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Phenolic composition, Antioxidant activity and FT-IR Spectroscopic Analysis of Halophyte *Sesuvium portulacastrum* L. extract

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Abstract

Sesuvium portulacastrum L. commonly known as 'sea pursulane' of family Aizaceae is a good source of food and conventional medicine. The aim of the present work was to analyse the total phenolic compounds (TPC) and to observe its antioxidant capacity, in four different extract using hexane, dichloromethane (DCM), ethyl acetate and methanol solvents respectively. Results presented that extraction with Hexane (0.6 ± 0.03 gm) and DCM (0.5 ± 0.02 gm) showed the highest yield. The methanol extract revealed highest amount (40.75 mg/g dry wt. Gallic acid equivalent) of TPC. Extract in DCM showed maximum reducing power (0.350 ± 0.009)s and minimum (0.239 ± 0.009) in hexane extracts was observed. Also, DCM showed the highest antioxidant activity of 112.95 ± 0.087 mg/g dry wt. Ascorbic acid equivalent, followed by ethyl acetate (83.56 ± 0.25 mg/g dry wt. Ascorbic acid equivalent). Among the four extracts as mentioned above, in DPPH (2, 2-diphenyl-1-picryl hydrazyl) assay methanol extract revealed maximum efficacy with respect of scavenging ability (75.1%). Here DCM fraction was found powerful inhibitor (84.76%) in comparison to hydroxyl radical at 1000µg/mL concentration. The maximum hydrogen peroxide radical scavenging activity (83%) was showed by DCM fraction. The FT-IR spectrum confirmed its functional group, responsible for antioxidant potential of the halophyte S. portulacastrum. The findings of the present research work helped us to identify the proper solvent for extracting phenolic compounds from the halophyte which may provide a rich source of natural antioxidants in food industry.

Keywords: Halophyte; *Sesuvium portulacastrum*; FT-IR; Antioxidant; Phenolic compounds.

Introduction

Abbreviations: TPC-Total phenolic compounds; DCM-Dichloromethane; DPPH- 2, 2-diphenyl-1-picryl hydrazyl; FT-IR- Fourier transform infrared spectrophotometer

More than 80 % of the human population in the world depend on plants based food materials. As such different antioxidants and phenolic compounds such as proanthocyanidins, flavonoids and phenolic acids are passively taken[1,2]. Plant phenolic compounds are synthesized through phenylpropanoid pathway. These compounds play an important role during abiotic and biotic stress and protect the plants during this period[3]. Salinity, which is considered as an important abiotic stress, influences the growth and reproduction of the plants[4]. This generates oxidative stress in the plant tissue[5-7], due to which cytotoxic reactive oxygen species (ROS), such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals are generated[8]. ROS may disrupt normal metabolic activity due to degradation of lipids, protein, and nucleic acids[4,9,10], which may result in tissue injuries, cellular damage, metabolic disorder and senescence processes[11].

The enzymatic and non-enzymatic components are present among the halophytes in the form of antioxidant system, due to which they withstand and reduce toxic ROS, owing to which they survive in different saline habitats[12]. The polyphenols found in different halophytes contain higher level of bioactive compounds which help then in stress conditions. The medicinal value of halophytes are due to the above powerful compounds. As the polyphenols are used in different industries viz. functional food, cosmetic, pharmaceutical and medicine, so the economics impotence of the halophytes has considerable increased now a day[13].

Polyphenols from halophytes such as *Mesebryanthemum edule* are potentially useful as traditional medicine against bacterial and fungal infections as well as other disease like tuberculosis, sinusitis, diarrohea and infantile eczema[14]. Whereas polyphenols from *Tamarix gallica* are utilised as astringent, detergent, diuretic, expectorant and laxative[11]. Aqueous extract of *Suaeda fruticosa* are found to be suitable for hypoglycaemic and hypolipidaemic activities[15,16]. Similarly methanolic extracts of *Suaeda pruinosa* has been used to detect antimicrobial activity[17].

As the polyphenolic contains several -OH groups so they are being used as chemopreventive agents against cardiovascular and degenerative diseases[18]. So are consider as cardio protective and also acts against cancer, antibacterial and antiviral activities[19,20]. Thus the antioxidant properties may be considered for the protection of cells against oxidative stress[19]. The

biomolecular structure proanthocyanidins enables these to act as radical scavenging accents. This conformation and their intramolecular and intermolecular changes also help in the above activites[21].

Sesuvium portulacastrum L. belongs to the family Aizoaceae, commonly known as "sea purslane" that grows naturally in the coastal areas of sub-tropical, Mediterranean and warmer areas around the world[22,23]. This halophyte has been used as conventional medicine for the treatment of epilepsy, conjunctivitis, dermatitis, haematuria, leprosy and purgative and also used to cure toothache for a long time[24]. This halophyte is also being used to treat many infectious diseases and kidney problems by the conventional healers in Zimbabwe and South Africa[25]. *S. protulacastrum* expresses fatty acid methyl esters or FAME extracts which is used as a potential antimicrobial and antifungal agent[26]. The oil from the fresh leaves of this halophyte exhibit antibacterial, antifungal and antioxidant activity[27]. We did not get any reference related with other biological activity of this halophyte. Keeping the economic importance of the halophytes, the present work was carried with a view to quantify the phenolic present in *S. portulacastrum* and to determine its efficacy as an antioxidant. Other chemicals were analysed with the help of FT-IR in all four extracts.

