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Isolation and Structural Elucidation of three Substituted Flavones from *Croton Zambesicus* (Mull) Fruit and Leaves

Mustafa S.Koya[1], Nada A. Elamin[2], Zeinab A. Elrabei[3]

[1]Department of Chemistry, Faculty of Education, university of Nyala Sudan
Corresponding Author E mail: *mustafakoya2[at]gmail.com*

[2]Department of Biology, Faculty of Education, University of Nyala, Sudan

[3]Department of Chemistry, Faculty of Education, University of Nyala, Sudan

Abstract: ***Three substituted flavones were isolated from fruit and leaves of C. Zambesicus Mull. The dried plant material was separately soxhlet extracted in 70% ethanol and then re-extracted with petroleum ether, diethyl ether and ethyl acetate. The fractions were concentrated and set to a silica gel column chromatography using different solvent systems. In our continuing phytochemical study of the medicinal plants indigenous in Sudan, we have examined phenolic constituents of Croton Zambesicus. This paper will report our first isolation of three Flavone derivatives from fruits and leaves of Croton Zambesicus, namely 5, 7, 3', 4'-tetramethoxy-flavone, apigenin-8-C-β-D-glucopyranoside (Vitexin) and 5, 7, 4'-trihydroxyflavone glycoside (Isovitexin). The structure of these compounds were elucidated based on the analysis spectrum of UV, IR, MS and NMR including ^{1}D and ^{2}D NMR.***

Keywords: *C. Zambesicus, substituted flavones, tetramethoxy flavones, Vitexin isovitxin*

1. Introduction

Medicinal plants

Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances and medicines (Cragg and Newman, 2005). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The potential for finding more compounds is enormous as to date only about 1% of tropical species have been studied for their pharmaceutical Potential (Cragg and Newman, 2005). This proportion is even lower for species confined to the tropical rain forests.

Several species of the genus Croton (Euphorbiaceae) showed excellent results when crude extracts were tested for antioxidant activity previously

Of the species tested the leaves of *C. Zambesicus*, had the best activity and was therefore selected for isolation of the active principles in this study (Koya, 2014).

Medicinally, despite , they are used for ailments such as malaria, hepatic and kidney disorders, obesity, hypertension, fever, dysentery, convulsions, snakebite, chest pains, gastrointestinal disturbances, sterility, eye and respiratory complaints (Pooley, 1993; Ngadjui et al., 2002; Suarez et al., 2006). Chemically, the genus contains very diverse compounds including alkaloids, flavonoids and triterpenes. Many structurally diverse diterpenes have also been isolated from the genus (Prozesky, 2004).

This species has not being well studied plus the interesting results reported previously by our team led to the conclusion that it contains more active principles and was reselected for further studies. The hypothesis of this study is therefore that C. *Zambesicus* contains compounds with valuable bioactivity.

Flavonoids are a group of about4000 naturally occurring polyphenolic compounds, found universally in food of plant origin (Harborne, 2000). These are primarily recognized as the pigments responsible for the colours of leaves, especially in autumn. Flavonoids are widely distributed in fruits, vegetables, nuts seeds, herbs, spices, flowers as well as tea. They are usually subdivided according to their substituent into flavanols flavones flavonones anthocyanins and Chalcones. Flavonoids display a remarkable array of biochemical and pharmacology actions viz., anti-inflammatory, antioxidant, hepatoprotective, antiviral and anticarcinogenic activities (Meena and Vida, 2008).

2. Materials and Methods

1) General procedure

Proton and ^{13}C-NMR was recorded using Mercury- 200BB (400Hz), at the department of plant sciences University of Gifu-Japan. UV spectra were measured on UV. Perkin Elmer Lambda. (Germany). I. R spectra were recorded in KBr discs using FTIR (Perkin Elymer1600).The mass spectrum were determined on FAB-MS (VG70SE) Mass electronic U.K London. All chemicals and reagents were of analytic grade and were obtained from Fisher Scientific Springfield, Sigma and Merck.

2) Plant material

The fruit and leaves of C.Zambesicus were collected from rural areas of North Kordofan state around Al-Obeid town (ALdayer Hill) in July 2014. The plant was identified by a botanist and a specimen was deposited in botany lab.

University of Nyala faculty of education. The plant samples were dried, powdered and used for extraction.

