Anti-tyrosinase, total phenolic content and antioxidant activity of selected Sudanese medicinal plants

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ARTICLE INFO

Article history:
Received 22 May 2016
Received in revised form 17 September 2016
Accepted 13 December 2016
Available online xxx
Edited by AR Ndhlala

Keywords:
Sudanese medicinal plants
Phenolic content
FRAP
Tyrosinase inhibition
Acacia nilotica

ABSTRACT

The flora of Sudan is relatively rich in medicinal plants and represents an important component of traditional medicine. Fifty methanolic extracts of selected Sudanese medicinal plants were evaluated for their in vitro tyrosinase inhibitory effect, antioxidant activity and total phenolic content (TPC). The standard method of antioxidant evaluation, ferric reducing ability of plasma (FRAP), was employed to determine the antioxidant activity while the enzyme based tyrosinase inhibition was used for the anti-tyrosinase activity. Acacia nilotica (pods, bark) and Acacia seyal var. seyal (wood) demonstrated comparable anti-tyrosinase inhibitory activity using L-tyrosine as substrate (IC50: 31.93, 36.32 μg/ml) compared to positive control (IC50: 37.63 μg/ml). The results revealed significant differences in TPC between plants extracts. The highest level of phenolic content was found in Terminalia brownii (bark; 46.02 μg GAE/mg) while the lowest was in Ziziphus spinoca-christi (fruits; 09.63 μg GAE/mg). The study indicated significant differences in total antioxidant capacity between the extracts. Terminalia laxiflora (wood), A. nilotica (pods, bark), T. brownii (bark), A. seyal var. seyal (bark), Khaya senegalensis (bark), T. brownii (wood) Combretum hartmannianum (bark), Polygonum glabrum (leaves), Z. Spinoca-christi (bark) and Guiera senegalensis (leaves) extracts displayed the high antioxidant equivalent concentration (EC) values. A. nilotica (pods, bark) expressed promising activity that warrant further research since it has high tyrosinase inhibitory activity, antioxidant activity and could be a good source of phenolic compounds. To the best of our knowledge, this is the first data presenting comprehensive data on anti-tyrosinase, TPC, antioxidant activity of the Sudanese medicinal plants.

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1. Introduction

Tyrosinase (EC 1.14.18.1; PPO) is known to be a key enzyme in melanin biosynthesis and is widely distributed in plants and mammalian cells. Tyrosinase enzyme catalyzes two different reactions: the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity). o-Quinone is transformed into melanin in a series of non-enzymatic reaction (Sánchez-Ferrer et al., 1995). Melanogenesis is a physiological process resulting in the synthesis of melanin pigments, which plays a crucial protective role against skin photocarcinogenesis. In humans and other mammals, the biosynthesis of melanin takes place in a lineage of cells known as melanocytes, which contain the enzyme tyrosinase (Robb, 1984). Melanin synthesis inhibitors are topically used for treating such localized hyperpigmentation in humans as lentigo, nevus, ephelis, post inflammatory state and melanoma of pregnancy (Tomita et al., 1990). Melanin formation is considered to be deleterious to the color quality of plant-derived food. Prevention of this browning reaction has always been a challenge to food scientists (McEvily et al., 1992). Tyrosinase is also one of the most important key enzymes in the insect molting process therefore it could be used as alternative insecticide (Miyazawa and Tamura, 2007). Therefore the inhibitors of this enzyme may lead to novel skin whitening agents, anti-browning substances or compounds for insect control. Recently applications of tyrosinase-inhibiting agents are increasingly used in cosmetic products for maintaining skin whiteness (Kadekaro et al., 2003). Plants and their extracts are inexpensive and rich resources of active compounds that can be utilized to inhibit tyrosinase activity as well as melanin production (Montaz et al., 2008).

The idea behind using antioxidants for skin-lightening activities lies in the hypothesis that the oxidative effect of UV-irradiation contributes to activation of melanogenesis. UV irradiation can produce reactive oxygen species (ROS) in the skin that may induce melanogenesis by activation tyrosinase as the enzyme prefers superoxide anion radical...
(Wood and Schallreuter, 1991). Additionally these ROS enhance the damage of DNA and may induce the proliferation of melanocytes (Yasui and Sakurai, 2003). Therefore ROS scavenger such as antioxidants may reduce the hyperpigmentation (Ma et al., 2001). Total phenolic and flavonoid contents were significantly correlated with free radical-scavenging and tyrosinase-inhibiting activities. Thus, the strong free radical-scavenging and tyrosinase-inhibiting properties increased proportionally with the level of antioxidants in Sorghum distillery residue extracts (Wang et al., 2011).

