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Full Length Research Paper

Phytochemical and biological investigation of *Eugenia uniflora* L. cultivated in Egypt

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Eugenia uniflora L. (Myrtaceae) is a plant species used in folk medicine for treatment of various disorders. This study aims to quantify the phenolic and flavonoid contents of *E. uniflora* aqueous methanolic extract (AME), identification of its major constituents, as well as the evaluation of its biological activity. Quantification was performed using colorimetric assays. Column chromatographic separation was used for isolation of the major phenolic constituents while their structures were elucidated by 1D- and 2D Nuclear magnetic resonance spectroscopy (NMR) spectra. Alkaloids were identified using gas chromatography-mass spectrometry (GC-MS) analysis. The antidepressant activity of *E. uniflora* AME in mice was evaluated using the tail suspension test (TST). The weight control effect was evaluated by serial weighing. The results showed high phenolic and flavonoid contents of *E. uniflora* AME. The chromatographic investigation identified a new flavonoid, myricetin 3-O-(4", 6"-digalloyl) glucopyranoside, for first time in this genus, along with four flavonoids and phenolic acids. Integerrimine alkaloid was identified through GC/MS analysis. Administration of *E. uniflora* AME significantly reduced the immobility time in mice (P value of < 0.0001) in a dose-dependent manner for doses of 1, 10, 50 and 100 mg/kg. Also, a non-significant weight reduction in mice chronically treated with the *E. uniflora* AME was observed. Our study reports the isolation of myricetin 3-O-(4", 6"-digalloyl) glucopyranoside from *E. uniflora*. It confirms that *E. uniflora* leaf extract has an antidepressant and anti-obesity effect.

Key words: *Eugenia uniflora*, flavonoid, alkaloid, depression, obesity.

INTRODUCTION

Obesity and depression are escalating global and societal problems (McArdle et al., 2013; WHO, 2006). Associated environmental factors such as poor dietary habits, and sedentary lifestyle, that impair hormone

secretion and metabolism result in obesity (McArdle et al., 2013). There is an increasing interest by researchers to identify food items or dietary components that may control weight. On the other hand, conventional therapy

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of depression has been reported to be effective only in 50% of cases due to this, thus the necessity for a new treatment has risen (Kessler et al., 2003). The use of herbal therapy may be preferred in depression as it could provide a safer and potentially a more effective therapeutic option (Elhwuegi, 2004; Halliwell, 2006).

The genus *Eugenia* is considered one of the largest genera belonging to family Myrtaceae with about 500 species of trees and shrubs in tropical and subtropical America (Faqueti et al., 2013). *Eugenia uniflora* L. (Pitanga cherry or Brazilian cherry) is a putative candidate for management of both diseases. *E. uniflora* L. is a semi-deciduous shrubby tree with edible, cherry-like fruits, native to Brazil. The leaves and fruits of *E. uniflora* have been used in folk medicine for treatment of diarrhea, inflammation, rheumatic pains, fever, stomach problems and hypertension (Almeida et al., 1995; Consolini et al., 1999). In addition, it was reported to possess a wide range of medicinal properties including alleviation of mood disorders, antibacterial, antifungal, cytotoxic as well as radical scavenging activities (Alves, 2008; Auricchio et al., 2007; Auricchio and Bacchi, 2003; Franco and Fontana, 2004; Rattmann et al., 2012; Samy et al., 2014a; Saravanamuttu, 2012; Schapoval et al., 1994).

Among its wide range of biological activities, *E. uniflora* showed hypoglycemic as well as hypolipidemic effects (Saravanamuttu, 2012). Also, oral administration of essential oils (EO) from other *Eugenia* species (*E. brasiliensis*, *E. catharinae* and *E. umbelliflora*) produced antidepressant-like effects in mice (Colla et al., 2012; Victoria et al., 2013).

The leaves of *E. uniflora* were the subject of many phytochemical reports and the plant itself is deemed of interest as it is rich in tannins, flavonoids, triterpenoids and alkaloids. The identified alkaloids have been correlated with its anti-diabetic activity (Auricchio and Bacchi, 2003; Consolini et al., 1999; Fortes et al., 2015; Lee et al., 1997; Samy et al., 2014a; Saravanamuttu, 2012).

Our objectives were to identify the major constituents of *E. uniflora* aqueous methanolic extract (AME) using different chromatographic techniques, quantifying its phenolic and flavonoid contents, as well as investigating its antidepressant and weight control activity in mice.

