



Original Paper

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Antioxidant and HPTLC profile of the leaf and fruit extracts of *Lagenaria siceraria*

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ABSTRACT

Lagenaria siceraria Standl. (Cucurbitaceae) is a widely used medicinal plant in Africa. It is used for both medicinal and nutritional purposes. The health promoting ability of the fruits and leaves of this species might be related to the antioxidant properties of its constituents. The antioxidant effect of the leaves and fruits of *L. siceraria* was evaluated by comparing the DPPH radical scavenging and reducing capacity of the methanol leaf extract with ethyl acetate and *n*-butanol extracts of fresh fruits. The comparison was further emphasized by HPTLC analysis where the chemical profiles of each extract was established. Results indicated that, at 0.1 mg/ml, ethyl acetate extract of the fresh fruits has higher DPPH radical scavenging effect, of up to 81.6% compared to 77.4% and 72% for methanol and *n*-butanol extracts respectively. The order of activity was; Bt fresh fruit < MeOH leaves < EA fresh fruits < Gallic acid. In the reducing capacity assay, the absorbencies of extracts increased with an increase in concentration. The *n*-butanol fresh fruit extract had higher reducing power than other screened extracts. At the maximum concentration of 0.1 mg/ml, the trend of activity was; Gallic acid < MeOH leaves < EA fresh fruits < Bt fresh fruits. The HPTLC analysis revealed significant differences in the chemical profiles of extracts. The disparity confirmed the variation in the antioxidant properties within the extracts of the fresh fruits and between leaves and fruits extract of *L. siceraria*.

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Keywords: Antioxidant activity, DPPH radical scavenging, HPTLC profile, *Lagenaria siceraria*.

INTRODUCTION

Lagenaria siceraria Standl. (Cucurbitaceae) is an important food and medicinal plant found throughout sub-Saharan Africa. It grows on fertile and moist soil as a climber with angular, ribbed and thick soft hairy stem of about 5 m long (Watt and Breyer-Brandwijk, 1962). In Tanzania, the fresh fruits of this species are eaten raw and also added as ingredients in various food

stuffs. Ethnomedically, fresh fruits and leaves are used in the treatment of diabetes. The herbal drug may be made from fresh fruits or the leaves alone but in case of chronic diabetes an infusion of both fruit and leaf extracts is used. Furthermore, leaves and fruits have laxative, analgesic and diuretic properties (Kakrani and Saluja, 1994; Al-khalil, 1995; Coe and Anderson, 1996). In Asia and some parts of Africa, fruits of *L.*

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siceraria are used in the treatment of cough, asthma, jaundice, kidney stones, colds, measles, skin rashes, diabetes and lipomatosis (Jain and Sharma, 1967; Han et al., 1984; Okoli, 1984; Al-khalil, 1995).

Several secondary metabolites have been isolated from the fruits of *L. siceraria*, including steroids such as campsterol, fucosterol and triterpenes cucurbitacin B, D and G while leaves yielded cucurbitacin B and D (Watt and Breyer-Brandwijk, 1962; Guha and Sen, 1975; Shiwaikar and Sreenivasan, 1996). There is, however, limited information on the pharmacological properties of the fruits and leaves of *L. siceraria*. Some documented biological activities of the fruits include antimicrobial, cytotoxicity, anticancer and antihepatotoxicity properties (Answar et al., 1984; Desta, 1993; Furukawa et al., 1995; Shiwaikar and Sreenivasan, 1996). Despite the fact that fruits and leaves of *L. siceraria* are used as food and for medicinal purposes, there is no information on their antioxidant properties, yet, it has been suggested that the etiology of complications of some physiological ailments such as diabetes involves oxidative stress, perhaps as a result of hypoglycemia (Hunt et al., 1990; Govindarayan et al., 2005). This paper, therefore, presents the antioxidant properties and HPTLC chemical profile of the extracts of leaves and fresh fruits of *L. siceraria*. This aims at consolidating further the medicinal potential of leaves and fruits of this plant species by comparing the activities within roots extracts and between leaves and roots extracts.

MATERIALS AND METHODS

Chemicals

Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Potassium ferricyanide [$K_3Fe(CN)_6$], Trichloroacetic acid (Cl_3CCO_2H), Ferric chloride ($FeCl_3$) were purchased from Sigma Aldrich (South Africa). Pre-coated silica gel glass plate 60F-254 (20 cm \times 10 cm with 250 μ m thickness) was purchased from E. Merck, Darmstadt,

Germany. All solvents were supplied by Unilab Kenya Limited, Nairobi.

