Free Radicals and Antioxidants 3 (2013) 52–54

Contents lists available at SciVerse ScienceDirect

Free Radicals and Antioxidants

journal homepage: www.elsevier.com/locate/fra

Short communication

Higher antioxidant activity in decoction than raw fruit parts of *Lagenaria siceraria* (Mol.) Standley as determined by pulse radiolysis

Padmashree Joshi [a], Mukula Kulkarni [b], Sushama Joag [b,*]

[a] Department of Physics, University of Pune, Pune 411007, Maharashtra, India
[b] Department of Chemistry, Modern College of Arts, Science and Commerce, Pune 411053, India

ARTICLE INFO

Article history:
Received 7 March 2013
Accepted 22 March 2013
Available online 10 April 2013

Keywords:
Radical scavenging activity
Folin–Ciocalteu reagent
Phenolic content
Bottle gourd
Decoction

ABSTRACT

Antioxidant activity of decoctions and fresh juice samples of raw fruit parts of *Lagenaria siceraria* (Mol.) Standely, (*L.S.*), viz., fruit pulp, whole fruit and fruit skin, has been evaluated in terms of radical scavenging ability by pulse radiolysis method and phenolic content by Folin–Ciocalteu assay. The decay of $ABTS^{\bullet-}$ radical anion generated by pulse radiolysis has been monitored in presence of samples. The trend in the radical scavenging ability of six samples recorded in terms of ascorbic acid equivalent (AAE) values (4.650–41.230 AAE μg/g fresh weight) have been found to correlate well with the trends in phenolic content of these samples expressed as gallic acid equivalent (GAE) values (0.417–2.000 GAE mg/g fresh weight). The result also shows that the antioxidant activity of decoctions of all the three fruit parts is higher, apparent from their higher AAE and GAE values than the corresponding fresh juice samples.

1. Introduction

In the recent times there has been a lot of interest all over the world in antioxidant activity of various fruits and vegetables[1–4] due to the role they play in controlling the hazardous effects of oxidative stress created by reactive oxygen species formed in human body as a consequence of aerobic metabolism. Epidemiological studies[5,6] have demonstrated the protective role of antioxidants in cardiovascular diseases and also correlated the risk of cardiovascular diseases with low plasma level of essential antioxidants. To obtain health benefits that include cardiotoning and cardioprotection, consumption of vegetable bottle gourd (*Lagenaria siceraria* (Mol.) Standley, *L.S.*) either as fresh juice or in cooked form has been recommended in current practices of naturopaths[7] and traditional Indian medicinal system, Ayurveda.[8] Recent studies have demonstrated the cardioprotective effects of *L.S.* fruit on doxorubicin induced cardiotoxicity in rats.[9] The cardioprotective property is suggestive of antioxidant activity of *L.S.* fruit. The antioxidant activity of the extracts of fresh fruit of *L.S.* in organic solvents has been evaluated as DPPH radical scavenging ability.[10,11] Organic solvent extracts of *L.S.* have also been tested for assay of total phenolics, flavonoid and DPPH radical scavenging ability.[12] In traditional as well as naturopathic way of ingestion of *L.S.* fruit the medium is aqueous. Therefore, the use of aqueous medium in evaluation of overall antioxidant activity of fruit of *L.S.* is of great practical interest. Traditionally, *L.S.* fruits are skinned, washed and then cooked with added water before ingestion. In naturopathic practice the raw juice of the whole fruit of *L.S.* is consumed within a few minutes of extraction. The use of aqueous medium in the evaluation of the overall antioxidant activity of fruit of *L.S.*, believed to be a synergistic effect of various ingredients is, therefore, important.