MATERIALS AND METHODS

General experimental procedure

The NMR spectra were recorded at 400 (^1H) and 100 (^{13}C) MHz, on Varian Mercury 300, Bruker APX-400 and JEOL GX-500 NMR spectrometers and δ -values are reported as parts per million. (ppm) relative to TMS in the convenient solvent. For column chromatography, Sephadex LH-20 (Pharmacia, Uppsala, Sweden), microcrystalline cellulose (E. Merck, Darmstadt, Germany) was used. For paper chromatography, Whatman No. 1 sheets

(Whatman Ltd., Maidstone, Kent, England) were used. The solvent systems were: S1: n-BuOH-HOAc-H₂O (4:1:5, upper layer) and S2: 15 % aqueous HOAc. All solvents used for separation processes were of analytical grade.

Plant material

Fresh leaves of *E. uniflora* L. were harvested in April 2014 from El Zohria garden, Cairo, Egypt and were identified according to Prof. Abdel-Haleem Abdel-Mogaly, Department of Plant Taxonomy, Herbarium of Horticultural Research Institute, Agricultural Research Centre, Dokki, Cairo, Egypt. A voucher specimen (RS 0018) was deposited in the herbarium of the Faculty of Pharmacy, MSA University.

Extraction and isolation

Fresh leaves of *E. uniflora* (1 kg) were dried, ground and defatted in petroleum ether under reflux. The extracts were combined and dried under vacuum, while the leaves were refluxed with 70% methanol till exhaustion. The extracts were filtered off, combined, and dried under reduced pressure, giving rise to a crude aqueous methanolic extract (AME) (110 g). Seventy grams of the AME were applied on a cellulose column (1.5 m × 10 cm, 1000 g), elution being carried out with water-methanol mixtures of decreasing polarity and monitored by paper chromatography and detection by UV light. Fractions of 100 mL each were collected. The similar fractions were gathered yielding four main fractions according to their chromatographic properties (fluorescence-UV light, and responses towards different spray reagents on PC). Fraction I (0-20% MeOH, 15 g) was phenolic free. Fraction II (30-40% MeOH, 4 g) was subjected to Sephadex LH-20 column using 50% MeOH to yield compound 1 (20 mg). Fraction III (40-60% MeOH, 2 g) was purified on a Sephadex column using saturated butanol for elution, to yield compound 2 (15 mg). Fraction IV (80-100% MeOH, 2.5 g) was chromatographed on a Sephadex column with 50% MeOH to yield compounds 3, 4 and 5 (7 mg, each). For alkaloid estimation, ten grams of the AME extract was mixed with 200 mL distilled water, acidified with 5% sulfuric acid solution, and then fractionated with dichloromethane. The aqueous extract was further alkalized with ammonia, then extracted with dichloromethane. The process was repeated until the dichloromethane extract was negative to Dragendorff's reagent (Yubin et al., 2014). The combined dichloromethane extract was concentrated under vacuum and kept for GC/MS analysis. 0.25 g of total alkaloid extract was obtained with a percentage yield 2.5%.

GC/MS analysis

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a nonpolar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness). The carrier gas was helium with the linear velocity of 1 mL/min. The identification of components was based on the comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY libraries as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Santana et al., 2013).

Quantitative colorimetric estimation of phenolic and flavonoid contents

The total phenolic content of *E. uniflora* AME was quantified using

Folin-Ciocalteu Reagent (FCR) and gallic acid as standard (Sellappan et al., 2002) measured at λ_{\max} of 765 nm. Calculations were based on gallic acid calibration curve where the total phenolics were expressed as milligram of gallic acid equivalents (GAE) per gram dry extract. The total flavonoid content was determined using aluminum chloride colorimetric assay (Kosalec et al., 2004) where the measurement was performed at λ_{\max} of 415 nm. Calculations were based on quercetin calibration curve and the total content was expressed as milligram of quercetin equivalent (QE) per gram dry extract. All measurements were carried out in triplicate.

HPLC analysis

Identification of flavonoids in *E. uniflora* AME was performed using HPLC (Mattila et al., 2000). Dry plant extract (0.1 g) was mixed with 5 mL methanol and centrifuged at 10,000 rpm for 10 min and the supernatant was filtered through a 0.2 μm Millipore membrane filter, after which then 1 to 3 mL was collected in a vial for injection into HPLC Hewlett-Packard (series 1050) equipped with auto-sampling injector; solvent degasser, ultraviolet (UV) detector set at λ_{\max} of 330 nm and quarter horsepower pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 mL/min. Phenolic compounds were determined according to Goupy et al., (1999) with the same sample preparation with the exception that the UV detector was set at λ_{\max} of 80 nm and quarter HP pump (series 1100). Flavonoids and phenolic acid standards from Sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for calculation of flavonoids and phenolic acid concentrations by the data analysis of Hewlett-Packard software.

