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Anti-Oxidant and Anti Tyrosinase Effect of *Zizyphus spina-christi* Seed Extract

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Abstract: Medicinal plant such as *Ziziphus Spina-Christi* (ZSC) has been used for the treatment and prevention of several ailments in human and animals.It is recommended for the management of diseases in which free radical species are produced as a result of oxidative stress. However, there is lack of systematic study on the antioxidant and antityrosinase capacities of ZSCF from Nigeria. The present study quantifies the anti-oxidant and anti tyrosinaseability of the ZSCF grown in the Gwaski, Southern Borno state Nigeria. Antioxidant activity was assessed by using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), Superoxide-Radical Scavenging Activity (SRSA), Ferric Reducing/Antioxidant Power (FRAP assay). Overall the ZSCF showed strong antioxidant ability and high anti tyrosinase effect. There is variation in the oxidation power (DPPH) with 15.5% at the concentration of 7.8 μg mL^{-1}-88.4% at 1000 μg mL^{-1} and 22.5% at 7.8 μg mL^{-1} as well as 63.5% at 1000 μg mL^{-1} for FRAP assay. The percentage antityrosinase activities varied from 4.1% at 7.8 μg mL^{-1} -70.5% at 1000 μg mL^{-1} for plant crude extract. The observed antioxidantand anti tyrosinase ability of ZSCF may be due to abundant presence of phenolic contents and high electron donating ability to neutralize free radicals.

INTRODUCTION

Ziziphus spina-christi ordinarily known as Christ's Thorn Jujube is a persistence tree commonly found in warm temperate zone, subtropical region including North and West Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and middle East[1, 2]. It is member of Rhamnaceae family in the order of Rosales that contains up to 60 genera and >850 species[3]. There are about 100 species of deciduous or evergreen trees and shrubs under the genus *Ziziphus* all over the world[4, 5]. The tree and its parts have been used in Pharaonic industry (carpentry) as well as dietary supplement, antixodant and in folk medicine as a demulcent, depurative, anodyne, emollient, stomach-ache for toothaches, astringents and as a mouth wash[6, 7].

Scientific finding reveal that some plants contained peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds[8-10]. Some works also, shows that the plant contain betulic and ceanothic acid, three cyclopeptide alkaloids as well as four saponin glycosides[11, 12] and several flavonoids have been isolated from the leaves of *Z. spina-christi*[13, 14]. The present study aimed to evaluate the antioxidant and antityrosinate effect of 80% methanolic extracts of *Z. spina-christi* seed.

MATERIALS AND METHODS

Plant

Plant collection and identification: *Ziziphus spina-christi* seeds were collected from Gwaski, Hawaul LGA Borno state and identified at Department of Botany, Faculty of science, University of Maiduguri. Seed were washed with clean water to separate it from the white membranes and allowed to dry room temperature for four weeks and ground to semi-powder form.

Plant extraction: Plant seed was grounded into semi powder (40-60 mesh) with a blender. A total of 70 g of samples was immersed for 3 days subsequently in 1000 mL of 80% methanol in 2.5 L flat bottom flask. The sample was shaking ceaselessly for 3 days at 25°C to acquire 80% methanolic extract. The extract obtained was then filtered with what-man filter paper after which was concentrated to semi-solid form with a rotary evaporator at the temperature of 42°C. Crude extracts obtained were then weighed, transferred to the sample bottle and stored at 4°C.

Plant sample dilution and dose preparation: A stock solution (100 mg mL^{-1}) was prepared by dissolving (100 mg) in 1 mL of 100% DMSO. Sub-stocks were prepared in a microliter (μg mL^{-1}) using the formulae $M_1V_1 = M_2V_2$ and diluted to the concentration of interest by twofold serial dilution using 100% methanol at eight concentrations ranging from 7.81-1000 μg mL^{-1} prepared in a 96-well microplate. DMSO (vehicle) was maintained at 0.1% in all concentration of samples.

Antioxidant assay

DPPH radical-scavenging activity assay: The free radical scavenging activity of sample extracts was evaluated by 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH) according to the method previously reported by Shen etc. with minor modifications. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of methanol and then exposed to different concentration plant extract (7.81-1000 μg mL^{-1}). DPPH reagent (270 μL) and sample solutions (30 μL) were mixed in 96-well plates shake vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-VIs spectrophotometer. Trolox and quercetin were used as the reference. Lower absorbance values of reaction mixture indicate higher free radicalscavenging activity. The capability of scavenging the DPPH radical was calculated by using the following equation:

$$\text{DPPH scavenging effect}(\text{inhibition}\%) = \frac{A_0\text{-}A_1}{A_0} \times 100$$

Where:

A_0 : The absorbance of the control reaction

A_1 : The absorbance in the presence of all of the extract samples and reference

All the tests were performed in triplicates and the results were averaged.