In the present work aqueous samples from *L.S.* fruit were generated without use of any organic solvents. Two assays, compatible with the aqueous nature of samples, employed were: pulse radiolysis to assess the ability of samples to scavenge $ABTS^{\bullet-}$ radical anion[13,14] and Folin–Ciocalteu Reagent assay (FCR assay) for determination of the total phenolic content.[15,16]

2. Materials and methods

2.1. Preparation of samples

2.1.1. Representative portion of fresh fruit part

The fresh *L.S.* fruit, purchased from local market, was washed with water, wiped dry and cut longitudinally into wedges. One of the wedges was used as the representative sample of the whole fruit. The skin peeled off from a few wedges was used as the representative sample of the fruit skin. Skinned wedge (after

* Corresponding author. Tel.: +91 20 25535927, +91 9423568922 (mobile); fax: +91 20 25536075.
E-mail address: joagsd@gmail.com (S. Joag).

2231-2536/$ – see front matter
http://dx.doi.org/10.1016/j.fra.2013.03.006

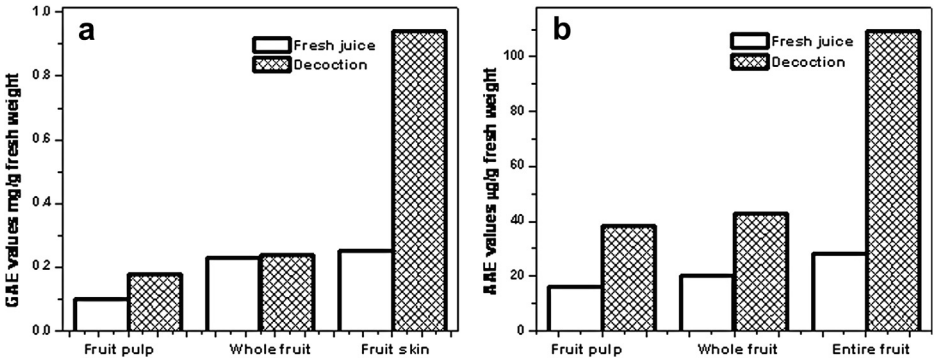

Fig. 1. Phenolic content (a) and radical scavenging ability (b) of fresh juice samples and decoctions of L.S. fruit parts.

peeling off the skin) was used as the representative sample of the fruit pulp.

2.1.2. Preparation of fresh juice samples and decoctions of fruit parts

Fresh juice samples of the three fresh fruit parts were obtained by homogenizing their representative portions with water followed by filtration through bolting cloth and three times washing of solid residue. Decoctions of the three fresh fruit parts were obtained by refluxing their representative portions with water for 30 min. Hot filtration through bolting cloth followed by three washings gave a clear decoction. The ratio of fresh weight of the fruit part to the volume of fresh juice or decoction was kept at 0.1 g/ml.

2.2. Assays

2.2.1. FCR assay

The FCR assay was carried out by the following schematic protocol; the reduced FCR was observed at 765 nm ($A_{765\ nm}$) with UV_VIS_NIR Spectrophotometer (V-670 JASCO).

$$1.55\ \text{ml FCwRgt} + 15\ \mu\text{l samples/water} \xrightarrow{3\ \text{min standing}} + 0.3\ \text{ml carbonate} \xrightarrow{30\ \text{min at}\ 37\ ^{\circ}\text{C}} A_{750\ nm}$$

Working Folin–Ciocalteu reagent (FCwRgt) was obtained by ten times dilution of FCR with water. Sodium carbonate was employed as 80% saturated solution. Gallic acid (10 mg) in water (10 ml) was used as stock solution for calibration. The phenolic content of the samples determined by FCR assay was expressed as gallic acid equivalent value, GAE mg/g fresh weight.

2.2.2. Pulse radiolysis

In pulse radiolysis experiments, the Pune University Linear Accelerator Facility (PULAF) LINAC 7 MeV, 50 ns[17] was used to measure the kinetics and the transient absorption spectra. The dose rate was determined using KSCN dosimetry, where $(SCN)^{\bullet -}$ has absorption maximum at 480 nm with a molar absorption of 7600 l mol^{-1} cm^{-1}. The dose rate was kept 7.16 Gy per pulse. The following schematic protocol was used in the pulse radiolysis assay.

$$\left.\begin{array}{l}1\ \text{ml sample/standard}\\ +1\ \text{ml ABTS (20 mM)}\\ +1\ \text{ml azide (0.5 M)}\end{array}\right\} \rightarrow 10\ \text{ml} \xrightarrow{\text{Nitrous oxide purging}} \text{pulse} \rightarrow \text{decay monitoring at 655 nm}$$

The radical scavenging ability as determined by pulse radiolysis assay was expressed as AAE µg/g fresh weight.

