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Preliminary Phytochemical Sereening and in *Vitro* antioxidant Activity of Extracts of whole Plant of *Sonchus Oleraceus Asteraceae*

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Abstract

Plant drug are being increasingly utilized to treat a wide variety of clinical diseases many oxidative stress related diseases are as a result of free radical in body. A lot of research are going on worldwide directed towards natural antioxidant of herbal origin. The Present study was conducted to ascertain the folkloric claim of its antioxidant protential of Crude powdered drug extracted with petroleum ether and ethanol (90%) named as PEX and ALX. Preliminary photochemical screening revealed the presence of cardiac glycoside, steroid, tannis, phenolic compound, flavonoid and carbohydrate. As these compounds are known to support antioxidant acivities hence Further DPPH, H_2O_2 assay and OH-scavenging activity of ALX and PEX showed IC-50 as 38.37 µg/ml, 52.27 µg /ml, 55.27 µg/ml, 202.79µg/ml, 203.85µg/ml respectively our findings provide evidences that the crude extracts of the plant is a potential source of natural antioxidants and this justified its uses in folkloric medicines.

Keywords: Antioxidants, scavenging, photochemical, sox let extraction.

Introduction

Living cell may generate free radical and other reactive oxygen species as a result of physiological and biochemical changes. Free radical can cause oxidative damage to lipid protein and DNA eventually leading to many chronic disease such as diabetes, aging and other degenerative disease in human[1]. Plants are endowed with free radical scavenging molecules such as vitamins, terpenoid, phenolic acids, lignins , tannis, flavonoids, quinines, alkaloid, amines and other metabolites which are rich in antioxidant activity[2,3]. Studies have shown that many of these antioxidant compound possessanti inflammatory, antiatheroscleratic, antitumor, antimutagenic, anticarcinogenic, antibacterial, antiviral, and other activity[4,5], The ingestion of natural antioxidant has been associated with reduced risk of cancer, cardiovascular diseases, diabetes and other diseases associated with aging[6,7]. In recent year there has been worldwide trends towards the natural phytochemical present in the berry crops, teas, herbs, oil seed, beans, fruits, vegetables[8,9].

The plant *sonchus oleraceus* belongs to family *Asteraceae* is an erect annual herb with simple branches. One particular feature about this sow thistle is that most of the plant is smooth and glabrous without ant hair or bristles. The stems is hollowed and have a milky sap and its lower part usually gets a purple brown colour later in spring. The leaves differ according to age, the old leaves are stalked elongated and deeply lobde. Colour of the leaves vary from pale green and green blue and may have a serrated outline but on prickles or hair. The fruits are simple achene, brownish in colour and oval/oblong in shape .The shape of involucral fruit is vase like round bottomed with tapering apes and so differs from the cylindrical shape of the bud. Plant has various use like anticancer, digestive, purgative, emollient, blood purifire and also used by tribals of Rajsthan as liver tonic.

Material and Methods

Plant Material: Plant material ***sonchus oleraceus*** were collected in the month of March 2011 from nearby area of jodhpur district of Rajasthan (India) and authenticated by botanical survey of India Jodhpur with herbarium no LMC/PH/SKJ/02 and specimen deposited to L.M. College of Pharmacy Jodhpur (Rajasthan). Whole plant than washed with water to remove soil and dirt, than reduced to small size and shade dried after than it was pulverized in to mill and stored in an airtight container for further use.

Prepration of extracts: 500 gms of powderd drug was extracted by using soxlet appratus with petroleum ether and than successiv extraction with alcohal (90%) both the extracts concentrated and dried by using vaccum dryer alcohalic and petroleum ether extracts named as **ALX and PLX** respectivly.

Chemicals: All chemical were of higest purity (≥ 99.0%) Feric chloride, HCL, dregen- droff's reagent, gallic-acid purchased from BDH and other chemicals Na_2Co_3,vanillin, phosphasate – buffer, trichloracetic - acid (TCA), 2, thiobarbituric - acid (TBA), thiocyanate (FTC), butylated hydroxy toluene (BHT), 2.,2- diphenyl-1-picryhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzthiazoline - 6 – sulphonic – acid (ABTS), sodium nitroprusside, hydrogen peroxide, sulfanillicacid, glacial - acitic acid, potassium metabisulphite (PMS) and other purchased from Merck, India LTD.

Phytochemical screening: Small portion of extracts was used for phytochemical screening for compounds which included tannins, flavonoid, alkaloid, saponins and steroids in accordance with the method[11-12].

Determination of total phenolic content: The total phenolic content of all extracts were determined by using folin Ciocalteu's assay. An aliquot (0.4ml) of extract or standard solution of Gallic acid (1, 5, 10, 15 and 20 $\mu g/mL^{-1}$) was added to 10 ml volumetric flask, containing 3.6ml of double distilled water. Folin-Ciocalteu's phenol reagent (0.4 ml) was sadded to the mixture. The solution was diluted with 10 ml of DD water and mixed. After incubation of 90 min at room termperature, the absorbance against prepared regent blank was determined at 750 nm with an UV-Visible spectrophotometer shimadzu 1700. Total phenolic content of various extract were expressed as Mg Gallic acid Equivalent (GAE)/100 g of extract .all samples were analysed in triplicate[3B].

Antioxidant assay: Antioxidant activity of the aqueous plant extract was determined using ferric thiocynate (FTC) and thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxied at the beginning of peroxidantion while TBA method was used to measure free radical present after peroxide oxidation.