Ferric Reducing/Antioxidant Power (FRAP) assay: The FRAP assay was carried out according to the previously reported procedure by Wojdylo with slight modifications. Sample extracts were dissolved in 0.1% DMSO-methanol (v/v) to prepare the stock solution (1 mg mL^{-1}). Briefly, the working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), a solution of 10 mM 2, 4, 6-Tripyridyl-s-Triazine (TPTZ) in 40 mM hydrochloric acid and 20 mM ferric chloride at 10:1:1 (v/v/v). The working FRAP reagent (270 μL) and sample solutions (30 μL) were mixed in 96-well plates and warmed at 37°C in constant temperature oven for 5 min. The absorbance was taken at 593 nm. Trolox and quercetin were used as a standard.All determinations were performed in triplicate:

$$\text{FRAP scavenging activity}(\%)\frac{(\text{Abs}_{\text{control sample}})\text{-Abs}_{\text{control}}}{\text{Abs}_{\text{control}}} \times 100$$

Where:

$\text{Abs}_{\text{control}}$: The absorbance of FRAP+methanol

$\text{Abs}_{\text{sample}}$: Absorbance FRAP+Sample extract/standard

L-DOPA and mushroom tyrosinase were purchased from Sigma Chemical. The 20 μL of mushroom tyrosinase (1000 U mL^{-1}), 20 μL of 0.1 M phosphate buffer (pH 6.8) and 100 μL of the test sample solution (20%) containing 20 μL of plant extracts were mixed (called sample solution with enzyme). Sample solutions without enzyme were also, prepared by repeating all previous steps but with no plant extracts added. Blank solutions with and without enzyme were also prepared with no test sample solution added. We also prepared positive controls of 0.5 mg mL^{-1} kojic acid solutions (with water) with and without enzyme. The 20 μL of 0.85 mM L-DOPA solution as the substrate was added into every sample and blank. These assay mixtures were incubated at 25°C for 10 min. The amount of dopachrome

produced in the reaction mixture was measured at 475 nm (e475 = 3600/m/cm) using microplate reader (Zenyth 2000, Anthos Labtech Instrument). Percent inhibition of tyrosinase activity was calculated as the following: % tyrosinase:

$$\text{Inhibition} = (\text{A-B})-(\text{C-D})\times 100/(\text{A-B})$$

Where:

A : Absorbance of blank solution with enzyme
B : Absorbance of blank solution without enzyme
C : Absorbance of sample solution with enzyme
D : Absorbance of sample solution without enzyme

RESULTS AND DISCUSSION

Present of phenolicsin the fruits and vegetables have been extensively studied due to their potential biological activities. Phenolic compounds such as flavonoids, phenolics acid and tannins possess diverse biological activities including anti-inflammatory, anti-carcinogenic and antiatherosclerotic activities. These activities might be related to their antioxidant activity.

Yield of 35.2 g was obtained following extraction of 100 g seed samples with 80% methanol and concentrated to semisolid form with a rotary evaporator at a temperature of 42°C (Table 1). Percentage scavenging of *Zizyphus spina-christi* (seed) on DPPH with different concentration 7.8-10000 μg mL^{-1} of plant crude extract as compared with Quercetin and Trolax (reference). Percentage of inhibition (mean±SD) (n = 3) is shown versus concentration of the tested sample. The antioxidant activity of *Zizyphus spina-christi* seed extract showed variation in the oxidation power with 15.5% at the concentration of 7.8 μg mL^{-1}-88.4% at 1000 μg mL^{-1} for *Zizyphus spina-christi* seed extract. While the percentage scavenging power for trolax varied from 56.4% at the concentration of 7.8 μg mL^{-1}-96.5% at 1000 μg mL^{-1}. For quercetin the percentage scavenging power varied from 89.1% at 7.8 μg mL^{-1}-100% at 1000 μg mL^{-1}. The largest capacity to neutralize DPPH radicals was found at 1000 μg mL^{-1} for both *Zizyphus spina-christi* seed extract, Trolax and quercetin (Fig. 1).

Percentage inhibition of *Zizyphus spina-christi* (seed) on FRAP revealed differences in activities at concentration range 7.8-1000 μg mL^{-1} of plant crude extract compared with Trolax and Quercetin (reference). Percentage of inhibition (mean±SD) (n = 3) is shown versus concentration of the tested sample. The antioxidant activities values varied from 22.5% at the concentration 7.8 μg mL^{-1}-63.5% at 1000 μg mL^{-1} for *Ziziphus spina-christi*. For Trolax the percentage inhibition activities vary from 46.3% at 7.8 μg mL^{-1}-100% at 91.5 μg mL^{-1}. Percentage antioxidant power of quercetin differed from 79.1% at 7.8 μg mL^{-1}-100% at 1000 μg mL^{-1}. The largest capacity to inhibit FRAP was found at 1000 μg mL^{-1} (Fig. 2).

