Antioxidant and metal chelating activities of *Lagenaria siceraria* (Molina) Standl peel, pulp and aerial parts in relation to their total phenol and flavonoid content

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ABSTRACT

Objective: The aim of the present study was to evaluate the antioxidant activity and total phenol and flavonoid content of different parts (peel, pulp and aerial parts) of *Lagenaria siceraria.* **Method:** Successive extraction was done by cold percolation method using solvents of different polarity viz. petroleum ether, toluene, ethyl acetate, acetone, water. Total phenol content was determined by Folin-Ciocalteu's reagent method and flavonoid was determined by aluminium chloride colorimetric method. The antioxidant assays evaluated were 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, 2, 2′-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity and Ferric reducing antioxidant power. **Results:** *L. siceraria* peel possessed maximum phenol and flavonoid content in acetone and toluene extracts respectively. FRAP was maximum in acetone extract of peel. The peel extract demonstrated stronger DPPH activity with IC_{50} value of 111 μg/ml followed by pulp extract. The same extract was effective in scavenging ABTS radical with an IC_{50} value of 39 μg/ml while other parts were ineffective. **Conclusion:** This work demonstrated good antioxidant activity of *L. siceraria* vegetable cultivated in India and recommends that the peel of this vegetable may be of interest from a functional point of view as a major source of natural antioxidant.

Key words: *Lagenaria siceraria*, antioxidant activity, DPPH, ABTS, FRAP, total phenol.

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological process. However, oxygen-centre free radicals and other reactive oxygen species (ROS) which are continuously produced *in vivo,* results in cell death and tissues damage. Free radicals are derived from two sources: endogenous source such as nutrient metabolism, aerobic respiration, stimulated polymorphonuclear leucocytes and macrophages, ageing process, action of peroxisomes and activation of inflammatory cells[1] and exogenous sources such as environmental agents (tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc.). Scientific evidence has suggested that under oxidative stress conditions, oxygen radicals such as superoxide anions ($O2^-$), hydroxyl radical (OH) and peroxyl radicals (H_2O_2) are produced in biological system. These reactive oxygen species can damage DNA which causes mutation and chromosomal damage. It also oxidizes cellular thiols and extracts hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membranes lipids.[2] Moreover, the production of excessive free radicals stimulates the oxidative damage and such situation contribute to more than one hundred disorder in humans including atherosclerosis, coronary heart disease, neurodegenerative disorder, cancer and they play major role in the aging process.[3]

Antioxidants have the ability to protect organisms from

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damage caused by free radical-induced oxidative stress. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. The commercial development of plants as source of antioxidants to enhance health is of current interest. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease. Various herbs and spices have been reported to exhibit antioxidant activity, for example *Ocimum sanctum, Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica, Camellia sinensis* Linn., *Zingiber officinale* and several Indian and Chinese plants. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. Therefore, antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals induced oxidative stress.[4] Synthetic antioxidants also available in market, such as butylated hydroxy anisole (BHA), butylated hyroxy toluene (BHT) but they are gaining less importance due to their side effects and carcinogenicity for this reasons increasing attention has been directed towards natural antioxidants.[5]

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. *Lagenaria siceraria* is well documented as a food source but there is still not much known about the antioxidant potential of its different parts. Therefore, in the present study we have evaluated the free radical scavenging activity of different parts of *Lagenaria siceraria* in different solvents like petroleum ether, toluene, ethyl acetate, acetone and water and also estimated total phenol and flavonoid contents of the crude extract.

Lagenaria siceraria (Molina) Standl. Belongs to the family Cucrbitaceae. It is widely cultivated as a vegetable crop in tropical countries such as India, Japan and Thailand. The fruit is reported to contain the tritereperiodecucurbitacine B, D, G, H and 22-deoxy cucurbitacin the bitter principal of cucurbitaceae. Two sterols i.e., fucosterol and campesterol, aerpenebyonolic acid (an allergic compounds, flavones-c glycosides, ribosome inactivating protein) .Lagenin (antiproliferative, mmunosuppressive, antifertility).[6] reported a new a new phenolic glycoside from fruit. They are widely used in Ayurveda and other folk medicines; traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons, scorpion strings, alternative purgative, scorpion strings, and cooling effects. It cures pain, ulcers and fever and used for pectoral cough, asthma and other bronchial disorders especially syrup prepared from the tender fruits.[7] Some of the reported activities are Hypolipidemic and antihyperlipidemic effects,[8] Diuretic activity,[9] Cardioprotective effect,[10,11] Cytotoxic activity,[12] Antioxidant activity,[13-16] Antimicrobial activity,[17] Lipid-lowering and antioxidant activity,[18] hepatoprotective activity,[19] antinociceptive and antioxidant from root,[20] antithrombotic potential of the fruits.[21]

MATERIALS AND METHODS

Plant Collection

The seeds, peel and aerial part of *Lagenaria siceraria (*Molina) standl. were collected from Jamnagar, Gujarat, India in August, 2011. The various parts were washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in air tight bottles.