3) Extraction and Isolation

Methanol extract correspond to 30g was subjected to fractionation over silica gel VLC using EtOAC-MeOH mixture as an eluent system to yield eight fractions ($Fr._1$-$Fr._8$) after combination on the basis of TLC analysis, fraction eight (315 mg)from the first silica gel column was applied to sephadex column chromatography and eluted with the DCM- MeOH mixtures in the ratio (20, 30, 40and50%) to afford (10mg) of yellow- brown amorphous powder which washed repeatedly with acetone and coded as compound1. Combined fractions 5, 6, and7from the first silica gel column chromatography were rechromatographed on silica gel column chromatography using EtOAc-MeOH in ratio (50: 50, 70:30, 80:20, 90:10) to give five sub-fractions (Fr.a-.Fr.e). Fractions (Fr.a and Fr.b) were pooled together due t o TLC profile similarity and rechromatographed on sephadex LH-20 column chromatography, eluted with MeOH-H_2O (1:1) to afford three sub-fractions ($fr.ab_1$-$fr.ab_3$)sub fraction (ab_2)was purified by repeated washing with MeOH to give (9mg) brown amorphous powder insoluble in MeOH coded as compound**2**on the basis of TLC analysis sub-fractions (Fr.c, Fr.d, and Fr.e) which obtained from the first silica gel column chromatography were combined and subjected to further purification over sephadex LH-20 column using MeOH: H_2O mixtures as an eluent system. (9:1v/v) to afford 25 mg of yellow amorphous powder coded as compound **3.**

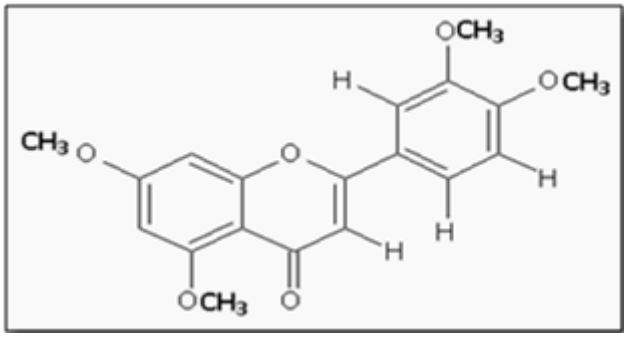


Figure 1: Structure of Compound1 5, 7.3', 4'-tetramethoxy-flavone.

Compund**1**was obtained by fractional crystallization of 70%EtOH extract on Sephadex LH-20 column as pale yellow needles powder, m.p.183-185°C.Its FAB-MS exhibited a molecular ion peak ($[M]^+$ at 374 m/z, calc. for $C_{19}H_{18}O_6$, and characteristic fragmentation ion peaks due to loss of methyl units at m/z 359 (M-CH3), 344 (M-2 × CH_3), 329 (M-3× CH3), 314 (M-4× CH_3). The UV spectrum of compound 1exhibited absorption bands at 339 nm (band I) 270 nm (band II) to suggest a flavones structure (Mabry, et al, 1970). Its UV spectrum in MeOH was unaffected by the shift reagents NaOMe, NaOAc and AlCl3/HCl indicating the absence of free hydroxyl at C-4', C-7 and C-5 positions (Bernard *et al.*, 1983)**.** The IR-spectrum of compound 1 showed absorptions at 2928, 1661, 1614and 1471 cm-1, along with the negative ferric chloride test also indicated that compound**1** had no free hydroxyl groups.

3. Results and Discussion

Table: ^{1}H and ^{13}C NMR data of compound1 from C.ZamF, CDCl3, δ in ppm, J in Hz

DEPT	δH	δC	HMBC selected	DEPT	δH	δC	HMBC selected
C (2)		164.0	H-3, H, H-6'	C (1')		123.2	
CH (3)	6.57 (1H, s)	108.3		CH (2')	7.39 (1H, d, 1.7)	103.8	
C (4)		182.9		C (3')		135.7	
C (5)		152.5		C (4')		146.9	4'-OMe
C (6)	6.35 (1H, d, 2.0 Hz)	136.9		CH (5')	7.03 (1H, d, 8.5)	115.1	
C (7)		132.9		CH (6')	7.52 (1Hdd, 1.7, 8.5	120.8	
C (8)	6.50 (1H, d, 2.0H)	106.9		3'-OMe	3.93 (3H, s)	56.3	
C (9)		149.0		4'-OMe	3.98 (3H, s)	61.7	
C (10)		95.7		5-OMe	4.09 (3H, s)	62.1	
				7-OMe	3.90 (3H, s)	61.6	

