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Extraction, separation and identification of compounds from leaves of *Solanum elaeagnifolium* Cav. (Solanaceae)

*Fakhri A. Elabbar, Nawil M.A. Bozkeha, Ashraf T. M. El-Tuonsia

Department of Chemistry, University of Benghazi, Benghazi, Libya

ABSTRACT

Aim of the present study was to identify some phenolic compounds isolated from the dried powder leaves of *Solanum Elaeagnifolium* Cav. (Solanaceae family) collected from Benghazi area, Libya originally native in the Americas. The extraction was carried out with methanol at room temperature and treated by acidification (H_2SO_4, pH = 4) & basification (NH_4OH, pH = 10). The compounds were separated by chromatographic method through a wet glass silica gel column and purification by medium pressure liquid chromatography (MPLC). The experiment yielded one novel compound named 2-(2-hydroxyphenoxy)-3,6,8-trihydroxy-4H-chromen-4-one [A_3] and three previously known phenolic which were isolated for the first time from this plant under study (Quercetin [A_1], Rutin [A_2] & Mangiferin [A_4]). The structures were determined using modern spectroscopic techniques (IR, EI-mass, ^{1}H-NMR, ^{13}C-NMR, APT, HMQC & COSY) using DMSO-d_6 solvent in magnetic resonance application.

***Key Words*:** Solanaceae, Libya, 2-(2-hydroxyphenoxy)-3,6,8-trihydroxy-4H-chromen-4-one, quercetin, rutin, mangiferin.

INTRODUCTION

The Solanaceae, or *nightshades* are plant family contains about 94 genera (Hepper, 1998) and comprises more than 3000 species (Quattrocchi, 2000), herbs, shrubs or small trees. The chief centers of distribution are Central and South America, but it is generally distributed in tropical and temperate regions all over the world (Hepper, 1998; Quattrocchi, 2000). It is represented by 10 genera and 24 species in Libya (Siddiqi, 1978). The nightshade family is certainly one of most economically important plant families as food (e.g. potato and tomato genus), medicine (e.g. *Atropa Belladonna*), ornaments (e.g. *Physalis Alkekengi*), and several noxious weeds (Siddiqi, 1978; Hickey and King, 1981; Hussein, 1985). The family contains a wide range of alkaloids which are of great taxonomic interest. Types of alkaloid recorded are tropane, alkaloidal amine indole, isoquinoline, purine, pyrazole, pyridine, pyrrolidine, quinazolizidine, steroid alkaloids and glycoalkaloids. Other constituents include steroidal saponins, coumarins, flavones, carotenoids and anthraquinones (Evans, 2002).

Solanum elaeagnifolium Cav. is a perennial herb introduced and cultivated in Benghazi area however was not reported in the flora of Libya (El-sherif, 1988). Having a common name of Silverleaf nightshade, *S. elaeagnifolium* is a branched (multi-stemmed), broadleaved (leaves are dark green to pale grayish green) and perennial herbaceous.

EXPERIMENTAL

The analytical thin layer chromatography (TLC) was carried out on precoated 0.25 mm silica gel plates with florescent indicator (Macherey-Nagel GF254). Wet column chromatography was carried out using RDH silica gel S (230 - 400 mesh ASTM). Preparative TLC was conducted on glass plates (20 × 20 cm) coated with silica gel 60. The spots were visualized under UV light, by ammonia or I_2 vapor. Medium pressure liquid chromatography (MPLC) was performed on Sepacore® (BÜCHI) using silica gel for flash chromatography (70 - 230 mesh ASTM). A column with 150 mm length and 40 mm diameter was used at pressure range from 10 to 50 bar controlled by pump manger C-615 (BÜCHI). UV photometer C-635 detector was used with wavelength range of 190 to 740 nm. IR (vmax in cm^{-1}), spectra were recorded in KBr discs using Unicam Mattson FTIR, 1000 series spectrometer. The NMR spectra were recorded on a Varian Mercury VX-300 & Jeol-500 NMR spectrometer. 1H spectra were run at 300 & 500 MHz and 13C spectra were run at 75.46 & 125 MHz in deuterodimethylsulfoxide (DMSO-d6). Chemical shifts are given as ppm (δ value) and coupling constants in Hz. Mass spectrum (MS) Finnigan mat SSQ700 ionization mode EI were measured at 70 eV.

Plant material

The plant material of *S. elaeagnifolium* was collected by Azza M. Habel from University of Benghazi, filed in June/July 2006 and identified by Dr. Imhamed M. El-Sherif, Botany Department of University of Benghazi. The leaves were allowed to dry in air and then grounded into a powder state using a commercial blender and finally used for the preparation of different extracts.

