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Phytochemical profiling, Antioxidant Properties and Characterization of Aqueous extract of Palmyra Sprout: A Forgotten Food

Reshma U.S [1], Annie Abraham [2]

Department of Biochemistry, University of Kerala, Kariavattom, Kerala, India

[1] Research scholar, Department of Biochemistry, University of Kerala, Kariavattom, Kerala, India.

[2] Professor, Department of Biochemistry, University of Kerala, Kariavattom, Kerala, India.

Corresponding Author: Prof (Dr). Annie Abraham

Address: Department of Biochemistry, University of Kerala, India

Email: annieab2013@gmail.com

Mob: +91 9447246692

Off: +91 471 2308078

ABSTRACT

Plants have been one of the important source of medicines since the dawn of the human civilization and still remain the major sources of drugs in modern as well as in traditional systems of medicine. Palmyra sprout (panamkizhangu) is obtained from *Borassus flabellifer* commonly known as palmyra palm, toddy palm, is the state tree of Tamilnadu, India. Sprouts are produced from palm seed. It is a forgotten traditional food. The present study was anticipated to evaluate the nutritional and phytochemical screening and antioxidant potential of aqueous extract of palmyra sprout. Phytochemical screening was carried out using standard methods of precipitation and colour reactions. In addition total phenolic, flavonoid and tannin were determined by using spectrophotometric methods. Finally extract was assayed to evaluate its antioxidant properties by using DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, reducing power activity and total antioxidant activity were calculated. Results revealed the presence of various bioactive molecules and its antioxidant properties. Aqueous extract of palmyra sprout revealed promising antioxidant potential so it can be considered as an alternative to synthetic antioxidant and helps to promote its value as nutritious food.

Keywords: Palmyra sprout, *Borassus flabellifer*, Extraction, Phytochemical screening, CHNS analysis, Antioxidant, FTIR, LCMS

INTRODUCTION

Phytochemicals are ecologically derived secondary metabolites synthesized by the plant [1]. These secondary metabolites are known to bring out significant pharmacological and beneficial effects to alleviate chronic diseases such as cancer, diabetics, cardiovascular diseases etc due to their antioxidant regulatory actions. Therefore phytochemical screening is the crucial and initial step in the discovery of bioactive compounds [2, 3]. Palmyra sprout (panam kizhangu) is obtained from *Borassus flabellifer* commonly known as toddy palm, is native to the Indian subcontinent and Southeast Asia [4]. *Borassus flabellifer* considered to be a nature's perennial gift that could flourish well in arid conditions and also could withstand many adverse climatic conditions and natural calamities. In India, it has been cultivated mainly in Tamilnadu, Karnataka, Andhrapradesh and Kerala [5]. Palm tree can grow up to 30 meters and has a life span above 100 years. Sprouts are produced from palm seed. During sprouting, phytonutrient content increases as compared to seeds and consumption of these sprouts is the best way to gain all the health benefits [6, 7]. For cultivating the sprout, a shallow trench is dug, then palm seeds are planted very close together and it is irrigated on a regular basis. Within a month or so, it began to germinate. The trench is the dug up to remove the palmyra sprout. Their seasons last primarily from February to April. Palmyra sprout can be eaten raw, roasted, baked or boiled [1]. It is a forgotten traditional food and one of the oldest fiber source found in southern Indian dishes. This sprout is known as food for poor in south India. Its high fiber content regulate bowel movements, lower blood cholesterol, reduce risk of cardiovascular diseases, curbs hunger, prevent overeating and help to maintain healthy weight. The sprout is considered cooling, restorative, diuretic and anthelmintic. Some tribal groups in South India is still using palmyra sprout to treat liver diseases, diabetes and the decoction from the sprout have been used for gastric problems and hiccups. Based on this information, a systematic study was essential to assess the therapeutic efficacy of palmyra sprout.

MATERIALS AND METHODS

Sample collection and preparation

Palmyra sprout (Figure 1) or panam kizhangu were collected from kuzhithura, Tamilnadu. Peel was removed from the sprout. Fresh sprout was washed with distilled water, chopped into small pieces and dried under the sun for 7-10 days. Dried sprouts were grounded thoroughly.

Figure 1: Image of palmyra sprout or panam kizhangu

Preparation of extract

About 50gm of palmyra sprout was soaked in 500ml distilled water in refrigerator for 72hrs. The extract was filtered using Whatman No. 1 filter paper and the filtrate was then concentrated by rotator evaporator. The dried extract was weighed, and stored in sterile containers at 4^0 C in a refrigerator for further studies.

Nutritional analysis

The nutritional analysis was analyzed based on the A.O.A.C.lnternational 21st Edition 2019 and quantified the total fat, total ash, total protein, total energy, and total carbohydrates present in 100g of the sample.

CHNS analysis

Quantification and analysis of CHNS were carried out at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of aqueous extracts of the palmyra sprout was carried out for the detection of phytoconstituents using standard protocols (Trease and Evans, 1989; Tiwari et .al., 2011).

Test for alkaloids: A small amount of aqueous extracts were dissolved in dilute HCl and filtered. The filtrate was used for the following tests:

Mayer's test: To 1 mL of the filtrate, 1 mL of Mayer's reagent (potassium mercuric iodide solution) was added. Formation of pale yellow colour indicated the presence of alkaloids.

Hager's test: To few mL of filtrate, add 1mL of Hager's reagent (saturated picric acid) was added. The development of yellow colour indicates the presence of alkaloids.

Test for flavonoids: A small amount of aqueous extracts were dissolved in methanol and performed the following tests:

Alkaline reagent test: To the extract, added a few drops of 2% sodium hydroxide solution. An intense yellow colour was obtained, which turned colorless on addition of dilute acid indicated the presence of flavonoids.

Lead acetate test: Extracts were treated with 1mL of 10% lead acetate solution. Formation of yellow coloured precipitate indicated the presence of flavonoids.

Test for steroids

Salkowski's test: To 2mL of the extract 2mL chloroform was added, mixed well and concentrated sulphuric acid was added along the side of the test tube. A reddish brown ring at the interface of two liquids indicated the presence of steroid.

Acetic acid test: A small amount of the extract was dissolved in 1mL of acetic acid. It was gently warmed, cooled under tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green color indicated the presence of steroids.

Test for glycosides: A small amount of the aqueous extracts were dissolved in methanol and were performed the following tests:

Keller-Killiani test: 2 mL of the extract was mixed with 3.5% ferric chloride solution and glacial acetic acid. Concentrated sulphuric acid was added along the sides of the test tube. A brown ring at the interface of two liquids indicated the presence of cardiac glycosides.

Balget's test: To 1 mL of the test extract, 1 mL of sodium picrate solution was added. Formation of yellow color revealed the presence of glycosides.

Test for diterpenes: A small amount of aqueous extracts were dissolved in methanol to perform the following tests:

Copper acetate test: Extract was dissolved is treated with 3-4 drops of copper acetate solution. Formation of an emerald green indicates the presence of diterpenes.

