Investigation of Aloe vera extract for antimutagenicity and Antioxidant Activities

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Abstract- In the present investigation , Antimutagenicity, antioxidant and phytochemicals investigations have been carried out. The 50 % methanolic extract of Aloe vera showed the presence of Alkaloids, Carbohydrate, Resin, Saponins, Glycoside and Tanins whereas Protein, Starch. Flavonods and Steroids were absent .The different concentration of Resin (2.5 %) , Tannin (1.95%) , Carbohydrate (1.34 %) and Alkaloids (0.67 %) were quantified. In Micronuceus assay , single application of Aloe Vera ext. at the dose of 125 , 250 and 375 mg/kg b.wt.,24hours prior the i.p. administration of Cyclophosphamide have significantly prevented the micronucleus formations in bone marrow cells of mice. It was also observed that single application of aloe vera extract at the dose of 250 mg/kg have not significantly induced micronuclei formation as compared to solvent control. The dose dependent inhibition of TBARS formation at the different concentration of Aloe vera extract ranging from 100 ug/ml to 1,000 ug/ml was studied using T Bars method.. The Ascorbic acid was used as positive control. The IC 50 values of Ascorbic acid and of Aloe vera extract was found to be 40 µg/ml and 60µg/ml, respectively.

Index Terms- Aloe vera; Micronucleus ; Antioxidant, , Phytochemicals

I. INTRODUCTION

Aloe belonging to family Liliaceae that originated in South Africa, but have been indigenous to dry subtropical and tropical climates, including the southern USA, (Reynolds et al., 1999). Aloe vera has been used for medicinal purposes in several cultures for millennia (Foster et al., 2011). There is broad list of the therapeutic claims of different parts of Aloe vera due to its Pharmacological activities which are employed in traditional management of diverse veterinary and human diseases, (Blumenthal et al., 1998). The herb is used internally to combat most digestive problems, including constipation, poor appetite, colitis, irritable bowel syndrome as well as, asthma, diabetes, immune system enhancement, peptic ulcers, (Brusick et al., 1997; Mansour et al., 2013; Ezuruike et al., 2014; Kavyashree et al., 2015; Pandey et al., 2016). Aloe vera has many biological and pharmacological activities. These effects are due to the variety of the chemical compounds including anthraquinones, glycoproteins, polysaccharides, vitamins and enzymes contained (Choi and Chung 2003; Du Plessis and Hamman 2014; Akev et al. 2015; Shrestha et al. 2015). Many of the medicinal effects have also been attributed to the immunomodulatory properties of the inner gel (Im et al. 2010), but it is also believed that synergistic action of the compounds contained in the whole leaf extracts is responsible for the multiple and diverse beneficial properties of the plant (Eshun and He 2004). The mucilageneous gel part of A. vera is used commercially as a softener in various cosmetic preparations, soaps and shampoos due to its glycoprotein content. The first known effects of A. vera after its cathartic activity, is the wound and burn healing effect of the leaves gel portion, widely supported in scientific literature (Capasso et al. 1998; Chithra et al. 1998; Heggers et al. 1995). Because of this effect, the gel is also added to many preparations used for skin treatment.

Material and Method

Chemicals

The leaves of Aloe vera were collected from local areas of Satna M.P.(India). Authenticated by the botanist Dr.Manoj Tripathi of Deen Dayal Research Centre, Chitrakoot, Madhya Pradesh (India), where the herbarium was deposited .

Animals:

Random bred male Swiss Albino mice of (6-8 weeks old) of 15- 20 grams body weight were obtained from the animal Colony of our research centre. They were kept on synthetic pellet diet and water ad libitum. The experiment was approved by the institutional animal ethics committee before conduction of experiments.

Preparation of *Aloe vera* extract-

Pulp were taken out from leaves and mixed with distilled water and methanol (1:1) and kept in separating funnel for 6 hours. The supernatant was collected and this process was repeated until cleared solution of supernatant was obtained. All supernatant was pulled together and dried to the power of 40º C water bath. The powder was dissolved in double distilled water before the each treatment at required concentrations.

Preliminary Phytochemical Screening

Standard screening test for various phytochemicals of methanoic extracts of Aloe vera were carried out. The crude extracts were screened for the presence or absence for primary and secondary metabolites such as flavonoids, steroids, alkaloids, phenolic compound, saponin, tannins etc.

