Research Article

In vitro comparative studies of antioxidant action of different parts of sweet and bitter variety of *Lagenaria siceraria*

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ABSTRACT

Objective: The object of the present investigation is to verify the antioxidant activity of sweet and bitter variety of *Lagenaria siceraria* (LS) and to investigate the medicinal properties of whole plants. **Materials:** Ascorbic acid, 1, 1-Diphenyl-Picryl-2-hydroxyl (DPPH), hydrogen peroxide (H_2O_2), trichloroacetic acid, ferric chloride, potassium ferricyanide, and petroleum ether were obtained from Merck Limited, Mumbai, India. All other reagents used were of analytical grade. The leaves of LS were procured from the local market of Pusad and authentification was made from the Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur. **Methods:** The dried and coarsely powdered plant material was extracted with petroleum ether (60–80°) by hot percolation in Soxhlet apparatus. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. Standard methods were used for preliminary phytochemical screening of the extract to recognize the phytoconstituents present in the extract. **Results:** In the present study, identification collection and authentification of sweet and bitter verities of LS were successfully done. The extract of bitter LS has high antioxidant activity; antioxidant activity of the extract was located by DPPH free radical scavenging activity and H_2O_2 method. **Conclusion:** The results obtained in the present study indicated that LS both variety all parts extract exhibited free radical scavenging activity against H_2O_2 and DPPH. The overall antioxidant activity of the ethanolic extract of LS might be attributed to its polyphenolic content and other phytochemical constituents.

Keywords: Antioxidant, free radical, *Lagenaria siceraria*, medicinal properties

Introduction

Lagenaria siceraria (LS) is the traditional plant mostly useful in the Ayurveda on various diseases, such as tonic, anthelmintic, antibacterial, stomachic, and carminative. Medicinally, it has isotropic, hypoglycemic, hypolipidemic,[1] analgesic, and antioxidant properties.[2]

In Hindi, it is called Lauki or Kaddu; in English, it is bottle gourd or calabash gourd; in Marathi, it is Bhopala or Dudhya; in Sanskrit, it is Tumbi; in Telugu, it is Sorrakaya; in Tamil, it is Shorakkai; in Gujrathi, it is Dudhi or Tumada; in Assamese, it is Lau or Bogalau; in Bengali, it is Lau; and in Punjabi, it is called Ghiya. It is a climbing herb distributed throughout India in wild habitat and cultivated as a vegetable.

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It is a large pubescent, climbing or trailing herb, with stout 5-angled stems and bifid tendrils. Leaves are long petiolated, 5-lobed; flowers large, white, solitary, monoecious or dioecious, Fruits are large, up to 1.8 m long, usually bottle or dumble shaped, almost woody when ripe. Seeds are numerous, long white, smooth, 1.6–2.0 cm long, horizontally compressed with the marginal groove. LS is mentioned in Ayurvedic pharmacopeia for treatment of Jvara, Kasa, Svasa, Visa roga, Sopha, Vraṇa, and Sula (Anonymous Ayurvedic Pharmacopoeia, 2001).

Antioxidants are defined as the chemical compounds disposing of the free radicals, scavenging them, suppressing their formation or opposing their action. Free radicals can be defined as chemical species possessing an unpaired electron, which is formed either by hemolytic cleavage of the covalent bond of a molecule or by the loss of a single electron from the normal molecule or by the addition of single electron to the normal molecule.[3]

The antioxidants and disease prevention[4]

Antioxidants and prevention of atherosclerosis

Lipoprotein oxidation is a key early stage in the development of atherosclerosis. Oxidized low-density lipoprotein is known to

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Table 1: Showing the results of phytochemicals screening of various parts of sweet LS extracts used in the study

Phytochemical	Fruits	Stems	Leaves	Roots	Seeds
Flavonoids	−−	−−	++	−−	−−
Glycosides	−−	−−	−−	−−	−−
Cardiac glycosides	++	++	++	++	++
Terpenoids	++	++	++	++	++
Carbohydrates	++	++	++	++	++
Saponins	++	++	++	++	++
Alkaloids	−−	−−	−−	+	−−
Steroidal terpenes	−−	−−	−−	−−	−−
Phytosterol	++	++	++	++	++

+/++: Indicates the presence of phytochemical, −−: Indicates the absence of phytochemical, LS: *Lagenaria siceraria*

Table 2: Showing the results of phytochemicals screening of various parts of bitter LS extracts used in the study

Phytochemical	Fruits	Stems	Leaves	Roots	Seeds
Flavonoids	−−	−−	+	−−	−−
Glycosides	−−	−−	−−	−−	−−
Cardiac glycosides	++	++	++	++	++
Terpenoids	++	++	++	++	++
Carbohydrates	++	++	++	++	++
Saponins	++	++	++	++	++
Alkaloids	−−	−−	−−	++	−−
Steroidal terpenes	−−	−−	−−	−−	−−
Phytosterol	++	++	++	++	++

+/++: Indicates the presence of photochemical, −−: Indicates the absence of phytochemical, LS: *Lagenaria siceraria*

promote atherogenesis through foam cell formation and inflammatory responses.

