

Cooking Effect on Anti-oxidative and Alpha-amylase Inhibitory Potential of Aqueous Extract of *Lagenaria siceraria* Fruit and its Nutritional Properties

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ABSTRACT

Objective: To evaluate effect of boiling for different durations on total phenolic content, total flavonoid content, antioxidant activity and alpha-amylase inhibitory activity of water extract of *Lagenaria siceraria* fruit, and to compare nutritional properties of its epicarp, mesocarp, seeds pulp and pedicle. **Methods:** The bioactivities of aqueous extract of fruit was evaluated after boiling for 10, 20, and 30 min. Moisture, ash, fiber, protein, carbohydrate and lipid contents and vitamin C of epicarp, mesocarp, seeds pulp and pedicle were determined using standard methods. Mineral elements (Fe, Cr, Cu, Ag, Mn, Zn, Ni, Co and Pb) were estimated by atomic absorption spectrophotometer. **Results:** DPPH (1,1-diphenyl-2-picrylhydrazyl) antioxidant and alpha-amylase inhibitory activities, and phenolic content drastically decreases with increase in duration of boiling. Flavonoid content and antioxidant capacity in phosphomolybdate assay exhibited a slight increase. Epicarp had higher percentage of protein, carbohydrate and vitamin C than other parts of the fruit, while pedicle had the highest content of fiber, zinc, silver and chromium. Epicarp had the highest content of Copper. Iron was in the range of 3.4-5.13, cobalt 0.14-0.31 and lead 0.5-1.8 mg/100 g. Amount of zinc in pedicle was highest among all the trace elements (6.5%) in all parts. **Conclusion:** The study revealed that boiling duration affects antioxidant and alpha-amylase inhibitory activities of the fruit of *L. siceraria*. Peel and pedicle of the fruit should not be discarded as they are rich in nutritional properties.

Key words: Anti-oxidative, Anti-diabetic, Cooking effect, *Lagenaria siceraria*, Nutritional.

INTRODUCTION

Fresh fruit of *Lagenaria siceraria* (family Cucurbitaceae) is a popular vegetable in many parts of world including Pakistan. It is quite renowned for its immense ethnomedicinal efficacy, and is considered to have cardioprotective, hepatoprotective, immunomodulatory, diuretic antihyperlipidemic, analgesic, and anti-inflammatory activities.¹⁻² The fruit is commonly prescribed for such ailments by traditional healers or hakeems. Fresh juice of the fruit, in various forms and formulations, is advised for the treatment of diabetes and cardiovascular disorders.³⁻⁴ The phytochemical research on the plant has shown the presence of glycosides of polyphenols, flavonoids and triterpenoids and their

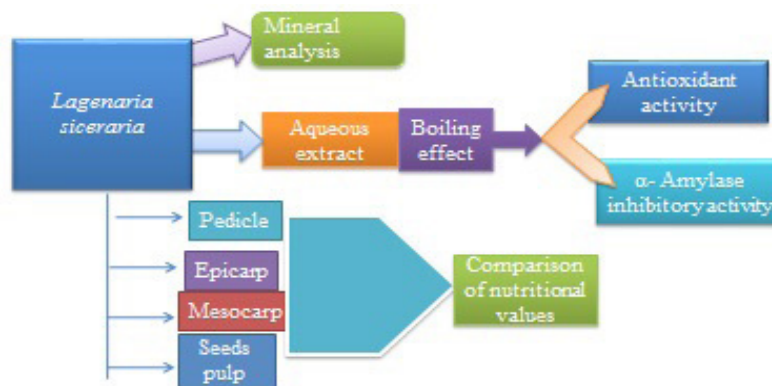
aglycones, different vitamins, sterols and beta-carotene.⁵⁻⁸

Antioxidants are required to combat free radicals which are highly reactive species and can damage cells that may lead to cancer and other disastrous health conditions.⁹ Fruits and vegetables are rich dietary sources of natural antioxidants.¹⁰ The natural products that have antioxidant potential include vitamins A, C and E, lycopene, beta-carotene, phenolics and flavonoids. *L. siceraria* fruit contains a number of these phytochemicals.⁶ Although, considerable work has been done on antioxidant activities of the fruit,¹¹ no study has yet been conducted, as far as we could explore the literature, to evaluate the effect of boiling on bioactivities of aqueous extract of the fruit. In the present work, therefore, we are reporting study of the effect of boiling for different durations on anti-oxidative and alpha-amylase inhibitory activities of fresh water extract of *L. siceraria* fruit. A comparative analysis of nutritional properties of different parts of the fruit and their mineral elements have also been conducted.