Materials and Methods

Plant extract preparation: The species of *S. portulacastrum*, growing in Vellar estuary (Lat. 11° 29'N and Long. 79°49'E) at Parangipettai, Tamil Nadu, India was identified with the help of manual published by Kathiresan K.[28]. From there this plant specimen was collected and brought to the laboratory for further study. The specimen was cleaned properly to remove all the waste particles and was air dried at 25-30^{0}C for 4-5 days. The well cleaned and dried mortar and pestle were used to prepare the powder of the above dried specimen. The powder was sieved in 22 mm pore size sieve to get fine powder, which was stored in amber glass container and kept in dark.

Twenty gram of above powder was taken in a beaker containing three fold volume of the solvent. Separate extraction was done on orbital shaker for all the four solvent viz., hexane, dichloromethane (DCM), ethyl acetate and methanol respectively. They have different polarity and increasing hydrophilic properties. The duration of extraction was maintained for 48 h. Extraction was repeated twice and the extracts were concentrated in rota-evaporator (IKA- RV 10, USA) at a reduced pressure at <40°C. Above concentrated extracts were separately dissolved in dimethylsulfoxide (DMSO) and stored at 4^{0}C for further analysis.

Total Phenolic Content Determination: All the four crude extracts were used for the estimation of total phenolic content after slightly modified method as adopted by Tagaet, 1984[31]. Briefly, 100 µl of above crude extract was mixed with 2.0 ml of 2% sodium carbonate (Na_2CO_3). This experiment was done separately for each extract. The tube were kept vertically at room temperature. Above tube 100 µl of 50% Folin-Ciocalteau's phenol reagent was added in each tube separately and was made homogenous by thorough shaking of the tube. All the tubes were stored at room temperature in dark for 30 min. With the help of LAMBDA 25 UV/Vis spectrophotometer (PerkinElmer) the absorbance of above solution was determined at 720 nm. Gallic acid equivalent per gram (GE/g) was used as standard solution.

Estimation of reducing power capacity: Slightly modified method used by Oyaizu, 1986[32] was used to determine the reducing efficacy of all the four crude extract, through transformation of $Fe3^+$ to Fe^{2+}. The extract was diluted so that the concentration become 50, 100, 250, 500 and 1000µg. 10 ml each from the different extract was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The reaction mixture were incubated at 50^{0}C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (10%) was added to the above mixture and centrifuged at 650g for 10 min. The supernatant was taken and 2.5 ml of this was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%). Absorbance was taken at 700 nm with the help of Spectrophotometer, mentioned above. Increased absorbance is revealed increased reducing power.

Determination of Total antioxidant capacity: Protocol devised by Prietoet[33] was used to determine the antioxidant activity of all the above crude extract dissolved in DMSO. 0.3 ml of sample was mixed with 3.0 ml reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate), and the tubes containing the reaction mixtures were incubated at 95^{0}C for 90 min. in the water bath. Absorbance at all the mixture was taken at 695 nm. The total antioxidant activity was expressed as the number of equivalent of ascorbic acid.

Estimation of Radical scavenging ability on DPPH: Radical scavenging capability of the above extract was determined by slightly modified Mensor[34] technique. 2.5 ml of each of the above extracts were taken in a tube. To the above 1.0 ml from 0.3mM methanol solution of 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) was added. These tubes were stored in dark at room temperature for 30 min. Optical density was taken at 518 nm and the antiradical activity (AA) was determined by the formula mentioned below.

$$AA\% = 100 - \{[(Abs._{Sample} - Abs._{Empty\ Sample}) \times 100] / Abs._{control}\}$$

Where: Empty samples = 1 ml ethanol + 2.5 ml of different extract of the plant used here; Control sample = 1 ml 0.3mM DPPH + 2.5 ml methanol. The O.D. of the samples, the control and the empty samples were measured in comparison with methanol.

Detection of hydroxyl radicals by deoxyribose assay: Method

suggested by Halliwell *et al.*,[35] was slightly modified for the detection of hydroxyl radical by deoxyribose assay.
1 ml of reaction mixture had 100 µl of 28mM 2-deoxy-D- ribose (dissolved in KH_2PO_4–K_2HPO_4 buffer, pH 7.4), 500 µl extract of different concentrations extracted from *S. portulacastrum*, 200 µl of 200 µM $FeCl_3$ and 1.04mM EDTA (1:1 v/v), 100 µl H_2O_2 (1.0mM) and 100 µl ascorbic acid (1.0mM). The tube was incubated for 1 h at 37^0C. After this the extent of deoxyribose mortification was measured by TBA reaction for which 1.0 ml of TBA (1% in 50mM NaOH) and 1.0 ml of TCA were added to the above mixture and the tubes were heated at 100^0C for 20 min.

The tube was taken out and kept on ice for stopping the reaction of the mixture.

Absorbance was taken at 532 nm against the blank (buffer + deoxyribose). Experiment were repeated thrice and ascorbic acid was used as a positive control.

The following formula was used to calculate the percent of inhibition in hydroxyl radical.
I (%) = $[(A_0 – A_1) / A_0] \times 100$,
Where: A_0= absorbance of the control, A_1 = absorbance of the sample. The data obtained at each point was the average of three measurements.

Hydrogen peroxide radical scavenging assay: For the determination of H_2O_2 radical scavenging of the halophytes, 1 ml each of different concentration as prepared earlier of the extract was added to 2 ml solution of 10 mM H_2O_2 in 0.1 M phosphate buffer, pH 7.4.