Antioxidants and prevention of cancer

The underlying cause of cancer is thought to be damage to DNA, much of which is oxidative in nature. These oxidative processes, the mechanisms of which not fully understood, occur during the promotional stage of carcinogenesis. Therefore, it is plausible that antioxidants may be able to interfere with the metabolic activation of chemical carcinogens, cause regression of pre-malignant lesions or inhibit their development into cancer.

Antioxidants and prevention of ocular disease

Oxidative processes are thought to be an important contributing factor in the development of both cataracts and the age-related disorder of the retina, maculopathy. Oxidation, induced mainly by exposure to ultraviolet light, is believed to be a major cause of damage to the proteins of the lens. The oxidized protein precipitates and causes cloudiness of the lens. Antioxidants and antioxidant enzymes inactivate harmful free radicals and proteases degradation and remove the damaged portion from the lens, but the oxidative damage occurs at a faster rate.

Antioxidants and prevention of skin aging

The reactions which add hydroxyl groups to the amino acids proline and lysine in the collagen molecule, through prolyl hydroxylase and lysyl hydroxylase, both require Vitamin C as a cofactor. Hydroxylation allows the collagen molecule to assume its triple helix structure, making Vitamin C essential to the development and maintenance of scar tissue, blood vessels, and cartilage. In addition, topically applied Vitamin C seems to enhance the mRNA level of Collagens I and III, their processing enzymes, and the tissue inhibitor of matrix metalloproteinase 1 in the human dermis.

Materials and Methods

Ascorbic acid, 1, 1-Diphenyl-Picryl-2-hydroxyl (DPPH), hydrogen peroxide (H_2O_2), trichloroacetic acid, ferric chloride, potassium ferricyanide, and petroleum ether were obtained from Merck Limited, Mumbai, India. All other reagents used were of analytical grade. The leaves of LS were procured from the local market of Pusad and authentification was made from the Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur.

Extraction of following parts of sweet and bitter variety of LS was done by hydroalcoholic solution:

- Fruit
- Seed
- Leaves
- Stems
- Roots.

Preparation of extract

The dried and coarsely powdered plant material was extracted with petroleum ether (60–80°) by hot percolation in Soxhlet apparatus. The defatted plant material was then extracted with methanol until it became colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. The last traces of the solvent were evaporated under reduced pressure in the rotatory evaporator. Standard methods were used for preliminary phytochemical screening of the extract to recognize the phytoconstituents present in the extract.[5]

Phytochemicals screening

Flavonoids

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.[6]

Terpenoids (Salkowski test)

About 5 ml of extract was mixed with 2 ml of chloroform and carefully added conc. H_2SO_4 (3 ml) to form a layer. A reddish-brown coloration at the interface shows positive results for the presence of terpenoids.[7]

Cardiac glycosides (Keller-Kiliani test)

Crude extract 2 ml was mixed with 2 ml of glacial acetic acid containing 1–2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.[8]

Table 3: Absorbance of a sweet variety of LS of fruit, leaves, stem, seed, and root

Plant part	Conc. (μg/ml)	Abs ascorbic acid	Absorbance	% Inhibition
Fruit	10	1.1368±0.012	1.0631±0.058	6.48±0.65
	20	1.2056±0.098	1.0341±0.064	14.22±0.058
	30	1.2729±0.098	1.0191±0.069	19.93±0.032
	40	1.3396±0.036	0.9763±0.014	34.12±0.047
	50	1.4191±0.014	0.9131±0.036	49.65±1.025
	60	1.4911±0.015	0.8230±0.045	59.80±0.065
	70	1.5430±0.31	0.8091±0.123	61.56±0.047
	80	1.6039±0.35	0.7631±0.078	64.42±0.069
	90	1.7931±0.098	0.6149±0.321	65.70±0.032
	100	1.8011±0.16	0.5066±0.0312	71.87±0.039
Leaves	10	1.1368±0.098	1.0994±0.12	3.28±0.031
	20	1.2056±0.032	1.0632±0.031	11.81±0.065
	30	1.2729±0.014	1.0394±0.085	18.34±0.045
	40	1.3396±0.036	1.0134±0.98	24.35±0.036
	50	1.4191±0.017	0.9741±1.32	31.35±0.032
	60	1.4911±1.020	0.8331±0.36	44.12±0.014
	70	1.5430±0.167	0.7655±0.014	50.38±0.025
	80	1.6039±0069	0.6021±0.096	62.46±0.096
	90	1.7931±0.030	0.5530±0.78	68.99±0.041
	100	1.8011±0.011	0.5460±0.96	69.68±0.011
Stem	10	1.1368±0.96	1.0975±0.0321	3.45±1.021
	20	1.2056±1.32	1.0937±0.031	9.28±0.025
	30	1.2729±0.032	1.0532±0.036	17.25±0.085
	40	1.3396±0.0321	1.0391±0.014	22.43±0.047
	50	1.4191±0.96	1.0131±0.025	28.60±0.069
	60	1.4911±0.98	1.0112±0.052	32.184±0.14
	70	1.5430±0.321	0.9813±0.095	36.40±0.321
	80	1.6039±0.032	0.9133±0.095	43.05±1.36
	90	1.7931±0.078	0.7313±0.36	59.21±0.98
	100	1.8011±0.987	0.6764±0.085	62.44±0.96
Seed	10	1.1368±0.098	1.0831±0.074	4.72±0.56
	20	1.2056±0.096	1.0822±0.069	10.23±0.31
	30	1.2729±0.014	1.0596±0.036	16.75±1.39
	40	1.3396±0.0321	1.0169±0.32	24.08±0.96
	50	1.4191±0.052	1.0120±0.085	28.68±0.34
	60	1.4911±0.045	0.9321±0.321	37.58±0.96
	70	1.5430±0.065	0.902 9±0.014	41.48±0.34
	80	1.6039±0.096	0.8039±0.015	49.87±0.16
	90	1.7931±0.047	0.7319±0.052	59.18±0.85
	100	1.8011±0.096	0.6930±0.025	61.52±0.31
Root	10	1.1368±0.096	1.0593±0.0056	6.81±0.065
	20	1.2056±0.069	1.0342±0.036	14.21±0.054
	30	1.2729±0.014	1.0133±0.025	20.39±0.310
	40	1.3396±0.054	1.0109±0.014	24.53±0.031
–	50	1.4191±0.063	0.9143±0.098	35.57±0.65
–	60	1.4911±0.096	0.8239±1.02	44.74±01.32
–	70	1.5430±0.085	0.8041±1.036	47.88±0.031
–	80	1.6039±0.085	0.7323±0.018	54.34±0.045
–	90	1.7931±0.078	0.6621±0.38	63.07±0.321
	100	1.8011±0.36	0.6030±1.025	66.52±0.850