Medicinal plants represent an important component of traditional medicine in the world including in Sudan. The flora of Sudan is relatively rich in medicinal plants corresponding to a wide range of ecological habitats and vegetation zones of the country (Khalid et al., 1986). Due to the rich plant diversity existing in Sudan, it is very encouraging to explore the potential of Sudanese plants for cosmeceutical purposes. Despite few of these medicinal plants used for skin decoration and softening the authors decided to investigate the ability of some Sudanese medicinal plants as skin whitening which it could be useful for cosmeceutical industry. The ability of different extracts of Sudanese medicinal plants to act as a skin-lightening agents was tested as their ability to inhibit tyrosinase, the rate limiting enzyme in melanogenesis, initially using a cell-free mushroom tyrosinase system, which has commonly been employed for the testing and screening of potential skin-lightening agents (Song et al., 2009). In this study, fifty methanolic extracts of Sudanese medicinal plants were evaluated for their anti-tyrosinase, total phenolic content and antioxidant properties in order to find the most potential plant extracts for the skin-lightening agent.

2. Materials and methods

2.1. Plant materials

The plant materials which have medicinal values were randomly selected from Khartoum and Gadarif states of Sudan in March 2011. Identification of the plant materials was done at the University of Khartoum, Faculty of Agriculture and Faculty of Forest. Authentication voucher specimens are deposited in the Horticultural Laboratory, Department of Horticulture, Faculty of Agriculture, University of Khartoum.

2.2. Chemicals and reagents

Dimethylsulfoxide (DMSO), iron (III) chloride hexahydrate, Folin–Ciocalteau, L-tyrosine and L-dihydroxyphenylalanine (DOPA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Mushroom tyrosinase (2870 units/mg), and 2,4,6-Tris (2pyridyl)-s-triazine (TPTZ) were purchased from Sigma. Quercetin, butylated hydroxytoluene, ascorbic acid and gallic acid were purchased from Naka Lai Tesque, Inc. (Kyoto, Japan). Other chemicals were of the highest grade commercially available.

2.3. Extract material preparation

Plant materials were shade dried at room temperature and powdered before extraction; they were each extracted three times with absolute methanol (ratio of 1 g sample: 10 ml solvent) for 12 h three times. The extracts were filtered and then the solvent was removed under vacuum using rotary evaporator. All extracts were stored at 4 °C prior to analysis.

2.4. Inhibition of tyrosinase activity

The tyrosinase inhibitory activity was performed by the method described by Batubara et al. (2010). Briefly, the sample (70 μl) was added in 96-well plate. Tyrosinase (30 μl, 333 unit/ml in phosphate buffer 50 mM, pH 6.5) and 110 μl of substrates (L-tyrosine 2 mM or L-DOPA 12 mM) were added. After incubation at 37 °C for 30 min, the absorbance at 510 nm was determined using a micro plate reader. The percent inhibition of tyrosinase activity was calculated at the concentration of 125 and 500 μg/ml. The extracts showed activities up to 50% inhibition of the enzyme were expressed as (IC50). Kojic acid was used as a positive control.

2.5. Determination of total phenolic content

The total phenolics assay was performed as described previously by Ainsworth and Gillespie (2007). Plant extract was dissolved in 50% methanol and 100 μl was transferred into test tubes, followed by 200 μl 1 N Folin Ciocalteau reagent 10% (v/v). Then they were mixed with 800 μl of sodium carbonate (700 mM) and maintained at room temperature for 2 h. Two hundred microlitres of samples from the assay tube was transfer to 96-well microplate and read the absorbance at 765 nm using microplate reader. Total phenolic concentrations were expressed as microgram of gallic acid equivalents (GAE).