Absorbance was taken at 230 nm against blank solution without H_2O_2 after 10 min incubation. Inhibition percentage of H_2O_2 was calculated as suggested by Gulcinet, 2004[36].

(%) inhibition = $[(A_0 – A_1) / A_0] \times 100$,

Where: A_o= Absorbance of extract + reagent, A_1=Absorbance of extract only.

Analysis of Fourier transform infrared spectrophotometer (FT-IR) spectroscopy: Fourier transform infrared spectrophotometer analyse is used to analyse the functional groups present in a molecule based on their frequencies of vibration between bonds of the atoms. All crude extracts (10 mg/ml) of *S. portulacastrum* were characterized using Fourier transform infrared spectrophotometer (FT-IR; IR Affinity-1, Shimadzu, Tokyo, Japan) for FT-IR spectra measurement in the frequency range of 400 to 4,000 cm^{-1}.

Results and Discussion

Extraction and yield of extract: The different solvents having different polarity and considered best for extraction and isolation of biologically useful molecules from plants[37]. In the present study from different solvents having wide range of polarity were used. It was observed that out of the four solvent used here, hexane was the best for the phenolic extraction yield, where the extracted amount was 0.6 gm), followed by DCM extract that was 0.5 gm. Similarly extract from the leaves yielded higher percentage in hexane solvent because in the leaves and shoots of *S. portulacastrum* mostly having lipophilic compounds such as chlorophyll, waxes, and fatty acids are presented.

Determination of Total polyphenol content (TPC): Phenolic compounds are secondary metabolites and play an imperfect in profiting the plants from biotic and abiotic stresses[12,13]. For that of total phenolic compound present in *S. portulacastrum*, four different solvent were used for the extraction. The TPC were different in the above four solvents viz., hexane, DCM, ethyl acetate and methanol. The amounts of TPC were 6.75, 24.25, 20.06 and 40.75 mg/g dry weight (DW) GAE equivalent respectively (figure-1.). Total phenolic compound, in other medicinal halophytes like *Suaeda fruticosa* (31.8 mg GAE/g DW)[38] and *Salsola kali* (17.23 mg GAE/g DW)[39] have been reported. Among the glycophytic species, phenolic has been reported in *Nigella sativa* L. (10.04 mg GAE/g DW) [40]. The present finding clearly indicates that phenolic compound are much higher in *S. portulacastrum* in comparison to the above halophytes.

Reducing power ability: In the present work, it was noted that the antioxidants in the sample reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction[41] that involves single electron transfer. The reducing power indicates that the antioxidant compounds are electron donors and reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary antioxidants[42]. In this assay, there was a gradual increase in the reducing power ability of *S. portulacastrum* extracts with the increasing concentration of all the samples (figure-2). Same trend was observed by Kumaran and Karunakaran[43] in methanol extracts of higher plants. The maximum (0.350±0.009) reducing power value was observed in dichloromethane extracts and minimum (0.239±0.009) was obtained from hexane extracts. This property is associated with the presence of reductions that are reported to be terminators of free radical chain reaction[44]. Similar observation was seen in case of other halophytes like *Limoniastrum monopetalum, Cakil emaritima, Mesembryanthemum crystallinum, M. edule, Tamarix gallica* and *Salsola kali* also with different solvent extracts of their different parts[12].

Total antioxidant activity: The total antioxidant activity of plant extract was evaluated using four different solvents (hexane, dichloromethane, ethyl acetate and methanol) were evaluated. It was observed that in phosphomolybdenum method, green colour was demonstrated due to reduction of molybdenum VI (Mo^{6+}) to phosphate/Mo^{5+} complex. Similarly it was noted

that dichloromethane extract had the highest antioxidant activity of 112.95 mg/g DW ascorbic acid equivalent, followed by ethyl acetate (83.56 mg/g DW ascorbic acid equivalent). This activity was of less significant in the rest two solvent used in the present work (figure-3). The total antioxidant activity of extract taken from another higher plant has been formed to be 245– 376 mg/g DW ascorbic acid equivalent[43], has been reported. In comparison to brown seaweed (*Sargassum pallidum*) highest total antioxidants activity (30.50 µmol $FeSO_4$ /mg) in ethanol extract[45]. Higher activity observed in the fractions might be due to the presence of different compound in the crude extract. Similarly impact of extraction solvent may have striking effects on the chemicals present in the species[46].

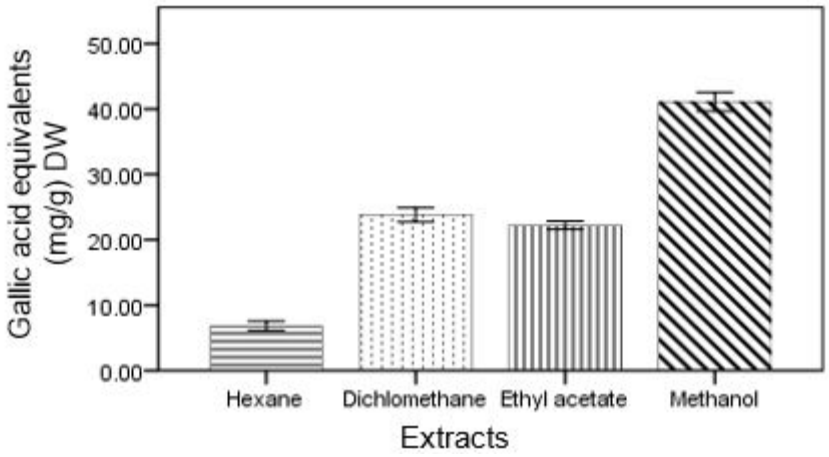


Figure-1
Total phenolic content of total extract from *S. portulacastrum*. The data are the mean ± SD of 3 replicates