(Contd...) / **Table 3: *Continued***

Data presented as±standard error mean of each triplicate test, LS: *Lagenaria siceraria*

Tannins about 0.5 g of the extract were boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added an observed for brownish green or a blue-black coloration.[9]

Steroids (Liebermann–Burchard reaction)

- To 200 mg plant extract add 10 ml chloroform. Take 2 ml of this filtrate and add 2 ml acetic anhydride and conc. H_2SO_4. Blue-green ring indicate steroids (Siddiqui *et al*., 2009).
- 2 ml of acetic anhydride was added to 0.5 g of each extract with 2 ml of H_2SO_4. The color change from violet to blue or green in some samples indicated the presence of steroids.[10]

Saponins

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.[10]

Phytosterols

About 2 ml of acetic anhydride was added to 1 ml extract + 2 ml conc. H_2SO_4. The color change from violet to blue or green indicated the presence of sterols.[7]

Alkaloids

Extracts (2 ml) were dissolved individually in 1% dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

- Mayer's test: Filtrates were treated with few drops of Mayer's reagent (potassium mercuric iodide). Formation of a yellow cream precipitate indicated the presence of alkaloids.
- Wagner's test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish brown precipitate indicated the presence of alkaloids.[11]

Carbohydrates (Molisch's test)

One drop of concentrated sulfuric acid was added to about 1 g of the extract, and then three drops of 1% α-naphthol in 80% ethanol were added to the mixture without mixing to form an upper phase. Formation of the brown or purple ring at the interphase indicated the presence of carbohydrates.[12]

Table 4: Absorbance of a bitter variety of LS of fruit, leaves, stem, seed, and root

