AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Characterization of antioxidant property and Chemical compositions of Lagenaria Siceraria stand. Fruit extract

Sarang Sunil Mahamuni*[1] ,Vishal Sanjay Savant[2]

1.Satara College of Pharmacy Satara

2. Sant Gajanan Mahajaj College of Pharmacy. Gadhinglaj Kolhapur

ABSTRACT

Last few decades, research data has prompted a passionate debate as to whether oxidation, or specifically, oxidative stress mediated by free radicals/reactive oxygen species (ROS)/reactive nitrogen species (RNS), is a primary or secondary cause of many chronic diseases. Lagenaria siceraria is fair source of ascorbic acid, beta carotene and different phytochemicals to heal many disorders. It shows ribonucleolytic, antihyperlipidemic, hepatoprotective, antioxidant, immunomodulatory, cardiotonic activity. The collection and identification of juice of Lgenaria siceraria and preparation of four fractions of concentrated juice were carried out and preliminary phytochemical and pharmacological screening of same fractions was done. Preliminary pharmacological screening of each fraction was investigated to find out desired in-vitro antioxidant activity. Aqueous fraction showed better yield because of polarity of compound. Preliminary phytochemical screening of four different fractions had various phytochemicals like steroids, flavonids, glycosides, saponins, etc. As we had followed the biological activity guided isolation of active constituents, the fractions were evaluated for antioxidant activity. Isolated aliphatic and saponins moiety may be responsible for the antioxidant activity.

Keywords: Lagenaria siceraria, antioxidant activity, phytochemical and pharmacological screening.

*Corresponding Author Email: sarang259@gmail.com

Received 14 August 2016, Accepted 26 August2016

Please cite this article as: Mahamuni SS *et al*., Characterization of antioxidant property and Chemical compositions of Lagenaria Siceraria stand. Fruit extract. American Journal of PharmTech Research 2016.

INTRODUCTION

Scientific resources have focused to a large extent on the role that antioxidants could play to delay or prevent oxidative stress and consequently the incidence of chronic disorders. Plant shows different chemical moiety including flavonoids [1-3], trepinods [1-3] and steroids [1-3] which have the pharmacological properties like Antiulcer [4], Antihyperlipidemic [5-6], antioxidant [7-8], cytotoxic [9] as well.

Lagenaria siceraria Standley, commonly known as bottle-gourd (in English), belongs to the Cucurbitaceae family. The plant is widely available throughout India. It is a climbing or trailing herb, with bottle- or dumb-bell shaped fruits. Both its aerial parts and fruits are commonly consumed as a vegetable. Traditionally, it is used as medicine in India, China, European countries, Brazil, Hawaiian island, etc. for its cardiotonic, general tonic and diuretic properties.

Lagenaria siceraria Standley Fruit has different biological activities, as traditional medicinal plants, such as Antihyperlipidemic, antidiabetic, antiulcer and prominently antioxidant activity. So the present communication deals with successive extraction of Lagenaria siceraria Standley Fruit. for antioxidant activity. This activity was screened by DPPH radical scavenging activity. The DPPH radical scavenging activity was selected because this is easy to done and give fastest promising results.

MATERIALS AND METHOD

The dry fruit of the plant *Lagenaria siceraria* Standley was collected by cutting the fruit from climbing plant which was stay on other big plant trunk from the local area of Vaduj District of Satara, Maharashtra, India. The plant was identified by botanist, Head of Department of botany, Yashawantrao chavan institute of science satara, Maharashtra. After proper identification, voucher specimen was deposited in department of Pharmacognosy, Satara college of pharmacy, Satara Near Midc, Satara, Maharashtra.

Equipment and Apparatus

Soxhlet, apparatus, Mettler analytical balance, Rotamentle (J-SIL), Rotary film evaporator (Evator). Petri plats, Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved in one liter of analytical grade methanol. Ascorbic acid solution in methanol (10**µ**g/ml.). Test samples of each dry fraction were prepared by dissolving in methanol in the concentration of 10**µ**g/ml.

Preparation of fresh juice and its fractions

Fresh fruits (7 kg) were cut in to small pieces and grinded in the mixer grinder. The resulting juice (5.5 lit.) was passed through muslin cloth and centrifuged at 2500 rpm for 15 minutes to obtain clear brownish juice. Extractive values are mention in table 1.[10]

Table 1: Percentage Yield of *Lagenaria Siceraria* Standely Fruit Extracts

Sr. No.	Solvent	Colour	Consistency	% Yield
1	Petroleum ether	Reddish brown.	Viscous	12.83
2	Chloroform	Reddish brown.	Viscous	2.78
3	Ethyl acetate	Pale brown.	Viscous	1.28
4	Aqueous	Faint brown.	Viscous	94.65

Phytochemical Screening

All the extracts obtained were subjected for Phytochemical screening using standard procedure. [10-11] The dried extracts were dissolved in sufficient amount of respective solvents and tested for various constituents. The results of the tests are mentioned in table 2.