^{1}H-NMR of componud1 showed the presence of four methoxyl signals at (δ_H 3.98, 4.09, 3.93, 3.95, each 3H, s) and (δ 56.3q, 61.7q, 61.6q, 62.1q) in the ^{13}C-NMR, .A set of *meta* coupled doublets (J=2.0) at δ_H 6.35 and 6.50 were assigned to H-6 and H-8 respectively (Table1). A sharp singlet integrating for one proton at δ_H 6.57correlated with C-3 was a characteristic C-3 proton of a flavone (Yerra, et al, 2003). Its ^{1}HNMR spectrum indicated that 3, 2'-, 5'-, 6'positions in the flavone skeleton were unsubstituted (Table 1). The ^{1}H-1H COSY showed that H-5' proton signal at δ 7.52 correlated with the H-6'signal at δ 7.03. In the HMBC the proton at δ 6.57 (H-3, s) was correlated with the carbons at δ 164.0 (s, C-2), 123.2 (s, C-1') and 120.8 (d, C-6**'**); the protons at δ 7.52 (H-5'), 7.39 (H-2') and 7.03 (H-6') with the carbon at δ 164.0 (s, C-2), respectively. It supported that the B-ring was connected to C-2 with 3', 4' substituted by methoxy or hydroxyl groups (Fig.4.55). The ^{13}C-NMR spectrum (Table I) showed three hindered methoxy groups δ 61.1q, 61.6q, 62.1q, and one non-hindered at δ 56.3q, which indicated that the carbon of the methoxyl at δ 56.3q is not bordered by the other methoxy groups (Lima *et al.*, (1996). ^{1}H-^{13}C correlations between δ 3.98 (s, H-OMe) and δ 56.3 (q, C-OMe) and the long-range correlations between δ 3.98 (s, H-OMe) and δ 146.9 (s, C-4') indicated that C-4' at the B-ring was substituted by the only unhindered methoxy group. Besides the four methoxyl signals, there were other 15 carbon signals in the ^{13}C-NMR, and 14 of them were aromatic carbons, a carbonyl signal appeared at δ 182.9 (s). From the foregoing spectral analysis the structure of compound**1** was characterized as 5, 7.3', 4'-tetramethoxy-flavone.

Compound **2** was isolated from MeOH extract *C.zambesicus* by repeated chromatographing on silica gel column, as Amorphous yellowish powder; mp; 250-252°C. FAB-MS spectrum of compound2 gave a molecular ion peak [M+H]+

at *m/z* 433 indicating a molecular mass of 432 (calc for **$C_{21}H_{20}O_{10}$**). A second prominent ion was obtained at *m/z* 271 $[M+H-162]^+$ resulting from the loss of a sugar chain consisting of one hexose. The presence of a peak at *m/z* 313 [M+H-120]+ suggests that hexose is linked to the aglycone by a C- linkage. (Fig1). UV spectrum of Compound2 displayed absorptions (UV λ_{max} MeOH) at 270, 339; +NaOMe 280, 395; +$AlCl_3$ 271, 343; +AlCl3/HCl 270, 343; +NaOAc 280, 383; +NaOAc/H_3BO_3, 271, 340. (Table 4.17 & fig.4.57).IR (KBr)V_{max}. 3383cm^{-1} (OH) 1655 (α, β-unsaturated carbonyl), 1614, 1508, 1429 (aromatic double bond) cm^{-1}.

Table 2: ^1H and ^{13}C NMR data of compound 2 (CDCl3), δ in ppm, *J* in Hz

DEPT	δH	δC	DEPT	δH	δC
C (2)		163.94	C (1')		121.60
CH (3)	6.74 (1H, s)	102.45	CH (2')	8.01 (2H, d, J=8.4)	128.96
C (4)		182.21	CH (3')	6.89 (2H, d, J=8.4)	115.80
C (5)		155.98	C (4')		161.12
C (6)	6.23 (1H, s)	98.12	CH (5')	6.89 (2H, d, J=8.4)	115.80
C (7)		162.55	CH (6')	8.01 (2H.d, J=8.4)	128.96
C (8)		104.59	CH (1'')	4.79 (1H, d, J=10)	78.64
C (9)		160.38	CH (2'')		73.37
C (10)		104.03	CH (3'')	3.40-4.00	70.82
-			CH (4'')		70.51
-			CH (5'')		81.84
-			$CH_{2\ (6''a)}$		61.27

