl methyl ketone-acetic acid-water (20:3:1:1, 5:3:1:1 v/v), benzene-methanol-acetic acid (45:8:4 v/v), ethyl acetate-methanol-water (8:1:1 v/v), and chloroform-methanol (3:1 v/v) showed it to be an intricate mixture of a number of compounds. Therefore, it was chromatographed over a silica gel column. The column was eluted successively with light petroleum ether, light petroleum ether–

benzene (9:1–1:1 v/v) mixtures, benzene, benzene–ethyl acetate (9:1–6:4 v/v) mixtures, ethyl acetate, and ethyl acetate-methanol (9:1–6:4 v/v) mixtures.

Fractions showing similar behaviour on TLC plates and the same IR spectra were pooled together. Repeated column chromatography followed by fractional crystallization afforded gallic acid, apigenin, and tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (**1**).

RESULTS AND DISCUSSION

Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (**1**) (165 mg), gallic acid (53 mg), and apigenin (145 mg) were isolated from mixed ethyl acetate and methanol extract (210 g) of the wood of *A. nilotica*. The gallic acid and apigenin were identified on the basis of TLC, co-TLC, melting point, mixed melting point, and spectral data of their authentic samples.

The elemental analysis of compound **1** agreed with the molecular formula $C_{32}H_{30}O_{14}$. The glycosidic nature of the compound **1** was evidenced by its paper chromatographic behavior (Mabry et al. 1970), high solubility in water, and positive Molish test (Koch and Hunke 1948). The compound **1** gave positive test with zinc and hydrochloric acid and sodium amalgam followed by acidification, indicating its flavone or flavanone nature (Shinoda 1928).

Further yellow color with Wilson boric acid reagent (Wilson 1939) was consistent with a flavone (Mabry et al. 1970; Agrawal et al. 1989). Also, the compound showed a purple spot on a paper chromatogram, which necessarily implies a 5-OH group present in a flavone nucleus.

Spectral Data of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The compound **1** was obtained from the column with ethyl acetate-methanol (8:2 v/v) eluate and crystallized from chloroform–methanol as yellow powder (165 mg); m.p. 176–177 ^{o}C. λ_{max} nm (MeOH): 244 (band II), 350 (band I); λ_{max} nm (MeOH+NaOAc): 263, 350; λ_{max} nm (MeOH+ $AlCl_3$/HCl): 265, 350; IR (KBr), υ cm^{-1}: 3416, 1620, 1600, 1582, 1512, 1128, 1055, 1024, 889; ESI-MS m/z: 638 ($C_{32}H_{30}O_{14}$) (3.2), 492 (2.8), 330 (94.7), 315 (22.3), 300 (34.4), 272 (31.1), 181 (4.5), 178 (36.5), 153 (5.6), 152 (12.2). ^{1}H-NMR (DMSO-d_6): δ; 7.16 (s, H-3), 6.52 (d, H-6, J=2.0 Hz), 6.67 (d, H-8, J=2.0 Hz), 7.23 (s, H-2′, H-6′), 3.56 (s, 3′,5′-OCH_3), 5.32 (d, H-1″, J=7.2 Hz), 4.21 (t, H-2″, J=7.6 Hz), 3.97 (m, H-3″), 3.92 (m, H-4″), 4.33 (m, H-5″), 4.87 (d, H_a-6″, J=8.6 Hz), 5.21 (d, H_b-6″, J=8.6 Hz), 7.21 (d, H-2‴, H-6‴, J=7.8 Hz), 6.58 (d, H-3‴, H-5‴, J=7.8 Hz), 7.53 (d, H-7‴, J=16.1 Hz), 6.22 (d, H-8‴, J=16.1 Hz). ^{13}C-NMR (DMSO-d_6): δ; 162.3 (C-2), 103.6 (C-3), 181.7 (C-4), 163.4 (C-5), 102.5 (C-6), 166.3 (C-7), 96.6 (C-8), 159.8 (C-9), 106.4 (C-10), 118.2 (C-1′), 103.7 (C-2′), 151.4 (C-3′,5′), 137.2 (C-4′), 104. 6 (C-6′), 60.5 (3′,5′-OCH_3), 102.4 (C-1″), 78.2 (C-2″), 75.3 (C-3″), 71.6 (C-4″) 77.9 (C-5″), 69.4 (C-6″), 121.0 (C-1‴), 127.9 (C-2‴, C-6‴), 110.5 (C-3‴, 5‴), 155.2 (C-4‴), 130.7 (C-7‴), 128. 3 (C-8‴), 169.4 (C-9‴).

