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ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC AND FLAVONOID CONTENT OF ROOTS OF SEVEN *ASPARAGUS SPECIES* FROM NORTH-WEST INDIA

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ABSTRACT: The aim of current study was to compare the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of roots of seven *Asparagus* species from North-West India. Total phenolic content measured by Folin-Ciocalteu method ranged from 3.85 ± 0.25 (*A. racemosus*) to 7.74 ± 0.03 (*A. falcatus*), 3.42 ± 0.14 (*A. racemosus*) to 7.22 ± 0.12 (*A. falcatus*) and 1.12 ± 0.13 (*A. officinalis*) to 3.93 ± 0.54 mg/g of dry weight of root powder (*A. retrofractus*) in methanolic, ethanolic and aqueous extracts, respectively, expressed as gallic acid equivalents. The total flavonoid content determined by Aluminium chloride method ranged from 0.61 ± 0.03 (*A. officinalis*) to 1.86 ± 0.0303 (*A. densiflorus*), 0.39 ± 0.01 (*A. adscendens*) to 1.63 ± 0.0403 (*A. densiflorus*) and 0.31 ± 0.00 (*A. adscendens*) to 1.51 ± 0.02mg/gDW (*A. densiflorus*) in methanolic, ethanolic and aqueous extracts, respectively expressed as rutin equivalents. The results showed that TPC and TFC values were highest in methanolic extracts followed by ethanolic and lowest in aqueous extracts. The antioxidant capacity in the form of DPPH was evaluated by spectrophotometric method. The lowest radical scavenging activity was recorded in *A. adscendens* (149.6 µg/ml), while highest in *A. densiflorus* (116.2 µg/ml).

INTRODUCTION: Medicinal plants, as source of remedies, are universally used in modern society as alternative therapeutic tools to synthetic medicines for the prevention or treatment of many diseases [1]. Traditional herbs are usually cheaper, easily available and culturally more acceptable. Many synthetic drugs cause side effects, thereby increasing popularity of herbal medicine globally, even in regions with good healthcare systems. There has been a global boom in the incidences of cancer, hypertension and other diseases related to oxidative stress especially in developing countries.

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Medicinal plants are used in prevention and reducing risk of many diseases related to oxidative stress, such as cancer and hypertension, because they contain strong antioxidants [2].

Medicinal plants produce several secondary metabolites such as phenols, flavonoids, saponins, tannins, alkaloids and sterols which are important source of pharmaceutical drugs. These secondary metabolites vary significantly in their quality and quantity in different plant parts [3]. The antioxidant activity of plants is mainly due to phenolic compounds. Phenolics (phenolic compounds) are the most pronounced plant secondary metabolites, with many physiological functions in plants due to antioxidant properties having positive effects on human health, containing numerous varieties of compounds: phenolic acids, simple flavonoids, complex flavonoids, tannins *etc.* [1, 4, 5] Natural antioxidants have become objective of a great

number of research studies in finding the sources of potentially safe, effective and cheap antioxidants. Natural antioxidants in the plants scavenge harmful free radicals from our body. Accumulation of free radicals can cause severe pathological conditions and fasten ageing process. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the immune system of body or by supplementing with proven dietary antioxidants [6].

A huge number of plants are well known in traditional medicine system for their medicinal and therapeutic potentials worldwide alike *Asparagus*. The genus *Asparagus* belonging to the family *Asparagaceae* comprises about 300 species distributed all over the world, out of which 22 species have been reported in India. It is found to be grown throughout the temperate, tropical and subtropical parts of India [7, 8]. *Asparagus* species are grown as herbaceous perennials, tender woody shrubs and vines provided with short underground rhizomes from which the aerial shoots arise. Roots are often tuberous, sometimes fleshy. The most distinguishing feature of the genus is presence of cladodes (stem modifications) performing the function of leaves (reduced to scale-like bracts, often spiny) [9]. The major bioactive principals responsible for medicinal value of the genus are a group of steroidal saponins, polyphenols, flavonoids and sarsasapogenins. The *Asparagus* species show a great diverse of pharmacological activities: aphrodisiac, anti-oxidant, an immune-stimulant, anti-cancerous, anti-bacterial, anti-diabetic, anti-depressant, anti-inflammatory, anti-hepatotoxic, anti-tuberculosis, anti-diarrheal, cardio-protective, anti-aging, *etc*.[7, 10, 11]

Therefore, the purpose of this study is to evaluate the total phenols content, total flavonoids content and antioxidant potential of extracts of roots of seven *Asparagus species* by using three different solvents.