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Graphical Abstract

MATERIALS AND METHODS

Chemicals

All chemicals used in the study were of analytical grade. Sodium phosphate was purchased from BDH Chemicals, ammonium molybdate, sodium dihydrogen phosphate, disodium hydrogen phosphate, and gallic acid were purchased from Riedel-de-Haen. Ascorbic acid was bought from Fischer Chemicals. DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich, Rutin from Alfa-Aesar and Folin-Ciocalteu reagent from Merck. The absorbance of samples was measured on UV-Vis spectrophotometer (Labomed, Inc UVD-3200). Distillation of samples was conducted on Buchi R-210 rotary evaporator.

Collection and processing of plant material

Fresh young fruit of *L. siceraria* (round in shape) was collected from the farms of Pattoki, District Kasur, Punjab (Pakistan). The fruit was washed with distilled water and dried with a piece of cloth. It was further processed for different analyses according to the requirement of study.

For the analysis of boiling effect on bioactivities of the fruit of *L. siceraria*, the fruit was carefully peeled off and ground to a slurry. The slurry was used for the study of boiling effect on antioxidant and alpha-amylase inhibitory activities. Distilled water was added to a part of it and the content was boiled for 10 min. It was allowed to come to room temperature, and pressed to obtain its water extract. The solvent was evaporated to obtain dried extract. In the same manner, samples of the slurry were prepared after 20 min and 30 min boiling.

For the determination of nutritional properties, the fruit was carefully separated into four parts, namely, pedicles, epicarp, mesocarp, and seeds pulp. Each part was then

used to determine amounts of mineral elements in it and its nutritional properties.

Total phenolic content (TPC)

A reported method was used to determine total phenolic content in water extract of the fruit using Folin-Ciocalteu reagent.¹²⁻¹³ Each plant sample was prepared by dissolving the dried extract in methanol to obtain a solution with concentration 1 mg/mL. In a test tube, 40 μ L (1 mg/mL) extract was mixed with 3.16 mL distilled water and 200 μ L Folin-Ciocalteu reagent. To it, 600 μ L sodium carbonate solution (7%) was added after an interval of 8 min. The sample was incubated at 40°C for 30 min. The blank was prepared to contain 40 μ L of methanol in place of extract solution. Gallic acid was used as a standard and total phenolic content was expressed as μ g/mL of gallic acid equivalents (GAE). The absorbance was measured at 765 nm.

Total flavonoid content (TFC)

The total flavonoid content was determined by following a reported method.¹⁴ In a test tube, 300 μ L of sample (3 mg/mL) or standard solution was mixed with 3.4 mL of 30% aqueous methanol. To it, 150 μ L sodium nitrite (0.5 M) and 150 μ L aluminum chloride (0.3 M) were added. Finally, 1 mL of sodium hydroxide was added after 5 min interval. The mixture was mixed and absorbance of the clear solution was noted at 506 nm. Rutin was used as a standard, and total flavonoid content was expressed as μ g/mL of rutin equivalents (RE).

DPPH Scavenging Activity

The free radical scavenging activity of each extract was determined using a reported method.¹⁴⁻¹⁵ To prepare the stock solution of a water extract of the fruit, 100 mg of the dried extract was dissolved in methanol to obtain 10 mL final volume. Serial dilutions of the extract were made ranged from 1 mg/mL to 10 mg/mL. In a test tube covered

with aluminum foil, 3 mL of the DPPH radical solution [24 mg DPPH diluted with methanol to an absorbance of 0.98 (\pm 0.02) at 517 nm] was mixed with 100 μ L of the fruit extract. The absorbance was measured at 517 nm after 30 min incubation in dark. Control had 100 μ L methanol instead of fruit extract. The antioxidant ability of the sample was determined using the formula:

$$\% \text{ Antioxidant Activity} = 100 [(A_c - A_s) / A_s]$$

where A_s and A_c are absorbance of sample and control, respectively.