Extraction

The dried powdered leaves of S. elaeagnifolium (7 Kg) was completely defatted at room temperature by petroleum ether (40°-60°C), then extracted by methanol (3 × 14 L) at room temperature The methanol extract was filtered, then concentrated using rotary evaporator at less than 45°C. To the methanol extract (green color), charcoal was added to remove the colored pigments (to brown red). It was then acidified by H_2SO_4 (2 M, pH = 4), and was partitioned between water (1.5 L) and chloroform (3 × 500 mL). The chloroform extract obtained from partition contained phenolices and terpenioids, the aqueous layer was basified by NH_4OH solution (pH = 10), and extracted with chloroform: methanol 3:1 (3 × 500 mL). The chloro-

***Corresponding Author**:
Fakhri A. Elabbar, Associate Professor
Department of Chemistry
University of Benghazi
Benghazi, Libya
E-mail: *fakhri.dr@gmail.com*
Contact No.: +218925104284

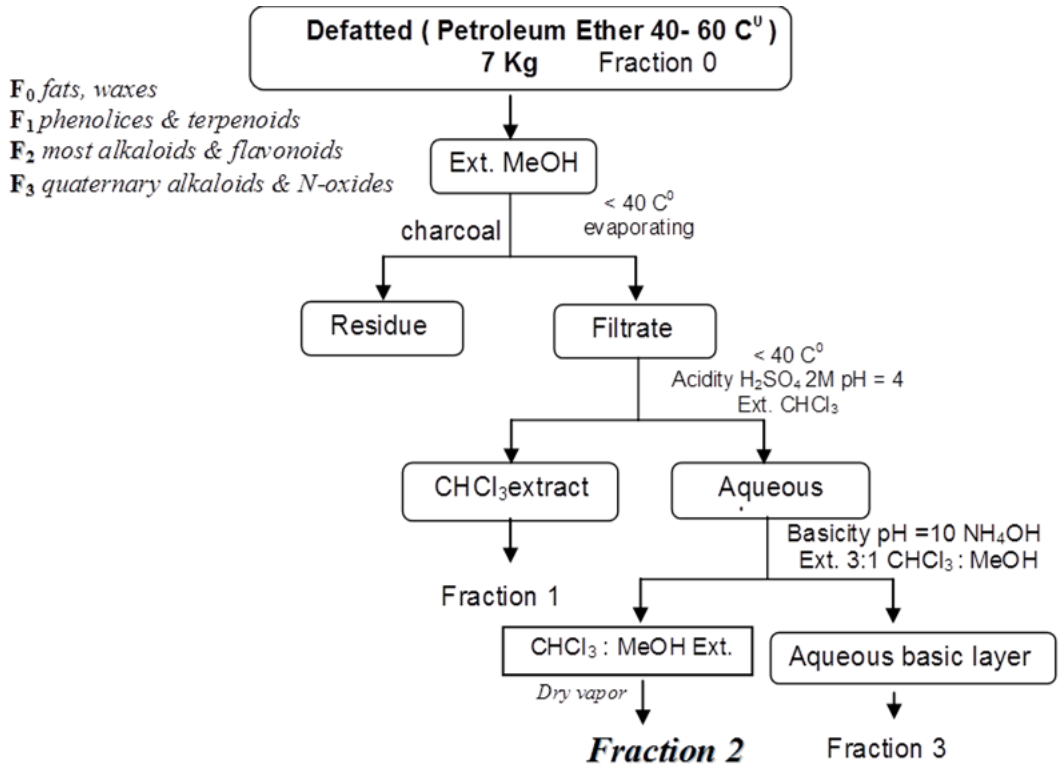

Figure 1: Separation and purification of chloroform: methanol extract.

form: methanol extract contained flavonoids (visual on TLC) and alkaloids (figure 1).

The extract was evaporated to dryness, and the residue (7.8 g) was chromatographed through a wet glass silica gel (350 g) column. Elution was carried out with solvent systems of increasing polarity starting with n-hexane and various mixtures of $CHCl_3$, EtOAc, and MeOH of increasing gradient, whereby 3494 × 15 mL fractions were collected. The fractions were examined by TLC using n-hexane, chloroform, methanol systems and similar fractions were combined. Fractions 47, 57 and 59 showed interesting phenolic behavior as green color on TLC after exposing to ammonia vapors, then was separated and the residue obtained after concentration was fractionated by medium pressure liquid chromatography technique using silica gel for flash column and mobile phase starting with chloroform. By this procedure, four phenolic compounds named Quercetin [A1], Rutin [A2], 2-(2-hydroxyphenoxy)-3,6,8-trihydroxy-4H-chromen-4-one [A3] and Mangiferin [A4], were isolated.

