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ANTIOXIDANT CAPACITY AND TOTAL POLYPHENOLICS FROM SEED OILS OF VARIOUS CULTIVARS OF *LAGENARIA SICERARIA*

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Abstract:
Lagenaria siceraria (Molina) Standl. is a vegetable food, also used as a traditional medicine. The present study was designed to investigate the flavonoids, total phenolics and antioxidant activities of seed oils extracted from six cultivars of Lagenaria siceraria. The total phenolics, flavonoids content and antioxidant activity were measured spectrophotometrically using standard procedures. The total phenolic contents of the oils ranged from 5.2-7.0 mg gallic acid/100g and total flavonoids, 4.1-6.1 mg quercetin equivalent/100g. The seed oils (120 µg/ml) free radical scavenging activity (DPPH assay) varied between 54.1-57.8% with EC_{50} values of 98.0-117.0 µg/ml; metal chelating activity and EC_{50} values of the oils were 65.5-83.6% and 19.0-59.0 µg/ml respectively; and ferric reducing power of seed oils varied from 0.528-0.580 with EC_{50} values of 91.0-104.0 µg/ml. Dietary polyphenolics from Lagenaria siceraria cultivars may supply substantial antioxidants, which may provide health-promoting advantages to the consumers.

Keywords: *Curcubitaceae, Lagenaria siceraria, Cultivars, Polyphenolics, Antioxidant activity.*

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INTRODUCTION:

An antioxidant is a molecule which can prevent effects of oxidation in tissues and can protect cell damage caused by free radicals [1]. Free radicals involving oxygen are termed reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide [2]. The oxidative damage caused by excess ROS may lead to development of many diseases such as congestive heart failure, hypertension, cerebrovascular accidents, ageing and several stress related diseases including cataracts, cognitive dysfunction, cancer, myocardial infection, diabetic complications and several heart diseases [2, 3]. Many antioxidants are plant based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress [4]. Phenolics and flavonoids are among the most important groups of secondary metabolites that are found in vegetables and fruits [5].

The *Lagenaria siceraria* (Molina, Standley), synonymous: the common bottle gourd *L. vulgaris* (Seringe), and the white-flowered *L. leucantha* (Rusby), is a creeping vine of the Cucurbitaceae family to which pumpkins and squashes belong [6]. The land races of *L. siceraria* show amazing diversity, particularly with respect to fruit shape and size. African and American land races (subsp. *siceraria*) are morphologically distinct from Asian land races (subsp. *asiatica*). In most local African communities, a number of landraces are cultivated for myriad of uses such as food and containers, corresponding to the characteristics of the fruit. 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 [6, 7].

Lagenaria siceraria fruit is traditionally used for its cardioprotective, cardiotonic, general tonic and aphrodisiac properties. It is also used in treatment of various allergic and inflammatory disorders like bronchial asthma, rhinitis, bronchitis and rheumatism. Seeds are nutritive, diuretic and anthelmintic [8, 9]. Esuoso *et al*. [10] reported that seeds of some species of Cucurbitaceae can be the edible oil sources to meet the increasing demands for vegetable oil. In some countries like Niger, seeds are used for dough, cakes and edible oils [11]. The nutritional, physicochemical and fatty acids composition of the seeds and seed oils of some *L. siceraria* cultivars have been reported [12, 13]. It is worthy to explore other value added potential of these oils, which include antioxidant activity, in order to enhance their ultimate and holistic utilization as a functional medicinal food.

MATERIALS AND METHODS:

Samples collection and extraction:

Mature fruits of *L. siceraria* cultivars: long hybrid bottle (LHB), short hybrid bottle (SHB), cave man's club (CMC), tobacco box (TB), bottle gourd (BG) and long handle dipper (LHD) were collected in October, 2012 from different farms in Mkpat Enin, Ika, Ikono, Ibesikpo, Essien Udim and Uruan Local Government Areas of Akwa Ibom State, Nigeria. 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 seeds were washed, dried, dehulled, pulverized and extracted with *n*-hexane using a Soxhlet apparatus. All chemicals, solvents and fatty acid methyl esters (FAMEs) standards 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. 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 Singleton and Rossil [14] 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*. [15] 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 method of Blois [16]. 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:

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

Metal chelating activity:

The method of Dinis *et al*. [17] 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:

$$\% \text{ inhibition of ferrozine} - Fe^{2+} = \frac{A_{control} - A_{sample}}{A_{control}} X \ 100$$

Ferric reducing capacity:

The reducing power of each sample was determined according to the method of Oyaizu [18]. 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 ºC 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:

