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Antioxidant evaluation and gas chromatography–mass spectrometry (GC–MS) profiling of aqueous dried tuberous roots of *Asparagus racemosus* Willd.: The queen of herbs

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Abstract
In the present investigation, the bio-efficacy of dried tuberous roots of Shatavari was investigated with special reference to its antioxidant potential. The Shatavari is used as "Rasayanas" to enhance the body resistance against infections and improve the immune system. It is widely used for the treatment of various ailments as it contains many different phytochemicals. The notable medicinal properties of Shatavari are antispasmodic, anti-oxidant, anti-diabetic, anti-allergic, anti-malarial, hepato-protective, anti-neoplastic activities, enhance immune responses. Antioxidant effect of aqueous extract of the dried tuberous roots of *Asparagus racemosus* (Asparagaceae family) were tested on the basis of DPPH˙ (1,1-diphenyl-2- picrylhydrazyl), Superoxide (O_2˙-) radical, Hydroxyl (OH•) radical, ABTS•+ radical cation and reduction process such as Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activity. The maximum DPPH˙ radical, Superoxide (O_2˙-) radical, Hydroxyl (OH•) radical scavenging activities were 49.32±0.41%, 56.83±0.28% and 77.98±0.15% at 120 µg/mL concentration and the IC_{50} values were 121.65 µg/mL, 106.83 µg/mL and 75.82 µg/mL concentrations respectively. The maximum Mo^{6+} reduction and Fe^{3+} reduction were 88.42±0.42% and 80.24±0.36% at 120 µg/mL concentration and the RC_{50} values were 15.45 µg/mL and 19.40 µg/mL concentrations respectively. The maximum ABTS•+ radical cation scavenging activity was 83.56±0.43% at 12 µg/mL concentration and the IC_{50} value was 4.22 µg/mL concentration respectively. The GC-MS analysis of aqueous extract of the dried tuberous roots of *Asparagus racemosus* revealed the presence of 2-Phenyl-1,3-Cyclohexadiene, Flavone, Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl]-, Piperazine-2,5-dione, 1,4-(4-methylphenyl)- exhibiting therapeutic activities such as Antimicrobial activity, Antioxidant activity, Antimalarial activity, Immunomodulatory effect, Antiproliferative activity, Anxiolytic activity, Antialzheimer activity, etc.

Keywords: Antioxidant, Steroids, DPPH˙ radical, ABTS•+ radical cation, Mo^{6+} reduction and GC-MS analysis

Introduction
Asparagus racemosus is being used from Pre-Vedic times and mentioned in ayurvedic literature. Ayurveda systems are originated from India around 5000 years ago. It is purely based on natural herbal system. Initially it is restricted to some regions but now it's spreaded around the globe and has occupied a prime position in medicine system. The ancient history of India is very rich in herbal medicine and one of the oldest. It offers a rich, comprehensive outlook to a healthy life [1, 2]. The genus *Asparagus* comprises of more than 250 species distributed throughout the world out of which 22 species of *Asparagus* are recorded in India. The plant ranges up to 1500 m of altitude range and present in tropical and subtropical regions. The plant is under-shrub and grows to up to 3 metre in height. It is a spinous herb bearing numerous succulent short rootstocks [3]. The roots are elongated, tuberous brown in colour with tapering ends at both sides. It is 1-2 cm in thick and 25-90 cm long that appears ash silver white colour internally or externally (Figure 1). The plant is a woody climber known as liana bearing brown or may be whitish to grey coloured and small protective spines [4, 5].
The Shatavari plant contains a large group of isoflavones, polysaccrides and steroidal saponins. The saponins are present in predominant form such as Shatavarin I-IV (Figure 3). Others phytoconstituents are 8-methoxy-5, 6, 4'- trihydroxyisoflavone 7-O-beta-D-glucopyranoside. Asparagamine, Racemosol, 9, 10- dihydrophenanthrene), Shatavaroside, Secoisolariciresinol Shatavari Immunoside this is a glycoside of Sarsasapogenin, Racemoside A Ursolic Acid, Beta-Sitosterol and Stigmaterol Genistein and Daidzein, Racemosides A-C [2, 6, 7]. Roots of *Asparagus racemosus* containing active molecules exhibit therapeutic activities such as Estrogenic, Antioxytoxin Immunomodulators, Antidyspepsia, Antiallergic, Anticancer,

Anti-inflammatory, Antidiabetic, Antioxidant, Antitussive, Hepatoprotective, Antibacterial, Antiulcer, Anti-diarrhoeal, Antilithiatic (Figure 2) [7]. The plant extract of *Asparagus racemosus* exhibits enhanced antioxidant effects on mitochondria membrane of rat liver induced by generating free radicals induced by gamma radiation under in vitro condition. It enhances the GPX and GSH enzyme activity and inhibits the oxidation of protein and lipid peroxidation [8, 9, 10].

Taxonomic Classification of *Asparagus racemosus*

Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Order: Asparagales
Family: Asparagaceae
Genus: Asparagus
Species: Racemosus
Botanical name: *Asparagus racemosus* Willd.

(a)-Whole plant of *Asparagus racemosus* (b)-Dried tuberous roots of *Asparagus racemosus*

