Evaluation of antioxidant, anti-tyrosinase potentials and phytochemical composition of four Egyptian plants

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Abstract

The aim of this present research is to evaluate antioxidant and antityrosinase potentials and to investigate the phytoconstituents of methanol (70%) extract from four Egyptian plants, Solanum rantonnetii, Tilia cordata, Cichorium intybus (L.), and Lagerstroemia tomentosa. Antioxidant activity was measured by using two different free radical scavenging methods, 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), whereas polyphenols and flavonoids contents were evaluated by using Folin-Ciocalteau, and aluminum nitrate methods respectively. Tyrosinase inhibition was evaluated using commercial enzyme and dihydroxyphenylalanine (DOPA) as substrate and methanol extract of each plant was investigated by phytochemical analysis. The results showed that all the plants had good antioxidant activity where Cichorium intybus exhibited the highest antioxidant activity determined as total content of free-radical scavenging, polyphenol and flavonoid molecules, and showed the best tyrosinase inhibition activity compared with the other plants extracts studied. Chromatographic separation and NMR analysis of Cichorium intybus methanol extract revealed the identification of two coumarins, scopoletin and esculetin, and seven flavonoids, dihydroquercetin 7-4’-dimethyl ether, blumeatin, diosmetin, tamarixetin, quercetin, quercetin 3-O-β-galactoside and kaempferol 3-O-rutinoside. These findings suggest that Cichorium intybus is rich with bioactive compounds and could be used as a good source of potentially natural antioxidants and antityrosinase molecules.

Keywords

Medicinal plants
Cichorium intybus
Aerial parts
Phytoconstituents
Antioxidants
Tyrosinase inhibition

Introduction

The medicinal plants represent an interesting contribution to modern therapeutics due to the presence in their composition of molecules with pharmacological and antioxidant action. Research on natural antioxidants has become increasingly active in various fields due to the action of antioxidant molecules on protection from the damage induced by oxidative stress. It is well known that free radicals may be involved in a number of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer’s disease, Parkinson’s disease. Thus, many studies have been carried on antioxidants in order to identify some bioactive compounds from natural sources (Pintus et al., 2013). Plant materials could be also a great source of powerful skin-whitening agents in medicinal and cosmetic fields (Chang, 2009). Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme that catalyzes the first steps of melanin synthesis: hydroxylation of tyrosine and the oxidation of 3, 4-dihydroxyphenylalanine (L-DOPA) to o-dopaquinone. Abnormal production of melanin is the cause of various dermatological disorders such as melasma, age spots and post-inflammatory hyperpigmentation. Because of their abilities to decrease melanin production, there is a big demand for tyrosinase inhibitors in order to treat dermatological disorders associated with skin hyperpigmentation. Four Egyptian medicinal plants including Solanum rantonnetii, Tilia cordata, Cichorium intybus, and Lagerstroemia tomentosa have been selected for the present study on the basis of their antioxidant and tyrosinase inhibitory properties. Solanum is a large and diverse genus of flowering plants, including two food crops of the highest economic importance, the potato and the tomato. Solanum rantonnetii is a shrub from Solanaceae family. The roots have soothing effects of the plant upon ingestion (Quattrocchi, 2000). Tilia cordata is a deciduous tree from Tiliaceae family. Active ingredients in the Tilia flowers include flavonoids (which act as antioxidants) and volatile oils. The plant also contains tannins that can act as
an astringent (Bradley, 1992). Infusions obtained from the flowers of Tilia cordata are widely used for the treatment of fever and anxiety problems in folk medicine. Recently some research have been done about the antioxidant capacity of flower extracts of this plant (Vinha et al., 2013) but nothing it’s known about the other aerial parts of the plant, object of this research. Lagerstroemia tomentosa from Lythraceae family is a medicinal and ornamental plant. The bark is considered as stimulant and febrifuge, leaves and flowers are used as purgative (Chopra et al., 1958), and the roots are astringent (Kirtikar and Basu, 1935). Cichorium intybus (L.) known as chicory is a plant from Asteraceae family. This plant is native to Europe and Asia. It has been widely used in folk medicine for treatment of gallstones, appetite loss, gout, jaundice, skin swellings, rheumatism, and liver inflammation (Gazzani et al., 2000). Historically, chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, and vegetable crop and was occasionally used for animal forage. Important phytochemicals are distributed throughout the plant, but the main contents are present in the root (Bais and Ravishankar, 2001). The seeds extract of C. intybus has high antioxidant and antidiabetic activities (Ghamarian et al., 2012), also it has ameliorating effects on non-alcoholic fatty liver disease (Ziamajidi et al., 2013). Methanolic extract of C. intybus and its various fractions have revealed wound healing effects. The methanolic extract of the plant has demonstrated some anticancer and apoptosis inducing effects (Suntar et al., 2012). The objective of this research is to explore phytoconstituents and potential activities of four Egyptian plants to contribute to the knowledge of these plants that might represent a potential source of bioactive natural molecules.

