South African Journal of Botany 109 (2017) 9–15

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

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 30 December 2016

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 (08.61, 10.47 and 10.77 μg/ml respectively) to Kojic acid (10.02 μg/ml) which was used as a positive control. *A. nilotica* (bark) and *Acacia seyal* var. fistula (bark) exhibited good tyrosinase inhibitory activity using L-DOPA as substrate (IC_{50}: 31.93, 36.32 μg/ml) compared to positive control (IC_{50}: 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 spina–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. Spina-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.

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 (Momtaz 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 activating tyrosinase as the enzyme prefers superoxide anion radical

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http://dx.doi.org/10.1016/j.sajb.2016.12.013
0254-6299/

(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 cosmaceutical 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 cosmaceutical 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 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 (IC_{50}). 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 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. Ethanolic solutions of known Fe (II) concentration, in the range of 50–500 μM ($FeSO_4$), 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 $FeSO_4$. Quercetin, ascorbic acid and butylated hydroxytoluene (BHT) were used as positive controls.

2.7. Statistical analysis

The IC_{50} 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*, *Acacia 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 IC_{50} values. The study revealed that 36 and 24% of extracts presented a good tyrosinase inhibitory activity which inhibited more than 50% inhibition for both