HYPOURICEMIC EFFECT OF *ANTIDESMA BUNIUS* (L.) LEAVES EXTRACT AND IDENTIFICATION OF FLAVONOID CONTENT

Nermin D. Soliman[1], Azza Shafei[2], Walid H. El-Tantawy[3], Dina M. Elnaggar[2,4]*,

[1]Al-Tawfik Hospital, Nasr City, Cairo, Egypt.

[2]Dapartment of Pharmacognosy, Faculty of Pharmacy for Girls, Al-Azhar University, Nasr City , Cairo, Egypt.

[3]National Organization of Drug Control and Research, Cairo, Egypt.

[4] Dapartment of Pharmacognosy, Faculty of Pharmacy Egyptian Russian University, Badr City, Cairo, Egypt.

*Corresponding author: E-mail: DinaElnaggar.52@azhar.edu.eg

ABSTRACT

Phytochemical investigation of *Antidesma bunius* (L.) spreng leaf ethyl acetate extract led to the isolation and identification of amentoflavone 7-O-*β*-D-glucoside, kaempferol 3-O-*β*-D-glucoside (Astragalin) and quercetin 7-O-*α*-L-rhamnopyranosyl-(1‴→6″)-O-*β*-D-glucopyranoside (Quercetin-7-O-rutinoside) isolated for the first time from *A. bunius* leaf together with amentoflavone which is previously reported in *A.bunius* leaf. Detailed phenolics profile in *A. bunius* leaves ethyl acetate extract using HPLC-DAD-ESI- MS / MS analysis revealed a total of 12 metabolites belonging to flavonoids and phenolic acids. Xanthine oxidase inhibition and hypouricemic effect *in vitro* bioassays recommends for the potential use of *A. bunius* leaf extract for the treatment of gout.

Key words: *Antidesma bunius;* biflavone glycoside; flavonoids; xanthine oxidase; hypouricemic

1. Introduction

Antidesma is a genus of tropical plants belonging to family Phyllanthaceae which comprises ca. 200 species (Jangid and Gupta 2017). It is native to tropical Asia, Africa, Australia, and Islands of the Pacific (Hans 1970). *Antidesma bunius* (L.) spreng is a fruit-producing tree native to India, Singapore, Sri Lanka and Pakistan, and is widely cultivated in Philippines and Thailand (Li, Dressler et al. 2008, Lim 2016).The ripe fruits are used traditionally in Thailand for the treatment of gastric intestinal problems i.e., dysentery, indigestion and constipation (Hamidu, Ahmad et al. 2018). In Vietnam and China, *A. bunius* was used for the treatment of inflammation and infectious diseases (Chi 2012). Additionally, the leaves are used for the treatment of skin disorders, syphilis and for snake bites (Lim 2012). Besides , it is used for relieving cough, stomachache and to exhibit a hepatoprotective effect (Sosef, Hong et al. 1998, Zaman, Islam et al. 2018). Methanol extract of *A bunius* leaves and fruits was showed to have a potential cytotoxic activity (Zaman, Islam et al. 2018). Also, the hypoglycemic effect of *A. bunius* methanol extract was investigated in type 1 diabetes reavealing antidiabetic activity via enhancement of hepatic glycogen storage and regeneration of the islets of Langerhans (El-Tantawy, Soliman et al. 2015).

A wide range of phytochemicals have been reported in *A. bunius (L)* spreng including flavonoids as flavan-3-ols, flavanones (Butkhup and Samappito 2008, Pongnaratorn, Kuacharan et al. 2017, Hamidu, Ahmad et al. 2018), bioflavonoids, flavones (Kassem, Hashim et al. 2013, Hang, Nhiem et al. 2016), flavanones , anthocyanins, in addition to tannins, phenolic acids (Jorjong, Butkhup et al. 2015, Hamidu, Ahmad et al. 2018), Triterpenes (Hui and Sung 1968), vitamins and Essential oils (Zhang, Yin et al. 2017).

As part of our ongoing search of bioactive phytoconstituents with potential biological effects; investigation of *A. buinus* leaves ethyl acetate extract using isolation techniques and HPLC-DAD-ESI- MS / MS led to the annotation of 12 peaks, with full characterization of **1** new biflavonoid glycoside which is identified for the first time in natute alongside with 3 known flavonoids. Ultimately, the ethyl acetate extract exhibited potential xanthine oxidase inhibition and hypouricemic effect posing it for future incorporation in nutraceuticals used for gout treatment.

2. Results and discussion

2.1. HPLC-DAD-ESI-MS/MS analysis

To provide a comprehensive profile of phenolics in *A. bunius* (L.) leaf ethyl acetate fraction, HPLC coupled to DAD and MS detection in negative ion ESI mode was employed, with chromatogram represented in **Figure 1**. The identities, retention times, observed molecular and fragment ions for the individual components are presented in **Table 1.** Twelve compounds representing different classes of polyphenol were identified, **Figure 2, 3 & 4.** Identification was based on comparison of tandam MS/MS fragmentation pattern with that reported in literature.