Plant part	Conc. (μg/ml)	Abs ascorbic acid	Absorbance	% Inhibition
Fruit	10	1.1368±0.098	1.0321±0.085	9.2±0.0126
	20	1.2056±0.096	1.0129±0.096	15.98±0.321
	30	1.2729±1.025	1.0172±0.094	20.08±0.095
	40	1.3396±0.025	0.9541±0.064	48.77±1.023
	50	1.4191±1.032	0.8941±0.050	52.99±0.32
	60	1.4911±0.032	0.7834±0.045	54.46±0.014
	70	1.5430±0.078	0.6421±0.031	58.38±0.014
	80	1.6039±0.087	0.5899±0.064	63.22±0.069
	90	1.7931±0.021	0.5421±0.034	69.76±0.014
	100	1.8011±0.147	0.4022±0.035	77.66±0.096
Leaves	10	1.1368±0.012	1.0463±0.034	7.96±0.032
	20	1.2056±0.078	1.0191±0.036	15.46±0.004
	30	1.2729±0.014	0.9829±0.034	22.78±0.014
	40	1.3396±0.078	0.9148±0.058	31.71±0.098
	50	1.4191±0.045	0.9090±0.095	35.94±0.032
	60	1.4911±0.064	0.8083±0.031	45.81±0.056
	70	1.5430±0.034	0.7240±0.054	53.07±0.032
	80	1.6039±0.031	0.6019±0.018	62.47±0.074
	90	1.7931±0.048	0.5021±0.095	71.99±0.098
	100	1.8011±0.064	0.4249±0.098	76.40±0.147
Stem	10	1.1368±1.023	1.0231±0.098	10.00±1.20
	20	1.2056±0.032	1.0210±0.095	15.31±0.98
	30	1.2729±0.031	1.0191±0.50	19.93±0.54
	40	1.3396±0.096	0.9021±0.050	32.65±0.065
	50	1.4191±1.032	0.8834±0.060	37.74±1.02
	60	1.4911±0.036	0.8234±0.047	44.77±0.96
	70	1.5430±0.0321	0.7036±0.069	54.40±0.12
	80	1.6039±0.016	0.6246±0.056	61.05±0.078
	90	1.7931±0.015	0.5341±0.024	70.21±0.097
	100	1.8011±0.024	0.4321±0.016	76.00±0.32
Seed	10	1.1368±0.069	1.0993±0.098	3.29±0.047
	20	1.2056±0.031	1.0831±0.21	10.16±0.98
	30	1.2729±0.036	1.0801±0.015	20.38±0.63
	40	1.3396±0.0345	1.0591±0.023	20.93±0.32
	50	1.4191±0.0254	1.0413±0.025	26.62±0.97
	60	1.4911±0.0.031	1.0129±0.014	32.67±1.36
	70	1.5430±0.031	0.9941±0.036	35.57±1.069
	80	1.6039±0.031	0.9539±0.033	40.52±0.96
	90	1.7931±0.068	0.9131±0.069	49.07±0.25
	100	1.8011±0.031	0.8421±0.075	53.24±1.36
Root	10	1.1368±1.032	1.09421±0.098	3.74±0.69
	20	1.2056±0.012	1.079±0.045	10.48±0.69
	30	1.2729±0.096	1.0632±0.034	16.47±0.36
	40	1.3396±0.031	1.0243±0.036	23.53±1.25
	50	1.4191±0.058	1.0148±0.098	28.48±1.30
	60	1.4911±0.068	0.9621±0.058	35.47±1.96
	70	1.5430±0.098	0.9033±0.23	41.45±0.36
	80	1.6039±0.065	0.8341±0.96	47.99±1.89
	90	1.7931±0.031	0.8099±0.034	54.83±0.98
	100	1.8011±0.034	0.7541±0.039	58.13±0.025

(Contd...)

Table 4: *Continued*

Data presented as±standard error mean of each triplicate test, LS: *Lagenaria siceraria*

Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicated the presence of glycosides.[10]

In vitro antioxidant activity

Antioxidant activity should not be concluded based on a single antioxidant test model. Moreover, in practice, several *in vitro* test procedures are carried out for evaluating antioxidant activities with the samples of interest. Another aspect is that antioxidant test models vary in different respects. Therefore, it is difficult to compare fully one method to another one. To some extent comparison among different *in vitro* methods has been done. In general, *in vitro,* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (i.e.,not involved with many steps and reagents) and inexpensive in comparison to other test models.

DPPH assay

The ability of the extracts to scavenge DPPH radicals (DPPH) was determined according to the method prescribed (Zeyep *et al.*, 2007) with minor modifications. Different concentrations of plant extract and standard ascorbic acid solution, namely, 10–100 μg/ml prepared in alcoholic solution. A 50 μl aliquot of extract in 50 mm Tris–HCl buffer (pH 7.4) was mixed with 450 μl of Tris–HCl buffer and 1.0 ml of 0.1 mm DPPH in methanol. After 30 min incubation at ambient temperature. The resultant absorbance was recorded at 517 nm against corresponding blanks (0.01 mm DPPH in methanol), and ascorbic acid was used as a standard. All the tests were performed in triplicate, and the graph was plotted with ± standard error mean of three observations.

H_2O_2 radical scavenging activity

H_2O_2 scavenging activity of the extract was estimated by a previously prescribed method. A solution of H_2O_2 (20 mm) was prepared in phosphate buffer saline (pH 7.4). Different concentrations of plant extract and standard ascorbic acid solution, namely 10–100 μg/ml in methanol (1 ml) were added to the H_2O_2 solution (2 ml). The absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer

Table 5: Absorbance of a sweet variety of LS of fruit, leaves, stem, seed, and root