Test for saponins

Foam test: A small amount of the extract was diluted with water and shaken vigorously. Formation of stable foam indicated the presence of saponins.

Test for tannins: The extracts were dissolved in methanol to perform the following tests:

Gelatin test: To a little of the extract, 1% gelatin solution containing 10% sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

Potassium hydroxide test: To 0.5 g of the extract, freshly prepared potassium hydroxide solution was added and shaken to dissolve. A dirty precipitate indicated the presence of tannins.

Test for phenols

Ferric chloride test: Extract is treated with 3-4 drops of 2% ferric chloride solution. A bluish or greenish black revealed the presence of phenols.

Qualitative phytochemical analysis

Total flavonoid

Total flavonoid content was measured using aluminum chloride colorimetric assay. The reaction mixture consists of 1 mL extract, 4 mL distilled water, was taken in a 10 mL volumetric flask. To the flask, add 0.30 mL 5 % sodium nitrite and after 5 minutes, add 0.3 ml 10 % aluminum

chloride. After 5 minutes, add 2 mL 1M Sodium hydroxide and make up with 10 mL with distilled water. Quercetin is used as standard (20-100 µg/mL) were prepared in same manner. The test and standard solutions were determined against blank and absorbance was measured at 510 nm using UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g extract.

Total phenol

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE) (Lim et al., 2006). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/mL of plant extract were also prepared in methanol and 0.5mL of each sample were introduced into test tubes and mixed with 2.5mL of a 10 fold dilute Folin-Ciocalteu reagent and 2mL of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined (Savitree et al., 2004).

Antioxidant assays

DPPH radical scavenging assay

The free radical scavenging activity of the methanolic extracts was determined using DPPH assay. Various concentrations of methanolic extract of the sample (1 mL) were mixed with 1 mL of methanolic solution containing 1, 1 Diphenyl-2-picrylhydrazyl radical (DPPH) radicals resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken dynamically and left to stand for 30 mins, and the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. The percentage of DPPH decolorization of the sample was calculated using the following formula:

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Extract concentration providing 50 % inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration.

Hydroxyl radical scavenging activity

The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the fe^{3+}-ascorbate-EDTA-H2O2 system (the Fenton reaction). The reaction mixture contained, in a final volume of 1ml, 2-deoxy-2-ribose (3mM), phosphate buffer 20mM, fecl3 (0.1mM), EDTA (0.1mM), H_2O_2 (1.0mM), ascorbic acid (0.1mM) and various concentrations of test sample. The reaction mixture was incubated at 37^0C for 1hr. The TBARS (Thiobarbituric reactive substances) formed were measured by treating with 1.0 ml of TBA and 1.0mL of TCA (at 90^0C for 20min). After cooling, the absorbance was measured at 532 nm against a control. Percentage inhibition was evaluated by comparing the test and blank solution.

Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazinemethosulfate (PMS) under aerobic condition. TrisHCl buffer (3 mL, 16 mM, pH 8.0) containing 1 mL NBT (50 µM) solution, 1 mL NADH (78 µM) solution and a sample solution of extract (10–500 µg/mL) in distilled water mixed. The reaction was started when 1 mL of PMS solution (10 µM) was added to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance was read at 560 nm against the corresponding blank samples. Ascorbic acid was used as a standard. The decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

Measurement of reducing power

The reducing power of aqueous extracts was determined by slight modification of the method of Oyaizu. Substances, which have reduction potential reacts with ferricyanide (Fe^{3+}) to form potassiumferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex, that has an absorption maximum at 700 nm. 1.5mL of various concentrations of the plant extract (200-1000 µg) was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was kept in a water bath at 50°C for 20 minutes. After

cooling 2.5 mL of TCA (trichloro acetic acid) was added and centrifuged at 3000 rpm for 10 minutes, whenever necessary. The upper layer of solution (2.5mL) was mixed with 2.5 mL distilled water and 0.5 mL of freshly prepared ferric chloride solution. The absorbance was read at 700nm in a UV-VIS spectrophotometer (Hitachi U- 500). Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations (10-100 µg) was used as standard. Increased absorbance of the reaction mixture indicated increase in reducing power.

Determination of total antioxidant activity

The total antioxidant capacity of the methanol extract was determined by the phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate complex at acid pH. 0.3 mL extract was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 90°C for 90 mins. Then, after cooling the absorbance of the solution was estimated at 695 nm using a spectrophotometer against the blank. Methanol (0.3 mL) in the place of the extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (10–500 µg/mL) with methanol.

RESULT AND DISCUSSION

Nutritional Composition of palmyra sprout

The nutritional composition of palmyra sprout is depicted in table 1 and it showed that sprout possesses an excellent macronutrient composition with a total energy of 131.32 Kcal

Sl.No.	Component	Quantity/100g
1	Total Energy	131.32 Kcal
2	Total Fat	0.3 g 100 g $^{-1}$
3	Total Protein	9.87 g 100 g $^{-1}$
4	Total Carbohydrates	18.4 g 100 g $^{-1}$
5	Crude fiber	11.5 g 100 g $^{-1}$

6	Moisture	54.92 g 100 g $^{-1}$
7	Total ash	5.35 g 100 g $^{-1}$

Table 1: Nutritional composition of palmyra sprout

3.3.3 CHNS Analysis

Carbon, hydrogen and nitrogen are basic elements and these all are found actively participating in most of the metabolic reactions occurred in living beings.Analysis were carried out to find the organic composition of carbon, hydrogen, nitrogen and sulphur. CHNS of palmyra sprout, found to be 30.49% carbon, 6.83% hydrogen, 1.07% nitrogen and 0.74% sulphur. The higher percentage of carbon and hydrogen means the higher amount of carbohydrates, which provide energy to the consumers. The values of nitrogen and sulphur are within limits as compare to the necessary elemental composition which should be 1% for nitrogen and <1 % for sulphur (Ravichandran et al.,2015). The nitrogen is a structural component of proteins and sulphur is also present in proteins and vitamins.

Preliminary phytochemical screening of Palmyra sprouts

The phytochemical characteristics of aqueous extracts of palmyra sprout were summarized in tables 2. The results revealed the presence of various bioactive molecules such as alkaloids, flavonoids, glycosides, phenols, and tannins. The amount of phytochemical substances varies considerably from species to species, depending on the age and various ecological and climatic factors [8].