Materials and Methods

Materials

All chemicals were obtained as pure commercial products and used without further purification. 2,2’-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), aluminum nitrate, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), Folin-Ciocalteu phenol reagent, gallic acid, quercetin, mushroom tyrosinase and L-3,4-dihydroxyphenylalanine (L-DOPA) were from Sigma Chemical Co (St. Louis, USA).

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt), and Ultrospec 2100 spectrophotometer (Biochrom Ltd, Cambridge, England) using cells with a 1 cm path length. 1H-NMR and 13C-NMR spectra were collected on Inova NB Varian instrument (Varian Scientific Instruments, Palo Alto, CA), MS (Finnigan MAT SSQ 7000, 70 ev). Silica gel (0.063-0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F254 plates. Solvent mixtures, BAW (n-butanol:acetic acid: water 4:1:5, v/v/v), upper phase, 15% acetic acid). Paper Chromatography (PC) Whatman No.1 (Whatman Led. Maid Stone, Kent, England) sheets for qualitative detection of flavonoids and sugars.

Plants identification and collection

Aerial parts (leaves and stems) from Solanum rantonnetii were collected from Zoo garden, Giza, Egypt. Tilia cordata and Cichorium intybus aerial parts were collected from the Agricultural Research Centre, Giza, Egypt, while aerial parts of Lagerstroemia tomentosa were collected from Al-Zohiriya garden, Giza, Egypt in May 2012. All the plants were identified by Dr. Mohammed El-Gebaly, Department of Botany, National research centre (NRC) and by Mrs. Tereeza Labib consultant of plant taxonomy at the ministry of agriculture and director of Orman botanical garden, Giza, Egypt.

Preparation of the plants extracts

Air dried aerial parts of S. rantonnetii (280 g), T. cordata (340 g) C. intybus (640 g) and L. tomentosa (480 g) were extracted with methanol (70%) several times at room temperature by maceration method. Each extract was concentrated under reduced pressure to give 22.5 g, 25.5 g, 32 g and 28 g, respectively and immediately dissolved in DMSO before every experiment in order to have a 1mg/ml solution. Each extract was phytochemically screened according to (Yadav and Agarwala, 2011) and the antioxidant capacity, polyphenols, and flavonoids content were determined. Moreover, the ability of these extracts to inhibit tyrosinase activity was also evaluated.

ABTS•⁺ assay

This method (Re et al., 1999) is based on the capacity of an antioxidant to scavenge the free radical ABTS•⁺. The ABTS•⁺ was generated by reacting 7 mMABTS with 2.45 mM potassium persulfate (final concentration) in aqueous solution and the mixture was kept in the dark at room temperature for 24 h before use. The ABTS•⁺ solution, which has an absorption maximum at 734 nm, was diluted in the appropriate solvent to obtain an A734 of about 0.70. Extracted samples at different concentration (10 µL) were added to 1 mL of diluted ABTS•⁺ solution and
mixed vigorously. After reaction at room temperature for 1 min, the absorbance at 734 nm was measured. The decrease in $A_{375}$ was calculated and the results are presented as the ability of antioxidant to scavenge 50% of free radical ABTS $^\cdot$ (IC50).