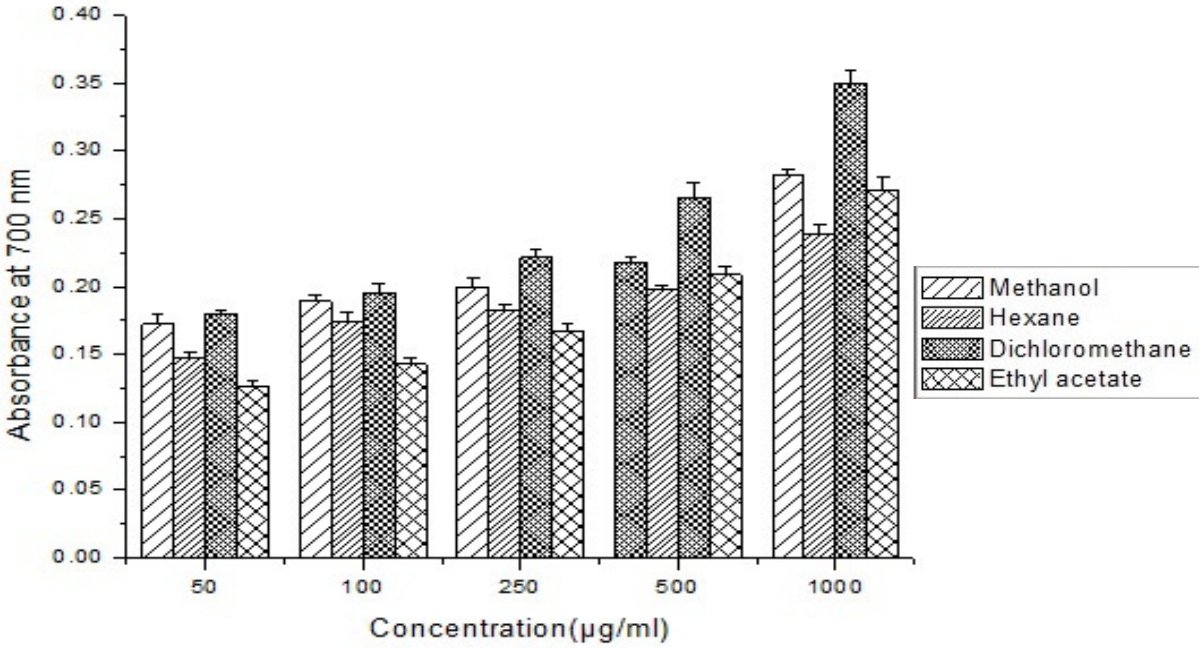


Figure-2
Reducing power of total extract from *S. portulacastrum*. The data are the mean ± SD of 3 replicates

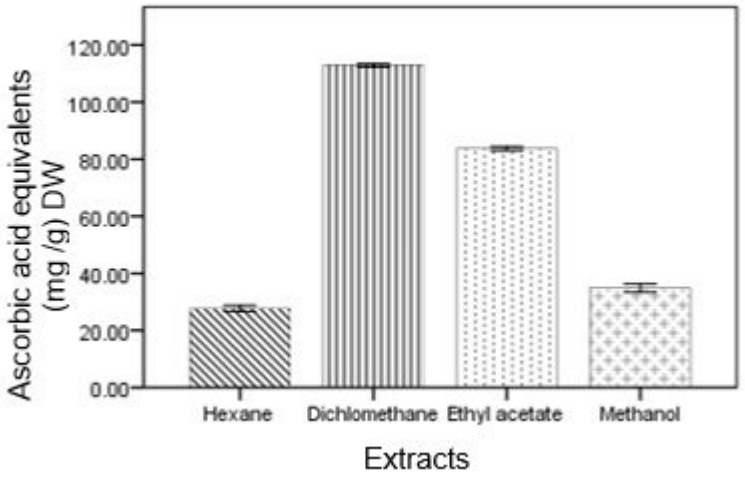


Figure-3
Total antioxidant activity of total extract from *S. portulacastrum*. The data are the mean ± SD of 3 replicates

It was found in the present work that different parts of *S. portulacastrum* had variability with respect to antioxidant activity. Parts such as leaves and stems reduced strongest antioxidant activities. Such type of different organ related variability for the total antioxidant activity has been reported for other halophytes[12] and in *Salvia* spp.[47].

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH): The antioxidant capability of the plants extracts having phenolic compounds are due to their capability to donate electrons and capture the free radicals. Due to activity of DPPH as free radical, the compound is being used to assess the reducing ability of an unknown plant extract. Therefore it is considered as a valuable agent for determining the free radical scavenging activity of unknown solution[48,49]. In present study also, DPPH was used to examine the scavenging efficacy of four different extracts of *S. portulacastrum,* treated in four different solvents. From the data presented in figure-4, it is clear that extract having high TPC had high DPPH free radical scavenging capability. This may suggest that polyphenolics are responsible for this activity among the extracts of halophyte. This may be correlated that different solvents having different polarity may often the specific group of the antioxidant compounds that influenced the scavenging capability of the extracts in different solvents taken from the same plants[50].