Antidepressant activity

Animals

Forty male albino mice (4 to 5 weeks) were used for this study. Animals were housed randomly in-groups of 4 rats per cage, kept at room temperature and provided with rodent chow and water ad libitum. Mice were kept under a 12:12 h light: dark cycle (lights on at 07:00 h). Mice were allowed to acclimatize to the holding room for at least 24 h before the behavioral procedure. All manipulations were conducted in the light phase, with each animal used only once ($n = 7$ animals per group). Animal care and handling was performed in conformance with approved protocols of the MSA research ethics committee.

Treatment

The *E. Uniflora* AME was dissolved in distilled water and administered orally acutely (just once at the beginning of the experiment) or chronically (for 10 days) at doses 1, 10, 50 and 100 mg/kg by oral route (p.o.) 60 min before the tail suspension test. A control group received distilled water only.

Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described previously (Steru et al., 1985). Mice were suspended about 50 cm above the floor by adhesive tape placed around 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively or stay completely

motionless. Conventional antidepressants decrease the immobility time in this test (Cunha et al., 2008; Steru et al., 1985).

Statistical analysis

All experimental results are given as the mean \pm S.E.M. Comparisons between experimental and control groups were performed using one-way ANOVA. A value of P of 0.05 was considered to be significant.

RESULTS

Phytochemical investigation

Column chromatographic investigation of *E. uniflora* AME results in the isolation of five compounds identified as: Myricetin 3-O-(4'', 6''-digalloyl glucopyranoside (1), myricetin 3-O-glucopyranoside (2) (Figure 1), quercetin (3), gallic acid (4) and ellagic acid (5). Compounds (1) and (2) were identified by comparing their spectroscopic data with that reported (Samy et al., 2014b) while compounds 3 to 5 were identified using Co-chromatography, by comparing with standards.

Myricetin 3-O-(4'', 6''-digalloyl glucopyranoside (1))

Yellow amorphous powder, purple fluorescent turned into yellow by long UV light. ^1H NMR (400 MHz, CD_3OD): ppm 3.2-4.3 (sugar protons), 5.3 (1H, br s, H-1''), 6.12 (1H, d, $J=2\text{Hz}$, H-6), 6.31 (1 H, d, $J=2\text{Hz}$, H-8), 7.14 (2H, d, $J=2\text{Hz}$, H-2'-6'), 7.22 (2 H, s, H-2'''/6'''), 7.29 (2H, s, H-2'''/6'''), ^{13}C -NMR (100 MHz, CD_3OD): 63 (C-6''), 71.5 (C-4''), 72.1 (C-2''), 74 (C-5''), 75 (C-3''), 94.6 (C-8), 99.8 (C-6), 101 (C-1''), 107.06 (C-10), 109.6 (C-2'-6'), 109.6 (C-2'''-6'''), 121 (C-1'''), (122 (C-1'), 136 (C-3), 137 (C-4'), 141.1 (C-4'''), 143.4 (C-3'-5'), 143.4 (C-3'''-5'''), 156.92 (C-9), 159 (C-2), 163 (C-5), 165 (C-7), 168 (C-7'''), 168.2 (C-7'''), 179 (C-4).

Myricetin 3-O-glucopyranoside (2)

Yellow amorphous powder, purple fluorescent turned into yellow by long UV light. ^1H NMR (400 MHz, CD_3OD): 3.2-4.3 (sugar protons), 5.05 (1H, d, $J=7.6\text{ Hz}$, H-1''), 6.1 (1H, d, $J=2\text{Hz}$, H-6), 6.31 (1 H, d, $J=2\text{Hz}$, H-8), 7.23 (2H, d, $J=2\text{Hz}$, H-2'-6'), ^{13}C -NMR (100 MHz, CD_3OD): 61 (C-6''), 70 (C-4''), 72 (C-2''), 73 (C-5''), 76 (C3''), 94 (C-8), 99 (C-6), 104 (C-1''), 105 (C-10), 109 (C2',6'), 109 (C-2'''-6'''), 122 (C-1'), 136 (C-3), 138 (C-4'), 146 (C-3'-5'), 158 (C-9), 162 (C-5), 166 (C-7), 179 (C-4).

Compound 3, 4 and 5

These were identified as quercetin, gallic acid and ellagic acid by Co-PC.

