Original Article

Thai Journal of Pharmaceutical Sciences

Antioxidant potential of wild *Lagenaria siceraria* (Molina) Standl.

Suraj B. Patel, Usmangani A. Attar, S. G. Ghane

Department of Botany, Shivaji University Kolhapur, Kolhapur, Maharashtra, India

Corresponding Author:
S. G. Ghane, Department of Botany, Shivaji University Kolhapur, Kolhapur, Maharashtra, India.
E-mail: sgg.botany@unishivaji.ac.in

Received: Dec 27, 2017
Accepted: Apr 02, 2018
Published: May 17, 2018

Keywords:
2, 2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid, antioxidant, *Lagenaria siceraria*, metal chelating, phenolic compounds, phosphomolybdenum

ABSTRACT

Objective: The aim of the present study was to evaluate the antioxidant potential of leaf and fruit of wild bottle gourd. **Methods:** Successive extraction of dried material was done by Soxhlet apparatus, and total phenolics, total flavonoids, and total tannins were determined. The antioxidant assays were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, metal chelating activity, ferric reducing antioxidant power (FRAP), and phosphomolybdenum assay. **Results:** The present study revealed highest phenolics (195.15 ± 11.64 tannic acid equivalent [TAE]/g extract) and flavonoids (76.62 ± 0.34 catechin equivalent [CE]/g extract) in leaves extracted with ethanol. However, tannins were recorded higher in acetone leaf extract (225.98 ± 10.56 CE/g extract). In fruit, maximum phenols (111.18 ± 1.21 TAE/g extract) and tannins (47.14 ± 2.23 CE/g extract) recorded in acetone and aqueous extracts, respectively. FRAP activity was maximum in ethanol extract of the leaf (395.79 ± 2.00 mg Fe (II)/g extract) and fruit (196.00 ± 3.18 mg Fe (II)/g extract). Among all the extracts, acetone extract of fruit displayed the strongest DPPH activity with IC_{50} values 711 ± 3.63 μg/mL. ABTS radical scavenging was highest in ethanol (379.81 ± 9.21 mg trolox equivalent [TE]/g extract) and methanol (274.64 ± 2.8 mg TE/g extract) extract of leaf and fruit, respectively. On the other hand, aqueous extract exhibited the highest metal chelating and phosphomolybdenum activity. Overall, ethanol and acetone were most suitable for the extraction of phytochemicals from the leaf. The antioxidant activities of leaf and fruit recorded significantly higher in the ethanol and methanol. **Conclusion:** The work demonstrated that *Lagenaria siceraria* showed good antioxidant activity and may be the source of natural free radical scavenger.

INTRODUCTION

Plants are the rich source of natural antioxidants which attract the food, pharmaceutical, and cosmetic industries for novel source of antioxidants from plants. The interest is increasing day by day due to the connection of reactive oxygen species (ROS) to several oxidative stress-related disorders such as arthritis, ischemia, atherosclerosis, reperfusion injury of many tissues, cardiovascular, neurodegenerative disorders, cancer, and AIDS.[1] ROS is formed in the body as part of normal cell metabolism, but an excess of ROS causes damage of biological molecules such as lipids, proteins, and nucleic acids and leads to oxidative stress.[2,3] Antioxidants are only compounds that inhibit or delay the oxidation process by blocking the initiation of oxidizing chain reactions and inhibit the formation of ROS. Nowadays, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and tert-butylhydroquinone are widely used in the industry, but they are toxic and carcinogenic.[4,5] Therefore, development and utilization of more effective antioxidants which are cheap as well as natural in origin are an urgent need. These natural antioxidants exhibited enormous biochemical activities, including inhibition of ROS and alteration of intracellular redox potential.[6] Among the natural antioxidant, phenolics are non-toxic and biodegradable products and received much attention due to the best alternative to currently available synthetic antioxidant.[7,8]

Family Cucurbitaceae contains about 122 genera and 940 species. In India, it is represented by 94 species from 31 genera.[9] Conventionally, many plants of this family are used to cure diseases. Most of the members are found in wild and may contain an enormous source of bioactive compounds. Exploration of nutraceutical properties of such wild resources assists us to find novel source bioactive metabolites and functional foods. As well as such potential and unique wild resources help us to alleviate malnutrition, hunger, and illness within society. *Lagenaria siceraria* is the most popular vegetable commonly called as bottle gourd or Lauki. Due to its curative properties, it has been utilized for the treatment of various disease, including jaundice, diabetes,

ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases.[10] The plant also possesses diuretic, laxative, cardioprotective, hepatoprotective, antioxidant activity, hypolipidemic, central nervous system stimulant, anthelmintic, antihypertensive, immunosuppressive analgesic, and adaptogenic properties.[11] Leaves, fruits, and seeds are edible and also used in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. Decoction obtained from *L. siceraria* and *Rivina humilis* leaves found effective in stomach problems during pregnancy. Along with garlic, decoction of bottle gourd leaves taken to relief gas pains in the heart area. Salt or coconut oil and its leaves are also used as poultices to treat skin irritation and tumor.[12,13] Leaf extract of *L. siceraria* exhibited free radical scavenging activity against various ROS.[14] The fruits are also good sources of dietary and phytochemical constituent. However, However, fruits are rich source of choline which is a precursor of acetylcholine; a neuro-transmitter and hence used in various neurological disorders.[11] Fruits are widely used in ayurveda, and it is traditionally used as general tonic, diuretic, aphrodisiac, and antidote for scorpion strings poisons, cardioprotective, alternative purgative and showed cooling effects. In addition, it cures fever, pain, and ulcers and used in the treatment of asthma, pectoral cough, and other bronchial disorders. Fruit contains various triterpenoids, cucurbitacins B, D, G, H, and 22-deoxy cucurbitacin, which are well known for their diverse biological activities. HPLC analysis shows a presence of flavone-C glycosides.[15] A ribosome inactivating protein, Lagenin, was isolated from seeds of *L. siceraria* having immune protective, antitumor, anti-HIV, and antiproliferative properties.[16] Instead of all such valuable properties, wild *L. siceraria* is explored very less for phytochemicals and antioxidant activities. In view of its medicinal value, the current study was designed to explore the antioxidant potential of *L. siceraria*.

MATERIALS AND METHODS

Plant Material and Extraction Procedures

L. siceraria leaves and fruits were collected from Amboli, Sindhudurg, Maharashtra, India, during January 2016. The location lies between N15°57'52.97" latitude and E74°00'12.85" longitude. The plant material was identified and a specimen was deposited at Herbarium of Department of Botany, Shivaji University, Kolhapur, Maharashtra, India (Voucher specimen No. UAA03). Plant material was washed under running tap water and dried in shade. The dried leaves and fruits were ground in electric grinder. Further, powdered samples (3 g each) were extracted with four different solvents such as acetone, ethanol, methanol, and water using Soxhlet extraction method. Finally, all solvent extracts were evaporated to dry at room temperature, dissolved in known volume of respective solvent, and used for antioxidant assays.

Total Phenolic Content (TPC)

TPC was determined according to the Folin–Ciocalteu method.[17] Samples (100 μl) were added to 1.0 ml of Folin–Ciocalteu's reagent (1:10 diluted in water) and 0.8 ml of sodium carbonate (7.5%). The tubes were mixed and allowed to stand for 60 min at room temperature. Absorption at 765 nm was measured using UV-visible spectrophotometer (Shimadzu UV-1800, Japan). Tannic acid was used as a standard for plotting calibration curve (20–200 μg/ml). TPC was expressed as mg tannic acid equivalent (TAE)/g extract.

Total Tannin Content (TTC)

Tannins were determined by the method given by Bhat *et al.*[18] with slight modifications. In brief, 200 μl plant extract (1 mg/ml) or catechin standard solution (50–300 μg/ml) added to 1 ml reagent (4% vanillin and 8% concentrated HCl in 1:1 concentration in methanol) and then incubated at room temperature for 20 min. Finally, absorbance was measured at 500 nm. TTC was expressed as mg catechin equivalent (CE)/g extract.

Total Flavonoid Content (TFC)

TFC was determined using the method of Sakanaka *et al.*[19] Plant extracts 200 μl (1 mg/ml) were treated with 75 μl of $NaNO_2$ solution and 1.25 ml of distilled water and then incubated for 6 min. It was followed by the sequential addition of 150 μl of 10% $AlCl_3$ solution and 500 μl of 1 M NaOH. The mixture was diluted to 2.5 ml with distilled water and absorbance was measured immediately at wavelength 510 nm. Catechin (50–300 μg/ml) was used to plot calibration curve. TFC was expressed as mg of catechin equivalent (CE)/g of plant extract.

