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PHYTOCHEMICAL SCREENING AND PHARMACOGNOSTICAL STUDY ON FOUR ASPARAGUS SPECIES (ASPARAGACEAE) OF NEPAL

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ABSTRACT

The present study is aimed at the development and evaluation of pharmacognostical and physicochemical characteristics of four medicinally valued Asparagus species (Asparagaceae) found in Nepal. Of the 300 species of Asparagus found worldwide, Nepal harbors 7 species, viz. *Asparagus lycopodineas* (Baker) F.T. Wang & Tang, *Asparagus adscendens* Roxb., *Asparagus curillus* Buch-Ham. ex Roxb., *Asparagus filicinus* Buch.-Ham. ex D. Don (var. *brevipes* Baker, and var. *filicinus*), *Asparagus penicillatus* H. Hara (Endemic species from Dolpa), *Asparagus racemosus* Willd. (var. *racemosus*, and var. *subacerosus* Baker), and *Asparagus tibeticus* F.T. Wang & C. Chen. One of the species, *Asparagus racemosus* commonly known as Wild asparagus (Shatavari) is an important medicinal plant used against various disorders in indigenous and Ayurveda system of medicine. Besides this *Asparagus curillus, A. adscendens* and *A. penicillatus* are also used as substitutes for *A. racemosus*. There are very few reports on microscopic evaluation, standardization parameters and chemo profile of other species of Nepal rather than *Asparagus racemosus*, to check the identity and purity of the drug. The present work exemplifies the studies carried out for quality control of drugs as per WHO guidelines which includes macro- and microscopic features, physicochemical parameters like loss on drying, foreign matter, extractive value, ash value and preliminary phytochemical screening for the quality assurance of the drug.

Key words: Medicinal plants, Physicochemical parameters, Microscopic evaluation, Quality assurance.

INTRODUCTION

Of the 300 species of Asparagus found worldwide, Nepal harbors 7 species so far viz. *Asparagus lycopodineas* (Baker, and var. filicinus), F.T. Wang & Tang, *Asparagus adscendens* Roxb., *Asparagus curillus* Buch.-Ham. ex Roxb. *Asparagus filicinus* Buch.-Ham. ex D. Don (var. *brevipes* Baker, and var. *filicinus*), *Asparagus penicillatus* H. Hara (Endemic species from Dolpa), *Asparagus racemosus* Willd. (var. *racemosus*, and var. *subacerosus* Baker), and *Asparagus tibeticus* F.T. Wang & C. Chen (New record from Mustang), in the wild. *Asparagus racemosus* (Kurilo, Shatavari) is one of the top ten most traded high value MAP species having therapeutical and nutraceutical importance (Acharya, 2005; Tiwari, 2004). It is also one of the prioritized species for conservation viz. Vulnerable, Cultivation Priority (Bhattarai NK *et al*, 2002; IUCN, 2004; GoN, 2006). Other species *Asparagus curillus, Asparagus adscendens* are also reported to have medicinal value and used as substitutes or adulterant for Wild asparagus (*Asparagus racemosus*). According to WHO (World Health Organization) the macroscopic and microscopic descriptions, phytochemical, biochemical and physiochemical characteristics are some steps towards establishing the identity and degree of purity for plant material (WHO, 1998).

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Pharmacognostic characteristics are studied so far for *Asparagus racemosus* Willd. *(*Mandal SC *et al.*, 1996; Battu & Kumar, 2010; Alok S *et al.*, 2013). Nepalese Satavar (Pili Satavar) is considered as best quality drug in the Indian market in relation to its efficacy. But its pharmacognostic study and phytochemical screening of different traits from different ecological habitats is still lacking. While considerable information on *A. officinalis* and *A. racemosus* are available from Nepal, the same are lacking for other species of the genus. In fact, some of the *Asparagus* species have been found adulterated or substituted in raw material trade but their merit or harmfulness in terms of their chemical constituents have not so far been investigated. So it is very urgent need to characterize the species to prevent frequent adulteration of Asparagus raw materials. Thus, this study was to explore the drug potentialities of other species of Asparagus and to provide referential information for the correct identification of the crude drug on the basis of these standardization parameters.

MATERIAL AND METHODS

In the present study, four species of Asparagus L. viz. *Asparagus racemosus*, *Asparagus curillus*, *Asparagus lycopodineus* and *Asparagus penicillatus* were selected on the basis of availability and accessibility to compare there pharmacognostic and physico- chemical characteristics so that they can be differentiated from each other even in their drug form to avoid adulteration and maintain purity of the drugs. These four species were collected from Makwanpur, Rasuwa, Ilam and Rukum districts respectively. The voucher specimens were identified from KATH, a National Herbarium of Nepal and deposited there. Fresh tuberous roots from all these species were collected, washed and dried and were powdered for further physico-chemical and phytochemical analyses.

