Appendix I: Higher Antioxidant Activity in decoction than raw fruit parts of *Lagenaria Siceraria* (Mol.) Standley as determined by Pulse Radiolysis

I.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 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 bottlegourd (*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.

*Note: The pulse radiolysis experiments were carried out using the Pune University Linear Accelerator Facility (PULAF) at the National Centre for Free Radical Research (NCRFF), Savitribai Phule Pune University. The author acknowledges the centre for providing the facility.*
In the present work aqueous samples from *L.S.* fruit were generated without use of any organic solvents. Two assays, compatible with aqueous nature of sample, employed were: pulse radiolysis to assess the ability of samples to scavenge ABTS*−* radical anion\textsuperscript{13,14} and Folin-Ciocalteu Reagent assay (FCR assay) for determination of the total phenolic content.\textsuperscript{15,16}

**I.2. Experimental techniques**

**I.2.1. Preparation of samples**

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

**I.2.2. Preparation of fresh juice samples and decoctions of fruit parts**

Fresh juice samples of 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 three fresh fruit parts were obtained by refluxing their representative portions with water for 30 minutes. 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 0.1g/ml.

**I.2.3. FCR assay**

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

\[
1.55\text{ml FCwRgt} + 15\mu l \text{sample/water} \xrightarrow{\text{after 3 min}} + 0.3\text{ml carbonate} \xrightarrow{30 \text{ minute at } 37^\circ C} A750\text{nm}
\]

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.

I.2.4. Pulse radiolysis

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

\[
\begin{align*}
\{1\text{ml sample/standard} + 1\text{ml ABTS (20 mM)} + 1\text{ml azide (0.5 M)}\} &\rightarrow 10\text{mL N}_2\text{O purging} \rightarrow \text{pulse} \rightarrow \text{decay monitoring at 655 nm}
\end{align*}
\]

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

I.3. Results and Discussion

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\textsuperscript{•−} (4.650-41.230 AAE µg/g fresh weight).

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

*Fig.I.1. Phenolic content (a) and radical scavenging ability (b) of fresh juice samples and decoctions of L.S. fruit parts*
The trends observed in the phenolic content of the three fruit parts and their radical scavenging ability are similar (Fig. I.1 (a) and (b)). Thus the skin samples have highest GAE values among the three fruit parts and their AAE values are also 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 (Fig. I.1 and I.2). 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 or 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.

![Graph showing decay of ABTS•− (a), decay of ABTS•− in the presence of: skin juice (b), skin decoction (c), 10 mg/ml ascorbic acid (d)](image)

Fig. I.1. *Decay of ABTS•− (a), decay of ABTS•− in the presence of: skin juice (b), skin decoction (c), 10 mg/ml ascorbic acid (d)*

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. During the process of refluxing with water hydrolysis of glycosides
would occur to release free phenolics, thereby increasing the phenolic content and radical scavenging ability of the decoctions.

I.4. Conclusion

From the present study, it is concluded that the fruit 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.
References