3. Results

It is found that all the six samples obtained from *L.S.* fruit, viz., fresh juice samples and decoctions of fruit pulp, whole fruit and fruit skin, contain phenolics (0.417–2.000 GAE mg/g fresh weight) and also have ability to scavenge $ABTS^{\bullet -}$ (4.650–41.230 AAE µg/g fresh weight). The trends observed in the phenolic content of the three fruit parts and their radical scavenging ability are similar (Fig. 1a and b). Thus the skin samples have the highest GAE values among the three fruit parts and their AAE values are also the highest.

A comparison of the two modes of sample preparation indicates that all the decoctions have higher GAE values as well as AAE values than those of the fresh juice samples (Figs. 1 and 2).

4. Discussion

The two parameters monitored in the present study of antioxidant activity of fresh juice samples and decoctions of fruit parts of *L.S.* are the radical scavenging ability using pulse radiolysis method and the phenolic content by FCR assay. Skin samples (fresh juice as well as decoctions) have the highest phenolic content and also the highest radical scavenging ability, while pulp samples have the lowest phenolic content and also the lowest radical scavenging ability. Phenolic content is, thus, a major contributing factor of antioxidant activity of the samples.

Decoctions of the three fruit parts have higher antioxidant activity than the corresponding fresh juice samples, as indicated by the trends in their GAE values and AAE values. The higher phenolic content and the corresponding higher radical scavenging ability of the decoctions than the fresh juice samples may have its origin in the method of preparation. A fresh juice sample is expected to have the same phenolic content as the raw fruit part. A decoction on the other hand, is obtained by refluxing the fruit part with water. *L.S.* fruit is known to contain bonded phenolics such as glycosides of 4-hydroxymethyl phenol and 4-hydroxymethyl catechol.[12] During the process of refluxing with water hydrolysis

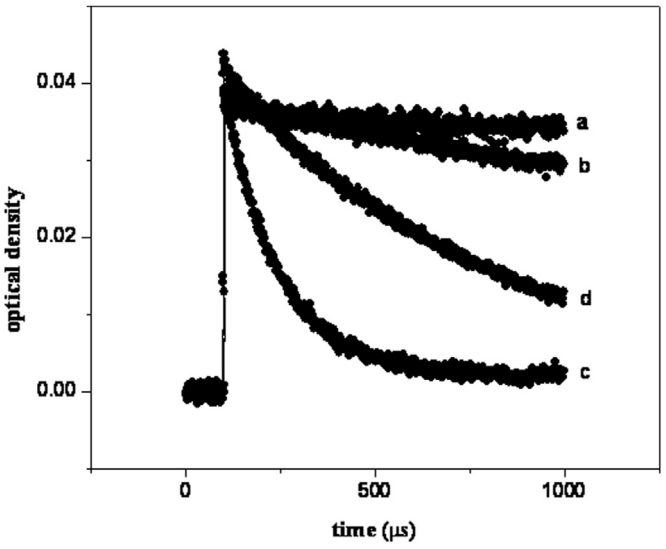

Fig. 2. Decay of $ABTS^{\bullet-}$ (a), decay of $ABTS^{\bullet-}$ in the presence of: skin juice (b), skin decoction (c), 10 μg/ml ascorbic acid (d).

of glycosides would occur, releasing free phenolics, and thereby increasing the phenolic content and radical scavenging ability of the decoctions.

5. Conclusion

From the present study, it is concluded that the decoctions of fruit pulp, whole fruit and skin of *L.S.* fruit have higher antioxidant activity than the corresponding raw fruit parts as determined by FCR assay and pulse radiolysis.