Total flavonoids and phenolics contents:

The total phenolics and flavonoids contents of seed oils of *L. siceraria* cultivars are presented in Table 1. A calibration curve ($y = 0.0058x + 0.1584$, $R^2 = 0.9653$) and R^2 value of 0.9694 were obtained for flavonoids and total phenols respectively. The total phenolic contents of the oils ranged from 5.2-7.0 mg gallic acid/100 g and total flavonoids, 4.1-6.1 mg quercetin equivalent/100 g. Among the various extracts, LHD had the highest total phenolic content (7.0 mg GAE/100 g), followed by BG (6.2 mg GAE/100g), CMC (6.0 mg GAE/100g), SHB (5.9 mg GAE/100g), TB (5.6 mg GAE/100g) and LHB (5.2 mg GAE/100g).

Table 1: Total phenolics and flavonoids content of seed oils of *L. siceraria* cultivars

	Cultivars of *L. siceraria* seed oils					
	Long hybrid bottle	**Cave man's Club**	**Tobacco box**	**Bottle gourd**	**Long handle dipper**	**Short hybrid Bottle**
Total flavonoids (mg QE/100 g)	4.1±0.1	5.1±0.2	4.7±0.1	4.6±0.4	6.1±0.7	4.5±1.0
Total phenolics (mg GAE/100 g)	5.2±1.2	6.0±0.3	5.6±0.1	6.2±1.5	7.0±0.3	5.9±0.5

Data were expressed as means ± standard deviation of triplicate experiments

DPPH radical scavenging activity

The DPPH radical scavenging activity of seed extract of *L. siceraria* cultivars were determined and compared with ascorbic acid and BHA. The percentage inhibition at various concentrations (20-120 µg/ml) is shown in Fig 1; LHB (18.9-54.1%), CMC (28.1-55.7%), TB (26.7-55.3%), BG (24.8-54.7%), LHD (26.2-56.8%), SHB (20.9- 56.6), BHA (37.2-74.2%) and ascorbic acid (36.1-71.7%). The EC_{50} values were obtained between 98.0 - 117.0 µg/ml) for seed oils, BHA (51.0 µg/ml) and ascorbic acid (56.0 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).

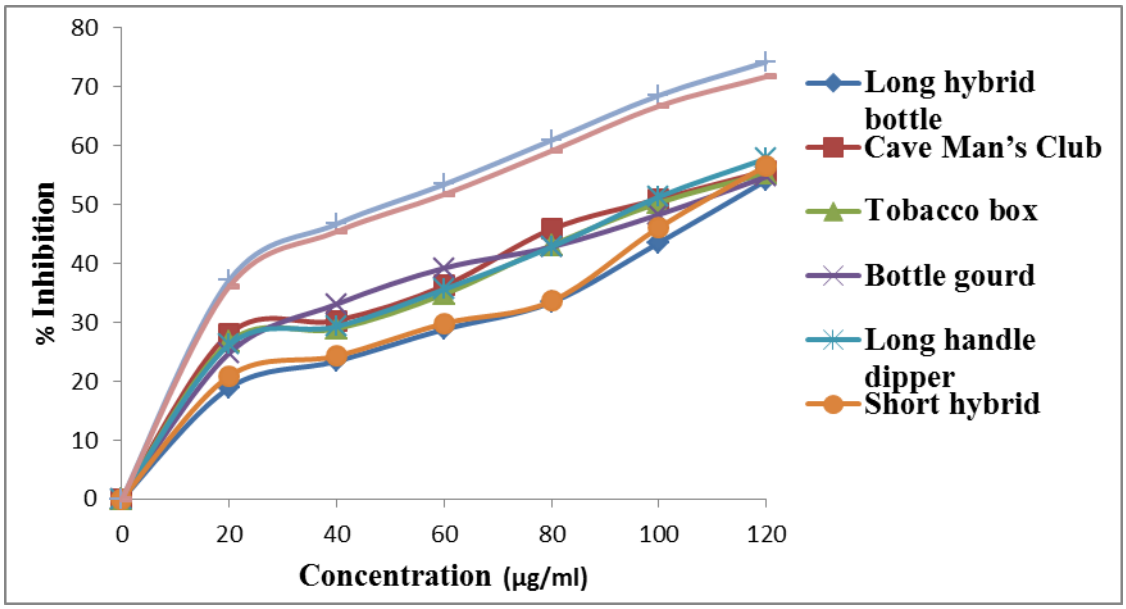

Fig 1: Percentage DPPH scavenging activity of seed oils of *L. siceraria* cultivars and standard