Fig 1: Habitat of *Asparagus racemosus*

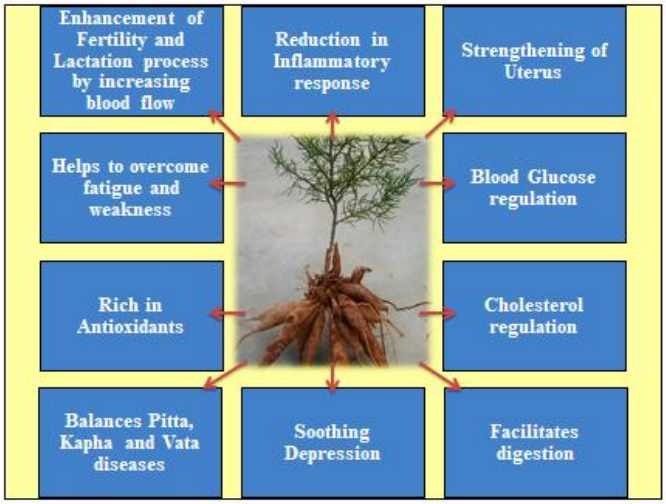


Fig 2: Important Pharmacological activities of *Asparagus racemosus*

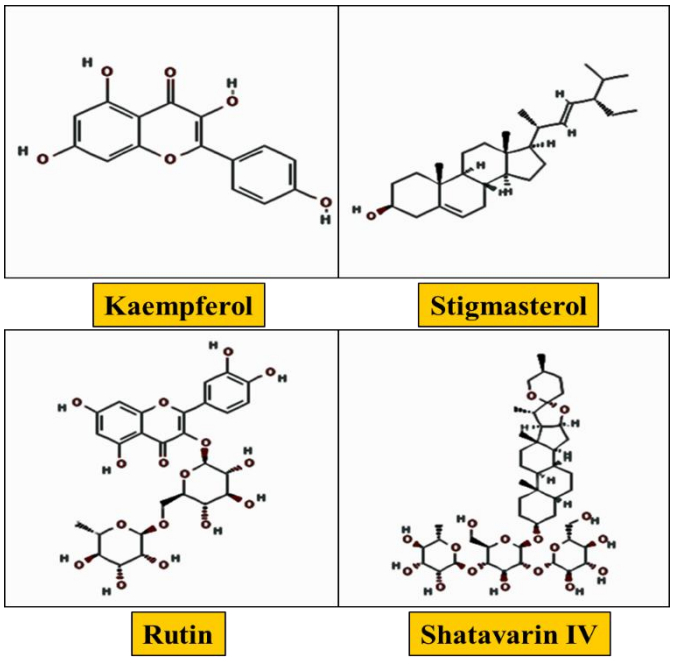


Fig 3: Active Phytoconstituents of *Asparagus racemosus*

In modern Ayurvedic practices the roots of plant are considered to be effective as antispasmodic, appetizer, stomach tonic, aphrodisiac, galactogogue, astringent, antidiarhoeal, antidysentiric, laxative, anticancer, anti-inflammatory, blood purifier, antitubercular, antiepileptic and also in night blindness, kidney problems and in throat complaints [11]. Further, it is mentioned as medhya- the plants which increase intelligence and promote learning and memory. The rejuvenator herbs improve health by increasing immunity, vitality and resistance, imparting longevity as well as protection against stress [12]. Among approximately 300 species distributed around the world, 22 species of *Asparagus* have been recorded in India where, *Asparagus racemosus* is most commonly used as a medicinal plant in traditional medicine [13].

Free radicals attack three main cellular components.

Lipids - Peroxidation of lipids in cell membranes can damage cell membranes by disrupting fluidity and permeability. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzymes and receptors.

Proteins - Direct damage to proteins can be caused by free radicals. This can affect many kinds of protein, interfering with enzyme activity and the function of structural proteins.

DNA - Fragmentation of DNA caused by free radical attack causes activation of the poly (ADP-ribose) synthetase enzyme. This splits NAD+ to aid the repair of DNA. However, if the damage is extensive, NAD+ levels may become depleted to the extent that the cell may no longer be able to function and dies. The site of tissue damage by free

radicals is dependent on the tissue and the reactive species involved. Extensive damage can lead to death of the cell; this may be by necrosis or apoptosis depending on the type of cellular damage. When a cell membrane or an organelle membrane is damaged by free radicals, it loses its protective properties. This puts the health of the entire cell at risk [14].

Materials and Methods

Collection of plant material and Extraction process

The tuberous roots of *Asparagus racemosus* were collected from Indian herbal market. The collected roots were washed thoroughly with distilled water to remove the sandy particles and other impurities. The clean tuberous roots were air-dried completely and the dried roots were subjected to hot decoction method (or) aqueous extraction. Then the extract was cooled and the supernatant was filtered using sterile filter paper and condensed by using rotary evaporator at 50°C, which yields pale brown coloured gummy extract [15, 16]. This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in preparation of Ayurvedic extracts called "quath" or "kawath". The starting ratio of crude drug to water is fixed and the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure.

Thin layer chromatography analysis

Thin layer chromatography (TLC) analysis was carried out for aqueous dried tuberous root extract of *Asparagus racemosus* on silica gel aluminium sheet [17] (Merck Silica gel 60 F254). The aqueous extract was spotted at 0.5 mm above from the bottom of the TLC plate. The spotted TLC plate was placed in a 100 mL beaker containing optimized solvent mixture. The chromatogram was developed and the spots were visualized under UV light at 254 nm as well as in iodine vapour chamber. The ratio in which distinct coloured bands appeared was recorded and the R_f values were calculated.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Phytochemical Analysis of aqueous dried root extract of *Asparagus racemosus*

The aqueous dried tuberous root extract of *Asparagus racemosus* was subjected to preliminary qualitative phytochemical screening using standard methods for analysing different classes of phytoconstituents using specific reagents [16].

Quantitative estimations of total phenols, flavonoids and steroids

Determination of phenolic content

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds [18] with slight modifications. One hundred µL (1 mg/mL) of aqueous dried tuberous root extract of *Asparagus racemosus* were mixed with 900 µL of methanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of Na_2CO_3 (20% w/v) solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a standard reference compound.

Determination of flavonoids content

The total flavonoid content of aqueous dried tuberous root extract of *Asparagus racemosus* (1 mg/mL) were determined by aluminium chloride reagent method with slight modification [19]. Five hundred µL of aqueous extract were mixed with 0.5 mL of methanol and 0.5 mL of (5% w/v) sodium nitrite solution. Then, 0.5 mL (10% w/v) aluminium chloride solution was added followed by 1 mL of 1 M Sodium hydroxide solution was added. The mixture was incubated for 30 min at room temperature and the absorbance was measured using UV-Vis spectrophotometer at 510 nm. The total flavonoid content was expressed in terms of quercetin equivalent (µg/mg of extract), which is a standard reference compound.