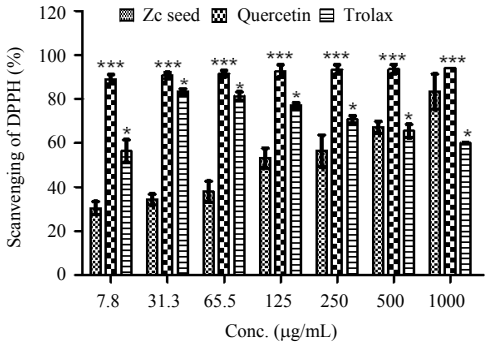

Fig. 1: Antioxidant activities of *Ziziphus spina-christi* crude extracts (2 mg mL^{-1}), quercetin and trolox (500 μg mL^{-1}). Free radical scavenging inhibitory activities was measured using DPPH assay. Absorbance was taken at 517 nm with quercetin and trolox as standards. ***p<0.0001 represented significantly different values from standards and tested plants. The values represent mean±SD from three independent experiments

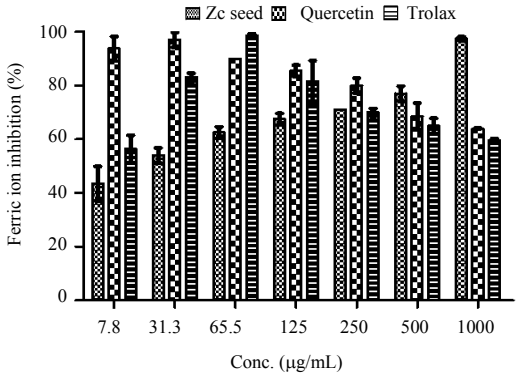

Fig. 2: Antioxidant activities of *Ziziphus spina-christi* crude extracts (2 mg mL^{-1}), quercetin and trolox (500 μg mL^{-1}). Free radical scavenging inhibitory activities was measured using DPPH assay. Absorbance was taken at 517 nm with quercetin and trolox as standards. There is no significant different value from standards and tested plants. The values represent mean±SD from three independent experiments

Table 1: Result of seed samples

Plant's	Part	Weight of samples in powder form (g)	Weight of samples crude extract (g)	Percentage yield/100 g (DW)
Ziziphus spina-christi	Seed	100	67.1	39.74

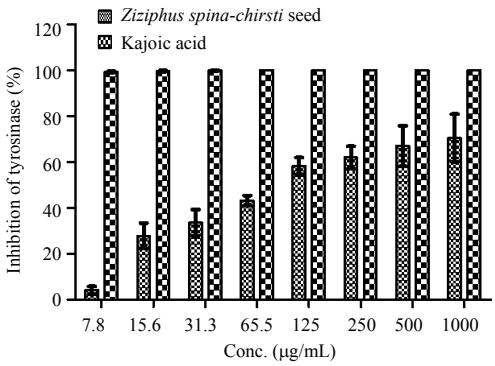

Fig. 3: Antityrosinase activities of *Ziziphus spina-christi* crude extracts (1000 µg mL^{-1}), kajoic acid (500 µg mL^{-1}). A tyrosinase inhibitory activity was measured using and the absorbance was taken at 517 nm with kajoic acid as standards

Percentage inhibition of *Zizyphus spina-christi* (seed) on tyrosinase shows variation in activities with increased concentration 7.8-1000 µg mL^{-1} of plant crude extract as compared to Kajoic acid (reference). Percentage of inhibition (mean±SD) (n = 3) is shown versus concentration of the tested sample. The percentage antityrosinase activities varied from 4.1% at 7.8 µg mL^{-1} -70.5% at 1000 µg mL^{-1} for plant crude extract. Percentage kajoic acid activities was observed to be 99.3% at 7.8 µg mL^{-1} to 100% at 1000 µg mL^{-1}. The largest capacity to inhibit tyrosinase was found at 1000 µg mL^{-1} for both plant extracts and Kajoic acid (Fig. 3).

Phytochemicals including phenolic compounds, present in many herbs have received much attention in recent years due to their many health benefits including antioxidant and anti-inflammatory activities[15, 16]. For this reason there are interests in using some herbs not only as culinary products but also as a supplement to functional products [17-19]. Therefore, the study on the stability of some bioactive compounds in the plant material during some processing (drying, freezing, etc.) or preparation of plant extracts would be useful in the selection of the bioactive compounds extraction procedure[20-22]. For extracting phenolic compounds typically from plant material previously used solvents are methanol, ethanol, acetone and ethyl acetate[23-25]. In the present study for extracting phenolic compounds from the seed 80% methanol was used.

