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LAGENARIA SICERARIA (MOLINA) STANDLEY. TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF SEED OILS OF BOTTLE GOURD CULTIVARS

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ABSTRACT

The polyphenols content and antioxidant activity of *Lagenaria siceraria* seed oils of four bottle gourd cultivars (water jug-globose head, African wine gourd, speckled swan and water jug- hourglass shape) were evaluated. The total phenolics and flavonoids content of the seed oils were measured spectrophotometrically using the Folin-Ciocalteu assay and aluminum chloride colorimetric method respectively. The total phenolic contents of the seed oils ranged from 8.0-10.5 mg gallic acid/100g and total flavonoids, 5.6-7.7 mg quercetin equivalent/100g. The seed oils (100 µg/ml) free radical scavenging activity (DPPH assay), varied between 47.2-68.5% with EC_{50} values of 51.0-108.0 µg/ml; metal chelating activity of seed oils ranged from 63.8-80.1% with EC_{50} of 17.0-57.0 µg/ml; and ferric reducing power of seed oils was 0.479-0.607 with EC_{50} values of 50.0-103.0. The results suggest that seeds of these bottle gourds are a veritable source of potential edible medicinal oils and may be used in different food applications to provide nutrition and health benefits.

KEYWORDS: Cucurbitaceae, *Lagenaria siceraria*, cultivars, seed oil, antioxidant activity.

INTRODUCTION

Many oils for human consumption or for industrial purposes are derived from plants. These oils consist mainly of mono-, di-and tri-acylglycerols which act as solvent for minor constituents such as sterols, fat-soluble vitamins, pigments including chlorophylls and carotenoids, phenolic compounds, phospholipids and free fatty acids.[1] These minor constituents can have either pro-oxidative (e.g., free fatty acids and hydroperoxides) or

antioxidative (e.g., tocopherols, pigments, phenols and phospholipids) effects.[2] Antioxidant compounds are gaining in importance due to their dual role in food and pharmaceutical industries as lipid stabilizers.[3] Nutritionally important antioxidants such as tocopherols improve the stability of oils.[4] Phenolic compounds may prevent deterioration through the quenching of reactions responsible for lipid rancidity.[5] Moreover, the higher antioxidant activity of some crude oils is partly due to polar lipids, especially phospholipids. Indeed, these compounds are usually considered free radical scavengers, antioxidant synergists and extenders for the action of primary antioxidants.[6] With regard to carotenoids, it is accepted that they can act as primary antioxidants by trapping free radicals or as secondary antioxidants by quenching singlet oxygen.[7]

The Lagenarias include the hard, thin-shelled "utility" bottle/birdhouse gourds which have smooth stems; soft, large leaves; and white flowers. The *Lagenaria* gourds (family: Cucurbitaceae) are tan to brown when mature with long, narrow hard stems and have many distinct shapes and sizes. They are climbers or trailers, and the most important is the cultivated *L. siceraria* (Molina) Standley.[8] Worldwide, bottle gourd is grown for its fruit either being harvested young and used as a vegetable or harvested mature and used as a bottle, utensil, or pipe. Another recent utilization of bottle gourd is as rootstocks for watermelon against soil-borne diseases and low soil temperature.[9] Ibiok *et al*.[8] also showed that variation exist in the morphology of leaves, fruits and seeds of the five investigated cultivars grown in Southern Nigeria. As the morphological variation in *L. siceraria* is diverse and continuous, it is difficult to classify the land races into distinct groups. However, they are generally distinguished by the size and shape of their fruits with common names.[10]

Ethno-medicinal usage reveals that *L. siceraria* seed is diuretic, anthelmentic and is also used to reduce inflammation and pain. In addition, it is used in treatment of boils, aching teeth and gums, diabetes mellitus, cough, fever and skin diseases.[11,12] Lagenin - a novel ribosome protein was isolated from the lyophilized water extract of *L. siceraria* seeds and shown to possess antitumour, immunosuppressive, antiviral, antiproliferative and anti-HIV activities.[13]

There is paucity of information on the biological activity of seed oils of *L. siceraria* gourds. Interestingly, we noted slight morphological variations in the leaves, fruit shape and size, seed colour, seed shape and size of these cultivars. In our earlier works, the seed oils quality and fatty acids composition of fourteen cultivars of *L. siceraria* were reported.[14,15] The

results obtained showed that the seed oils of the oleaginous gourds are a good source of polyunsaturated fatty acids (PUFAs) - linoleic acid, an essential fatty acid - for human diet and could also be used by the food industry for formulating functional foods enriched with PUFAs. Sunil *et al.*[16] found in their study of diversity *of L. siceraria* in India that, the germplasm has high variation for all traits including seed oil content and fatty acid profile. They concluded that apart from its use as vegetable and medicinal plants, the other potential use of the crop as an edible minor oil-seed, having up to 28% seed oil and composition at par with sunflower oil, may be tapped. Furthermore, in some communities in Nigeria, the seeds of the mature bottle gourds are used for cakes, edible oils and as food thickeners. Therefore, the present study was undertaken to determine the total polyphenols and antioxidant activity of seed oils of four bottle gourd cultivars.