It is conformed in the present findings as maximum scavenging capability was 75.10% in methanol extract followed by dichloromethane (52.30%). Weak scavenging ability was noted in the rest two extracts used in the present work. Again the finding of the present work reveals that *S. portulacastrum* contains high polyphenols contents. The scavenging capability of a solution having polyphenolics with DPPH radicals have also been reported by other workers[51,52].

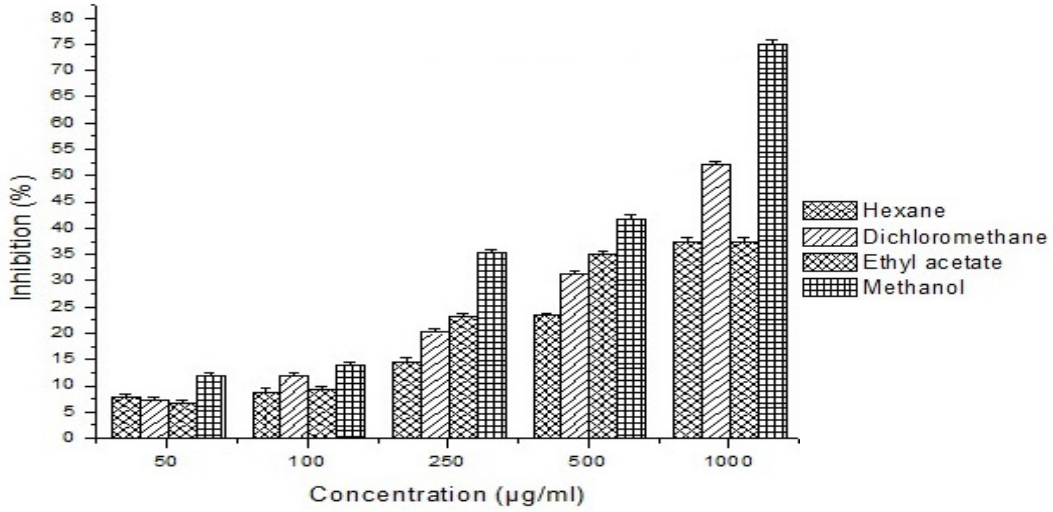


Figure-4
Scavenging effects of *S. portulacastrum* extract on DPPH radical. The data are the mean ± SD of 3 replicates

Conformation of hydroxyl radicals by deoxyribose assay: Hydoxyl radical (-OH) formed in living systems are highly damaging free radicals[53]. This may attach to the nucleotides of DNA, due to which breakage may be caused. This is considered as one of the cause of carcinogenesis, mutagenesis and cytotoxicity[54-56]. In the present work Ferric-EDTA was mixed with H_2O_2 (Fenton reaction) and ascorbic acid at pH 7.4. Hydroxyl radicals formed in the above solution were different detected by adding 2-deoxy-D-ribose and heating with TBA at low pH, which gave pink colour chromogen[35,57]. The hydroxyl radical scavenging activity of different extracts of *S. portulacastrum* was added separately to the reaction mixture. This prevented that degradation of sugar as the hydroxyl radicals were removed fluid. From the results figure-5, it is clear that DCM fraction had strongest inhibition (84.76%) against hydroxyl radical at 1000 µg/mL concentration in comparisons to other different extracts. The scavenging activity of the different extracts was in this order of DCM > ethyl acetate > methanol> hexane.

Hydrogen peroxide radical scavenging assay: Hydrogen peroxide is a weak oxidizing agent and can deactivate a few enzymes directly, generally due to oxidation of thiol (-SH) groups. It can cross cell membranes rapidly and enter inside the cell. H_2O_2 possibly reacts with Fe^{2+} and probably Cu^{2+}ions to form hydroxyl radical which may be the origin of many different type of its toxic effects[58]. It is therefore biologically useful for cells to control the amount of hydrogen peroxide that is getting accumulated inside the cells. The scavenging activity of the different fractions of *S. portulacastrum* is displayed in figure-6. In this study, all fractions exhibited appreciable scavenging activity. Here also maximum scavenging activity (83%) was exhibited by DCM extract.

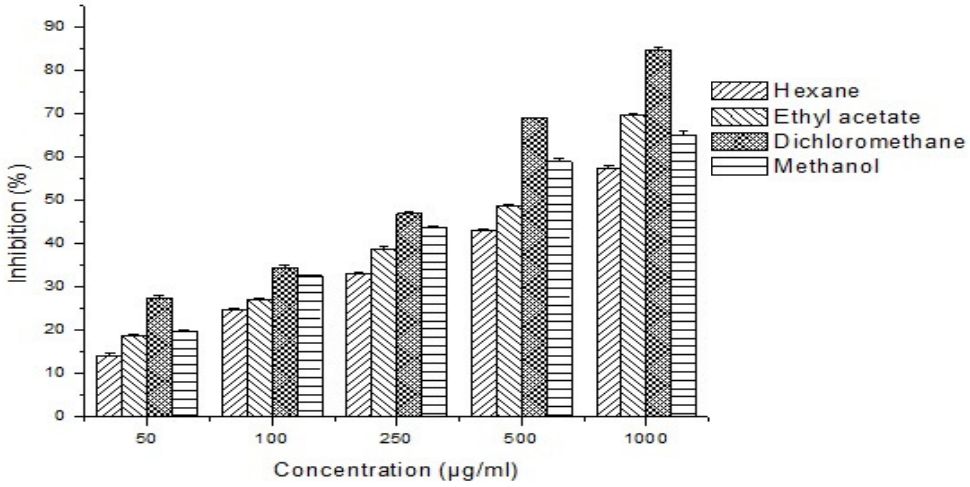


Figure-5
Inhibition of the radical degradation of 2-deoxy-D-ribosa of *S. portulacastrum* extract. The data are the mean ± SD of 3 replicates