Collection of plant materials

The leaves and fruits of *L. siceraria* were collected in May 2008 from a cultivated garden in Geita, Mwanza Tanzania. The plant was authenticated by Mr Haji O. Suleimani, a Taxonomist in the Department of Botany, University of Dar es Salaam and the voucher specimen, HOS 128, was deposited in the herbarium of the Institute of Traditional Medicine.

Preparation and extraction of plant materials

The leaves of *L. siceraria* were air dried at room temperature before grinding using an electric miller. 500 g of fresh fruits were crushed into fine pulp using a blender (Waring blender, USA). The macerates of leaves and fruits were shaken in 100% MeOH for 18 hours, then filtered and extracts concentrated under *vacuo* at 50 °C using a rotary evaporator. Since the fruits were fresh and succulent, the extracts did not dry completely. Therefore, the remaining aqueous solution was partitioned with ethyl acetate followed by *n*-butanol to give 5.8 g and 15.7 g of ethyl acetate (EA fresh fruit) and the *n*-butanol (Bt fresh fruits) extracts respectively. The leaves afforded 9.3 g of methanol extract. The extracts were kept in the refrigerator until further use.

Determination of DPPH radical scavenging activity

The method described by Liyana-Pathirana and Shahidi (2005) was used to assess the DPPH radical scavenging activity of leaf and fruit extracts of *L. siceraria*. An amount of 0.5 ml of 0.12 mM DPPH solution in methanol was separately mixed with 2 ml of 0.01, 0.025, 0.05 and 0.075 mg/ml of the extracts in methanol and vortexed thoroughly. The absorbance of the mixture at ambient temperature was recorded for 60 minutes at 10 minutes intervals. Gallic acid (GA) was used as a reference antioxidant compound. The

absorbance of the remaining DPPH radicals was read at 519 nm using a Jenway 6505 UV/Vis spectrophotometer (Essex, UK). The analysis of each assay solution was replicated thrice. The scavenging of DPPH radical was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})] \times 100$$

Where A_{control} is the absorbance of DPPH radical in methanol, A_{sample} is the absorbance of DPPH radical + sample extract/standard.

Reducing capacity

Adopting the method of Oyaizu (1986), 0.01, 0.025, 0.05 and 0.075 mg/ml of the leaf and fruit extracts were separately mixed with 2.5 ml of 0.02 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$]. The mixture was then incubated at 50 °C for 20 min. Aliquots (2.5 ml) of 10% trichloroacetic acid were added to the mixture followed by centrifugation at 1000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl_3 , and the absorbance was measured at 700 nm in a Jenway 6505 UV/Vis spectrophotometer. Gallic acid was used as a standard antioxidant compound. The analysis of each assay solution was done in triplicate.

High performance thin layer chromatography (HPTLC) analysis of the extracts

The samples were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60F-254 (20 cm × 10 cm with 250 μm thickness; E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed using methanol and activated at 60 °C for 5 min prior to chromatography. A constant application rate of 0.1 $\mu\text{l/s}$ was employed and space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm/s scanning speed was employed. The monochromatic bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase

consisted of dichloromethane-methanol (10:1, v/v) for EA and Bt fresh fruit extracts while dichloromethane-methanol (9:2, v/v) was used for leaves methanol extract. 15 ml of mobile phase was used in each chromatographic development of plates. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated by the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C ± 2). The length of chromatogram run was 8 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 250 nm and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Data analysis

All determinations were conducted in triplicate and results presented as mean ± standard deviation (SD). Statistical analysis was performed using Microsoft Excel 2003.

RESULTS AND DISCUSSION

DPPH radical scavenging

All samples exhibited high DPPH radical scavenging effect at all tested concentrations (Figure 1), with the ethyl acetate extract from fresh fruits (EA fresh fruits) being the most active of all the samples. At 0.05 mg/ml, the activities of MeOH leaves and EA fresh fruits extracts after 60 minutes were of the same order. The overall order of activity at this concentration being Bt fresh fruits < MeOH leaves ≤ EA fresh fruits < gallic acid (Figure 1). A slight change of activity particularly for MeOH leaves and EA fresh fruits extracts was observed at the concentration of 0.1 mg/ml. At this level, the latter exhibited higher DPPH radical scavenging activity than the former.