Phytochemical constituents	Tests	Observation
Alkaloids	Mayer's test	+
	Hager's test	+
Flavonoids	Alkaline reagent test	+
	Lead acetate test	+
Glycosides	Keller kelliyani test	+
	Baljet's test	+
Tannins	Gelatin test	ND
	KOH test	ND
Steroids	Salkowiski's test	+

	Acetic acid test	+
Phenols	Ferric chloride test	+
Saponins	Foam test	ND
Diterpenes	Copper acetate test	+

Table 2: Preliminary phytochemical analysis of aqueous extracts of palmyra sprouts '+' indicated the presence, ND - not detected

Quantitative phytochemical analysis

On the basis of phytochemical screening, some of the present constituents are qualitatively analyzed and summarized in Table 3. The total phenolic content of aqueous extracts of palmyra sprout measured by Follin-Ciocalteu reagent in terms of gallic acid equivalent. The value obtained for the concentration of total phenol is 29.04±1.2 mg/g dry weight. The flavonoid content was expressed in terms of quercetin equivalent and the concentration is about 22.11±0.3 mg/g. The results strongly show that the phenol is important components of this sprout and some of pharmacological effects could be attributed to the presence of this component.

Total flavonoid content (mg of QE/g of extract)	Total phenol content (mg of GAE/g of extract)
22.11±0.3	29.04±1.2

Table 3: Quantitative phytochemical analysis of aqueous extracts palmyra sprout

In vitro antioxidant assay

To evaluate the antioxidant potential of palmyra sprout, various assays like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, reducing power activity and total antioxidant activity were calculated.

DPPH radical scavenging activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was

determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progress, results in the scavenging of the radical by hydrogen donation. The degrees of discoloration of DPPH by its reduction indicated the radical scavenging activity of the extract. The DPPH radical scavenging activity of aqueous extract of palmyra sprout was shown in Figure 2. Palmyra sprout exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentrations tested. There was a dose dependant increase in the percentage antioxidant activity for all concentrations tested. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. A graded increase in percentage of inhibition was observed for the increase in the concentration of ascorbic acid. DPPH radical scavenging activity was compared with the standard ascorbic acid (IC_{50} 2.952µg /mL). Sprout extract showed an IC_{50} value of 7.536µg/mL. All determinations were done in triplicates and the mean values were determined

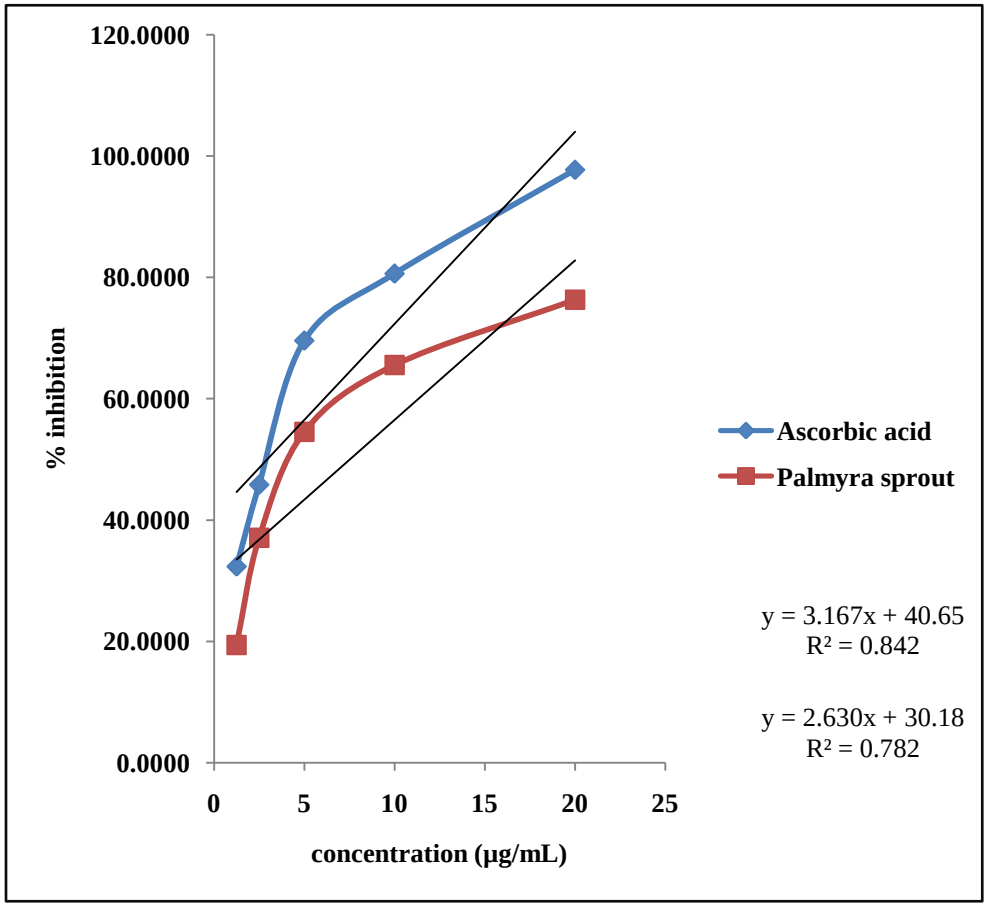

Figure 2: DPPH radical scavenging activity of aqueous extract of palmyra sprout

Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid. Fig. 3 represents the hydroxyl radical scavenging activity of aqueous extract of palmyra sprout. The sprout extract exhibited the minimum activity of 28.56% at 100µg/mL and the maximum activity of 71.48 at 500µg/mL and IC_{50} was 18.931µg/mL. The scavenging of the hydroxyl radicals may be due to the presence of hydrogen donating ability phenolic compounds in the extracts.