**DPPH assay**

The assay was carried out as previously described (Pintus et al., 2013). DPPH· radical shows a strong absorption maximum at 515 nm and goes through a change of color after the transfer of a hydrogen atom from an antioxidant. An aliquot of extracts at different concentration (25 μL) was added to 975 μL of $6 \times 10^{-5}$ M DPPH· solution. The reaction mixture was incubated in the dark for 15 min and, thereafter, the $A_{375}$ was recorded against DPPH· solution alone as a control. The decrease in absorbance of DPPH· after addition of test extracts compared to the control, was used to calculate the antioxidant activity which was expressed as ability of antioxidant to scavenge 50% of DPPH radical (IC$_{50}$).

**Determination of total polyphenol content**

Total polyphenol content in the extracts were determined according to the Folin-Ciocalteau method using a calibration curve obtained with gallic acid as polyphenolic reference standard (Mansouri et al., 2005). Different concentrations of the extracts (10 μL) were mixed with 50 μL of the Folin-Ciocalteu reagent and 790 μL of distilled water. After 1 min, 150 μL of saturated sodium carbonate (20% aqueous solution) was added to the mixture. The mixture was kept in the dark for 45 min, and then the A750 was measured and compared to the gallic acid calibration curve. The polyphenol content was expressed as gallic acid equivalents (GAE; mM).

**Determination of total flavonoid content**

Total flavonoid content was determined according to the methods of Moreno et al., 2000. The extracts (0.5 mL) were added to 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M sodium acetate and 4.3 mL of 80% EtOH. After 40 min at room temperature, the absorbance was determined at 415 nm. Flavonoid concentration was calculated by a calibration curve obtained using quercetin as reference standard and expressed as quercetin equivalent molarity (QE, mM).

**Mushroom tyrosinase activity**

The inhibitory effect of the plant extracts on tyrosinase activity was determined spectrophotometrically with the degree of inhibition of mushroom tyrosinase-catalysed oxidation of L-DOPA (Rahaman et al., 2001). The reaction mixture contained the extracts and L-DOPA (1.25 mM) in 25 mM phosphate buffer (pH 6.8). Then, tyrosinase (100 U/ml) was added into the mixture and the activity was determined by following the increase in absorbance at 475 nm resulting from the formation of the dopachrome product. The inhibition of tyrosinase activity was calculated with the following formula: 

$$\text{Inhibition (\%) = (1 - (A_{375} \text{ in sample}/A_{375} \text{ in control})) \times 100\%.}$$

**Isolation of the components of methanol (70%) extract from Cichorium intybus aerial parts**

Methanol (70%) extract of C. intybus (28 g) was subjected to silica gel column chromatography eluting with n-hexane, dichloromethane, ethyl acetate and methanol gradually. One hundred and twenty fractions of 100 ml conical flask were collected. The fractions that showed similar paper chromatography in two solvent systems, butanol–acetic acid–water (BAW, v/v/v) 4:1:5 and 15% acetic acid were combined to give four main fractions. Fraction 1 (4.55 g) was subjected to sub–column of silica gel eluted with n-hexane: dichloromethane where elution with n-hexane-dichloromethane (60:40) yielded compound 1 and further elution with n-hexane:dichloromethane (80:20) yielded compound 2. Fraction 2 (3.85 g) was subjected to sub–column of silica gel eluted with dichloromethane:ethyl acetate. Elution with dichloromethane: ethyl acetate (90:10) gave compound 3 and further elution with dichloromethane:ethyl acetate (85:15) yielded compound 4. Fraction 3 (6.45 g) was subjected to sub–column of silica gel eluted with dichloromethane:methanol. Elution with dichloromethane:methanol (98:2) afforded compound 5 while elution with dichloromethane:methanol (95:5) yielded compound 6 and further elution with dichloromethane:methanol (90:10) gave compound 7. Fraction 4 (5.65 g) was subjected to sub–column of silica gel eluted with ethyl acetate:methanol (95:5) afforded compound 8 and further elution with ethyl acetate:methanol (70:30) yielded compound 9. All the isolated compounds were purified on sephadex LH–20 column using methanol, ethyl alcohol and different systems of methanol and distilled water (methanol: distilled water, 1:1, 2:1, v/v).

**General method for acid hydrolysis**

Compounds 8, and 9 (5 mg) was heated in 5 ml 10% HCl for 5 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in

$$\text{Inhibition (\%) = (1 - (A_{375} \text{ in sample}/A_{375} \text{ in control})) \times 100\%}.$$
Results and Discussions

This study focused on antioxidant and antityrosinase activities of four methanol (70%) extracts of Solanum rantonnetii, Tilia cordata, Cichorium intybus and Lagerstroemia tomentosa and also phytochemical analysis was done.