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

Activity	LHB	CMC	TB	BG (µg/ml)	LHG	SHB	EDTA	BHA	AA
DPPH	117.0	99.0	103.0	103.0	98.0	112.0		51.0	56.0
Metal Chelating	54.0	45.0	50.0	52.0	59.0	48.0	19.00		
Ferric Reducing	100.0	103.0	104.0	102.0	100.0	91.0	51.0		

LHB = Long hybrid bottle; CMC = Caveman's club; TB = Tobacco box; BG = Bottle gourd; LHD = Long handle dipper; SHB = Short hybrid bottle; EDTA = Ethylene diamine tetraacetic acid; BHA = Butylated hydroxyanisole; AA = Ascorbic acid

Metal chelating activity:

The metal chelating activity of seed extract of *L. siceraria* cultivars in this study was determined and compared with EDTA. The percentage metal chelating activity at various concentrations (20-120 µg/ml) is shown in Fig. 2. 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: LHB (54.0 µg/ml), CMC (45.0 µg/ml), TB (50.0 µg/ml), BG (52.0 µg/ml), LHD (59.0 µg/ml), SHB (48.0) and EDTA (19.0 µg/ml) (Table 2).

Ferric reducing power:

The results of absorbance in ferric reducing power at various concentrations (20-120 µg/ml) are presented in Fig. 3; LHB (0.301-0.557), CMC (0.268-0.544), TB (0.261-0.528), BG (0.261-0.541), LHD (0.280-0.563), SHB (0.278-0.580) and BHA (0.438-0.651). The EC_{50} values results indicated that the reducing capacity of the seed oils decrease in the order: TB (104.0 µg/ml), CMC (103.0 µg/ml), BG (102.0 µg/ml), LHB (100.0 µg/ml), LHD (100.0 µg/ml), SHB (91.0µg/ml); the standard BHA exhibited higher activity with EC_{50} value, 51.0 µg/ml (Table 2).

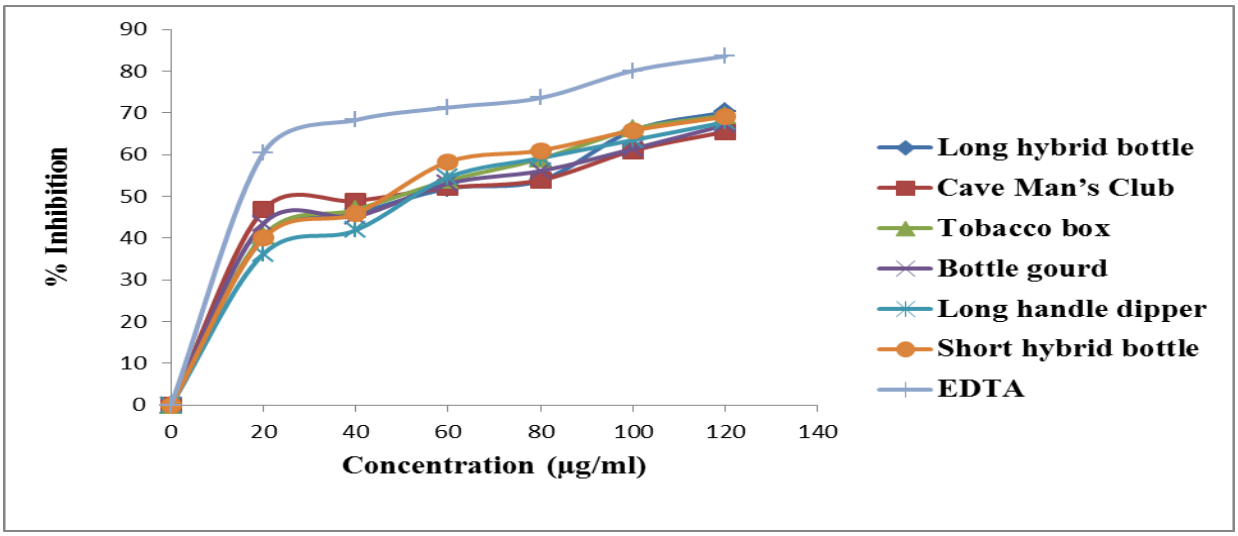

Fig 2: Percentage metal chelating activity of seed oils of *L. seciraria* cultivars and EDTA