FTC method: The standard described by Kikuzaki et al.[14] was used for FTC determination. The absorbance of the resulting mixture (Red colour) was measured at 500 nm every 24h unitl the absorbance of the control reached its maximum butylated hydroxyl toluene (BHT) was used as positive controls while the mixture without the plant extract was used as the nagative control[15].

TBA method: The method of Ottolenghi (1959) modified[16] by Kikuzaki and Nakatani[16] was used for the determination of free radicals present after peroxide oxidation of aqueous leaf extract.The final sample of 0.02% w/v from the same samples prepared for FTC assay was used. Two millilitres of 20% trichloroacetic acid and 2ml of 0.67% of thiobrabituric acid were added to 2 ml of sample solution followed the FTC Method .The mixture was placed in a boiling water bath for 10 min and the centrifuged after cooling at 3000 rpm for 20 min the absorbance activity of the supernatant was measured at 552 nm and recorded after it has reached its maximum.

DPPH assay: The method of Liyana-Pathiana and Shahidi was used for the determination of scavenging activity of DPPH free radical. To 1 ml of 0.135 mM DPPH prepared in methanol was mixed with 1 ml of aqueous extract ranging from 0.2-0.8 mg/ml The reaction mixture was votexed thoroughly and left in dark at room temprature for 30 min the absorbance was measured spectrophotometrically at 517 nm the scavenging activity of the plant extract was calculated using this equation.

DPPH Scavenging activity (%)=$[(Abs_{control}--Abs_{sample})]\times100$ where $Abs_{control}$ is the absorbance of DPPH + methanol; Abs_{sample} is the absorbance of DPPH radical + sample (i.e.extract or standard)

Scavenging activity of superoxide anion: The scavenging activity of the superoxide anion was determined by the method of Yen and Chen[17]. The reaction mixture consists of 1 ml of plant extract (1mg/ml), 1 ml of PMS (60µm) preprared in phosphate buffre(0.1 M pH 7.4) and 1 ml of NADH (phosphate buffer) was incubated at 25°C for 5 min, the absorbance was read at 560 nm against blank samples:

Hydrogen peroxide scavenging activity: Scavenging activity of hydrogen peroxide by the plant extract was determined by the method of Reche. Plant extract (4ml) prepared in distilled water at various concentrations was mixed with 0.6 ml of mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2 .

Results

Prelimnary phytochemical test of the extract ALX reveated the presence of carbohydrate, protein, steroid, tannis, phenolic compand and extract PEX revealed the presence of glycoside (table-1).

Discussion: As phytochemical analysis conducted on ***sonchus oleraceus*** extract revealed the presences of tannins, flavonoids, steroid. They may have remarkable activity in cancer prevention. Flavonoid have been shown to exhibit their action through effect on membrane permeability and by inhibition of membrance bound enzyme[18] this property may explain the mechanism of antioxidant action.

Flavonoids serve as health promoting compound as a result of its anion radicals. These observation support the usefulness of this plant in folklore remedies in the treatment of stress-related ailment.

The result of DPPH scavenging activity assay in this study indicates that the plant was Roll Potential active This suggest that the plant extracts contain compounds that are capable of donation hydrogen to free radical reactivity Super oxide anion radical is are of the strongest reactive oxygen species among the free redials generated.

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membrane .Thus extract was capable of scavenging hydrogen peroxide in a concentrated dependent manner.Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free redical known as major contribution to serval clinical disorders such as diabetes mellitus, cancer, liver, disease renal failure and degenerative disease as a result of deficient natural antioxidant defense mechanism mechanism. This kind of study also done by Jain Sanjay Kumar et al.[19]

Table–1
Study of Alcoholic Extract and Pet ether Extract

S.No.	Test	Result	
		Alcoholic Extract	**Pet ether Extract**
Test for carbohydrates			
1	Molish test	Positive	Negative
Test for reducing sugars			
2	Fehling's test	Positive	Negative
3	Benedict's test	Positive	Negative
Test for monosaccharides			
4	Barfoed's test	Positive	Negative
Test for proteins			
6	Biuret test	Positive	Negative
7	Millon's test	Positive	Negative
8	Ninhydrin test	Positive	Negative
Test for steroids			
9	Salkowski's test	Positive	Positive
10	Liebermann-burchard reaction	Positive	Positive
Test for glycosides:			
Test for cardiac glycosides			
11	Legal's test	Negative	Positive
12	Keller-killiani test	Negative	Positive
Test for anthroquinone glycosides			
13	Borntrager's test	Negative	Negative
Test for saponin glycosides			
14	Foam test	Negative	Negative
Test for flavonoids			
15	Shinoda's test	Positive	Positive
16	Lead Acetate test	Positive	Positive
17	Alkaline test	Positive	Positive
Test for fats and oils:			
Solubility test			
18	Chloroform	Negative	Negative
19	90% ethanol	Negative	Negative
Test for alkaloids			
20	Dragendorff’s test	Negative	Positive
21	Mayer's test	Negative	Positive
22	Hager's test	Negative	Positive
23	Wagner's test	Negative	Positive
Test for Tannin and Phenolic compounds			
24	Ferric chloride test	Positive	Positive
25	Lead acetate test	Positive	Positive
26	Gelatin test	Positive	Positive
27	Dilute Iodine solution test	Positive	Positive