MATERIALS AND METHODS:

Chemicals: HPLC grade methanol and ethanol were procured from the Merck Specialties Pvt. Ltd. Gallic acid, rutin, aluminium trichloride, sodium nitrite, sodium carbonate, Folin-Ciocalteu reagent, DPPH sodium hydroxide *etc*. were purchased from Sigma-Aldrich.

Collection of Plant Material: The fresh roots of seven *Asparagus* spp. *viz*., *A. adscendens*, *A. racemosus*, *A. retrofractus*, *A. officinalis*, *A. densiflorus*, *A. falcatus* and *A. sprengeri* were collected from various regions of North-West India when the plants were in their full bloom in the year 2015-16. The species were grown and maintained in the Botanical Garden of Punjabi University, Patiala. The species were identified by consulting different floras and comparing with the samples available in the Herbaria, Department of Botany, Punjabi University, Patiala (PUN), Botanical Survey of India (BSI), Dehra Dun, and Forest Research Institute (FRI), Dehra Dun. The plant specimens were submitted to the Herbarium, Department of Botany, Punjabi University, Patiala. The accession numbers of seven species of *Asparagus* are *A. racemosus* Willd. (62456), *A. adscendens* Roxb. (62457), *A. officinalis* L. (62458), *A. sprengeri* Regel (62459), *A. retrofractus* L. (62460), *A. densiflorus* (Kunth) Jessop (62461) and *A. falcatus* L. (62462). The data for locality and altitude are given in **Table 1**.

TABLE 1: DATA OF LOCALITY, ACCESSION NUMBER AND ALTITUDE OF SEVEN *ASPARAGUS* SPECIES

S. no.	Species	Locality	Altitude (m)
1	*A. racemosus* Willd.	Udaipur, Rajasthan	600
2	*A. adscendens* Roxb.	Udhampur, J&K	755
3	*A. officinalis* L.	Solan, H.P.	1502
4	*A. sprengeri* Regel	Bhiwani, Haryana	225
5	*A. retrofractus* L.	Jammu, J&K	305
6	*A. densiflorus* (Kunth) Jessop	Patiala, Punjab	244
7	*A. falcatus* L.	Patiala, Punjab	244

Extraction Procedure: The roots were well washed, air-dried and powdered. The root extracts were prepared by soaking 10 g of powder in 100 mL of each; double distilled water, methanol and ethanol followed by vigorous shaking. The mixtures were left at room temperature for 72 h and then filtered with Whatman's filter paper. The extracts so obtained were stored at 4 °C until further use.

Determination of Total Phenolic Content (TPC): Total phenolic content (TPC) was determined by spectrophotometer using Folin-Ciocalteu reagent according to a procedure described by Singleton and Rossi, 1965 with some modifications [12]. 300 µL of diluted sample was treated with 5 mL of Folin-Ciocalteu reagent (diluted with distilled water 1:10 v/v). Then 4 mL of 7% sodium carbonate solution was added after 4 min. The tubes were vortexed for few seconds and were left at 40 °C for 30 min. The absorbance was read at 765 nm. Gallic acid was used as a reference standard. Then the content of phenolics in extracts was expressed as milligram of gallic acid equivalent (mg GAE/g of dried root).

Determination of Total Flavonoid Content (TFC): Aluminum chloride method (Park *et al*., 2008) was used for flavonoid content determination [13]. To 0.3 mL of diluted sample 0.15 mL of $NaNO_2$ (0.5 M), 0.15 mL of 0.3 M $AlCl_3.6H_2O$ and 3.4 mL of 30% methanol were added in a test tube. After 5 min, 1 ml of NaOH (1 M) was added to the solution. Then it was mixed gently and absorbance was read at 506 nm spectrophotometrically. Rutin was used as a standard solution to obtain standard curve. The content of flavonoids in extracts was expressed in terms of rutin equivalents (mg of RU/g of dried root).