Successive extraction method

Successive extraction[22,23] was done by cold percolation method. 10 g of dried powder was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h, it was then filtered through eight layers of muslin cloth, centrifuged at 5000 g for 15 minutes and the supernatant was collected and air dried under reduced pressure to obtain the dried residue. Petroleum ether was evaporated from the powder. This dry powder was then taken successively in 100 ml of each solvent (toluene, ethyl acetate, acetone, water) and was kept on a rotary shaker at 120 rpm for 24 h. Then the procedure followed was same as above, and the residues were weighed to obtain the extractive yield of all the extracts and were stored in air tight bottles at 4°C.

Quantitative phytochemical analysis

Determination of total phenol content

The amount of total phenol content of different solvent extracts was determined by Folin-Ciocalteu's reagent method.[24] The extract (0.5 ml) and 0.1 ml of Folin-Ciocalteu's reagent (0.5 N) were mixed and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm using a digital spectrophotometer (Systronic, India), against a blank sample. The calibration curve was made by preparing gallic acid (10 to 100 μg ml^{-1}) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g^{-1} of extracted compounds).

Determination of flavonoid content

The amount of flavonoid content of different solvent extracts was determined by aluminium chloride colorimetric method.[25] The reaction mixture (3.0 ml) consisted of 1.0 ml of sample (1 mg ml^{-1}), 1.0 ml methanol, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm using a digital spectrophotometer (Systronic, India), against a blank sample. The calibration curve was made by preparing a quercetin (5 to 60 μg ml^{-1}) solution in methanol. The flavonoid content is expressed in terms of standard equivalent (mg g^{-1} of extracted compound).

Antioxidant activity

Ferric reducing antioxidant power (FRAP)

The reducing ability of different solvent extracts s of *Lagenaria siceraria* was determined by FRAP assay.[26] FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2,4,6- tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM $FeCl_3$] and the reaction mixture is incubated at 37°C for 10 min and then the absorbance was measured at 593 nm. $FeSO_4$ (100 to 1000 μM ml^{-1}) was used as a positive control. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M $FeSO_4$ equivalents per gram of extracted compound.

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

The free radical scavenging activity of different solvent extracts was measured by using DPPH by the modified method described by McCune and Johns.[27] The reaction mixture 3.0 ml consisting of 1.0 ml methanol, 1.0 ml DPPH (0.3 mM) and 1.0 ml of solvent extracts of different concentrations of the *Lagenaria siceraria* diluted by methanol, was incubated for 10 min, in dark, after which the absorbance was measured at 517 nm using digital spectrophotometer (Systronic, India), against a blank sample. Ascorbic acid (2 to 16 μg ml^{-1}) was used as positive control (Liu *et al.,* 2011). The percentage inhibition was determined by comparing the results of the test and the control. Percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [1 - (A/B)] \times 100$$

Where, B is the absorbance of the blank (DPPH plus methanol) and A is absorbance of the sample (DPPH, methanol, plus sample).

Determination of superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of different solvent extracts of *Lagenaria siceraria* was measured following the method described by Robak and Gryglewski.[28] Superoxide radicals are generated by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture 3.0 ml consisted of 1.0 ml of the solvent extracts of different concentrations of the *Lagenaria siceraria,* diluted by distilled water, 0.5 ml Tris HCl buffer (16 mM, pH 8), 0.5 ml NBT (0.3 mM), 0.5 ml NADH (0.93 mM) and 0.5 ml PMS (0.12 mM). The superoxide radical generating reaction was started by the addition of PMS solution to the mixture. The reaction mixture was incubated at 25°C for 5 min and then the absorbance was measured at 560 nm using digital spectrophotometer (Systronic, India), against a blank sample. Gallic acid (50 to 225 μg ml^{-1}) was used as a positive control (Robak and Gryglewski, 1988). Percentage of inhibition was calculated as described earlier.