For example, peak **1** showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 515, as well as a base peak at *m/z* 341 corresponding to the loss of quinic acid moiety (-*m/z* 174 u), and further loss of hexosyl moiety (-*m/z* 162 u) and a fragment ion at *m/z* 179 for caffeoyl acid residue. Consequently, peak 1 was identified as chlorogenic acid-O-hexoside (Abu-Reidah, Arráez-Román et al. 2013).

Free caffeic acid was detected in peak **3** [M-H]− at (*m/z*) 179 with daughter ion peaks at (*m/z*) 135 [M-H-44]− and (*m/z*) 107 [M-H-44-28]− (Biesaga and Pyrzynska 2009). Another phenolic acids were detected in peak **2** $[M-H]^-$ at *m/z* 169 and yielding a fragment at *m/z* 125 with a $[M-H-CO_2]^-$ identified as gallic acid (Hamed, Refahy et al. 2017) and its methyl ester in peak **4** $[M-H]^-$ at *m/z* 183 and MSn ions at *m/z* 169, and 125 (Hamed, Refahy et al. 2017).

Flavonoid glycosides were identified in several peaks **6**, **7**, **8**, **11**, and **12** mostly based on aglycone mass loss after sugar cleavage. For example peak **6** $[M-H]^-$ at *m/z* 463 showed a loss of hexosyl moiety (-*m/z* 162 u) yielding quercetin aglycone ion at m/z 301. Other key fragment ions were also observed at *m/z* 271, 255, 179, and 151and annotating peaks 6 as quercetin-*O*-hexoside (Bravo, Goya et al. 2007, Al-Rawahi, Edwards et al. 2014). Quercetin aglycone was detected in peak **5** at *m/z* 301 showing characteristic key fragment ions at *m/z* 271, 255, 179, and 151 (Abu-Reidah, del Mar Contreras et al. 2014).

Peak **7** [M-H]− at *m/z* 593, showed a loss of rhamnosyl moiety (-*m/z* 146 u) gave a fragment ion at *m/z* 447 assigned to kaempferol hexoside. Further loss of the hexosyl moiety (-*m/z* 162 u) yielding kaempferol aglycone ion at *m/z* 285 which finally confirmed through the appearance kampferol rutinoside ***kaempferol-3-O-rutinoside*** (Al-Rawahi, Edwards et al. 2014). Peak 8 at [M-H]− at *m/z* 447 was assigned to kaempferol hexoside from the loss of hexosyl moiety (-*m/z* 162 u) gave a fragment ion at *m/z* 285 which was assigned to kaempferol aglycone with other key fragment ions at *m/z* 255, 227, 179 and 151. Therefore, it was identified as ***kaempferol-3-O-hexoside*** (Bravo, Goya et al. 2007, Abliz, Mijit et al. 2015).

Peak **9** showed a pseudomolecular ion peak at *m/z* 167 and MSn ions at *m/z* 153 [M-H-CH3]−, 137 [M-H- OCH3]− and 123 [M-H-CO2]−; it was identified as ***Vanillic acid*** (Biesaga and Pyrzynska 2009). Peak **10** [M-H]− at *m/z* 537, it produced the $[M-H-C_6H_6O]^-$ ion at *m/z* 443, $[M-H-C_7H_4O_2]^-$ ion at *m/z* 417, $[M-H-C_7H_6O_3]^-$ ion at *m/z* 399 & $[M-H-C_9H_6O_3]^-$ ion at *m/z* 375 corresponding to the base peak, while $[M-H-C_{10}H_6O_5]^-$ ion appeared at *m/z* 331, and $C_8H_5O^-$ ion at *m/z* 117 in the mass spectrum , Therefore, it was identified as ***Amentoflavone*** (Yao, Chen et al. 2017).

Peak **11** [M-H]− at *m/z* 699 which was assigned to amentoflavone glucoside, the loss of glucosyl moiety (-*m/z* 162 u) gave a fragment ion at *m/z* 537, it also produced the $[M-H-C_6H_6O]^-$ ion at *m/z* 443, $[M-H-C_7H_4O_2]^-$ ion at *m/z* 417, $[M-H-C_7H_6O_3]^-$ion at *m/z* 399, $[M-H-C_9H_6O_3]^-$ ion at *m/z* 376 corresponding to the base peak, $[M-H-C_{10}H_6O_5]^-$ion at *m/z* 331, and $C_8H_5O^-$ ion at *m/z* 117 in the mass spectrum. Therefore, it was identified as ***Amentoflavone -O-hexoside*** (Negm, Ibrahim et al. 2016). Peak **12** at [M-H]− at *m/z* 609, while the loss of rhamnosyl moiety (-*m/z* 146 u) led to generation of MS/MS fragment ion at *m/z* 463 which was assigned to quercetin-

O-hexoside, the removal of water molecule (-*m/z* 18 u) produced a fragment ion at *m/z* 445, while the elimination of the hexosyl moiety (-*m/z* 162 u) yielded quercetin aglycone at *m/z* 301 with other key fragments at *m/z* 271, 255, and 179. Therefore, it was identified as ***quercetin-3-O-rutinoside (rutin)*** (Abu-Reidah, Ali-Shtayeh et al. 2015).