The antioxidant ability of the extracts was screened by ABTS and DPPH assays and IC$_{50}$ values are reported in Table 1. S. rantonnetii and L. tomentosa showed the same scavenging capacity in each assay with an IC$_{50}$ of about 50 and 100 μg/mL for ABTS and DPPH methods, respectively. T. cordata has the lowest amount of antioxidant, whereas C. intybus showed the best antioxidant activity with lower IC$_{50}$ values of 21.56 and 30.06 μg/mL using ABTS, and DPPH methods.

These values are high if compared with trolox (IC$_{50}$~3.2 μg/mL); this is not surprising since analyzed samples are complex extracts and they most likely contain pro-oxidants agents which may compete with the antioxidants in the reaction with ABTS and DPPH radicals. Thus IC$_{50}$ values are the results of the balance between pro-oxidant and antioxidant molecules. In Figure 1A., the total polyphenolic content of the plants extracts is reported, expressed as gallic acid equivalent and measured by Folin-chocalteu method. Starting with the better extract, the amount of polyphenols follows the order C. intybus (2.33 mM) > L. tomentosa (1.02 mM) > S. rantonnetii (0.69 mM), and T. cordata (0.46 mM). The total flavonoid content also varies in the different extracts as shown in Figure 1B., L. tomentosa gave the highest value (0.5 mM) followed by C. intybus (0.25 mM) whereas S. rantonnetii, and T. cordata showed the lower values (0.113 mM, and 0.132 mM). Moreover, in vitro tyrosinase assay was performed to evaluate if these plants extracts showed any inhibitory activity toward this enzyme. Melanin synthesis is carried out by a specific enzymatic pathway controlled by tyrosinase. The inhibition of tyrosinase is therefore the most common approach for the identification of new depigmenting agent. There is no any observed inhibitory effect for S. rantonnetii, and L. tomentosa extracts whereas, in presence of the extracts from T. cordata and C. intybus, the activity of mushroom tyrosinase was markedly decreased with the latest showing the strongest inhibitory effect (Table 2). Moreover, as compared with arbutin (IC$_{50}$ value of 0.13 mg/mL), a well known potent tyrosinase inhibitor, extracts from T. cordata and C. intybus had almost similar activity with IC$_{50}$ values of 0.67 and 0.34 mg/mL, respectively (Loizzo et al., 2012). According to these results, our attention was focused on identification and characterization of the bioactive compounds present in C. intybus methanol extract. Phytochemical analysis of the four methanol (70%) extracts was done and the results are reported in Table 3, showing their phytoconstituents. S. rantonnetii extract proved the presence of triterpenes, flavonoids, tannins, and carbohydrates. T. cordata extract showed the presence of coumarins, triterpenes, flavonoids, tannins, saponins, and carbohydrates. L. tomentosa extract revealed the presence of triterpenes, flavonoids, tannins, and carbohydrates, while C. intybus extract showed that it contained triterpenes, flavonoids, tannins, carbohydrates, and coumarins (Table 3). Chromatographic separation of C. intybus methanol (70%) extract revealed the identification of two coumarins, scopoletin and esculetin, and seven flavonoids dihydroquercetin 7-4’-dimethyl ether, blumeatin, diosmetin, tamarixetin, quercetin, quercetin 3-O-β-galactoside and kaempferol 3-O-rutinoside (Figure 2). The NMR parameters for the identification of the isolated compounds are reported in the next paragraph

**Structure elucidation of the isolated compounds**

Scopoletin (1): 12 mg, 1H-NMR (CDCl$_3$, 300...
MHz) δ ppm 3.76 (3H, s, 6-OCH$_3$), 6.34 (1H, d, J=9.7 Hz, H-3), 7.08 (1H, s, H-5), 7.14 (1H, s, H-8), 7.72 (1H, d, J=9.7 Hz, H-4). $^{13}$C-NMR (CDCl$_3$, 100 MHz) δ ppm 56.34 (-OCH$_3$), 104.14 (C-8), 109.32 (C-5), 110.46 (C-10), 112.42 (C-3), 144.28 (C-4), 146.28 (C-6), 151.42 (C-7), 151.68 (C-9), 160.82 (C-2). It was obtained as colourless needles. NMR spectral data was very similar to that described by Kang et al., (2003).