Total antioxidant capacity through phosphomolybdate assay

A reported method was used to conduct this assay.¹⁶ In a test tube, 3 mL phosphomolybdate reagent [made by mixing 100 mL each of sulfuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate solution (4 mM)] and 300 μ L of fruit extract (3 mg/10 mL) or ascorbic acid (standard) were added. The reaction was protected from light and incubated at 95°C for 90 min. A blank without sample was also run. The mixture was cooled and absorbance was measured at 765 nm. The antioxidant activity was reported in μ g/mL of ascorbic acid equivalents.

Evaluation of alpha-amylase inhibitory activity

Alpha-amylase inhibitory activity was determined by a reported method.¹⁷⁻¹⁸ To 100 mL sodium phosphate buffer (pH 6.9), 0.001 g alpha-amylase was mixed to make 0.5 unit/mL enzyme solution. Fresh starch solution (0.5% w/v) was prepared by boiling 0.125 g starch in 25 mL distilled water for 15 min. DNS (3,5-dinitrosalicylic acid) reagent (96 mM) was prepared by dissolving the acid in distilled water. The mixture containing 1 mL fruit extract (1 mg/mL) and 1 mL enzyme solution, in a test tube, was incubated for 30 min at 25°C. To it, 1 mL starch solution (0.5 %) was added. The mixture was again incubated for 3 min at 25°C, and 1 mL DNS was added. It was heated at 85°C for 15 min and, then, allowed to come to room temperature. Distilled water (9 mL) was added, and absorbance was recorded at 540 nm against a blank. The blank was prepared in the similar manner except that DNS was added after 30 min incubation instead of starch, and starch was added after 3 min incubation instead of DNS. The control contained 1 mL DMSO in place of fruit extract. Rest of the procedure was same as used to prepare sample. Percent alpha-amylase inhibitory activity of an extract was calculated using the formula given below:

$$\% \text{ Alpha-amylase inhibitory activity} = [1 - A_s / A_c] \times 100$$

Here, A_s and A_c are absorbance of sample and control, respectively.

Determination of nutritional properties

Proximate analysis of moisture, ash, protein, fats, fiber and carbohydrate contents in different parts of fruit was conducted according to the standard protocols.¹⁹ Vitamin C was estimated by titration method.²⁰

Evaluation of mineral elements

A reported method was used to determine amounts of various mineral elements in fruit of *L. siceraria* using atomic absorption spectrophotometer.²¹ For mineral elements estimation, a weighed amount of each part of the fruit was allowed to dry in an oven at 50°C. The dried material was crushed and ground into a powder. Each sample was taken in a crucible and heated in a furnace at 550°C for 24 h to convert into ash. The ash so obtained was dissolved in 10% aqua regia (1 mL). It was transferred into a 100-mL volumetric flask, and diluted with distilled water up to the mark. This solution was used for estimation of mineral elements.

Statistical analysis

All the measurements were made in triplicates (n=3), and the values were averaged with \pm SD using Excel 2010, Microsoft Corporation (USA).

RESULTS AND DISCUSSION

Fruits and vegetables are rich sources of natural antioxidants. The chemical substances responsible for anti-oxidative action include polyphenols and flavonoids. The heat provided through cooking has the potential to make structural changes in the phytochemicals, thereby, affecting their antioxidant activities. *L. siceraria* fruit is known for its multifarious therapeutic properties. It is valuable to know the extent to which boiling may bring changes in antioxidant activities of the fruit. Recently, Joshi *et al.* (2013)²² studied antioxidant activity of decoction and fresh fruit samples of this fruit. There was, however, a need to study the effect of duration of boiling on antioxidant properties. The present work aimed to fill up this knowledge gap.