MATERIALS AND METHODS

Samples collection and extraction

Mature fruits of *L. siceraria* cultivars: water jug-globose head (seed is flat, smooth surfaced and spear-headed, 3.5-4.0 cm long, 1.2-1.5 cm wide and brown in colour) and African wine kettle (huge gourd with round head, short neck and large round base) were collected in October, 2012 from different farms in Mkpat Enin and Ika Local Government Area of Akwa Ibom State; speckled swan (smooth, dark green, curved neck, swan head shaped) and water jug- hourglass shape (seed is compressed or flat with two ridges running down the flat surfaces, 2.5-3.0 cm long and 1.5-2.0 cm wide) were purchased in a local market in Kano State of Nigeria in October, 2012. The plants were identified and authenticated by Dr. (Mrs.) M. E. Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria where voucher specimens were deposited.

The mature pods of *L. siceraria* cultivars were beaten and allowed to soften after which the seeds were removed. The seeds were washed, sun dried, dehulled, pulverized and extracted with *n*-hexane. Rotary evaporator was used to separate the seed oil from the filtrate. All chemicals and solvents used in this study were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO). Standard antioxidant compounds used were obtained from laboratory stock, acquired from commercial sources, or isolated, purified, and characterized from natural sources. All solutions were made in distilled water.

Determination of total phenolics

The concentration of phenolics was expressed as µg gallic acid equivalent per mg of the extract. The method of Spanos and Wrolstad[17] was used. Solution (1 ml) containing extract (1 mg) in methanol was added to distilled water (46 ml) and Folin Ciocalteu Reagent (1 ml) then mixed thoroughly. After 3 mins, sodium carbonate (2%, 3 ml) was added to the mixture and shaken intermittently for 2 hrs at room temperature. The absorbance was read at 760 nm. Gallic acid was used as a standard and a calibration curve was plotted.

Determination of total flavonoids

Measurement of flavonoid concentration of extracts was based on the method of Park *et al.*[18] expressed as quercetin equivalent. An aliquot of the solution (1 ml) containing the oil extract (1 mg) in methanol was added to test tubes containing aluminium nitrate (10%, 0.1 ml), potassium acetate (1 M, 0.1 ml) and ethanol (3.8 ml). After 40 mins at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard and a calibration curve was plotted.

DPPH radical-scavenging activity

DPPH radical scavenging activity of each seed oil extract was determined according to the methods of Blois[19] and Pise *et al.*[20] Seed oil (3 ml) was added to DPPH solution (1 ml, 0.2 mM in methanol) as the free radical source. The mixture was shaken and kept for 30 mins at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517 nm. Ascorbic acid and Butylated hydroxyanisole (BHA) were used as the positive control. The DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} X \quad 100$$

Metal chelating activity

The method of Dinis *et al.*[21] was used. Crude extract (0.5 ml) was mixed with $FeCl_2$ (2 mM, 0.05 ml) and Ferrozine (5 mM, 0.4 ml). The total volume was diluted with methanol (2 ml). The mixture was shaken vigorously and left standing at room temperature for 10 mins. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a Unicam uv/vis spectrophotometer, model 8700. The percentage inhibition of ferrozine Fe^{2+} complex was calculated using the formula:

$$\% \text{ inhibition of ferrozine} - Fe2+ = \frac{A_{control} - A_{sample}}{A_{control}} X \quad 100$$

Ferric reducing capacity

The reducing power of each sample was determined according to the method of Oyaizu.[22] Sample solutions of different concentrations were mixed with phosphate buffer (pH 6.6, 0.2 M, 0.5 ml) and potassium ferric cyanide (1%, 2.5 ml). After the mixture was incubated at 50°C for 20 mins, trichloroacetic acid (TCA) (10%, 2.5 ml) was added and the mixture was centrifuged for 10 mins. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.1%, 0.5 ml); the absorbance was measured at 700 nm against water as a blank. Higher absorbance of the reaction mixture indicated greater reducing power. BHA was used as positive control.