RESULTS AND DISCUSSION

Identification of compound A_1

Compound A_1 was isolated from the dried powdered leaves of *s. elaeagnifolium*. The spectral date were in agreement with a *Quercetin* type skeleton, with IR spectrum showing an absorption for hydroxyl group at 3406.73 cm^{-1} and strong interamolecular hydrogen bonding, the resulting absorption at 2750 - 3150 cm^{-1} was broad, carbonyl group 1666.69 cm^{-1} and aromatic double bond 1610.44 cm^{-1}The ^{1}H-NMR spectrum showed an AMX spin system for the Ring B protons whose pattern indicated a 3`,4`-disubstiluted ring since a doublet spotted at δ_H 6.90 ppm, $^3J_{AM}$ = 8.47 Hz, which is typical of H-5` for this system, a doublet at $\delta_H$ 7.68 ppm, $^4J_{MX}$ = 2.20 Hz, and a doublet of doublet at $\delta_H$ 7.54 ppm, $J$ = 2.20, 8.47 are due to the H-2` & H-6` respectively. The spectrum also displayed AB system with meta coupled protons which identified a tetra substituted benzene Ring A at δ_H 6.19 ppm, $^4J_{AB}$ = 2.03 Hz & δ_H 6.41ppm, $^4J_{AB}$ = 2.04 Hz these data implied flavonol with 5,7-dioxygenated Ring A and these signals are due to H-6 & H-8 respectively and the spectrum showed the absorptions of five hydroxyl groups at δ_H 9.09, 10.65 & 12.40. The absorption at 12.40 ppm indicated the presences of hydroxyl at C-5.(Harborne, 1984) The ^{13}C-NMR spectrum of compound A_1 pointed fifteen carbon signals, which were resolved through APT experiment into five methines and ten quaternary carbons. The spectrum showed signals at δ_C 175.77, 146.84 & 135.65 ppm were typical of C-4, C-2 & C-3 respectively. (Markham, 1982) The chemical shifts at δ_C 160.70, 163.85, 145.01 & 147.64 ppm for four aromatic carbons connected to hydroxyl group at C-5,7,3` & 4` respectively. ^{13}C-NMR spectrum signals at δ_C 98.24, 93.39, 115.17, 115.63 & 120.06 ppm for =C-H aromatic at C-6, 8, 2`, 5` & 6` respectively. The flavonoids ring junctions appeared at $\delta_C$ 156.19 & 103.05 ppm for carbons C-9 & C-10 respectively and other aromatic carbons quaternary appeared C-1` at 122.07 ppm. The attachment of each carbon to hydrogen was confirmed by the HMQC experiments, which also confirmed the quaternary carbon signals at 2, 3, 4, 5, 7, 9, 10, 1`, 3`, & 4`, Which were uncorrelated to the proton spectrum. Carbons signals at δ_C 93.39 & 98.24 ppm were a directly correlated to proton signals at δ_H 6.41 & 6.19 ppm in the HMQC spectrum respectively and δ_C 115.17, 115.63 & 120.06 ppm appear to be correlated with proton signals at δ_H 7.68, 6.90 & 7.54 ppm respectively. The position of carbonyl group, position of hydroxyl group at C-3 and substituted aromatic double bond, thus suggesting that

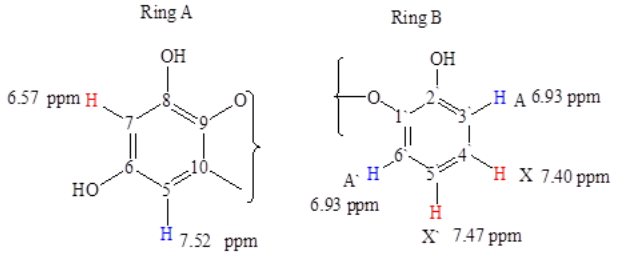

Figure 2: Rings A & B systems by 1H-NMR in compound A3.

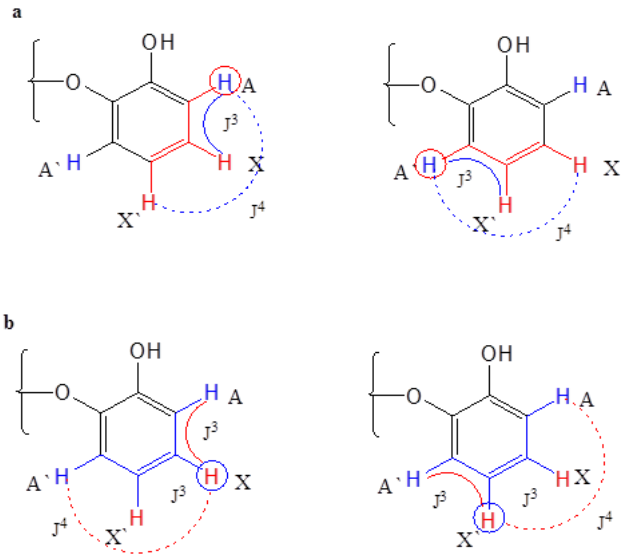

Figure 3: a. Coupling A & A`, b. coupling X & X` by 1H-NMR in compound A3.