Antioxidant Assays

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH free radical scavenging activity of acetone, methanolic, ethanol, and aqueous (water) extracts was determined according to the method reported by Brand-Williams[20] with slight modifications. The stock solution of the radical, prepared by dissolving 25 mg DPPH in 1000 ml methanol, was kept in a refrigerator for further use. Samples were also diluted with respective solvent (50–400 μg/ml) for the analysis. In a test tube, 1 ml DPPH working solution was mixed with 500 μl plant extract (1 mg/ml). The absorbance was measured at 515 nm for 30 min. The control contained 500 μl methanol in place of the plant sample. Absorbance was measured using methanol as blank. IC_{50} values of the extract, i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% were calculated.

ABTS+ radical scavenging activity

The free radical scavenging capacity was also studied using the 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay,[21] which is based on the reduction of ABTS+• radicals by antioxidants of the plant extracts tested. ABTS radical cation (ABTS+•) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate (1:1 ratio) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the assay, the ABTS+• solution was diluted in ethanol (1:89 ratio) to obtain an absorbance 0.7 (±0.02) at 734 nm. Samples were prepared in different concentrations (50–400 μg/ml) as well as standard Trolox having concentrations (20 to 120 μg/ml). In

test tube, 1 ml ABTS solution and 100 μl of extract or standard were used. The reaction mixture was incubated at 30°C and absorbance taken at 730 nm exactly after 30 min using ethanol as blank. All solutions were used on the day of preparation, and all determinations were carried out in triplicate. The results were expressed as mg Trolox equivalent (TE)/g extract.

Metal chelating activity

The chelation of ferrous ions by extracts was estimated by the method of Dinis *et al.*[22] with slight modifications. Briefly, 50 μl of 2 mM $FeCl_2$ was added to 200 μl extract or standard EDTA (20–120 μg/ml). The reaction was initiated by the addition of 100 μl of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. Na_2EDTA was used as positive control. The results are expressed as mg EDTA equivalent (EE)/g extract.

Ferric reducing antioxidant property assay (FRAP)

FRAP assay was determined according to the method described by Benzie and Strain.[23] The assay was based on the reducing power of antioxidant compounds. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex ($Fe^{2+}TPTZ^{-1}$), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl and 20 μM $FeCl_3$ at a ratio of 10:1:1 (v/v/v). The reagent (300 μl) and sample solutions (200 μl) or standard $FeSO_4.7H_2O$ (20–120 mg/ml) were added, mixed thoroughly, and incubated at 37°C in a water bath. Absorbance was taken at 593 nm. All solutions were used on the day of preparation. All determinations were performed in triplicates and results expressed as in mg Fe (II) equivalent/g extract.

Phosphomolybdate assay

Total antioxidant capacity (TAC) was determined as the method proposed by Prieto *et al.*[1] with slight modifications. In brief, test tube with 300 μl plant extract (400 μg/ml) or standard ascorbic acid (20–120 μg/ml) was mixed with 1 ml phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tube was covered and incubated at 95°C for 90 min. The mixture was then allowed to reach room temperature when its absorbance was recorded at 765 nm. Blank was run using the same procedure but contain an equal volume of methanol in place of the plant sample. The antioxidant capacity was reported as mg of ascorbic acid equivalent (AAE)/g extract.

Statistical Analysis

All the analysis was done in triplicate, and values are represented as mean ± standard error. The data were subjected to one-way analysis of variance and significant differences between mean values were determined by Duncan's multiple range test ($P < 0.05$); correlation coefficient was determined between TPC, TFC, TTC, and antioxidant activities using SPSS (version 16.0).

RESULTS AND DISCUSSION

TPC

Phenolics are considered as widest secondary metabolites in the plant kingdom. Due to redox properties such as reducing agents, hydrogen donators, and singlet oxygen quenchers, phenolics serve as better antioxidants. In addition, they have a metal chelation potential.[24] The amount of total phenolics varied with the plant part and solvent used in the present study. Total phenolics were ranged from 135 to 163 mg TAE/g extract. The highest phenolic content was found in leaf ethanolic extract (195.15 ± 11.64 mg TAE/g extract) [Table 1]. However, in fruit, it ranges from 72 to 111 mg TAE/g extract, but the highest phenolic content was noted in acetone extract (111.18 ± 1.21 mg TAE/g extract) [Table 1]. In contrast, very less amount of phenolics (11.9 ± 2.2 TAC unit) was recorded in fruits of *L. siceraria* by Agrawal *et al.*[25] Mohan *et al.*[26] reported slightly high content in the ethanolic extract of fruit (233.4 ± 3.0 mg TAE/g extract). Similarly, total phenolics from the seeds of *Citrullus colocynthis* (202.80 ± 7.50 mg GAE/100 g DW) and *L. siceraria* (141.35 ± 24.80 mg GAE/100 g DW) were reported by the method of Sabo *et al.*[27] We report higher TPC content in the studied material than *Diplocyclos palmatus*.[28]