Plant part	Conc. (µg/ml)	Abs ascorbic acid	Absorbance	% Inhibition
Fruit	10	1.1012±0.15	0.9763±0.095	11.34±0.261
	20	1.1091±0.056	0.9163±0.156	17.39±0.054
	30	1.1278±0.021	0.8316±0.089	26.26±0.087
	40	1.1989±0.098	0.8036±0.056	32.97±1.051
	50	1.2167±1.023	0.7943±0.541	48.71±1.065
	60	1.3730±1.025	0.7130±0.078	51.06±0.089
	70	1.5019±1.056	0.6310±0.048	57.98±0.087
	80	1.5916±1.045	0.6039±1.021	62.05±1.051
	90	1.6204±1.021	0.5210±1.056	67.84±0.083
	100	1.6629±0.513	0.4139±1.054	75.10±1.042
Leaves	10	1.1012±0.15	1.0145±0.139	7.89±0.012
	20	1.1091±0.056	0.9923±0.140	10.53±0.014
	30	1.1278±0.021	0.9251±0.081	17.97±0.096
	40	1.1989±0.098	0.8931±0.025	25.50±0.091
	50	1.2167±1.023	0.8291±0.014	31.85±0.047
	60	1.3730±1.025	0.7793±0.054	43.25±0.025
	70	1.5019±1.056	0.7231±0.052	51.85±0.097
	80	1.5916±1.045	0.6941±0.030	56.38±0.023
	90	1.6204±1.021	0.6029±0.005	62.79±0.031
	100	1.6629±0.513	0.5531±0.011	66.73±0.056
Stem	10	1.1012±0.15	1.0653±0.102	3.26±0.36
	20	1.1091±0.056	1.0134±0.360	8.62±0.12
	30	1.1278±0.021	0.9854±0.012	12.60±0.032
	40	1.1989±0.098	0.9128±0.036	23.93±0.065
	50	1.2167±1.023	0.8721±0.058	28.32±0.096
	60	1.3730±1.025	0.8591±0.012	37.42±0.085
	70	1.5019±1.056	0.8953±0.14	40.53±0.039
	80	1.5916±1.045	0.8231±0.066	48.28±0.065
	90	1.6204±1.021	0.7714±0.036	54.39±0.014
	100	1.6629±0.513	0.7328±0.010	55.93±0.012
Seed	10	1.1012±0.15	1.0879±0.32	1.2±0.034
	20	1.1091±0.056	1.0643±0.012	3.67±0.064
	30	1.1278±0.021	1.0461±0.032	5.63±0.090
	40	1.1989±0.098	1.0386±0.086	13.37±0.015
	50	1.2167±1.023	1.0234±0.069	15.70±0.032
	60	1.3730±1.025	1.0965±0.34	20.23±0.033
	70	1.5019±1.056	1.1896±0.314	20.79±0.051
	80	1.5916±1.045	1.1896±0.001	24.68±0.031
	90	1.6204±1.021	1.1891±0.097	25.30±0.015
	100	1.6629±0.513	1.2103±0.034	27.08±0.014
Root	10	1.1012±0.15	1.0361±0.018	5.91±0.045
	20	1.1091±0.056	1.0139±0.310	8.58±0.020
	30	1.1278±0.021	0.9821±0.63	12.91±0.31

(Contd...)

Table 5: *Continued*

Plant part	Conc. (µg/ml)	Abs ascorbic acid	Absorbance	% Inhibition
	40	1.1989±0.098	0.9130±0.064	23.84±0.045
	50	1.2167±1.023	0.8396±0.034	30.99±0.080
	60	1.3730±1.025	0.8791±0.025	35.97±0.035
	70	1.5019±1.056	0.9061±0.085	39.66±0.096
	80	1.5916±1.045	0.8221±0.014	48.34±0.36
	90	1.6204±1.021	0.7596±0.060	53.12±0.21
	100	1.6629±0.513	0.6930±0.18	58.32±0.092

Data presented as±standard error mean of each triplicate test, LS: *Lagenaria siceraria*

without H_2O_2. For each concentration, a separate blank sample was used for background subtraction. The experiment was performed in triplicate.[13]

Reducing power assay

The Fe3 + reducing the power of the extract was determined by a previously described method. The methanolic extract (10–100 µg/ml) was mixed with 2.5 mL of phosphate buffer (0.2 m, pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe(CN)6] (1%), and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5 mL FeCl3 (0.1%), and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate, and the graph was plotted with the average of three observations. Increased absorbance of the reaction mixture indicated increased reducing power.[14]

Results and discussion

The results are expressed as mean ± standard error of three observations. The percentage inhibition of various radicals was calculated by comparing the results of the test with those of control using the formula (Shirwaikar *et al*., 2004).

% inhibition=absorbance (control)−absorbance (test)/absorbance (control)×100.

Phytochemicals screening

Several concentrations ranging from 10 to 100 µg/ml of the ethanolic extract were compared for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extracts in a concentration-dependent manner (within the predetermined concentration range) in all the models [Tables 1 and 2].

DPPH radical scavenging activity

Free radicals scavenging activity of DPPH has been widely used to evaluate the antioxidant activity of natural products obtained from plant and microbial sources. In DPPH scavenging activity model, it was observed that EELS (10–100 µg/ml) significantly scavenged DPPH,

Table 6: Absorbance of bitter variety of LS of fruit, leaves, stem, seed, and root