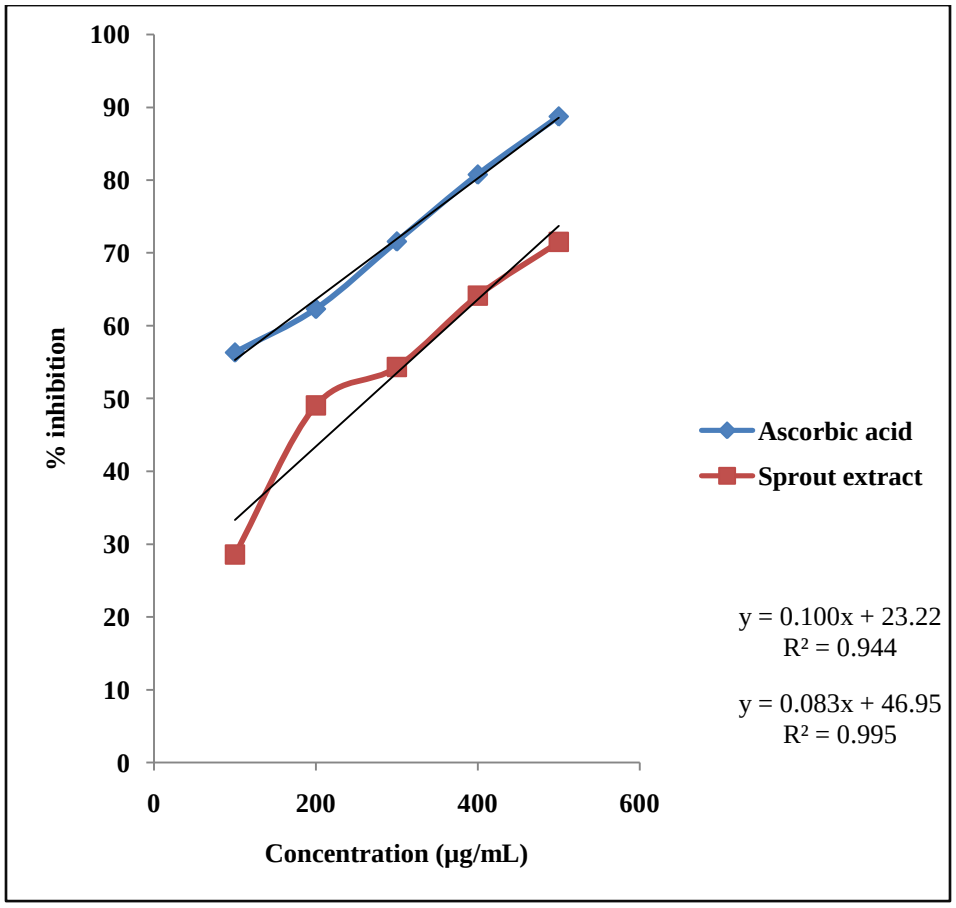

Figure 3: Hydroxyl radical scavenging activity of aqueous extract of palmyra sprout

Superoxide radical scavenging activity

The superoxide radical reduced NBT to blue colored formazan that can be measured at 560 nm. At 100–500 µg/mL, the superoxide scavenging activity of aqueous extract of palmyra sprout was 40.85% to 86.4%. The result shows the concentration-dependent radical scavenging activity is increased with sample concentration. Sprout extract exhibit good superoxide scavenging activity and the IC_{50} value obtained was 66.038µg/mL.

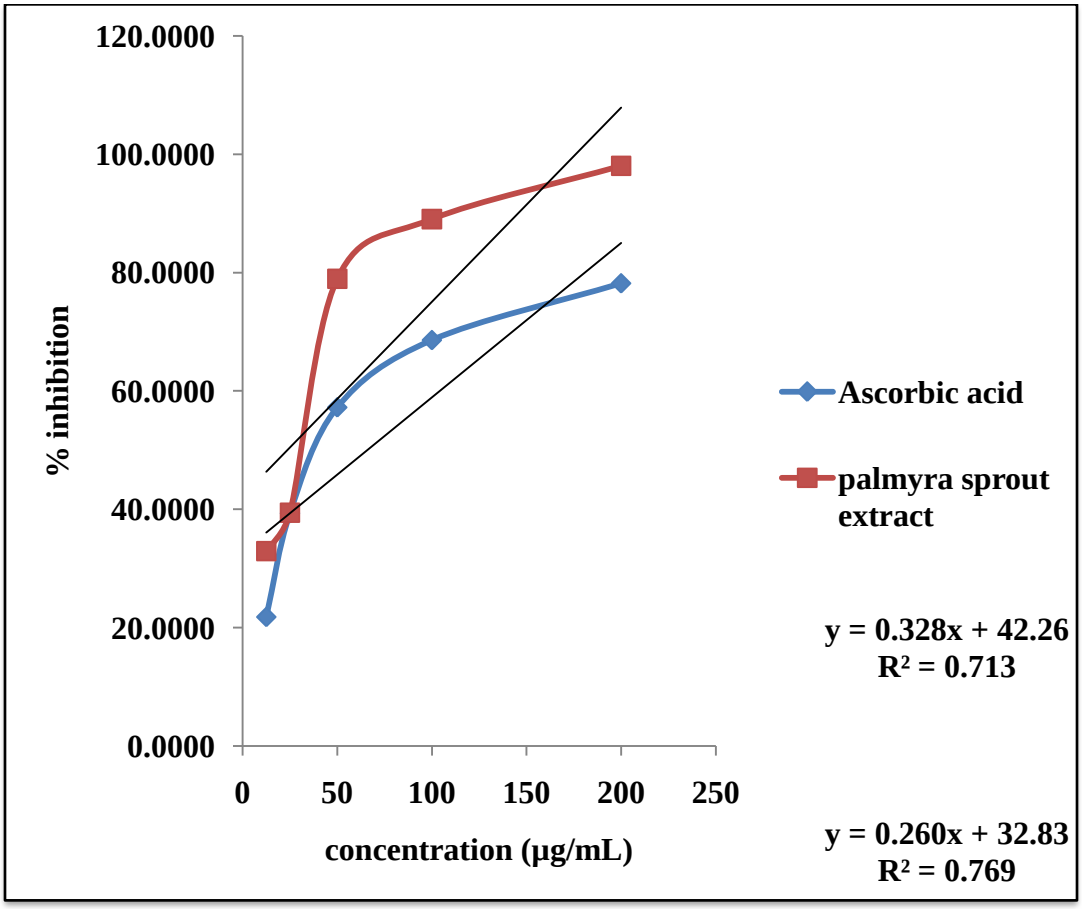

Figure 4: Superoxide scavenging activity of aqueous extract of palmyra sprout

Parameters	IC_{50} of sprout extract (µg/mL)	IC_{50} of ascorbic acid (µg/mL)
DPPH radical scavenging	7.536	2.952
Hydroxyl radical scavenging	18.931	3.065
Superoxide anion radical scavenging	66.038	23.597

Table 4: IC_{50} values of the free radical scavenging activities of the aqueous extract of palmyra sprout

Reducing power activity

The reducing power has significant correlation with the antioxidant activity. The concentration of Fe^{2+} formed by the reduction of Fe^{3+} ferricyanide complex was monitored by measuring the formation of Prussian blue at 700nm. Reducing power of aqueous extract of palmyra sprout was shown to increase linearly with increasing concentration. Sprout possesses significant reducing power, when compared with the standard.

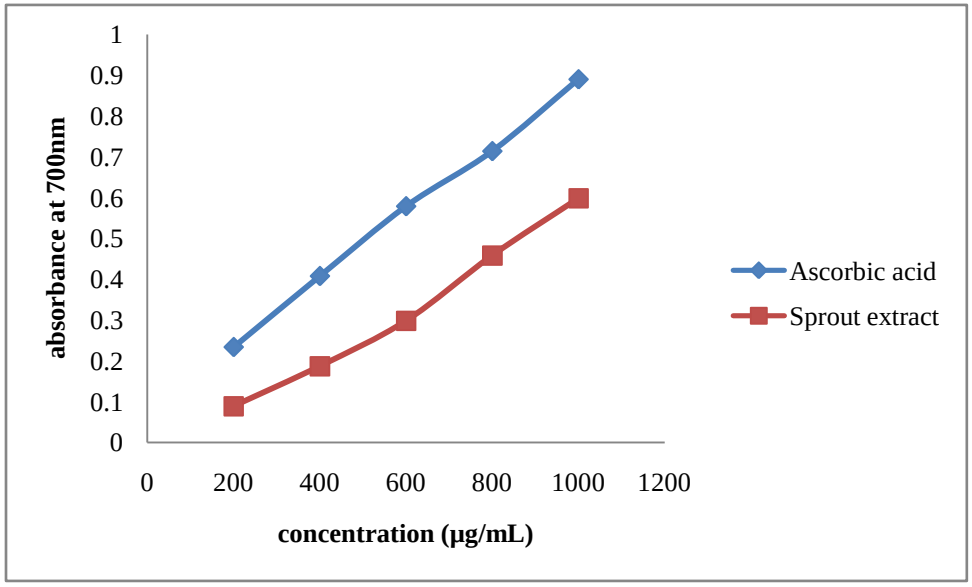

Figure 5: Reducing power activity of aqueous extract of palmyra sprout

Total antioxidant activity

Total antioxidant activity is an important parameter to scavenge free radical generation. The total antioxidant activity of palmyra sprout extract was evaluated by based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate complex at acid PH. The antioxidant activity of the plant extract is expressed as mg equivalents of ascorbic acid (mg of AAE/g). The extract showed high antioxidant capacity (139.33± .12)