Investigation of Phytochemical Analysis of Aloe Vera extract Test for Phytochemical Analysis

The extracts were analyzed for the presence of various phytochemicals by applying the standard methods in 50% and 100% methanolic extract.

Test for carbohydrate

A small quantity of the extract was dissolved in 4 ml of distilled water and filtered. The Molisch's test was performed with filtrate to detect the presence of carbohydrates and further addition of Fehling's reagent if showed the brick red colour confirming the presence of reducing sugar.

Tests for glycosides

Extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides. Ammonia layer acquires pink colour confirming the presence of glycoside.

Test for alkaloids

A 100 mg of an extract was dissolved in dilute hydrochloric acid solution was subjected to filtration filtrate was tested with Dragendorff's and Mayer's reagents the treated solution was observed for precipitation.

Confirmatory test

Five grams of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly appears alkaline to Litmus Paper, and then extracted twice with 10 ml portion of chloroform then chloroform extracts were combined and concentrated to about 5 ml. Chloroform extract then spotted on thin layer plates solvent system(n-hexane-ethyl acetate, 4:1) was used to develop chromatogram and protected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. An orange or dark colour spots against a pale yellow background was confirmatory evidence for the presence of alkaloids.

Test for proteins

2 ml of filtrate was treated with 2 ml of 10 % sodium hydroxide solution in a test tube and heat it for 10 minutes. A drop of 7% to copper sulphate solution was added in the above mixture formation of purple violet colour indicates the presence of proteins

Test for flavonoids

0.5 g of the extract dissolved in water and added with 5 ml of Ethyl acetate then shaked well and allowed to settle down. After sometime inspected for the production of yellow colour in the organic layer, which is confirmatory for free flavonoids

Lead acetate test

0.5g extract dissolved in water and mixed about 1 ml of 10% lead acetate solution. Production of yellow precipitate is considered as positive for flavonoids.

Reaction with sodium hydroxide
Salkowski's Test

0.5 g e extracts were dissolved into 2 ml of chloroform in a test tube concentrated sulphuric acid was bored on the wall of test tube to form a lower layer further examined for the appearance of a reddish brown colour ring at the interface which indicate the presence of Steroids (i.e., the aglycone portion of the glycoside).

Lieberman's Test 0.5g extract was dissolved in 2 ml of acetic anhydride and allowed to cool in an ice-bath. Concentrated sulphuricacid was then carefully added. Purple colour turned in to blue-green which indicated the presence of a steroid nucleus, i.e., aglycone portion of the cardiac glycosides

Test for saponin
Froth test

0.5 g extracts were dissolved in 10 ml of double distilled water for about 30 seconds and shaken vigorously for about 30 seconds. The test tube was put in a vertical position and observed over 30 minutes period of time. If a "honey comb" fraud above the surface of liquid persist after 30 minutes. Frothing confirmed presence of saponin.

Test for tannins
Ferric chloride test

Some amount of the extracts were dissolved in water and solution was filtered. 10% ferric chloride solution was added to the clear filtrate and observed for change in colour to bluish black confirming the presence of chloride.

Formaldehyde test

0.5 g extract in 5 ml water, three drops of formaldehyde, and 6 drop of dilute hydrochloric acid were added and heated to boil for 1 minute, then allowed to cool. The precipitate formed(if any) was washed with hot water, warm alcohol, and warm 5% potassium hydroxide successively.A balki precipitate, which leaves a coloured Residue after washing, indicated the presence of phlobatannins.

Test for anthraquinone

0.5 gm of crude powder was taken with 10 ml of benzene and was filtered 0.5 ml of 10% Ammonia solution was added to the filtrate and the mixture was shaken well and the presence of the violet colour in the layer face indicated the presence of the anthraquinones.

Test for fixed oils
Spot test

Small quantities of plant extracts were separately present between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of various extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1 to 2 hours. Formation of soap or partial neutralization of alkali indicate the presence of fixed oil and fats.

Test for phytosterol 0.5 gm of extract was treated with 10 ml chloroform and filtered. The filtred was used to test the presence of phytosterols and triterpenoids. The extract was refluxed with alcoholic potassium hydroxide solution until complete saponification. The mixture was diluted allowed by extracted with ether.Ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of dilute acetic acid, 3 ml of acetic anhydride followed by few drops of concentrated sulphuric acid.