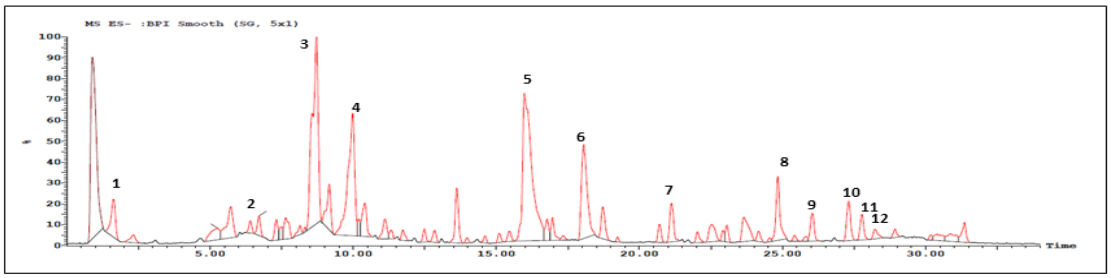

Figure 1 Representative of HPLC-DAD-MS analysis of *A. bunius* leaves ethyl acetate extract showing, chlorogenic acid-O-hexoside, gallic acid, caffeic acid, methyl gallate, quercetin, quercetin-*O*-hexoside, kaempferol-*O*-rutinoside, kaempferol-*O*-hexoside, vanillic acid, amentoflavone, amentoflavone-*O*- hexoside, and rutin from 1 to 12 respectively.

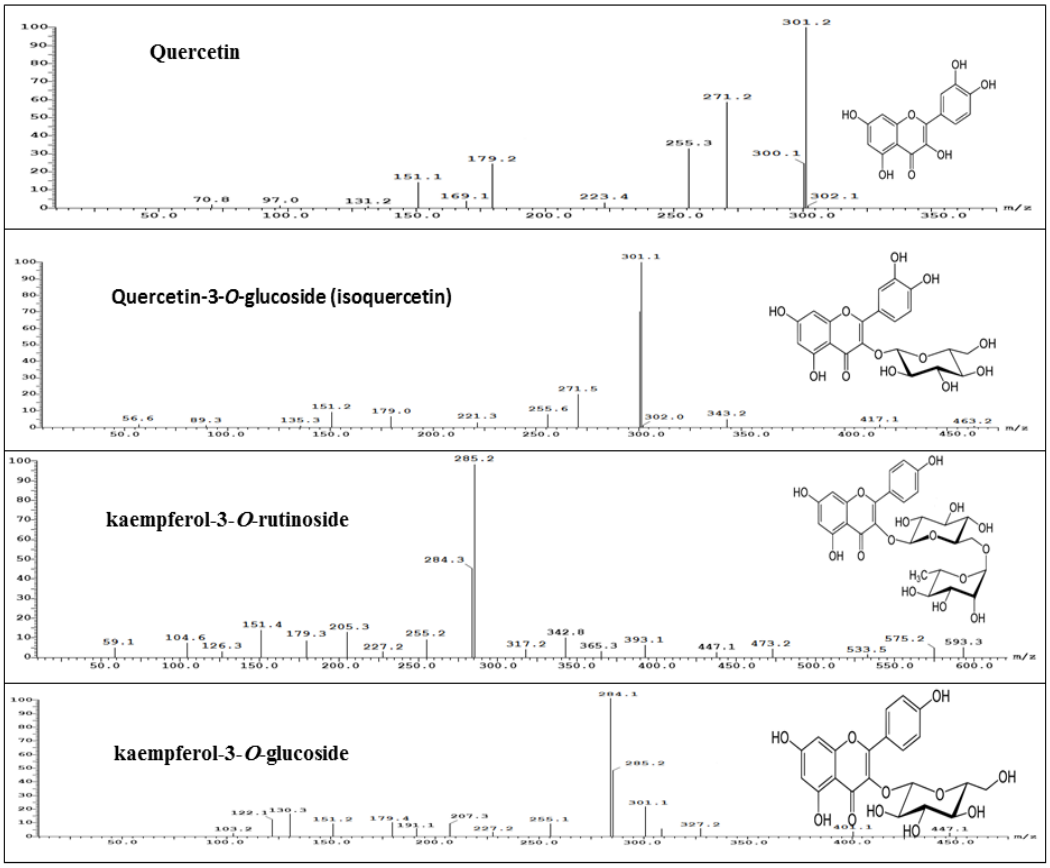

Figure 2: MS/MS spectra and postulated fragmentation patterns of some selected phenolic compounds detected in the EtOAc extract of *A. bunius* (L.) Spreng leaves using HPLC-DAD-ESI-MS/MS in negative ionization mode.