Table 2: Preliminary Phytochemical Screening of *Lagenaria Siceraria* Standely Fruit

Tests	PE	CH	EA	AQ
Tests for Sterols				
2. Salkowski's test	+	–	–	–
4. Libermann Burchard's Test	+	–	–	–
Test for Glycosides				
1. Baljet's test	–	–	+	+
2. Keller–killiani test	–	–	+	+
Tests for Saponins				
1. Foam Test	–	–	–	+
Test for Carbohydrates				
1.Molish's Test	–	–	+	+
2. Fehling test (for reducing sugars)	–	–	+	+
3. Barfoed's test (for monosaccharides)	–	–	–	+
4. Bials arcinol test (for pentose sugar)	–	–	–	–
5.Selvinoff's test (for hexose sugar)	–	–	–	+
6. Non reducing sugars	–	–	–	–
Tests for Alkaloids				
1. Mayer's test.	–	–	–	–
3. Dragendorff's test	–	–	–	–
Tests for Flavonoids				
1. Ferric chloride test.	–	–	+	+
2. Shinoda test.	–	–	+	+
Tests for Tannins				
1. Ferric chloride test.	–	–	–	+
Tests for Amino acid				
1.Millions test	–	–	–	+
2.Ninhydrin test	–	–	–	+

Test for Triterpenes				
1. Salkowski's test	_	_	_	+
2. Libermann Burchard's test	_	_	_	+

Preliminary pharmacological screening

After the preliminary phytochemical screening biological activity of each fraction was investigated to find out which fraction shows the promising results. For this purpose two biological activities are targeted which indicates the beneficial effects of sample on cardiovascular diseases such as *In vitro* antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method.

Procedure

0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of test sample. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at the same concentration (10**µ**g/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = A control – A test/ A control X 100

Where, A control is the absorbance of the control reaction and **A test** is the absorbance in the presence of sample.

Separation of tannins

As we know tannins are the most unstable components to environmental conditions and they cause interference with the other constituents during the process of isolation so tannin are separated by treating aqueous fraction with the 10 % lead acetate solution. Total 100 gm of dried aqueous fraction was dissolved in 600 ml of distilled water and treated with 50 ml of 10 % lead acetate solution. The resulting ppt. of tannins was filtered through filter paper. Remaining filtrate was again treated with 50 ml of 10 % lead acetate again precipitation occurred which was separated by filtration. Now small amount of the filtrate was further tested for precipitation by treating with 10 % lead acetate solution. It was observed that there is no precipitation and so concluded that total tannins have been removed.

Hydrolysis, fractionation, and pharmacological studies of aqueous fraction

Hydrolysis

It was observed that the fractions made without hydrolysis are very small in quantity because they might be present in glycosidic form and have more affinity for the aqueous solvent. Hence the most of the active constituents are present in aqueous fraction. To remove sugar moiety from its

aglycone part hydrolysis of the aqueous fraction was done by a conventional method of refluxing the aqueous fraction with 2M HCL for two hours.

Fractionation

Total six different fractions of the aqueous fraction were prepared by using solvents of increasing polarity such as petroleum ether, chloroform, diethyl ether, ethyl acetate, n- butanol and aqueous using separating funnel. All the fractions were dried using rotary vacuum evaporator weighed and percent yield of fraction (w/v) were calculated.

Pharmacological screening

Now again the fractions obtained are evaluated for the same biological activity as above to find out the most active fraction.

Antioxidant activity

Similar method was used for the evaluation of antioxidant activity as above using different concentrations such as 2, 4, 8, 16µg/ml of standard and samples and the antioxidant activity of the fractions were expressed as IC_{50} and compared with standard. The IC_{50} value was defined as the concentration of fractions that inhibits the formation of DPPH radicals by 50 %.

RESULTS AND DISCUSSION

The present study explores Preliminary phytochemical screening of four different fractions of Lagenaria siceraria fruit shows presence of various phytoconstituents like carbohydrates, steroids, glycosides, tannins, saponins, flavonoids. Aqueous fraction shown to have pentose, hexose and non reducing sugars, steroids, flavonoids, and tannins. As aqueous extract has all types of phytoconstituents and better extraction yield, have further scope for isolation and pharmacological studies of plant. As we had followed the biological activity guided isolation of active constituents the fraction were evaluated for antioxidant activity. The activity is compared with that of fresh juice of the fruit and it was observed that all the fractions has antioxidant but the fresh juice and the aqueous fraction have the most prominent antioxidant activity (41.62%) The reason behind the equivalent activity of aqueous fraction to that of fresh juice may be the same as above that most of the active constituents were present in to the aqueous fraction. Antioxidant activity of aqueous fraction made before hydrolysis has the maximum activity of 41.62% as compared to the other fractions but lesser activity than that of standard ascorbic acid.