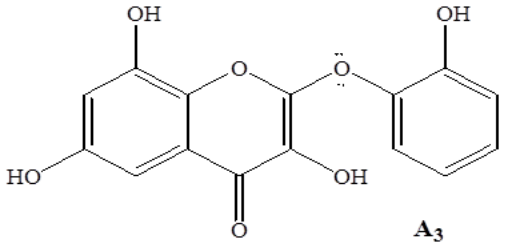

Figure 4: 2-(2-hydroxyphenoxy)-3,6,8-trihydroxy-4H-chromen-4-one.

the compound might be flavonol with 5,7-dioxygenated in Ring A. Support for this was further provided by characteristic Retro-Diels-Alder fragmentation (RDAF) of flavonol skeleton giving rise to peaks at m/z = 153 (6.72%) indicating the presence of carbonyl group, and m/z = 149 (0.12%) this also indicate the presence of hydroxyl group in Ring C. The EI-mass spectrum of compound A_1 showed a molecular ion peak **[M+]** at m/z = 302 (100%) which corresponds to the molecular formula of ($C_{15}H_{10}O_7$). The spectrum of compound also undergoes retrocyclization to give m/z = 286 (4.96%) and loss of hydrogen radical m/z = 301 (15.74)%. With the help of the spectral data discussed above and comparative literature report (Wawer and Zielinska, 2001; Barber *et al.*, 1986) the compound was identified as 5,7,3`,4`-tetrahydroxylflavonal *i.e. Quercetin* **[A_1]**. *Quercetin* had never been reported from *solanum* genus.

Identification of compound A_2

This compound was isolated from the early fractions of the column chromatograph of the ethyl acetate: methanol 6.5:3.5 and MPLC chloroform: methanol 1:9. The IR spectrum of the compound A2 exhibited absorptions at 3450 - 3423, 2938, 1665 & 1599 cm^{-1} corresponding for hydroxyl group, hydrogen-carbon bond for sp^3, carbonyl & olefinic functions in the structure. The ^{1}H-NMR spectrum displayed a pair of broad singles in the aromatic region resonated at δ_H 6.19 & 6.38 ppm, these signals are due to H-6 & H-8 respectively, revealed the presence of AB system of Ring A. The ^{1}H-NMR spectrum also displayed signals due to three ABX system type protons at δ_H 6.86 ppm (1H, d $^3J_{AB}$ = 8.65 Hz) and range at δ_H 7.5 - 7.59 ppm (2H, m). These data indicated a flavone with 3`,4`-dioxygenated in the Ring B and from their pattern the signals are due to H-5` & H-2`, H-6` respectively. The ^{1}H-NMR also showed evidence of typical signals of fifteen sugar protons range at δ_H 0.99 - 5.34 ppm indicated the presence of two sugar units in the molecule.(Wawer and Zielinska, 2001) The anomeric proton of the first sugar moiety resonated as one proton as a doublet at δ_H 5.33 ppm with coupling constant $J_{1``,2``}$ = 5.12 Hz (J > 5 Hz) indicating a β-configuration for the glycosyl D nature.(Mabry *et al.*, 1970) The signal of second anomeric proton revealed a broad singlet at 4.41 ppm with a methyl group as the terminal carbon at δ_H 1.00 ppm (3H, d $J_{6```,5```}$ = 5.85 Hz), in ^{1}H-NMR this suggests the presence of a rhamnosyl residue.(Teng *et al.*, 2002) The α-configuration of the anomeric center could be deduced broad singlet comparative with data reported literature.(Shahat *et al.*, 2005; Duan *et al.*, 2006) The signals for two protons appeared overlapping at a range of δ_H 3.o7to 3.13 ppm as a triplet like corresponding to H-4`` & H-4```. The signal at 3.43 ppm showing broad singlet for one proton assigned for H-2```, and six protons showing multiple at range 3.20 - 3.38 ppm corresponding to H-2``, 3``, 5``, 6``a, 3``` & 5```, finally one proton for H-6``b at 3.5 ppm. The spectrum showing broad singles for four hydroxyl groups at δ_H 9.03, 9.52, 10.72 & 12.56 ppm, the occurrence hydroxyl group at 12.56 ppm confirm the presence of OH at carbon five, with hydrogen-bonding to the C-4 carbonyl.(Harborne, 1984) The ^{13}C-NMR data of the compound displayed twenty seven carbon signals, twelve of them attributed to the sugar part and fifteen to the *Quercetin* aglycone, which were resolved through APT experiments into a methyl, a methylene, fifteen methines, and ten quaternary carbons. The results of the full analysis of ^{13}C-NMR confirmed the structure of compound A_2 as Quercetin-3-O-α-Lrhamnose[1→6]-O-β-D-glucose, the The main features of the ^{13}C-NMR for aglycone is the signals at δ_C 177.36 ppm due to C-4 carbonyl group, and δ_C 133.37 ppm should be located the C-3 O-glycoside. (El-sherif, 1988; Teng *et al.*, 2002) The carbon signals of flavonol Ring A at δ_C 161.18, 98.72, 164.01, 93.63, 156.65 & 104.3 ppm indicated from C-5 to C-10 respectively, were confirmed through HMQC correlation, proton H-6 at δ_H 6.19 ppm with carbon C-6 appeared at δ_C 98.72 ppm and the interaction of H-8 at δ_H 6.38 ppm with carbon C-8 resonated at 93.63 ppm. Further proof of the ortho 3`,4`-dioxy-substiution of Ring B appeared by connectivity