FT-IR and UV-vis Spectroscopy of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The IR spectrum showed absorption bands at 3416 cm^{-1} (OH), 1620 cm^{-1} (CO), 1600, 1582 cm^{-1}, and 1512 cm^{-1}, which indicated the presence of hydroxyl groups, carbonyls conjugated with double bonds, and aromatic rings respectively. A broad band in the region 1055 cm^{-1} and 1128 cm^{-1} was attributed to O-glycosylation.

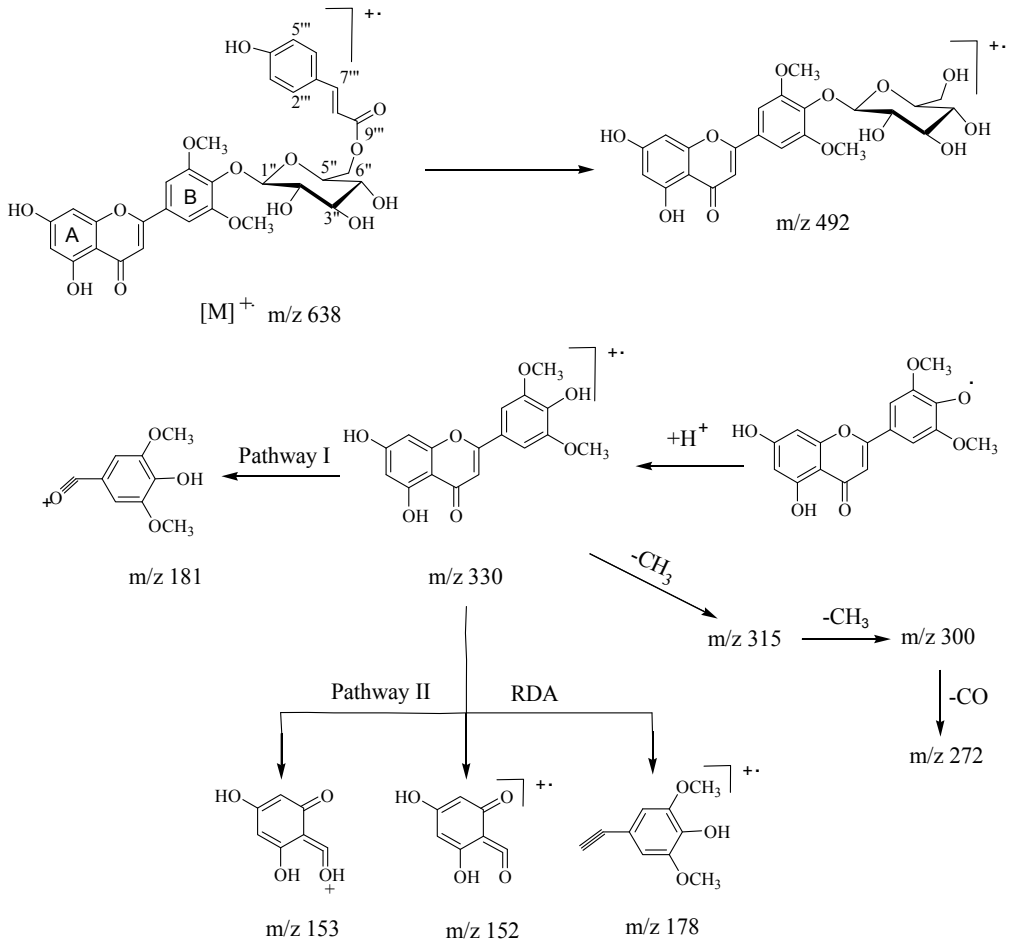
The UV bands at 244 (band II) and 350 (band I) nm in MeOH also confirms that the compound **1** belongs to the flavone group (Mabry et al. 1970; Agrawal et al. 1989). On addition of diagnostic shift reagents to the methanolic solution of the compound **1**, a bathochromic shift of 19 nm with NaOAc and 21 nm with $AlCl_3$/HCl, in band II was observed, suggesting the presence of free hydroxyl groups at C-5 and C-7 positions (Mabry et al. 1970; Markham and Mabry 1975). Furthermore, the complete acid stability of $AlCl_3$-complex together with negative borate reaction ($NaOAc/H_3BO_3$) of **1** ruled out the presence of ortho-dihydroxyl grouping in either A or B ring. The absence of a shift with NaOMe showed that there was no free hydroxyl group at 4′ position (Mabry et al. 1970). This suggests either 4′ position is occupied by some moiety or 4′-hydroxy is completely absent.