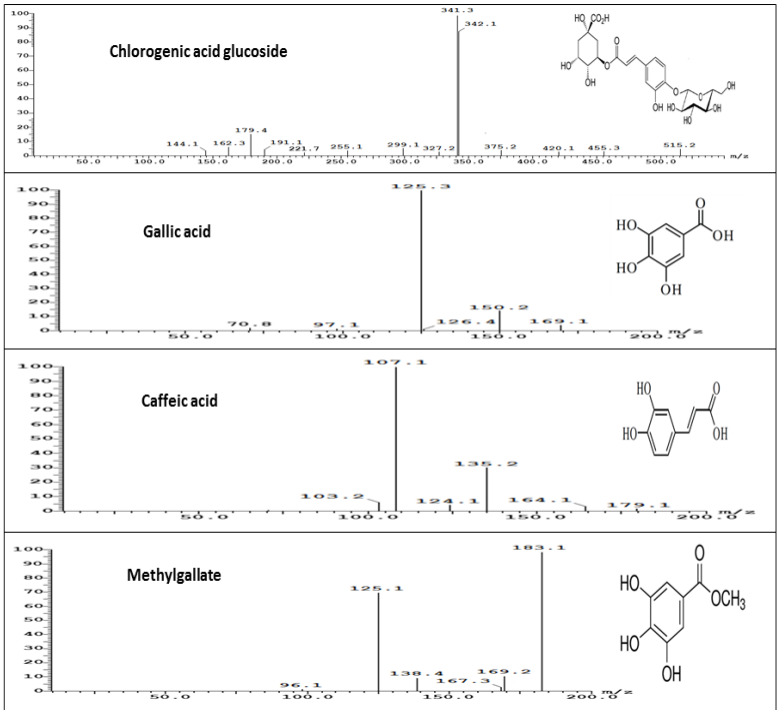

Figure 3: MS/MS spectra and postulated fragmentation patterns of some selected phenolic compounds detected in the EtOAc extract of *A. bunius* (L.) Spreng leaves using HPLC-DAD-ESI-MS/MS in negative ionization mode.

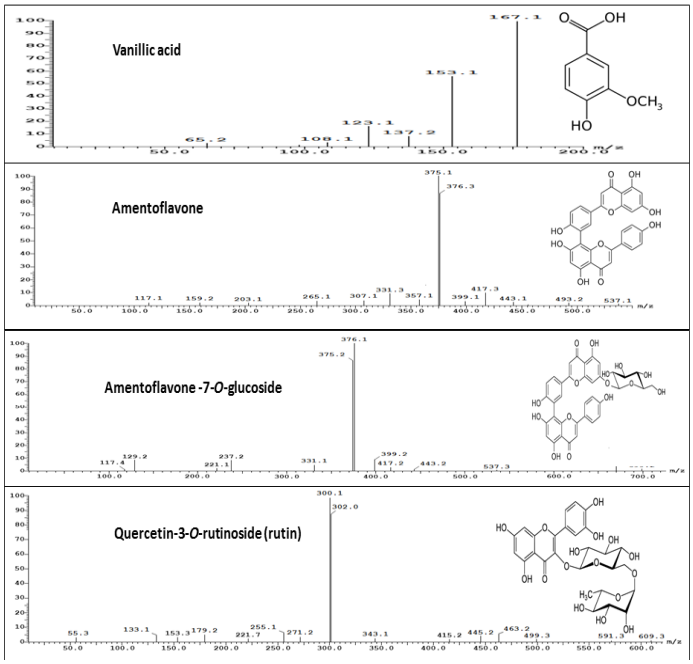

Figure 4: MS/MS spectra and postulated fragmentation patterns of some selected phenolic compounds detected in the EtOAc extract of *A. bunius* (L.) Spreng leaves using HPLC-DAD-ESI-MS/MS in negative ionization mode.