Determination of Steroids content

The total steroids content of aqueous dried tuberous root extract of *Asparagus racemosus* (1 mg/mL) were determined by potassium hexacyanoferrate (III) method [20]. 500 µL of aqueous extract of steroid solution was made upto 1 mL with methanol. The reagents are freshly prepared and 1 mL of 4N Sulphuric acid, 1 mL of iron (III) chloride (0.5% w/v) were added, followed by 500 µL of potassium hexacyanoferrate (III) solution (0.5% w/v). The mixture was incubated in water bath and maintained at 70°C for 30 min with occasional shaking and diluted to the markings made with distilled water. The absorbance was measured using UV-Vis spectrophotometer at 780 nm. The total steroids content was expressed in terms of cholesterol equivalent (µg/mg of extract), which is a standard reference compound.

Antioxidant activities

DPPH˙ radical scavenging activity

The antioxidant activity of aqueous dried tuberous root extract of *Asparagus racemosus* was measured based on the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical [21]. One mL of 0.1 mM DPPH solution in ethanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of aqueous extract. The mixture was then allowed to stand for 30 min incubation in dark. One mL ethanol mixed with 1 mL DPPH solution was used as control. The decrease in absorbance was measured at 517 nm using UV-Vis Spectrophotometer. Ascorbic acid was used as standard reference. The percentage of DPPH˙ radical inhibition was calculated as:

$$\%\ \text{of DPPH}^{\cdot}\ \text{radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

Superoxide ($O_2^{\cdot}$-) radical scavenging activity

Superoxide radical scavenging activity was carried out in which different concentrations of aqueous dried tuberous root extract (20-120 µg/mL) of *Asparagus racemosus* was mixed with 50 mM of phosphate buffer ((made upto 1 mL), 0.2 M, pH 7.8), 200 µL of 1.5 mM riboflavin, 200 µL of 12 mM EDTA and 100 µL of 50 mM NBT (pre-chilled solution) in a sequence. The reaction was started by illuminating the reaction mixture for 15 min. After illumination, the absorbance was measured at 590 nm using UV-Vis Spectrophotometer [22]. Ascorbic acid was used as standard reference. The percentage of Superoxide ($O_2^{\cdot}$-) radical inhibition was calculated as:

$$\% \text{ of Superoxide } (O_2^{\bullet-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

Hydroxyl radical ($OH^{\bullet}$) scavenging activity

Hydroxyl radical scavenging activity was measured by the salicylic acid method with some modifications [23]. One mL of aqueous dried tuberous root extract of *Asparagus racemosus* in different concentrations (20-120 µg/mL) was mixed with 500 µL of 9 mM salicylic acid, 500 µL of 9 mM ferrous sulphate and 50 µL of 9 mM H_2O_2 solution. The reaction mixture was incubated for 60 min at 37ºC in water bath after incubation the absorbance of the mixtures was measured at 510 nm using UV-Vis spectrophotometer. Ascorbic acid was used as standard reference. The percentage of hydroxyl ($OH^{\bullet}$) radical scavenging activity was calculated as:

$$\% \text{ of Hydroxyl } (OH^{\bullet}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

Phosphomolybdenum reduction activity

The total antioxidant capacity of aqueous dried tuberous root extract of *Asparagus racemosus* was assessed in which the aqueous extract in different concentrations (20-120 µg/mL) was combined with 1 mL of reagent solution containing 4 mM Ammonium molybdate, 28 mM Sodium phosphate and 0.6 M Sulphuric acid. The reaction mixture was incubated in water bath at 95ºC for 90 min. The absorbance of the coloured complex was measured at 695 nm using UV-Vis spectrophotometer [24]. Ascorbic acid was used as standard reference. The percentage of Mo^{6+} reduction was calculated as:

$$\% \text{ of phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] * 100$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of aqueous dried tuberous root extract of *Asparagus racemosus* was determined by slightly modified method [25]. One mL of aqueous extract of different concentrations (20-120 µg/mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixture was then incubated in water bath at 50°C for 30 min. 500 µL of trichloroacetic acid (10% w/v) was added to each mixture, mixed well and 100 µL of freshly prepared $FeCl_3$ (0.1% w/v) solution and shaken well. The absorbance was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as standard reference. The percentage of Fe^{3+} reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] * 100$$

$ABTS^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was determined in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity [26]. $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{\bullet+}$ solution (stable for 2 days) was diluted with distilled water and set an absorbance of 0.70±0.02 at 734 nm. Then 1 mL of different concentrations (2-12 µg/mL) of aqueous dried tuberous root extract of *Asparagus racemosus* was mixed with 500 µL of diluted $ABTS^{\bullet+}$ solution. The mixture was then allowed to stand for 10 min incubation and the absorbance was measured at 734 nm using UV-Vis spectrophotometer. Ascorbic acid was used as standard reference. The percentage of $ABTS^{\bullet+}$ radical cation scavenging activity was calculated as:

$$\% \text{ of } ABTS^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

Gas Chromatography–Mass Spectrometry (GC–MS) Profiling

In GC-MS analysis, the aqueous dried tuberous root extract of *Asparagus racemosus* was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200 °C and column oven temperature was programmed as 50-250 °C at a rate of 10 °C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250 °C; interface temperature of 250°C; and mass range of 50-600 mass units [27].

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Results and Discussion

Thin layer chromatography analysis

The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. TLC is used in the identification, purity testing and determination of the concentration of active ingredients, auxiliary substances and preservatives in drugs and drug preparations, process control in synthetic manufacturing7 processes. Various pharmacopoeias have accepted TLC technique for the detection of impurity in a drug or chemical [28].

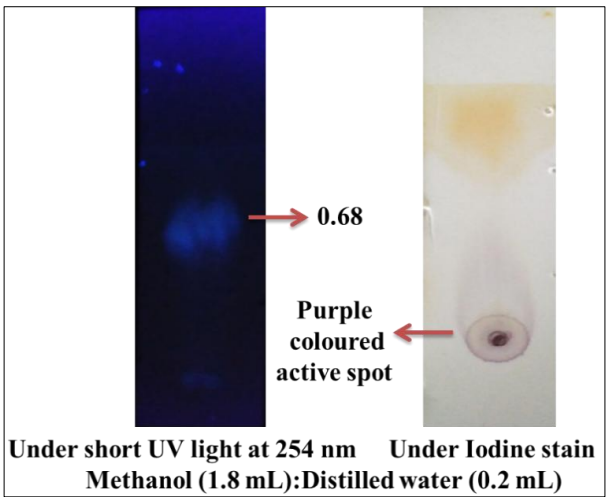


Fig 4: Thin layer chromatography analysis of aqueous dried tuberous root extract of *Asparagus racemosus*

The optimized solvent system was methanol and distilled water in the ratio of 1.8 mL:0.2 mL in which stable distinct band was well observed as depicted in Figure 4. Iodine has high vapour pressure and when the eluted TLC plate is kept in iodine chamber the plate becomes saturated with iodine vapour and turn light brown. The compound spot will appear as a dark brown spot in the TLC plate. Iodine has a high affinity for unsaturated and aromatic compounds. Thus spots get visible when they absorb iodine.