The macroscopic characters of roots were recorded and photographed using digital camera (DSC W220, Sony Corp, Japan). Size measurements were made using scale and the microscopic observations were recorded under stereoscopic photo-microscope (Amscope software) using a micrometer. For anatomical study of each species, transverse section of fresh root sample was made using sliding microtome (Radical RMT-45) and free hand sections whatever suitable and stained with safranin 5 % and fast green. Cell types were recorded under a compound microscope. Root powder of each species were also examined microscopically and photographed under different magnifications. Photographs were taken by Amscope microscope eyepiece camera (MD35).

Physico-chemical parameters such as percentage of total ash, acid-insoluble ash, water soluble ash, extractive values of water and alcohol were calculated as per the methods of Indian Pharmacopoeia (Anonymous 1996).

For the preliminary phytochemical analysis, 15gm powdered drug was extracted in soxhlet apparatus with petroleum ether, ethanol and water successively. The extracts were dried and weighed. The presence or absence of different phyto-constituents viz., triterpenoids, alkaloids, steroids, sugar, tannin, glycosides and flavonoids, etc. were detected by usual prescribed methods (Ciulei I, 1985).

RESULTS AND DISCUSSION

Species selection

Among 7 species reported in Nepal, four species namely *A. penicillatus, A. curillus, A. lycopodineus* and *A. racemosus* were selected for present study. Their distribution, altitudinal range of occurrence and ecological habitat are presented in Table 1, Figure 1.

Morphological analysis

Macro-morphological features of four species are described in Table 2. Among the four species, *A. racemosus* and *A. curillus* are subshrub with basal portion of stem woody and distal portion herbaceous. Other two species, *A. lycopodineus* and *A. penicillatus* are herbaceous with not more than 1m plant height. Root tubers are long and slender in *A. curillus*, while in *A. lycopodineus* swollen parts are not more than 5 cm and are blackish brown in color (Figure 2).

Microscopic analysis (Figure 3)

Anatomical characters of roots

Transverse section of root of *Asparagus* reveals outermost single layered piliferous layer followed by few layered thick walled hexagonal parenchymatous exodermis. Below exodermis there lies spherical parenchymatous cortex. Endodermis is single barrel shaped layered cells which is followed by single to few layered pericycle. Vascular bundle is as in all monocot, a radial type. Central core is filled with thin walled spherical parenchymatous pith. Besides this, root anatomical parameters in different species of *Asparagus* showed some specific structural modifications.

In *A. racemosus* also piliferous layer is devoid of hairs. It is followed by 6-8 rows of thick walled hexagonal parenchymatous cells of exodermis. Here also in the cortical zone few layers below the exodermis, 2-3 layers of distinct stone cells are present which are not present in other 3 species. Few cortical cells also possess raphids of crystals. Above endodermis there lie 2-3 layers of thick walled pitted parenchyma cells. Pericycle is single layered. The number of radial vascular bundle ranges from 35 to 45 and the diameter of xylem vessel is 17.52-65.53µm (Figure 1 a, b).

In *A. currilus*, piliferous layer is devoid of hairs followed by 8-10 rows of thick walled hexagonal

parenchymatous cells of exodermis. In the cortical zone, scattered and isolated or groups of stone cells were found. Above endodermis 2-3 layers of thick walled stone cells are found which are not present in other 3 species. Pericycle is single layered. The number of radial vascular bundle ranges from 38 to 40 and the diameter of xylem vessel is 14.54-118.45µm. Metaxylem vessels are more prominent in this species (Figure 1c, d).

In *A. lycopodineus*, piliferous layer consist of numerous unicellular root hairs. Exodermis is composed of 5-6 layered hexagonal parenchymatous cells. Cortex is differentiated into outer and inner cortex. Outer cortex consists of thick walled spherical parenchyma with few scattered isolated stone cells and abundant raphids of crystals embedded. Inner cortex consists of thin walled spherical and large parenchymatous cells. Pericycle is single layered with large cells. Vascular region possess minimum xylem and phloem area as compared to rest three species of *Asparagus*. The number of vascular bundle ranges from 30-35 and the diameter of xylem vessel is 3.9-12.17 µm. The xylem vessels are surrounded by thick walled sclerenchymatous cells (Figure 1 e, f).

In *A. penicillatus* piliferous layer is devoid of root hairs. Outer cortex consists of thick walled spherical parenchyma with few scattered isolated stone cells. Inner cortex consists of thin walled hexagonal large parenchymatous cells. Pericycle is double layered. Vascular region possess minimum xylem and phloem area as compared to rest three species of *Asparagus*. The number of vascular bundle ranges from 30-35 and the diameter of xylem vessel is 70.32µm in average. The xylem vessels are surrounded by thick walled sclerenchymatous cells (Figure 1 e, f). These microscopic features can be used as a rapid inexpensive botanical identification technique and is useful in standardization (Kaikade & Ingole, 2015; Nawaz *et al*., 2012; Raycheva and Stojanov, 2013). In addition, structural modification in different species is due to environmental impacts. More stone cells in *A. currilus* and *A. racemosus* than in other two species may be due to their growth in steep slopes where water retaining power of soil is comparatively low.

