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Chemical composition and *in vitro* antioxidant potential of pili and safed shatavar (*Asparagus racemosus*)

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Abstract
The chemical composition of methanolic extracts from pili and safed shatavar (*Asparagus racemosus*) was analysed through GC/GCMS. Both the plant type exhibited the presence of several bioactive compounds. In each roots extracts, the major 9 and 14 compounds were identified in pili and safed shatavar respectively. The rest of the compounds were identified as minor or trace compounds. The methanolic extract of pili and safed shatavr was analysed for antioxidativ assay. The extract obtained from the pili and safed shatavar were displayed good radical scavenging potential with the IC_{50} value of 536.66±0.92 and 612±0.95 respectively, as compared with the standard antioxidant ascorbic acid. Pili shatavar methanolic extract (RP_{50}=79.06±1.69) posses highest reducing power with respect to gallic acid (RP_{50}=80.24±1.19). Pili shatavar methanolic extract (IC_{50}=271.21±0.21) and safed shatavar methanolic extract (IC_{50}=599.78±10.10) exhibited highest metal chelating effect in comparison to standard chelating agent EDTA. The extract due to its antioxidant activity could be utilized in pharmaceutical sector and food industries.

Keywords: *Asparagus racemosus*, methanol extracts GC-MS, antioxidative activity

1. Introduction
The use of plants to fight or cure disease is possibly as old as mankind. For decade native people of diverse cultures have been used medicinal plants for all kind of healing [1]**.** The active compounds in most parts of the medicinal plants have indirect or direct therapeutic effects and are used as medicinal agents [2]. Therapy with natural origin bioactive medicine is increasing concern about harmful synthetic medicine [3]. Increasing dependence on the use of medicinal plants in industry has been attributed to the removal and synthesis of various drugs and chemotherapeutic products from these plants, as well as traditional medicinal herbal remedies [4]. Natural antioxidants are required to prevent and/or cure the disorders caused by free radicals. The free radicals are highly reactive chemical species produced in the body and have the potential to damage cells, organelles, DNA, and other biomolecules, resulting in diseases such as cancer and cardiovascular and neurodegenerative ailments [5]. The treatment of such diseases has serious efficacy and safety issues. In addition, it is often highly expensive and many people cannot afford it. Thus, there is an urgent need of natural additives as potential antioxidants having an important role in preventing a variety of stress related diseases [6]. The genus Asparagus comprises about 150 species distributed worldwide out of which 17 species present in India. It is considered of medicinal value due to the presence of sapogenins and steroidal saponins present in different parts of the plants [7]. Many species are considered to be diuretic and have soothing effect [8, 9]. *A. racemosus* one of the important medicinal species of Asparagus [10]. *Asparagus racemosus* is an important medicinal of the tropical and subtropical region commonly called Shatavari, Shatavar or Satmuliand belongs to the family Asparagaceae. Several medicinal properties are attributed to the root of *Asparagus racemosus* and it is used as a galactagogue to stimulate the secretion of breast milk [11]. Other uses of the plant are aphrodisiacs, diarrhea, tuberculosis, diabetes, antioxidants, antitussives, hyperacidity, general weakness, habitual abortion and safe delivery [12]. The presence of flavonoids, considered a good sequestrant of free radicals, indicates that this plant could have antioxidant properties. Saponins are related to antibacterial activity and glycosides reported for lowering blood pressure [8, 7]. This plant is ideal for all-weather cultivation in Uttrakhand and will become a source of income for many young people [13].
Therefore, the present study focused on evaluation of chemical composition of the rhizmatous extracts from Pili shatavar and safed shatavar by GC/GC–MS and to analysed the antioxidative properties of the methanolic extracts.

2. Material and methods

2.1. Source of plant material

The rhizomes of pili shatavar and safed shatavar were collected from M.R.D.C.Pantnagar. Uttarakhand in the month of September 2018 and verified by Dr. D.S. Rawat (Plant taxonomist) G. B. Pant University of Agriculture andTechnology, Pantnagar.

2.2 Preparation of extracts

The shade dried rhizomes were coursly powdered and subjected to successive extraction using soxhlet apparatus. The extraction was done using methanol as solvent. The extracts was concentrated using distillation. The yield of each extracts were recorded in table 1

2.3. GC analysis of extracts

GC analysis of the methanolic extracts was performed in Nucon-GC 5765 system.