Phytochemical Analysis of aqueous dried tuberous root extract of *Asparagus racemosus*

The phytochemical analysis of aqueous dried tuberous root extract of *Asparagus racemosus* showed the presence of terpenoids, steroids, phenolic compounds, flavonoids, glycosides and saponins (Table 1) indicating various therapeutic activities.

Table 1: Qualitative analysis of aqueous dried tuberous root extract of *Asparagus racemosus*

S. No	Phytochemicals	Tests	Results
1	Alkaloids	(a) Wagner's reagent	-
		(b) Mayer's reagent	
2	Terpenoids	$CHCl_3$ + conc. H_2SO_4	+
3	Steroids	Liebermann–Burchard test (acetic anhydride+ Con. H_2SO_4)	+
4	Flavonoids	NaOH solution	+
5	Phenols	$FeCl_3$ solution	+
6	Tannins	Lead acetate solution	+
7	Glycosides	Sodium nitroprusside solution + Con. H_2SO_4	+
8	Saponins	Foam test	+

Table 2: Quantitative estimations of aqueous dried tuberous root extract of *Asparagus racemosus*

S. No	Phytochemicals	Value (µg/mg)
1	Phenols	412.6±0.42 GAE
2	Flavonoids	56.77±0.19 QE
3	Steroids	27.5±0.33 Cholesterol

(*Average value of 3 replicates)

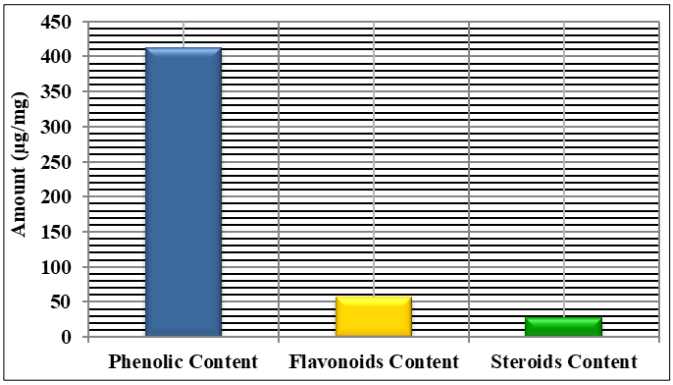


Fig 5: Quantitative estimations of aqueous dried tuberous root extract of *Asparagus racemosus*

The total phenols and flavonoids were quantified for aqueous dried tuberous root extract of *Asparagus racemosus* and seemed to be responsible for the antioxidant activity (Table 2 and Figure 5). Phenolic compounds possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenol molecule to that of a complex high-molecular weight polymer, are widespread groups of substances occurring in all vegetative organs such as fruits, vegetables, cereals, grains, seeds. Plant foods are the most significance source of natural antioxidants and its flavonoids and phenolic acids have attracted the most attention as potential therapeutic agents against cancer. Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, antihelminthic, cytotoxic and cardiotonic activity. These results provide a comprehensive profile of the antioxidant activity of *Asparagus racemosus* with respect to their phenols, flavonoids and steroids content [29].

Antioxidant activities

DPPH˙ radical scavenging activity

The ability of aqueous dried tuberous root extract of *Asparagus racemosus* to scavenge free radicals was assessed by using 1, 1-diphenyl-2picrylhydrazyl (DPPH) radical. The maximum DPPH˙ radical scavenging activity of aqueous dried tuberous root extract of *Asparagus racemosus* was 49.32±0.41% at 120 µg/mL concentration. The aqueous dried tuberous root extract of *Asparagus racemosus* has capacity to scavenge free radicals by reducing the stable purple colour DPPH˙ (1,1-diphenyl-2- picrylhydrazyl) radical to yellow colour 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract (Table 3 and Figure 6) [30]. The IC_{50} of DPPH˙ radical scavenging activity was 121.65 µg/mL concentration and was compared with standard ascorbic acid (IC_{50} = 13.57 µg/mL concentration).

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to blue formazan, which is measured at 590 nm using UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture (Table 3 and Figure 6) [31]. The maximum superoxide ($O_2^{\cdot-}$) radical scavenging activity of aqueous dried tuberous root extract of *Asparagus racemosus* was 56.83±0.28% at 120 µg/mL concentration and the IC_{50} was 106.83 µg/mL concentration. It was compared with the standard of ascorbic acid (IC_{50} = 12.83 µg/mL concentration).

Hydroxyl radical (OH•) scavenging activity

Hydroxyl radical has a short half-life and the most reactive, known to be capable of abstracting hydrogen atoms from cell membranes and induce oxidative damage to DNA, lipids and protein. Hydroxyl radical (OH•) is the neutral form of hydroxyl ion and the most reactive free radical in biological systems generated from free metal ions (copper or iron) catalysed breakdown of H_2O_2 (Fenton reaction) which was scavenged by aqueous extract in a concentration dependent manner (Table 3 and Figure 6) [32]. The maximum hydroxyl radical (OH•) scavenging activity of aqueous dried tuberous root extract of *Asparagus racemosus* was 77.98±0.15% at 120 µg/mL concentration and the IC_{50} was 75.82 µg/mL concentration, which was compared with standard ascorbic acid (IC_{50} = 10.68 µg/mL concentration).