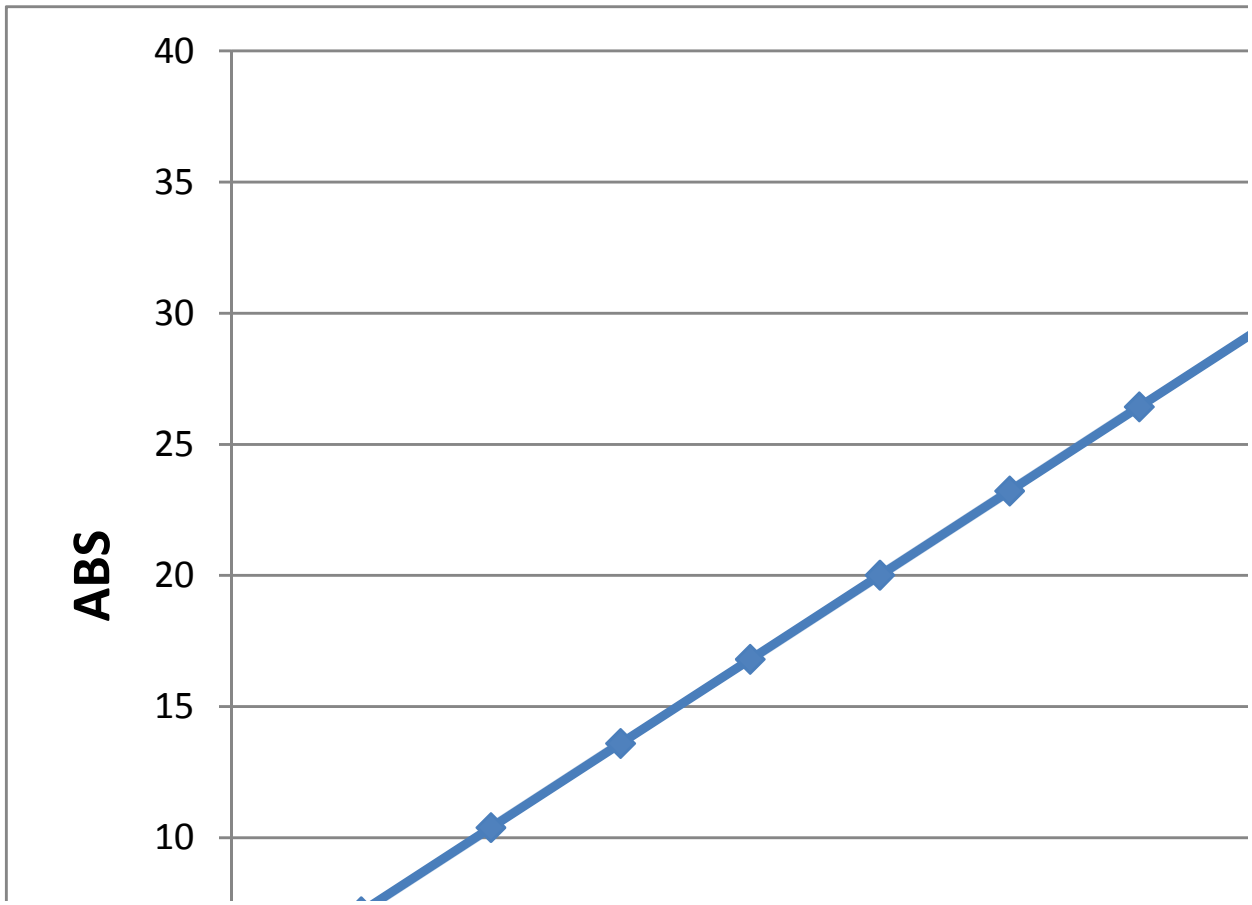

Figure-1
Standard Curve of Ascorbic Acid

Table-2
Study of DPPH-Assay

S.NO	Cone	With DPPH	Without DPPH	With-without	Control	%inhibition	
1	10	0.071	0.009	0.062	0.079	0.21519	21.51899
2	20	0.067	0.01	0.057	0.079	0.278481	27.8481
3	30	0.065	0.011	0.054	0.079	0.316456	31.64557
4	40	0.061	0.012	0.049	0.079	0.379747	37.97468
5	50	0.058	0.015	0.043	0.079	0.455696	45.56962
6	60	0.052	0.016	0.036	0.079	0.544304	54.43038
7	70	0.05	0.017	0.033	0.079	0.582278	58.22785
8	80	0.046	0.018	0.028	0.079	0.64557	64.55696
9	90	0.042	0.021	0.021	0.079	0.734177	73.41772
10	100	0.038	0.023	0.015	0.079	0.810127	81.01266