RESULTS AND DISCUSSION

Total flavonoids and phenolics contents (TPC)

The total phenolics and flavonoids contents of seed oils of *L. siceraria* cultivars are presented in Table 1. Calibration curves ($y = 0.0058x + 0.1584$, $R^2 = 0.9653$) and ($y = 0.0004x + 0.0937$, $R^2 = 0.9694$) were obtained for flavonoids and total phenols respectively. The total phenolic contents of the oils range from 9.1-10.5 mg gallic acid/100g and total flavonoids, 5.6-7.7 mg quercetin equivalent/100g. Among the various extracts, water jug-globose head (GHWJ) cultivar had the highest TPC (10.5 mg GAE/100g), followed by hourglass shaped water jug (HGWJ) cultivar (9.2 mg GAE/100g), African wine kettle (AWK) gourd (9.1 mg GAE/100g) and speckled swan (SW) gourd (8.0 mg GAE/100g). Differences in phenolic contents of seed oils in this study may be attributed to the slight morphological differences which characterize these cultivars. Total phenolics content in pumpkin oil was 15.9 mg CAE/g and 22.7 mg CAE/g in soybean oil.[23] *n*-Hexane extract of *C. lanatus* seed at 1000 µg/ml contained 76.28 mgGAE/g of polyphenols while ethanol and chloroform extracts respectively gave 42.34 and 27.71 mgGAE/g.[24]

The results from this study were also compared with other conventional seed oils: sunflower seed oil (1.20 mgCAE/100g), rapeseed oil (1.31 mgCAE/100g), corn oil (1.26 mgCAE/100g), grapeseed oil (0.51 mgCAE/100g), hemp oil (2.45 mgCAE/100g), flax oil (1.14 mgCAE/100g), rice bran oil (1.44 mgCAE/100g).[25] Medicinal plants are rich sources for naturally occurring antioxidants. Among these substances, the phenolic compounds have the ability to scavenge free radicals, super oxide and hydroxyl radicals through single-electron transfer reactions.[26] Flavonoids are the most common groups of polyphenolic compounds in the human diet and are ubiquitous in plants. The results of the flavonoids

content in seed oils obtained for various cultivars in this study were lower than those reported for some Curcubitaceae seeds, although with the use of other polar solvents: *n*-hexane (88.12 mg QE/g), chloroform (43.09 mg QE/g) and ethanol (113.53 mg QE/g) of *C. lanatus*; *L. siceraria* (17.9 mg QE/g), *L. cylindrica* (6.3 mg QE/g) and *C. pepo* (2.1 mg QE/g).[24,27]

Table 1: Total phenols and flavonoids content of seed oils of *L .siceraria* (bottle gourds) cultivars

	Cultivars of *L. siceraria* seed oils			
	Water Jug (Hour Glass Shape)	**Speckled Swan**	**Water Jug (Globose Head)**	**African Wine Kettle**
Total flavonoid (mg QE/100g)	6.1±0.80	5.6±0.15	7.7±1.20	6.4±1.50
Total phenolic (mg GAE/100g)	9.2±0.20	8.0±0.10	10.5±0.5	9.1±0.30

Values are mean± S.D of triplicate determinations

DPPH free radical scavenging activity

The DPPH radical scavenging activity of seed oils of bottle gourd cultivars were determined and compared with ascorbic acid and BHA. The seed oils of *L. siceraria* cultivars exhibited significant DPPH radical scavenging activity *in vitro*. The percentage inhibition at various concentrations (20-100 µg/ml) is shown in Fig 1; HGWJ (23.6-47.2%), SW (25.3-49.9%), GHWJ (21.9-49.1%), AWK (30.1-53.6%), BHA (37.2-68.5%) and ascorbic acid (36.1-66.7%). The highest antioxidant activity was displayed by the extract obtained from AWK (53.6%), and decreased in the order: SW (49.9%), GHWJ (49.1%) and HGWJ (47.2%) at concentration of 100 µg/ml; comparable with BHA (68.5%) and ascorbic acid (66.7%). The standards demonstrated higher DPPH activity than the seed oils indicating relative lower EC_{50} values. The EC_{50} values (96.0-108.0 µg/ml) were obtained for seed oils, BHA (51.0 µg/ml) and ascorbic acid (55.0 µg/ml) (Table 2). Correlation of R^2 = 0.9624-0.9851 corresponding to cultivars seed oils were obtained from the regression equation of the calibration curve, 0.9945 and 0.9957 respectively for ascorbic acid and BHA. The various calibration curves obtained from the graphs were extrapolated for the EC_{50} values (effective concentration at 50% inhibition).