Esculetin (2): 10 mg. 1H NMR (DMSO, 400 MHz): δ ppm 7.84 (1H, d, J = 9.5 Hz, H-4), 6.98 (1H, s, H-5), 6.78 (1H, s, H-8), 6.14 (1H, d, J= 9.5 Hz, H-3). $^{13}$C-NMR (DMSO, 100 MHz) δ ppm 160.84 (C-2), 150.48 (C-7), 149.12 (C-9), 144.58 (C-4), 143.28 (C-6), 112.85 (C-5), 111. 86 (C-3), 116.38 (C-3), 110.86 (C-10), 103.16 (C-8). It was obtained as colourless needles. Its spectral data was in agreement with that described by Fazilatun et al., (2004).

Dihydroquercetin 7-4'-dimethyl ether (3): 18.5 mg. UV max (MeOH): 208, 229, 290, 358sh; (NaOAc/H$_3$BO$_3$): 232, 290, 332sh. 1H- NMR (DMSO, 400 MHz): δ ppm 3.79 (3H, s, 4'-OCH$_3$); 3.82 (3H, s, 7-OCH$_3$), 4.58 (1H, dd, J=6.4, 11.4 Hz, H-2), 5.12 (1H, d, J =11.4 Hz, H-3), 5.88 (1H, d, J=6.4 Hz, H-5), 6.14 (1H, d, J= 4 Hz, H-6), 6.92 (1H, d, J =1.6 Hz, H-6'), 6.96 (1H, s, H-2'), 6.98 (1H, d, J =1.6 Hz, H-5'). $^{13}$C-NMR: (DMSO, 100 MHz): δ ppm 197.85 (C-4), 167.95 (C-7), 163.15 (C-5), 162.95 (C-9), 148.76 (C-4'), 146.38 (C-3'), 129.66 (C-1'), 119.48 (C-6'), 115.24 (C-2'), 112.35 (C-5'), 101.58 (C-10), 95.14 (C-6), 94.26 (C-8), 84.28 (C-2), 71.76 (C-3), 56.18 (7-OCH$_3$), 55.84 (4'-OCH$_3$). It was obtained as a white powder. Its spectral signals were in agreement with those described by Blumeatin (4): 14.5 mg. UV max (MeOH): 208, 229, 290, 358sh; (NaOAc/H$_3$BO$_3$): 232, 290, 358sh. 1H- NMR (DMSO, 400 MHz): δ ppm 2.74 (1H, dd, J =2.9, 17.2 Hz, H-3a), 3.26 (1H, dd, J= 12.6, 17.2 Hz, H-2), 6.08 (1H, d, J =2 Hz, H-6), 6.10 (1H, d, J =2 Hz, H-8), 6.78 (2H, s, H-2', H-6'), 6.89 (1H, s, H-4'); 12.15 (1H, s, 5-OH). 13C-NMR (DMSO, 100 MHz): δ ppm 101.58 (C-10), 95.14 (C-6), 94.26 (C-8), 84.28 (C-2), 71.76 (C-3), 56.18 (7-OCH$_3$), 55.84 (4'-OCH$_3$). It was obtained as a white powder.
ppm 197.15 (C4), 167.76 (C7), 163.38 (C5), 162.95 (C9), 145.9 (C3', C5'), 129.85 (C1'), 118.16 (C4'), 115.48 (C6'), 114.52 (C2'), 102.78 (C10), 94.75 (C6), 93.94 (C8), 78.82 (C2), 42.28 (C3), 56.45 (7-OCH₃). It was obtained as white powder. Its spectral signals were in agreement with those described by Fazilatun et al., (2004).