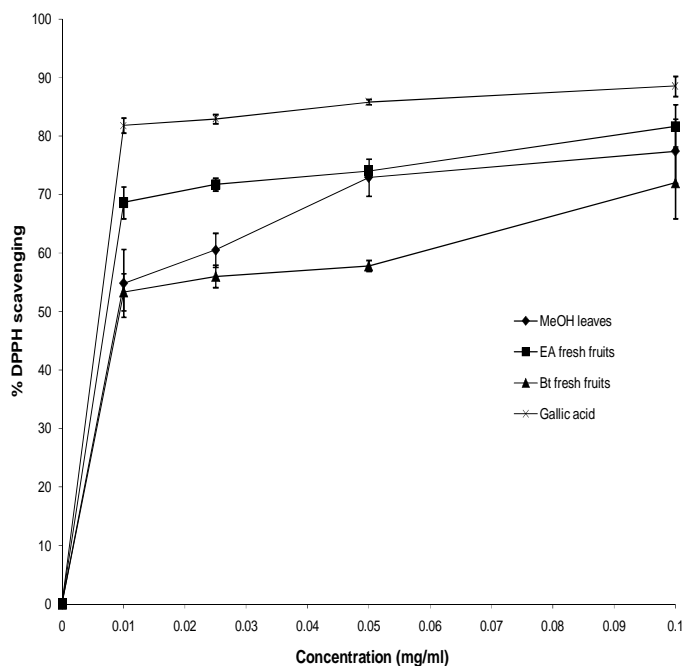


Figure 1: The DPPH radical scavenging activity methanol leaf (MeOH leaves) and ethyl acetate (EA) and n-butanol (Bt) fresh fruit extracts of *Lagenaria siceraria* compared with gallic acid after 60 min of reaction. Each value is expressed as mean \pm SD ($n = 3$).

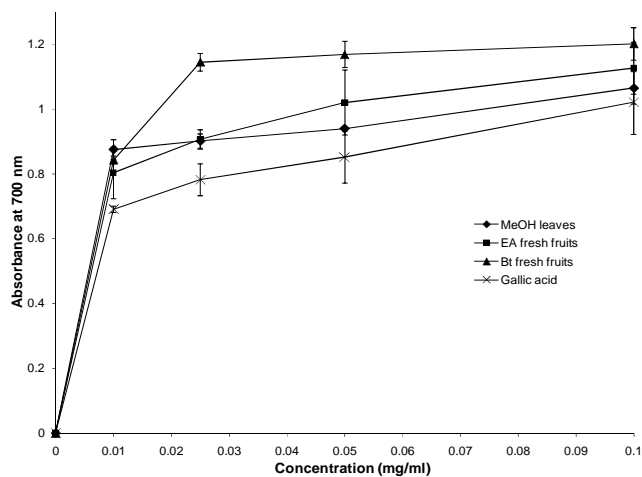


Figure 2: Reducing capacity of different amounts of methanol leaf extract (MeOH leaves) and ethyl acetate (EA) and n-butanol (Bt) fresh fruits extracts of *Lagenaria siceraria* compared with gallic acid (a standard antioxidant compound) using spectrophotometric detection of the $Fe^{3+} \rightarrow Fe^{2+}$ transformation. Each value is expressed as mean \pm SD ($n = 3$).