2.6. Antioxidant capacity

The total antioxidant potential of extracts was determined by using a ferric reducing ability of plasma (FRAP) assay described by Tachakittirungrod et al. (2007). Briefly, the FRAP reagent was freshly prepared. The extracts were dissolved in ethanol to a concentration of 1 mg/ml. An aliquot of 20 μl of test of solution was mixed with 180 μl of FRAP reagent. The absorption of the reaction mixture was measured at 590 nm by a microplate reader. Ethanol solutions of known Fe (II) concentration, in the range of 50–500 μM (FeSO4), were used as calibration curve. The reducing power was expressed as equivalent concentration (EC). This EC was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM FeSO4. Quercetin, ascorbic acid and butylated hydroxytoluene (BHT) were used as positive controls.

2.7. Statistical analysis

The IC50 values of tyrosinase inhibitory, total phenolic content and antioxidant activities were expressed as the mean (mean ± standard deviation). The significant differences between extracts and positive controls were assessed by one-way analysis of variance (ANOVA) followed by pairwise comparison of the mean with positive control using Tukey’s multiple comparison test. Values were determined to be significant when p was less than 0.05 (p < 0.05).

3. Results and discussion

Table 1 summarizes the botanical name, family, voucher specimen and summary of traditional uses of the investigated plant species. Within these selected Sudanese medicinal plants Lawsonia inermis, Combretum hartmannianum, Accacia seyal var. fistula, Solanum dubium, Citrullus colocynthis and Acacia tortilis are used for preventing the dryness and bacterial infection of the skin in addition to other uses. The 50 plant extracts used in this research belong to 39 plant species which are distributed among 27 families from different plant parts.

3.1. Tyrosinase inhibitory activity

L-tyrosine and L-DOPA were used as the substrate to determine the monophenolase and diphenolase activities of mushroom tyrosinase. The tyrosinase inhibitory activities of all extracts are presented in Table 2 as percentage of L-tyrosine and L-DOPA inhibition at the concentration of 125 and 500 μg/ml as well as IC50 values. The study revealed that 36 and 24% of extracts presented a good tyrosinase inhibitory activity which inhibited more than 50% inhibition for both
substrates (L-tyrosine and L-DOPA, respectively). At the concentration of 500 μg/ml of extracts with L-tyrosine and L-DOPA substrates; Z. spinachristi (bark), A. digitata, A. nilotica (pods and bark), K. senegalensis, G. senegalensis, T. brownii (bark), A. seyal var. seyal (bark), A. seyal var. fistula (bark) and P. glabrum showed inhibition levels more than 70%. In addition to that Kojic acid which was used as a positive control showed an inhibition level of 99.65 and 94.40 to L-tyrosine and L-DOPA, respectively. The IC50 values of A. nilotica (pods- bark), A. seyal var. seyal (wood) and kojic acid (positive control) demonstrated significantly lower IC50 for L-tyrosine substrate inhibition (8.61, 10.47, 10.77 and 10.02 μg/ml respectively) than other extracts. Moreover A. nilotica (bark), A. seyal var. fistula (bark) and positive control exhibited the lowest IC50 for L-DOPA substrate inhibition (31.93, 36.32 and 37.63 μg/ml respectively) and it's significantly different from other extracts.