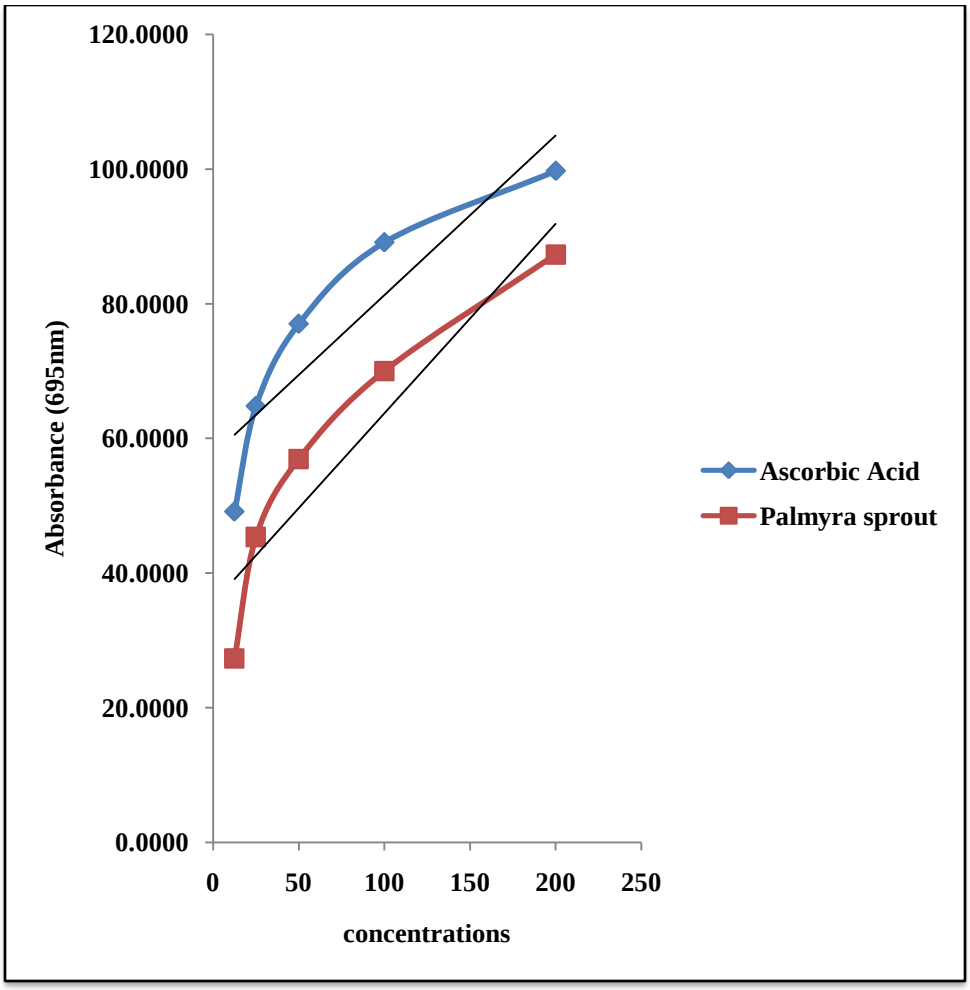

Figure 6: Antioxidant capacity of aqueous extract of palmyra sprout

FTIR Spectrum analysis of palmyra sprout

The palmyra sprout was subjected to analysis of the different surface groups or functional groups present in the plant sample by Fourier transmission infrared spectroscopy- PerkinElmer Spectrum Two FTIR spectrometer, UK in the frequencies of 4000- 500 cm^{-1} RT wavelength range. The FTIR spectrum shows a broad spectrum 3261cm^{-1}corresponds to X-H stretch (X is C, O, N), indicating the presence of alkanes, hydroxyl group, or imides. Peak at 2934 is due to the The C= C stretch at 1617$cm^{-1}$is due to the presence of alkenes. The C-N stretch observed at 1052cm^{-1}indicates the presence of aliphatic amines. The peaks 522 and 414 indicate the presence of ethers and alkyl halides.