Plant part	Conc. (μg/ml)	Abs ascorbic acid	Absorbance	% Inhibition
Fruit	10	1.1012±0.15	1.0413±0.15	5.43±0.0113
	20	1.1091±0.056	1.0306±0.014	17.07±0.074
	30	1.1278±0.021	0.9763±0.032	37.92±0.023
	40	1.1989±0.098	0.8441±0.032	47.93±0.014
	50	1.2167±1.023	0.7831±0.058	51.52±0.052
	60	1.3730±1.025	0.7011±0.065	55.13±0.032
	70	1.5019±1.056	0.6730±0.078	60.10±0.14
	80	1.5916±1.045	0.6350±0.034	60.10±0.005
	90	1.6204±1.021	0.6129±0.045	62.17±0.031
	100	1.6629±0.513	0.5531±0.09	63.73±0.014
Leaves	10	1.1012±0.15	1.0793±0.012	1.98±0.010
	20	1.1091±0.056	1.0541±0.021	4.95±0.032
	30	1.1278±0.021	1.0192±0.0321	9.62±0.025
	40	1.1989±0.098	1.0106±0.0325	15.70±0.065
	50	1.2167±1.023	0.9831±0.032	34.29±0.056
	60	1.3730±1.025	0.9021±0.06	44.49±0.036
	70	1.5019±1.056	0.8337±0.005	51.75±0.98
	80	1.5916±1.045	0.7043±0.025	55.75±0.032
	90	1.6204±1.021	0.6321±0.014	60.99±0.15
	100	1.6629±0.513	0.5950±0.034	64.21±0.014
Stem	10	1.1012±0.15	1.0639±0.012	3.38±0.033
	20	1.1091±0.056	1.0331±0.24	6.85±0.0325
	30	1.1278±0.021	0.9940±0.13	11.78±0.031
	40	1.1989±0.098	0.9151±0.025	23.67±0.16
	50	1.2167±1.023	0.9069±0.036	39.32±0.24
	60	1.3730±1.025	0.8331±0.098	48.33±0.015
	70	1.5019±1.056	0.7621±0.025	52.23±0.04
	80	1.5916±1.045	0.6913±0.014	56.56±0.096
	90	1.6204±1.021	0.6534±0.0231	59.67±0.054
	100	1.6629±0.513	0.6243±0.085	62.45±0.012
Seed	10	1.1012±0.15	1.0691±0.015	2.9±0.014
	20	1.1091±0.056	1.0331±0.069	6.85±0.036
	30	1.1278±0.021	0.0101±0.007	10.43±0.01
	40	1.1989±0.098	0.9931±0.025	17.16±0.032
	50	1.2167±1.023	0.9136±0.12	24.91±0.064
	60	1.3730±1.025	0.9063±0.013	33.99±0.085
	70	1.5019±1.056	0.8534±0.098	43.17±0.065
	80	1.5916±1.045	0.8334±0.065	47.63±0.16
	90	1.6204±1.021	0.8013±0.10	50.54±0.13
	100	1.6629±0.513	0.7734±0.30	55.49±0.019
Root	10	1.1012±0.15	1.0510±0.13	4.5±0.045
	20	1.1091±0.056	1.0121±0.014	8.74±0.021
	30	1.1278±0.021	0.9934±0.31	11.91±0.096
	40	1.1989±0.098	0.9311±0.09	22.33±0.10
	50	1.2167±1.023	0.8944±0.030	26.48±0.036
	60	1.3730±1.025	0.8511±0.15	38.01±0.015
	70	1.5019±1.056	0.8019±0.13	46.60±0.014
	80	1.5916±1.045	0.7869±0.09	50.55±0.085
	90	1.6204±1.021	0.7402±0.33	54.31±0.069
	100	1.6629±0.513	0.7109±0.25	57.24±0.15

(Contd...)

Table 6: *Continued*

Data presented as±standard error mean of each triplicate test, LS: *Lagenaria siceraria*

in a concentration-dependent manner. However, extract showed weak scavenging activity in lower concentrations the higher concentrations exhibited promising DPPH scavenging activity. DPPH is a relatively stable free radical and the assay determines the ability of EELS to reduce DPPH to the corresponding hydrazine by converting the unpaired electrons to form pairs. This conversion is the action of the antioxidant.

DPPH assay; sweet LS

The result of DPPH scavenging activity assay in this study indicated that the plant was potently active and the fruit sweet variety consist lesser antioxidant activity as that of the bitter variety [Table 3].

DPPH assay; bitter LS

The result of DPPH scavenging activity assay in this study indicated that the plant was potently active and the fruit of the bitter veriety possese strong antioxidant activity. This suggested that the plant extract did contain compounds that could be capable of donating hydrogen to a free radical in order to remove the odd electron which is responsible for the radical's reactivity [Table 4].

H_2O_2 radical scavenging activity of sweet LS

Ethanolic extract of LS also demonstrated H_2O_2 decomposition activity in a concentration-dependent manner. The decomposition of H_2O_2 by ethanolic extract of LS might have partly resulted from its antioxidant and free radical scavenging activity [Table 5].

H_2O_2 radical scavenging activity of bitter LS

Ethanolic extract of lagenaria siceraria also demonstrated H2O2 decomposition activity in a concentration dependent manner. The decomposition of H2O2 by ethanolic extract of lagenaria siceraria might have partly resulted from its antioxidant and free radical scavenging activity. (SAME AS THAT OF SEWWT LS) [Table 6].