Table 1

| Botanical name | Family | Voucher specimen | Traditional uses and references |
|---------------|--------|-----------------|---------------------------------|
| Ammi visnaga (L) Lam. | Apiaceae | SD-OD-38 | It relaxes smooth muscles and lowers the urethral toxicity. A decoction is used to ease the passage of kidney calculi (Khalid et al., 2012). |
| Carum carvi L. | Apiaceae | SD-OD-48 | Antispasmodic, carminative, galactagogue and anthelmintic properties (Khalid et al., 2012). |
| Hyphaene thebaica (L.) Mart. | Arecaceae | SD-OD-41 | Against splenomegaly, alimentary system disorders and bacterial eye infections (El Ghazali et al., 2003). |
| Aristochia bracteolata Lam. | Aristochiaceae | SH-04 | Analgesic, treatment of scorpion bite and malaria. It is also used against-tumors (El-Tahir et al., 1998). |
| Solenostemma argol (Delile) Hayne | Asclepiadaceae | SH-23 | The leaves are used as an antispasmodic, carminative and as an anti-diabetic (Kanel et al., 2000). |
| Xanthium brasiliacum Vell. | Asteraceae | SH-12 | Antiprotozoal (Nour et al., 2009). |
| Balanites aegyptiaca (L.) Delile | Bignoniaceae | SH-15 | Venerable diseases, rheumatism, digestion problems, dysentery and bilharzias (Iwu, 1993; El Ghazali et al., 1997; Van Wyk et al., 1997). |
| Kigelia africana (Lam.)Benth. | Combretaceae | SH-21 | Stomach problem, cough, dysentery and venereal diseases (Neuwinger, 1996; El Ghazali et al., 1997). |
| Adamsonia digitata L. | Bombacaceae | SD-OD-27 | Used as a cold beverage and added to yogurt for treatment of diarrhoea and amoebic dysentery (Mangan, 1988). |
| Lepidium sativum L. | Brassicaceae | SH-02 | Used as anti-asthmatic, anti-scorbutic, aperient, diuretic, galactagogue and stimulant (Adam et al., 2011). |
| Tambussindus indicus L. | Capparaceae | SH-18 | Fever, malaria and laxative (El Kamali and El-Khalifa, 1999). |
| Capparis decidus (Forssk.) Edgew. | Capparaceae | SH-17 | Jaundice, rheumatic arthritis and to treat swell (El Ghazali et al., 1997). |
| Maersus oblongifolia (Forssk.) A.Rich | Capparaceae | SH-45 | Malaria (El Kamali and El-Khalifa, 1999). |
| Combretum hartmannianum Schweinf. | Combretaceae | SH-04 | Arthritis rheumatism, skin dryness, jaundice and bacterial diseases (El Ghazali et al., 1997; Al Magboul et al., 1988). |
| Guiera senegalensis J.F.Gmel var. seyal (wood) | Leguminosae | SD-OD-01 | Anti-pyretic, anti-diabetic, anti-hypertension and febrifuge (El Ghazali et al., 1994, 1997). |
| Terminalia brownii Fresen. | Combretaceae | SD-OD-40 | Cough bronchitis and anti-rheumatic (El Ghazali et al., 1994, 1997). |
| Terminalia laxiflora Engl. | Combretaceae | SD-OD-02 | Cough treatments and fumigant (Musila et al., 2011). |
| Ambrosia maritima L. | Compositae | SH-03 | Urinary tract infections, elimination of kidney stones, anti-diabetic and anti-hypertensive (Mahmoud et al., 1999). |
| Cirrulus colynthis (L) Schrad. | Cucurbitaceae | SD-OD-05 | Purgative and skin diseases (El Ghazali et al., 1997). |