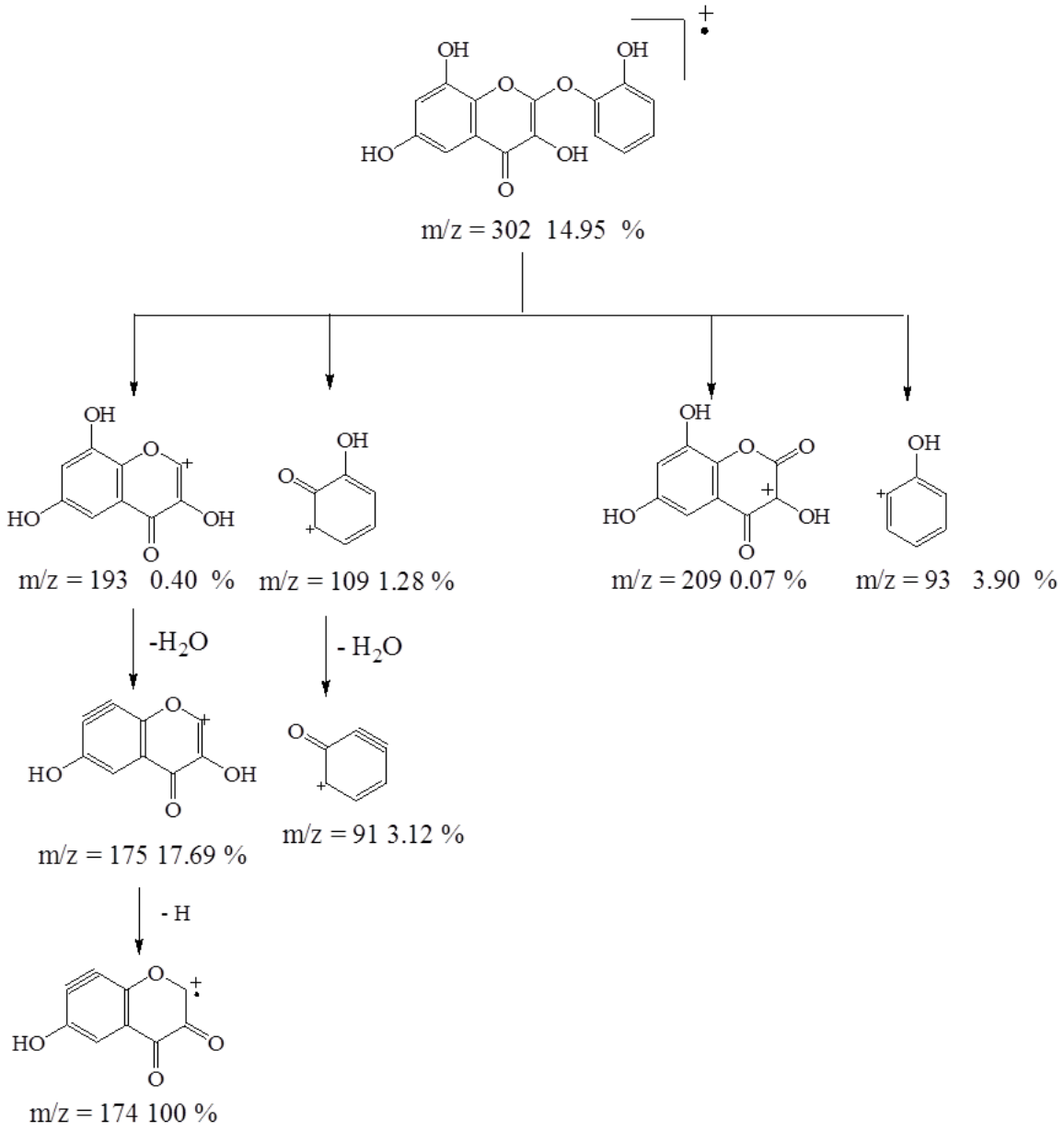

Figure 5: Mass fragmentation of compound A_3.