2.4. GC-MS analysis of extracts

The GC-MS data were analysed GC MS-QP 2010 plus. The compounds were identified by matching their mass spectra and GC retention indices with those in NIST-MS Wiley Library, comparing with literature reports and published data [14].

Table 1: Percent yield of methanolic extracts of pili and safed shatavar.

Plant part	% yield (safed shatavar)	% yield (pili shatavar)
Rhizomes	5.64%	7.66%

2.5 Determination of antioxidant activity

2.5.1. Free radical scavenging activity

The free radical scavenging activity of the methanolic extracts were determined, by the method described earlier [15] with slight modifications, based on the ability of sample to neutralize DPPH radical. DPPH (2,2-diphenyl-2-picrylhydrazyl) is a stable free radical that can accept hydrogen radical or an electron to convert it into a stable diamagnetic molecule. The reaction mixture contained 5mL of 0.004% methanolic solution of DPPH and different amount of extract samples ranging from 50µg/mL to 250µg/mL. The solution was rapidly mixed and scavenging capacity was measured by spectrophotometer of Thermo Scientific Evolution 201 series by monitoring the continuous decrease in absorbance at 517nm. Standard antioxidants ascorbic acidwas used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. Inhibition of free radical by DPPH in percent (IC %) was calculates by using the equation.

$$IC\ \% = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where,
A_0 = Absorbance value of Control sample
A_t = Absorbance value of Test sample
IC = Inhibitory concentration

2.5.2 Reducing power activity

The reducing power activity of methanolic extracts was determined by the method proposed earlier. Varying concentration of the tested samples (50 to 250 µg/mL) andstandard were mixed with 2.5 mL of phosphate buffer (200 mM, pH= 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3[FeCN_6]$). After 20 min incubation at 50 ± 1^0C, trichloroacetic acid was added in the amount of 2.5mL to the prepared mixtures, followed by centrifugation at 650 RPM for 10 to 15 min. the upper layer (1 mL) was mixed with 5 mLditilled water and 1 mL of 0.1% ferric chloride and absorbance of the resultant solution were deliberated at 700nm using spectrophotometer. All the readings were taken as triplicate with respect to gallic acid was used as the standard antioxidant. The reducing power of samples was calculated using the formula given below:

$$\text{Reducing Power } \% = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where,
$A_{0=}$ Absorbance value of control sample
A_t= Absorbance value of test sample

2.5.3 Effect on the chelating activity of Fe^{2+}

The metal chelating activity of Fe^{2+} by constituents of extracts was examined by spectrophotometric method. Based on the principle of Fe^{2+} chelating ability of the antioxidant by measuring the absorbance of the ferrous ion-ferrozine complex formed at 560 nm [16]. 0.1 mL of 2mM $FeCl_2.4H_2O$, 0.2mL of 5mM ferrozine and 4.7 mL of methanol was added to different concentrations of tested samples (50,100,150,200,250 µg/mL). The solutions were mixed and incubated for 10 min. The absorbance of test sample was measured at 562 nm. using spectrophotometer. All the readings were taken as triplicate; EDTA (0.01 mM) was used as the standard. The metal-chelating activity of tested samples was calculated using the following formula:

$$IC\ \% = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where
$A_{0=}$ Absorbance value of control sample
A_t= Absorbance value of test sample

3. Results and discussion

3.1 GC and GC-MS analysis of extracts

The components present in roots of *Asparagus racemosus* were identified by GC/GC-MS analysis. The identified compounds listed in table.2. In pili shatavar percentage (%) content of various compounds ranged from 0.01%(2-butanone, 1-pentyl-3,3-d2 acetate, methylbutyrate - d2, baking soda, o-methylisourea, methyl isobutyrate, ethyl propionate, dimethyl fumarate, 1-piperidino-2,4,6-trinitrobenzene, glutamic acid hydrochloride, 2-methoxyoxolane) to 22.76% (1-heptanol) and major 9 compound constitude total 82.47% of total compound. These major compound identified 1,3:4,6-dimethylene-d-glycero-d-mannoheptitol (22.8%), 3-deoxy-d-mannoic lactone (19.5%), 5-(hydroxymethyl)-2-furaldehyde (18.9%), 1,3 dichlorocyclopentane (12.5%), methyl sorbate (7.2%), dimethyl fumarate (3.4%), oleic Acid (3.5%), diisopropyl 2-oxomalonate (2.3%) dimethyl maleate (2.2%) and the remaining compounds together constitute 17.52% responsible for the several biological activities. The chromatogram of the

compounds was mentioned in Fig 1.