^1H NMR spectrum, showed two doublet signals at δ 8.01 (d, *J* = 8.40 Hz, H- 2', 6') and δ 6.89 (d, *J* = 8.40 Hz, H-3', 5') suggested the typical A_2B_2 splitting pattern of flavonoid B-ring. In addition, two singlet signals were detected at δ 6.74 and 6.23, which were characteristics of an apigenin moiety missing H-3 or H- 8 peak (Oh *et al.*, 1994). The existence of two signals with no splitting in the ^1H NMR was an evidence that the two protons to which these signals were attributed are found apart from each other in the structure (geminal protons).Thus, the signal at δ_H 6.74 ppm is probably from the proton in position 3 of the C- ring, and the signal at δ_H 6.23ppm would be the proton located in C-6 position of the A- ring. The typical signal of a β- anomeric proton is detected at δ 4.79 (1H, d, *J* = 10 Hz, 1'') and other protons of D-glucose are detected as a multiplet at 3.40~4.00 ppm. (Table 2).

In the ^{13}C-NMR spectrum, carbonyl carbon was shown at δ_H182.1 ppm, and the signals of the fourteen aromatic carbons are detected at δ 99.00 to 165.00. Compound **2** was C-glycoside, and six *sp*3 carbon signals of the sugar moiety were confirmed at δ 82.6 (C-5), 79.9 (C-3), 74.4 (C-1), 71.9 (C-2), 71.6 (C-4), and 62.4 (C-6). In addition, there was no reaction upon attempted acid hydrolysis. Therefore, the anomeric carbon of the sugar would be expected to bind directly with the carbon of the flavonoid.^{13}C NMR (DMSO-*d*6) signals were assigned as follow: 182.10 (C-4), 163.94 (C-2), 162.55 (C-7), 161.12 (C-4'), 160.38 (C-9), 155.98 (C-5), 128.96 (C-2' and C-6'), 121.60 (C-1'), 115.80 (C-3' and C-5'), 104.59 and 104.03 (C-8 and C-10), 102.45 (C-3), 98.12 (C-6), 81.84 (C-5''), 78.64 (C-1'') 73.37 (C-2''), 70.82 (C-3''), 70.51 (C-4''), 61.27 (C-6'') ppm. (Table 2).

From these results, the chemical structure of the isolated compound2 was identified as a flavone glycoside and its spectral data is identical to that of apigenin-8-C-β-D-glucopyranoside (Vitexin). The spectral properties of compounds 2 including UV, ^1H NMR, ^{13}C NMR, were verified by comparison of its spectral data with those previously described in the literature (Mabry *et al.*, 1970; Markham *et al.*, (1994); Agrawal, (1989); Kartnig *et al.* (1991); Harborne, (1998). To the best of our knowledge's this is the first report of these compounds from *croton zambesicus*