Phosphomolybdenum reduction activity

The total antioxidant activity of aqueous dried tuberous root extract of *Asparagus racemosus* was measured by phophomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate Mo (V) complex at acidic pH, with a maximum absorption at 695 nm (Table 3 and Figure 6). The maximum phosphomolybdenum reduction of aqueous dried tuberous root extract of *Asparagus racemosus* was 88.42±0.42% at 120 µg/mL concentration and the RC_{50} was 15.45 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} = 7.94 µg/mL concentration). Total antioxidant activity is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand. It involves in thermally generating auto-oxidation during prolonged incubation period at higher temperature [33].

Ferric (Fe^{3+}) reducing power activity

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the aqueous dried tuberous root extract of *Asparagus racemosus* and the subsequent formation of ferro-ferric complex. The reduction capacity increases with increase in concentration of the extract (Table 3 and Figure 6). The maximum Fe^{3+} reduction of aqueous dried tuberous root extract of *Asparagus racemosus* was 80.24±0.36% at 120 µg/mL concentration and the RC_{50} was 19.40 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} = 10.36 µg/mL concentration). Also in this assay, higher absorbance of the reaction mixture indicates higher reduction potential. The reducing capacity of the extract was performed using Fe^{3+} to Fe^{2+} reduction assay as the yellow colour changes to green or blue colour depending on the concentration of antioxidants [34]. The antioxidants such as phenolic acids sand flavonoids were present, considerable amount in aqueous dried tuberous root extract of *Asparagus racemosus* and showed the reducing capacity in a concentration dependent manner.

Table 3: Antioxidant activities of aqueous dried tuberous root extract of *Asparagus racemosus*

S. No	Concentration (µg/mL)	Percentage of inhibition*			Percentage of reduction*	
		DPPH$^{\bullet}$ radical	Superoxide ($O_2^{\bullet-}$) radical	Hydroxyl ($OH^{\bullet}$) radical	Mo^{6+} reduction	Fe^{3+} reduction
1	20	17.34±0.17	8.2±0.20	15.59±0.17	64.69±0.40	51.54±0.22
2	40	19.78±0.25	12.76±0.13	25.68±0.21	78.18±0.16	60.45±0.18
3	60	23.03±0.38	20.06±0.39	39.9±0.30	85.75±0.31	68.74±0.24
4	80	28.45±0.10	35.25±0.45	52.75±0.39	87.07±0.27	76.07±0.38
5	100	37.39±0.43	46.8±0.16	71.1±0.12	88.07±0.48	78.52±0.17
6	120	49.32±0.41	56.83±0.28	77.98±0.15	88.42±0.42	80.24±0.36

(*Average value of 3 replicates)

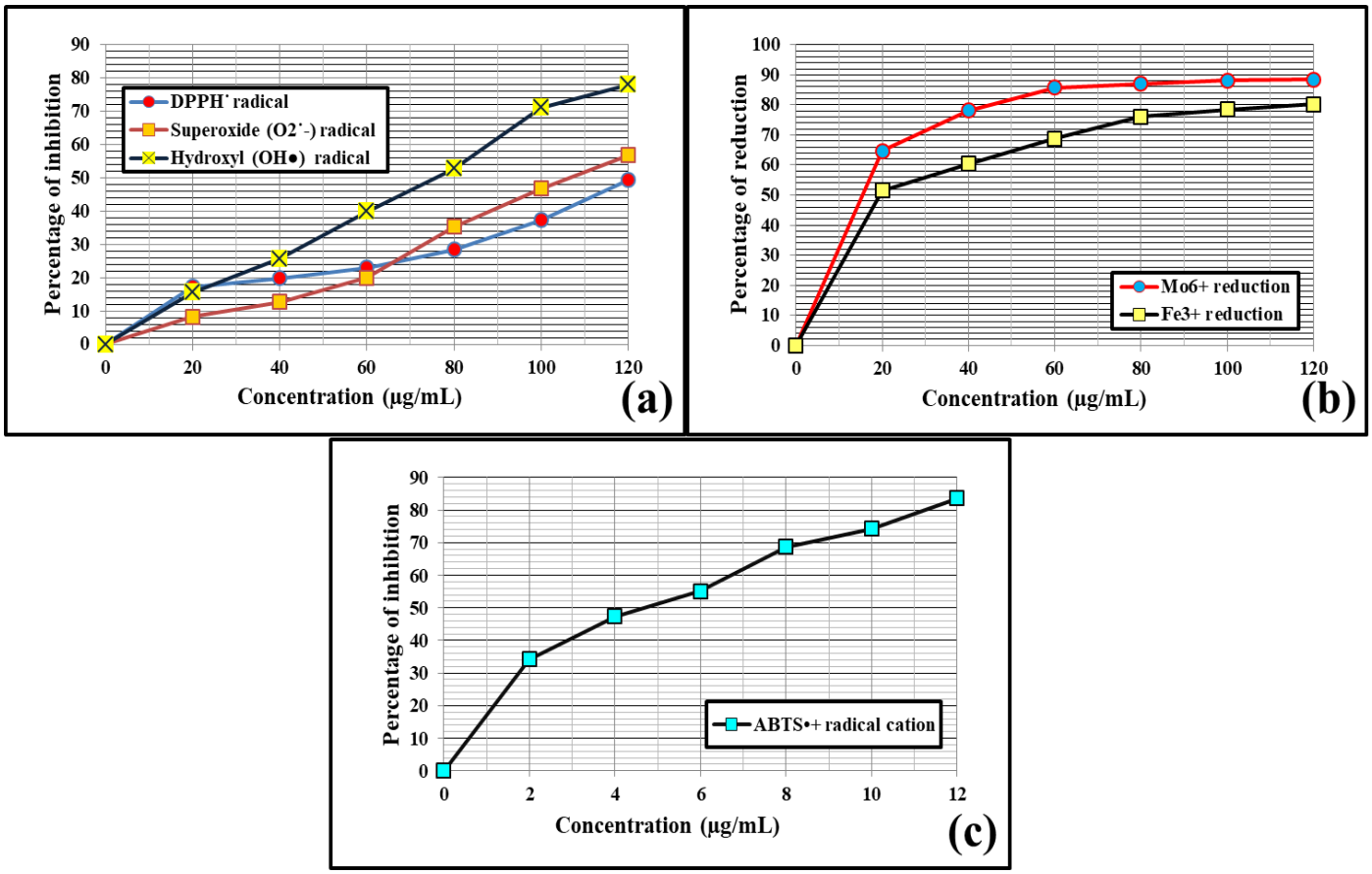
$ABTS^{\bullet+}$ radical cation scavenging activity