DPPH Radical Scavenging Assay: DPPH (2, 2-diphenyl- 2- picrylhydrazyl hydrate) radical scavenging activity of root extracts was determined spectrophotometrically by slightly modified method of Miliauskas *et al*., 2004 [14]. 2.8 mL of 80 µM methanolic solution of DPPH was added to 500 µl of diluted extract solutions prepared using methanol. The determination was carried out in triplicates. The blank sample was containing the same amount of methanol and $DPPH^{\cdot}$ The reaction mixtures were kept in darkness at room temperature for 15 min. and then absorbance (A) was read at at 515 nM. The percent inhibition of DPPH was calculated by the following formula:

$$\% \text{ inhibition} = \frac{(\text{A of blank} - \text{A of test sample})}{\text{A of blank}} \times 100$$

RESULTS:

Total Phenolic Content: In the present work, we have examined the total phenolic, flavonoid content and DPPH radical scavenging activity of root extracts prepared in three solvents. Total phenolic content was expressed as gallic acid equivalents (mg GAE/g of dried root). All the studied species of *Asparagus* have good amount of phenols as shown in **Table 2**. TPC of methanol extracts of roots of *Asparagus* ranged from 3.85 ± 0.25 mg GAE/gDW (*A. racemosus*) to 7.74 ± 0.03 mg GAE/gDW (*A. falcatus*). In case of ethanol and aqueous extracts, lowest was recorded in *A. racemosus* (3.42 ± 0.14 mg GAE/gDW) and *A. officinalis* (1.12 ± 0.13 mg GAE/gDW) and highest in *A. falcatus* (7.22 ± 0.12 mg GAE/gDW) and *A. retrofractus* (3.93 ± 0.54 mg GAE/gDW), respectively. It was noticed that the highest concentration of phenolic compounds was found in the root extracts obtained using solvent of high polarity *i.e.* methanol followed by ethanol while lowest concentration was found in aqueous extracts.

TABLE 2: TOTAL PHENOLIC CONTENT OF ROOT EXTRACTS OF THREE DIFFERENT SOLVENTS OF SEVEN *ASPARAGUS* SPECIES (mg GAE/gDW)

S. no.	*Asparagus* species	Methanol	Ethanol	Aqueous
1	*A. adscendens*	5.49 ± 0.31	4.95 ± 0.16	1.94 ± 0.07
2	*A. officinalis*	6.12 ± 0.40	4.91 ± 0.45	1.12 ± 0.13
3	*A. retrofractus*	5.12 ± 0.18	4.18 ± 0.20	3.93 ± 0.54
4	*A. racemosus*	3.85 ± 0.25	3.42 ± 0.14	1.67 ± 0.19
5	*A. sprengeri*	6.17 ± 0.17	4.91 ± 0.05	2.28 ± 0.12
6	*A. densiflorus*	6.16 ± 0.01	5.59 ± 0.03	3.10 ± 0.14
7	*A. falcatus*	7.74 ± 0.03	7.22 ± 0.12	3.51 ± 0.07

Values are Mean ± S.E. of three replicates

Total Flavonoid Content: TFC content ranged from 0.61 ± 0.03 mg RU/gDW (*A. officinalis*) to 1.86 ± 0.03 mg RU/gDW (*A. densiflorus*) in methanol extracts, while in ethanol extracts it ranged between 0.39 ± 0.01 mg RU/gDW (*A. adscendens*) to 1.63 ± 0.04 mg RU/gDW (*A.*

densiflorus). In the aqueous extracts of roots, quantity of flavonoids was recorded low and ranged between 0.31 ± 0.00 mg RU/gDW (*A. adscendens*) to 1.51 ± 0.02 mg RU/gDW (*A. densiflorus*) as shown in **Table 3**.

TABLE 3: TOTAL FLAVONOID CONTENT OF ROOT EXTRACTS OF THREE DIFFERENT SOLVENTS OF SEVEN *ASPARAGUS* SPECIES (mg RU/gDW)

S. no.	*Asparagus* species	Methanol	Ethanol	Aqueous
1	*A. adscendens*	0.78±0.02	0.39±0.01	0.31±0.00
2	*A. officinalis*	0.61±0.03	0.53±0.01	0.49±0.02
3	*A. retrofractus*	1.48±0.04	0.84±0.02	0.69±0.02
4	*A. racemosus*	1.38±0.04	0.72±0.01	0.49±0.01
5	*A. sprengeri*	1.44±0.06	1.22±0.04	0.86±0.03
6	*A. densiflorus*	1.86±0.03	1.63±0.04	1.51±0.02
7	*A. falcatus*	0.89±0.02	0.97±0.02	0.77±0.03

Values are Mean ± S.E. of three replicates

DPPH Radical Scavenging Activity: As clearly seen in the results, the maximum quantity of phenols and flavonoids were recorded in the methanol extracts. So, they were further subjected to DPPH radical scavenging assay to test their antioxidant potential. The lowest radical scavenging activity among *Asparagus* species was recorded in *A. adscendens* (149.6 µg/ml), while highest in *A. densiflorus* (116.2 µg/ml). The other two species *i.e. A. officinalis* (128.3 µg/ml) and *A. falcatus* (127.7 µg/ml) have moderate antioxidant activity **Fig. 1**.