ESI-MS Spectroscopy of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The molecular formula of compound **1** was further confirmed to be $C_{32}H_{30}O_{14}$ by high resolution ESI-MS showing molecular ion peak at m/z 638 (Fig. 1). The presence of ion peaks at m/z 638 and m/z 492, in mass spectra indicate the presence of hexose moiety in the molecule. The mass spectrum of **1** exhibited base peak at 330 $[M\text{-gly}]^{+\cdot}$. The RDA (retro diel's alder) fragmentation pattern (Kingston 1971) made it possible to deduce the presence of free hydroxyls and methoxyls groups. Due to ring A, peaks appear at m/z 153 $[A_1 + H]^+$, 152 $[A_1]^{+\cdot}$ and ring B, at m/z 181 $[B_1]^+$, 178 $[B_1]^{+\cdot}$, which are indicative of the presence of two hydroxyl groups in ring A and two methoxyl and one hydroxyl group in ring B of the aglycone. The signals at m/z 315 and m/z 300 also support the presence of two methoxyl groups. Thus, the above studies confirmed the position of hydroxyl groups at C-5 and C-7 positions in ring A.

NMR Spectroscopy of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The ^{1}H-NMR spectrum displayed a signal at δ 5.32 (J=7.2 Hz), corresponding to an anomeric proton (H-1″), which again supported the O-glycosidic nature of compound **1**. The larger coupling constant confirmed hexose as a β-glucopyranoside. The compound **1** exhibited two peaks of one proton each at δ 6.67 (J=2.0 Hz) and δ 6.52 (J=2.0 Hz), which were attributed to H-8 and H-6 protons, respectively. The positions of methoxyl groups in B ring were detected as two proton singlets in ^{1}H-NMR at δ 7.23, attributed to a pair of degenerate H-2′ and H-6′ protons. This type of situation is consistent with C-3′, C-4′, and C-5′ oxygenation. The H-2′ and H-6′ protons are similar only in the case when C-2′ and C-6′ positions are occupied by identical groups, and this confirms the position of methoxy groups at C-3′ and C-5′, as well as a glucose moiety at C-4′. Also, a sharp singlet was integrated for six protons at δ 3.56, ascribed to two methoxy groups at C-3′ and C-5′. Thus, on the basis of above evidence, aglycone of compound **1**, was confirmed as 5,7,4′-trihydroxy-3′,5′-dimethoxy flavone (tricin) (Duarte-Almeida et al. 2011).



Scheme. 1. Mass Fragmentation pattern of compound **1**

The compound **1** exhibited two proton *ortho* coupled doublets at δ 7.21 (J=7.8 Hz) and 6. 58 (J=7.8 Hz) corresponded to H-2‴, H-6‴ and H-3‴, H-5‴, respectively. This justified the presence of a *p*-hydroxycinnamic moiety. Two doublets each integrating for one proton at δ 7.53 (J= 16.1 Hz) and 6.22 (J=16.1 Hz) were assigned to H-7‴ and H-8‴, suggesting the presence of *trans*-oriented conjugated vinyl hydrogens. Collectively, these assignments showed the presence of a *p*-hydroxy-*trans*-cinnamoyl moiety. Furthermore, two one-proton doublets at δ 4.87 (J=8.6 Hz) and δ 5.21 (J= 8.6 Hz) were attributed to oxygenated H_2-6″ methylene protons, and shifting of these protons signals in the deshielded region suggested that acyl group is linked to C-6″ of glucose.

The ^{13} C-NMR signal at δ 102.4 (C-1″) also indicated that the glucose unit is O-linked. The position of glucose residue is also confirmed by ^{13}C-NMR signals. The peaks for C-3′ and C-5′ appear at δ 151.4 and for C-4′ and C-7, at δ 137.2 and δ 166.3, respectively consistent with the glycosylation at C-4′ position. The presence of acyl group as *p*-hydroxycinnamic unit confirmed by ^{13}C-NMR signal, which resonate at δ 127.9 and δ 110.5 assigned to C-2‴, C-6‴ and C-3‴, C-5‴, suggesting 1,4 disubstituted phenyl group. The peaks at δ 128.3 and δ 130.7 attributed to vinylic carbons of *p*-

hydroxycinnamoyl unit. The carbonyl carbon (C-9‴) at δ 169.4, assigned to *p*-hydroxycinnamic ester.