Table 1: Phenolic compounds identified in *A. bunius* (L.) Spreng leaf ethyl acetate extract using HPLC-DAD-ESI-MS/MS (negative ion mode)

NO.	R*t*	M.wt.	$[M-H]^-$	MSn fragments	Identification	References
(1)	1.92	516	515	341, 179, 173	Chlorogenic acid-O-hexoside	Abu-Reidah *et al*.2013
(2)	6.23	170	169	125 [M-H-CO_2]-, 97 [M-H-CO_2-CO]-	Trihydroxybenzoic acid (Gallic acid)	Hamed *et al*.2017
(3)	8.72	180	179	163, 135[M-H-CO_2]- , 125, 118, 107	3,4-Dihydroxy-cinnamic acid (Caffeic acid)	Biesaga and Pyrzynska 2009
(4)	9.82	184	183	169, 167, 138, 125	Methyl gallate	Hamed *et al*.2017
(5)	16.23	301	300	301, 300, 271, 255, 179, 169, 151	Quercetin	Abu-Reidah *et al*.2014
(6)	18.34	464	463	301, 271, 255, 179, 151	Quercetin-*O*-hexoside	Bravo *et al*. 2007 & Al-Rawahi *et al*. 2014
(7)	21.12	594	593	447, 429, 285, 255, 227, 179, 169	Kaempferol-*O*-rutinoside	Al-Rawahi *et al*. 2014
(8)	24.51	448	447	285, 284, 255, 227, 151	Kaempferol-*O*-hexoside	Bravo *et al*. 2007 & Abliz *et al*.2015
(9)	26.24	168	167	153 [M-CH_3]-, 137 [M-OCH_3]-, 123 [M-H- CO_2]-	Vanillic acid	Biesaga and Pyrzynska 2009
(10)	27.14	538	537	443, 417, 399, 375, 331, 117	Amentoflavone	Yao *et al*.2017
(11)	28.24	700	699	537, 443, 417, 375, 331,117	Amentoflavone-*O*- hexoside	Negm *et al*.2016
(12)	29.11	610	609	463, 300, 301, 271, 255, 179	Quercetin-3-O-rutinoside (rutin)	Abu-Reidah *et al*.2015

2.2. Isolated compounds

The investigation of phytoconstituents with potential biological activity; of *A. buinus* leaves ethyl acetate extract using isolation techniques led to characterization of 1 new biflavonoid glycoside identified as amentoflavone 7-O-glucoside **(2)** in addition to 3 known flavonoids identified as amentoflavone **(1)** (Aboul-Ela et al. 1999) previously reported, kaempferol 3-O-β-D-glucoside (Astragalin) **(3)** (Mabry, Markham et al. 2012), and quercetin 7-O-α-L-rhamnopyranosyl-(1‴→6‴)-O-β-D-glucopyranoside (Quercetin-7-O-rutinoside) **(4)** (Mabry, Markham et al. 2012) isolated for the first time from Antidesma.

Compound 2 was purified as yellow amorphous powder (15 mg), (R_f =0.54), m.p. >300 ºC. ^{1}H-NMR spectrum (400 MHz, DMSO-d_6) and ^{13}C-NMR with the high resolution electron spray ionization mass spectrometry (HR-ESI-MS) at m/z 699 $[M-H]^-$ all confirmed The molecular formula to be $C_{36}H_{28}O_{15}$.The signals of *meta*-coupled proton at 6.15 and 6.28 (*d, J*=2.1 Hz) were assigned for H-6 and H-8, respectively, whereas the signals at δ H 5.96 (1H, s) accounted for H-6″.The signals at δ H 6.76 ppm (s) and 6.67 (s) corresponded for H-3 and 3″, respectively. ^{1}H-NMR spectrum also showed an ABX system represented by signals at δ H 8.30 (*d, J*=2.1 Hz) for 2′ , 6.87 (*d,*

J=9.6 Hz) for 5′ & δ H 7.87 (*dd*, *J*= 9.6, 2.1 Hz) for H-6` . The presence of AA` BB` system was confirmed from signals at δ H 6.55 (*d*, *J*=9.6 Hz) for 3‴ & 5‴ and δ H 7.70 (2H, *d*, *J*=9.6 Hz) for H-2‴, 6‴. Acid hydrolysis of compound 2 yielded amentoflavone as the aglycon part isolated amentoflavone in this work by co-chromatography. Whereas, sugar part resulting from acid hydrolysis showed that revealed for glucose a the glycone moiety against authentic. ^{13}C-NMR revealed the signals of 30 carbons including two carbonyl at C 182.4 and 182.5 ppm including 16 non-protonated and 12 methine carbons indicating the presence of two flavone units (Hang, Nhiem et al. 2016). ^{13}C signals at δ C 99.69, 70.0, 74.49 , 69.61 , 70.34 & 63.42 ppm confirmed the presence of one sugar moiety (O-glucose) (Agrawal, Bansal et al. 1989). Glycosylation position in compound 2 was revealed from the anomeric proton appearing at δ H 5.40 with *J*=4.4 Hz, suggestive for its attachment at C-7 position (Markham *et al*.1978), and further confirmed from key HMBC correlation crosspeak between anomeric proton of glucose at δ H 5.4 and C-7 at δ C 159.75 concurrent with a downfield shift of the ortho-related carbon C-6 (δ C 99.37 ppm) (Agrawal, Bansal et al. 1989) and(Markham, Ternai et al. 1978). Another key HMBC cross peaks form H-2′ (δ-H 8.3) to C-3′ (δ C 121.76) and C-8′′(δ C 104.33), as well as the long range correlation between C-8″ (δ C 104.33) with H-6′′(δ-H 5.96) and C-3′ (δ-C121.76) with H-5′ (δ H 6.87) indicated the linkages between the two flavone units at C-3` and C-8˝consequently (Hang, Nhiem et al. 2016). From these data compound 2 was identified as amentoflavone 7-*O*-glucoside), first time to be reported in *A. bunius*, **Figure 5**.