$ABTS^{\bullet+}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and $ABTS^{\bullet+}$ radical cation gets reduced in the presence of aqueous extract and the remaining radical cation concentration was then quantified at 734 nm. It can be prepared using $K_2S_2O_8$ as an oxidant. The blue-green colour of aqueous ABTS solution is formed by the loss of an electron by the nitrogen atom of ABTS (2, 2-azinobis (3ethylbenzothiazolin-6-sulfonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom) (Table 4 and Figure 6) [35]. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity of aqueous dried tuberous root extract of *Asparagus racemosus* was 83.56±0.43% at 12 µg/mL concentration and the IC_{50} was 4.22 µg/mL concentration, which was compared with standard ascorbic acid (IC_{50} = 3.14 µg/mL concentration).

Table 4: $ABTS^{\bullet+}$ radical cation scavenging activity of aqueous dried root extract of *Asparagus racemosus*

S. No	Concentration (µg/mL)	Percentage of inhibition*
		$ABTS^{\bullet+}$ radical cation
1	2	34.19±0.28
2	4	47.36±0.32
3	6	55.08±0.17
4	8	68.65±0.45
5	10	74.24±0.20
6	12	83.56±0.43

(*Average value of 3 replicates)



(a) - DPPH˙ radical scavenging activity, Superoxide ($O_2^{\cdot-}$) radical scavenging activity and Hydroxyl radical ($OH^{\bullet}$) scavenging activity
(b) - Phosphomolybdenum reduction activity and Ferric (Fe^{3+}) reducing power activity
(c) - $ABTS^{\bullet+}$ radical cation scavenging activity

Fig 6: Antioxidant activities of aqueous dried root extract of *Asparagus racemosus*

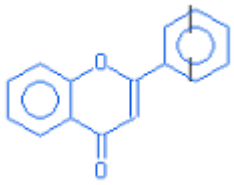
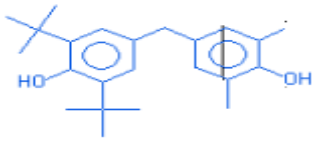
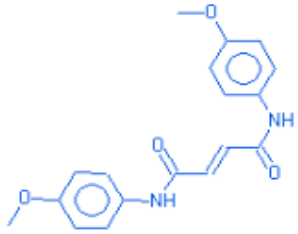
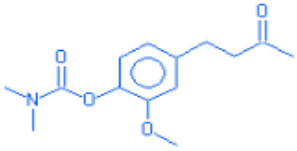
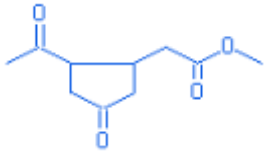
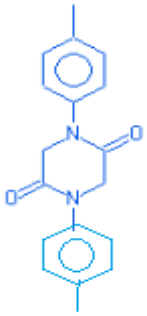
Gas Chromatography–Mass Spectrometry (GC–MS) Profiling

Gas chromatography (GC) is a widely applied technique in many branches of science and technology. For over half a century, GC has played a fundamental role in determining how many components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e., its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule [36].

The GC-MS analysis of aqueous dried tuberous root extract of *Asparagus racemosus* (Table 6) revealed the presence of ten different bioactive compounds (phytochemical constituents) that could contribute the antioxidant and several therapeutic benefits of Shatavari. The identification of the phytochemical compounds was confirmed based on the peak area, retention time, molecular weight and molecular formula (Table 5 and Figure 7).

Table 5: GC–MS Profiling of aqueous dried tuberous root extract of *Asparagus racemosus*

S. No	RT	COMPOUND NAME	COMPOUND STRUCTURE	MOLECULAR WEIGHT (g/mol)	MOLECULAR FORMULA
1	13.2	2-Phenyl-1,3-Cyclohexadiene		156.15	$C_{12}H_{12}$
2	15	Benzidine		184.04	$C_{12}H_{12}N_2$
3	16.7	Pentadecane		211.98	$C_{15}H_{32}$

4	17.78	Flavone		222	$C_{15}H_{10}O_2$
5	23.72	Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl]-		340	$C_{23}H_{32}O_2$
6	21.83	But-2-endiamide, N,N'-bis (4-methoxyphenyl)-		325.24	$C_{18}H_{20}O_3$
7	18.48	Pentadecane-2,4-dione		239.87	$C_{15}H_{28}O_2$
8	19.17	Formic acid,1-dimethylamino-,[2-methoxy-4-(3-oxobutyl)]phenyl ester		265.55	$C_{14}H_{19}NO_4$
9	16.13	Cyclopentanone,3-acetyl-4-(methoxycarbonylmethyl)-		198.03	$C_{10}H_{14}O_4$
10	20.4	Piperazine-2,5-dione, 1,4-(4-methylphenyl)-		293.51	$C_{18}H_{18}N_2O_2$