The effectiveness of antioxidant properties is inversely correlated with EC_{50} values. The scavenging effect increased with corresponding increment in the concentration of the seed oil. DPPH scavenging activities of studied seed oils were compared with published data at 100 µg/ml for *C. melo* (57.59%), *L. cylindrica* (48.75%), *L. siceraria* (35.13%) and *L.*

breviflora (43.15%).[28,29] EC_{50} values were also reported for related seed oils of *L. acutangula* (104.12 µg/ml) and *C. lanatus* (149.09 µg/ml).[24,30]

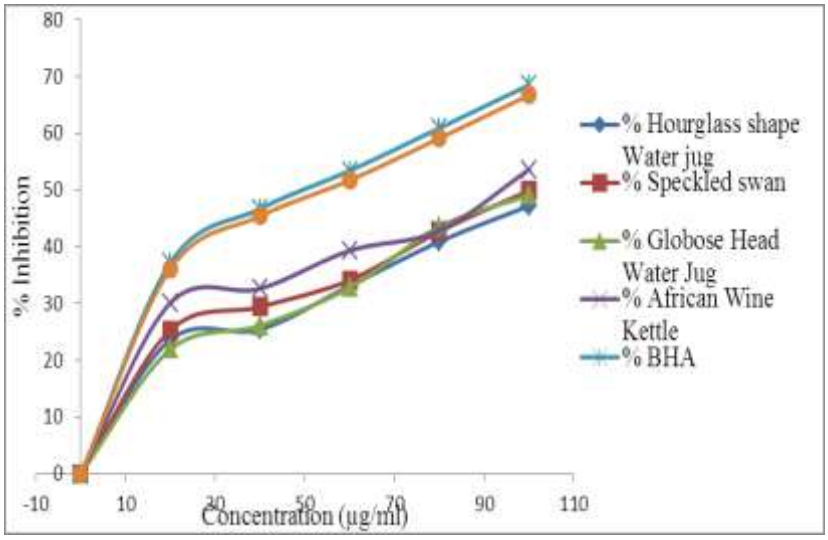

Fig. 1: Percentage DPPH scavenging activity of seed oils of *L. siceraria* cultivars and standards

Table 2: EC_{50} (µg/ml) for antioxidant activity of *L. siceraria* seed oil extracts

Activity	HGWJ	SW	GHWJ	AWK	EDTA	BHA	AA
DPPH	108.0	104.0	103.0	96.0	ND	51.0	55.0
Metal Chelating	52.0	46.0	57.0	32.0	17.0	ND	ND
Ferric Reducing	103.0	97.0	94.0	95.0	ND	50.0	ND

HGWJ = Hourglass shaped water jug; SW = Speckled swan; GHWJ = Globose head-water jug; AWK = African wine kettle; EDTA = Ethylene diamine tetraacetic acid; BHA = Butylated hydroxyanisole; AA = Ascorbic acid; ND = Not detected.

Metal chelating activity

The metal chelating activity of seed extracts of *L. siceraria* cultivars in this study were determined and compared with EDTA. The percentage metal chelating activity at various concentrations (20-100 µg/ml) is shown in Fig. 2. The metal chelating activity of the seed oils (100 µg/ml) ranged from 63.8-69.4%; the highest metal chelating activity was detected in the oil obtained from AWK (69.4%), then GHWJ (64.5%), SW (63.9%), HGWJ (63.8%) and EDTA (80.1%). Correlation of R^2 = 0.9524-0.9927 corresponding to the various seed oils were obtained from the regression equation of the calibration curve and 0.9732 for EDTA. The various calibration curves obtained from the graphs were extrapolated for the EC_{50}. The

EC_{50} values obtained comprised: HGWJ (52.0 µg/ml), SW (46.0 µg/ml), GHWJ (57.0 µg/ml), AWK (32.0 µg/ml) and EDTA (17.0 µg/ml) (Table 2).

EC_{50} values show that the seed oils contain potential metal chelators as compared with EDTA. Iron is an essential mineral for normal physiological activity of the human body, but excess can cause cellular damage and injury. The ferrous ions are the most effective pro oxidants in food systems; good chelating effect would be beneficial and removal of free ion from circulation could be a promising approach to prevent oxidative stress induced disease.[31]