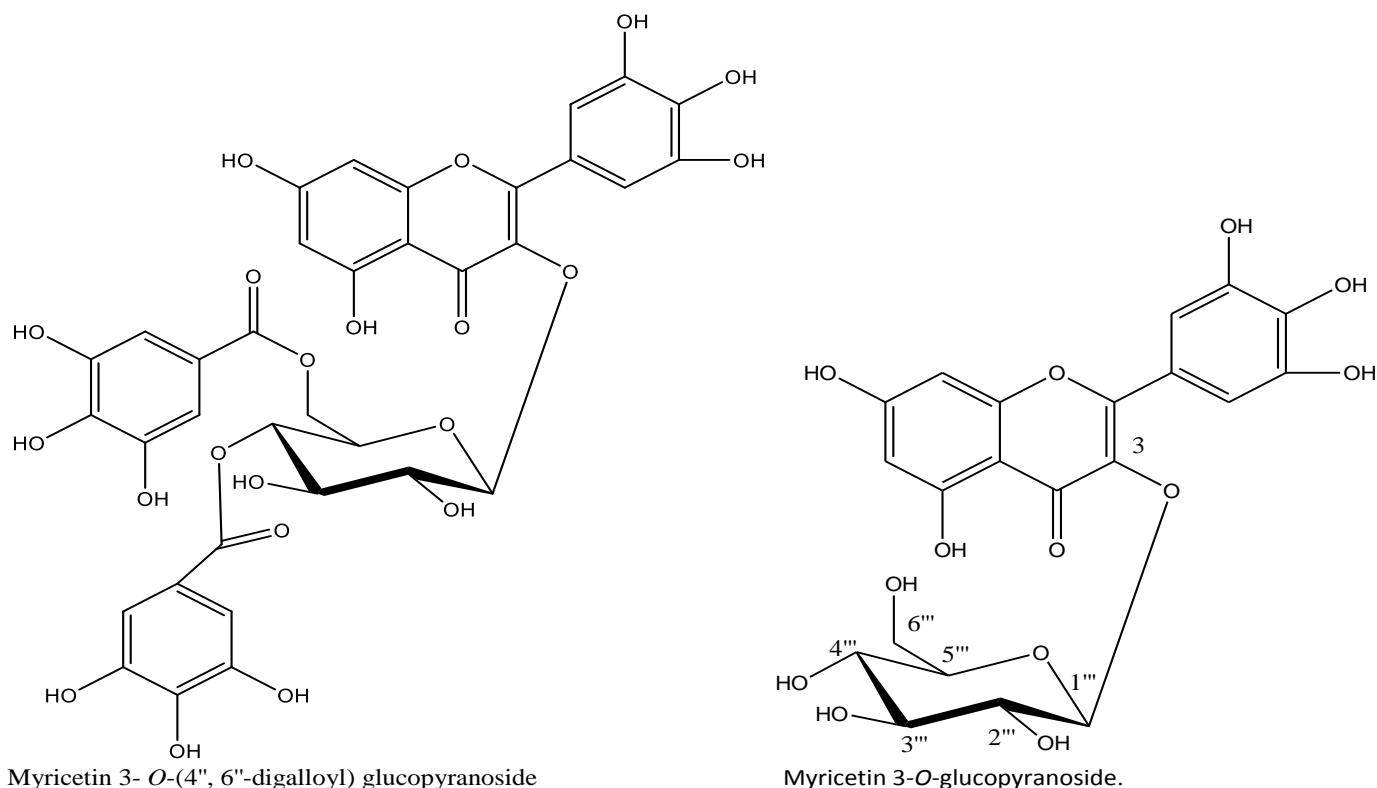


Figure 1. Chemical structures of *E. uniflora* main constituents.

GC/MS analysis

Investigation of the dichloromethane fraction resulted in the identification of ten compounds (Figure 2 and Table 1) including one main alkaloid, nitrogenated derivatives and some fatty acids. The identification was based on comparison of the MS characteristics and molecular weight (MW) with the mass spectra of the available databases and previous reports. Dodecanoic, oleic acid, in addition to hexadecanoic, heptadecanoic and octadecanoic acid derivatives have been identified. Compound 10 was identified as Integerrimine according to its fragmentation pattern (Figure 3).

Quantitative colorimetric estimation of phenolic and flavonoid contents

E. uniflora appeared rich in phenolic content expressed as 98.17 ± 0.35 mg/g GAE (standard curve equation: $y = 0.0011x + 0.0009$, $r^2 = 0.9867$). In addition, flavonoid content represented 8.1 ± 0.27 mg/g QE (standard curve equation: $y = 0.005x - 0.0198$, $r^2 = 0.9774$).

HPLC analysis

HPLC analysis was employed to identify the main

phenolic and flavonoid contents based on comparison of spectra with those of available standards. Results were presented in Tables 2 and 3. The results showed that gallic acid (10.85 mg/g) was the most abundant phenolic acid followed by benzoic acid (5.23 mg/g) then isoferulic acid (2.96 mg/g), while flavonoid analysis revealed quercetin (2.17 mg/g) as the main identified flavonoid followed by naringin (1.92 mg/g).