proton with carbon established through HMQC experiments which showed that the H-2`, H-6` at δ_H 7.52 ppm and H-5` at 6.86 ppm were linked with the C-2` at δ_C 115.27 ppm , C-6` at 121.28 ppm and C-5` at δ_C 116.37 ppm. The APT experiment confirmed the signal at δ_C 156.43 & 121.62 ppm which was assigned to quaternary carbons for C-2 & C-1` respectively. The ^{13}C-NMR spectrum showed two anomeric carbons at δ_C 101.3 & 100.68 ppm assigned for C-1`` & C-1``` corresponding through HMQC experiments with H-1`` & H-1``` at δ_H 5.33 & 4.41 ppm respectively. The *HH*-COSY spectral data showed correlation between protons resonating at δ_H 6.19 ppm (H-6) & 6.38 ppm (H-8), and showed coupling between δ_H 7.52 ppm (H-2`, H-6`) & 6.87 ppm (H-5`). In the *HH*-COSY spectrum, the signal at δ_H 5.33 ppm (H-1``) also showed correlation with signal at δ_H 3.20 - 3.38 ppm (multiple). The *HH*-COSY spectrum indicated that the proton signal at δ_H 4.41 ppm (H-1```) coupled to protons resonating at δ_H 3.43 (H-2```), 3.20 - 3.38 (H-6``a) & 3.5 ppm (H-6``b), the appearance of two signals for H-6`` further proved the non-equivalent protons environment resulting a *geminal* coupling in the *HH*-COSY representing H-6``a & H-6``b. The molecular ion peak of compound A_2 was not observed through EI-mass spectrum, at m/z = 302 (100%) which corresponding to the molecular formula of *Quercetin* aglycone ($C_{15}H_{10}O_7$). The compound A_2 produced second generation at m/z = 464 (0.01%) due to the loss of a rhamnose and resulting fragment at m/z = 153 (3.09%) according to undergoes Retro-Diels-Alder fragmentation (RDAF). The fragmentation of Rutin gave intense ion at m/z = 301 (24.06%) corresponding to the loss of the rutinose and hydrogen radical, another peaks at m/z = 286 (3.51%), m/z = 285 (2.17%), m/z = 274 (2.15%) & m/z = 273 (4.53%) resulted from the loss of oxygen radical,

hydroxyl group, carbonyl group and the loss of carbonyl with hydrogen respectively. The spectroscopic evidence and comparative studies (Shahat, etal 2005) were in conformity with structure (A_2) and the compound was identified as Quercetin-3-O-α-Lrhamnose[1→6]-O-β-D glucose i.e. *Rutin* **[A_2]**, has never been reported from the Solanaceae family.

Identification of compound A_3

Compound A_3 was also isolated from fraction 57, through column chromatograph eluted by ethyl acetate: methanol 6.5:3.5, followed by MPLC using chloroform: methanol 3:7. The electron impact mass spectrum of compound A_3 showed a molecular ion peak **[$M^{+\bullet}$]** at m/z = 302 (14.95%) in agreement with the molecular formula ($C_{15}H_{10}O_7$), this was corroborated by the decoupled ^{13}C-NMR spectrum which showed signals for all the fifteen carbons of the molecule. Here the molecular formula permits for four phenolic OH groups of shift values at δ_H 9.38 ppm in the ^{1}H-NMR spectrum. The IR spectrum of compound A_3 hydroxyl group at 33368.49 cm^{-1} and carbonyl group at 1618.13 cm^{-1} were seen to indicate the presence of double bond conjugated with carbonyl group.(Wang *et al.*, 2007) The IR spectrum showed absorption of two strong bands at 1212.44 & 1190.96 cm^{-1} typical to a ether functions C-O-C, with a comparative theoretical data (1210 & 1192) indicating two types of ether bands .The absorptions at 1579.3, 1522 & 1466 cm^{-1} are suggestive to aromatic double bonds and aliphatic conjugation with carbonyl group. The absorption of the =C-O enolic group at 1070 cm^{-1}. Combinative study of ^{1}H-NMR and APT spectra of compound A_3 showed signals assignable to six aromatic protons. In the ^{1}H-NMR spectrum an AB spin system at δ_H 6.57 & 7.52 ppm with meta coupling J = 3.3 Hz indicates a tetra substituted benzene of Ring A, the signal at δ_H 7.52 ppm of H-5 wasn't perfectly coupled with the proton of C-7 (broad distorted doublet) as a result of the interaction with the hydrogen on the hydroxyl group at C-6 and the carbonyl group at C-4. The absorption of Ring B displayed AA`XX` spin system at δ_H 7.47, 7.40 & 6.93 ppm these chemical shifts are characteristic of four protons ortho-meta coupling similar to para disubstiluted in the benzene ring, (Williams and Fleming,1980) (Silverstein, R. M. & Webster, 1996) the protons at H-4` & H-5` (approximately chemically equivalent but not magnetically equivalent) didn't couple to each other. Both protons H-4` & H-5` at δ_H 7.40 & 7.47 ppm with coupling constant J = 8.63, 3.53 & 8.32, 2.1 Hz respectively distorted apparent form double of double. The other two protons at 6.93 ppm spotted as distorted a triplet form duo to the overlapping of two double of doublet.