TFC

Flavonoids are a class of antioxidant agents which act as a free radical scavengers. They have a preventative role in the

Table 1: Total phenolics, total tannins, and total flavonoids in leaf and fruit extracts of wild *L. siceraria*

Plant material	Solvents	Extract yield (%)	Phytochemicals		
			Total phenolics*	Total flavonoids**	Total tannins**
Leaf	Acetone	5.7	135.93±3.79^{c}	60.52±1.41^{b}	225.98±10.56^{a}
	Ethanol	3.5	195.15±11.64^{a}	76.62±0.34^{a}	88.21±2.50d
	Methanol	5.4	192.07±6.62^{a}	73.92±0.81^{a}	117.81±6.80^{c}
	Aqueous	14.36	163.38±3.06^{b}	19.80±1.34^{f}	202.60±4.70^{b}
Fruit	Acetone	6.7	111.18±1.21^{d}	50.63±1.59^{c}	30.14±2.61^{f}
	Ethanol	5.2	110.43±4.05^{d}	26.47±2.16^{e}	14.71±1.05^{g}
	Methanol	7.5	81.19±2.57^{e}	41.92±1.81^{d}	35.40±2.85^{f}
	Aqueous	20.46	72.61±1.98^{e}	14.47±1.30^{g}	47.14±2.23^{e}

*mg tannic acid equivalent/g extract, **mg catechin equivalent/g extract. Values were the means of three replicates±standard error. Mean value with different alphabets in column was showed statistically significant differences (P<0.05) according to Duncan's multiple range test. *L. siceraria: Lagenaria siceraria*

development of cancer and heart diseases.[29] In the present work, ethanol leaf extract showed highest amount of flavonoids (76.62 ± 0.34 mg CE/g extract), but in fruit, acetone extract revealed highest quantity (50.63 ± 1.59 mg CE/g extract) [Table 1]. In our results, higher flavonoid content recorded than ethanolic fruit extract of wild bottle gourd.[30] Acetone extract of bitter melon showed fewer flavonoids content than our recorded value.[31]

TTC

Tannins are the second most abundant polyphenols after lignins found in plants.[19] They are known for the treatment of ulcerated tissues and effective as antimicrobial and anticarcinogenic agents. It can be served as defensives against pathogens and herbivory.[32] Our results showed that leaf extracts were superior to that of the fruit. Acetone and water proved to be the best solvent for the extraction of tannins from leaf (225.98 ± 10.56 mg CE/g extract) and fruit (47.14 ± 2.23 mg CE/g extract), respectively [Table 1]. In contrast, TTC was lower in leaf and fruit extract of *D. palmatus* (22.07 ± 0.06 and 6.99 ± 0.10 mg CE/g extract, respectively).[28]

Antioxidant Assays

DPPH radical scavenging activity

DPPH is present as a stable free radical in different extracts that accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is generally used as a substrate to evaluate the antioxidant potential of the phytochemicals. The concentration of sample at which the inhibition percentage reaches 50% is its IC_{50} value. IC_{50} values are negatively correlated to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC_{50} value represents the higher antioxidant activity of the tested sample.[2] Among the all tested solvents, the leaf showed higher radical scavenging potential than the fruits [Figure 1]. Both leaf and fruit of *L. siceraria* showed maximum scavenging activity in methanol extract (IC_{50} 251 μg/ml and 711 μg/ml). In contrast, methanolic seed extract showed quite higher IC_{50} in *L. siceraria*.[33] In the present study, all the extracts showed comparable activity against Trolox (IC_{50}–100 μg/ml) which is lined with Chanda *et al.*[34]