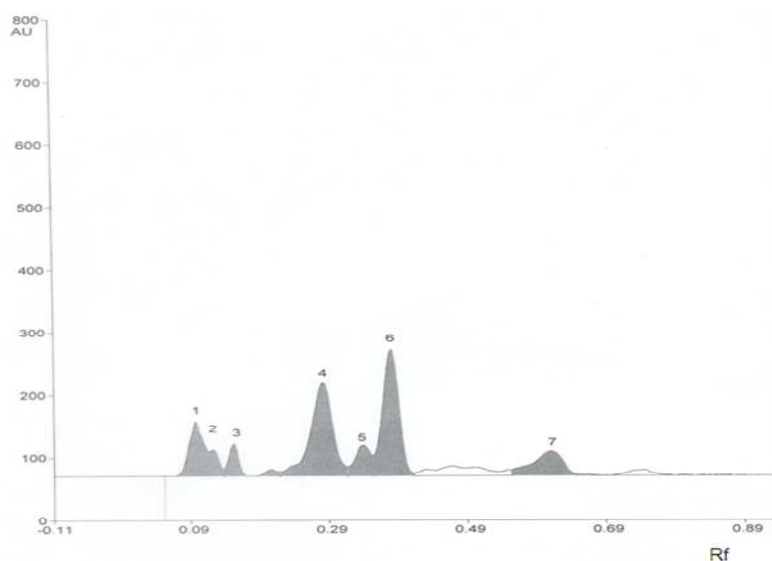


Figure 3: HPTLC profile of ethyl acetate extract of fresh fruits (EA fresh fruits) of *Lagenaria siceraria* as developed in dichloromethane/methanol (10:1, v/v) mobile phase. The Rf values and peak areas are given below.

Peak No	Rf value	% area
1	0.1	2.56
2	0.16	0.77
3	0.21	1.1
4	0.29	6.78
5	0.35	4.14
6	0.39	6.83
7	0.64	5.51

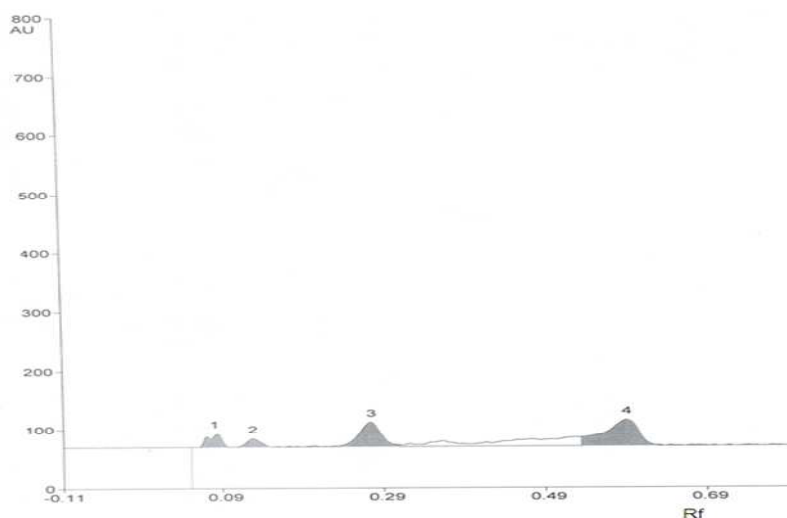


Figure 4: HPTLC profile of *n*-butanol extract (Bt fresh fruits) of fresh fruits of *Lagenaria siceraria* as developed in dichloromethane/methanol (9:2, v/v) mobile phase. The Rf values and peak areas are given below.

Peak No	Rf value	% area
1	0.09	5.7
2	0.13	2.93
3	0.28	9.19
4	0.6	9.37

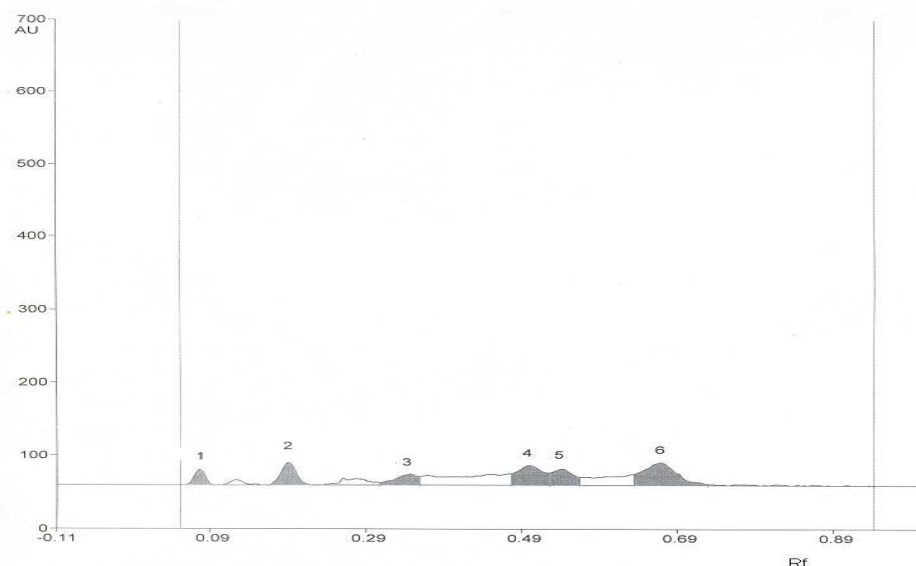


Figure 5: HPTLC profile of methanol extract of the leaves (MeOH leaves) of *Lagenaria siceraria* as developed in dichloromethane/methanol (10:1, v/v) mobile phase. The Rf values and peak areas are given below.