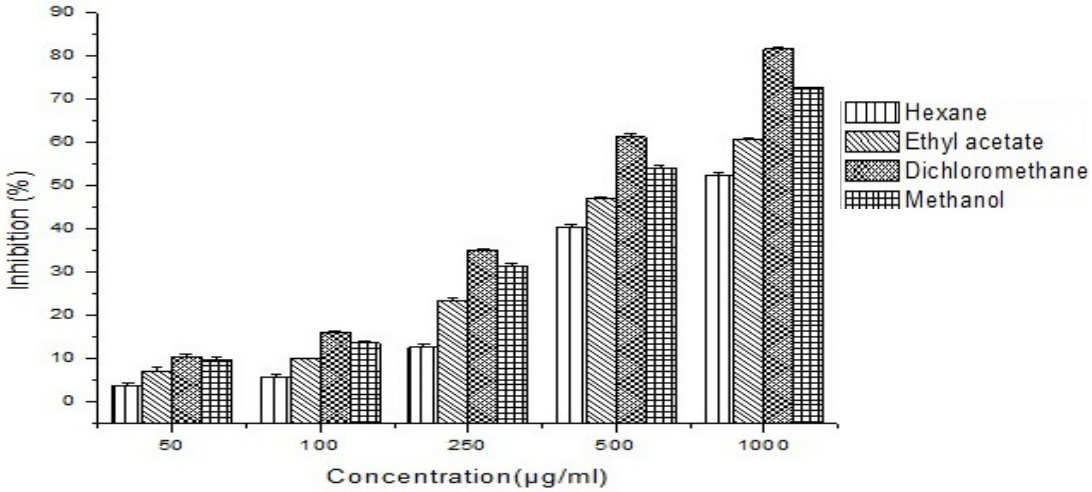
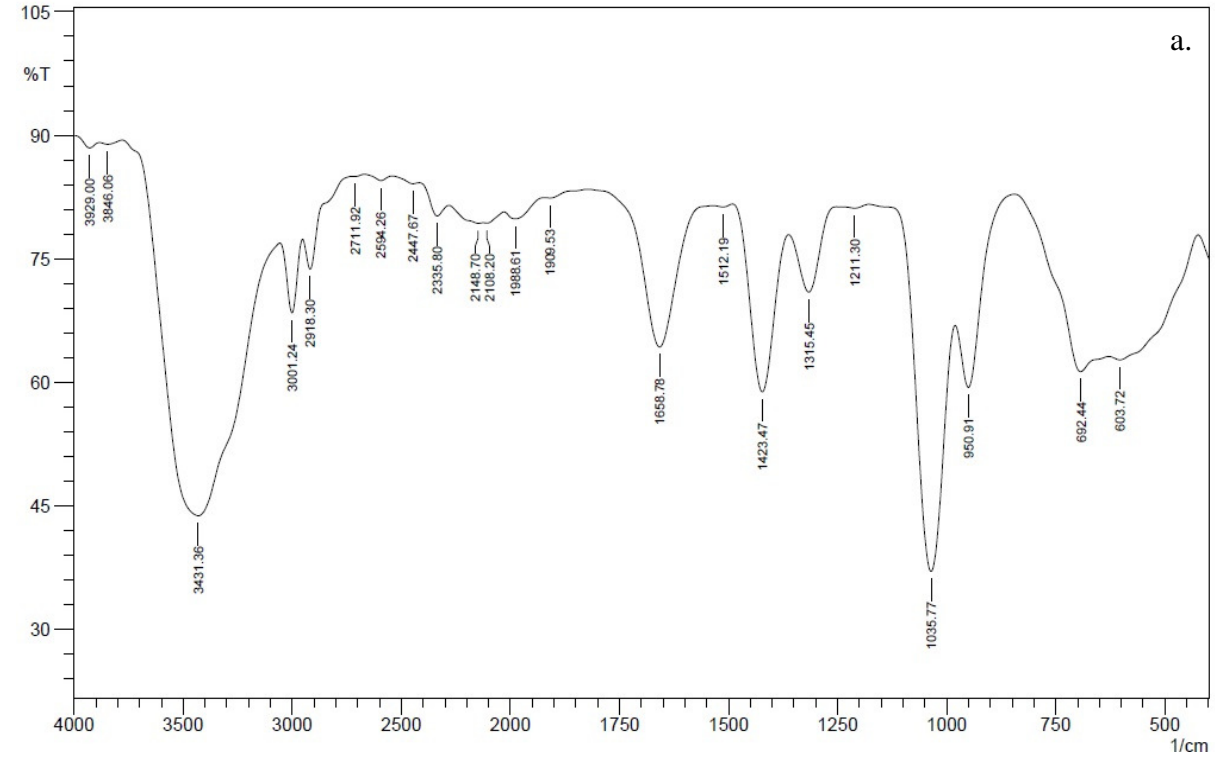
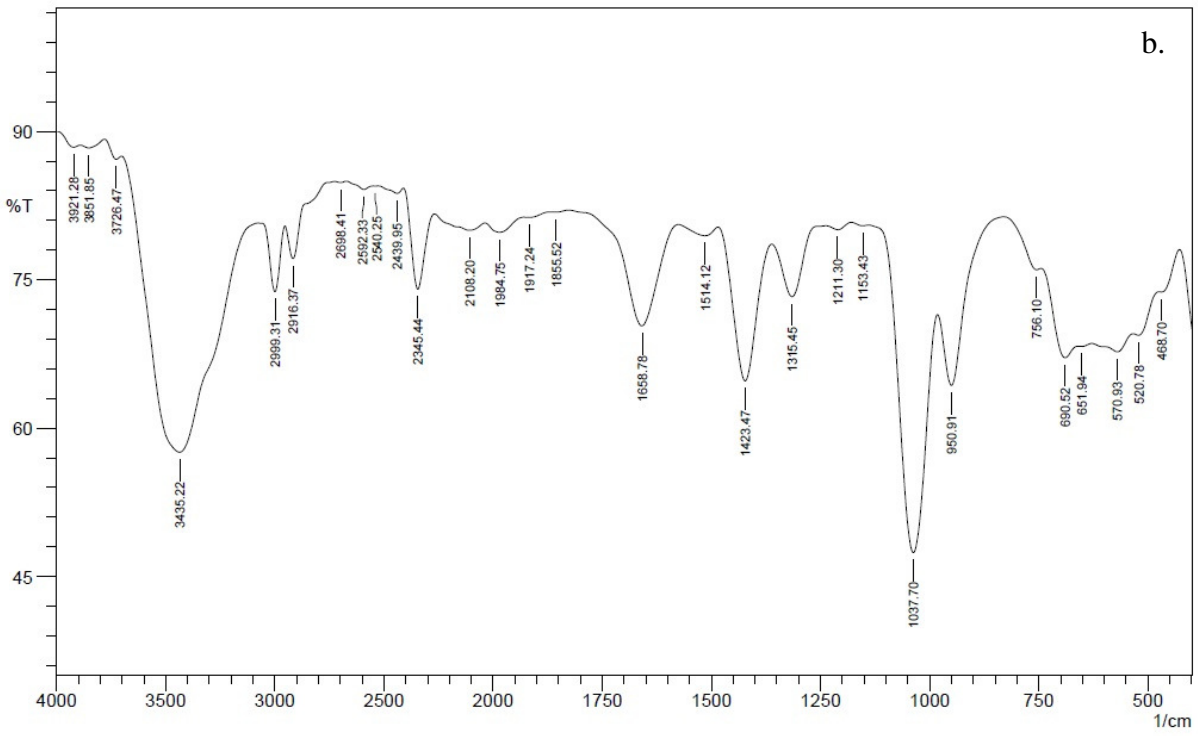