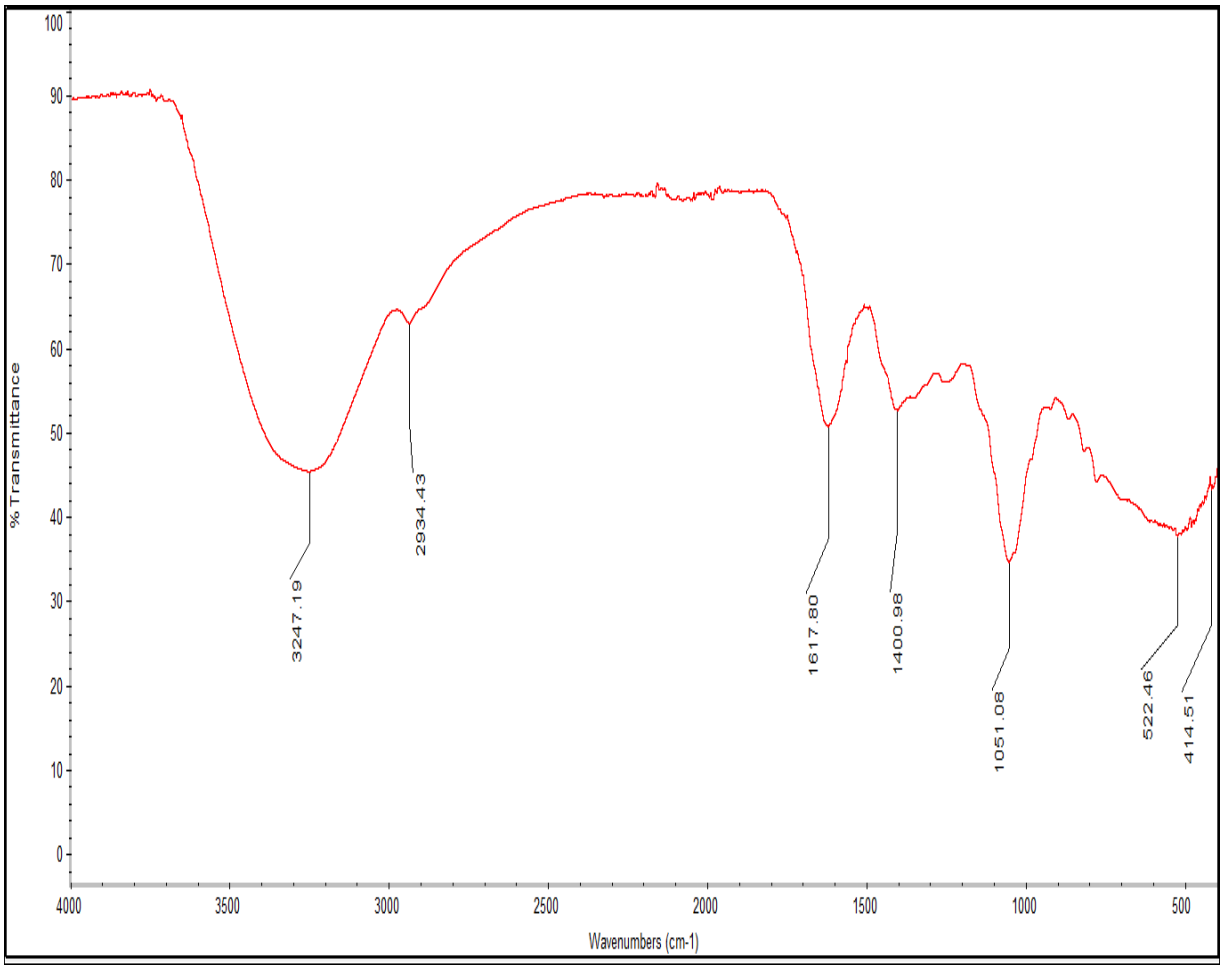

Figure 7: FTIR spectrum: palmyra sprout shows different functional groups of biomolecules

FTIR Peak Values	**Functional group**
3261	X-H stretch (X is C, O, or N)
2934	C-H stretch (alkane)
1617	C=C stretch(alkene)
1400	C-F stretch (alkyl halides)
1052	C-N stretch(aliphatic Amines)

522	C-Br(alkyl halides)
414	C-O(ethers)

Table 4: List of Functional Groups present in palmyra sprout identified through Infrared Spectroscopy.

LCMS Analysis of palmyra sprout

LCMS (MS Q-TOF) is the superior and standard technique used to identify phytoconstituents. The title of phytoconstituents was based on mass to charge ratio, retention time, molecular weight, peak area, molecular formula, polarity, and score.

Sl. No	Name	Molecular Formula	Molecular Weight	RT	Peak area	Score	Biological activities
1	Choline	$C_5 H_{13} N O$	103.0997	28.62	2126	97.1	Hepatoprotective, memory booster, enhance athletic performance, reduce choelsetrol,
2	Betaine	$C_5 H_{11} N O_2$	117.0790	28.78	1073	95.9	Liver function regulation, cellular reproduction, helps to make carnitine, metabolize homocystine.
3	Gentisic acid	$C_7 H_6 O_4$	154.0266	13.34	1610	92.8	Hepatoprotective, anti-inflammatory, antimicrobial and antioxidant
4	Tomatidine	$C_{27} H_{45} N O_2$	415.3450	11.275	1055	99.8	Antioxidant, Reduce muscle atrophy
5	Mannitol	$C_6 H_{14} O_6$	182.078	2.703	4212	92	Diuretic treats swelling from liver and kidney.
6	L-Threonic acid	C4 H8 O5	136.036	1.678	9634	82.8	Endogenous Metabolites, used as a mineral chelating agent able to greatly enhance bioavailability of minerals.
7	Pyridoxal	C8 H9 N O3	167.058	7.24	1.09	81.4	Natural available form of vitamin B_6, dietary shortage treatment, production of RBC,
8	Diosgenin	C27 H42 O3	414.3134	11.362	9133	89.2	Natural antioxidant,
9	Nicotinamide	C6 H6 N2 O	122.0480	28.553	1.95	86.6	Endogenous Metabolites prevent vitamin B_3 deficiency, diabetes, cancer.
10	Sorbitol	C24 H30 O6	414.2034	16.327	2343	96.1	Endogenous Metabolites, laxative

11	Matairesinol	C20 H22 O6	358.1416	1.024	3389	88.9	Anticancer properties
12	Trehalose	C11 H27 N3 O3 P2 S	343.1255	1.233	2881	89.6	Protect cellular membranes and labile proteins against damage and denaturation
13	Azelaic acid	C9 H16 O4	188.104	10.63	1112		Anti-inflammatory
14	Acetyl-L-lysine	C8 H16 N2 O3	188.116	1.063	4011	83.5	Anti-inflammatory, anti-cancer
15	Ethyl anthraquinone	C16 H12 O2	236.0837	2.139	1069	84.9	Anti-inflammatory, anti-cancer, Laxative
16	Protocatechuic acid	C7 H6 O4	154.0266	1.207	1173 2556	72.8	Anti-inflammatory, anti-cancer, anti-hyper glycemic