Appearance of bluish green colour confirms the presence of phytosterol.

II. Results

The results of phytochemical screening of Hydromethanolic leaf extract of Aloe vera are depicted in table no. 4.1. The extracts were screened for preliminary phytochemical testing to detect for the presence of different chemical group of phytocompounds. The 50 % methanolic extract of Aloe vera showed the presence of Alkaloids, Carbohydrate, Resin, Saponins, Glycoside and Tanins whereas Protein, Starch. Flavonods and Steroids were absent (Table 1) The different concentration of Resin (2.5 %) , Tannin (1.95%) , Carbohydrate (1.34 %) and Alkaloids (0.67 %) were quantified (Table 2)

Table-1: Preliminary phyto-chemical screening of (*Aloe vera* (Linn.) (Leaf))

S. No.	Name of Experiments	Observation	Result
1.	**Alkaloids**		
	a) Mayer' test	Yellow colour appear	Present
	b) Wagner's test	Brown colour appear	Present
	c) Dragendorff's test	Orange colour appear	Present
2.	**Carbohydrate**		
	a) Anthrone's test	Dark colour appear	Present
	b) Fehling's test	Green colour appear	Present
	c) Molisch's test	No red – violet ring disapper	Present
3.	**Proteins**		
	a) Bieuret's test	Green colour appear	Absent
	b) Millon's test	White ppt are not appear	Absent
5.	**Resins**	Turbidity are seen	Present
6.	**Saponins**	Honey comb – like structure are form	Present
7.	**Starch**	Red colour is formed	Absent
8.	**Flavonoid**		Absent
	a) Ferric chloride test	Reddis pink colour is appear	Absent
	b) Alkaline reagent test	On addition of dilute acid yellow colour disappear	Absent
9.	**Steroid**		
	a) Salkowski's reaction	A red colour is disappear in the chloroform layer	Absent
10.	**Glycoside**		
	a) Borntrager's Test	Colour is change	Present
11.	**Tannin**	Greenish colour appear	Present
	a) Lead acetate Test	Reddish brown bulky ppt. are formed	Present

Table-2. : Quantitative Phyto-chemical Analysis of Sample-1 *(Aloe vera* (Linn.) (Leaf)

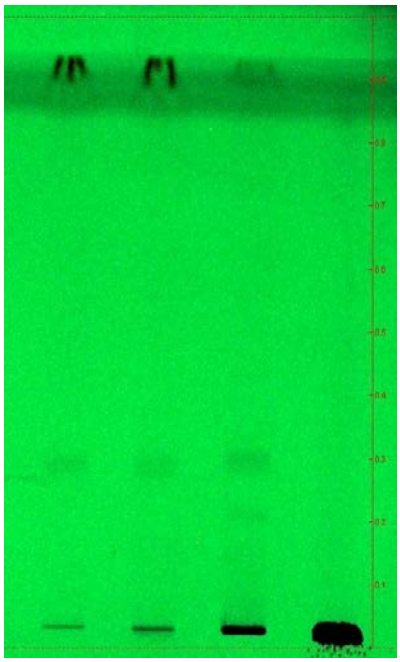
S. No.	Name of tests	Sample-1 *Aloe vera* (Linn.) (Leaf)
1	Resin	2.5%
2	Tannin	1. 95%
3	Carbohydrate	1.34%
4	Alkaloid	0.67%

Table-III : Rf values of HPTLC Fingerprints profile of Extract-1, 2, 3 & -4 *(Aloe vera* (Linn.) (Leaf)
Plate-1: HPTLC Fingerprint Profile of Sample- 1 *(Aloe vera* (Linn.) (Leaf)