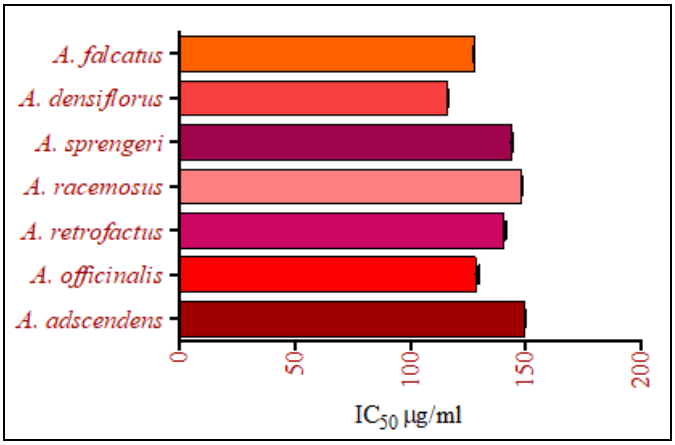

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACTS OF ROOTS OF SEVEN ASPARAGUS SPECIES (IC_{50} mg/ml)

DISCUSSION: Phenols, flavonoids (polyphenols) are the important secondary metabolites found in the plant kingdom. They are simple molecules (phenolic acids, phenylpropanoids, flavonoids) or can be found in highly polymerized form (lignins, tannins). They have been associated with various types of fields like biological, agricultural, and medical studies [15]. The consumption of these compounds in the human diet regularly can help to protect from various disorders generated by excess of free radicals and reactive oxygen species [16]. Phytochemical investigations of *Asparagus* have revealed the presence of phenolic compounds and flavonoids in high concentrations which can be of potential use for fighting against various diseases [17]. Extraction of phenols and flavonoids varied with the different solvent systems and methanol was found best for the yield in the present study. This is in confirmation with the previous reports which supports methanol as optimum extraction solvent [18]. Many workers (Rodriaguez *et al.*, 2005; Papoulias *et al.*, 2009 and Lee *et al.*, 2014) have studied the polyphenol content in different cultivars of *A. officinalis* and co-related the concentration of these with the antioxidant activities [19, 20, 21]. As the concentration increases, the antioxidant potential also amplified. In the present study we have seen that *A. falcatus* and *A. densiflorus* have highest quantity of phenols and flavonoids, respectively. *A. densiflorus* also found to have highest radical scavenging activity among the seven species studied. *A. falcatus* has almost same level of antioxidant activity as compared to *A. officinalis*.

Survey of literature also reveals that there is no report on the analysis of polyphenolic content in the two species of *Asparagus* (*A. densiflorus* and *A. falcatus*), while there were few reports of biological activities. Hewawasam *et al.*, 2008 reported anti-oxidative effect of tuberous extracts of *A. falcatus* on the acetaminophen toxicity in mice [22]. Ghalib *et al.*, 2012 isolated a novel caryophyllene type sesquiterpene lactone from the leaves of *A. falcatus* which shows anti-angiogenic activity on human umbilical vein endothelial cells [23]. El-Deeb *et al.*, 2016 reported molluscicidal activity of *A.*

densiflorus against *Biomphalaria alexandrina* snails [24]. Further research is needed in the other species of *Asparagus* to find out valuable bioactive compounds for pharmaceutical and nutraceutical industry.

CONCLUSION: It can be concluded from the current study that water is less efficient as compared to organic solvents- methanol and ethanol for extraction of phenolic compounds from roots of *Asparagus*. Highest concentrations of phenols and flavonoids in the extracts were obtained with solvent of high polarity *i.e.*, methanol. This may be due to more solubility of bioactive compounds of *Asparagus* with methanol during the extraction process as compared to other solvents. It was found that *A. falcatus* and *A. densiflorus* had highest quantity of phenols and flavonoids, respectively. *A. densiflorus* also found to have highest antioxidant potential suggesting that with increase in concentration of phenols and flavonoids, antioxidant potential also increases. *Asparagus* species have good amount of phenols and flavonoids. They are natural sources of antioxidant substances of high significance and are of great value for use in pharmacy and phytotherapy.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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