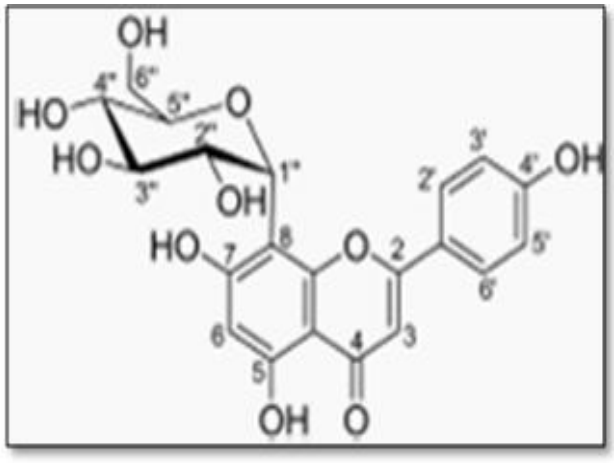


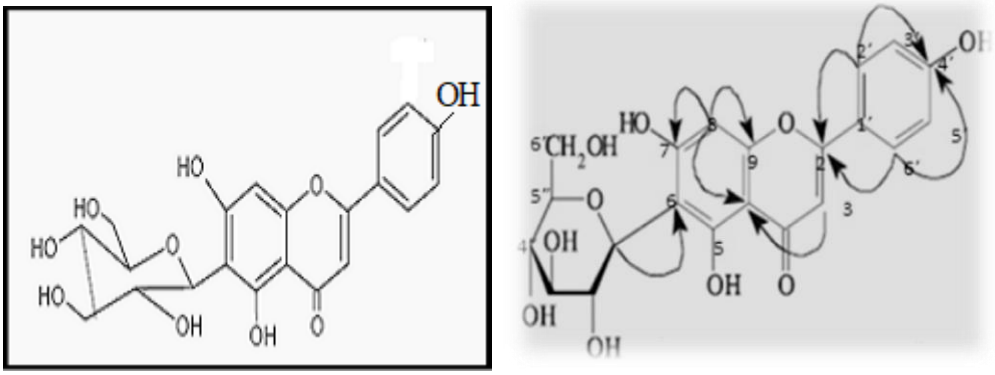
Figure 2: Structure of compond2 apigenin-8-C-β-D-glucopyranoside (Vitexin)

Compound **3** was isolated as a yellow amorphous powder. m.p. 255-257 °C. The FAB mass spectrum of compound **3** in positive ion mode gave a molecular ion peak [M+H]+ at *m/z* 433, , suggesting M_r = 432 compatible with the molecular formula $C_{21}H_{20}O_{10}$. An aglycone peak $[A]^+$ was observed at *m/z* 281.4. This ion was formed by the loss of ($C_5H_{12}O_5$) from the molecular ion the peak at313cm^{-1} was evidence that the sugar moiety is linked to the aglycone by C- linkage. The UV- spectrum of compound3 showed UV λ_{max} MeOH 270, , 337 nm ; +NaOMe 278, 383 nm , 405; +AlCl3 277, 348; +AlCl3/HCl 277, 343; +NaOAc 280, 395;+NaOAc/H_3BO_3 270, 345 nm. Its UV absorptions in MeOH and the shifts observed upon addition of diagnostic shift reagents were consistent with the presence of a 5, 7, 4'-trihydroxyflavone structure Markham, (1982).IR spectrum of the compound 3 showed absorptions at 3395cm^{-1} (OH), 1660 cm^{-1} (C=O), 1611 cm^{-1} (C=C), 1509, 1490 cm^{-1} (Aromatic group), 1117 cm^{-1} (C-O).

Table 3: 1H and 13C NMR data of compound 3 (C.z3/Y), (DMSO-*d*6) δ in ppm, *J* in Hz. (isovitexin)

DEPT	δH	δC	DEPT	δH	δC
C (2)		166.19	C (1')		123.10
CH (3)	6.59 (1H, d, J=6.84)	103.88	CH (2')	7.83 (2H, dj=8.22)	129.45
C (4)		184.05	CH (3')	6.92 (2H, d, J=8.28).	117.03
C (5)		162.04	C (4')		162.80
C (6)		109.18	CH (5')	6.92 (2H, d, J=8.28)	117.03
C (7)		164.86	CH (6')	7.83 (2H, d, J=8.94)	129.45
CH (8)	6.49 (1H, d, J=10)	95.20	CH (1'')	4.49 (1H, d10.98)	75.29
C (9)		158.71	CH (2'')		72.58
C (10)		105.21	CH (3'')	3.30-3.50 (m)	80.12
-			CH (4'')		71.79
-			CH (5'')		82.64
-			$CH_{2\ (6''a)}$	3.74 (1H, dd.5.5, 4.8)	62.87
-			$CH_{2\ (6'', b)}$	3.88 (1H, dd, J=2.1, 2, 0)	62.87

