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Evaluation of antioxidant potential of Asystasia chelonoid var. chelonoides Nees. (Acanthaeae).

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Abstract

The ethanolic leaf extract of *Asystasia chelonoides* var. *chelonoides* Nees. was evaluated using various *in vitro and ex 0vivo* assays. Evaluations were made for DPPH radical scavenging activity, NO radical scavenging activity, Super oxide radical scavenging activity, Ferric Reducing Antioxidant Potential (FRAP) assay, Total antioxidant activity, Reducing Power activity and Lipid Peroxidation inhibition. Over all findings revealed that *A.chelonoides* leaf exhibited four extracts namely (AC HEX, AC CHL, AC ETH and AC CRD) and were selected for the study, AC ETH and AC CRD showed potent antioxidant activity by inhibiting DPPH radical in a concentration dependent manner . The nitric oxide radical scavenging activity of all the samples increased as their concentration increased from 20 µg/mL to 320 µg/ mL. AC CRD showed its maximum superoxide radical scavenging of 78.56 % at 320 µg/mL and the EC_{50} was found to be 93.26 µg/mL. The extracts exhibited a dose dependent reducing power activity within the applied concentrations (20 µg/mL to 100 µg/mL) and AC CRD at 100 µg/mL concentration showed highest activity, which was almost comparable to standard ascorbic acid. In FRAP assay the crude extract of *A. chelonoides* (AC CRD) showed 138.14 µg trolox equivalent/g of dry extract respectively. Total antioxidant activity of AC CRD, AC HEX, AC CHL and AC ETH were found to be 98.27, 14.86, 20.16 and 72.56 µg of ascorbic acid equivalent/mg of dry extract respectively. *A.chelonoides* crude extract and ethanolic fraction showed potent inhibition of $FeCl_2$AA stimulated rat liver lipid peroxidation in concentration dependent manner.

Keywords: *Asystasia chelonoides*, antioxidant potential, lipid peroxidation, DPPH, FRAP.

Introduction

Liver provides protection to an organism against foreign particles by eliminating them, through the process of excretion (Shaker *et al*., 2011). Liver damage caused by various hazardous chemicals such as carbon tetrachloride (CCl_4), thioacetamide, chronic alcohol consumption, and even some drugs used to treat various diseases, may accumulate in the liver tissue and cause alteration in the normal physiological function of the liver by generating free radicals, which is the major reason for the cause of heart related problems like stroke, autoimmune diseases like rheumatoid arthritis, diabetes, and cancer (Halliwell *et al*., 1989). Plants are rich in biologically active compounds like antioxidants and phytochemicals, and they play an essential role in the treatment of diseases (Xavier *et al*., 2017) . Many diseases like cancer,

inflammation, lesions, degenerative diseases are caused due to excess of Reactive Oxygen Species (ROS) in the cellular body. Patients with liver injury reported to have oxidative stress. The imbalance in the level of pro-oxidants and antioxidants leads to oxidative stress leads to potential damage for the organism (Halliwell *et al* ., 1990). Antioxidants act as scavenging reactive oxygen species by inhibiting their formation by binding transition metal ions and preventing formation of OH and decomposition of lipid hydro-peroxides, by repairing damage. All of these conditions, along with the ageing process are associated with OS due to elevation of ROS or insufficient ROS detoxification (Lemon –Pacheco *et al*., 2009). Currently available synthetic drugs used for the treatment of liver diseases are inadequate and known for various side effects. Earlier scientific studies reported that, the use of plant phenolics, reduces the risk of liver diseases by acting as antioxidants (Gaur *et al*., 2006). Recently, interests in consuming edible plants that are rich in antioxidants and health-promoting phytochemicals have been increased worldwide. Oxidative stress generates dangerous free radicals, which affect human health and exposing them to disease by altering the naturally structured cellular lining of human body subsequently causing lipid peroxidation and effects on enzyme activity as well as producing carcinogenesis. Antioxidants benefit human body by neutralizing and removing free radicals in the blood stream. Secondary metabolites like phenol and flavonoids play an important in plant growth as well as reproduction. These compounds also have antioxidant properties as well as anti-cancer properties (Mathur *et al*., 2017) .

Materials and method

Evalutaion of *in vitro* antioxidant activity

Antioxidant activity of crude and various fractions of *A chelonoides were* evaluated using standard procedures as mentioned below.

Estimation of DPPH radical scavenging activity.

The effect of crude and various fractions of *A chelonoides* on DPPH radicals were assayed using the standard method of Blois, 1958. A methanolic solution of 2ml of DPPH (0.025g/l) was added to 200ml of the different concentrations (20mg/ml to 320mg/ml) of plant extract, allowed to react at room temperature for 30m in dark and the absorbance was measured at 517 nm. Methanol served as the blank. 200ml of methanol was added to DPPH in positive control tubes, instead of plant extract. Ascorbic acid was used as standard.

% of inhibition= Absorbance of control- Absorbance of sample/Absorbance of control x 100

From the estimated DPPH radical scavenging activity the EC_{50} was calculated, which represents the concentration of the scavenging compound that caused 50% neutralization.

Estimation of Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured by using the standard procedure of Mondal *et al*., 2006. Sodium nitroprusside (1ml of 10mM) was mixed with 1ml of different concentration (20-320mg/ml) of plant extract in phosphate buffer (pH 7.4). The mixture was incubated at 25°C for 150 min . To 1ml of the incubated solution, 1ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 1% Napthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage of inhibition was calculated using the formula

% of NO radical scavenging = $(A_0 - A_1/A_0) \times 100$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of sample. Ascorbic acid was used as standard.

Estimation of Super oxide radical scavenging activity

The effect of super oxide radical scavenging activity was determined by the Nitroblue tetrazolium reduction method, described by Fu *et al.*, 2010. 1ml of NBT solution (156mm) in 100m M phosphate buffer, pH 7.4), 1ml of NADH solution (468M NADH in 100mM phosphate buffer, pH 7.4) and o.1 ml of different concentrations of the plant extract were mixed. The reaction was started by adding 100 µl of phenazine methosulphate (PMS) solution (60mM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25ºC for 5min and the absorbance at 560 nm was measured against blank, containing all the reagents except the PMS. All readings were taken in triplicate and Trolox was used as standard. % of inhibition was calculated by the formula

[1 - (absorbance of sample/absorbance of control)] × 100.