Reducing power activity

For the measurements of the reducing ability the Fe3+–Fe2+ transformation was investigated in the presence of EELS. Such reducing capacity of a compound might serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants would have been assigned to various mechanisms such as

Table 7: Absorbance of different parts of sweet and bitter LS

Conc. μg/ml	Absorbance of different parts of sweet LS					
	Ascorbic acid	Fruit	Leaves	Stem	Root	Seed
10	4.2029±0.05	5.3039±0.02	4.3129±0.01	4.1019±0.08	3.3316±0.02	3.0931±0.08
20	4.2836±0.03	5.3136±0.02	4.4036±0.03	4.2233±0.04	3.4689±0.09	3.696±0.15
30	4.3996±0.08	5.3941±0.03	4.4301±0.01	4.3641±0.04	3.4951±0.03	4.1016±0.34
40	4.4139±0.06	5.5615±0.02	4.4915±0.09	4.3055±0.09	3.5359±0.05	4.3214±0.15
50	4.4256±0.03	5.6193±0.01	4.5306±0.01	4.4132±0.08	3.6116±0.04	4.3316±0.34
60	4.4649±0.03	5.6210±0.08	4.5501±0.01	4.5913±0.08	3.8019±0.01	4.3901±0.96
70	4.5653±0.03	5.7989±0.03	4.5916±0.04	4.6661±0.03	3.8913±0.01	4.4056±0.07
80	4.596±0.07	5.9634±0.02	4.663±0.03	4.893±0.06	3.9011±0.07	4.5318±0.34
90	4.6163±0.09	6.8689±0.01	4.80±0.03	4.9±0.036	4.6013±0.03	4.653±0.39
100	4.697±0.02	6.9013±0.09	4.337±0.09	4.931±0.01	4.7018±0.07	4.7019±0.37
Absorbance of different parts of bitter LS						
10	4.3317±0.01	4.9613±0.07	4.2788±0.09	4.0913±0.06	4.0611±0.09	4.0101±0.06
20	4.2137±0.07	4.9906±0.03	4.3488±0.03	4.1052±0.09	4.1216±0.02	4.1096±0.02
30	4.3624±0.03	4.3012±0.03	4.4906±0.01	4.3631±0.03	4.2019±0.98	4.3929±0.03
40	4.5821±0.03	4.3906±0.03	4.5013±0.10	4.4143±0.03	4.3656±0.06	4.5601±0.03
50	4.6964±0.01	4.3909±0.06	4.5096±0.15	4.5802±0.06	4.5994±0.03	4.5796±0.03
60	4.6001±0.03	4.4601±0.09	4.6301±0.03	4.6143±0.03	4.6399±0.03	4.6031±0.03
70	4.6211±0.04	4.4994±0.05	4.8839±0.03	4.6633±0.05	4.6143±0.02	4.6316±0.08
80	4.7042±0.01	4.5113±0.06	4.9401±0.03	4.6931±0.03	4.6997±0.01	4.6718±0.09
90	4.7182±0.03	4.5688±0.03	4.9602±0.09	4.8259±0.08	4.8319±0.03	4.9339±0.06
100	4.8911±0.03	4.6369±0.06	4.9713±0.03	4.8396±0.01	4.8936±0.01	4.9396±0.02

Data presented as ±standard error mean of each triplicate test, LS: *Lagenaria siceraria*

Table 8: % Inhibition of DPPH free radicals of a sweet and bitter variety of LS

Conc. μg/ml	% Inhibition of DPPH free radicals of sweet variety of LS				
	Fruit	Leaves	Stem	Seed	Root
10	6.48±0.65	3.28±0.031	3.45±1.02	4.72±0.56	6.81±0.06
20	14.22±0.05	11.81±0.06	9.28±0.02	10.23±0.31	14.21±0.05
30	19.93±0.03	18.34±0.04	17.25±0.08	16.75±1.39	20.39±0.31
40	34.12±0.04	24.35±0.03	22.43±0.04	24.08±0.96	24.53±0.03
50	49.65±1.02	31.35±0.03	28.60±0.06	28.68±0.34	35.57±0.65
60	59.80±0.06	44.12±0.01	32.184±0.14	37.58±0.96	44.74±01.32
70	61.56±0.04	50.38±0.02	36.40±0.32	41.48±0.34	47.88±0.031
80	64.42±0.06	62.46±0.09	43.05±1.36	49.87±0.16	54.34±0.04
90	65.70±0.03	68.99±0.04	59.21±0.98	59.18±0.85	63.07±0.32
100	71.87±0.03	69.68±0.01	62.44±0.96	61.52±0.31	66.52±0.85
% Inhibition of DPPH free radicals of bitter variety of LS					
10	9.2±0.0126	7.96±0.032	10.00±1.20	3.29±0.047	3.74±0.69
20	15.98±0.32	15.46±0.00	15.31±0.98	10.16±0.98	10.48±0.69
30	20.08±0.09	22.78±0.01	19.93±0.54	20.38±0.63	16.47±0.36
40	48.77±1.02	31.71±0.09	32.65±0.05	20.93±0.32	23.53±1.25
50	52.99±0.32	35.94±0.03	37.74±1.02	26.62±0.97	28.48±1.30
60	54.46±0.01	45.81±0.05	44.77±0.96	32.67±1.36	35.47±1.96
70	58.38±0.01	53.07±0.03	54.40±0.12	35.57±1.09	41.45±0.36
80	63.22±0.06	62.47±0.07	61.05±0.08	40.52±0.96	47.99±1.89
90	69.76±0.01	71.99±0.09	70.21±0.07	49.07±0.25	54.83±0.98
100	77.66±0.09	76.40±0.14	76.00±0.32	53.24±1.36	58.13±0.05