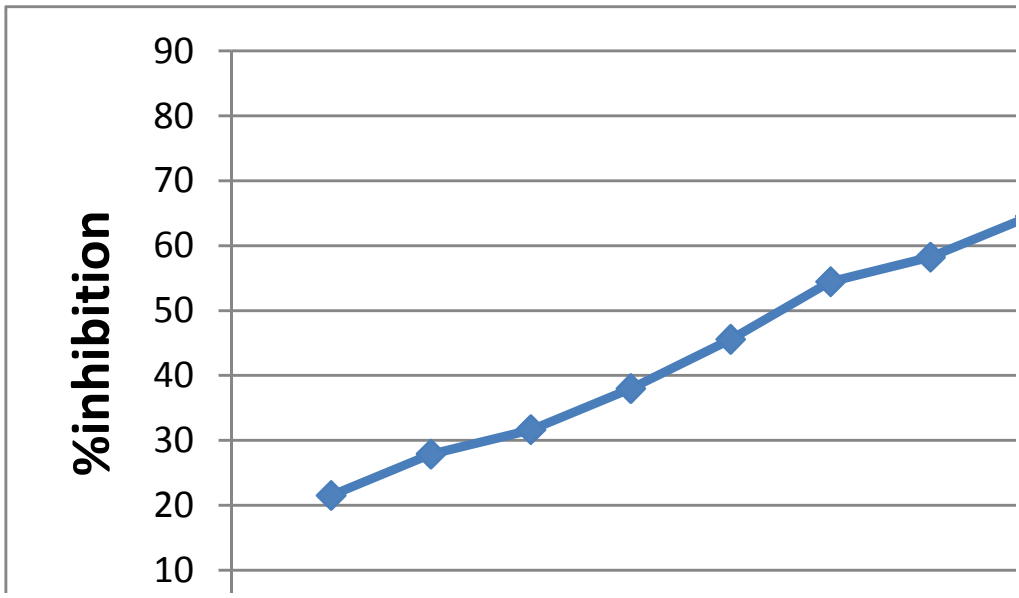

Figure-2
DPPH – PEX Curve

Table-3
Study of IC50 of Sample

S.No	IC50 of sample
1	55.64
2	59.35
3	57.89
Mean±SD	57.62±1.86
Ascorbic Acid IC-50	14.34

Table-4
Study of DPPH % inhibition

H_2O_2 .. Assay

S. No.	Cone	With DPPH	Without DPPH	With-without	Control	%inhibition	
1	5	0.921	0.008	0.913	1.24	0.26371	26.37097
2	10	0.884	0.009	0.875	1.24	0.294355	29.43548
3	15	0.862	0.012	0.85	1.24	0.314516	31.45161
4	20	0.821	0.013	0.808	1.24	0.348387	34.83871
5	25	0.795	0.014	0.781	1.24	0.370161	37.01613
6	30	0.736	0.016	0.720	1.24	0.419355	41.93548
7	35	0.718	0.016	0.702	1.24	0.433871	43.3871
8	40	0.663	0.017	0.646	1.24	0.479032	47.90323
9	45	0.618	0.019	0.599	1.24	0.516935	51.69355
10	50	0.602	0.019	0.583	1.24	0.529839	52.98387

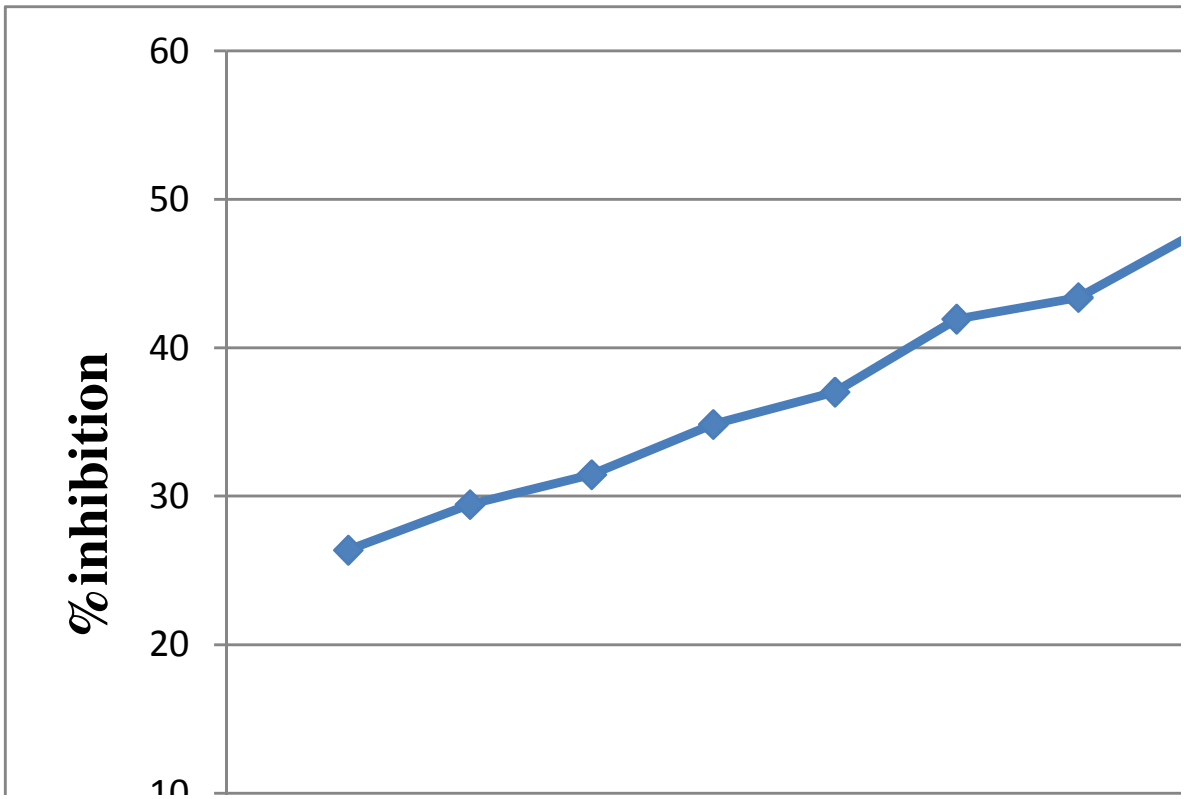

Figure-3
PEX Curve

Table-5
Study of IC50 of Sample

S. No	IC50 of sample
1	44.29
2	51.15
3	70.38
Mean±SD	57.27±13.52

Table-6
Study of OH- Scavenging Activity

S. No.	Cone	Abs	Control	%inhibition	
1	10	0.221	0.245	0.097959	9.795918
2	20	0.216	0.245	0.118367	11.83673
3	30	0.202	0.245	0.175510	17.55102
4	40	0.191	0.245	0.220408	22.04082
5	50	0.188	0.245	0.232653	23.26531
6	60	0.175	0.245	0.285714	28.567143
7	70	0.168	0.245	0.314286	31.42857
8	80	0.157	0.245	0.359184	35.91837
9	90	0.152	0.245	0.379592	37.95918
10	100	0.148	0.245	0.395918	39.59184