The ^{1}H and ^{13}C NMR spectra of compound **3** exhibited resonances due to aromatic systems and a *β*-D-glucopyranose moiety (Jinyong et al., 2005). The ^{13}C NMR signals of compound **3** were assigned with the help of an HMQC experiment. The connectivity of the molecular fragments was established by a hetero-nuclear multiple-bond correlation experiment (HMBC). The singlet ^{1}H NMR resonance at *δH* 6.49 was assigned to the H-8 proton of the A-ring, due to the long-range correlations observed from H-8 to C-10 (*δC* 105.21), C-7 (*δC* 164.86) and C-9 (*δC* 158.71). Two doublets at *δH* 7.83 and 6.92 (each 2H, *J* = 8.8 Hz) were characteristic of the H-2*′*/6*′* and H-3*′*/5*′* protons, respectively, of the *para*-substituted B-ring. A singlet proton resonance at *δH* 6.59, which correlated to the carbon resonance at *δC* 103.88 (*d*) in the HMQC spectrum, was assigned to H-3 of the aglycone. HMBC correlations observed fromH-3 to C-2 (*δC* 166.19), C-10 (*δC* 105.21) and C-1*′* (*δC* 123.10) supported this assumption. On the other hand, both the chemical shift value of the anomeric carbon atom (*δC* 75.29) and the coupling constant value of H-1*′′* (*J* = 10.98 Hz) indicated that the linkage of the glucose was through a *C*-bond (Mabry et al (1970) and Harborne (1998). The information concerning the linkage of the sugar moiety was obtained from the HMBC spectrum. A prominent long-range correlation between the anomeric proton (*δH* 4.49) of the glucose unit and C-6 (*δC*109.18) of the aglycone showed the attachment of the sugar moiety at the C-6 position. Further proof for this assignment came from the HMBC cross-peaks observed from H-1*′′* to C-5 (*δC* 162.05) and C-7 (*δC* 164.86). Based on the NMR data and comparison of the data given in the literature, the structure of compound**3**was characterized as 5, 7, 4'-trihydroxyflavone glycoside. Isovitexin (Mabry et al., 1970).



(a) HMBC correlations of cpd3 (b) The structure of cpd3

Figure 3: HMBC correlations and the proposed structure of compound 3. 5, 7, 4'-trihydroxyflavone glycoside. Isovitexin

References

[1] **Cragg, G.M. and Newman, D.J.** (2005.) Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem. 77 (1):7-24.

[2] **Mahesh ChandMeena and Vidya Panti** (2008) Isolation and Identification of flavonoid (Quercetin) from Citrullus Colocynthis (linn) Asian J. Exp. Sci. Vol.22No.1; 137-142.

[3] **Mustafa S.Koya .Nada A. Elamin and Zeinb A. Elrabei** (2014) Qualitative and quantitative analysis of phytochemicals in some Sudanese Medicinal plants. Nyala University journal for applied science.

[4] **Harborne, J. B**. and Williams, C.A. (2000). Advances in flavonoid research since *1992*. *Phytochemistry*. (55): 481-504.

[5] **Mabry, T.J**.; Markham, K.R. and Thomas, M.B. (1970). *The Systematic Identification of Flavonoids*. (1st edition). Springer-Verlag, New York:p13-19.

[6] **Pooley, E. 1993.** The complete field guide to trees of Natal, Zululand & Transkei.P.222. Natal Foral Publications Trust, Durban.

[7] **Prozesky, E.A. 2004**. Antiplasmodial and chloroquine resistance reversal properties of a new diterpene from Croton steenkampianus. PhD thesis. University of Pretoria, South Africa.

[8] **Ngadjui, B.T., Abegaz**, B.M., Keumedjio, F., Folefoc, G.N., & Kapche G.W.F. 2002. Diterpenoid from the stem bark of Croton zambesicus. Phytochemistry 60:345-349.

[9] **Suarez, A. I., Blanco, Z., Compagnone**, R.S., Salazar-Bookaman, M.M., Zapata V., Alvarado C. 2006. Anti-inflammatory activity of Croton cuneatus aqueous extract. J. Ethnopharmacol. 105: 99-101.

[10] **Bernard** J.D. (1983). UV spectral differentiation of 5-hydroxy-and 5-hydroxy-3-methoxyflavoneswith mono- (3'), di- (3', 4') or tri- (3', 4', 5')-substituted B ring .*Phytochemistry (***37**):2107-2145.

[11] **Lima** M.A.S., Silveira E.R., Maroues M.S.L., Helena R., Santos A., and Gambardela M.T.P. (1996).Biologically active flavonoids and terpenoids from Egletes viscosa. Phytochemistry (**41**): 217-223.

[12] **Agrawal P**. K., Bansal M. C., (1989b), "The Carbon-13 NMR of Flavonoids," ed. by Agrawal P. K., Elsevier, Amsterdam, pp: 96—116.

[13] **Markham** K.R. and H. Geiger (1994).1H-NMR spectroscopy of flavonoids. Advance in research since 1986. (Harbrone, J.K.) ed.; Chapman and Hall. London: pp: 670-676.

[14] **Kartnig Th**., Bucar F., Wagner H. and Seligmann O. (1991), Flavonoids from the above ground parts of *Ruscus aculeatus*. *Planta Med*. (**57**): 85.