Determination of 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity

The ABTS radical cation scavenging activity of different solvent extracts of *Lagenaria siceraria*, determined by the method described.[29] ABTS radical cations are produced by reaction of ABTS (7 mM) with potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 h. The working solution obtained was further diluted with methanol to give an absorbance of 0.85 ± 0.20. 1.0 ml of different concentrations of solvent extracts and fractions of the *Lagenaria siceraria* diluted by methanol was added to 3.0 ml of ABTS working solution. The reaction mixture was incubated at room temperature for 4 min and then the absorbance was measured at 734 nm using digital spectrophotometer (Systronic, India), against a blank sample. Ascorbic acid (1 to 10 μg ml^{-1}) was used as a positive control. Percentage of inhibition was calculated as described earlier.

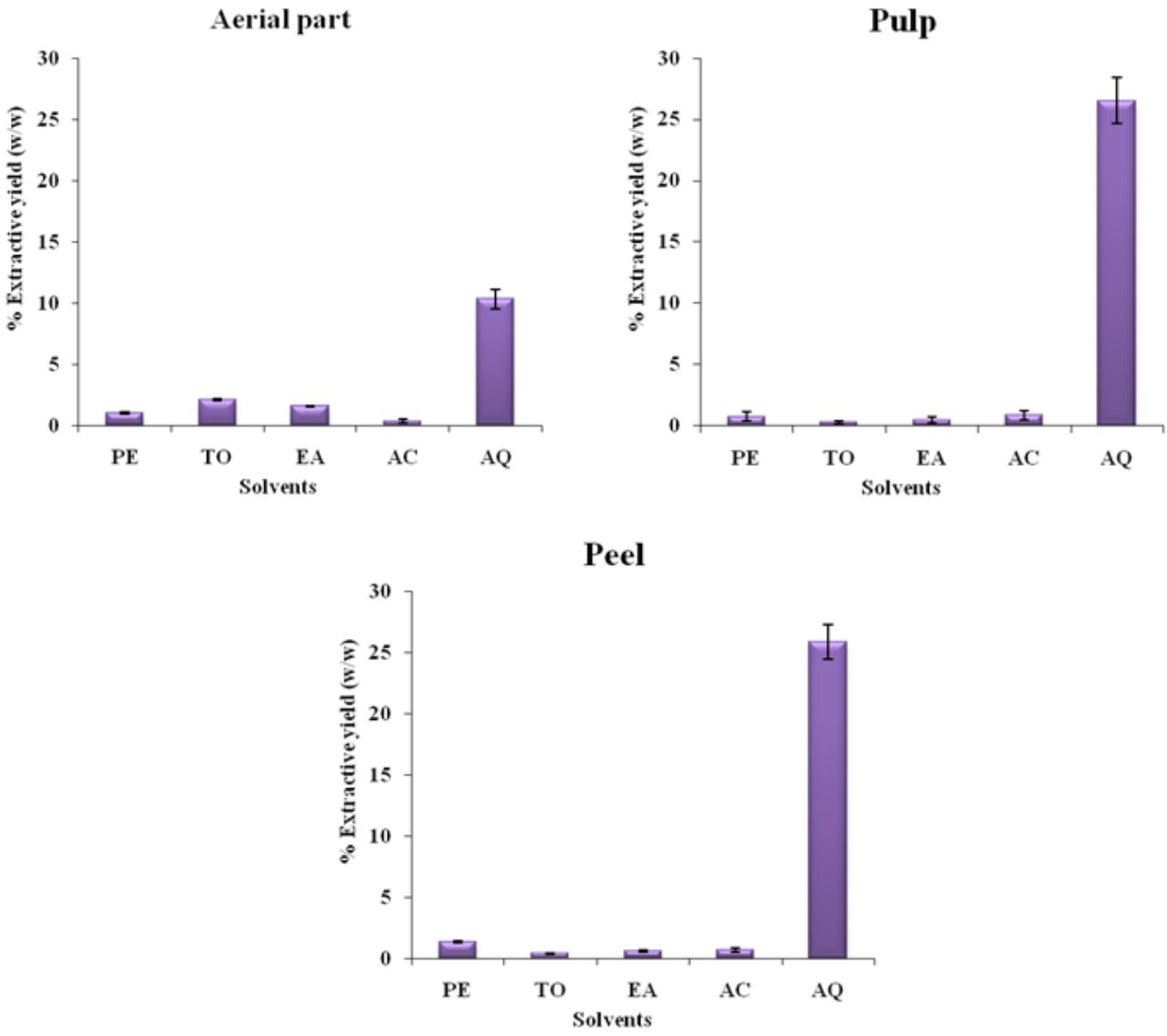

Figure 1: Extractive yield of different solvent extracts of different part of *L. siceraria*

RESULTS AND DISCUSSION

Extractive yield

The extraction yield of antioxidant compounds from plant materials is influenced mainly by the conditions under which the process of liquid solid extraction is achieved, the type of the solvent used to separate the soluble fraction from the permeable solid the degree of polymerization of phenolics and their interaction with the other components. The selection of an appropriate solvent is one of the most important factors in this operation.