The detailed analysis of HMBC spectrum showed ^{3}J correlation of anomeric proton (H-1″) at δ 5.32 with signal at C-4′ (δ 137.2) and ^{2}J correlation with signal at C-2″ (δ 78.2). Also, H-2′ and H-6′ (δ 7.23) showed correlation with signal at C-4′ (δ 137.2) and ^{3}J correlation with methoxy carbon (δ 60.5), indicated the position of glucose moiety at 4′ carbon (Wang et al. 2004) . The HSQC spectrum correlated all protons to their respective carbons.

Acid Hydrolysis of Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The glycoside **1** (80 mg) was dissolved in water and acidified with 2N hydrochloric acid. The reaction mixture was refluxed over water bath for four hours and left overnight at room temperature. The separated precipitate was filtered, washed well with water, and dried. On TLC examination, the precipitate displayed a mixture of two spots. The precipitate was further loaded on to silica gel column. It gave purified aglycone, which was crystallized from chloroform-methanol as yellow crystals of tricin (32 mg); m.p. 291-93^{o}C and the other spot came out to be *p*-hydroxycinnamic acid (10 mg); m.p. 213-214 ^{o}C. Their structures were confirmed by comparing melting point, mixed melting point, co-TLC, spectral values with that of authentic samples. The presence of *p*-hydroxycinnamic acid indicated that acylation was present in the compound **1** as a *p*-hydroxycinnamoyl moiety.

Identification of Sugar in Tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside (1)

The acidic filtrate, left after filtering the precipitate, was extracted with ether and then ethyl acetate to ensure the complete removal of any residual precipitate. The solution was concentrated to syrup in vacuum over NaOH pellets. The concentration was continued until the syrup was neutral to litmus paper. The syrup was chromatographed on Whatman No. 1 filter paper using n-butanol-water-ethanol (60:28.5:16.5 v/v) as solvent systems, employing descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying, were sprayed with aniline phthalate and *p*-anisidine phosphate solution. The chromatograms on drying at 100-105 ^{o}C showed the presence of D-glucose only.

UV-vis Spectroscopy of Tricin (aglycone)

The total acid hydrolysis of **1** gave tricin (aglycone), D-glucose, and *p*-hydroxycinnamic acid. The UV spectrum of aglycone again confirmed the position of a glucose moiety. The aglycone of **1** showed a bathochromic shift of 52 nm without decrease in intensity of band I by addition of NaOMe. While glucoside **1** showed no shift with NaOMe. The above bathochromic shift of band I suggests the presence of free 4′-hydroxyl in aglycone, thus indicating that D-glucose is linked to the 4′ position of glycoside **1**.

Thus, on the basis of these evidences, the structure of **1** could be assigned as tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside.

CONCLUSIONS

1. In the present study our results provide evidence that *A. nilotica* is a source of gallic acid, apigenin, and tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside.
2. Among the isolated polyphenols, tricin glucoside is present in considerable amount in cultivated *A. nilotica* wood as compared to the gallic acid and apigenin.
3. The novel tricin-4′-O-β-(6″-hydroxycinnamic)-glucoside constitutes up to 0.0786 % of mixed ethyl acetate and methanol extract, while gallic acid and apigenin make up to 0.0252 % and 0.0690 %, respectively.
4. The potential of *A. nilotica* as a source of varied new secondary metabolites is still largely unexplored and can be exploited for future prospects. Therefore, we conclude that *A. nilotica* wood in addition to engineering and structural applications, can be considered as a promising source of flavonoids.

REFERENCES CITED

Abdul Khalil, H. P. S., Bhat, I-U-H., and Awang. K. B. (2010). "Preliminary study on enhanced properties and biological resistance of chemically modified *Acacia sp*.," *BioResources* 5(4), 2720-2737.

Abdul Khalil, H. P. S., Khairul, A., Bakare, I. O., and Bhat, I-U-H. (2011). "Thermal, spectroscopic, and flexural properties of anhydride modified cultivated *Acacia spp*.," *Wood Sci Technol*., In Press.

Agrawal, P. K., Thakur, R. S., and Bansal, M. C. (1989). *Carbon-13 NMR of Flavonoids*, Agrawal, P. K. (ed.), Elsevier Science Publishers, New York.

Basu, N. M., Ray, G. K., and De, N. K. (1947). "On the vitamin-C and carotene of several herbs and flowers used in Ayurvedic system of medicine," *J. Indian Chem. Soc.* 24, 358-360.

Bhat, I-U-H., Abdul Khalil, H. P. S., Awang, K. B., Bakare., I. O., and Issam A. M. (2010). "Effect of weathering on physical, mechanical and morphological properties of chemically modified wood materials," *Mat. Design*, 31, 4363-4368.

Chakraborti, N., Mandal, L., and Banerjee, G. C. (1988). "Chemical composition of some common tree leaves," *Indian Vet. J.* 65, 145-149.