Figure-6
Hydrogen peroxide scavenging activity of *S. portulacastrum* extract. The data are the mean ± SD of 3 replicates

Functional groups identification through FTIR: The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed through the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines and halogen compound (figure-7, and table-1). The polyphenolic compounds having several hydroxyl groups exhibit cardio protective, anticancer, antibacterial and antiviral activities[19].





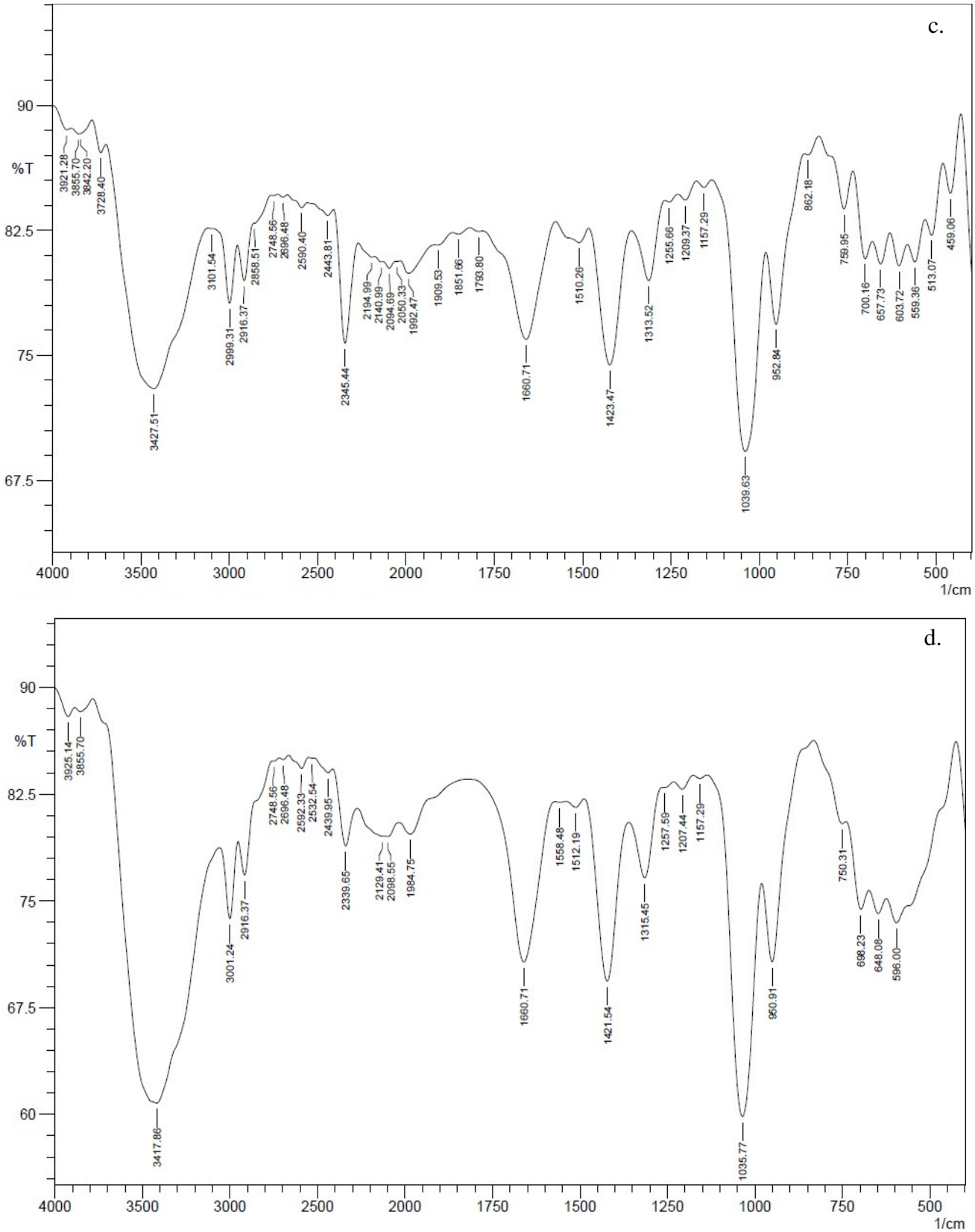


Figure-7
FTIR Spectrum of a. hexane, b. dichloromethane, c. ethyl acetate and d. methanol of *S. portulacastrum*

Table-1
FTIR peak values and functional groups of different extracts of *S. portulacastrum* (a. Hexane, b. Dichloromethane, c. Ethyl acetate, d. Methanol)

a. Hexane

Peak values	Functional Groups	Peak values	Functional Groups
603.72	Alkyl halide	2108.2	Alkyne
692.44	Alkyl halide	2148.7	Alkyne
950.91	Alkene	2335.8	Unknown
1035.77	Ester	2447.67	Unknown
1211.3	Ether, Acid, Ester	2594.26	Acid
1315.45	Amine	2711.92	Acid
1423.47	Aromatic	2918.3	Aldehyde
1512.19	Nitro	3001.24	Aromatic
1658.78	Alkene	3431.36	Amine
1909.53	Unknown	3846.06	Unknown
1988.61	Unknown	3929.0	Unknown

b. Dichloromethane

Peak values	Functional Groups	Peak values	Functional Groups
468.7	Unknown	1917.24	Unknown
520.78	Alkyl halide	1984.75	Unknown
570.93	Alkyl halide	2108.2	Alkyne
651.94	Alkyl halide	2345.44	Unknown
690.52	Alkyl halide	2439.95	Unknown
756.1	Alkyl halide	2540.25	Unknown
950.91	Alkene	2592.33	Acid
1037.7	Alcohol	2698.41	Acid
1153.43	Alcohol, Ester	2916.37	Acid
1211.3	Ester	2999.31	Acid
1315.45	Amine	3435.22	Amine
1423.47	Aromatic	3726.47	Alcohol
1514.12	Nitro	3851.85	Unknown
1658.78	Alkene	3921.28	Unknown
1855.52	Ketone		

c. Ethyl acetate