The dry powder of different parts of *L. siceraria* was extracted in water (aqueous) and organic solvents like petroleum ether, toluene, ethyl acetate, acetone by cold percolation successive method. The results are shown in (Figure 1). In aerial part, maximum extractive yield was in aqueous extract and minimum was in acetone extract (Figure 1a). In pulp, maximum extractive yield was in aqueous and minimum was in toluene (Figure 1b). In peel, maximum extractive yield was in aqueous and minimum was in toluene (Figure 1c). The extractive yield depends on solvents time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature condition, the solvent used and the chemical property of sample are the two most important factors.[30] There are many reports in the literature where extractive

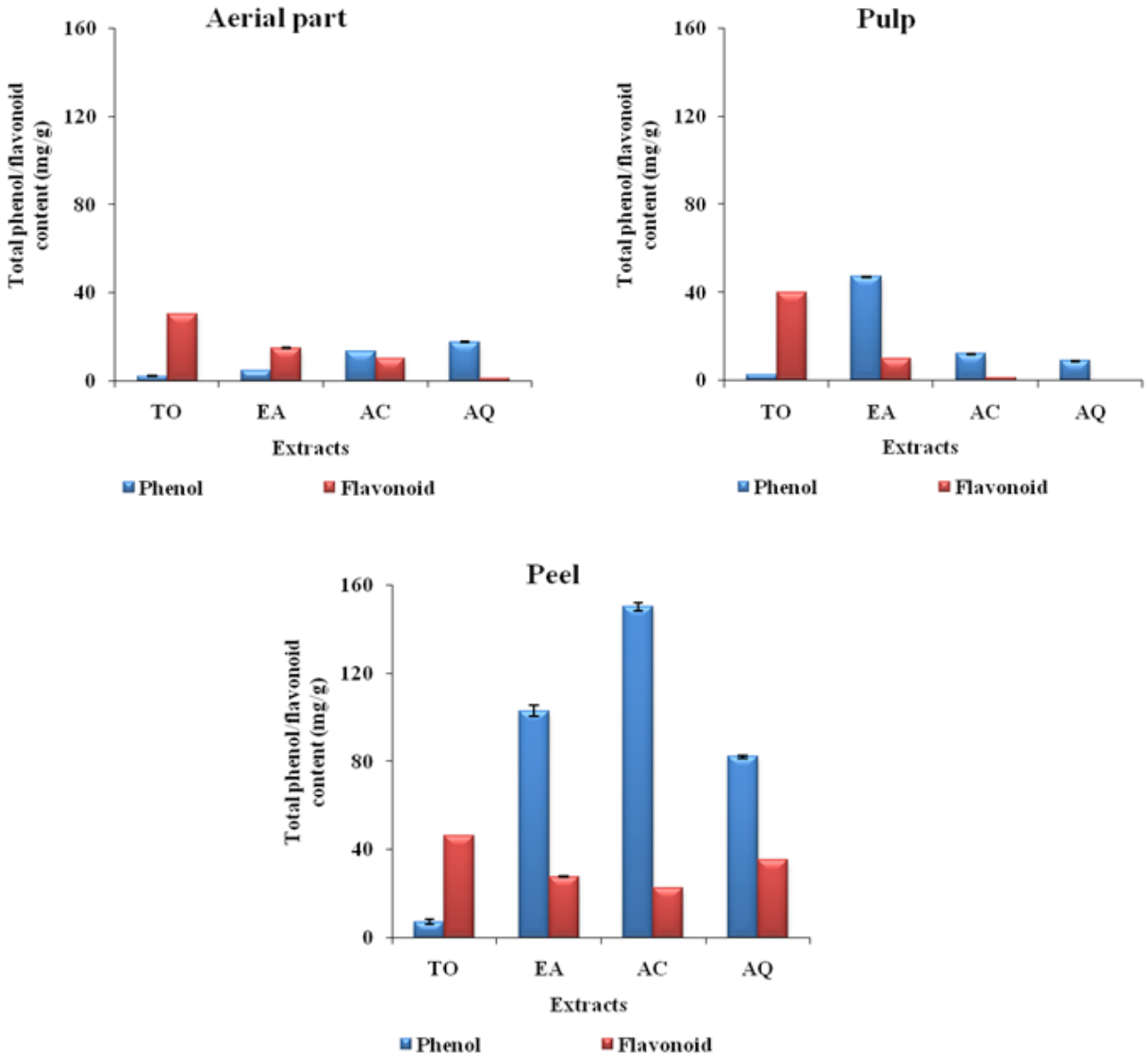

Figure 2: Total phenol and flavonoid content of different parts of *L. siceraria*

yield varied with different solvents .[31-35]