Ferric reducing antioxidant potential (FRAP) assay

The ability of the plant extract to reduce the ferric ion was measured using the modified version of the method used by Benzie and Strain 1996. Plant extract, 200ml was added to 3ml of FRAP reagent(10 part 300mM Sodium acetate buffer at pH 3.6, 1 part 10mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution and one part 20 mM $FeCl_2 6H_2O$ solution) and the reaction mixture was incubated in a water bath at 37°C for 30 min. The absorbance was measured at 593 nm. The antioxidant capacity of the plant extract was calculated from the calibration curve of Trolox and expressed as µ mol Trolox equivalent/g of extract.

Determination of total antioxidant activity

Total antioxidant activity of the plant extract was determined by the method of Umamaheswari and Chatterjee , 2008. 200µl of extract solution (mg/mL) in respective solvent was mixed with 2 mL of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95ºC for 90 min. The blank solution contained 2 mL of the reagent solution and approximate volume of the same solvent used for preparing plant extract. Absorbance of the sample was measured at 635 nm both for the sample and the standard, Ascorbic acid. The Total antioxidant capacity of the plant extract was calculated from the calibration curve of Ascorbic acid and expressed as µg Ascorbic acid equivalents /g of dry extract.

Evaluation of reducing power activity.

The reducing power (Ferric to Ferrous reduction) was investigated by the Ferric (Fe^{3+}) - Ferrous (Fe^{2+}) transformations in the presence of fractions as described by Fejas *et al* ., 2000 . The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 7 00nm (Meir *et al.*, 1995).The plant extract (2mg/ml) at different concentrations (10-500ml) were mixed with 25ml of Phosphate buffer (0.2m, pH 6.6) & 2.5ml of 1% Pottassium ferricyanide ($K_3Fe(CN)_6$) and incubated at 50ºC for 20 min . Trichloroacetic acid solution (2.5ml of 10%w/v) was added to the reaction mixture at room temperature. After centrifugation at 3000 rpm for 10min, 2.5 ml of supernatant was mixed with equal volume of distilled water and 0.5 ml of freshly prepared Ferric chloride(0.1%). A blank was prepared without adding the extract .The absorbance at 700nm was measured, and an increase in absorbance indicated increase in reducing power. All measurements were made in triplicate and the graph was plotted with the average of the three determinations. Ascorbic acid was used as the reference compound.

***.Ex-vivo* antioxidant study**

Anti- Lipid peroxidation Studies.

Anti-lipid peroxidation effects of plant extracts were studied by the method of Okhawa, 1979. 1 gm rat liver tissue is homogenized in 0.1 M KCl-Tris HCl buffer pH 7.4. Lipid peroxidation in this homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily.

Tissue homogenate (0.2 mL), 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid and 1.5 mL of 8% TBA are added. The volume of the mixture is made up to 4 mL with distilled water and then heated at 95ºC on a water bath for 60 min. After incubation, the tubes are cooled to room temperature and final volume was made to 5 mL in each tube. Five mL of butanol: pyridine (15:1) mixture is added and the contents are vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer is taken and its OD is taken at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides can be expressed as n moles of Thiobarbituric acid reactive substances (TBARS) /mg protein using an extinction coefficient of 1.56 X 105 ML-1

$$LP = \frac{ODX \text{ Total volume of Homogenate X } 100 \text{ X} 10}{21.56 \text{ X } 105 \text{ X tissue wgt. in Gram X volume of homogenate taken}}$$

$$\% \text{ Inhibition of MDA} = \frac{OD \text{ Toxin} - OD \text{ Sample X } 100}{OD \text{ Toxin}}$$

RESULT

In vitro Antioxidant Studies

In- vitro antioxidant studies of *A.chelonoides* were carried out using standard procedures and the results are as shown below.

DPPH radical scavenging activity

From the extracts (AC HEX, AC CHL, AC ETH and AC CRD) selected for the study, AC ETH and AC CRD showed potent antioxidant activity by inhibiting DPPH radical in a concentration dependent manner (Fig. 1). AC HEX showed maximum DPPH radical scavenging of 32 % at 320 µg/mL and the EC_{50} was found to be 678.5 µg/mL. AC CHL shows maximum DPPH radical scavenging of 45 % at 320 µg/mL and the EC_{50} was found to be 337.9 µg/mL. AC ETH shows maximum DPPH radical scavenging of 68 % at 320 µg/mL and the EC_{50} were found to be 76.7µg/mL. Among the four samples AC CRD showed most potent DPPH radical scavenging of 81 % at 320µg/mL and the EC_{50} was found to be 33.73 µg/mL which is comparable to the ascorbic acid standard with EC_{50} value of 13.32 µg/mL.

Nitric oxide (NO) radical scavenging activity

The results of NO radical scavenging activity of the plant extracts were represented as percentage of NO scavenging in (Fig. 2). The nitric oxide radical scavenging activity of all the samples increased as their concentration increased from 20 µg/mL to 320 µg/mL. AC CRD showed maximum nitric oxide radical scavenging of 72.34 % at 320 µg/mL and the EC_{50} of the extract was found to be 58.48 µg/mL. AC CHL showed maximum nitric oxide radical scavenging of 41.9 % at 320 µg/mL and the EC_{50} of the extract was found to be 350 µg/mL. AC HEX showed maximum nitric oxide radical scavenging activity of 36.24 % at 4.21 µg/mL and the EC_{50} of the extract was found to be 453.1 µg/mL. AC ETH showed maximum nitric oxide radical scavenging of 65.34% at 320 µg/mL and the EC_{50} of the extract was found to be 105 µg/mL. The EC_{50} of standard Ascorbic acid was 25.32%. The NO radical scavenging was observed to be concentration dependent and AC HEX and CHL fractions failed to evoke significant response.

Superoxide radical scavenging activity.

The super oxide radical scavenging activities of all the samples and fractions were determined by the Nitroblue tetrazolium reduction method and represented in the (Fig. 3). Of all the extracts used for the study, AC ETH and AC CRD showed significant superoxide radical scavenging activity in a concentration dependent manner from 20 µg/mL to 320 µg/mL. AC CRD showed its maximum superoxide radical

scavenging of 78.56 % at 320 µg/mL and the EC_{50} was found to be 93.26 µg/mL. AC HEX showed its maximum Super oxide radical scavenging of 37.67% at 320 µg/mL and the EC_{50} was found to be 37.31 µg/mL. AC CHL showed its maximum superoxide radical scavenging of 48.34% at 320 µg/mL and the EC_{50} was found to be 48.47 µg/mL. Among the four extracts, AC CRD showed maximum superoxide radical scavenging of 78.56% at 320 µg/mL and the EC_{50} was found to be 93.26 µg/mL. Here, the standard used for reference was Ascorbic acid which showed an EC_{50} value of 24.58 µg/mL comparable to the EC_{50} of AC CRD.