Rf Values	**Before derivatization**			
	At 254nm			
	Track-A (Extract-Methanol)	**Track-B (Extract-Ethanol)**	**Track-C (Extract -Benzene)**	**Track- D (Extract-Chloroform**
R_f-1	0.30(black)	0.30(black)	0.30(black)	-
R_{f-2}	0.90(black)	0.90(black)	0.90(black)	-
	Before derivatization			
	At 366nm		**At visible light**	
	Track-A (Extract-Methanol)	**Track-B (Extract-Ethanol)**	**Track-C (Extract -Benzene)**	**Track- D (Extract-Chloroform**
R_f-1	-	0.06(pink)	0.06(pink)	0.06(pink)
R_{f-2}	-	0.30 (brown)	0.30(brown)	-
R_{f-3}	-	0.40(brown)	0.40(pink)	-
R_{f-4}	-	0.56 (reddish brown)	0.56(reddish brown)	-
R_{f-5}	-	0.62 (reddish brown)	0.62 (reddish brown)	-
R_{f-6}	-	0.80(red)	0.80(red)	0.80(red)
R_{f-7}	-	0.90 (red)	0.90 (red)	0.90 (red)

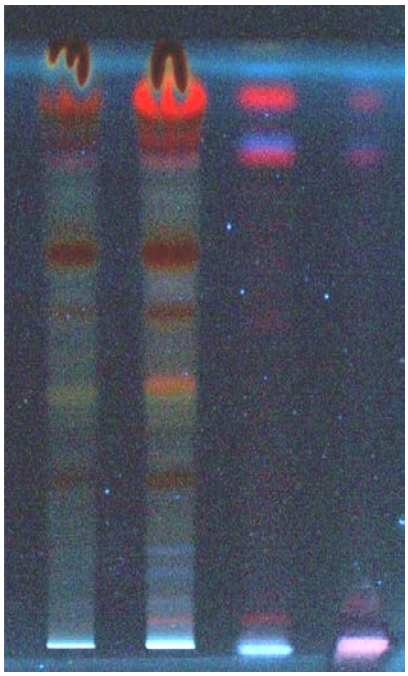
Rf Values	**After derivatization**			
	At 366nm			
	Track-A (Extract-Methanol)	**Track-B (Extract-Ethanol)**	**Track-C (Extract -Benzene)**	**Track- D (Extract-Chloroform**
R_f-1	0.08 (white)	0.08 (white)	0.08 (white)	-
R_{f-2}	0.30 (light black)	0.30 (light black)	0.30 (light black)	-
R_{f-3}	0.50(sky blue)	0.50(sky blue)	0.50(sky blue)	-
R_{f-4}	0.60(brown)	0.60(brown)	0.60(brown)	-
$R_{f\,5}$	0.68(sky blue)	0.68(sky blue)	0.68(sky blue)	-
$R_{f\,6}$	0.72(brown)	0.72(brown)	0.72(brown)	-
$R_{f\,7}$	0.78(sky blue)	0.78(sky blue)	0.78(sky blue)	-
$R_{f\,8}$	0.80(red)	0.80(red)	0.80(red)	-
$R_{f\,9}$	0.92(pink)	0.92(pink\)	0.92(pink)	-
	After derivatization			
	Day Light		**At visible light**	
	Track-A (Extract-Methanol)	**Track-B (Extract-Ethanol)**	**Track-C (Extract -Benzene)**	**Track- D (Extract-Chloroform**
R_f-1	0.08(light black)	0.08(light black)	0.08(light black)	-
R_{f-2}	0.30(light black)	0.30(light black)	0.30(light black)	-
R_{f-3}	0.50(light black)	0.50(light black)	0.50(light black)	-
R_{f-4}	0.60(black)	0.60(black)	0.60(black)	-
R_{f5}	0.70(brownish black)	0.70(brownish black)	0.70(brownish black)	-
R_{f6}	0.72(brownish black)	0.72(brownish black)	0.72(brownish black)	-
R_{f7}	0.78(brownish blue)	0.78(brownish blue)	0.78(brownish blue)	-
R_{f8}	0.92(black)	0.92(black)	0.92(black)	-

Fig 1-Where Track A: Methanol extract; Track B: Ethanol extract; Track C: Benzene extract; Track D: Chloroform extract