Total phenolic and flavonoid contents

Total phenolic and flavonoid contents of the aqueous extract of *L. siceraria* fruit were determined using standard protocols and the findings are shown in Table 1. To analyse the cooking effect on these contents, they were determined after boiling the extract samples for 10, 20 and 30 min.

Table 1: Total phenolic (TPC) and total flavonoid (TFC) contents, total antioxidant capacity (TAC), and alpha-amylase inhibitory activity of *Lagenaria siceraria* fruit extract before boiling and after boiling for different durations

Extract	TFC (µg/mL of rutin equivalents)	TPC (µg/mL of gallic acid equivalents)	TAC as measured by phosphomolybdate assay (µg/mL of ascorbic acid equivalents)	Alpha-amylase inhibitory activity (%)*
10 min boiling	1.52	527.27	14.96	14.04
20 min boiling	1.52	445.00	15.76	10.44
30 min boiling	1.57	441.36	16.36	7.39

*Concentration of each extract in this study was 1 mg/mL.

Table 2: DPPH Antioxidant Activity of aqueous extract of fruit of *Lagenaria siceraria* after boiling for different durations of time

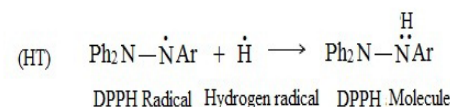
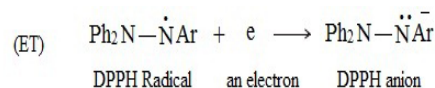
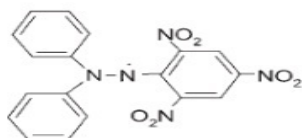
Concentration mg/mL	%Activity		
	10 min	20 min	30 min
1	0.00	2.45 ± 0.005	0.00
2	0.94 ± 0.005	2.82 ± 0.010	1.75 ± 0.005
3	2.63 ± 0.010	7.38 ± 0.005	3.39 ± 0.010
4	16.88 ± 0.010	5.97 ± 0.005	7.03 ± 0.010
5	21.10 ± 0.010	8.32 ± 0.006	8.73 ± 0.005
6	27.33 ± 0.010	9.60 ± 0.005	8.68 ± 0.006
7	29.54 ± 0.005	12.72 ± 0.010	7.54 ± 0.005
8	34.77 ± 0.010	13.60 ± 0.005	9.12 ± 0.005
9	36.32 ± 0.010	15.12 ± 0.030	11.84 ± 0.010
10	39.90 ± 0.060	16.06 ± 0.050	12.18 ± 0.005

The fruit of *L. siceraria* has significant amount of phenolics as well as flavonoids. The content of former, however, was much higher than that of latter. There is a clear decrease in total phenolic content with boiling. The total flavonoid content, on the other hand, remained almost unaffected by boiling. It therefore may be inferred that non-flavonoid phenolics are more sensitive to heat. The findings of the work are in agreement with the outcome of other studies.²³

Free radical scavenging activity

Aqueous extract of the fruit of *L. siceraria* was subjected to evaluation of free radical scavenging or antioxidant activity using DPPH radical method and the results are exhibited in Table 2. The activity of extract samples boiled for 10, 20 and 30 min were determined in order to find out effect of cooking on this activity of the fruit. DPPH is a stable free radical having an odd electron on the nitrogen bearing picryl moiety. The radical can accept an electron to become an anion. This is called electron transfer (ET) pathway. It can also accept a hydrogen radical, through a hydrogen transfer mechanism (HT), to form a neutral molecule.²⁴

DPPH radical



The purple color of DPPH radical (having intense absorption at 517 nm) changes into yellow and the change can be monitored spectrophotometrically. Phenolics, flavonoids and other compounds with antioxidant ability can readily cause this reduction in DPPH radical. As the Table 2 shows, boiling drastically lowered the activity. For instance, at the sample concentration of 7 mg/mL, the activity of the 10 min boiled, 20 min boiled and 30 min boiled extracts were 29.54 ± 0.005 , 12.72 ± 0.010 , and 7.54 ± 0.005 , respectively. The trend can be explained on the basis of decrease in total phenolic content with the increase in the duration of boiling (Table 1). The cooking for prolong duration, therefore, had an adverse effect on antioxidant activity of this vegetable. Studies on other vegetables showed similar results.²⁵ As a whole, the antioxidant activity was dose-dependent.