Ferric Reducing Antioxidant Potential (FRAP) assay.

The ability of plant extract to reduce the ferric ion was determined from standard graph drawn from known concentrations of Trolox (Fig. 4) and expressed as µg of Trolox equivalent/g of dry extract. The crude extract of *A. chelonoides* (AC CRD) showed 138.14 µg trolox equivalent/g of dry extract respectively.

Determination of Total antioxidant activity.

Total antioxidant capacity of the extracts was estimated by phosphomolybdenum method and the results were quantitatively expressed as of Ascorbic acid equivalents/g of dry extract. Total antioxidant activity of AC CRD, AC HEX, AC CHL and AC ETH were calculated from the calibration curve of ascorbic acid (Fig. 5). Total antioxidant activity of AC CRD, AC HEX, AC CHL and AC ETH were found to be 98.27, 14.86, 20.16 and 72.56 µg of ascorbic acid equivalent/mg of dry extract respectively.

Reducing power activity.

The reducing powers (iron (III) to iron (II) reduction) of the extracts are shown in (Fig.6) with ascorbic acid as the positive control. The extracts exhibited a dose dependent reducing power activity within the applied concentrations (20 µg/mL to 100 µg/mL) and AC CRD at 100 µg/mL concentration showed highest activity, which was almost comparable to standard ascorbic acid.

***Ex vivo* antioxidant study.**

Anti-lipid peroxidation studies.

A.chelonoides crude extract and ethanolic fraction showed potent inhibition of $FeCl_2$AA stimulated rat liver lipid peroxidation in concentration dependent manner (Table 1). The degree of lipid peroxidation in the liver tissue was determined in terms of malondialdehyde (MDA) produced as nmol/g wet liver. There was a significant increase of MDA in $FeCl_2$-AA treated rat liver homogenate, compared to normal control without $FeCl_2$-AA. Treatment of $FeCl_2$-AA stimulated rat liver homogenate with AC HEX, AC CHL, AC ETH and AC CRD showed a maximum % of inhibition in MDA production of 22.34%, 32.19%, 61.19% and 66.09% respectively at 200 µg/mL dose compared to toxin control. Significant decrease ($p \leq 0.05$) of MDA production in hepatic cells from toxin control was found in the drug treated doses of AC ETH and AC CRD 200µg/mL. The IC_{50} values of *in vitro* anti-lipid peroxidation in terms of MDA inhibition of AC HEX, AC CHL were much increased when compared to AC ETH and AC CRD which showed maximum protective effect against lipid peroxidation. The IC_{50} value shown by AC CRD is 99.42 µg/mL.

Table: 1. Inhibitory effect of *A.chelonoides* extract on $FeCl_2$-Acetic acid (AA) induced lipid peroxidation in rat liver homogenate *in vitro*.

Lipid peroxidation assay				
SL.NO.	Samples	MDA(n mol/g Wet Liver)	% of MDA inhibition	
1	Normal Control	0.74 ± 0.02		
2	$FeCl_2$ +AA	2.92 ± 0.12		
3	$FeCl_2$ +AA+AC HEX 25	2.78 ± 0.10	4.79 ± 1.02	
4	$FeCl_2$+AA+AC HEX 50	2.62± 0.08	10.27 ±1.44	
5	$FeCl_2$+AA+AC HEX 100	2.39 ± 0.05	18.15±1.23	IC_{50}=456.81±3.58
6	$FeCl_2$+AA+AC HEX 150	2.25 ± 0.03	20.94±1.05	
7	$FeCl_2$+AA+AC HEX 200	2.18 ± 0.04	22.34±1.18	
8	$FeCl_2$+AA+AC CHL 25	2.41 ± 0.02	17.46± 0.98	
9	$FeCl_2$+AA+AC CHL 50	2.30 ± 0.02	21.23± 1.25	
10	$FeCl_2$+AA+ AC CHL 100	2.17 ± 0.01	25.68± 1.48	IC_{50}=413.28±5.58
11	$FeCl_2$+AA+AC CHL 150	2.08 ± 0.03	28.76± 1.02	
12	$FeCl_2$+AA+AC CHL 200	1.98 ± 0.04	32.19± 0.87	
13	$FeCl_2$+AA+AC ETH 25	2.29 ± 0.03	21.57± 1.87	
14	$FeCl_2$+AA+AC ETH 50	2.06 ± 0.03	29.45± 1.38	
15	$FeCl_2$+AA+AC ETH 100	1.89 ± 0.08	35.27± 1.53	
16	$FeCl_2$+AA+AC ETH 150	1.44 ± 0.06	50.68± 1.92	IC_{50}=151.50± 2.26
17	$FeCl_2$+AA+AC ETH 200	1.11 ± 0.02	61.19± 1.07	
18	$FeCl_2$+AA+AC CRD 25	2.01 ± 0.02	31.16± 1.08	
19	$FeCl_2$+AA+AC CRD 50	1.79 ± 0.10	38.69± 1.15	
20	$FeCl_2$+AA+AC CRD 100	1.31 ± 0.01	56.13± 0.96	
21	$FeCl_2$+AA+AC CRD 150	1.06 ± 0.06	63.69± 1.49	IC_{50}= 99.42 ±3.64.
22	$FeCl_2$+AA+AC CRD 200	0.99 ± 0.09	66.09± 1.65	

Fig.1. DPPH radical scavenging activity of *A. chelonoides* crude extrcat and fractions.