Total phenol and flavonoid content

Phenolic compounds such as flavonoids or phenolic acids are responsible for antioxidant activity due to their free radical scavenging capacity, although, there are several other mechanisms that contribute to the antioxidant activity like electron donor, metal chelating and scavenger of singlet oxygen.[36] In aerial part, maximum phenol content was in aqueous extract and minimum was in toluene extract while maximum flavonoid content was in toluene extract and minimum was in aqueous extract (Figure 2a). In pulp, maximum phenol was in ethyl acetate extract and minimum was in aqueous extract while maximum flavonoid content was in toluene extract and minimum was in acetone extract (Figure 2b). In peel, maximum phenol content was in acetone extract and minimum was in toluene extract while maximum flavonoid content was in toluene extract and minimum was in acetone extract (Figure 2c). When different parts are compared both phenol and flavonoid content was maximum in peel; maximum phenol content was in acetone extract while flavonoids was in toluene extract. Similar reports are found in guava,[37] *Mangifera pajang*,[38] *Annona cherimola* ,[39]etc.

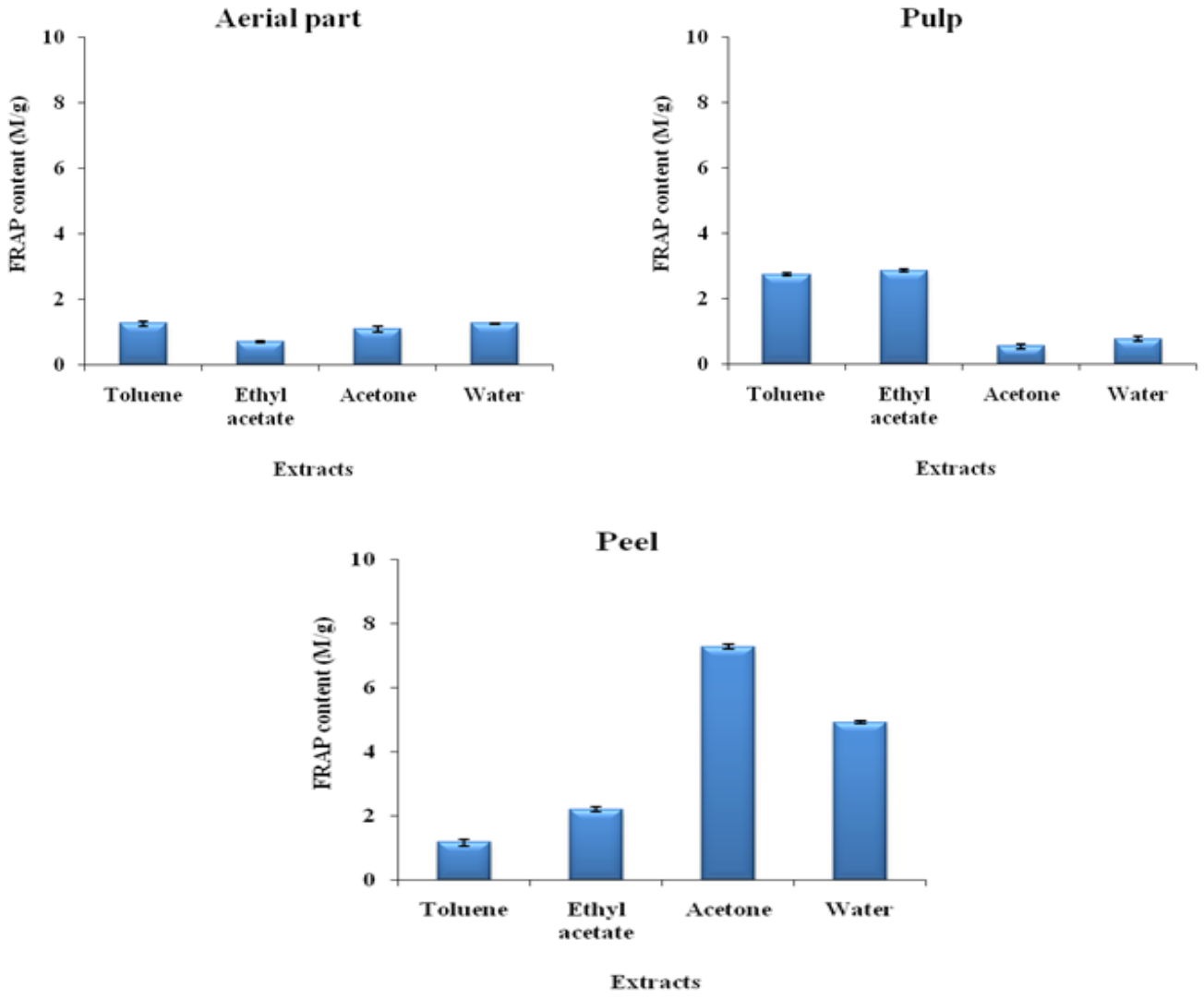

Figure 3: FRAP of different solvent extracts of different parts of *L. siceraria*

Antioxidant

Primary antioxidants, when present in trace levels, the response of antioxidants to different radical or oxidant sources may be different. Therefore, no single assay accurate reflects the mechanism of action of all radical sources or all antioxidants in a complex system, at least two methods should be employed in order to evaluate the total antioxidant activity, due to various oxidative processes. In this study, antioxidant activity was determined by three different *in vitro* antioxidant assays.

FRAP assay

The principle of the FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous coloured form in the presence of antioxidants. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so that they can act as primary and secondary antioxidants. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. In aerial part, maximum FRAP was in aqueous extract and minimum was in ethyl acetate extract (Figure 3a). In pulp, maximum FRAP was in ethyl acetate extract and minimum was in acetone extract (Figure 3b). In peel, maximum FRAP was in acetone extract and minimum was in toluene extract (Figure 3c). There was a direct correlation between phenol content and FRAP. Acetone extract of peel had maximum phenol content and highest FRAP activity.