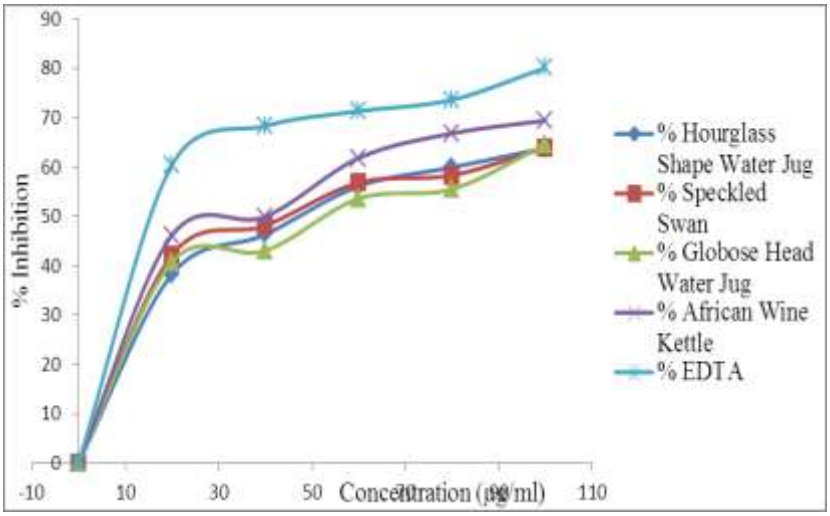

Fig. 2: Percentage metal chelating activity of seed oils of *L. siceraria* cultivars and EDTA.

Ferric reducing power

The results of absorbance in ferric reducing power at various concentrations (20-100 µg/ml) are presented in Fig. 3; HGWJ (0.239-0.479), SW (0.293-0.503), GHWJ (0.248-0.501), AWK (0.311-0.512). The EC_{50} values results indicate that the reducing capacity of the seed oils decreased in the order: HGWJ (103.0 µg/ml), SW (97.0 µg/ml), AWK (95.0 µg/ml), GHWJ (94.0 µg/ml); the standard BHA exhibited higher activity with EC_{50} value, 50.0 µg/ml (Table 2). The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity.[32] Phenolic compounds show reducing power and have ability to convert Fe^{3+} to Fe^{2+}.[33] The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain through donation of hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.[34,35] The ferric reducing potential

of the studied seed oils is concentration dependent (Fig. 3). Therefore, the studied *L. siceraria* oils are suggested to act as electron donors, reacting with free radicals and converting them to more stable products, which can terminate radical chain reaction.

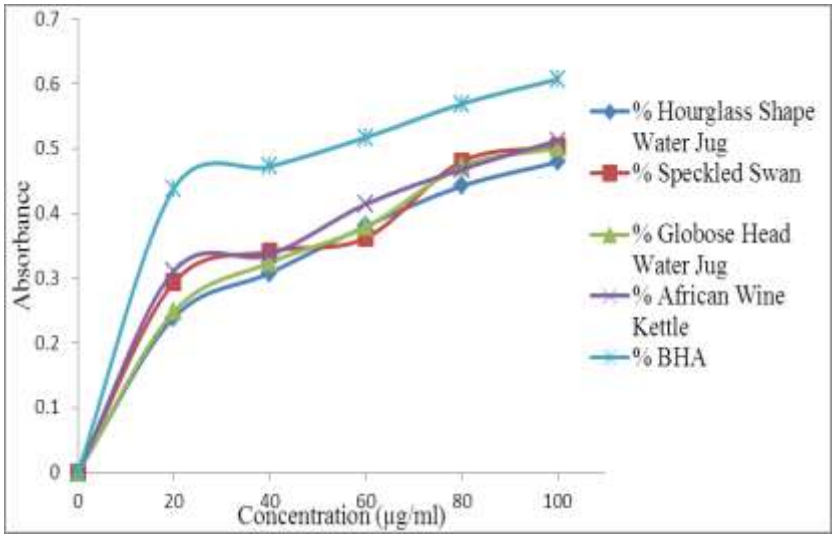

Fig. 3: Ferric reducing power of seed oils of *L. siceraria* cultivars and BHA

CONCLUSION

The seed oils *L. siceraria* cultivars contain substantial amount of phenolic compounds especially flavonoids. The various oils exhibit significant antioxidant activity – DPPH radical scavenging, metal chelating and ferric reducing when compared with standard compounds. The antioxidants in the seed oils also revealed promising medicinal potentials when compared with other conventional seed oils. This work demonstrates that seeds of *L. siceraria* cultivars are a veritable source of potential medicinal oils. These edible oils rich in natural antioxidants may play a role in reducing the risk of chronic diseases. Thus, the oils examined may be used in different food applications to provide nutrition and health benefits. It will also reawaken interest in the re-cultivation of these plant cultivars, especially in Nigeria, which hitherto were cultivated solely for their mature fruits which served as containers.

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