Fifteen peaks were observed in the ^{13}C-NMR spectrum the most downfield-shifted ^{13}C peak at 176.71 ppm was assigned to C-4 carbonyl carbon, this value indicated that the carbonyl group conjugation with double bond (Harborne, 1984). In the spectra of compound A_3 the resonances of C-2 at δ_C 162.54 ppm & C-3 at δ_C 133.12 ppm are high-frequency shift, because of two oxygen connected with C-2, double bond and 3-hydroxyl (Breitmaier, 2002). The aromatic oxygenated carbons will appear at range of δ_C 165 - 155 ppm in the case of the absent oxygenated ortho or para carbons, however oxygenated ortho or para will appear at δ_C 150 - 130 ppm (Harborne, 1984). Therefore peaks at δ_C 150.24, 149.08, 146.64, 145.61, & 122.56 ppm indicated the oxygenated aromatic carbons. The carbons absorption at a range of 125 - 90 ppm are characteristic for aromatic non oxygenated carbons with ortho- or para- with hydroxyl groups (Harborne, 1984), therefore absorption at δ_C 118.74, 115.95, 115.07, 113.77, 113.58 & 104.04 ppm indicated the aromatic carbons non-oxygenated. The non-substituted aromatic carbons appeared at δ_C 118.74 & 104.04 ppm indicated by APT experiments corresponding to C-5, C-7 respectively, and in the HMQC correlated this signals with direct connectivity to proton signals at δ_H 7.52 & 6.57 ppm, while aromatic carbons attached to hydroxyl groups appeared at C-6 & C-8 with δ_C 122.56 & 150.24 ppm respectively in Ring A. The ring junctions appeared at δ_C 145.61 & 117.05 ppm for carbons C-9 & C-10, respectively.

The peaks at δ_C 113.58 & 115.95 ppm indicated the non-substitution on these aromatic carbons, but the ortho position with oxygen substitution in the HMQC correlation these signals showed direct connectivity with proton signals overlapped at δ_H 6.93 ppm for C-3` & C-6`, absorptions at δ_C 115.07 & 113.77 ppm also indicated the non-substitution on aromatic carbons with occurring oxygen in para position, this signals showed interactive in HMQC correlation with proton signals at δ_H 7.40 & 7.47 ppm for C-4` & C-5`, respectively. From APT spectrum displayed the two oxygen substituted aromatic quaternary carbons apparently at δ_H 146.64 & 149.08 ppm corresponding with C-2` & C-1` respectively. These data are in consistent with the proposed structure and further supporting the presence of the ether linkage between C-2 & C1` carbons. The mass spectrum of the compound A_3 pointed up molecular ion peak at m/z = 302 14.95%, break at the ether bonding resulting fragmentation at m/z = 193 0.40% & 109 1.28%, which indicate the loss of water (m/z = 175 17.69%) and hydrogen molecules fragment at 193 to give the base peak at m/z = 174 100%. The fragment at m/z = 93 3.90% was also characteristic of ether bonding for the phenolic ion. The structure proposed and confirmed by spectral data, was not found in any previous published data, concluding that this compound is novel, named as 2-(2-hydroxyphenoxy)-3,6,8-trihydroxy-4H-chromen-4-one. Registration of the desired compound is now under processing.

Identification of compound A_4

The compound was obtained from the latterly fractions of the column chromatograph of the ethyl acetate: methanol 6.5:3.5 and MPLC chloroform: methanol 1:9. The spectral data were in agreement of a *Mangiferin*, with the broad absorption at 3366 cm^{-1} in the IR spectrum of the compound appeared due to the hydroxyl function in the structure, and absorption at 2938 cm^{-1} of hydrogen-carbon bond for sp^3. The intense absorptions at 1649, 1594 cm^{-1} of the compound indicated the presence of conjugated carbonyl, aromatic function, and interamolecular hydrogen bonding at rang 3050 - 3500 cm^{-1}. The ^{1}H-NMR spectrum revealed the presence of three aromatic proton signals at δ_H 6.33, 6.82 & 7.33 ppm one hydrogen each representing protons at H-4, H-5 & H-8 respectively. The signal at δ_H 4.64 ppm is due to the anomeric proton, and the signal for the anomeric carbon was observed at δ_C 73.23 ppm. Both chemical shift values are typical of C-glycoside, with large coupling constant ($J_{1`,2`}$ 9.77 Hz) of the signal for the anomeric proton indicated a β-C-glycoside. (GÜvenalp *et al.*, 2006; Zhang *et al.*, 2006) The signal for H-2` appeared at $\delta_H$ 4.04 ppm as triplet with coupling constants of 8.17, 8.91 Hz which can be assigned to trans position of H-1` to H-2` and trans position of H-2` to H-3`, while H-3`, H4` & H-5` appeared at 3.21 - 3.44 ppm as multiple. The signals for H-6`a & H-6`b appeared at 3.71 & 3.47 ppm one proton appeared at 3.71 ppm as doublet with a coupling constant of 11.54 Hz, while other