| Albuinum pannosum (L.) (G.Forst.) Sclidti. | Leguminosae | SD-OD-22 | Oral contraceptives, against chronic nephritis and diabetes (Manago and Alumanan, 2005; Garaniya and Napostra, 2014). |
| Acacia nilotica (L) Delile | Leguminosae | SD-OD-01 | Diarrhea, cold, pharyngitis, used as anti-septic and tooth ache (Eldeen et al., 2010). |
| Acacia seyal var. seyal Del. | Leguminosae | SD-GF-05 | Arthritis rheumatism and rheumatic fever (El Ghazali et al., 1997). |
| Acacia seyal var. fistula (Schweinf.) | Leguminosae | SD-GF-06 | Fumigant for rheumatic pain. It is also used to protect women from fever after childbirth and soften the skin (Khalid et al., 2012). |
| Acacia tortilis (Forssk.) Hayne | Leguminosae | SH-07 | Vermifuge, skin infections, oedema and allergic dermatitis (http://www.fao.org/ag/aggc/doc/data/PF000139.htm). |
| Parkinsonia aculeata L. | Leguminosae | SD-SH-02 | Fever, malaria, abortifacient and rheumatism (Orwea et al., 2009). |
| Lawsonia inermis L. | Lythraceae | SD-SH-07 | Anti-asthmatic, anti-scorbutic, aperient, diuretic, galactagogue and stimulant (Adam et al., 2011). |
| Abutilon pannosum (L.) (G.Forst.) Sclidti. | Malvaceae | SD-SH-43 | Muscular pains, antispasmodic and against heart burn (El Ghazali et al., 1986). |
| Grewia texana (Forssk) Fiori | Malvaceae | SD-SH-42 | Hepatoprotective, antispasmodial and hypoglycemic activities (Cho-kang et al., 2007). |
| Hibiscus sabdariffa L. | Malvaceae | SH-07 | Malaria and iron deficiency anemia (Khalid et al., 2012). |
| Khaya senegalensis (Desv.) A. Juss. | Meliaceae | SH-14 | Tonic, fever, vermifuge, taenicide, emmenagogue, venereal diseases and antiprotozoal (Beever, 1986; Iwu, 1993; Kayser and Abreu, 2001). |
| Moringa oleifera lam. | Moringaceae | SH-08 | Antimicrobial, febrile, pulmonary diseases (Neuwinger, 2000; Anwar et al., 2007). |
| Polygnum glabrum Wild. | Polygonaceae | SH-03 | Anthelmintic (Muddathir et al., 1987). |
| Nigella sativa L. | Ranunculaceae | SH-06 | Anti-inflammatory, allergies, eczema and malaria (Ahmed et al., 2010). |
| Ziziphus spina-christi (L) Desf. | Rhamnaceae | SH-06 | Ulcers, gonorrhea, sore throat, chest pain, dysentery, venereal diseases and wounds (El Ghazali et al., 1997; Dafni et al., 2005). |
| Halophyllum tuberculatum (Forssk.) A. Juss. | Rutaceae | SH-KH-32 | Used against diarrhea and spasms (Khalid et al., 2012). |
| Salvadoraperruca L. | Salvadoraceae | SH-09 | Gingivitis, malaria, liver swellings and HIV-1 (El Kamali and Khalid, 1996; Hussein et al., 1999; Neuwinger, 2000). |
| Solanum dubium Fresen | Solanaceae | SH-34 | Applied to wounds and skin tumors as a dressing (El Kheir and Salih, 1980). |
| Tamarix nilotica | Tamaricaceae | SH-OD-10 | Febrile, colds and hemorrhoids (El Ghazali et al., 1994, 1997). |
| Fagonia cretica L. | Zygophyllaceae | SD-OD-26 | Muscular pains, antispasmodic and against heart burn (El Ghazali et al., 1997; El Ghazali, 1986). |
Table 2 
Tyrosinase inhibitory activities of selected Sudanese medicinal plants extracts.