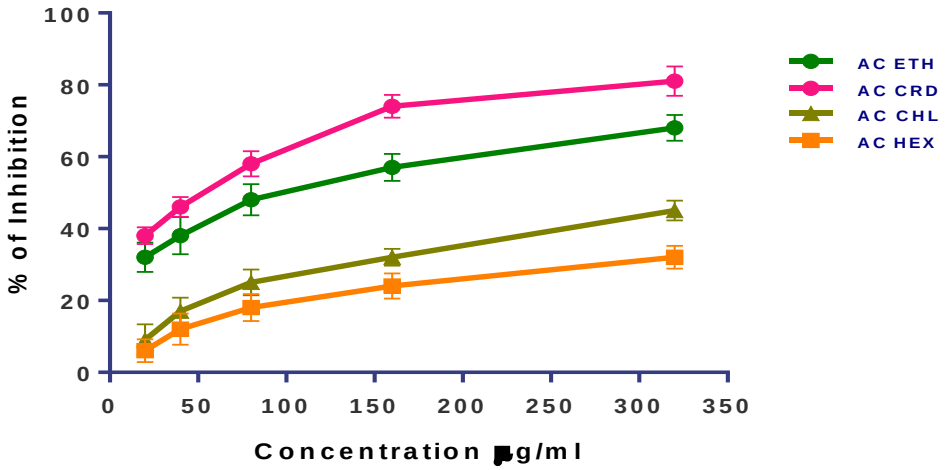
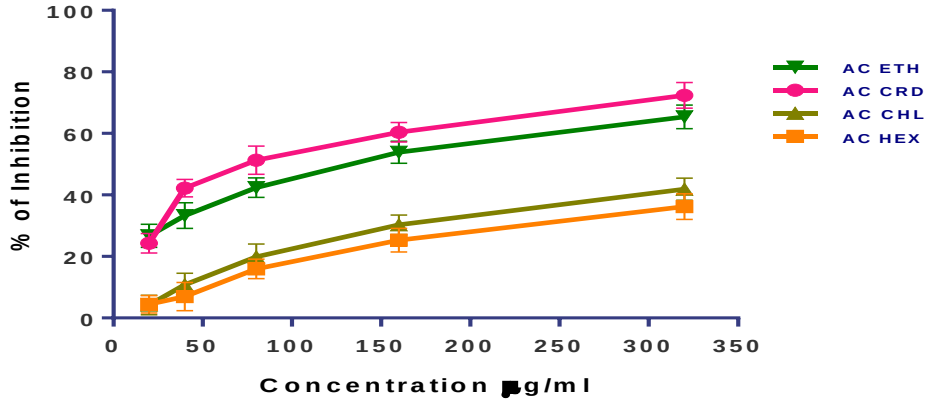


Fig.2. Nitric oxide radical scavenging activity of *A. chelonoides* crude extrcat and fractions.



Values are mean ± SD of 3 replicates.

Fig. 3. Superoxide radical scavenging activity *A. chelonoides* crude extrcat and fractions.

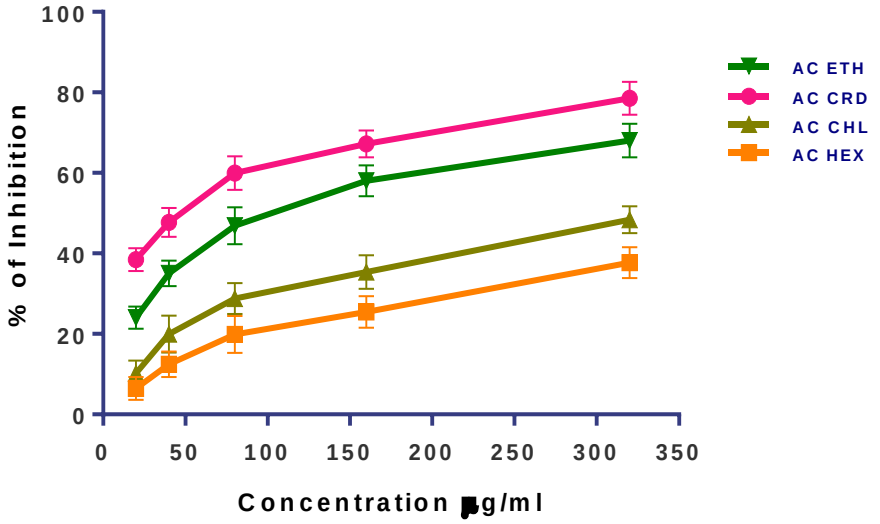


Fig. 4. FRAP assay Standard Curve.

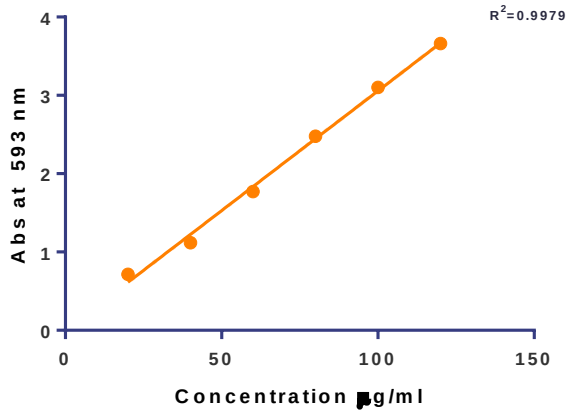
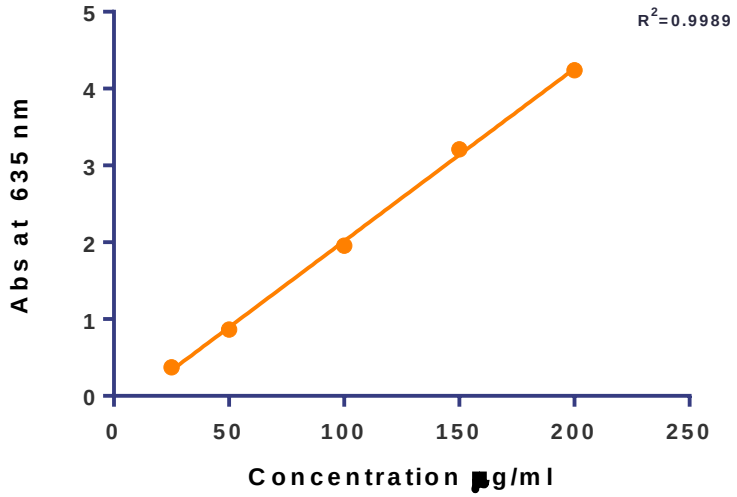
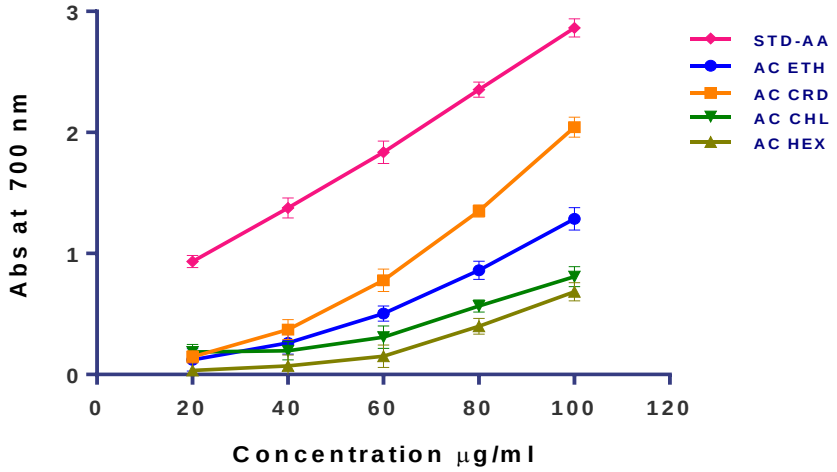


Fig. 5. Total antioxidant assay Standard Curve.