Peak No	Rf value	% area
1	0.08	3.56
2	0.19	4.91
3	0.35	2.37
4	0.49	5.1
5	0.53	4.19
6	0.67	5.34

The order of activity was; Bt fresh fruit < MeOH leaves < EA fresh fruits < Gallic acid (Fig. 1). At all concentrations, Gallic acid a standard antioxidant natural product exhibited higher DPPH radical scavenging activity than the tested plant extracts. When comparing the activities of the leaves and fruits of *L. siceraria*, the fruit extracts had more DPPH radical scavenging activity than leaves extract. These observations corroborate the previous

report which indicated that fruits have high antioxidant activity, and that the antidiabetic efficacy of the fruits may be attributed to its radical scavenging activity. Additional benefits of using *L. siceraria* for medicinal and nutritional purposes, is that its fruits and leaves have been found to be non-toxic in animal models, even at higher concentrations (Deshpande et al., 2008; Jadhar et al., 2010). This further provides the basis for continuous

use of the fruits of this plant species in various culinary preparations.

Reducing capacity

The antioxidant activity of the leaves and fresh fruit extracts of *L. siceraria* was further manifested through their reducing capacity as shown in Figure 2. In this assay, the $Fe^{3+} \rightarrow Fe^{2+}$ transformation was established as reducing capacity. The Bt fresh fruit extract had higher reducing capacity than other samples at all concentrations. However the activities of MeOH leaves and EA fresh fruit extracts at 0.025 mg/ml were of the same order. The trend of activity at this concentration was; gallic acid < MeOH leaves \leq EA fresh fruit < Bt fresh fruits (Fig. 2). At the highest concentration of 0.1 mg/ml, the order of activity remained the same with the exception of EA fresh fruit extract which had higher reducing capacity than the MeOH leaves extract. At this concentration, the order of activity was; gallic acid < MeOH leaves < EA fresh fruits < Bt fresh fruits (Fig. 3). In this assay, the fresh fruit extracts exhibited higher reducing capacity than the leaves extract. These results confirms further that the fruits extracts of *L. siceraria* have higher antioxidant activity, which may be contributing to its ethnomedical efficacy.

HPTLC profile of the extracts

The leaves and fruit extracts of *L. siceraria* were subjected to HPTLC analysis. This was done with the aim of comparing the chemical profiles of the leaves and fruits of this species in relation to their antioxidant activities. The results show that chemical profiles of the two parts of this plant species are different. However, a slight similarity was observed in the profile of EA fresh fruits chromatogram and that of MeOH leaves, in which they both have a compound with an RF value of 0.35 (Fig. 3 & 5). Their corresponding peaks are peak 5 for EA fresh fruit and 3 for MeOH leaves spectra. In terms of the number of compounds, fresh fruits have more compounds in abundance than leaves. The EA fresh fruit spectra showed 7

chromatograms (peaks) whereas the MeOH leaves had only 6 peaks. Conversely, the Bt fresh fruits extract had only 4 peaks (Fig. 4), which is less than the previous two extracts. The variation in the number of compounds and the chemical profiles of extracts could be responsible for the differences in their antioxidant activities.

Conclusion

The results of this study have confirmed the antioxidant properties of the leaves and fresh fruits of *L. siceraria*. The significant differences in DPPH radical scavenging effect, and reducing capacity of the extracts were further confirmed by their respective HPTLC chemical profiles. Therefore, leaves and fruits of *L. siceraria* possess antioxidant activity, a property which adds value to the medicinal and nutritional potential of this species. It can therefore be suggested that, consumption of fruits or leaves of this species may reduce a risk of developing certain physiological diseases by arresting excessive free radical species in the body.

ACKNOWLEDGEMENTS

Authors thank the Pacific Way Foundation (PWF, Vancouver) for financial support. Mr. Danstan Shewiyo and Makaranga of Tanzania Food and Drugs Authority are appreciated for their technical support during HPTLC analysis of samples.

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