Antidepressant activity

Administration of *E. uniflora* AME reduced the immobility time in the TST in a dose-dependent manner for doses 1 and 10 mg/kg. The average immobility time for control mice was 3.7 ± 0.4 seconds, while it was 2.3 ± 0.8 and 0.9 ± 0.3 s for doses 1 and 10 mg/kg consequently. This reduction was statistically significant with the P value of < 0.0001 . At doses of 50 and 100 mg/kg the extract still significantly reduced (P value of < 0.0001) the immobility time to 1.6 ± 0.4 , and 2.4 ± 0.3 seconds respectively (Figure 4).

On the other hand, administering *E. uniflora* AME chronically for 10 days significantly (P < 0.0001) reduced the immobility time in TST at all doses tested. The immobility times for doses 1, 10, 50 and 100 mg/kg were 1.4 ± 0.2 , 2.3 ± 0.3 , 2.9 ± 0.5 and 2.1 ± 0.4 s, respectively.

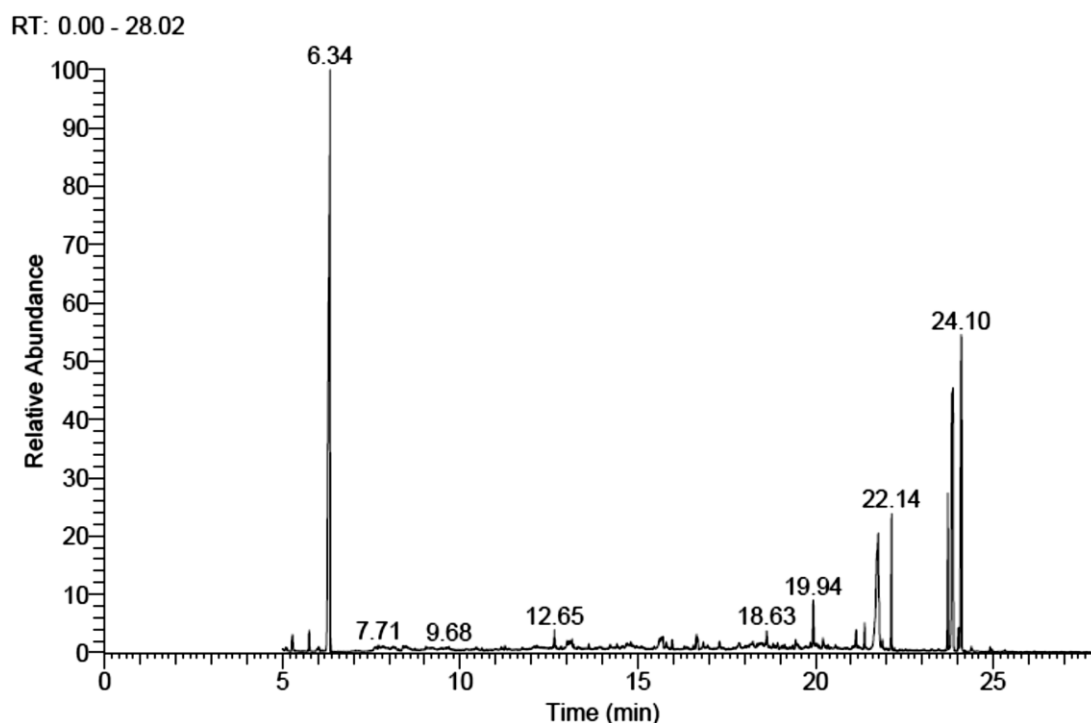


Figure 2. Fragmentation pattern of integerrimine identified in *E. uniflora* dichloromethane fraction.

Table 1. Identification of the main constituents in the dichloromethane fraction (GC/MS analysis).

S/N	Rt ¹	Constituent	M+	Mass spectral data (m/z ² , intensity %)
1	5.28	Pyridine	79	79 (100%) 52 (80%) 51 (60%) 50 (48%)
2	5.75	3 methyl Pyrrol	81	81 (75%) 80.1 (100%) 53 (30%)
3	6.33	2 butoxy ethanol	118	57.1 (100%) 45 (46%) 87.1 (30%) 75 (15%) 100 (10%)
4	15.70	Dodecanoic acid	200	55.1 (100%) 43 (92%) 73 (90%) 60 (85%) 129 (30%) 85 (30%)
5	16.66	Dihydromethyl jasmonate	226	83 (100%) 55 (30%) 67 (20%) 105 (20%) 155 (15%)
6	21.38	Heptadecanoic acid methyl ester	298	74 (100%) 87.1 (80%) 43 (60%) 55 (50%) 298.3 (30%) 143.1 (10%) 109 (10%)
7	21.76	Oleic acid	282	55.1 (100%) 69 (70%) 83 (69%) 97.1 (50%) 111.1 (30%) 129 (15%) 264.2 (15%) 226.1 (10%)
8	22.14	Octadecyl acetate	312	43 (100%) 83.1 (90%) 97.1 (80%) 111.1 (50%) 125.1 (20%) 252.2 (10%) 139.1 (10%) 165.1 (10%)
9	23.73	Hexadecanoic acid bis (2ethylhexyl)ester	370	129 (100%) 57 (50%) 112 (50%) 70.1 (30%) 83 (27%) 147 (27%) 241 (8%) 259.1 (8%)
10	23.86	Integerrimine	335	120.1 (100%) 136 (98%) 94.1 (80%) 80.1 (50%) 43 (22%) 67 (20%) 220.1 (20%) 335.1 (20%) 246 (18%)

Rt = Retention time; m/z = Mass to charge ratio; M+ = Molecular ion.