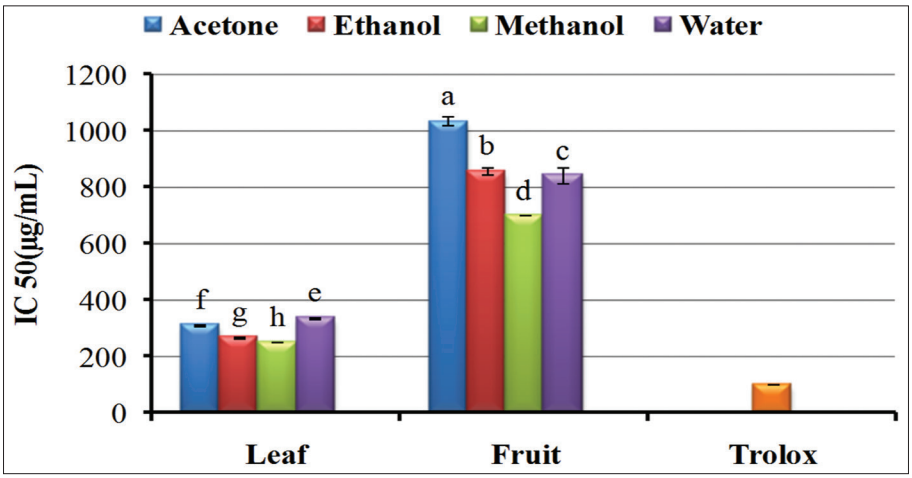

Figure 1: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity of leaf and fruit extract of *Lagenaria siceraria*. Values are mean of three replicates ± standard error. Bars having different alphabets showed statistically significant differences ($P < 0.05$) according to Duncan's multiple range test

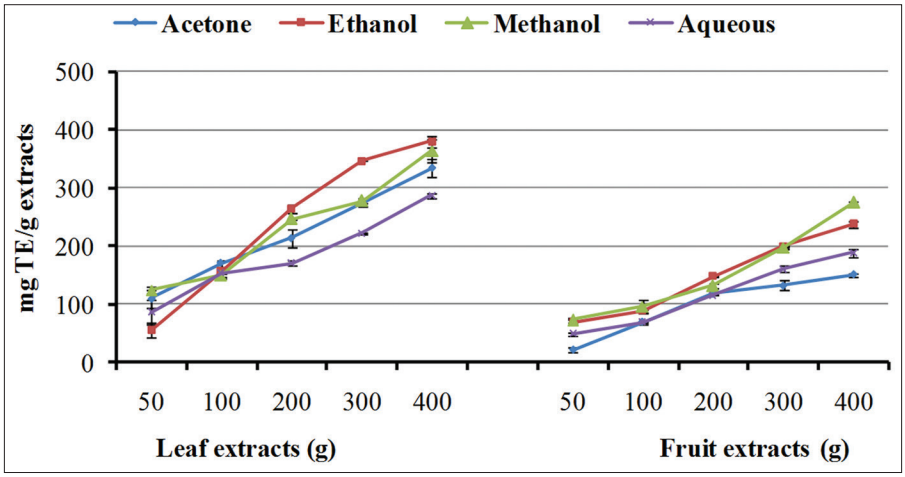

Figure 2: 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid radical scavenging activity of leaf and fruit extract of Lagenaria siceraria. Values are mean of three replicates ± standard error

Table 2: Correlation between antioxidant activity and TPC, TTC, and TFC of *L. siceraria*

Studied parameters	DPPH	Metal chelating	FRAP	Phosphomolybdate	ABTS
TPC	−0.797*	−0.014	0.689	−0.768*	0.780*
TFC	−0.512	−0.659	0.920**	−0.483	0.664
TTC	−0.792*	0.306	0.295	−0.653	0.574

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). TPC: Total phenolics content, TTC: Total tannins content, TFC: Total flavonoids content, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant property assay, *L. siceraria: Lagenaria siceraria*

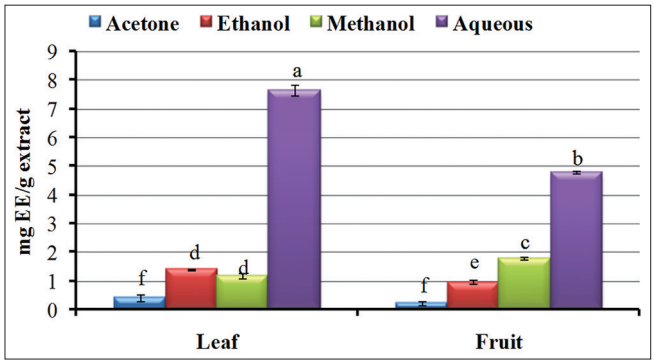

Figure 3: Metal chelating activity of leaf and fruit extract of *Lagenaria siceraria*. Values are means of three replicates ± standard error. Bars having different alphabets showed statistically significant differences (P < 0.05) according to Duncan's multiple range test