In safed shatavar percentage (%) content of various compounds ranged from 0.02% (hexanoic acid, 5-hydroxy-, methyl ester, 4-penten-1-ol, succinic acid, 3-methylbut-2-yl 2-methoxyphenyl ester, 2-pentadecanol and fumaric acid, 2-methylphenyl dodec-2-en-1-yl ester) to 24.4% (5-(hydroxymethyl)-2-furaldehyde) and major 14 compound constitude88.80% of total compound. These major compound were 3-deoxy-d-mannoic lactone (20.7%), pentanoic-3,3-D2 acid (10.1%), cyclopentane, 1-acetyl-1,2-epoxy(7.5), oleic acid (4.5%), 2-butanone, 4-hydroxy-3-methyl (4.4%), pyranone (3.5%), tetradecanoic acid (2.5%), diethyl methoxymalonate (2.3%), glycerol (1.5%), pedilstatin (1.4%) sucrose (1.4%), tridecanedial (1.4%), oleoyl chloride (1.4%), and the remaining compounds together constitute 11.16% together constitute minor compounds responsible for the several biological activities. The chromatogram of the compounds was mentioned in Fig 2.

Table 2: Comparative phytochemical composition of rhizomes of pili and safed shatavar with earlier investigation.

		Peak area % Contribution				
		Previous finding			Our study	
S. No	**Name of Compound**	**Panneer Selvam *et al*., 2014**	**Sivakumar and Gajalakshmi (2014)**	**Janani. *et al*., 2014**	**PSME**	**SSME**
		Ethanol	**Methanol**	**Ethanol**		
1.	ethyl propionate	-	-	-	t	2.3
2.	methyl sorbate	-	-	-	7.2	-
3.	diisopropyl 2-oxomalonate	-	-	-	2.7	-
4.	dimethyl fumarate	-	-	-	3.3	T
5.	5-(hydroxymethyl)-2-furaldehyde	-	-	-	18.9	24.4
6.	dimethyl maleate	-	-	-	2.2	-
7.	1,3-dichlorocyclopentane	-	-	-	12.5	10.1
8.	3-deoxy-d-mannoic lactone	-	-	-	19.4	20.7
9.	oleic acid	-	-	1.7	3.2	4.5
10.	2-hexadecanoyl glycerol	2.5	-	-	0.9	T
11.	1-Heptanol	-	-	-	22.7	-
12.	2-etradecanol octanoate	2.4	-	5.1	0.31	T
13.	hexanoic acid, 5-hydroxy-, methyl ester	t	-	9.9	0.9	T
14.	cyclopentane, 1-acetyl-1,2-epoxy-	-	-	-	t	7.4
15.	4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-	-	2.6	-	3.5
16.	2-butanone, 4-hydroxy-3-methyl	-	-	-	-	4.4
17.	1,2,3-propanetriol	-	-	-	-	1.5
18.	ethyl 3-(acetyloxy)-2-(hydroxymethyl)propanoate	-	-	-	t	2.3
19.	3-deoxy-d-mannoic lactone	-	-	-	19.4	20.7
20.	tetradecanoic acid	0.7	-	2.2		2.5
21.	Tridecane	-	-	-	-	1.4
22.	oleoyl chloride	-	-	-	-	1.4
23.	1,2,3,5-tetraisopropylcyclohexane	19.7	-	-	-	1.2
24.	Sucrose	-	-	-	-	1.4
25.	neotigogenin acetate	2.7	-	-	-	0.1
26.	1-allyl-2-methylenecycloheptano	-	-	-	-	1.5
27.	2-propanone, 1,3-dihydroxy	-	10.9	-	-	-
28.	2-fruancarboxy aldehyde, 5- (hydroxymethyl)	-	76.6	39.5	-	-
29.	hexadecanoic acid	-	2.2		t	-
30.	n-hexadecanoic acid	-	4.5	7.9	t	-
31.	ethanol,2(octyloxy)-	-	3.9	-		-
32.	1,9-nonanediol	-	1.8	-	-	-
33.	tetranorlipoic acid]	-	-	18.2	-	-
34.	1,6-anhydro-β-d-talopyranase	-	-	14.0	-	-
35.	propane, 1,1,3- triethoxy	-	-	1.2	-	-
36.	dodecanoic acid		-	6.2	-	-