| Botanical name   | Part used          | Tyrosinase inhibition (%) | Tyrosinase inhibition | Tyrosinase inhibition |
|------------------|--------------------|---------------------------|-----------------------|-----------------------|
|                  |                    | L-Tyrosine (μg/ml) | L-DOPA (μg/ml) | IC50 μg/ml | L-Tyrosine (μg/ml) | L-DOPA (μg/ml) | IC50 μg/ml |
|                  |                    | 125 | 500 | 125 | 500 | 125 | 500 |
| A. visnaga (L.) Lam. | Fruits         | 18.55 | 42.10 | 02.82 | 28.90 | nd | nd | 51.71 ± 4.27| 91.25 ± 5.97 |
| C. carvi L.         | Fruits          | – | 05.38 | – | 06.93 | nd | nd | 386.89 ± 11.29| nd |
| H. thebaica (L.) Mart. | Fruits          | 19.32 | 26.54 | 01.58 | 08.40 | nd | nd | 198.51 ± 14.18| 240.35 ± 10.17 |
| A. bracteolata Lam. | Aerial parts     | – | 10.91 | – | 13.26 | nd | nd | 119.14 ± 9.39| 120.66 ± 10.22 |
| S. argel (Delile) Hayne | Leaves       | – | 25.55 | – | 30.32 | nd | nd | 415.44 ± 21.70| nd |
| X. brasiliicum Vell. | Leaves         | 08.86 | 19.30 | 14.30 | 27.23 | nd | nd | 78.38 ± 9.69| nd |
| B. aegyptiaca (L.) Delile | Bark     | – | 02.73 | – | 16.02 | nd | nd | 119.14 ± 9.39| 120.66 ± 10.22 |
| K. africana (Lam.) Benth. | Fruits | 02.54 | 04.25 | – | 06.00 | nd | nd | 78.38 ± 9.69| nd |
| A. digitata L.      | Fruit pulp      | 73.53 | 97.14 | 59.20 | 77.62 | nd | nd | 78.38 ± 9.69| nd |
| L. sativum L.       | Seeds           | 01.88 | – | – | – | nd | nd | 78.38 ± 9.69| nd |
| T. indica L.        | Leaves          | 54.38 | 72.50 | 37.65 | 49.79 | nd | nd | 78.38 ± 9.69| nd |
| C. decidue (Forsk.) Edgew. | Stems  | – | 10.11 | – | – | nd | nd | 78.38 ± 9.69| nd |
| M. oblongifolia (Forsk.) A.Rich | Stems | – | – | 09.4 | 18.0 | nd | nd | 78.38 ± 9.69| nd |
| C. hartmannianum Schweinf. | Wood  | 27.75 | 59.03 | 13.86 | 43.88 | nd | nd | 78.38 ± 9.69| nd |
| G. senegalensis J.F.Gmel | Leaves | 45.30 | 95.23 | 22.86 | 80.89 | nd | nd | 78.38 ± 9.69| nd |
| T. browni Fresen.   | Bark            | 31.81 | 95.50 | 51.70 | 70.18 | nd | nd | 78.38 ± 9.69| nd |
| T. laxiflora Engl.  | Wood            | 16.33 | 61.80 | 08.35 | 32.35 | nd | nd | 78.38 ± 9.69| nd |
| A. maritima L.      | Aerial parts    | 01.84 | 35.95 | – | 12.70 | nd | nd | 78.38 ± 9.69| nd |
| C. colombynus (L.) Schrad. | Dry fruits | 13.90 | 50.51 | – | 27.82 | nd | nd | 78.38 ± 9.69| nd |
| A. precatorius L.   | Seeds           | – | 09.60 | 26.14 | 69.40 | nd | nd | 78.38 ± 9.69| nd |
| A. nilotica (L.) Delile | Pods       | 96.39 | 98.30 | 64.7 | 81.9 | nd | nd | 78.38 ± 9.69| nd |
| A. seyal var. seyal Del. | Bark     | 94.20 | 94.23 | 70.9 | 84.5 | nd | nd | 78.38 ± 9.69| nd |
| A. seyal var. fistula (Schweinf.) | Bark | 84.03 | 95.45 | 69.77 | 83.06 | nd | nd | 78.38 ± 9.69| nd |
| A. tortilis (Forsk.) Hayne | Bark      | 91.80 | 91.82 | 38.50 | 48.91 | nd | nd | 78.38 ± 9.69| nd |
| A. aculeata L.      | Leaves         | 65.52 | 70.91 | 24.20 | 33.65 | nd | nd | 78.38 ± 9.69| nd |
| L. inermis L.       | Leaves         | 02.91 | 15.11 | – | 12.20 | nd | nd | 78.38 ± 9.69| nd |
| A. pannosum (G.Forst.) Schidl. | Leaves | 30.41 | 07.22 | 10.53 | 18.72 | nd | nd | 78.38 ± 9.69| nd |
| G. tenax (Forsk) Fiori | Fruits       | 01.93 | 03.00 | 02.54 | 08.20 | nd | nd | 78.38 ± 9.69| nd |
| H. sabdariffa L.    | Flowers        | 18.28 | 21.21 | 10.88 | 12.23 | nd | nd | 78.38 ± 9.69| nd |
| K. senegalensis (Desv.) A. Juss. | Bark | 93.1 | 97.9 | 64.2 | 85.7 | nd | nd | 78.38 ± 9.69| nd |
| M. oleifera Lam.    | Leaves         | 06.35 | 08.94 | 09.90 | 12.13 | nd | nd | 78.38 ± 9.69| nd |
| P. glabrum Wildld.  | Leaves         | 100 | 100 | 84.39 | 91.75 | nd | nd | 78.38 ± 9.69| nd |
| Nigella sativa L.   | Seeds          | – | 15.73 | – | 17.64 | nd | nd | 78.38 ± 9.69| nd |
| Z. spina –christi (L.) Desf. | Fruits | – | 09.68 | – | – | nd | nd | 78.38 ± 9.69| nd |
| H. tuberculatum (Forsch.) A. Juss. | Bark | 17.77 | 94.75 | 42.41 | 77.30 | nd | nd | 78.38 ± 9.69| nd |
| A. persica L.       | Leaves         | 20.00 | 67.68 | 11.86 | 46.57 | nd | nd | 78.38 ± 9.69| nd |
| S. dubium Fresen.   | Stems          | 08.84 | 41.58 | 14.01 | 32.83 | nd | nd | 78.38 ± 9.69| nd |
| T. niloteca (Ehrenb.) Bunge | Bark | 51.22 | 79.51 | 30.52 | 53.00 | nd | nd | 78.38 ± 9.69| nd |
| Fecreta L.          | Aerial parts   | 10.30 | 22.56 | – | 16.12 | nd | nd | 78.38 ± 9.69| nd |
| Kojic acid***       |             | 99.50 | 99.65 | 83.54 | 96.40 | nd | nd | 78.38 ± 9.69| nd |