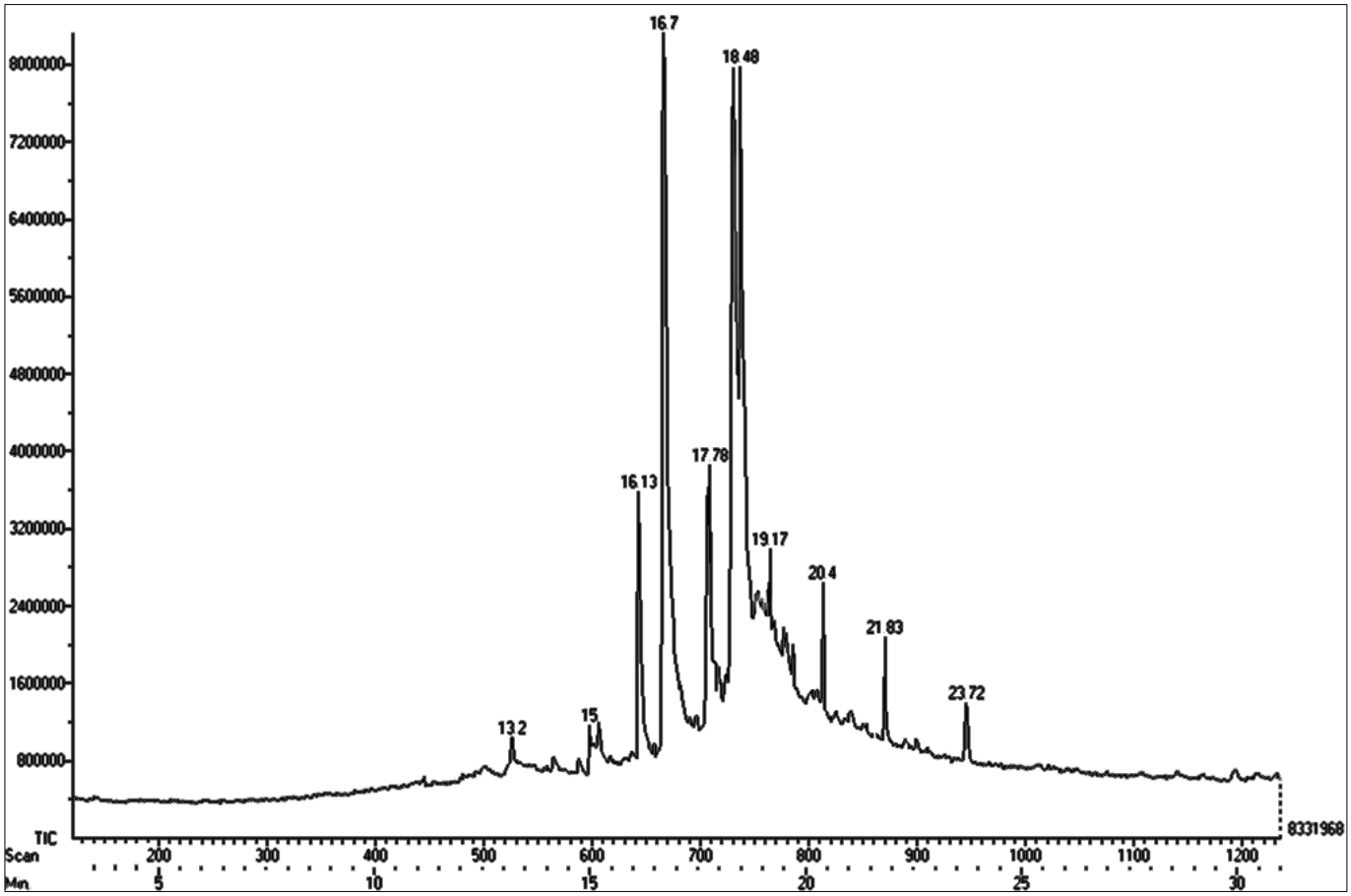


Fig 7: GC–MS Chromatogram of aqueous dried tuberous root extract of *Asparagus racemosus*

Table 6: Pharmacological properties of aqueous dried tuberous root extract of *Asparagus racemosus* from GC–MS analysis

S. No	Compound Name	Pharmacological activity [37-40]
1	Pentadecane	Sugar-phosphatase inhibitor, Acrocylindropepsin inhibitor, Chymosin inhibitor, Antibacterial activity
2	Flavone	Production of Reactive Oxygen Species (ROS) can be reduced by flavonoids Relevance of plant defense mode of action is highly possible by flavonoids Formation of oxygen radicals can be prevented by flavonoids thereby inhibiting the enzyme activity
3	Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl]-	Antimicrobial activity, Antioxidant activity, Antimalarial activity, Immunomodulatory effect
4	Piperazine-2,5-dione, 1,4-(4-methylphenyl)-	Anti-microbial activity, Anti-depressant activity, Anticonvulsant activity, Anti-parkinson activity, Antiinflammatory activity, Antipsychotic activity, Antioxidant activity, Antidiabetic activity, Antiarrhythmic activity, Antiproliferative activity, Anxiolytic activity, Antialzheimer activity, Antimalarial activity, Antihypertensive activity, Antiplatelet aggression and Anti-histaminic activity

Conclusion

The modern natural product research is undergoing a revolution due to recent advancements in combinatorial biosynthesis, microbial genomics and screening processes. Moreover, access to hyphenated techniques like Liquid Chromatography-Mass Spectrometry, Liquid Chromatography-Nuclear Magnetic Resonance have raised the hope of drastically reducing the time and cost involved in natural product research by using de-replication processes that are combination of techniques to avoid the already reported compounds. There is no doubt that plants are among the most perfect "natural laboratories" for the synthesis of various molecules ranging from simple skeleton to highly complex chemical structures. The research study revealed about the potent antioxidant activities of dried tuberous roots of Shatavari, making a pathway to study about anti-proliferative activity on the desired cancer cell lines. The successful isolation of resveratrol from grapes has remarked the benefits in promoting metabolic health by consuming red wines, suggesting the pharmaceutical values as a potential treatment for metabolic syndromes. There are approximately 40% of all medicines especially, 60% of anticancer agents and 80% of antimicrobials available in clinical use are either natural products or their semisynthetic derivatives. Natural products should remain as the major source to create molecule scaffolds or serve as building blocks in early-stage drug

discovery, but more efforts are needed to boost pharmaceutical output.