Values are mean ± SD of 3 replicates.

Fig. 6. Reducing power of *A. chelonoides* crude extrcat and fractions compared to Ascorbic acid.



Values are mean ± SD of 3 replicates.

DISCUSSION

In vitro antioxidant activity

Herbaceous plants have a long history of use as medicine, food and variety of daily needs. Many epidemiological studies suggest that an increased consumption of several medicinal plants containing antioxidants can protect against DNA damage and carcinogenesis, and often exhibit a wide range of pharmacological activities such as anti-inflammatory, anti- bacterial and anti- fungal properties (Rasineni *et al* .,2008). Flavonoids have good antioxidant efficiencies and are common in leafy vegetables. A number of phytochemicals commonly used in research have antioxidant activity that can protect cells from reactive oxygen species (ROS)- mediated DNA damage that results in mutation and subsequent carcinogenesis.

(Szeto *et al.*,2002 , Lazze *et al* .,2003). indicated that increased consumption of vegetables and fruits increases the plasma antioxidant capacity in humans.

Free radicals released during oxidative stress pose the major endogenous damage in the biological system. This type of damage is often associated with various degenerative diseases and disorders such as cancer, cardiovascular diseases and immune function decline and ageing. Free radicals are highly reactive molecules having unpaired electrons and produced by radiation or as by-products of the metabolic processes (Cheung *et al*.,2003) . To gain stability, free radicals capture the electrons quickly from other compounds and the attacked compound becomes a free radical itself, which continues to attack other compounds and leads to a chain reaction. These results in the disintegration of cell membranes and cell compounds, including lipid, protein and nucleic acids. Besides damage to living cells, free radicals are the major cause of food deterioration through lipid oxidation, which ultimately affects the organoleptic properties and edibility of foods (Kaur *et al.,* 2001). Recent research suggested that synthetic antioxidants could promote tumor formation as well as anticarcinogenic properties. Due to these contradictory properties, the application and exploration of natural antioxidants has received more attention (Halliwell,1989).

Ascorbic acid , the standard antioxidant used in the present study , acts as a chain breaking scavenging agent that impaires the formation of free radicals in the process of intracellular substances formation throughout the body, including collagen bone matrix and tooth.(Aquil *et al.,* 2006) . Several methods have been developed to estimate the antioxidant capacity of different plant materials (Guo *et al* .,2003). A single assay is not sufficient to evaluate the total antioxidant activity (Silva *et al* .,2006). Hence in the present study , the ethanolic extract of *A.chelonoides* were investigated for their antioxidant activity using DPPH radical scavenging activity, Nitric oxide scavenging activity, Super oxide radical scavenging activity, FRAP assay , Total antioxidant assay, Reducing power assay.

The DPPH radical scavenging assay is used for evaluation of antioxidant potential of natural products because of its stability in the radical form, accuracy and simplicity of the assay (Bozin *et al*., 2007).The radical scavenging activities of ethnolic extract of *A. chelonoides* were tested using the 'stable' free radical , DPPH. Unlike laboratory generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition.,(Amarowicz *et al* .,2004).DPPH is very popular for the study of natural antioxidants (Bhagya *et al* .,2013). DPPH radical scavenging is considered to be a good *in vitro* model widely used to asses antioxidant efficacy within a very short time. In its radical form, DPPH disappears, on reduction by an antioxidant compound or a radical species, to become a stable diamagnetic molecule resulting in the colour change from purple to yellow, due to the formation of diphenyl picryl hydrazine (DPPH), which could be taken as an indication of the hydrogen donating ability of the tested samples (Lee *et al*., 2007). The relatively stable organic radical , DPPH, has been widely used in the determination of antioxidant activity of a single compound, as well as of different plant extracts(Katalinic *et al*.,2006).In the present study , among the solvents tested , the AC CRD extract exhibited the highest DPPH radical scavenging activities. The result indicate that the plant extracts with their proton donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants (Marxen *et al*.,2007). DPPH containing an odd electron, gives maximum absorption at 517 nm. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, the absorption strength is decreased and the resulting decolorisation (Violet to yellow) is stoichiometric with respect to the number of electrons quenched (Blois , 1958).During this study, the stabilized DPPH radical produces an intense violet colour

in ethanol solution and the antioxidants present in *A.chelonoides* reacted with the DPPH free radicals and convert them into reduced form either by donating a hydrogen atom or transferring an electron followed by proton. The decrease in absorbance is taken as a measure of the extent of radical scavenging. These IC_{50} values obtained for AC CRD is (33.73 µg/mL) and is almost comparable to the standard Ascorbic acid (13.32 µg/mL) used. The result obtained shows that AC CRD contain phytochemical constituents that are capable of donating hydrogen or transferring an electron to free radicals, thereby acting as potent free radical inhibitors or radical scavengers which can be correlated with previous studies. A similar study is reported in different species of genus *Asystasia*. In *A. travancorica* the scavenging effect of all the extracts and also the standard increased with the increase in the concentration. Among the solvents tested, whole plant ethanol extract exhibited highest DPPH radical scavenging activity of 96.36%, the maximum at 800 µg/mL concentration. The concentration of *A.travancorica* whole plant ethanol extract needed for 50% inhibition (IC) was found 50 to be 20.13µg/mL, whereas 20.94µg/mL needed for ascorbic acid.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation, regulation of cell mediated toxicity (Hagerman *et al*.,1998) and prevention of cardiovascular diseases(Cannon *et al*.,1998). NO is generated by specific nitric oxide synthases (NOSs), which metabolizes arginine to citrulline with the formation of NO via. a five-electron oxidative reaction (Ross, 1993). Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4 and NO_3 are reactive and responsible for altering the structural and functional behavior of many cellular components resulting in DNA fragmentation, neuronal cell death and cell damage (Dawson *et al*., 1992). Incubation of sodium nitroprusside solution in PBS at physiological pH reacts with O_2 to form stable nitrite and nitrate ion. These nitrite ions further react with sulphanilamide present in the Griess reagent to produce diazotized molecule measured at 546 nm. The antioxidants present in the AC CRD effectively scavenged the free radicals and competed with oxygen for nitrous oxide, leading to the decrease in production of nitrite ion and finally the diazotized molecule. Maximum nitric oxide scavenging activity was shown by AC CRD followed by AC ETH when compared to other fractions. Potent NO scavenging activity shown by the *A.chelonoides* indicates that it may be useful in preventing the effects of excessive NO generation in inflammatory conditions and cellular damage. Even though the most active fraction AC CRD only showed 72.34% at 320 µg/mL and the EC_{50} of the extract was found to be 58.48 µg/mL. It is worth to note that in this study the NO radical scavenging activity of AC CRD reached equilibrium or steady state at the higher doses used which may help to protect the remaining NO for the proper maintenance of physiological processes and cellular integrity. Traditionally used medicinal plants and plant products has the ability to scavenge NO radicals effectively in inflammatory conditions. (Basu and Hazra, 2006).