Duarte-Almeida, J. M., Salatino, A., Genovese, M. I., and Lajolo, F. M. (2011). "Phenolic composition and antioxidant activity of culms and sugarcane (*Saccharum officinarum* L.)," *Food Chemistry* 125(2), 660-664.

Ebada, S. S., Ayoub, N. A., Nasser, A., and Al-Azizi, M. (2008). "Phytophenolics from *Peltophorum africanum* Sond. (*Fabaceae*) with promising hepatoprotective activity," *Phcog. Mag.* 4(16), 287-293.

EI-Tahir, A., Satti, G. M., and Khalid, S. A. (1999). "Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*," *Phytother Res.* 13, 474-478.

Eldeen, I. M. S., Van Heerden, F. R., and Van Staden, J. (2010). "*In vitro* biological activities of niloticane, a new bioactive cassane diterpene from the bark of *Acacia nilotica* subsp. *kraussiana*," *Journal of Ethnopharmacology* 128, 555-560.

Fagg, C. W. (1990). *Acacia nilotica – Pioneer for dry lands*, Department of Plant Science, University of Oxford, UK.

Gupta, S. K., and Bokadia, M. H. (1975). "Flavonoids from the flower of *Acacia Arabica*," *Vijnana Parishad Anusandhan Patrika*, 18, 41-45.

Harbone, J. B. (1967). *Comparitive Biochem. of the Flavonoids*, Acad. Press, London.

Jigam, A. A., Akanya, H. O., Dauda, B. E. N., and Okogun, J. O. (2010). "Polygalloyl-tannin isolated from the roots of *Acacia nilotica* Del. (Leguminoseae) is effective against Plasmodium berghei in mice," *J. Medicinal Plants Res.* 4(12), 1169-1175.

Kingston, D. G. I. (1971). "Mass spectrometry of organic compounds VI. Electron-impact spectra of flavonoid compound," *Tetrahedron* 27, 2691-2700.

Koch, F. C., and Hunke, M. E. (1948). *Practical Methods in Biochemistry*, The Williams and Wilkins Co., Baltimore.

Mabry, T. J., Markham, K. R., and Thomas, M. B. (1970). *The Systematic Identification of Flavonoids*, Springer Verlag, New York.

Markham, K. R., and Mabry, T. J. (1975). *The Flavonoids*, Harborne, J. B., and Mabry, T. J. (eds.), Chapman and Hall, London, p. 48.

Marohasy, J. (1995). "Prospects for the biological control of prickly acacia, *Acacia nilotica* (L.) Willd. ex Del. (Mimosaceae)," *Pl. Prot. Quart.* 10, 24-31.

New, T. R. (1984). *A Biology of Acacias*, Oxford University Press, Melbourne.

Seigler, D. S. (2003). "Phytochemistry of *Acacia*—sensu lato," *Biochem. Syst. Ecol.* 31, 845-873.

Shinoda, J. (1928). "A new biologically active flavone glycoside from the roots of *Cassia fistula* Linn," *J. Pharm. Soc. Jpn*. 48, 214-220.

Singh, B. N., Singh, B. R., Sarma, B. K., and Singh, H. B. (2009). "Potential chemopre-vention of *N*-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica* bark," *Chemico-Biological Interactions* 181, 20-28.

Singh, R., Singh, B., Singh, S., Kumar, N., Kumar, S., and Arora, S. (2010). "Umbelliferone – An antioxidant isolated from *Acacia nilotica* (L.) Willd. Ex. Del," *Food Chemistry* 120, 825-830.

Sotohy, S. A., Sayed, A. N., and Ahmed, M. M. (1997). "Effect of tannin-rich plant (*Acacia nilotica*) on some nutritional and bacteriological parameters in goats," *Dtsch. Tierarztl Wochenschr.* 104, 432-435.

Swain, T. (1965). "The tannins," In: *Plant Biochemistry*, J. Bonner and J.Varner (eds.), Acad Press, New York, 552-580.

Tybirk, K. (1989). "Flowering, pollination and seed production of *Acacia nilotica*," *Nord J. Bot.* 9, 375-381.

Wang, H. B., Yao, H., Bao, G. H., Zhang, H. P., and Qin, G. W. (2004). "Flavone glucosides with immunomodulatory activity from the leaves of *Pleioblastus amarus*," *Phytochemistry* 65, 969-974.

Wilson, C. W. (1939). "A study of the basic acid colour reaction of flavone derivatives," *J. Am. Chem. Soc*. 61, 2303-2306.

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