proton appeared at 3.47 ppm as doublet of doublet with a coupling constant of 3.23 & 11. 54 Hz. Additionally four singlet protons were recorded at 10.63 & 13.72 ppm, which represent four hydroxyl groups bonded with four different carbon atoms. The signal at 13.72 ppm is typical for the C-1 OH (Agrawal, 1992). The ^{13}C-NMR spectrum of the compound A_4 afforded nineteen carbon signals which were resolved through APT experiments into one methylene, eight methines and ten quaternary carbon signals .The xanthon ring junctions appeared at 156.29, 150.84, 111.84 & 101.41 ppm for carbons C-4a, C-4b, C-8a & C-8b respectively. The aromatic carbons attached to hydroxyl groups appeared at 163.75, 161.72, 153.98 & 143.68 ppm for C-3, C-1, C-6 & C-7 respectively, the other non-substituted aromatic carbons appeared at 93.47, 102.78 & 108.26 ppm assigned to carbons C-4, C-5 & C-8 respectively. The quaternary carbons appeared at 107.56 ppm assigned to carbon C-2, which further confirmed the site of the sugar linkage to the aglycone in C-2 position (Sun *et al.*, 2006) (Catalano *et al.*, 2006) The ^{13}C-NMR spectrum showed six signals in the range 61.50 - 81.40 ppm suggesting the presence of a sugar moiety, appearing at 70.51, 78.97, 70.65, 81.40 & 61.50 ppm assigned to carbons C-2`, C-3`, C-4`, C-5` & C-6` respectively, also shown a comparison of carbon resonances of isolated xanthon with the literature data of *Mangiferin*. (Sun *et al.*, 2006) (Catalano *et al.*, 2006) However, the carbons signal at 73.90, 71.20, 79.67, 71.36 & 82.09 ppm showed a direct connectivity with proton signals at δ_H 4.50, 3.90, 3.10, 3.10 & 3.10 ppm in the HMQC spectrum respectively and δ_C 61.50 ppm showed a direct connectivity with proton signals at δ_H 3.71 & 3.47 ppm. The carbons signals at δ_C 94.17, 103.35 & 108.90 ppm showed a direct connectivity with protons signals at δ_H 6.33, 6.82 & 7.33 ppm. The *HH*-COSY spectrum is valuable only for the sugar part and revealed the vicinal couplings in this part. The *HH*-COSY plot has cross peaks for the vicinal coupling of the anomeric proton H-1` at ($\delta_H$ 4.64) with H-2` (δ_H 4.04), and oxymethine proton H-2` correlated with range $\delta_H$ 3.21 - 3.44. The appearance of two signals for oxymethylene of H-6` indicated the *geminal* coupling representing H-6`a & H-6`b, while the aromatic ring protons appeared not correlated to each other. Compound A_4 exhibited a molecular ion $[M]^+$ at m/z = 422 (1.59%) in the EI-mass spectra , indicating a molecular formula of $C_{19}H_{18}O_{11}$. e EI-mass illustrated Retro-Diels-Alder fragmentation (RDAF) ion at m/z = 314 (4.19%) Ring A and m/z = 108 (1.52%) Ring B, indicative presence of the monosaccharide and two hydroxyl groups substituted in the Ring A, as well as presence of two hydroxyl groups in the Ring B. the base beak at m/z = 273 (100%) resulting from retrocyclization of sugar part. The spectrum demonstrated major fragments at m/z = 404 [M^+-H_2O] (22.98%), m/z = 386 [M^+-$2H_2O$] (6.47%), m/z = 368 [M^+-$3H_2O$] (8.69%), m/z = 260 [M^+-H_2O – Glc (-$H^\bullet$)] (94.92%), m/z = 259 [M^+-Glc] (3.90%). On the basis of the above spectral data and comparative study (Agrawal, 1992; Sun *et al.*, 2006), compound A_4 was identified as 2-*β*-glucopyransoyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-i.e. *Mangiferin* **[A_4]**. This is the first isolation of *Mangiferin* from the Solanaceae family.

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