The immobility time for the control was 2.1 ± 0.8 s (Figure 5).

In addition, a non-significant weight reduction in mice chronically treated with the *E. uniflora* AME was

observed. The average weight for the control mice was 17.75 ± 0.7 while for the treated groups it was 16.8 ± 0.9 , 18.3 ± 0.6 , 17.1 ± 0.8 and 16.4 ± 0.8 g for doses of 1, 10, 50 and 100 mg/kg respectively (Figure 6). For all

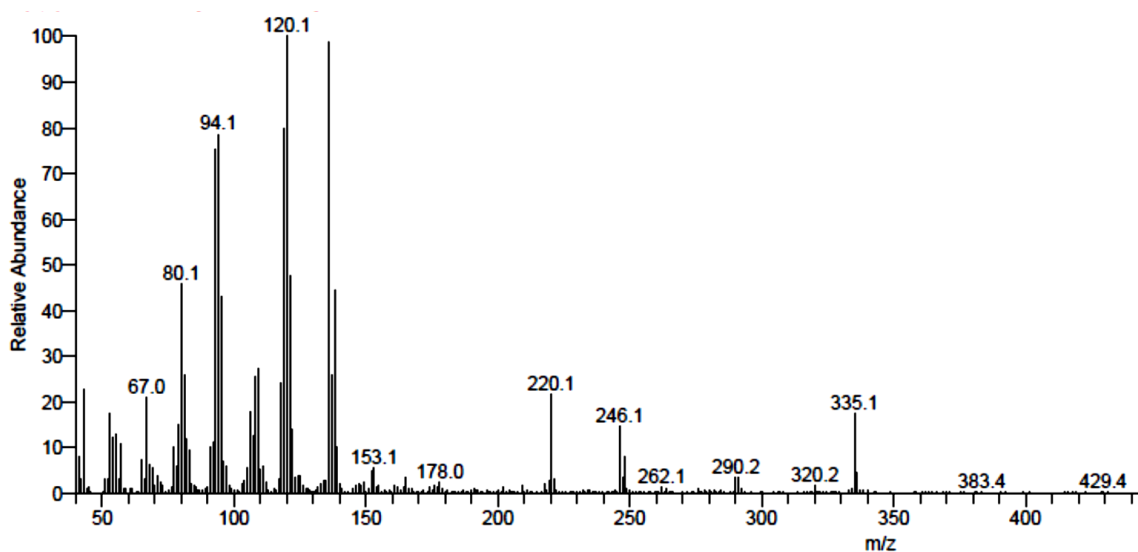


Figure 3. Fragmentation pattern of integerrimine identified in *E. uniflora* dichloromethane fraction using HPLC.

Table 2. Phenolic acid identified in *E. uniflora* AME using HPLC analysis.

Phenolic compound	Concentration (mg/g)
Gallic acid	10.85
Protocatechuic acid	0.238
Chlorogenic acid	1.364
Catechol	0.474
Catechin	0.289
P- hydroxy-benzoic acid	0.26
Caffeic acid	0.019
Vanillic acid	0.969
Ferulic acid	0.433
Isoferulic acid	2.96
Ellagic acid	1.383
Alpha coumaric acid	0.169
Benzoic acid	5.23
Salicylic acid	0.441
3, 4, 5 methoxy-cinnamic acid	0.066
P-Coumaric acid	0.162
Cinnamic acid	0.037

experiments N=7 for each experimental group.

DISCUSSION

This study confirmed high phenolic and flavonoid contents evaluated through HPLC and colorimetric assays, in agreement with reported literature (Suhendi et al., 2011). Beside the previously tentatively identified constituents, five compounds were isolated and identified.

Table 3. Flavonoid content in *E. uniflora* AME using HPLC analysis.