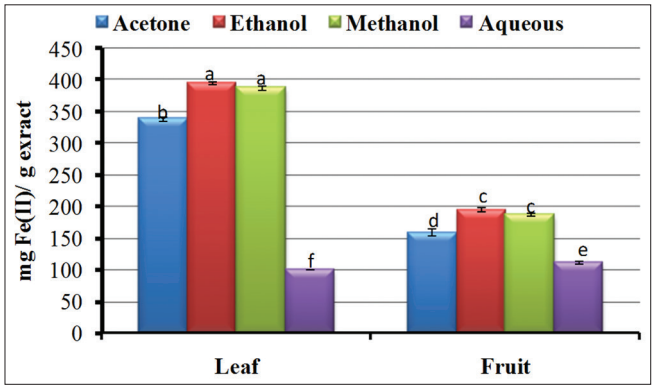

Figure 4: Ferric reducing antioxidant power assay of leaf and fruit extract of Lagenaria siceraria. Values are means of three replicates ± standard error. Bars having different alphabets showed statistically significant differences (P < 0.05) according to Duncan's multiple range test

ABTS radical scavenging activity

The ABTS cation radical (ABTS•+) is formed by the loss of an electron by the nitrogen atom of ABTS. In the presence of standard Trolox, the nitrogen atom quenches the hydrogen atom, yielding the solution decolorization. ABTS can be oxidized by potassium persulfate or manganese dioxide, giving rise to the ABTS cation radical (ABTS•+) whose absorbance diminution at 743 nm was monitored in the presence of Trolox, chosen as standard antioxidant. Increase in plant extract showed increased antioxidant activity in terms of the ABTS radical scavenging for both the leaf and fruit. In the present study, leaf extracted with ethanol (379.81 ± 9.21 mg TE/g

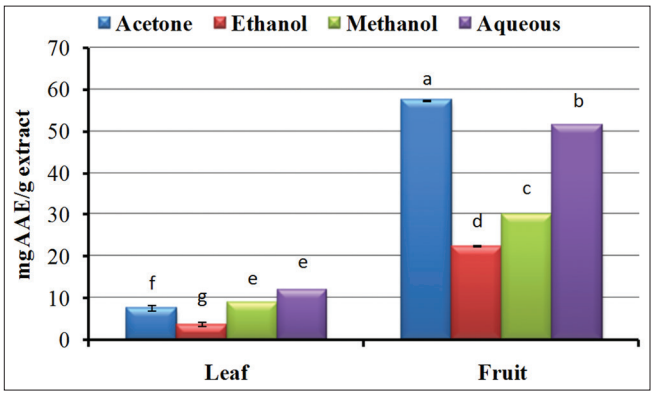

Figure 5: Phosphomolybdenum assay of leaf and fruit extract of *Lagenaria siceraria*. Values are means of three replicates ± standard error. Bars having different alphabets showed statistically significant differences (P < 0.05) according to Duncan's multiple range test

extract) and fruit extracted with methanol (274.64 ± 2.81 mg TE/g extract) exhibited highest radical scavenging capacity [Figure 2]. It was reported that fruit extract of *L. siceraria* possesses IC_{50} at 19 mg/ml which was comparatively lower than our investigation.[6]

Metal chelating activity

Chelation of transition metals is another mechanism of measurement of antioxidant activity, in which it prevents catalysis of hydroperoxide decomposition and Fenton-type reactions. These chelating agents stabilize transition metals and reduce their availability as catalysts and inhibit the first few free radicals and thus suppress lipid peroxidation in a living system. Standard antioxidants obstruct the formation of ferrozine complex which suggest their chelating activity and capture ferrous ion before formation of ferrozine complex. Ferrozine can quantitatively form complexes with Fe^{2+}. Antioxidant compounds act as chelating agents, disrupted complex formation, resulting in a decrease in red color of complex which allows an estimation of the metal chelating activity of the respective sample.[35] In the present investigation, aqueous extracts of leaf and fruit exhibited significant high antioxidant activity (7.67 ± 0.20 and 4.81 ± 0.05 mg EE/g extract respectively) compared to other extracts. Among all, leaf extract showed significantly higher activity than fruit extract [Figure 3]. These results are lined with Sulaiman *et al.*[36] who reported similar metal chelating activity in *L. siceraria*.