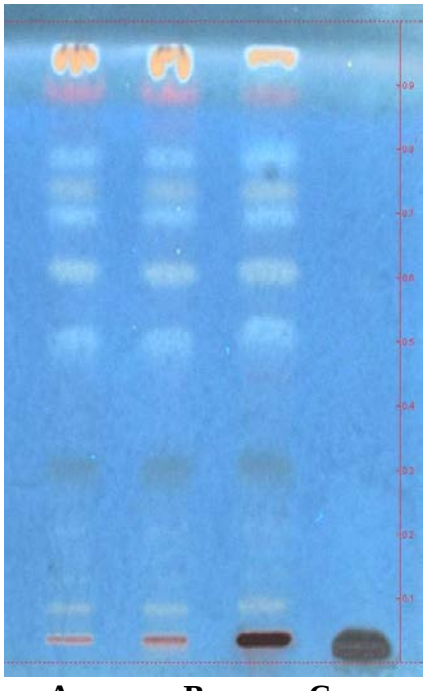
A B C D

At 254nm before derivatization



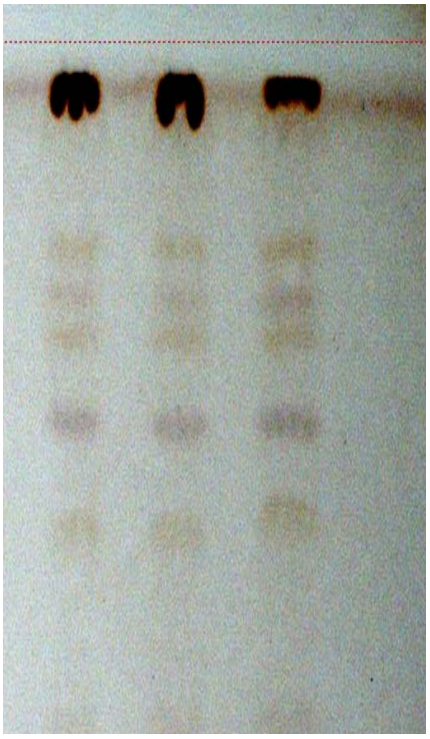
A B C D

At 366nm before derivatization



A B C D

At 366nm after derivatization



At day light after derivatization

2. Antioxidant activity of *Aloe vera* extract using DPPH methods

The free radical scavenging capacity of the extracts of *Aloe vera* was determined using TBARS method and the data was furnished in Table **5 .**The stock solution of *Aloe vera* extract was prepared at the concentration of 10 mg/ml. Extent of hydroxyl radical scavenging activity was determined by decreased intensity of pink colored chromospheres in the form of IC 50 values which was determined at 532 nm. Lower IC 50 value represents higher antioxidant activity. The dose dependent inhibition of TBARS formation at the different concentration of *Aloe vera* extract ranging from 100 mg/ml to 1,000 mg/ml was studied. The Ascorbic acid was used as positive control. The IC 50 values of Ascorbic acid and of *Aloe vera* extract was found to be 40 µg/ml and 60µg/ml, respectively.