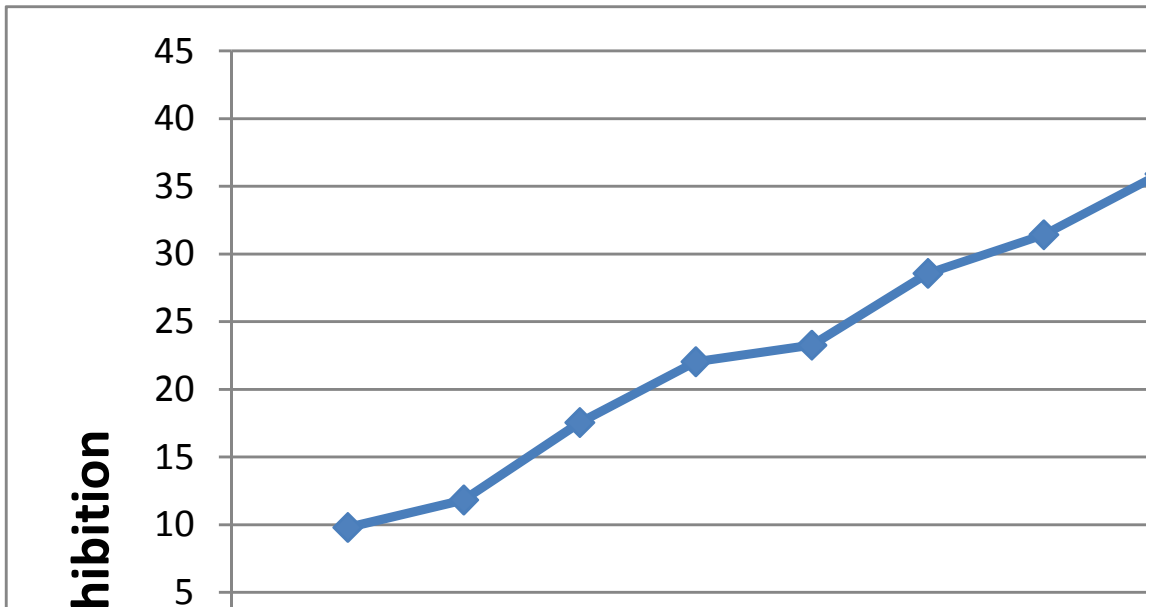

Figure-4
OH - ION- PEX Study

Table-7
Study of IC50 of Sample

S. No.	IC50 of sample
1	124.39
2	251.72
3	235.44
Mean±SD	203.85±69.29

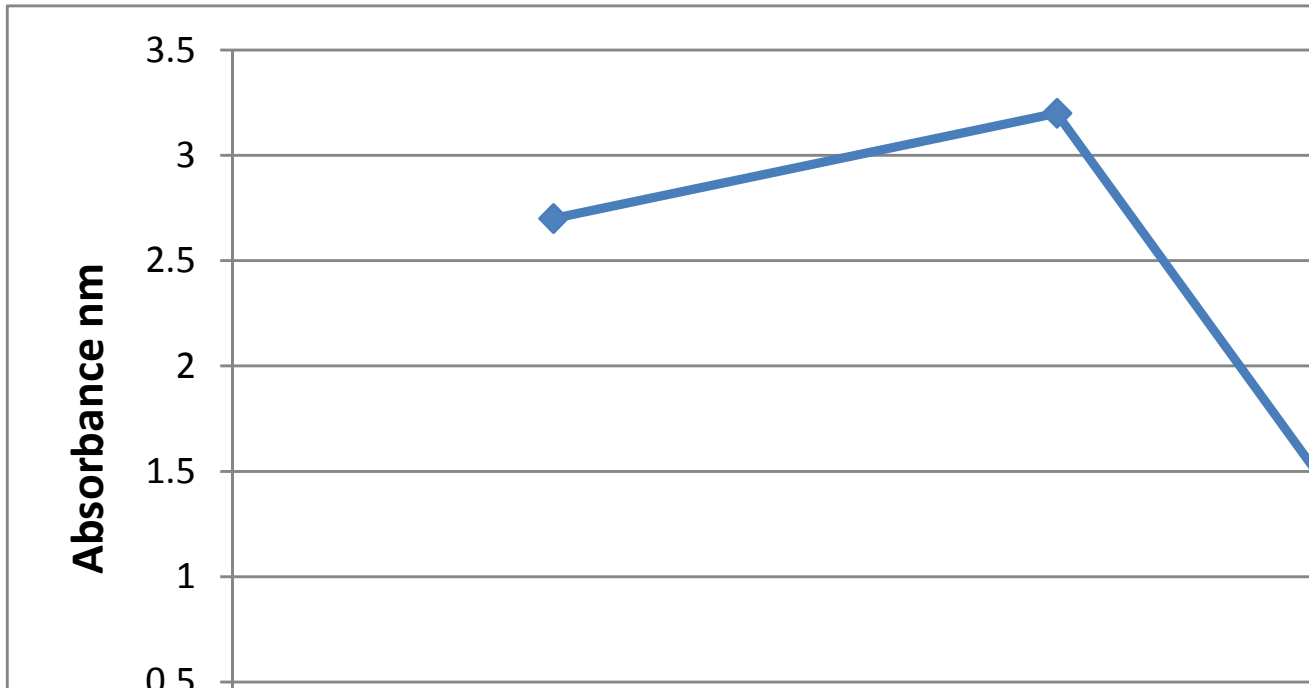

Figure-5
Standard curve of $FeSo_4$

Table-8
Study of mMEq/L (Fell)