Table 3: Observations of antioxidant activity of before hydrolysis fractions

Sr.No.	Name of sample	Conc. (mcg)	Absorbance*	% Activity
1	Blank	-----	0.0185	-----
2	PE	10	0.0162	12.43
3	CH	10	0.0143	22.70
4	EA	10	0.0139	24.86
5	AQ	10	0.0108	41.62
6	STD	10	0.0078	57.83

*mean of three determinations.

Table 4: Percent yield of after hydrolysis fractions

Sr. No.	Name of fraction	% Yield
1.	PE	1.14
2.	CH	5.42
3.	DE	4.85
4.	EA	8
5.	NB	20.94
6	AQ	58.71

PE- Petroleum ether, CH- Chloroform, AQ- Aqueous DE-Diethyl Ether, EA- Ethyl Acetate, NB - n-butanol

Table 5: Appearance of after hydrolysis fractions.

Sr. No.	Fraction	Consistency	Colour
1.	PE	Viscous	Reddish brown.
2.	CH	Viscous	Reddish brown.
3.	DE	Viscous	Reddish brown.
4.	EA	Viscous	Reddish brown.
5.	NB	Viscous	Reddish brown.
6	AQ	Viscous	Reddish brown.

Table 6 Observations for DPPH scavenging activity

Sr. No.	Name	Conc. (µg/ml)	Absorbance	% Activity*	I C $_{50}$ (µg/ml)
1	Std.	1	0.0143	25.13	
		2	0.0114	39.68	
		4	0.0087	53.96	4
		8	0.0044	76.71	
		16	0.0033	82.53	
2	PE	1	0.0164	13.22	
		2	0.0149	21.16	
		4	0.0126	33.33	8
		8	0.0094	50.26	
		16	0.0069	63.49	
3	CH	1	0.0175	07.40	
		2	0.0164	13.22	
		4	0.0151	20.10	16
		8	0.0119	37.03	
		16	0.0087	53.96	
4	DE	1	0.0160	15.34	

		2	0.0141	25.39	
		4	0.0128	32.27	8
		8	0.0096	49.20	
		16	0.0049	74.07	
5	EA	1	0.0149	21.16	
		2	0.0118	37.56	
		4	0.0079	58.20	3
		8	0.0043	77.24	
		16	0.0027	85.27	
6	NB	1	0.0172	8.99	
		2	0.0160	15.34	
		4	0.0136	28.04	11
		8	0.0103	45.50	
		16	0.0064	66.13	

Blank= 0.0189 *mean of three determinations.