nd: Not detected.
* IC50: Half minimal inhibitory concentration.
** No inhibition was detected.
*** Positive control.

Therefore the in vitro activity of A. nilotica pods and bark against tyrosinase, it may be due to the catechin derivatives compounds.

3.2. Total phenolic content

Polyphenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects (Kähkönen et al., 1999). Lin et al. (2008) mentioned that phenolics with ion reducing ability diminish the possibility of hydroxyl radical’s formation path from superoxide anion radicals and additionally inhibit enzymes due to their abilities to chelate copper at the active

catechin 5-O-gallate (Malan, 1991). Salem et al. (2011) demonstrate the isolation of gallocatechin 5-O-gallate compound from A. nilotica pods exhibiting in vitro activity and selectivity towards uveal melanoma cell lines comparable to that of the known compound epigallocatechin gallate (EGCG) from green tea. EGCG reported as potential whitening agent by reducing melanin production in mouse melanoma cells (Kim et al., 2004). Sato and Toriyama (2009) clarified that the catechin group inhibited melanin synthesis in B16 melanoma cells through inhibiting melanogenic protein, namely tyrosinase. Also he suggested the catechin group as a candidate for being anti-melanogenic agent and that it might be effective in hyperpigmentation disorders.
by Folin–Ciocalteu method. The phenolic content varied widely in our tested extracts and ranged from 46.02 to 0.28 mg GAE/mg (Table 3). The highest level of phenolic content was found in T. brownii (bark), while the lowest was in Z. spina-christi (fruits). There were significant differences in total phenolic content between plant extracts. The highest phenolic concentrations (more than 37.80 μg GAE/mg) were found in: T. brownii (bark), A. nilotica (bark), A. nilotica (pods), P. glabrum, Z. spina-christi (bark), T. brownii (wood), C. hartmannianum (bark), K. senegalensis, S. dubium, A. seyal var. seyal (bark), T. laxiflora (wood), G. senegalensis, A. seyal var. fistula (bark), A. precatorius and A. maritima extracts. Our findings agree with Cock (2015), and Sulaiman et al. (2014), who stated that Terminalia and Accacia species were rich in plant phenolic compounds respectively. Comparatively the moderate phenolic concentration (between 37.69 and 30.00 μg GAE/mg) was found in : L. sativum, A. digitata, C. carvi, A. tortilis (wood), X. brasiliicum, C. hartmannianum (wood), Z. Spina-christi (leaves), H. sabdariffa, A. seyal var. seyal (wood), T. indica, A. tortilis (bark), H. thebaica, S. persica (stems), M. oleifera, A. visnaga, L. inermis, S. argel, B. aegyptiaca (bark), H. tuberculatum, P. aculeate, K. africana and F. reticulata extracts.

G. tenax, B. aegyptiaca (leaves), M. oblongifolia, A. bracteolata, S. persica (leaves), A. pannosum, N. sativa, C. coloynthis, A. seyal var. fistula (wood) and C. decidus extracts, demonstrated relatively low level of phenolic content (between 26.54 to 18.50 μg GAE/mg). Whereas, B. aegyptiaca (fruits) and Z. spina-christi (fruits) revealed quite low phe-

nolic level (14.05 and 9.63 μg GAE/mg respectively). Karou et al. (2011) found that aerial parts of B. aegyptiaca had low content of phenolics. Although phenolic compounds are a diverse and ubiquitous group of secondary metabolites in the plant kingdom, their distribution and concentration vary across and within species (Robards et al., 1999).