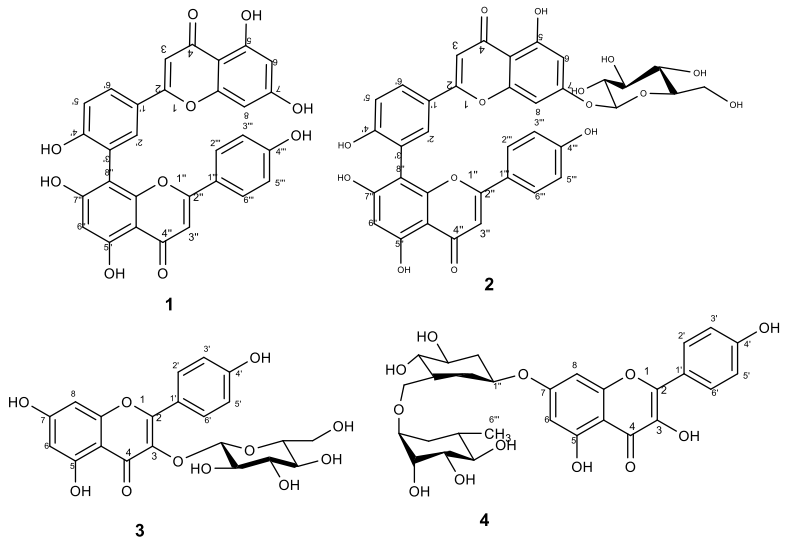

Figure5. Structures of the compounds isolated from *A. bunius leaves* ethyl acetate extract.

2.3. Hypouricemic activity of A. bunius

Previous studies revealed that the ethanol extract from *A. bunius* leaves contained flavonoids, tannins, saponins and phenols in their leaves. Many experimental studies have been conducted that have identified the association between the phenolic compounds and their anti-inflammatory activities. In the present work, administration of 30 % fructose to rats for 3 weeks resulted in a significant increase in the serum uric acid

level(uric acid was measured by colorimetric assay using a commercial kit purchased from Biodiagnostics, Cairo, Egypt) as compared to their corresponding controls, $P < 0.05$. The administration of *A. bunius* ethylacetate extract (at a dose of 250 mg/kg) to the hyperuricemic rats group led to a significant decrease in the serum uric acid level in comparison to that of fructose-treated group, $P < 0.05$. No significant difference was detected between the extract and reference drug (allopurinol)-treated rats, $P > 0.05$, Fig (6).

Allopurinol (used as a reference drug in the present study) is commonly used for gout treatment (Sabina, Nagar et al. 2011). The results of the current work indicated that *A. bunius* ethyl acetate extract exerts potential hypouricemic effect. Such effect is likely attributed to the antioxidant, anti-inflammatory and xanthine oxidase inhibitory activity of phytoconstituents such as flavonoids, phenolic acids in *A. bunius* extract(Ahmad, Farman et al. 2008, (Zhu, Wang et al. 2004, Song, Ki et al. 2017), Table 1.

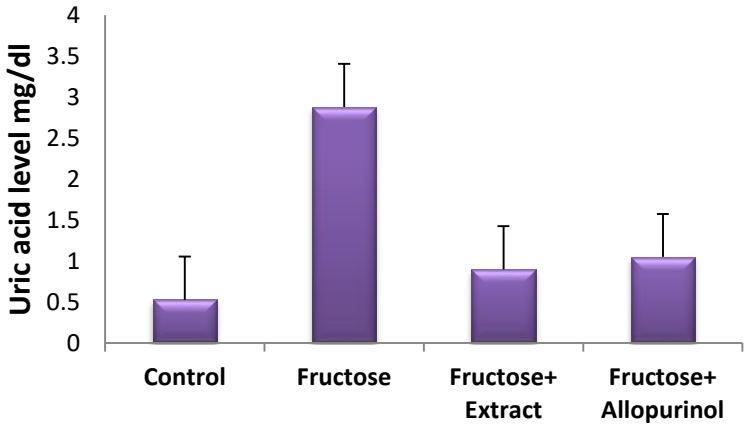

Figure 6. Uric acid level in control,fructose, fructose + 250 mg/kg *Antidesma bunius* extract and fructose + 10 mg/kg Allopurinol treated groups

3. Experimental

3.1.1 General experimental procedures

1D and 2D NMR spectra were acquired using a Bruker spectrometer operating at 400 MHz; DMSO-d_6 with TMS as internal reference. Mass spectrometer for HPLC-ESI-MS/MS; the analysis was performed on an Acquity UPLC system (Waters) equipped with a BEH (Ethylene Bridged Hybrid) C18 column (50 × 2.1 mm particle size 1.7 µm; Waters). Eluted compounds were detected from *m/z* 50 to 1000 using a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer).