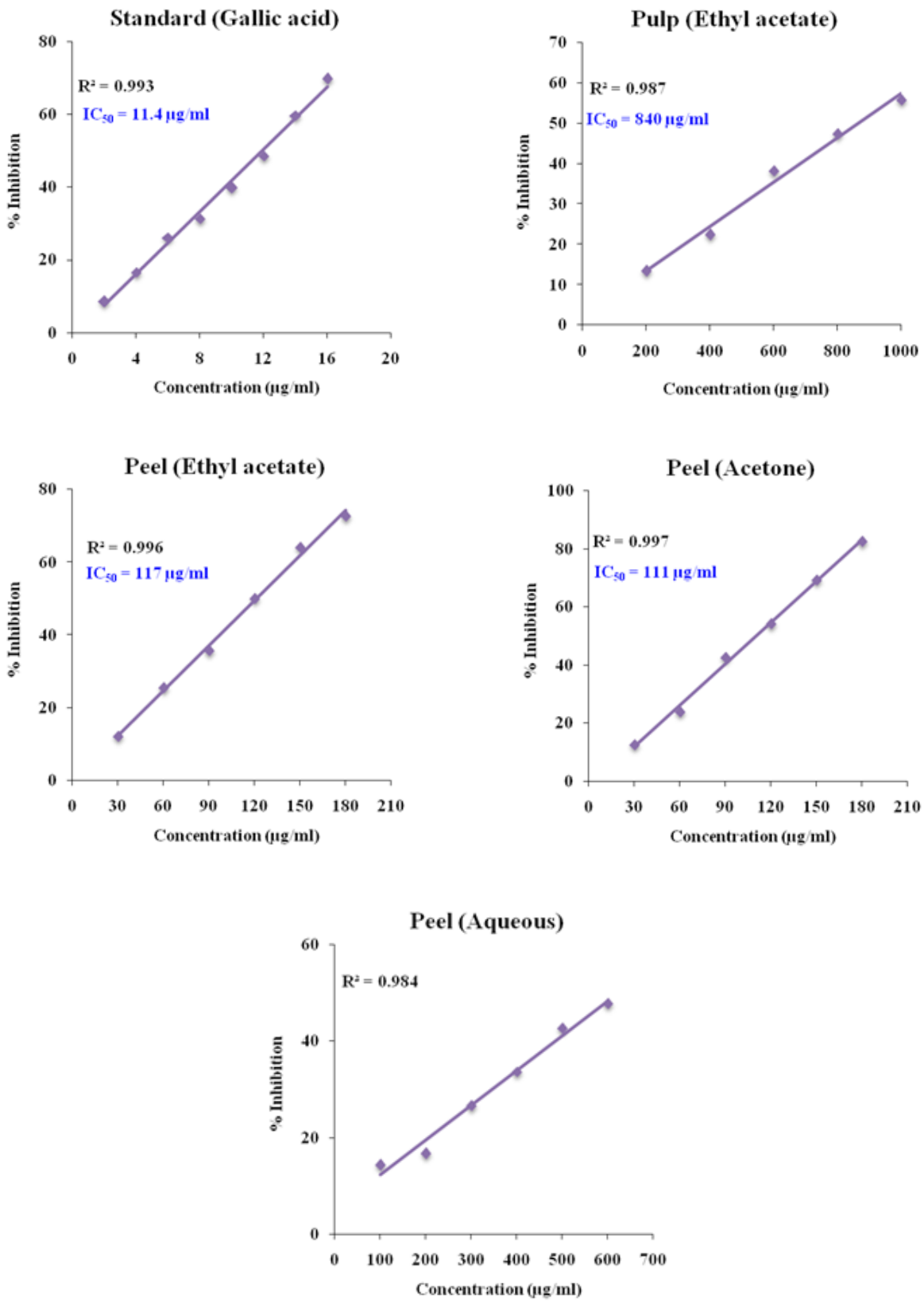

Figure 4: DPPH free radical scavenging activity of standard and different extracts of *L. siceraria pulp* and peel

DPPH assay

DPPH has been extensively used as a free radical to evaluate antioxidant substances that reduce DPPH. by donating hydrogen to form the non-radical DPPH – H. - DPPH• has an intense violet colour with a maximum absorbance at 517 nm, but turns colourless as unpaired electrons are scavenged by antioxidants.40 DPPH activity was present in L. siceraria peel (ethyl acetate, acetone, and aqueous extracts) and pulp (ethyl acetate extract) while it was absent in aerial part. The IC_{50} value of standard ascorbic acid was 11.4 µg/ml (Figure 4a). In pulp, maximum IC_{50} value was in ethyl acetate extract (Figure 4b) while in peel, minimum IC_{50} value was in acetone extract (Figure 4c). Aerial parts had low phenol content and it did not show any DPPH activity. In pulp, amongst four solvent extracts, ethyl acetate extract had maximum phenol content and it showed some DPPH activity. Amongst three parts evaluated, peel had maximum phenol content and acetone extract of peel had maximum phenol content (Figure 2) and it showed best DPPH activity (Figure 4d). The results support the popular view that there is a direct correlation between phenolic content and antioxidant activity, further supporting that phenols play an important role in beneficial effect of medicinal plants.[41-44]