Abs	mMEq/L(Fell)
0.04633	3.3095
0.044	3.142
0.048	3.428
	Mean±SD 3.293±0.143

Table-9
Study of DPPH-ALX Assay

S.NO	Cone	With DPPH	Without DPPH	With-without	Control	%inhibition	
1	10	0.056	0.003	0.053	0.083	0.361446	36.14458
2	20	0.054	0.008	0.046	0.083	0.445783	44.57831
3	30	0.051	0.012	0.039	0.083	0.530120	53.01205
4	40	0.049	0.014	0.035	0.083	0.578313	57.83133
5	50	0.048	0.014	0.034	0.083	0.590361	59.03614
6	60	0.046	0.016	0.030	0.083	0.638554	63.85542
7	70	0.045	0.017	0.028	0.083	0.662615	66.26506
8	80	0.044	0.018	0.026	0.083	0.686747	68.6747
9	90	0.043	0.018	0.025	0.083	0.698795	69.87952
10	100	0.041	0.020	0.021	0.083	0.746988	74.6988

Figure-6
DPPH- ALX Curve

Table-10
Study of IC50 of Sample

S.No	IC50 of sample
1	30.52
2	46.58
3	38.62
Mean±SD	38.37±8.03
Ascrobic Acid IC-50	14.34±2.84

Table-11
Study of DPPH With and Without Control

S. No.	Cone	With DPPH	Without DPPH	With-without	Control	%inhibition	
1	5	1.126	0.022	1.104	1.562	0.293214	29.32138
2	10	1.083	0.024	1.059	1.562	0.322023	32.2023
3	15	1.033	0.024	1.009	1.562	0.354033	35.40333
4	20	1.002	0.025	0.977	1.562	0.37452	37.45198
5	25	1.988	0.026	0.962	1.562	0.384123	38.41229
6	30	1.796	0.028	0.948	1.562	0.393086	39.30858
7	35	1.965	0.029	0.936	1.562	0.400768	40.07682
8	40	1.952	0.029	0.923	1.562	0.409091	40.90909
9	45	1.923	0.03	0.893	1.562	0.428297	42.82971
10	50	1.898	0.03	0.868	1.562	0.444302	44.43022

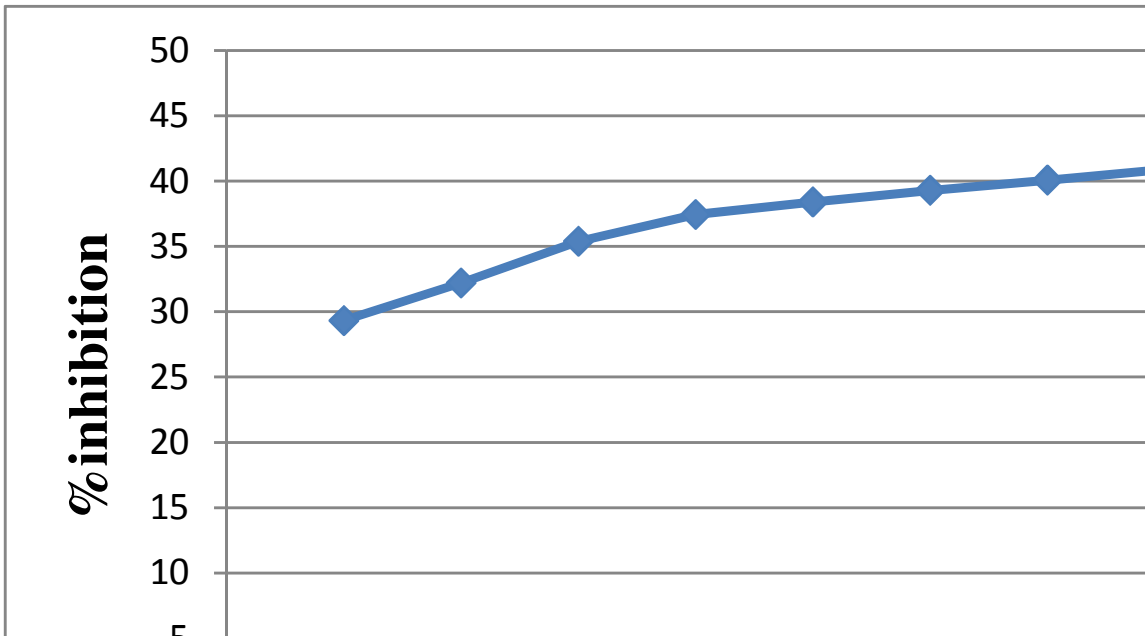

Figure-7
H_2O_2 ALX Curve

Table-12
Study of IC50 of Sample

S.No	IC50 of sample
1	67.52
2	100.93
3	87.37
Mean±SD	85.27±16.803

Table-13
Study of %inhibition

S.No.	Cone	Abs	Control	%inhibition	
1	10	0.154	0.172	0.104651	10.46512
2	20	0.15	0.172	0.127907	12.79070
3	30	0.148	0.172	0.139535	13.95349
4	40	0.145	0.172	0.156977	15.69767
5	50	0.141	0.172	0.180233	18.02326
6	60	0.139	0.172	0.191860	19.18605
7	70	0.136	0.172	0.209302	20.93023
8	80	0.132	0.172	0.232558	23.25581
9	90	0.127	0.172	0.261628	26.16279
10	100	0.124	0.172	0.279070	27.90698