PSME-pili shatavar methanolic extracts, SSME- safed shatavar methanolic extract, GC- Gas chromatography, MS- mass spectrometery, t- trace amount (contribution less than 1%)

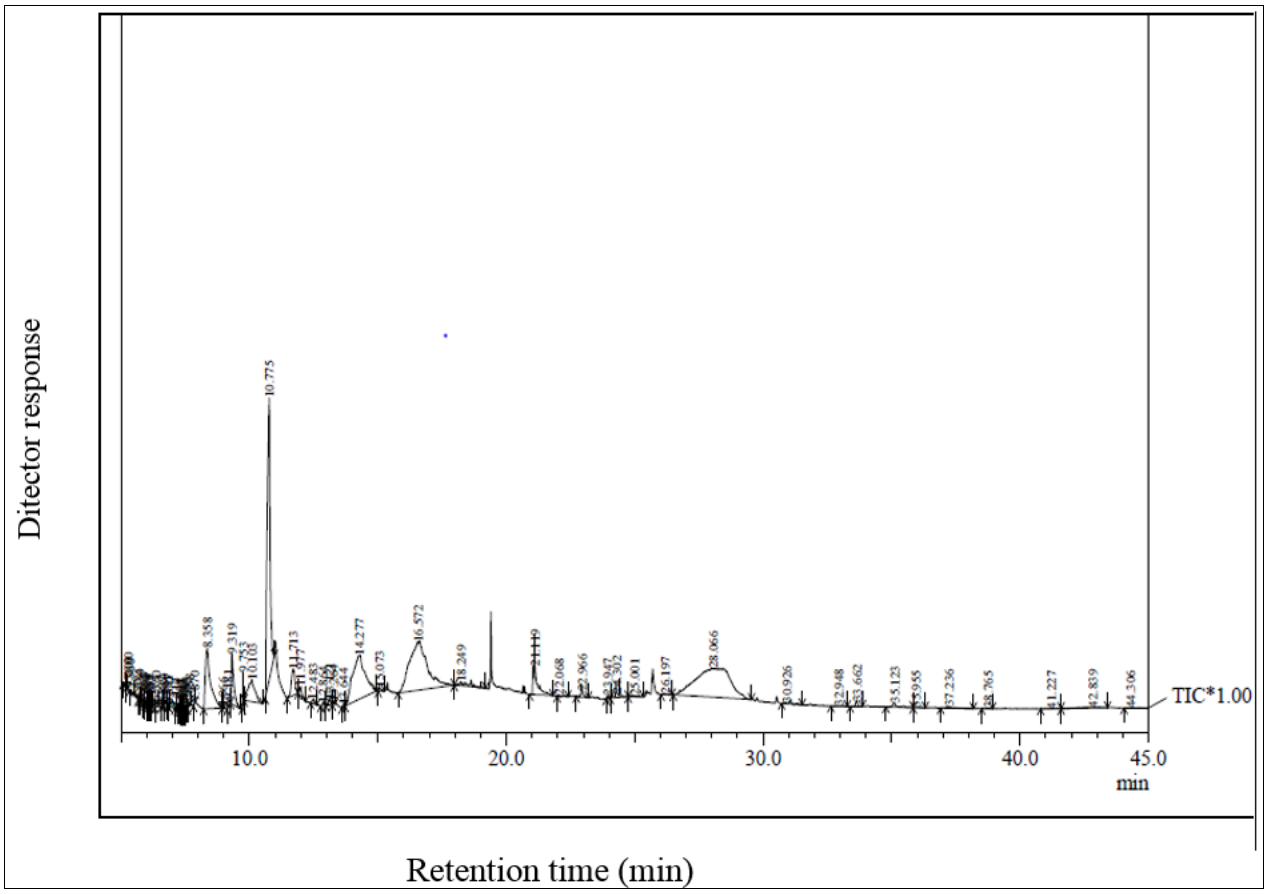

Fig 1: GC-MC chromatogram of roots extract of *A. racemosus* (pilishatavar)