Ferric reducing antioxidant potential (FRAP) assay measures the ability of antioxidants in plant extracts to reduce ferric ions. The antioxidant reacts with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and produces a colored ferrous tripyridyltriazine (Fe2+-TPTZ). The free radical chain breaking takes place through donating a hydrogen atom. At low pH of about 3.6, reduction of Fe^{3+}-TPTZ complex to blue colored Fe^{2+}-TPTZ takes place and the change is monitored spectrophotometrically at 593 nm (Huang *et al*., 2005). The ferric reducing or antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary polyphenols (Luximon-Ramma *et al*., 2005) . Antioxidant activity increased proportionally to the polyphenol content. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species

(Oktay *et al*., 2003). According to Hodzie *et al* ., 2009 , FRAP assay had been used to determine antioxidant activity as it is simple and quick. Besides that, the reaction is reproducible and associates with molar concentration of the antioxidants. Higher FRAP values give higher antioxidant capacity because FRAP value is based on reducing the ferric ion. AC CRD displayed the highest antioxidant potential of 142.32 µg trolox equivalent/g dry extract. Results of FRAP assay also revealed that *A.chelonoides* leaf crude extract is found to be most effective in reducing the ferric ion.

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical form mitochondrial electron transport systems. Mitochondria generate energy using electron chain reaction , reducing oxgen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion. It plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical or singlet oxygen in living systems. (Lee *et al*.,2004) . Superoxide is oxygen centred radical with selective reactivity. Although a relatively weak oxidant, superoxide exhibits limited chemical reactivity, but can generate more dangerous species, including singlet oxygen and hydroxyl radicals (OH-), which cause the peroxidation of lipids (Halliwell and Chirico; 1993). Superoxide is easily formed by radiolysis of water in the presence of oxygen, which allows accurate reaction rate constants that to be measured (Glucin and Dustan, 2007).It has been implecated in several pathophysiological processes due to its transformation into more reactive species such as hydroxyl radical. Also superoxide has been observed to directly initiate lipid peroxidation (Wickens, 2001).It has also been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical.O^{2-} is the precursor of H_2O_2, OH- and singlet oxygen, which induce 02 oxidative damage in lipids, proteins and DNA. Superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents(Pietta, 2000). Speroxide anions are the most common free radicals formed *in vivo* and are generated in a variety of biological systems and the concentration of superoxide anions increases under conditions of oxidative stress (Lee et *al*.,2002). It was therefore proposed to measure the comparative interceptive ability of the methanol extracts to scavenge the superoxide radical. In the present study , superoxide scavenging activities of extracts were measured by auto-oxidation of hydroxylamine in the presence of NBT. The reduction of MBT at 560 nm indicates the consumption (Khanamn *et al* .,2004). Overproduction of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences (Pervaiz and Clement, 2007). AC CRD under study were found to be an effective scavenger of S radical in a dose dependent manner. AC CRD showed maximum superoxide radical scavenging of 78.56%at 320 µg/mL and the EC_{50} was found to be 93.26 µg/mL, whereas the standard Ascorbic acid showed an EC_{50} value of 24.58 µg/mL revealing its potent superoxide radical scavenging ability. This result is in accordance with that of *Baccaharis grisebachii*(Tapia *et al* .,2004 and Calendula *et al*., 2006).

Reducing power assay measures the electron-donating capacity of an antioxidant (Hinneburg *et al* .,2006). In this assay the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe3+/ ferricyanide complex to the ferrous form which may serve as a significant indicator of its antioxidant capacity (Amarowicz *et al* .,2004). The existence of reductones is the key of the reducing power and the reductones exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Singh and Ranjini, 2004).The reduction of the Fe3+/ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution

(Siddhuraju *et al* .,2002).Antioxidant components and their activity are highly dependent on extracting solvent and concentration of solvent, but they also vary within the samples .In this study, higher absorption at higher concentration indicates the strong reducing power potential of the extracts. It is suggested that the extracts have high redox potentials and can act as reducing agents. Several reports shown a close relationship between total phenolic content and antioxidant activity of the plant extracts (Deighton *et al* .,2000). Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content , which also need their characterization(Heinonen *et al* .,1998). *A.chelonoides* showed a dosed dependent reducing potential depending on the antioxidant constituents present. AC CRD showed maximum absorbancy comparable to the standard Ascorbic acid. The results obtained in this study was in correlation with that of the study conducted by Umesh *et* al., 2014 where the methanolic extract of *Asystasia gangetica* showed highest activity which was appeared to be comparable to the activity of the reference standard Ascorbic acid that gave an absorbance of 3.12 and 2.10 at the same concentration.

Total antioxidant capacity was evaluated quantitatively using phosphomolybdenum assay which is based on reduction of Mo (VI) to Mo (V) by the analytes with the formation of green phosphate/ Mo V at low pH. The reducing power of a compound is associated with electron donating capacity and serves as an indicator of antioxidant activity (Renuka *et al* .,2012). This method is efficient in evaluating both water soluble and fat- soluble antioxidants. , usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids (Riaz *et al*., 2012). In the present study, total anti-oxidant capacity of the plant extracts was compared and expressed as Ascorbic acid equivalents. AC CRD showed the maximum antioxidant capacity of 106.48 µg of ascorbic acid equivalent/mg of dry extract. Umesh *et al*., 2014 studied total antioxidant activity of *Asystasia gangetica* and reported that methanol had higher total antioxidant activity with the order: Methanol(58.032±1.22)>Ethanol(45.229 ±1.383)>aqueous(36.229 ±1.708).

***Ex vivo* antioxidant study.**

Anti-lipid peroxidation studies.

Lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to the production of a variety of breakdown products, including alcohol, ketone, alkanes, aldehydes and ethers (Dianzani *et al*.,2008) . This process is initiated by hydroxyl and superoxide radicals leading to the formation of peroxy radicals that eventually propagate the chain reaction in lipids (Baratta *et al*., 1998). During this process, free radicals take electrons from the lipids in cell membranes, resulting in a loss of membrane fluidity, as well as an increase of membrane permeability and decrease in physiological performance leading to endanger cell viability (Geetha and Vasudevan., 2004).

Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reactions (Ak and Gülçin, 2008), which are important in the pathogenesis of various diseases and inflammatory conditions. Lipid peroxidation is a complex process known to occur in both plants and animals which involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products. The toxicity of lipid peroxidation products such as alcohols, ketones, alkanes, aldehydes and ethers (Dianzani and Barrera, 2008) may lead to neurotoxicity, hepatotoxicity and nephrotoxicity in mammals (Boveris *et al*., 2008). In biological systems, lipid peroxidation generates several aldehyde products, among which MDA is considered to be the most

important derivative and the elevated level of malondialdehyde is used as an indicator of lipid peroxidation (Rukmini *et al*., 2004).. AC CRD at 25 µg/mL to 200 µg/mL showed potent inhibition of MDA production indicating anti-lipid peroxidation activity. AC CRD at 200 µg/mL was found to be the most promising one with 66.09% of inhibition in MDA production and IC_{50} value of 99.42 ± 3.64µg. The potent anti-lipid peroxidation activity shown by the AC CRD can be directly correlated to their respective radical scavenging activities shown by antioxidant phytoconstituents. The results of *in vitro* antioxidant and anti-lipid peroxidation studies revealed that AC CRD of A. *chelonoides* showed potent activity by showing strong radical scavenging effects against various radicals tested and strong reducing potential activity. Although AC CRD showed promising activity, the detailed analysis of all the results revealed that AC CRD is the most potent sample with *in vitro* antioxidant and anti-lipid peroxidation activity. Based on these studies, it is evident that the leaf of *A.chelonoides* exhibited good radical scavenging, especially of those of peroxy type and probably have the ability to inhibit autoxidation of lipids and thus could be beneficial in the treatment of various diseases where lipid peroxidation is an important mechanism for pathogenesis. The free radical scavenging and anti-lipid peroxidation activity of the *A.chelonoides* is of great significances proving its various traditional medicinal uses against hepatic damage and associated diseases and becomes the base for the scientific validation of the tribal claim.

Conclusion

In conclusion, the crude extract of *Asystasia chelonoides* leaves was found to be an effective antioxidant in different *invitro* antioxidant assays including DPPH, Nitric oxide, Superoxide, FRAP, Total antioxidant and reducing power assays. Antioxidant rich plant extracts serves as sources of neutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases. An effort has been made to explore the antioxidant properties of commercial available herbal extracts. This indicates the potential of the extracts as a source of natural antioxidants or neutraceuticals with potential application to reduce oxidative stress with consequent health benefits .

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Reference

1. Ak, T., Gülçin, I., 2008. Antioxidant and radical scavenging properties of curcumin. Chem Biol. Interact. 17, 27-37.
2. Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B. and Weil, J. 2004. Free-radical scavenging capacity antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 84: 551-562.
3. Aqil, F., Ahmed, I. and Mehmood, Z. 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plant. Turk. J. Biol. 30: 177-183.
4. Basu, S., Hazra, B. 2006. Evaluation of nitric oxide scavenging activity, In vitro and ex vivo, of selected medicinal plants traditionall y used in inflammatory diseases. Phytother. Res. 20(10), 896-900.
5. .Baratta, T.M., Dorman, D.H.J., Deans, S.G. 1998. Chemical composition antimicrobial and antioxidative activity of Laurel, Sage, Rosemary, Oregano, and Coriander essential oils. J Essen Oil Res. 10(6), 618-27.

6. Benzie, I.F., Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239, 70-76.
7. Bhagya K.R, Chandrashekar .K.R. Evaluation of plant and callusextracts of Justicia gendarussa Burm.F for phytochemicals and antioxidant activity.Int J Phar Pharmaceu Sci 2013; 5(2):82-5.
8. Blios, M.S. 1958. Antioxidant determinations by the use of a stable Free Radical. Nature 181, 1199-1200.
9. Boveris, A., Repetto, M.G., Bustamante, J., Boveris, A.D., Valdez, L.B. 2008. The concept of oxidative stress in pathology. In: Álvarez, S.; Evelson, P. (ed.), Free Radical Pathophysiology, Transworld Research Network: Kerala, India. 1-17.
10. Bozin, B., Mimmkica-Dukic, N., Samojlik, I., Jovin, E. 2007. Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus offcinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J. Agric. Food Chem. 55, 7879-7885.ttary antioxidants on DNA
11. Cannell, R.J.P. 1998. Natural Products Isolation. Human Press Inc. New Jersey. 165-208.
12. Dawson, T.M., Dawson, V.L., Snyder, S.H. 1992. A novel neuronal messenger molecule in brain, the free radical, nitric oxide. Annu. Neural. 32, 297-311.
13. Deighton, N., Brennan, R., Finn, C. and Davies, H.V. 2000. Antioxidant properties of domesticated and wild Rubus species. J. Food Agric. Sci. 80: 1307-1313.
14. Dianzani, M., Barrera, G. 2008. Pathology and physiology of lipid peroxidation and its carbonyl products. In: Alvarez, S., Evelson, P., (Eds.), Free Radical Pathophysiology, Transworld Research Network, Kerala, India. 19-39.
15. Fejes, S., Blazovics, A., Lugasi, A., Lemberkovics, E., Petri, G., Kery, A. 2000. In vitro antioxidant activity of Anthriscus cerefolium L. (Hoffm.) extracts. J Ethnopharmacol. 69,259-265.
16. Fu, W., Chen, J., Cai, Y., Lei, Y., Chen, L., Pei, L., Zhou, D., Liang, X., Ruan, J. 2010. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. Et Sav.) Ching. J. Ethnopharmacol. 130(3), 521-528.
17. Gaur G, Alam MS, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of Cassia siamea flowers. J Ethnopharmacol. 2006;108:340–8.
18. Geetha, R. K., Vasudevan, D.M. 2004. Inhibition of lipid peroxidation by botanical extracts of Ocimum sanctum: in vivo and in vitro studies. Life Sci. 76(1), 21-8.
19. Gulcin, A. and Dastan. 2007. Synthesis of dimeric phenol derivatives and determination of in vitro antioxidant and radical scavenging activities. J. Enzy,. Inhib. Med. Chem. 22: 685- 695.
20. Guo, C., Yang, J., Wei, J., Li, Y., Xu, J. and Jiang, Y. 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutri. Res. 23: 1719- 1726.
21. Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agri. Food Chem. 46 (5), 1887-1892.
22. Halliwell B and Gutteridge JMC, Free radical in biology and medicine, 1989, Oxford: Clarendon Press; 1989. p. 96–8.
23. Halliwell B, Gutteridge JM. 1990. Role of free radicals and catalytic metal ions in human diseases: an overview. Methods Enzymol 1990;186:1–8.
24. Halliwell, B. and Chirico.S. 1993. Lipid peroxidation: its mechanism, measurement and significance. Am. J. Clin. Nut. 57: 715-725.