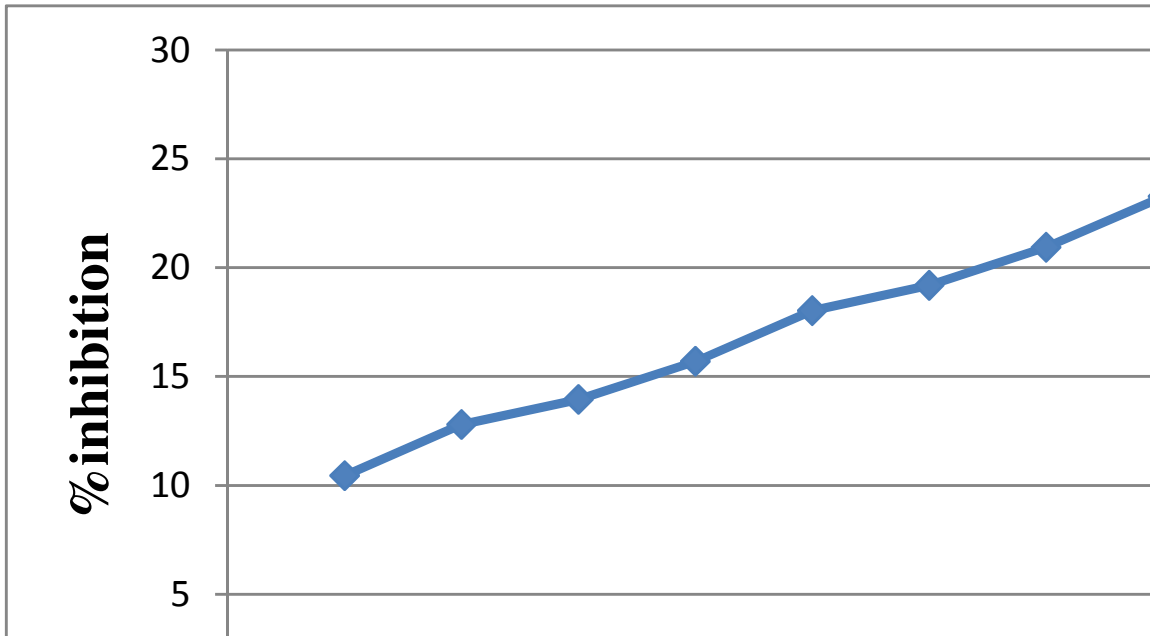

Figure-8
OH-ION Scavenging activity study

Table-14
Study of IC50 of Sample

S. No	IC50 of sample
1	219.09
2	183.80
3	205.49
Mean±SD	202.79±17.79

Table-15
Study of FRAP ASSAY

Absorbance	mMEq/L(Fell)
0.024	1.714286
0.026	1.857143
0.018	1.285714
	Mean±SD 1.619±0.297