Flavonoid	Concentration (mg/g)
Naringin	1.92
Rutin	0.38
Hesperidin	0.196
Quercetrin	2.17
Quercetin	0.024
Naringenin	0.069
Hesperetin	0.38
Apigenin	0.023
7-OH- flavone	0.007

Compound 1, Myricetin 3- O-(4'', 6''-digalloyl) glucopyranoside was identified for first time in this species, compared with myricetin 3-O-(4''-O-galloyl) α -L-rhamnopyranoside, previously identified (Samy et al., 2014a). $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ showed closely related data to myricetin 3 O-glucopyranoside (compound 2) except for the presence of additional signals at δ 7.22 and 7.29 ppm referring to the galloyl moieties. The location was determined to be at C-6'' and C-4'' on the basis of the downfield shift of C-6'' and C-4'' glucose compared with the resonance of the corresponding carbon in the spectrum of the free glucopyranose present in compound 2. The two equivalent Galloyl protons H-2 and H-6 appeared as sharp singlet integrated for two protons at δ 7.22 and 7.29 ppm. Our data are similar to previous literature (Suhendi et al., 2011).

Integerrimine, the main alkaloid identified through its fragmentation pattern (Figure 3), was compared with the

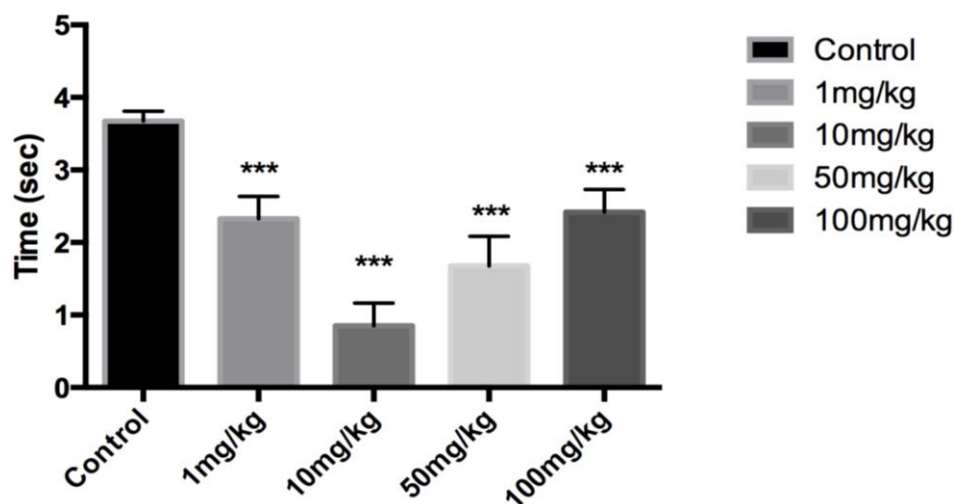


Figure 4. The effect of acute treatment with *E. uniflora* AME on the tail suspension test (TST) in mice. Administration of *E. uniflora* AME significantly reduced the immobility time in TST in mice treated for 60 min before the test at doses of 1, 10, 50 and 100 mg/kg. Results expressed as mean \pm SEM and analyzed using one-way ANOVA; (***) = P value was < 0.0001).

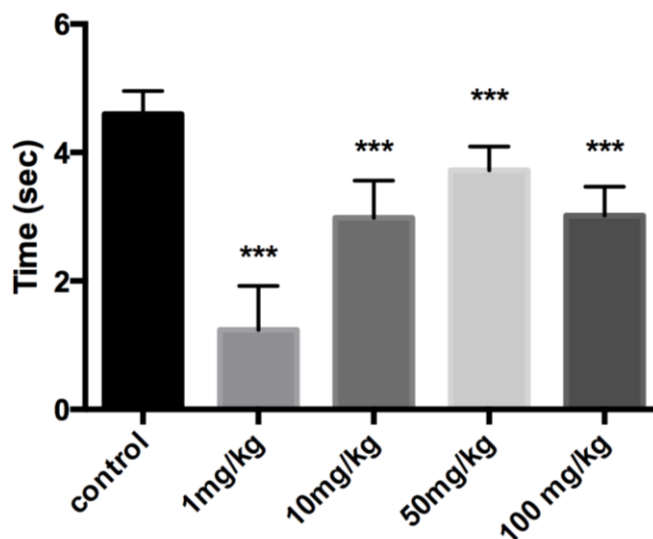


Figure 5. The effect of chronic treatment with *E. uniflora* AME on tail suspension test (TST) in mice. Administration of *E. uniflora* AME significantly reduced the immobility time in TST in mice treated for 10 days before the test at doses of 1, 10, 50 and 100 mg/kg. Results expressed as mean \pm SEM and analyzed using one-way ANOVA; (***) = P value was < 0.0001).

reported literature (El-Shazly et al., 1996; Zhu et al., 2015). The series of ions at m/z 136, 120, 119, 93, 94 and 80, are characteristic of 1, 2- unsaturated pyrrolizidine diesters. While the presence of a base peak at m/z 120 and 138 is denoting a retronecine-type of

pyrrolizidine alkaloid, where the ion fragment at m/z 220 is due to the cleavage of the weak allylic ester bond.