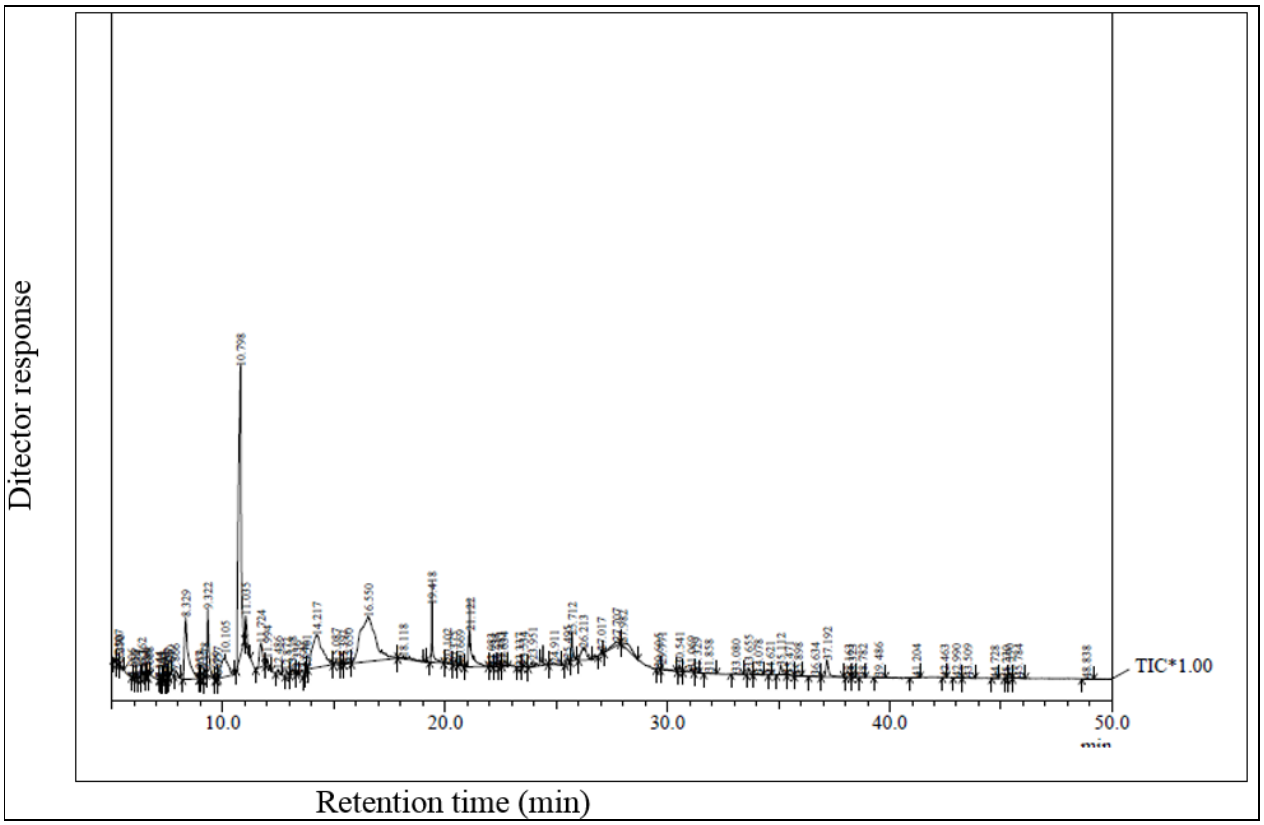

Fig 2: GC-MC chromatogram of roots extract of *A. racemosus* (safed shatavar)

In previous study the hexane extract of *Asparagus racemosus*roots, contains 21 different chemical components like disulfide, bis(1-methylpropyl), benzene, 1,3-bis(1,1 dimethylethyl), (E)-hex-3-enyl (E)-2-methylbut- 2-enoate, jatamansone, n-pentadecylcyclohexane, eicosane, squalene, heptacosane etc.[20]. The major phyto-constituents in *Asparagus racemosus* reported earlier di-n-octyl phthalate, pentadecanoic acid, 14-methyl-, methyl ester, 1,2-benzenedicarboxylic acid, butyl octyl ester etc [21].

3.2 Determination of antioxidant activity

3.2.1 DPPH free radical Scavenging activity

The 2,2'-diphenyl-1-picrylhydrazyl radical has been widly used to evaluate free radical scavenging capacity of the antioxidants [22]. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. The use of DPPH assay provides an easy and a rapid way to evaluate antioxidants by spectrophotometer [23].