FRAP assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ)

complex and forms a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). Normally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom.[20] In the present investigation, the ethanol extract of both leaf and fruit showed higher FRAP activity than any other extract (395.79 ± 2.00 and 196.00 ± 3.18 mg Fe (II) equivalent/g extract) [Figure 4]. However, the lowest activity was found in water extracts (100.29 and 112.27 mg Fe (II) equivalent/g extract). Similar results were obtained in other Cucurbitaceae members.[6,37]

Phosphomolybdenum assay

The assay is based on ability of antioxidant compound to reduce Mo (VI) complex into green colored Mo (V) complex having maximum absorption at 765 nm. In the present results, fruits showed better phosphomolybdenum activity than leaf extract [Figure 5]. Aqueous extract of leaf and acetone extract of fruit resulted with the highest activity (11.96 ± 0.5 and 57.31 ± 0.38 mg AAE/g extract). Similarly, hot water fruit extract of *Cucumis dipsaceus* noted with slightly more activity (67.8 mg AA/g extract) than our calculated value.[8]

Correlation between TPC, TFC, TTC, and Antioxidant Capacity

The Pearson correlation coefficients between antioxidant activities and TPC, TTC, and TFC were depicted in Table 2. TPC was significantly correlated with DPPH, phosphomolybdate, and ABTS assays (r = 0.797, 0.768 and 0.780) but not with metal chelating and FRAP, whereas TFC exhibited a strong correlation with FRAP activity (r = 0.920). In addition, considerable correlation was noted in TTC and DPPH assay (r = 0.792). Overall, the result revealed the existence of a positive and negative correlation between tested phytochemicals and antioxidant assays. A similar trend was noted by Annegowda *et al.*[38]

CONCLUSION

The presence of compounds such as phenolics, flavonoids, and tannins in *Lagenaria siceraria* contributed to the several biological activities that are of pharmacological significance. In leaf, ethanol and acetone were the most suitable for the extraction of phenolics, flavonoids, and tannins. However, in fruit, acetone and water were the ideal solvent for isolation of phenolics, flavonoids, and tannins. In antioxidant assay of leaf and fruit, FRAP, DPPH, and ABTS assay responded significantly with ethanol and methanol. However, highest metal chelating and phosphomolybdenum activity were recorded in the aqueous extract of both leaf and fruit. Remarkable amount of phenolics, flavonoids, tannins, and antioxidant potential indicated that *L. siceraria* may be serving as significant and novel source of bioactive compounds. Therefore, it is suggested that this plant could be used as an additive in the food industry providing protection against oxidative damage. Further, it may serve as a novel source of natural antioxidants and replace synthetic antioxidants from industries and may act as a vital source of food and other pharmaceutically important compounds. These plants may be considered as rich resources of ingredients which can be used in drug development and synthesis.

ACKNOWLEDGMENTS

Authors are thankful to Science and Engineering Research Board (SERB), New Delhi for their financial support. The help of Department of Botany, Shivaji University, Kolhapur and UGC- FIST and UGC- SAP programs are duly acknowledged.

REFERENCES

1. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
2. Luo H, Ren M, Lim KM, Koh YJ, Wang LS, Hur JS. Antioxidative activity of lichen *Thamnolia vermicularis in vitro*. Mycobiology 2006;34:124-7.
3. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anti-Carcinogens in Nutrition. 1st ed. UK: Health and Disease. Royal Soc Chem; 1999. p. 178-87.
4. Grice HC. Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem Toxicol 1986;24:717-23.
5. Wichi HP. Enhanced tumour development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chem Toxicol 1988;26:1127-30.
6. Mayakrishnan V, Veluswamy S, Sundaram KS, Kannappan P, Abdullah N. Free radical scavenging potential of *Lagenaria siceraria* (Molina) Standl fruits extract. Asian Pac J Trop Med 2013;6:20-6.
7. Babu D, Gurumurthy P, Borra SK, Cherian KM. Antioxidant and free radical scavenging activity of triphala determined by using different *in vitro* models. J Med Plant Res 2013;7:2898-905.
8. Nivedhini V, Chandran R, Parimelazhagan T. Chemical composition and antioxidant activity of *Cucumis dipsaceus* Ehrenb. Ex Spach fruit. Int Food Res J 2014;21:1465-72.
9. Renner SS, Pandey AK. The *Cucurbitaceae* of India: Accepted names, synonyms, geographic distribution and information on images and DNA sequences. Phytokeys 2013;20:53-118.
10. Minocha S. An overview on *Lagenaria siceraria* (bottle gourd). J Biomed Pharm Res 2015;4:4-10.
11. Yash P, Gill NS, Amber P. An updated review on medicinal properties of *Lagenaria siceraria*. Inter J Univ Pharm Biol Sci 2014;3:362-76.
12. Prajapati RP, Kalariya M, Parmar SK, Sheth NR. Phytochemical and pharmacological review of *Lagenaria siceraria*. J Ayurveda Integr Med 2010;1:266-72.
13. Rahman AS. Bottle gourd (*Lagenaria siceraria*) a vegetable for good health. Nat Prod Rad 2003;2:249-56.
14. Sharma NK, Yadav P, Singh HK, Shrivastava AK. *In Vitro* antioxidant activity of *Lagenaria siceraria* leaves. Mal J Pharm Sci 2013;1:1-11.
15. Upaganlawar A, Balaraman R. Bottle gourd (*Lagenaria siceraria*) A vegetable food for human health-a comprehensive review. Pharmacol Online 2009;1:209-26.
16. Wang HX, Ng TB. Lagenin, a noble ribosome inactivating protein with ribonucleolytic activity from bottle gourd (*Lagenaria siceraria*) seeds. Life Sci 2000;67:2631-8.
17. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
18. Bhat TK, Singh B, Sharma OP. Microbial degradation of tannins-A current perspective. Biodegrad 1998;9:343-57.
19. Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem 2005;89:569-75.

20. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995;28:25-30.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
22. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5- aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994;315:161-9.
23. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
24. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Rad Res 1995;23:375-83.
25. Agrawal S, Katare C. Antioxidant activity, total phenolic compound and flavonoid content of vacuum dried extract of *L. siceraria*. Glob J Multidiscip Stud 2015;4:302-8.
26. Mohan R, Birari R, Karmase A, Jagtap S, Bhutani KK. Antioxidant activity of a new phenolic glycoside from *Lagenaria siceraria* Stand. fruits. Food Chem 2012;132:244-51.
27. Sabo H, Sadou H, Alma MM, Sidikou RS, Saadou M, Amoukou IR. Antioxidant activity and phenolics content of the seeds of eighteen verities of Edible *Cucurbitaceae* of Nigeria. J Food Resour Sci 2014;3:1-11.
28. Attar UA, Ghane SG. Phytochemicals, antioxidant activity and phenolic profiling of *Diplocyclos palmatus* (L.) C. Jeffery. Int J Pharm Pharm Sci 2017;9:101-6.
29. Kalpna R, Mital K, Sumitra C. Vegetable and fruit peels as a novel source of antioxidants. J Med Plants Res 2011;5:63-71.
30. Tyagi N, Madan H, Pathak S. Phytochemical screening and estimation of total phenolics and total flavonoid content of *Lagenaria siceraria*, *Praecitrullus fistulosus* (50:50) fruit and their mixture. Int J Pharm Res Sch 2014;3:882-90.
31. Tan SP, Parks SE, Stathopoulos CE, Roach PD. Extraction of flavonoids from Bitter Melon. Food Nutr Sci 2014;5:458-65.
32. Adeolu A, Temitayo A, Margaret S. Phytochemistry, anti-inflammatory and analgesic activities of the aqueous leaf extract of *Lagenaria breviflora* (*Cucurbitaceae*) in laboratory animals**.** Rev Biol Trop 2013;61**:**281-90**.**
33. Prasad MP. *In vitro* phytochemical analysis and antioxidant activity of seeds belonging to *Cucurbitaceae* family. Indian J Adv Plant Res 2014;1:13-8.
34. Chanda S, Moteriya P, Padalia H, Rathod T, Baravalia Y. Antioxidant and metal chelating activities of *Lagenaria siceraria* (Molina) Standl peel, pulp and aerial parts in relation to their total phenol and flavonoid content. J Pharm 2015;7**:**64-73**.**
35. Flora SJ. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxid Med Cell Longev 2009;2:191-206.
36. Sulaiman SF, Ooi KL, Supriatno. Antioxidant and α-glucosidase inhibitory activities of cucurbit fruit vegetables and identification of active and major constituents from phenolic-rich extracts of *Lagenaria siceraria* and *Sechium edule*. J Agric Food Chem 2013;61:10080-90.
37. Irshad M, Ahmad I, Mehdi SJ, Goel HC, Rizvi MM. Antioxidant capacity and phenolic content of the aqueous extract of commonly consumed cucurbits. Int J Food Prop 2013;17:179-86.
38. Annegowda HV, Bhat R, Tze LM, Karim AA, Mansor SM. The free radical scavenging and antioxidant activities of pod and seed extract of *Clitoria fairchildiana* (Howard)- an underutilized legume. J Food Sci Technol 2013;50:535-41.