Conflicts of interest

All authors have none to declare.

Acknowledgment

The authors thank Prof. B.S. Madhava Rao and Prof. A.S. Kumbhar for providing PULAF at National Centre for Free Radical Research, University of Pune, Pune. PJ wishes to thank Prof. D.S. Joag for encouragement and discussion and Head, Department of Physics, University of Pune, for providing facilities. SJ acknowledges the financial support provided by BCUD, University of Pune, Pune. MK and SJ thank the Principals of MCASC, Ganeshkhind, Pune and MCASC, Shivajinagar, Pune, respectively for encourangement and support.

References

1. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity of total phenolics in selected fruits, vegetables and grain products. *J Agric Food Chem*. 1998;46: 4113–4117.
2. Szeto YT, Tomlinson B, Benzie IFF. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implication of dietary planning and food preservation. *Br J Nutr*. 2002;87:55–57.
3. Tilak JC, Jain JA, Devasagayam TPA. Cardioprotective and other beneficial effects of some Indian medical plants. *J Clini Biochem Nutri*. 2006;38:1–10.
4. Tarwadi K, Agte V. Antioxidant and micronutrient potential of common fruits available in the subcontinent. *Intern J Food Sci Nutr*. 2007;58:341–349.
5. Diplock AT, Charleux JL, Crozer W, et al. Funtional food science and defence against reactive oxygen species. *Br J Nutr*. 1998;80:S77–S112.
6. Harrison G, Griendling KK, Landmesser U, Hornig B, Drelex H. Role of oxidative stress in atherosclerosis. *Am J Cardiol*. 2003;91:7A–11A.
7. Deshpande JR, Choudhari AA, Mishra MR, Meghre SG, Wadodkar SG, Dorle AK. Beneficial effects of *Lagenaria siceraria* (Mol.) Standley fruit epicarp in animal model. *Indian J Exp Biol*. 2008;46:234–242.
8. Kirtikar KR, Basu BD. *Indian Medical Plants*. 2nd ed. vol. 2. Delhi: Periodicals Experts Book Agency, ISBN 978-81-7136-053-6; 2012:1116.
9. Fard MH, Naseh G, Bodhankar SL, Dikshit M. Cardioprotective effect of *Lagenaria siceraria* (Molina) Standley (*Cucurbitaceae*) fruit juice on doxorubicin induced cardiotoxicity in rats. *Am J Pharmacol Toxicol*. 2010;5:103–108.
10. Deshpande JR, Mishra MR, Wadodkar SG, Dorle AK. Free radical scavenging activity of *Lagenaria siceraria* (Mol.) Standl. fruit. *Natural Prod Rad*. 2007;6: 127–130.
11. Erasto P, Mbwambo ZH. Antioxidant activity and HPTLC profile of *Lagenaria siceraria* fruits. *Tanzania J Health Res*. 2009;11:79–83.
12. Mohan R, Birari R, Karmase A, Jagtap S, Bhutani KK. Antioxidant activity of phenolic glycoside from *Lagenaria siceraria* Stand. fruits. *Food Chem*. 2012;132: 244–251.
13. Khopde SM, Priyadarsini KI, Mohan H, et al. Characterizing the antioxidant activity of *amla (Phyllanthus emblica)* extract. *Curr Sci*. 2001;81:185–190.
14. Naik GH, Priyadarsini KI, Mohan H. Free radical scavenging reactions and phytochemical analysis of *triphala*, an ayurvedic formulation. *Curr Sci*. 2006;90: 1100–1105.
15. Singleton VL, Rossi Jr JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticult*. 1965;16:144–158.
16. Danny KA, Hong YJ, Diane MB, Alyson EM. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried *marionberry, strawberry*, and *corn* grown using conventional, organic, and sustainable agricultural practices. *J Agric Food Chem*. 2003;51:1237–1241.
17. Yadav P, Kulkarni MS, Shirdhonkar MB, Rao BSM. Pulse radiolysis: Pune University LINAC facility. *Curr Sci*. 2007;92:599–605.