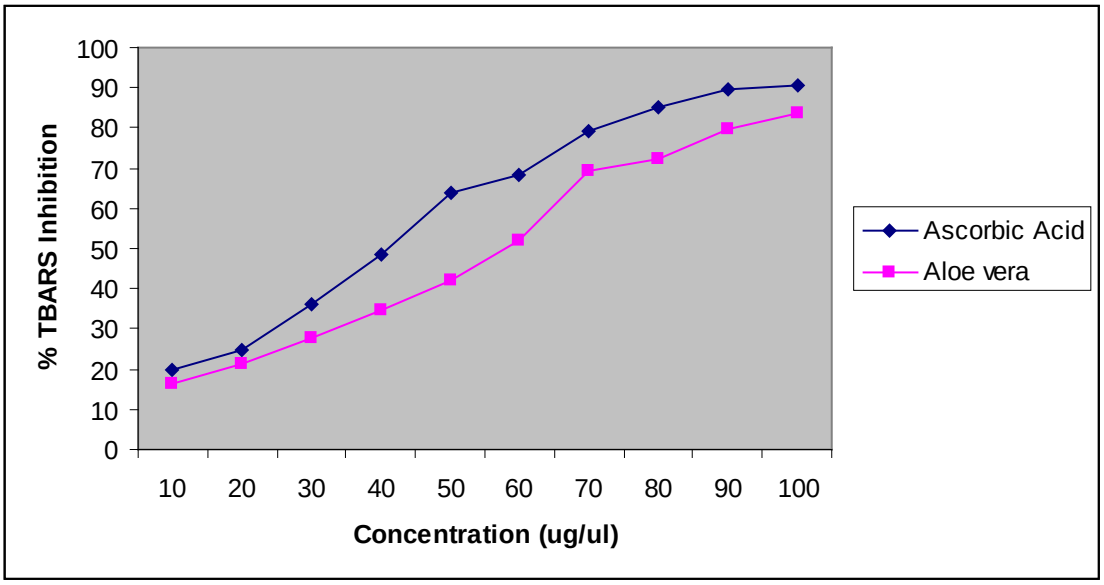


Table 4:- Antioxidant Activity of Aloe vera

S.No.	Concentration (ug/ ul)	Ascorbic Acid	*Aloe vera* Extract
1.	10	19.66	16.34
2	20	24.85	21.09
3.	30	36.14	27.92
4.	40	48.41	34.75
5.	50	63.86	42.18
6.	60	68.21	51.88
7.	70	79.00	69.40
8.	80	85.35	72.17
9.	90	89.70	79.60
10.	100	90.69	83.66

3. Micronucleus assay

Single application of Aloe Vera ext. at the dose of 125 , 250 and 375 mg/kg b.wt.,24hours prior the i.p. administration of Cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in bone marrow cells of mice as compared to Cyclophosphamide group. It was also observed that single application of aloe vera extract at the dose of 250 mg/kg have not significantly induced micronuclei formation as compared to solvent control

Table no: Effect of Aloe Vera Ext. on micronucleus formation in mouse bone marrow cells:

Group	MNPCE + SE RATIO	PCE/NCE RATIO
Cyclophosphamide (50kg/ml)	3.5±1.1	0.99±0.08
Aloe Vera Ext. (125mg/kg body wt) + CP (50 mg/kg)	0.4±0.3	0.25±0.03
Aloe Vera Ext. (250mg/kg body wt)+ CP (50 mg/kg)	0.33±0.18*	0.30±0.01*
Aloe Vera Ext. (375mg/kg body wt)+ CP (50 mg/kg)	0.8±0.9*	0.3±0.1*
Aloe Vera Ext. Alone (250mg/kg body wt)	0.33±0.21	0.948±0.02
Solvent (Water)	0.45±0.03	0.549±0.08

III. DISCUSSION

There iis a iworldwide ibelief ithat iherbal iremedies iare isafer iand iless idamaging ito ithe ihuman ibody ithan isynthetic idrugs. Our iphytochemical iscreening iof i*Aloe vera* igive iideas iregarding ivarious isecondary metabolites present iin ileaves . Phytochemical iscreening o of the 50 % methanolic extract of Aloe vera showed the presence of Alkaloids, Carbohydrate, Resin, Saponins, Glycoside and Tanins whereas Protein, Starch. Flavonods and Steroids were absent Qualitative idensitometric iHPTLC ifingerprint iprofile iof imethanolic iextract ican iprovide istandard ifingerprints iand ican ibe used as ia ireference ifor ithe iidentification iand iquality icontrol iof ithe lleaves. The ipresent istudy iwill iprovide ithe iinformation iwith irespect ito iidentification iand iauthentication iof i*leaves.*

Antioxidantsiare iknown ito iexhibit itheir ibiochemical ieffects ithrough inumerous imechanisms, iincluding ithe iprevention iof ichain iinitiation, ireductive icapacity iand iradical iscavenging imechanisms. Several I methods ihave ibeen ireported ito imeasure ithe iantioxidant iactivity iof ibiological imaterials. iIn ithe ipresent istudy, iDPPH ifree iradical iscavenging iactivity, isuperoxide ianion iradical iscavenging iactivity, iABTS iradical ication iscavenging iactivity, ihydroxyl iradical iscavenging iactivity, iferric ireducing iantioxidant ipower iand ireducing icapacity iassessment iwere idone i(Deshpande iJR, iet. ial. i2007).

Antioxidants helps organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability**.** Medicinal plants often represent the only therapeutic resource for antioxidants. Lipids, proteins and DNA are all susceptible to attack by free radicals (Mondal, 2006). Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc. Our present study shows the antioxidant activity of Lagenaria siceraria extracts using DPPH method and it showed promising free radical scavenging property. Ascorbic acid was used as reference standard.

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