Diosmetin (5): 16.5 mg. UV λmax (MeOH): 247, 264, 345, (NaOMe): 249, 302, 388, (AlCl₃): 272, 352, 400, (NaOAc): 252, 382, (NaOAc/H₂BO₃): 252, 382. 1H-NMR (DMSO–d₆, 500 MHz): δ ppm 7.65 (1H, d, J= 2.4 Hz, H2'); δ 7.35 (1H, dd, J=7.6, 2.4 Hz, H-6'); δ 6.85 (1H, d, J= 7.6 Hz, H-5'); δ 6.68 (1H, d, J=2.4 Hz, H-8); δ 6.45 (1H,d, J=2. 2 Hz, H6); δ 6.65 (1H,s,  H3); δ 3.9 (3H,s, OCH₃). 13C-NMR (DMSO-d₆,100 MHz): δ ppm 162.9 (C-2), 103.8 (C-3), 182.6 (C-4), 161.4 (C-5), 98.7.0 (C-6), 164.7 (C-7), 94.2 (C-8), 157.6 (C-9),104.2 (C-10),123.4 (C-1' ), 112.8 ( C-2' ), 147.3 (C-3' ), 151.4 (C-4' ), 112.7 (C-5'), 119.1 (C-6') and 55.6 (OCH₃). It was obtained as yellow powder. Its spectral data is in agreement with signals described by Lunesa et al., (2011).

Tamarixetin (6): 12.5 mg. 1H NMR (DMSO-d₆, 300 MHz): δ ppm 7.84 (1H, d, J= 1.6 Hz, H-2'), 7.83 (1H, dd, J= 1.6 Hz, H-2''), 6.98 (1H, d, J= 7.4 Hz, H- 5'), 6.56 (1H,d, J= 1.9 Hz, H-8), 6.26 (1H, d, J =1.9 Hz, H-6), 3.86 (3H, s, OMe). 13C- NMR (DMSO-d₆,100 MHz,): δ ppm 175.86 (C-4), 164.26 (C-7), 161.12 (C-5), 156.85 (C-9), 148.72 (C-4'), 146.68 (C-2), 146.62 (C-3'), 122.35 (C-10), 121.68 (C-6'), 115.54 (C-5'), 112.28 (C-2'), 103.65 (C-5), 98.25 (C-6), 93.64 (C-8). EI-MS: m/z 316. It was obtained as a yellow powder and it showed signals were very similar to that described by Benahmed et al., (2011).

Quercetin (7): 6 mg. UV λmax (MeOH): 255, 267, 371; (NaOMe): 270, 320, 420; (AlCl₃): 270, 455; (AlCl₃/HCl): 264, 303sh, 315sh, 428; (NaOAc): 257, 274, 318, 383; (NaOAc/H₂BO₃): 259, 387. EI-MS: m/z 302. It was obtained as yellow powder and its spectral data is in agreement with Manguro et al., (2005).

Quercetin 3-O-β-galactoside (8): 19 mg. UV λmax (MeOH): 254, 266, 289, 352; (NaOMe): 275, 329, 406; (AlCl₃): 275, 296, 362, 404; (NaOAc): 276, 327, 390; (NaOAc/H₂BO₃): 266, 298, 372. 1H NMR (CD3OD, 300 MHz): δ ppm 7.84 (1H, d, J= 2 Hz, H-2'), 7.83 (1H, dd, J= 2 Hz, H-8'), 6.87 (1H, d, J= 8 Hz, H-5'), 7.58 (1H, dd, J =2, 7.7 Hz, H-6'), 7.84 (1H, d, J = 2 Hz, H-2'), 5.08 (1H, d, J= 7.8 Hz, H-1''), 3.9-3.1 (rest of sugar protons, H-2′′-6′′). It was obtained as yellow needles. Acidic hydrolysis gave quercetin as an aglycone and galactose as sugar and spectral signals were in accordance with that described by (Guvenalp and Demirezer, 2005).