Antioxidants are exceptionally leading substances which influence the proficiency to defense the body system from detrimental effect ROS including superoxide anion, free radical, hydroxyl radical, hydrogen peroxide, nitrogen dioxide, nitric oxide radical induced by oxidative stress[26-28]. ROS can stimulate the production of melanin pigment cells and promote the generation of hyper pigmentation. The DPPH radical scavenging measure is a valuable technique for antioxidant evaluation[29, 30]. Neutralizing free radicals can repress the production of melanin in skin and other tissue which result in skin whitening.

Tyrosinase inhibition is desired as tyrosinase catalyzes the oxidation of phenolic compounds present in fruits and vegetables into quinone which gives an undesirable taste and color and also decreases the availability of certain essential amino acids as well as the digestibility of the products[31-33]. As such highly effective tyrosinase inhibitors are also needed by the body system[34-36]. The antioxidant and tyrosinase inhibition potential of *Z. spina-christi* 80% methanol extracts was investigated in the search for new bioactive compounds from natural resources. It has clearly shown that *Z. spina-christi* seed extract present high antioxidant and tyrosinase inhibition activities compared with reference quercetin and trolex for DPPH and FRAP assay as well as kajoic acid for anti tyrosinase assay. Previous studies reported that DPPH radical scavenging activity of fruit and seed extract showed good free radical scavenging activity when compared with ascorbic acid[37-39]. Presences of poly phenols such as flavonoid might be responsible for the antioxidant activities of the extract[40-42]. Scavenging of the free radical might be the major mode of action of this plant extract when use for the treatment of diseases[43]. Component with antioxidants activities are of interest to biologists and clinicians because they help in protecting human body against damages induced by reactive free radicals generated in atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and even in aging process[44]. Scientific finding has reveal that most of the natural products and their derivatives have efficient anti-oxidative characteristics, consequently linked to anti-cancer, hypolipidemic, anti-aging and anti-inflammatory activities[45, 46].

In human, skin it is the largest organ of the integument system. It covers the whole surface of the human body, representing around 15% of the body weight[47]. It connect the body with the outer environment, protect the basic structures against scraped spot and lack of hydration and assumes an essential part in invulnerability and other body defense mechanisms[48]. Intensity and skin pigmentation varies with environmental, state of origin, climate and gender. Individual skin color may varies with time or when exposed to climatic or environmental changes[49]. Thickness and melanin contained of the skin may alter hemoglobin and melanocytes resulting in minor changes in pigmentations such as carotenoids affect the perceived color and determination of skin color. Therefore, melanin synthesis is very vital in determining the skin functions and color formation[50]. The key enzyme that catalysis the synthesis of melanin and intervenes in

several intermediate stages of pigment formation is tyrosinase. Report shows that present of bioactive compounds such as (collagen gels in Aesculus hippocastanum) (Aloesin [2-acetonyl-8-beta-dglucopyranosyl-7-hydroxy-5-methylchromone], Tyrosine hydroxylase and 3, 4-dihydroxyphenylalanine oxidase in Aloe vera) (polysaccharides, flavonoids, hyaluronan synthase-3 and hyaluronan synthase-2 in Astragalusmembranaceus), (polyphenols catechin, epiglactocatechin, epiglactocatechin-3-gallate in *Camellia sinensis*) (triterpenoids, saponins, madecassoside, asiaticoside, centelloside and asiatic acid in Centellaasiatica) (phenolic compounds such as anthocyanins, flavanones, hydroxycinnamic acids and ascorbic acid in *Citrus sinensis*) competitively inhibit tyrosinase enzyme[51].

Microscopic differences for photo aged skin in contrast can be associated with either increased epidermal thickness or pronounced epidermal atrophy with histological changes like accumulation of elastin-containing material just below the derma lepidermal junction or disorganization of collagen may be responsible for microscopic differences for photoaged skin. For example, one of the main building blocks of human skin is cfollagen, it is synthesized from its precursor molecules called procollagen which is derived from dermal fibroblasts regulated by Transforming Growth Factor-β (TGF-β), a cytokine that promotes collagen production and Activator Protein-1 (AP-1), a transcription factor promotes collagen breakdown by up regulating enzymes called matrix metalloproteinases[51].

CONCLUSION

High antioxidant and tyrosinase inhibitory ability of 80% methanolic extract of *Ziziphus spina-christi* seed has been recorded in this study. Other scientific evaluation on the use of *Ziziphus spina-christi* for traditional medicinal used should be explored further based on different models. Fractions from the all part of the plant need to be evaluated for antioxidant and tyrosinase inhibition potential. Bioactive constituent from the all part of the plant should also be identified to find effective leads from natural resources useful in the treatment of skin wrinkling.

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