Table 5: Biologically active phytoconstituents in LCMS

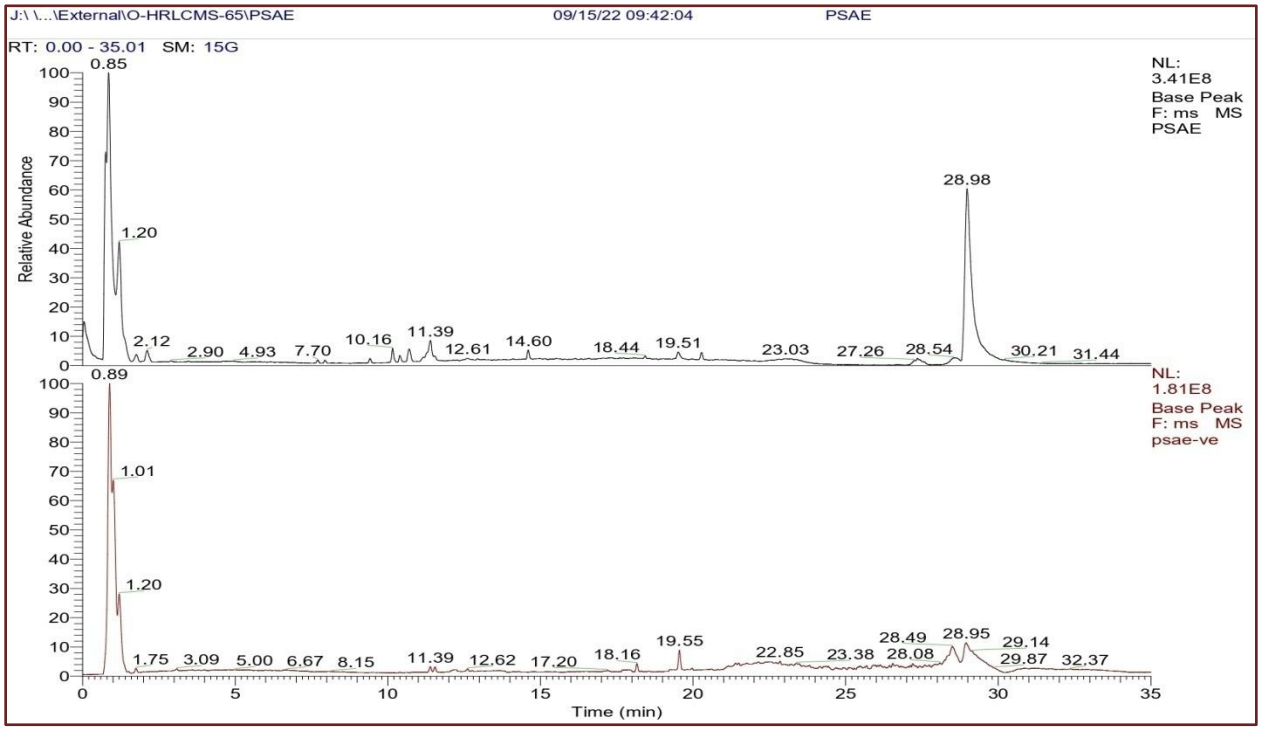

Figure 8: Representative LC Chromatogram (M+Z, M-Z)

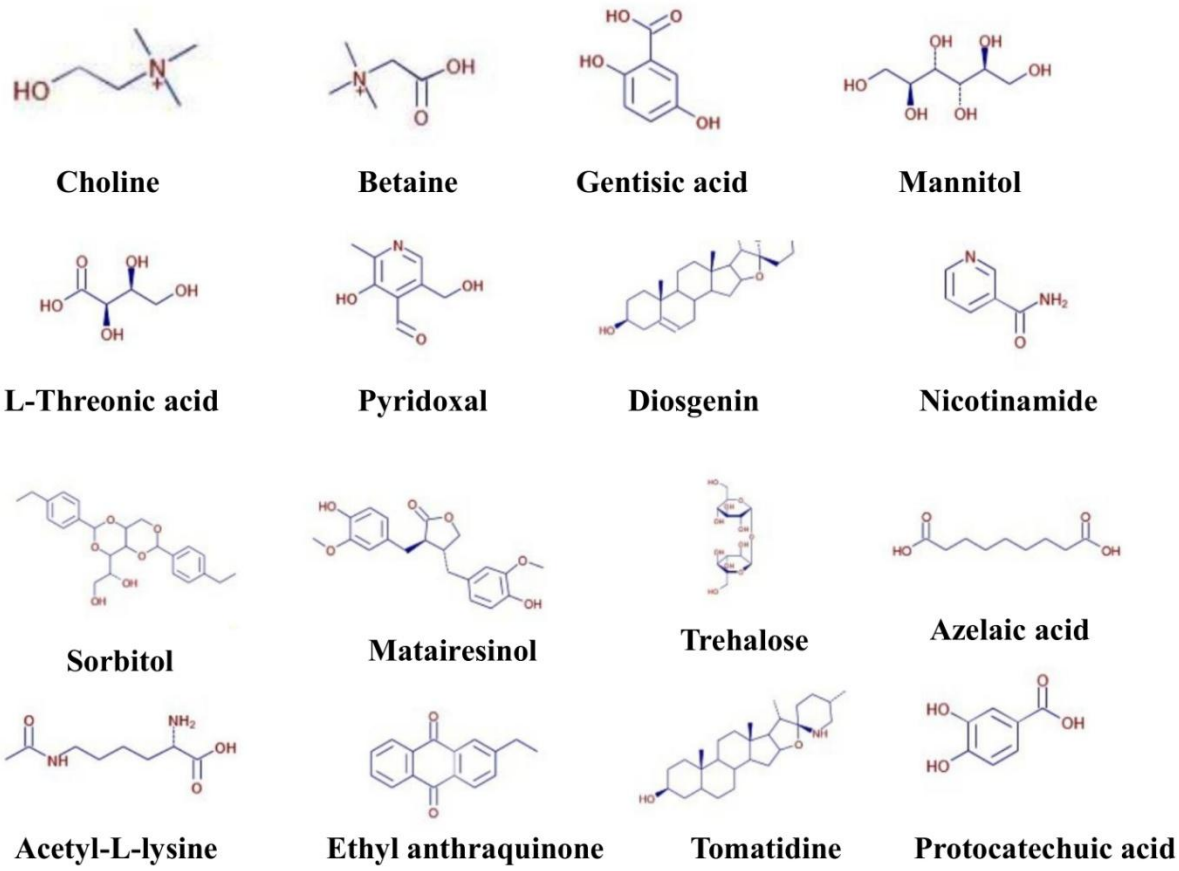

Figure 9: Structure of identified antioxidants and hepatoprotective compounds in aqueous extract of palmyra sprout by LC-MS

Discussion

Palmyra sprout is traditionally used to treat many diseases in folk practice without any scientific evidences. However, antioxidant potential and nutritional content of sprout extract could be relevant in the treatment of such diseases. In this study, aqueous extract was chosen. After the successful cold extraction, an investigation of nutritional content, preliminary phytochemical analysis, quantitative phytochemical analysis and antioxidant activity were conducted. Based on the nutritional content analysis, sprout is rich with total protein and crude fiber and the sprout was also found to be high in calorie content which is up to 131.32 Kcal. The carbohydrate content was not very high and fat content was negligible. The phytochemical screening of

aqueous extracts of palmyra sprout found that all the bioactive compounds are detected except saponnins and tannins. Phytochemicals are currently receiving the increased attention of interesting new findings regarding their biological activities. These compounds play some metabolic role and control development in a living system. So the phytochemical screening may be useful in the detection of the bioactive compounds and subsequently lead to the drug discovery and pharmacological formulation [9, 10, 11]. The alkaloids, flavonoids, phenols, steroids and diterpenes are detected in this extract could implicate these classes of phytochemicals as important bioactive agents of the sprout might be involved in the therapeutic action. The total phenolic content of aqueous extracts of palmyra sprout measured by Follin-Ciocalteu reagent in terms of gallic acid equivalent. The value obtained for the concentration of total phenol is 29.04±1.2 mg/g dry weight. The flavonoid content was expressed in terms of quercetin equivalent and the concentration is about 22.11±0.3 mg/g. Phenolic compounds are known as powerful chain breaking antioxidant, which may contribute directly to antioxidative action. Phenolic compound possess anticarcinogenic, antimutagenic as well as ability to modify the gene expression [12]. These phenolic compounds contribute to antioxidant activity, due to the arrangement of hydroxyl groups for hydrogen donation in order to stabilize radical molecules [13, 14]. Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in free state and as glycosides [15]. Their polyphenolic nature enables them to scavenge injurious free radicals such as superoxide and hydroxyl radicals [16]. The results strongly show that the phenol and flavonoid are the important components of this sprout and some of pharmacological effects could be attributed to the presence of these components. Different radical scavenging assays have been performed to analyze the antioxidant activity aqueous extract of palmyra sprout. IC_{50} value is defined as the concentration of substrate that causes 50% loss of the free radicals activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. In DPPH assay, the ability of aqueous extract of palmyra sprout to act as donors of hydrogen atom or electron was investigated. DPPH becomes diamagnetic molecule after gets reduced into its hydrazine form by electron donation by antioxidants. IC_{50} value was determined from a previously constructed standard curve and was found to be 2.952µg/mL. The high antiradical property of extract may be due to the presence of the phenolic compound. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage.