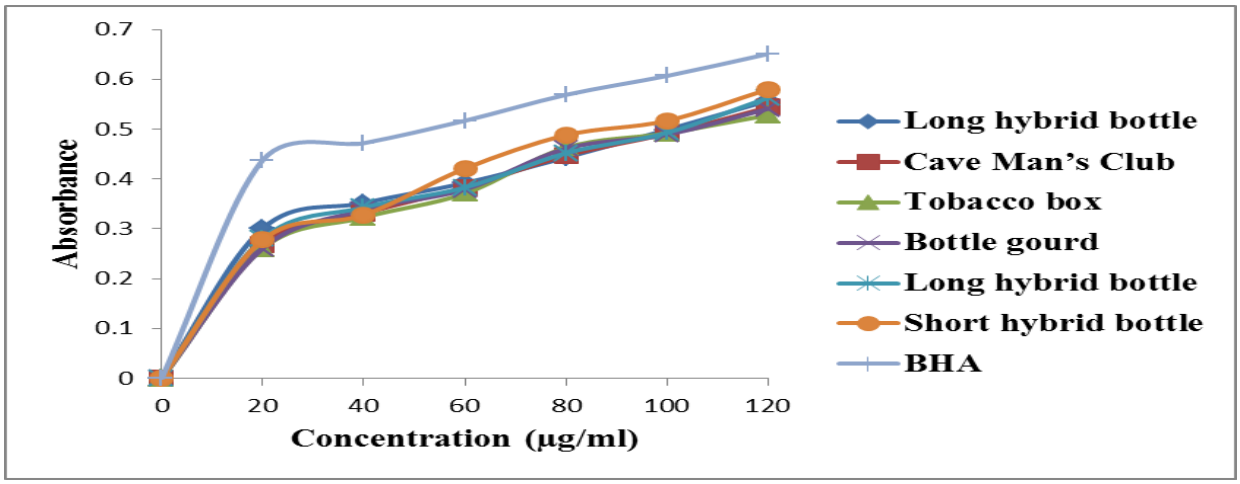

Fig 3: Ferric reducing power of seed oils of *L. siceraria* cultivars and BHA

DISCUSSION:

The highest total phenolic content was obtained in the LHD (7.0 mg/100g) while LHB seed oil was characterized with the lowest amount of total phenolics (5.2 mg/100g). Differences in phenolic contents of seed oils in this study may be attributed to the morphological differences which characterize them as cultivars. Total phenolics content in pumpkin oil was 15.9 mg CAE/g and 22.7 mg CAE/g in soybean oil [19]. *n*-Hexane extract of *C. lanatus* seed at 1000 µg/ml contained 76.28 mg GAE/g of polyphenols while ethanol and chloroform extracts respectively gave 42.34 and 27.71 mg GAE/g [20].

The results from this study were also compared with other conventional seed oils: sunflower seed oil (1.20 mg CAE/100g), rape seed oil (1.31 mg CAE/100g), corn oil (1.26 mg CAE/100g), grape seed oil (0.51 mg CAE/100g), hemp oil (2.45 mg CAE/100g), flax oil (1.14 mg CAE/100g), rice bran oil (1.44 mg CAE/100g) [21]. 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 [22].

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) extracts 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) [20, 23].

The seed oils of *L. siceraria* cultivars exhibited significant DPPH radical scavenging activity *in vitro*. The highest antioxidant activity was displayed by the extract obtained from LHD (57.8%), and decreased in the order: SHB (56.6%), CMC (55.7%), TB (55.3%), BG (54.7%) and LHB (54.1%) at concentration of 120 µg/ml; comparable with BHA (74.2%) and ascorbic acid (71.7%). The standards demonstrated higher DPPH activity than the seed oils (Table 2) indicating relative lower EC_{50} values. 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%)[24, 25].

The highest metal chelating activity was detected in the oil obtained from LHB (70.2%), then TB (69.4%), SHB (69.1%), followed by LHD (67.8%), BG (67.1%) and CMC (65.5%) at concentration of 120 µg/ml and were compared with EDTA (83.6%). 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 [26].

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [27]. Phenolic compounds show reducing power and have ability to convert Fe^{3+} to Fe^{2+} [28]. The reducing properties are generally associated with the presence of reductones which has been shown to exert antioxidant action by breaking the free radical chain through donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation [29] [30]. 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.

CONCLUSION:

The seed oils *L. siceraria* cultivars contain substantial amount of phenolic compounds especially flavonoids. The various oils exhibits significant antioxidant activity – DPPH radical scavenging, metal chelating and ferric reducing when compared with standard compounds. The antioxidants in the seed oils also reveal 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. 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.

Conflict of Interest

The authors declare no conflict of interest.

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