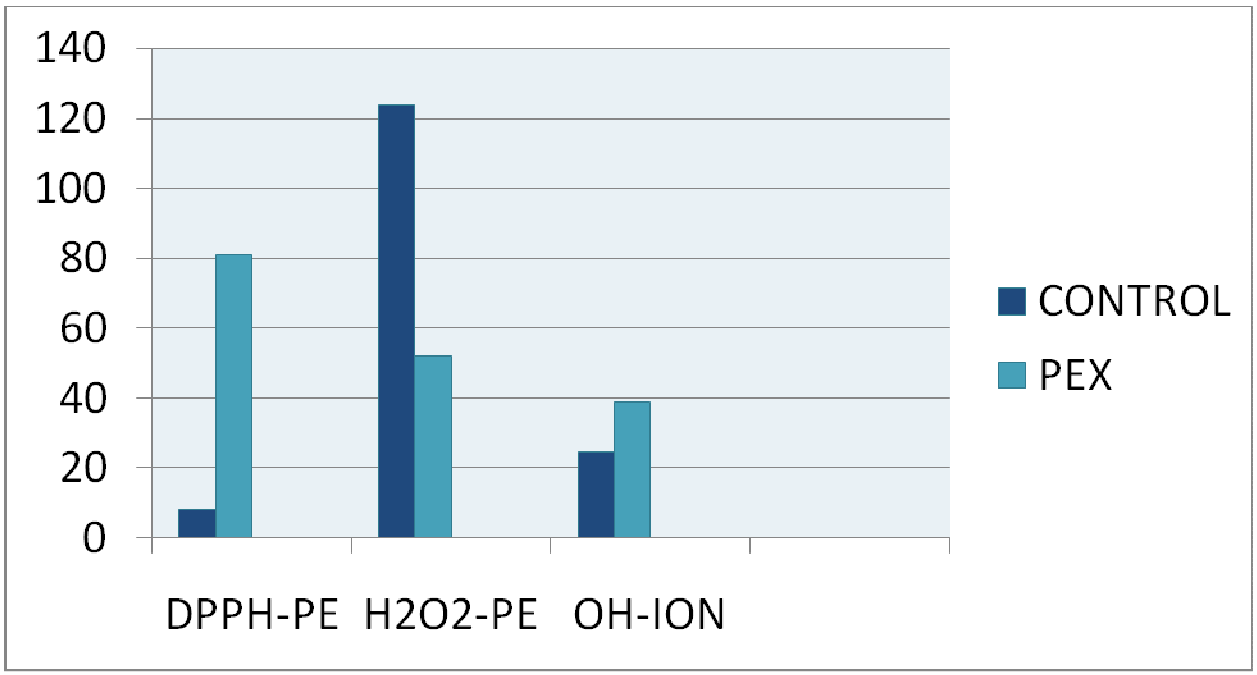

Figure-9
Percentage Inhibition PEX curve

Percentage Inhibition ALX

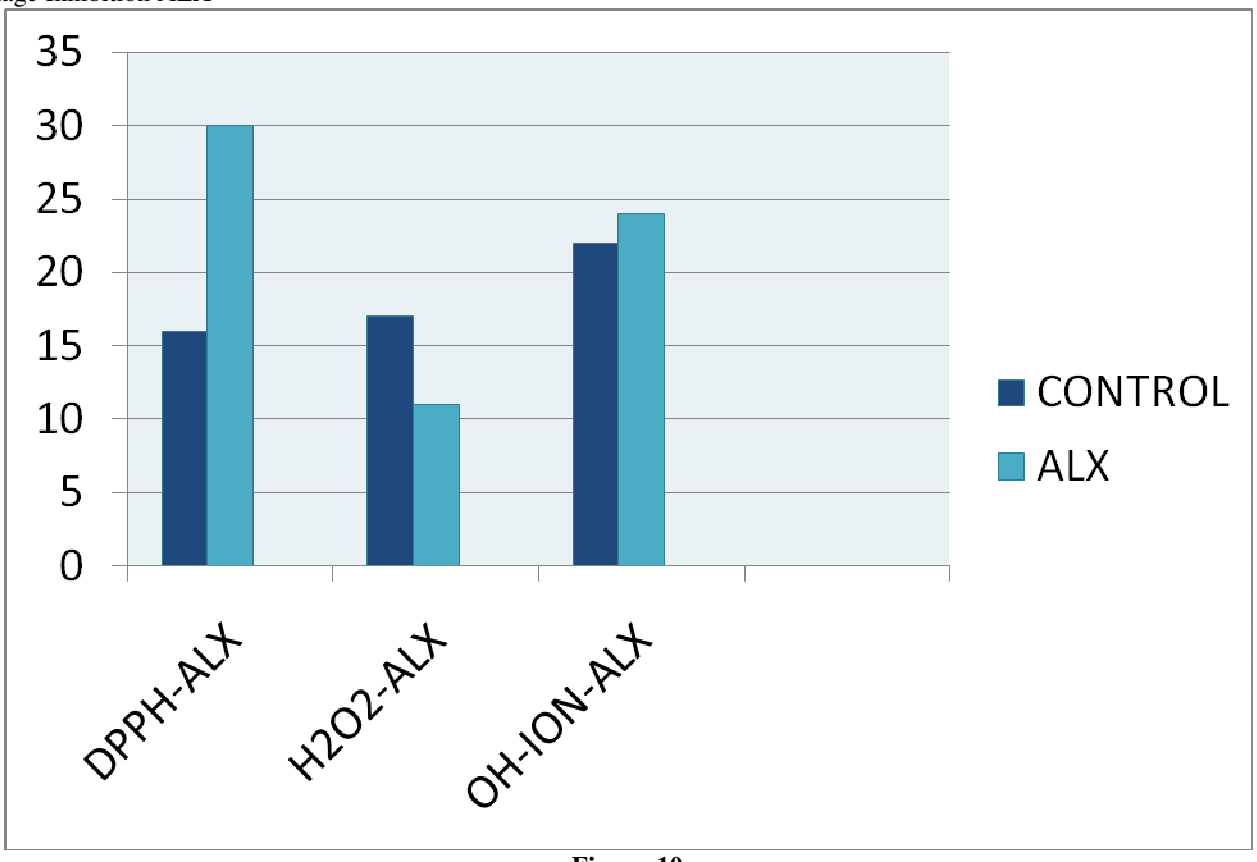

Figure-10
Percentage Inhibition ALX Curve

Table-16
Study of PLX-ALX

	PEX			ALX		
	DPPH	**H_2O_2 - PE**	**OH- ION**	**DPPH**	**H_2O_2 - PE**	**OH- ION**
1	55.64	44.29	124.39	30.52	67.52	219.09
2	59.35	51.115	251.72	46.58	100.93	183.80
3	57.89	70.38	235.44	38.62	87.37	205.49
MEAN±SD	57.62±13.52	55.27±13.52	203.85±69.29	38.37±8.02	85.27±16.803	202.79±0.297

Conclusion

This study offers the in *vitro antioxidant* potential of crude extract of ***sonchus oleraceus*** with result comparable to these of standard such as gallic acid and butylated hydroxyl toluene (BHT) further studies are needed to clarify the *invivo* potential of this plant in management of human disease resulting from oxidative stress. Alkaloid, tannis and phenolic compound which are known to support the antioxidant activity free radical scavenging activities,total antioxidant capacity.The *invitro* antioxidant potential compared with standard, BHT and gallic acid of both extract PEX and ALX.

DPPH, H_2O_2 assay and OH-scavenging activity of PEX showed IC-50 as 55.27µg/ml, 202.79 µg/ml,203.85 µg/ml And DPPH, H_2O_2 assay and OH-Scavenging activity of ALX showed IC-50 as 38.37 µg/ml ,52.27 µg/ml 85.27 µg/ml. Our findings provide evidence that extract of the plant is potential source of natural antioxidant and justified Its use in folkloric medicines and finding of the study further suggested to carry out the hepatoprotective activity of the plant.

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