DPPH: 1, 1-Diphenyl-Picryl-2-hydrazyl, LS: *Lagenaria siceraria*

Table 9: % Inhibition of H_2O_2 radical scavenging activity of sweet variety of LS

Conc. µg/ml	% Inhibition of H_2O_2 radical scavenging activity of sweet variety of LS				
	Fruit	Leaves	Stem	Seed	Root
10	11.34±0.261	7.89±0.012	3.26±0.36	1.2±0.034	5.91±0.045
20	17.39±0.054	10.53±0.014	8.62±0.12	3.67±0.064	8.58±0.020
30	26.26±0.087	17.97±0.096	12.60±0.032	5.63±0.090	12.91±0.31
40	32.97±1.051	25.50±0.091	23.93±0.065	13.37±0.015	23.84±0.045
50	48.71±1.065	31.85±0.047	28.32±0.096	15.70±0.032	30.99±0.080
60	51.06±0.089	43.25±0.025	37.42±0.085	20.23±0.033	35.97±0.035
70	57.98±0.087	51.85±0.097	40.53±0.039	20.79±0.051	39.66±0.096
80	62.05±1.051	56.38±0.023	48.28±0.065	24.68±0.031	48.34±0.36
90	67.84±0.083	62.79±0.031	54.39±0.014	25.30±0.015	53.12±0.21
100	75.10±1.042	66.73±0.056	55.93±0.0120	27.08±0.014	58.32±0.092
% Inhibition of H_2O_2 radical scavenging activity of bitter variety of LS					
10	5.43±0.0113	1.98±0.010	3.38±0.033	2.9±0.014	4.5±0.045
20	17.07±0.074	4.95±0.032	6.85±0.0325	6.85±0.036	8.74±0.021
30	37.92±0.023	9.62±0.025	11.78±0.031	10.43±0.01	11.91±0.096
40	47.93±0.014	15.70±0.065	23.67±0.16	17.16±0.032	22.33±0.10
50	51.52±0.052	34.29±0.0560	39.32±0.24	24.91±0.064	26.48±0.036
60	55.13±0.032	44.49±0.036	48.33±0.015	33.99±0.085	38.01±0.015
70	60.10±0.14	51.75±0.98	52.23±0.04	43.17±0.065	46.60±0.014
80	60.10±0.005	55.75±0.032	56.56±0.096	47.63±0.16	50.55±0.085
90	62.17±0.031	60.99±0.15	59.67±0.054	50.54±0.13	54.31±0.069
100	63.73±0.014	64.21±0.014	62.45±0.012	55.49±0.019	57.24±0.15

LS: *Lagenaria siceraria*, H_2O_2: Hydrogen peroxide

prevention of chain initiation, binding of transition metal ion catalysts decomposition of peroxides prevention of continued hydrogen abstraction reductive capacity and radical scavenging [Table 7].

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Similar to the antioxidant activity, the reducing power of EELS increased with increasing concentration. The result showed that EELS consists of hydrophilic polyphenolic compounds that might have caused the greater reducing power.

Determination of half maximal inhibitory concentration (IC_{50}) value

The concentration of the extract at which the intensity of DPPH solution is reducing to 50% to its original intensity is called IC_{50}. The IC_{50} value of the sweet extract was determined, and they were compared with the IC_{50} value of the bitter LS so that the free radical scavenging capacity of the sweet LS extracts as compared to bitter could be calculated. The free radical scavenging activity of sweet LS is shown in following table, in which the IC_{50} value was obtained on various concentrations in different parts. The percent inhibition of DPPH free radicals by the bitter fruit was obtained at 40 µg\ml concentration which is maximum as compared to other part.

The result showed that as the concentration of extract increase, percent inhibition against DPPH free radicals also increases, which clearly indicates the radical scavenging potential of the extract.

Data presented as IC_{50} value of various part of sweet and bitter LS.

Data presented as IC_{50} value of various part of sweet LS [Table 8 and 9].

Discussion

a. In present study, identification collection and authentication of sweet and bitter verities of LS were successfully done.
b. Defattation was carried out to remove the fat by petroleum ether.
c. Extraction of the following parts of sweet and bitter variety of LS was done by hydroalcoholic solution.
   - Fruit
   - Seed
   - Leaves
   - Stems
   - Roots.

The qualitative test represents the presence of various phytochemical constituent such as flavonoid, terpenoid, and phytosterol.

The extract of bitter LS has high antioxidant activity, antioxidant

activity of the extract was located by DPPH free radical scavenging activity and H_2O_2 method.

Conclusion

The results obtained in the present study indicated that LS both variety all parts extract exhibited free radical scavenging activity against H_2O_2 and DPPH. The overall antioxidant activity of ethanolic extract of LS might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggested that LS bitter fruit could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or solving the progress of reactive oxygen species and associated oxidative stress-related degenerative diseases.

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