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References

1. Lakhwinder Singh, Antul Kumar, Anuj Choudhary, Gurwinder Singh. *Asparagus racemosus*: The plant with immense medicinal potential. Journal of Pharmacognosy and Phytochemistry. 2018; 7(3):2199-2203.
2. Goyal RK, Singh J, Lal H. *Asparagus racemosus*-An update. Indian J Med Sci. 2003; 57(9):408-14.
3. Tou JC, Chen J, Thompson LU. Flaxseed and its lignan precursor, secoisolariciresinol diglycoside, affect pregnancy outcome and reproductive development in rats. J Nutr. 1998; 128(11):1861-8.
4. Palanisamy N, Manian S. Protective effects of *Asparagus racemosus* on oxidative damage in isoniazid-induced hepatotoxic rats: An in vivo study. Toxicol Ind Health. 2012; 289(3):238-44.
5. Rakesh KJ. *Asparagus racemosus* (Shatawari), phytoconstituents and medicinal importance, future source of economy by cultivation in Uttrakhand: A review. Int J Herb Med. 2016; 4(4):18-21.
6. Deepika C, Dimple S. A Phytopharmacological review on *Asparagus racemosus*. Int. J Sci Res. 2014; 3(7):742-46.
7. Santosh K, Mehla RK, Dang AK. Use of Shatavari (*Asparagus racemosus*) as a galactopoietic and therapeutic herb- A review. Agric. Rev. 2008; 29(2):132-138.
8. Thakur S, Sharma DR. Review on medicinal plant: *Asparagus adscendens* Roxb. Int J Pharma Sci and Health Care. 2015; 3(5):82-97.
9. Kumar MC, Udupa AL, Sammodavardhana K, Rathnakar UP, Shvetha U, Kodancha GP. Acute toxicity and diuretic studies of the roots of *Asparagus racemosus* Willd in rats. West Indian Med J. 2010; 59(1):3-6.
10. Noorul Hasan, Nesar Ahmad, Shaikh Zohrameena, Mohd Khalid, Juber Akhtar. *Asparagus racemosus*: for medicinal uses & pharmacological actions. International Journal of Advanced Research. 2016; 4(3):259-267.
11. Garde GK. Sarth Vagbhat (Marathia Translation of Vagbhat‟s Astangahridya). Uttarstana, Aryabhushana Mudranalaya, 1970, 40-48.
12. Srikantha M, Vagbhat‟s KR, Astanga Hrdayam. Text, English translation, Notes, Appendix and Indices. Krishnadas Academy; Varanasi, 1997.
13. Bopana N, Saxena S. *Asparagus racemosus*: Ethnopharmacological evaluation and conservation needs. J Ethnopharmacology. 2007; 110:1-15.
14. Abheri Das Sarma, Anisur Rahaman Mallick, Ghosh AK. Free Radicals and Their Role in Different Clinical Conditions: An Overview. International Journal of Pharma Sciences and Research. 2010; 1(3):185-192.
15. Trease GE, Evans WC. Pharmacognosy. 13th (ed). ELBS/Bailliere Tindall, London, 1989, 345-6, 535-6, 772-3.
16. Harborne JB. Phytochemical Methods, A guide to Modern Techniques of Plant analysis, second ed. Chapman and Hall, London, 1998, 54-84.
17. Stahl E, Thin Layer Chromatography, 2nd ed., Springer Pvt. Ltd., New Delhi, 2005, 85.
18. Liu X, Dong M, Chen X, Jiang M, Lv X, Yan G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. from *Ginkgo biloba*. Food Chemistry. 2007; 105:548-554.
19. Spanos GA, Wrosltad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry. 1990; 38:1565-1571.
20. Madhu M, Sailaja V, Satyadev TNVSS, Satyanarayana MV. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry. 2016; 5(2):25-29.
21. Khalaf NA, Shakya AK, Al-othman A, El-agbar Z, Farah H. Antioxidant activity of some common plant. Turk J Biol. 2008; 32:51-5.
22. Lokesh Deb SK, Dubey, Avijeet Jain, Amit Kumar Jain, Pandian GS. Free radical scavenging activity of aqueous n- butanol fraction of *Prunus Persica* aqueous extract. Journal of Natural Remedies. 2009; 9(2):152-158.
23. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 1989; 28:1057-1060.
24. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry. 1999; 269:337-341.
25. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J Nutr. 1986; 44:307-315.
26. Re R, Pellegrini N, Proteggente A, Pannal A, Yang M, Rice-Evan C. Antioxidant activity applying an improved $ABTS^{\bullet+}$ radical cation decolorization assay. Free Radic. Biol. Med. 1999; 26:1231-1237.
27. Saraswathi K, Sivaraj C, Arumugam P. Antioxidant Activities, Thin Layer Chromatographic Analysis and GCMS Analysis of *Capsicum annuum* L.: A Comparison of Green and Red Chilli. J Biol. Chem. Research. 2019; 36(1):184-197.
28. Sanjeet Kumar, Jyotirmayee K, Monalisa Sarangi. Thin Layer Chromatography: A Tool of Biotechnology for Isolation of Bioactive Compounds from Medicinal Plants. Int. J Pharm. Sci. Rev. Res. 2013; 18(1):126-132.
29. Petersen M, Simmonds MSJ. Rosmarinic acid. Phytochemistry. 2003; 62:121-125.
30. Awika M, Rooney LW, Wu X, Prior RL. Cisneros Zevallos L. Screening methods to measure antioxidant activity of Sorghum (*Sorghum ialmatei*) and Sorghum product. Journal of Agricultural and Food Chemistry. 2003; 51:6657-62.
31. Wickens AP. Aging and the free radical theory. Respiratory Physiology. 2001; 128:379-391.
32. Siddhuraju P and Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food. Chem. 2007; 105:950-958.
33. Yildirim A, Mavi A. Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem. 2001; 49:4083-4089.

34. Stadtman ER. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. Free Radical Biology and Medicine. 1990; 9:315-325.
35. Miller DD. Mineral. In: Fennema, O.R. (Ed.). Food Chemistry Marcel Deckker, New York, 1996, 618-649.
36. Elena Stashenko, Jairo Rene Martínez. Research Center for Biomolecules, Cibimol, Cenivam, Universidad Industrial de Santander, Bucaramanga, Colombia.
37. Sunita Arora and Sonam Meena. GC-MS Profiling of *Ceropegia bulbosa* Roxb. var. *bulbosa*, an endangered plant from Thar Desert, Rajasthan. The Pharma Innovation Journal. 2017; 6(11):568-573.
38. Justyna Mierziak, Kamil Kostyn, Anna Kulma. Flavonoids as Important Molecules of Plant Interactions with the Environment. Molecules. 2014; 19:16240-16265.
39. Elaiyaraja A, Chandramohan G. Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. Journal of Pharmacognosy and Phytochemistry. 2016; 5(6):158-171.
40. Kuldeep Singh, Siddiqui HH, Pragati Shakya, Paramdeep bagga, Arun Kumar, Khalid M *et al*. Piperazine – a biologically active scaffold. IJPSR. 2015; 6(10):4145-4158.