Kaempferol 3-O-rutinoside (9): 24 mg. UV λmax (nm): MeOH 268, 348; (NaOMe) 276, 326, 398; (AlCl₃): 275, 306, 357, 396; (AlCl₃/HCl) 275, 305, 354, 392sh; (NaOAc) 275, 314, 393; (NaOAc/H₂BO₃) 267, 356. 1H NMR (DMSO-d₆, 300 MHz): δ ppm 7.96 (2H, d, J = 8.5 Hz, H-2′, 6′), 6.86 (2H, d, J = 8.5 Hz, H-3′, 5′), 6.42 (1H, s, H-6'), 5.34 (1H, d, J = 2 Hz, H-6'), 4.42 (1H, s, H-1''), 3.0-4.1 (rest of rhamnose and glucose protons, 1.12 (3H, d, J = 6.5 Hz, -CH₃-rhamnosyl). 13C-NMR (DMSO-d₆, 100 MHz): δ ppm 156.44 (C-2), 133.15 (C-3), 177.28 (C-4), 161.85 (C-5), 98.75 (C-6), 164.45 (C-7), 93.85 (C-8), 156.74 (C-9), 103.78 (C-10), 120.75 (C-1′), 131.24 (C-2′, 6′), 115.26 (C-3′, 5′), 159.86 (C-4′), 101.38 (C-1”'), 74.16 (C-2'”), 76.35 (C-3'”), 69.84 (C-4’”), 75.62 (C-5’”), 66.84 (C-6’”), 100.75 (C-1””), 70.25 (C-2’”), 70.52 (C-3’’”), 71.74 (C-4’’”), 68.15 (C-5’’”), 17.62 (C-6’’”). It was obtained as yellow amorphous powder. Acidic hydrolysis afforded kaempferol as an aglycone, rhamnose and glucose as sugar moieties. All data were identical with that described by Bao et al., (2003). The results indicated that C. intybus exhibited the best antioxidant activity determined as total content of free-radical scavenging, and polyphenol molecules. Moreover, C. intybus extract

<table>
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<th>Phytoconstituents</th>
<th>S. rantonneti</th>
<th>T. cordata</th>
<th>C. intybus</th>
<th>L. tomentosa</th>
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<td>Triterpenes and/or Sterols</td>
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(+ ) the presence of the constituents, (- ) the absence of the constituents.
showed the highest value in terms of tyrosinase inhibitory activity. This observed activities can be explained by the properties of the isolated compounds and also phytoconstituents present in the extract, such as coumarins and flavonoids.

Plants contain a mixture of many kinds of secondary metabolism products, including phenols, which vary greatly in their antioxidant capacity. Coumarins are a large family of natural and synthetic compounds, which present different pharmacological activities. Recent studies paid special attention to their antioxidative, antiinflammatory, anticancer and enzymatic inhibition properties. In recent studies, some coumarins proved to be mushroom tyrosinase inhibitors (Borges et al., 2009). As showed in Table 3, coumarins are absent in S. rantonnetii and L. tomentosa extracts but are present in the extracts from T. cordata, and C. intybus, which are the only extracts that showed tyrosinase inhibition activity. Thus, we could assume that coumarins can be responsible for the tyrosinase inhibitory capacity of T. cordata and C. intybus extracts. Flavonoids have anti-inflammatory, anti-allergic, anticarcinogenic, antioxidant, and antiviral properties (Cook, 1996). Also flavonoids provide potent protection against oxidative and free radical damage (Świgło and Tyrakowska, 2003). Several reports have suggested that compounds yielding antioxidant properties could also serve as potential antityrosinase agents capable of blocking melanin synthesis (Niki et al., 2011). Compounds isolated from C. intybus methanol extract as dihydroquercetin 7-4′-dimethyl ether and blumeatin exhibited competitive inhibitor on tyrosinase activity (Saewan et al., 2011). Dietary phenolics have been shown to possess antityrosinase and antioxidant properties as quercitin compound which was isolated from our plant extract exhibited antityrosinase and antioxidant effects (Weerawon et al., 2014).

Conclusion

The results of this research indicated that methanol (70%) extract of aerial parts from four Egyptian plants possessed significant antioxidant activity determined as total content of free-radical scavenging, polyphenol, and flavonoid molecules and two of them showed a great tyrosinase inhibitory activity. Our results contribute to the knowledge of the components of these plants with a better characterization of the components of C. intybus extract which exhibits the best activities. The presence of antioxidant molecules and tyrosinase inhibitors make these plants a promising source of biomolecules for applications in the pharmaceutical field. Many therapeutic options exist, though treatment is often difficult, requiring lengthy therapy and the use of these substances is often limited because of skin irritation caused by toxicity or occurrence of dermatitis. Thus, it has become increasingly necessary to search for new drugs. The next step will be the evaluation of antioxidant and antityrosinase activities of each isolated compound and the skin-whitening activity in cellular and in vivo systems.

References