25. Heinonen, M., Lehtonen, P.J. and Hopla, A. 1998. Antioxidant activity of berry and fruit wines and liquor. J. Agric. Food Chem. 48: 25-31.
26. Hinneburg, I., H.J.D. Dorman, R. Hiltunen, Antioxidantactivities of extracts from selected culinary herbs and spices, Food Chem. 97 (2006) 122–129.
27. Huang, D., Ou, B., Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. J. Agri. Food Chem. 53(6), 1841-1856.
28. Kaur C Kapoor.H.C, Antioxidant in fruits and vegetables- the Millennium's health, *Intern J Food Sci Technol*, 2001, 36, 703-25.
29. Khanamn, S., Shivaprasad, H.N. and Kshama, D. 2004. In vitro antioxidative screening models: A review. Indian. J. Pharm Edu. 38: 180-3.
30. Lazze MC,Pizzala. R,Savio. M, Stivala.L.A, Prosperi.E,Blanchi.L, Anthocyanins protect against DNAdamage induced by tertbutyl- hydroperoxide in rat smooth muscle and hepatoma cells, Mutat Res 2003, 535: 103-115.
31. Lee, J., Koo, N. and Min, D.B. 2004. Reactive oxygen species, aging and antioxidative nutraceuticals. Comphre. Rev. Food Sci. Food Safety. 3: 21-33.
32. Lee, J.C., Kim, H.R., Kim, J. and Jang, Y.S. 2002. Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. J. Agric. Food Chem. 50: 6490-5496.
33. Lee, Y.R., Woo, K.S., Kim, K.J., Son, J.R. and Jeong, H.S. 2007. Antioxidant activities of ethanol extracts from germinated specialty rough rice. Food Sci. Biotechnol. 16: 765-770.
34. Limón-Pacheco J and Gonsebatt ME: The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res 2009; 674(1-2): 137-47.
35. M. Oktay, I. Gulcin, O. I. Kufrevioglu (2003). Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Lebensm.Wiss.Techol. 36, 263–271.
36. Marxen, K., Vanselow, K.H., Lippemeier, S., Hintze, R., Ruser, A. and Hansen, U.P. 2007. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. Sensors. 7: 2080–2095.
37. Mathur R and Vijayvergia R: Determination of total flavonoid and phenol content in Mimusops elengi Linn.International Journal of Pharmaceutical Sciences and Research 2017; 8(12): 5282-85.
38. Mondal, S.K., Chakraborty, G., Gupta, M., Mazumder, U.K. 2006. In vitro antioxidant activity of Diospyros malabarica Kostel bark. Ind. J. Exp. Biol. 44(1), 39-44.
39. Okhawa, H., Ohishi, N., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95(2), 351-359.
40. Pervaiz, S. and Clement, M. 2007. Superoxide anion: oncogenic reactive oxygen species? Int. J. Biochem. Cell Biol. 39: 1297-1304
41. Pietta, P.G. 2000. Flavonoids as antioxidants. J. Nat. Prod. 63: 1035-1042
42. Rasineni G.K, Siddavattam.D, Reddy.A.R. Free radical quenching activity and polyphenols in three species of Coleus. J.Med Plants Res 2008, 2:285-291.
43. Renuka, D., Amit, S., Malpathak, N 2012. Phytochemical composition and Antioxidant Potential of *Ruta graveolens* L. *In vitro* Culture Lines. J. of Botany; doi:10.1155 /685427.
44. Riaz T., Abbasi, M.A., Rehman, A.U., Shahzadi, T., Ajaib, M., and Khalid Mohammed Khan, K.M. 2012., Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of Dodonaea viscosa Jacq. J. Serb. Chem. Soc. 77(4), 423-435.

45. Ross, R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature 362, 801-809.
46. Rukmini, M.S., D'Souza, B., D'Souza, V. 2004. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. Indian J. Clini. Biochem 19(2), 114-118.
47. Shaker ME, Zalata KR, Mehal WZ, Shiha GE, Ibrahim TM. Comparison of imatinib, nilotinib and silymarin in the treatment of carbon tetrachloride induced hepatic oxidative stress, injury and fibrosis. Toxicol Appl Pharmacol. 2011;252:165–75.
48. Siddhuraju, P., Mohan, P. and Becker, K. 2002. Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. 79: 61-67.
49. Silva, E.M., Souza, J.N.S., Rogez, H., Rees, J.F. and Larondella, Y. 2006. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chem. 101: 1012-1018.
50. Singh, N. and Ranjini, P.S. 2004. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 85: 611-616.
51. Szeto Y.T, Collins.A.R, Benzie I.F. Effects of die ttary antioxidants on DNA damage in lysed cells using a modified comet assay procedure.Mutat Res 2002, 500: 31-38.
52. T.G.Umesh,Abhirami Dilkalal 2014; Evaluation of antioxidant potential and reducing power of callus induced from leaves of Asystasia gangetica (L.)T. Anderson ; International J.of pharmacy and pharmaceutical sciences, vol.6, Issue 8.
53. Tapia, A., Rodriguez, J., Theoduloz, C., Lopez, S., Feresin, G.E. and Schmeda-Hirshmann, G. 2004. Free radical scavengers and antioxidants from Baccharis grisebachii. J. Ethanopharmacol. 95: 155-161.
54. Umamaheswari., Chatterjee, TK. 2008. *In vitro* antioxidant activities of the fractions of *Coccinia grandis*. L. leaf extract. Afr J Traditional, complementary and Alternative Medicines. 5(1), 61-72.
55. Wickens, A.P. 2001. Aging and the free radical theory. Resp. physiol. 128: 379-391
56. Xavier J and Reddy J: A study on antioxidant and antibacterial activities of the fruit and seed extracts of two different cultivars of Momordica charantia Linn. J Pharmacogn Phytochem 2017; 6: 1182-87.