MS Data analysis: ESI-MS^n mass spectra were obtained from a LCQ Deca XP MAX system (ThermoElectron, San Jose, USA) equipped with an ESI source. Mass spectra were detected in the ESI negative ion mode between *m/z* 50–1000. Relative

identification of MS spectra and comparison of metabolic profiles with that reported in literature were processed using the Maslynx 4.1 software. Compounds were identified by comparing its retention time (R*t*) and mass spectrum with reported data.

Semi preparative separations were carried out with a PuriFlash 4100 system (Interchim; Montluçon, France). Column chromatography was run using Normal phase for silica gel G 60- 230 mesh (Merck, Germany) and Sephadex LH 20, 0.25 - 0.1 mm mesh (Merck, Germany). Precoated silica gel plates 60 GF254 (20 × 20 × 0.2 mm thick) on aluminum sheets (Merck, Germany) was used for fraction monitoring.

3.1. 2. Plant material

Fresh leaves of *A. bunius* were collected from tree cultivated in El-Orman Garden, Ministry of Agriculture, Cairo, Egypt, during May, 2013. The plant was identified and confirmed by Mrs. Treeze Labib, consultant of plant taxonomy in Ministry of Agriculture and ex-director of Orman Botanical Garden, Giza. A voucher speciment was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy (Girls), Al-Azhar University.

3.1.3. Extraction and isolation

The air-dried powdered plant material (2 Kg) was extracted with 70 % methanol. The methanol extract was evaporated under vacuum, then defatted with methylene chloride and extracted by ethyl acetate.

Ethyl acetate extract (2 g) was dissolved in methanol then introduced into the Puriflash 30 Silica Hp - 80.0 G (20 Bar) column and eluted with gradient elution with methylene chloride: methanol. PDA-UV-Vis detector allows separation of fractions which were concentrated and monitored by silica gel TLC. The fraction eluted with methylene chloride: methanol (65:35) was purified by successive precipitation and washing with solvents of different polarities to obtain Compound **1** (40 mg). Fraction eluted with methylene chloride: methanol (45:55) was further purified on Sephadex LH-20 column to yield Compound **2** (15 mg) .The fraction eluted with methylene chloride: methanol (40:60) was re-chromatographed on Sephadex LH-20 column to afford Compound **3** (11 mg). Fraction eluted with methylene chloride: methanol (30:70) was further purified on Sephadex LH-20 column followed by silica gel column to yield a yellowish white amorphous powder Compound **4** (30 mg).

3.1.4. Physical Data

Compound 2

Yellow amorphous powder (15 mg), (R_f =0.54), m.p. >300 ºC. ^{1}H-NMR (400 MHz, DMSO-d_6), 6.76 (1H, S, H-3), 6.15 (d, *J*= 2.1 Hz, H-6), 6.28 (d, *J*=2.1 Hz, H-8), 8.30 (d, *J*=2.1 Hz, H-2′), 6.87 (d, *J*=9.6 Hz, H-5′), 7.87 (dd, *J*=9.6, 2.1 Hz, H-6′), 6.67 (1H, S, H-3˝), 5.96 (1H, S, H-6˝), 7.70 (2H,d, J=9.6 Hz, H-2‴/ 6˝), 6.55 (d, J=9.6 Hz, H-3‴, 5˝), anomeric proton 5.40 (1H, d) and ^{13}C–NMR (DMSO-d_6 100 MHz), 164.50 (C-2), 103.02 (C-3), 182.38 (C-4), 161.09 (C-5), 99.37 (C-6), 159.75 (C-7), 94.35(C-8), 159.22(C-9), 104.32 (C-10), 127.93 (C-1′), 128.33 (C-2′, 6′), 121.76 (C-3′), 161.09 (C-4′), 121.69 (C-5′), 164.70 (C-2˝), 103.02 (C-3˝), 182.57 (C-4˝), 161.97 (C-5˝), 99.88 (C-6˝), 164.02 (C-7˝), 104.33 (C-8˝), 152.92 (C-9˝), 104.06 (C-10˝), 122.07 (C-1‴), 128.79 (C-2‴, 6˝), 116.52 (C-3‴/ 5˝), 161.72 (C-4˝), 99.69 (C- Glu-1), 70.0 (C- Glu-2), 74.49 (C- Glu-3), 69.61 (C- Glu-4), 70.34 (C- Glu-5), 63.42 (C- Glu-6).