Total antioxidant capacity by phosphomolybdate assay

Total antioxidant capacity (TAC) of fruit samples was

Table 3: Percent Proximate Nutritional Properties of Different Parts of *Lagenaria siceraria* fruit

Nutritional parameters	Epicarp	Mesocarp	Seeds pulp	Pedicele
Moisture	87.84 ± 0.06	90.25 ± 0.06	94.66 ± 0.02	87.60 ± 0.05
Ash	1.03 ± 0.05	1.03 ± 0.05	1.03 ± 0.05	1.03 ± 0.05
Fiber	2.00 ± 0.06	4.70 ± 0.01	1.42 ± 0.01	7.14 ± 0.01
Protein	2.63 ± 0.01	1.25 ± 0.06	2.13 ± 0.01	2.18 ± 0.01
Lipid	1.00 ± 0.00	0.12 ± 0.01	0.08 ± 0.01	1.31 ± 0.01
Carbohydrate	5.505 ± 0.01	2.65 ± 0.01	0.69 ± 0.01	0.74 ± 0.01
Vitamin C	9.0 ± 0.11	6.0 ± 0.05	6.0 ± 0.06	5.0 ± 0.11

Table 4: Amounts of Mineral Elements in different Parts of *Lagenaria siceraria* fruit (mg/100 g of sample)

Fruit part	Fe	Cr	Cu	Ag	Mn	Zn	Ni	Co	Pb
Epicarp	4.154	0.438	3.978	0.803	0.314	1.584	0.152	0.307	1.800
Mesocarp	5.129	nd	0.057	0.682	0.318	1.629	nd	0.137	0.500
Endocarp	3.416	0.481	0.541	0.744	0.163	1.895	0.263	0.224	0.900
Pedicele	4.187	0.565	0.098	2.179	0.236	6.505	0.055	0.224	0.700

nd, below detection level.

determined by phosphomolybdate assay according to a reported method, and the results are displayed in Table 1. The samples showed good activities, which range from 14.76 mg/mL to 16.36 mg/mL of ascorbic acid equivalents. Phosphomolybdate assay involves reduction of molybdenum(VI) to green colored molybdenum(V) by abstracting an electron from an antioxidant. The change is monitored through a spectrophotometer. The total antioxidant capacity (TAC) as measured by phosphomolybdate assay exhibited a slight increase as the time for boiling increased (Table 1). This difference between DPPH assay and this one may be due to their mechanism of action. Presumably, DPPH radical assay involves HT (hydrogen transfer) mechanism while phosphomolybdate assay works through ET (electron transfer) pathway.²⁴ Possibly also, the boiling might have assisted extraction of electron transferring antioxidant phytochemicals from the fruit material.

Alpha-amylase inhibitory activity

Alpha-amylase inhibitory activities of extract of the fruit after boiling for 10, 20 and 30 min were determined and the results are shown in Table 1. The alpha-amylase inhibitory activity decreased with increase in the boiling duration. In our body, alpha-amylase converts starch into maltose, which ultimately changes into glucose. Type II diabetes is one of the biggest health concerns in the 21st century.²⁶ According to estimates of WHO (World Health Organization), diabetes will be the 7th leading cause of death in 2030 if the present trend goes unchecked.²⁷ The fruit of *L. siceraria* is well known for its anti-hyperglycemic activity. As Table 1 reveals, there is a definite decrease in this activity with the increase in the duration of boiling. The trend has a positive correlation with total phenolic content (Table 1).