When sprout extract was added to the reaction mixture (Fenton reaction), it removed the hydroxyl radicals from the sugar (2-deoxy-2-ribose) and prevented the reaction [17]. The IC_{50} value indicates that the sprout extract is a better hydroxyl radical scavenger (18.931µg/mL). Superoxide anion is also very harmful to cellular compounds. As shown in figure 4, the superoxide radical scavenging activity of sprout extract and the reference compound are increased markedly with increasing concentrations. Reducing power assay is also widely used in evaluating antioxidant activity of plant polyphenols. The reducing power is generally associated with presence of reductants, which exert antioxidant action by breaking free radical change by donating hydrogen atom [18]. In this study sprout extract showed a reducing power capacity, which was concentration dependant (fig 5). The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. It evaluates both water soluble and fat soluble antioxidant [19]. The obtained value demonstrated that the extract is a potent antioxidant. The result of the study suggests that the aqueous extract of palmyra sprout can be used as a prospective source of natural antioxidant. The FTIR spectrum shows a broad spectrum 3261cm^{-1} corresponds to X-H stretch (X is C, O, N), indicating the presence of alkanes, hydroxyl group, or imides. Peak at 2934 is due to the C= C stretch at 1617$cm^{-1}$is due to the presence of alkenes. The C-N stretch observed at 1052cm^{-1}indicates the presence of aliphatic amines. The peaks 522 and 414 indicate the presence of ethers and alkyl halides. The LCMS Spectrum analysis of palmyra sprout also shows the presence of biologically significant compounds. Results were obtained by the mass spectral library of the Indian Institute of Technology, Bombay, and the unknown spectrum obtained was compared with the standard spectrum. There are many compounds with antioxidant, anti-inflammatory, hepatoprotective and anti-cancer properties have found (Table 5). Choline, betaine and gentistic acid belongs to the class of endogenous metabolites are reported to have a hepatoprotective effects [20, 21, 22]. Previous studies reported using choline as memory booster [23]. More than liver function regulation, betaine also promotes cellular reproduction, helps to make carnitine, metabolize homocystine. Anti-inflammatory, antimicrobial and antioxidant effect is also reported on gentisic acid [24]. Mannitol is a diuretic. It helps to make more urine and to lose salt and excess water from your body. It treats swelling from liver, heart and kidney diseases [25]. Diosgenin is a plant steroid. As a natural antioxidant, diosgenin is known to have neuroprotective effects to improve aging related deficits, memory improvement

[26]. Thus this steroid has potential interest in neuropathies such as neurodegenerative diseases, including Alzheimer's disease [27]. Phytosterols are plant sterols, which include plant-derived sterols and stanols. Phytosterols are widely used as a food additive and pharmaceutical industry with reported cholesterol-lowering effects and anti-inflammatory properties [28]. Most steroids possess satisfactory anti-inflammatory potential upon topical or systemic administration. Phytosterols can decrease cholesterol absorption efficiency, LDL-cholesterol, and total plasma cholesterol [29]. Plant sterols have an important role in fighting against bacterial infections via regulating the nutrient efflux and promoting plants' innate immunity. Threonic acid is a sugar acid derived from threose. The l-isomer is a metabolite of ascorbic acid (vitamin C). One study suggested that because l-threonate used as a mineral chelating agent able to greatly enhance bioavailability of minerals [30]. Pyridoxal is one of the natural forms available of vitamin B6, therefore, it is used for nutritional supplementation and for treating dietary shortage or imbalances. Pyridoxal is the precursor to pyridoxal phosphate. Matairesinol is a lignan that is gamma-butyrolactone in which the 3 and 4 positions are substituted by 4-hydroxy-3-methoxybenzyl groups (the 3R,4R-diastereomer). It has a role as a phytoestrogen, a plant metabolite, an angiogenesis inhibitor and an anti-asthmatic agent. It is a polyphenol, a lignan and a gamma-lactone. Matairesinol exhibits synergistic effects with conventional chemotherapeutic agents in Pancreatic cancer cells. Antiproliferative effect of matairesinol in the pancreatic ductal adenocarcinoma cells suppressed cell progression and migration, triggered apoptosis and mitochondrial dysfunction through MMP loss, and disturbed calcium regulation [31]. Nicotinamide Metabolizes several precarcinogens, drugs, and solvents to reactive metabolites. It inactivates a number of drugs and xenobiotics. An enzymatic system oxidizes nicotinamide to nicotinamide N-oxide. It is located in the endoplasmic reticulum of hepatocytes but the precise enzyme is unknown. We have used human liver microsomes in combination with selective cytochrome P450 inhibitors, specific substrates, and antibodies to identify CYP2E1 as the main activity producing nicotinamide N-oxide. Nicotinamide also inhibits CYP2E1 [32]. Niacinamide prevent vitamin B3 deficiency and related conditions such as pellagra. It is also used for acne, diabetes, cancer, osteoarthritis, aging skin, skin discoloration, and many other conditions, but there is no good scientific evidence to support most of these uses. Protocatechuic acid (PCA, 3,4-dihydroxybenzoic acid) is a phenolic compound found in many food plants. PCA content varies considerably depending on the type of food. Growing evidence suggests the significant

biological potential of PCA through the modulation of cellular signals involved in the control of oxidative stress and inflammation. Moreover, its antiapoptotic effects in normal cells and proapoptotic effects in cancer cells suggest definite benefits as a potential chemotherapeutic agent[33].

CONCLUSION

This study was anticipated to evaluate the nutritional and phytochemical screening, antioxidant potential and charecterization of aqueous extract of palmyra sprout. On the basis of the results obtained in the present study, it is concluded that palmyra sprout is nutritious and found to be high in calories content. Analysis of free radical scavenging activity, TPC and TFC revealed that palmyra sprout is a promising source of antioxidants.Various functional groups observed in FTIR endorses the presence of carbohydrate, glycogen, aminoacids, amides etc. The LCMS Spectrum analysis of palmyra sprout shows the presence of biologically significant compounds with with antioxidant, anti-inflammatory, hepatoprotective and anti-cancer properties. So aqueous extract of palmyra sprout can be used as a prospective source of natural antioxidant and can be considered as an alternative to synthetic antioxidant to promote its value as nutritious food.

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