3.3. Antioxidant capacity

The ferric reducing ability of plasma (FRAP) assay was used to evaluate the antioxidant potential of extracts of the selected Sudanese medicinal plants. The FRAP assay depends upon the reduction of ferric tripyridyltriazine [Fe(III)-TPTZ] to ferrous tripyridyltriazine [Fe(II)-TPTZ] at low pH. The [Fe(II)-TPTZ] complex has an intensive blue color and can be monitored at 590 nm. Ferric reducing ability of plasma assay is used in many studies because it is quick, simple to perform and reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present (Benzie and Strain, 1996). The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the oxidative damage process, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995).

As shown in Table 3, there were significant differences in total antioxidant capacity between the plant species and the part used of the plant extracts. The FRAP values can be divided in four groups: very low FRAP (less than 2.0 mM/mg) n = 29; low FRAP (2.0–2.62 mM/mg) n = 4; good FRAP (2.65–3.50 mM/mg) n = 6 and high FRAP (more than 3.50 mM/mg) n = 11. Quercetin, ascorbic acid and BHI as positive controls demonstrated antioxidant values of 3.96, 3.79 and 2.84 mM/mg respectively. The strongest antioxidant properties were observed on: T. laxiflora, A. nilotica (pods, bark), T. brownii (bark), A. seyal var. seyal (bark), K. senegalensis, T. brownii (wood), C. hartmannianum (bark), P. glabrum, Z. spina-christi (bark) and G. senegalensis extracts. This result is similar to the findings of Muddathir and Mitsunaga (2013), Siddiqui and Patil (2015) and Hassan et al. (2014). They demonstrated that, A. nilotica (pod, bark), T. laxiflora (wood), C. hartmannianum (bark), K. senegalensis, A. seyal var. seyal (bark), G. senegalensis and Z. sp. (bark) showed an excellent antioxidant activities when measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Similarly, high antioxidant activities measured by trolox equivalent antioxidant content (TEAC) assay have also been reported for the genus Acacia and Terminalia which seem to be correlated with their phenolic contents (Maldini et al., 2011; Cock, 2015). The extracts showed high EC values, it could be considered that compounds in these extracts were good electron donors and could
terminate oxidation chain reactions by reducing the oxidized intermediates into the stable form. It is worth to be mentioned that a significant relationship is observed between \( r = 0.944 \) total phenolic content and antioxidant components of the extracts that showed potent anti-tyrosinase activity (Table 2). This result is in agreement with Katalinic et al. (2006) who confirmed a significant linear correlation between total phenolic and related FRAP of medicinal plant extracts. The greater number of hydroxyl groups in the phenolics, the higher antioxidant activity (Prasad et al., 2005; Rangkdalik et al., 2007). The inhibition of tyrosinase activity might depend on the hydroxyl groups of the phenolic compounds of the extracts that could form a hydrogen bond to a site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors act through hydroxyl groups that bind to the active site on tyrosinase, resulting in steric hindrance or changed conformation. The antioxidant activity mechanism may also be one of the important reasons for tyrosinase inhibition activity (Baek et al., 2008; Kim et al., 2008).

Ma et al. (2001) suggested that ROS scavenger such as antioxidants may reduce the hyperpigmentation, therefore we proposed that the extracts with high EC value could be useful to reduce the hyperpigmentation process.

4. Conclusion

According to this study, a number of Sudanese medicinal plants revealed anti-tyrosinase activity, high phenolic content and antioxidant capacity. \textit{A. nilotica} (pod-bark) expressed a higher potency very promising for further research; since it has high tyrosinase inhibitory activity, antioxidant activity and a good phenolic content. Consequently these findings could be applied in the industry for the obtention of natural antioxidants and tyrosinase inhibitors. However, the results obtained need further investigation in pigment cell assays and in clinical studies.

Acknowledgments

The authors wish to thank Ms. Noah Mohamed from the Ministry of Agriculture and Forestry (Gadarif State), Mrs. Hamza Tag El-Sir, Botanist University of Khartoum, Faculty of Agriculture, Department of Botany (Herbarium) and Dr. Ashraf Mohamed from the Faculty of Forestry, University of Khartoum, Sudan. They are warmly thanked for their assistance in the plants identification and authentications. This project was supported financially by the Ministry of Education, Culture, Sports, Science and Technology Japan (MEXT Grant).

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