Peak values	Functional Groups	Peak values	Functional Groups
459.06	Unknown	1992.47	Unknown
513.07	Alkyl halide	2050.33	Unknown
559.36	Alkyl halide	2094.69	Unknown
603.72	Alkyl halide	2140.99	Alkyne
657.73	Alkyl halide	2194.99	Alkyne
700.16	Alkyl halide	2345.44	Unknown
759.95	Alkene	2443.81	Unknown
862.18	Alkene	2590.4	Acid
952.84	Alkene	2696.48	Acid
1039.63	Amine	2748.56	Acid, Aldehyde
1157.29	Alcohol, Ester	2858.51	Alkane, Acid
1209.37	Ether, Acid, Ester	2916.37	Aldehyde
1255.66	Ether, Acid, Ester	2999.31	Aromatic, Alkene
1313.52	Amine	3101.54	Acid
1423.47	Aromatic	3427.51	Amine
1510.26	Aromatic	3728.4	Unknown
1660.71	Alkene	3842.2	Unknown
1793.8	Carbonyl	3855.7	Unknown
1851.66	Ketone	3921.28	Unknown
1909.53	Unknown		

d. Methanol

Peak values	Functional Groups	Peak values	Functional Groups
596.0	Alkyl halide	1984.75	Unknown
648.08	Alkyl halide	2098.55	Unknown
698.23	Alkyl halide	2129.41	Alkyne
750.31	Alkyl halide	2339.65	Unknown
950.91	Alkene	2439.95	Unknown
1035.77	Amine	2532.54	Acid
1157.29	Alcohol, Ether	2592.33	Acid
1207.44	Ether, Ester	2696.48	Acid
1257.59	Ether, Acid, Ester	2748.56	Acid, Aldehyde
1315.45	Amine,	2916.37	Aldehyde
1421.54	Aromatic	3001.24	Aromatic
1512.19	Aromatic	3417.86	Amine
1558.48	Amide	3855.7	Unknown
1660.71	Alkene	3925.14	Unknown

Conclusion

It may be concluded from the present finding that *S. portulacastrum* contains phenolic which is an important source of antioxidant. These phenolic have been significant applications in pharmacological as well as in biological systems. Above phenolic was identified for the first time in this halophyte. These phenolic compound may be isolated at commercial scale for different use in source of health products for food and pharmaceuticalindustly.

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