3.2. Biological activity

3.2.1. Experimental design

Wister albino rats (24 male) weighing (140-160) g were used for this study. The rats were acclimatized in humidity controlled room with temperature (25±1°C), and a 12-h light-dark cycle (lights on at 0600 h).

3.2.2 Induction of hyperuricemia

Control rats (n= 6) received daily vehicle (water, 1 ml/kg). Another group of rats (N= 18) was given 30% fructose in drinking water with standard chow for 3 weeks. Fresh drinking water was replaced every 2 days. After 3-week from fructose feeding, fructose-fed rats were further divided into 3 groups: 1) Fructose-fed rats (hyperuricemic rats). 2) Fructose-fed rats + Extract: received oral treatment of extract at a dose of 250 mg/kg body weight, by gastric tube once daily for another one week. 3) Fructose-fed rats + Allopurinol, received oral treatment of Allopurinol (reference drug) at a dose of 10 mg/kg body weight, once daily for another one week.

At the end of experiment, blood samples were withdrawn from the retro-orbital vein of each rat by capillary tube. Serum uric acid level was measured by using commercial kit purchased from Biodiagnostic, Egypt.

3.2.3. Statistical analysis

The data are represented as Mean ± S. E.M., and statistical significance was done using one way analysis of variance (ANOVA) followed by LSD test. *P*-values <0.05 were considered as statistically significant.

3.3. Acid Hydrolysis and Determination of the Sugar Moiety

2 mg of pure compound was treated with 1.5 N HCl in aqueous methanol 50% for 2 hr at 100 ºC, then methanol was evaporated and extracted with ethyl acetate. Aglycone was traced in ethyl acetate fraction by co-chromatography alongside authentic aglycones or by using different spectroscopic analysis. The aqueous phase was neutralized with sodium bicarbonate (5 % aqueous solution) and used for investigation of the sugar moiety on co-chromatography against authentic sugar samples and spraying with aniline hydrogen phthalate reagen (Eskander, Haggag et al. 2014).

4. Conclusion

This study revealed for the enrichment of flavonoids and phenolic acid in *A. bunius* with the isolation of new biflavonoid glycoside & three known compounds **1, 3** and **4.** HPLC-DAD-ESI-MS/MS analysis identified 12 peaks in that extract likely to act synergistically and mediate for the observed hypouricemic effect. Identification of chemical relative activities should now follow using in vitro based assay targeting inhibition of xanthine oxidase enzyme.

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التأثير الخافض لمستوي حامض اليوريك في الدم لمستخلص أوراق انتيديزما بيونيس والتعرف علي مركبات الفلافونويد

نيرمين ضياء سليمان[١]، عزه حسن شافعي[٢]، وليد حمدي الطنطاوي[٣]، دينا محمد يسري النجار[٤،٢].

[١] مستشفي التوفيق- مدينه نصر- القاهره

[٢] قسم العقاقير، كليه الصيدله بنات- جامعه الازهر، القاهره

[٣] قسم الكيمياء الحيويه، الهيئه القوميه للرقابه و البحوث الدوائيه

[٤] قسم العقاقير،كليه الصيدله- الجامعه المصريه الروسيه

البريد الالكتروني للباحث الرئيسي : DinaElnaggar.52@azhar.edu.eg

أدى الفحص الكيميائي لخلاصة خلات الإيثيل ل أوراق انتيديزما بيونيس السبرينج إلي فصل أربع مركبات فلافونيدية وهم : Amentoflavone-7-O-glucoside: Kampferol-3-O-glucoside (Astragalin): Quercetin-7-O-rutinoside: لم يسبق فصله من قبل من جنس انتيديزما و Amentoflavone وقد سبق فصله من قبل من جنس انتيديزما.

أدي فحص ملف الفينولات المفصول من مستخلص خلات الإيثيل لأوراق نبات انتيديزما بيونيس باستخدام كروماتوجرافيا السائل ذو الأداء العالي وكاشف الكتلة الطيفي HPLC-DAD-ESI- MS / MS للتعرف علي ما مجموعه 12 مركب ينتمي إلى الفلافونويد والأحماض الفينولية .اسفرت دراسة النشاط الخافض لمستوي حامض اليوريك في الدم للمستخلص الكحولي عن التوصيه باستخدام مستخلص أوراق انتيديزما بيونيس لعلاج النقرس.

الكلمات الأساسية: انتيديزما بيونيس ؛ ثنائي فلافون جليكوسيد. الفلافونويد. أوكسيديز الزانثين المثبط حمض اليوري