Gallic acid was identified with HPLC analysis in high concentration in agreement with Schumacher et al. (2015), while benzoic acid derivatives have been

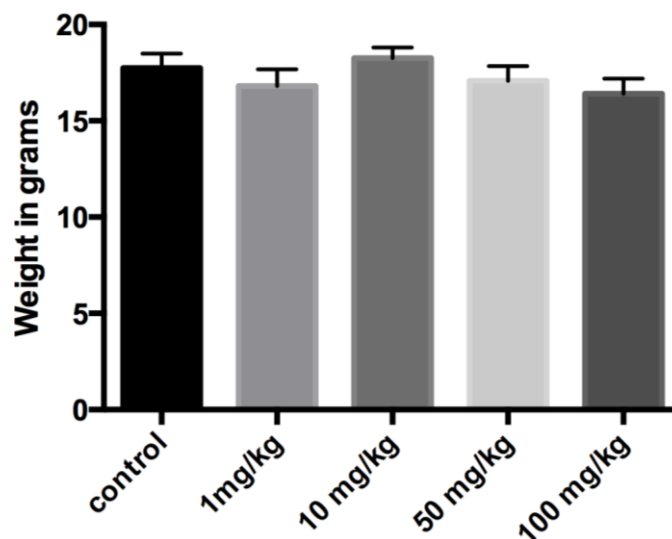


Figure 6. The effect of chronic treatment with *E. uniflora* AME on weight in mice. Administration of *E. uniflora* AME reduced the weight in TST in mice treated for 10 days at doses of 1, 10, 50 and 100 mg/kg. Results expressed as mean \pm SEM and analyzed using one-way ANOVA.

identified in *E. polyantha* (Lelono et al., 2013). Quercetrin which was the highest identified flavonoid was previously identified by Rattmann *et al.* (2012).

With respect to the antidepressant activity of *E. uniflora* AME extract the result of this study show that the extract significantly reduced the immobility time in a dose-dependent manner at the doses of 1 and 10 mg/kg. At higher doses the effect was less pronounced although the reduction was still significant when compared with the control. Our results come in agreement with Victoria et al., (Victoria et al., 2013) where they studied the antidepressant-like effect of *E. uniflora* essential oil in the TST at doses of 10 and 50 mg/kg. Furthermore, the administration of *E. uniflora* extract for one month still had antidepressant like activity (Figure 4) with the dose 1 mg/kg being highly significant compared to the other doses. These results could explain the traditional use of *E. uniflora* in folk medicine for the treatment of symptoms related to depression, as well as its use by Guarani Indians as a tonic stimulant (Alonso, 1998; Greinger, 1996). The stepwise fashion of the decrease in the reduction of immobility time at higher doses than 10 mg/kg could in fact support a receptor mediated mechanism for the antidepressant effect of *E. uniflora* AME (Colla et al., 2012).

The antidepressant activity demonstrated by *E. uniflora* AME can be attributed to its myricetin content. Previous report suggested the ability of myricetin to attenuate the depressant-like behaviors in mice exposed to repeated restraint stress by restoring the brain derived neurotropic

factor (BDNF) levels and attributed to the myricetin-mediated anti-oxidative stress in the hippocampus (Ma et al., 2015). Further studies however are needed to confirm this suggestion.

On the other hand, the different results obtained by Colla et al. (2012) who studied the hydro-alcoholic extract of different *Eugenia* species could be attributed to the difference in the plant constitution. Also, we used different species of mice and only male mice, which can account for different responses to the extract. Further studies are required to confirm the effective dose required for the antidepressant activity of the *E. uniflora* in different animal species as well as in human to detect the difference in response between males and females.

Although statistically non-significant, the *E. uniflora* AME produced a degree of weight loss in animals that received the extract for one month. This result could suggest a potential role for this extract in treatment of obesity especially that it has been reported that it has anti-hyperlipidemic and hypoglycemic effect (Ramalingum and Mahomoodally, 2014).

Conclusion

This study reports for the first time the identification of myricetin 3- O-(4", 6"-digalloyl) glucopyranoside and confirmed the high phenolic and flavonoid contents of Egyptian grown *E. uniflora* AME, beside the presence of alkaloid and fatty acids. In addition, this work supports the role for *E. uniflora* AME in the management of

depression and obesity. Further studies are required to correlate myricetin derivatives with the biological activities of *E. uniflora*, determine the effective dose, and the exact mechanism through which these effects are achieved.

ETHICS APPROVAL

Animal care and handling was performed in conformance with approved protocols of MSA University and Egyptian Community guidelines for animal care.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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