Nutritional properties

Various nutritional parameters were determined including moisture, ash, fiber, protein, lipid and carbohydrate contents, and the results are displayed in Table 3. Vitamin C was also estimated and the results are shown in the same table. As the Table 3 shows, fruit of *L. siceraria* contained high content of moisture, which is around 87-94% in all parts, with mesocarp and seeds pulp have slightly higher values than epicarp and pedicle. The fruit has good amount of fiber, proteins, carbohydrates and lipids. Fiber content was highest in pedicle, while epicarp had the highest content of carbohydrates. All the parts had almost equal percentage of ash. Hanif *et al.* (2006) analyzed the whole fruit of *L. vulgaris* and found (in %) 94.5, 1.2, 0.2, 3.75, 0.7 and 0.5 moisture, protein, fats, carbohydrates, fiber, and ash, respectively.²⁸ These results are comparable to the present findings.

The epicarp, which constitute the exposed part of the fruit, had highest percentage of vitamin C (9.0 %), while mesocarp, seeds pulp (endocarp) and pedicle had 6.0, 6.0 and 5.0 %, respectively. The finding suggests that epicarp (or peel) should not be removed while cooking the vegetable in order to get maximum amount of vitamin C.

Mineral elements analysis

A number of mineral elements were determined in different parts of the fruit of *L. siceraria* using atomic absorption spectrophotometer and the results are shown in Table 4. The fruit had excellent amount of iron (Table 4). Epicarp and pedicle had almost same amount of this important mineral element (around 4.1 mg/100 g of dried sample), while mesocarp had the highest value (5.13 mg/100 g). Epicarp had considerably higher amount of copper (3.98

mg/100 g) than the other parts. The fruit also had good amount of zinc, and pedicle had much higher value (6.5 mg/100 g) than the remaining parts (1.58-1.90 mg/100 g). Notably, amount of zinc in pedicle was even higher than iron in any part of the fruit. The fruit was also found to contain good amount of chromium and silver, pedicle being the richest in both the elements. Notably, the fruit parts were found to contain lead as well, and epicarp had the highest amount (1.8 mg/100 g). Manganese, nickel and cobalt were also found in fruit parts. Mercury was not detected in any part of the fruit.

CONCLUSION

Total phenolic content, antioxidant activity in DPPH assay and alpha-amylase inhibitory activity of aqueous extract of young fruit of *L. siceraria* was affected by boiling duration. Total antioxidant capacity measured via phosphomolybdate assay also increased slightly. Based on the study, fresh juice is recommended for maximum therapeutic benefit. Pedicles being rich in fiber and zinc should also be utilized.

Highlights of the paper

- Effect of boiling duration was determined on the phenolic and flavonoid contents, antioxidant activity and alpha-amylase inhibitory activity of water extract of *Lagenaria siceraria* fruit.
- DPPH (1,1-diphenyl-2-picrylhydrazyl) antioxidant and alpha-amylase inhibitory activities, and phenolic content drastically decreased with increase in duration of boiling.
- Flavonoid content and antioxidant capacity in phosphomolybdate assay exhibited a slight increase.
- Epicarp had higher percentage of protein, carbohydrate and vitamin C than other parts of the fruit, while pedicle had the highest content of fiber, zinc, silver and chromium. Epicarp had the highest content of Copper.

About Authors



Dildar Ahmed: *PhD, is a professor of organic chemistry. He did his doctorate from HEJ Research Institute of Chemistry, University of Karachi, Pakistan, in 1992 under the supervision of Professor Atta-ur-Rahman. His teaching interests include organic chemistry, natural products and spectroscopy. The interests his research group include isolation and characterization of phytochemicals, anti-enzymatic, antioxidant and antimicrobial activities. He has published over 50 research papers.*



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Ramsha Saeed: *Has recently obtained her MPhil degree in chemistry from Forman Christian College where she conducted research under the supervision of Dr Dildar Ahmed and worked on the roots of *Carissa opaca*. She learned many techniques during her BS (Hons.) and MPhil studies including chromatography, solvent extraction and antimicrobial, antioxidant and anti-enzymatic activities. Her future aim is to do PhD in Organic Chemistry.*



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CONFLICT OF INTEREST

All authors have none to declare.

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ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazyl

DNS: (3,5-dinitrosalicylic acid)

DMSO: Dimethyl sulfoxide

TPC: Total phenolic content

TFC: Total flavonoid content

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