DPPH radical scavenging activity was studied for extracts of at selected dose levels. Lower IC_{50} value indicates higher antioxidant activity. The radical scavenging activity of both the extracts samples were compared with the standard antioxidants, ascorbic acid which showed the activity in terms of mean of its IC_{50} value present as 153.69±0.09. The scavenging activity of both the extracts was found less in comparison to the standards. Both the extracts were found to possess strong DPPH radical scavenging activity with respect to the ascorbic acid used as standard. The extracts obtained from PSME displayed highest radical scavenging potential (IC_{50}=536.66±0.92) as compared to SSME (IC_{50}=612.95±0.95).With an increase in the concentration of extracts, an increase in the scavenging activity was observed for both the extracts (Table3).

Table 3: IC_{50} values of methanolic extracts of pili and safed shatavar.

S.N.	Sample Name	IC_{50} values (µg/mL) in triplicate			Mean IC_{50} values with standard deviation (µg/mL)
		1st	2nd	3rd	
1.	PSME	537.72	536.06	536.21	536.66±0.92
2.	SSME	612.27	612.87	612.77	612.95±0.95
3.	Ascorbic Acid	153.73	153.74	153.59	153.69±0.09

PSME= pili shatavar methanolic extract, SSME=safed shatavar methanolic extract Note: Values are given as mean ± SD of triplicate experiments.

3.2.2 Reducing power activity

A good reducing power activity was observed with the extracts of *Asparagus racemosus*. PSME (RP_{50}=79.06±1.69) observed to have highest reducing power as compared to SSME (RP_{50}=177.12±4.47), Gallic acid taken as standard antioxidant (RP_{50}=80.24±1.19). The reducing power activity of each extracts samples were evaluated by calculating the mean RP_{50} value with standard deviation of their triplicate readings listed in Table4. The results indicate that the antioxidant activity in terms of reducing power of the extracts of *A. racemosus*is lesser than that of ascorbic acid. It has been indicated that the antioxidant potential of certain compounds is related to their reducing power [24].

Table 4: IC_{50} values ofmethanolicextracts of pili and shatavar.

S. No.	Sample Name	IC_{50} values (µg/mL) in triplicate			Mean IC_{50} values with standard deviation (µg/mL)
		1st	2nd	3rd	
1.	PSME	537.72	536.06	536.21	536.66±0.92
2.	SSME	612.27	612.87	612.77	612.95±0.95
3.	Ascorbic Acid	153.73	153.74	153.59	153.69±0.09

PSME= pili shatavar methanolic extract, SSME=safed shatavar methanolic extract. Note: Values are given as mean ± SD of triplicate experiments.

3.2.3 Effect on the chelating activity of Fe^{2+}

The complex formation between the ferrozine (chelating agent) and Fe^{2+} ions is disrupted which results the change in the colour of the complex. By measuring the colour reduction spectrophotomitically, allows us to estimation of the metal chelating activity.

The chelating activity of Fe^{2+} of the extracts is shown in Table 5. The antioxidant activities increase significantly with the concentration of each extracts. In the present study roots extracts from PSME shows highest metal chelating effect (IC_{50}=271.21±0.20ug/ml) as compared to the SSME. The variation of chelating activity increases with the concentration of the extracts**.** The data obtained by performing the metal chelating antioxidant activity revealed that the extracts from roots of SSM exhibited moderate to goodFe^{+2} chelating activity so could function as Fe^{+2}chelator.

These extracts either chelated metal ions or suppressed reactivity by occupying all coordination sites of metal ions [25]. Therefore, these extracts can be used as effective chelating agents and could afford protection against oxidative damage.

Table 5: IC_{50} values of methanolic extracts of pili and safed shatavar.

S.N.	Sample Name	IC_{50} values (µg/ml) in triplicate			Mean IC_{50} values with standard deviation (µg/ml)
		1st	2nd	3rd	
1.	PSME	271.18	271.02	271.42	271.21±0.20
2.	SSME	610.61	598.11	590.62	599.78±10.10
3.	EDTA	136.85	137.00	138.01	137.29±0.63

PSME= pili shatavar methanolic extract, SSME=safed shatavar methanolic extract. Note: Values are given as mean ± SD of triplicate experiments.

4. Conclusion

The study revealed the potential antioxidant and radical scavenging activity of organic extracts and extract of *Asparagus racemosus* rhizomes indicates its protective role against oxidative damage and as an important natural antioxidant. The extract due to its antioxidant activity could be utilized in pharmaceutical sector and food industries. Further research in this direction will be utilized for strengthening its real potential in various sectors.

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