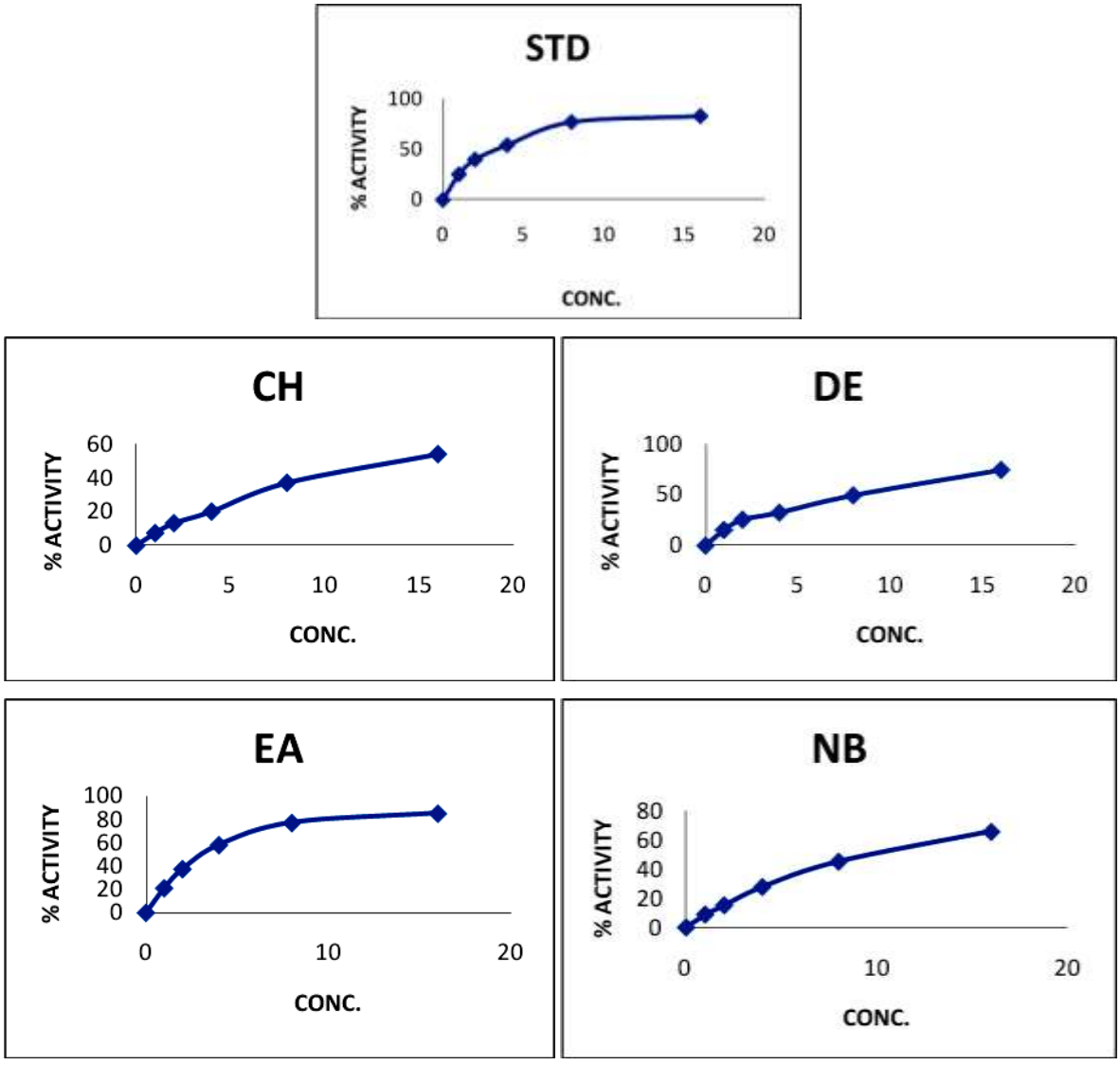

Figure 1: Plot of % DPPH radical scavenging activity versus concentration of standard and different fractions.

CONCLUSION

The activity is compared with that of fresh juice of the fruit and it was observed that all the fractions has antioxidant but the fresh juice and the aqueous fraction have the most prominent antioxidant activity. Antioxidant activity of aqueous fraction made before hydrolysis has the maximum activity. Further isolation of active moiety from extract for antioxidant activity by chromatographic techniques is almost completed.

ACKNOWLEDGEMENT

The authors are thankful to the Dr. N. H. Alurkar Principal, Satara College of Pharmacy, Satara, India, for providing facilities for carrying out work.

REFERENCES

1. Gennari, C., Castoldi D. and Sharon O. Pure and Appl. Chem., 2007; 79(2): 173-180.
2. Gangwal A., Parmar S. K., Sheth N. R. Scholars Research Library, 2010; 2 (1): 307-317.
3. Meenal S. K., Khadabadi S. S., Farooqui I. A., Deore S. L.Report And Opinion, 2010; 2(3).400-410
4. Prajapati R. P., Kalariya M, Paramar S. K., Sheth N. R,.2010; IP: 117. 241.248.98.
5. Shrivastav V., Dr. Rao Ch. V., Pasndy A., Yadav V. International journal of Pharmaceutical Research and Development (IJPRD), 2011;Vol. 3(7): 187-192.
6. Nainwal P., Dhamija K., Tripathi S. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; Vol 3, Issue 1: ISSN- 0975-1491.
7. Saha P., Mazumder U. K., Haldar P. K, Sen S.K., Naskar S. Original Research Article Accepted, 2011.Vol 4(5):203-217.
8. Deore S. L., Khadabadi S.S., Patel Q. R., Deshmukh S. P., Jaju M. S., Junghare N. R., Wane T.P. And. Jain R. G. Rasayan Journal, 2009; Vol.2: No.1, 129-132
9. Saha P., Mazumder U. K., Haldar P. K., Islam A. and. Suresh Kumaz R. B. International Journal of Pharmaceutical Sciences and Research, 2011; Vol. 2(6):748-753
10. Harborne, J. B. Phytochemical Methods. 1thed Chapman and Hall, London Springer publication: 1998, 10-23.
11. Khandelwal KR. Preliminary Phytochemical screening: Practical Pharmacognosy. 6th ed., Pune, India Nirali Prakashan: 2006, 49-539.

12. Nikhat F, D.Satynarayana, and Subhramanyam EVS. Isolation, Charectrisation and Screening of Antioxidant Activity of the Roots of Syzygiumcuminii (L) Skeel, Asian J. Research Chem. 2009; 2(2):218-221.