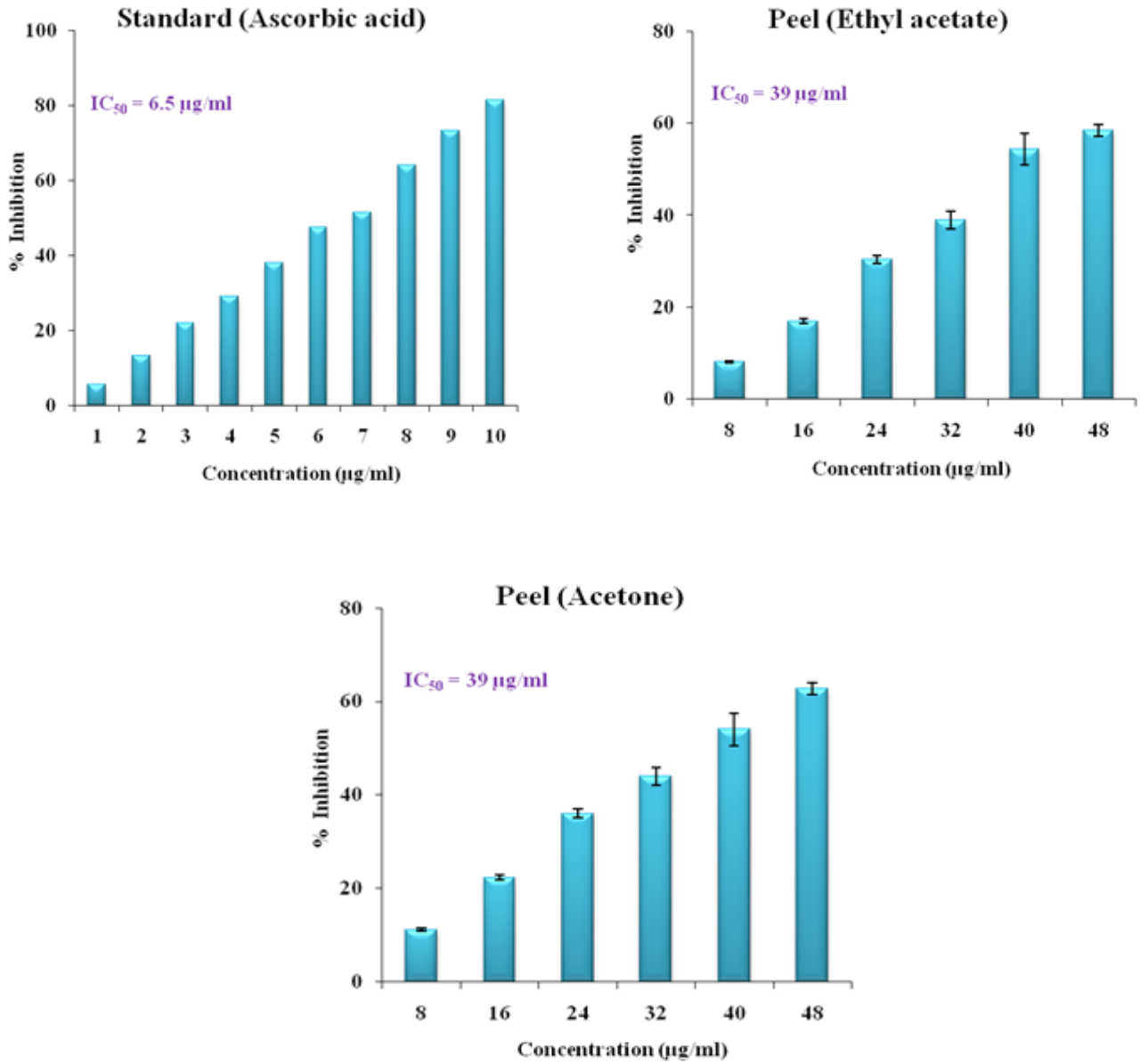

Figure 5: ABTS radical cation scavenging activity of standard and different extracts of *L. siceraria* peel

ABTS assay

ABTS assay is better to assess the antiradical capacity of both hydrophilic and lipophilic antioxidants because it can be used in both organic and aqueous solvent systems as compared to other antioxidant assays.[45] $ABTS^{+\bullet}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract, preformed cation radical gets reduced and employs a specific absorbance at 734 nm, a wavelength remote from the visible region and it requires a short reaction time. The ABTS radical cation scavenging activity of different solvent extracts of *L. siceraria* and standard ascorbic acid is shown in (Figure 5). The IC_{50} value of standard ascorbic acid was 6.5 µg/ml (Figure 5a). ABTS activity was found only in ethyl acetate and acetone extracts of peel. None of the solvent extracts of other two parts i.e. pulp and aerial parts of *L. siceraria* showed ABTS activity. The IC_{50} value of both the solvent extracts of peel was 39 µg/ml. (Figures 5b-5c). There was a direct correlation between phenol content and ABTS activity. Acetone extract of peel had maximum phenol content and highest ABTS activity.

There are no universal criteria for presence or absence of antioxidant activity in different plants or plant parts. It is imperative that one should evaluate more than one antioxidant methods and in more than one solvent in a single plant. This is necessary because plant is rich in secondary metabolites and it is not known which one predominates; and also the mechanism of action of different antioxidant assays is different.[46] Therefore in the present study different parts of the same plant were evaluated for their antioxidant potential in different solvents and different antioxidant assays were done. The peel of this commonly consumed vegetable has excellent potential as an antioxidant additive in foods because it showed good DPPH activity, FRAP, reducing capacity and excellent ABTS activity indicating multiple modes of antioxidant activity. It can be suggested that it has the ability to scavenge free radicals responsible for many chronic diseases and disorders. However, the phytochemicals responsible for antioxidant activity has to be identified and determines which may help in drug development.

CONCLUSION

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. In the present work, the high antioxidant capacity observed for acetone extract of L. siceraria peel suggest that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage. The plant is easily accessible in high quantities and therefore its application could be beneficial. These are novel, natural and economic sources of antioxidants which can be used in the prevention of diseases caused by free radicals. Therefore, our study will definitely open, scope for future utilization of these waste products for therapeutic purpose. However, conformation of its activity in in vivo models should be carried out. Such antioxidants could replace synthetic toxic antioxidants.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest

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