Powder analysis of roots

Root powder of Asparagus species are creamy yellow in color, except for *A. lycopodineus* which is blackish brown (Fig.4). Acicular raphides, pitted vessel elements with scalariform thickenings, stone cells are major elements found in powder analysis of all species. Similar features are found (Rajbhandary TK *et al.,* 1995 and Gupta *et al.,* 2003).

Physico chemical analysis

The physico-chemical parameters like moisture content, ash values and extractive values (both water soluble and alcohol soluble extractive value), were determined which are given in Table 3&4. These findings can be used to help in maintaining pharmacopoeial standards for the drug.

Phytochemical analysis

The phytochemical screening of root tubers of selected study species was done by using the different extracts that was extracted through successive solvent extraction with petroleum ether, ethanol and water. The yield of water extract (maximum 80.74% in *A. lycopodineus*) was more than that of alcohol extract (minimum 7.04 % in *A. penicillatus*). Both are in the range of standard value as given by Department of Plant Resources and By Indian Pharmacopoeia. Phytochemical study shows more or less similar phytoconstituents such as volatile oil, Steroidal glycoside, saponins, reducing compounds, tannins and coumarins. It was observed that maximum number of constituents were present in alcohol and aqueous extract (Table 5). In earlier studies also *Asparagus racemosus* showed the presence of steroids/ triterpenoids, alkaloids, flavonoids, carbohydrates and tannins (Nagamani T *et al.,* 2012; Janani & Singaravadivel 2014), while *A. curillus* showed presence of *Oligofurostanosides* (curillins G and H) and spirostanosides (curilloside G and H) (Negi T *et al*., 2010).

Table 1. Distribution, habitat and ecology of *Asparagus* species

S.No	Taxon Name	Distribution in Nepal	Altitude Range (M)	Ecology
1	*A. curillus*	West to Central region	700-2850	In forest, marginal land, plain, steep slope SW face
2	*A. lycopodineus*	Central to Eastern region	1530-2100	In mixed broadleaved forest
3	*A. penicillatus*	West	1500-1900	In forest, dry stony slope
4	*A. racemosus*	West to East	140-2100	In forest, plain or slopy area NE face, humous rich soil

Table 2. Morphological features of four study species

Species Name	Habit	Plant height (m)	Root tuber		Cladodes		Spiness size (cm)	Flower	
			length (cm)	Color	No	Size (mm)		Color	Size (mm)
A. curillus	Sub-shrub	1-3.5	8-60	Cream	3- 8	0.3-0.6	0.5-2.5	white	3.0-4.0
A. lycopodineus	Herb	0.4- 1.0	1.5-5	brownish black	1-5	0.3-0.5	0	White	3.0-4.0
A. penicillatus	Herb	0.3-0.9	5-15	light yellow	1-4	0.3-0.5	0.3-0.7	Lilac white	4.0
A. racemosus	Shrub	1.5-5	6-14	Yellow	3-11	0.3-3	0.5-1.5	White	3.0-5.0

Table 3. Physico-chemical properties of four targeted species

SN	Name of Species	Total Ash (%) (Wet basis)	Moisture (%)	Water Soluble Ash (%)	Acid Insoluble Ash (%)
1	Asparagus curillus (Rasuwa)	3.52	10.17	1.24	0.66
2	Asparagus lycopodineus (Ilam)	2.87	6.87	1.51	0.45
3	Asparagus penicillatus (Rukum)	1.5	7.67	1.5	0.073
4	Asparagus racemosus (Makwanpur)	5.88	6.01	2.34	0.96
	Standard value (Gupta *et al*. 2003)	<6	11	NA	<1

Table 4. Extract Values of four targeted species compared with Indian Standard value

Species names	Solvent	% of extractive values	Standard value (DPR)	Standard Value (Indian Pharmacopoea)
A. curillus	**Ethanol**	-	**>15**	**>9**
A. lycopodineus	**Ethanol**	-		
A. racemosus	**Ethanol**	19.48		
A. penicillatus	**Ethanol**	7.04		
A. curillus	**Water**	62.10	**>34**	**>34**
A. lycopodineus	**Water**	80.74		
A. racemosus	**Water**	53.32		
A. penicillatus	**Water**	48.74		

Table 5. Phytochemical screening of study species

Extract type	***Asparagus racemosus***	***Asparagus lycopodineus***	***Asparagus curillus***	***Asparagus penicillatus***
Petroleum Ether Extract	Volatile oil	Volatile oil, steroid, fatty acids	Steroids, fatty acids	Steroids, fatty acids
Alcoholic Extract	Coumarin, flavonoid, Catecholic tannin, reducing compound	Catecholic tannin, reducing compound	Catecholic tannin, reducing compound	Coumarin, Steroid, reducing compound
Aqueous Extract	Polyuronoid, reducing compound, plyoses, Saponin, Gallic tannin, catecholic tannin	Polyuronoid, reducing compound, plyoses, Saponin, Gallic tannin, catecholic tannin	Polyuronoid, reducing compound, plyoses, Saponin, Gallic tannin, catecholic tannin	Polyuronoid, reducing compound, plyoses, Saponin,

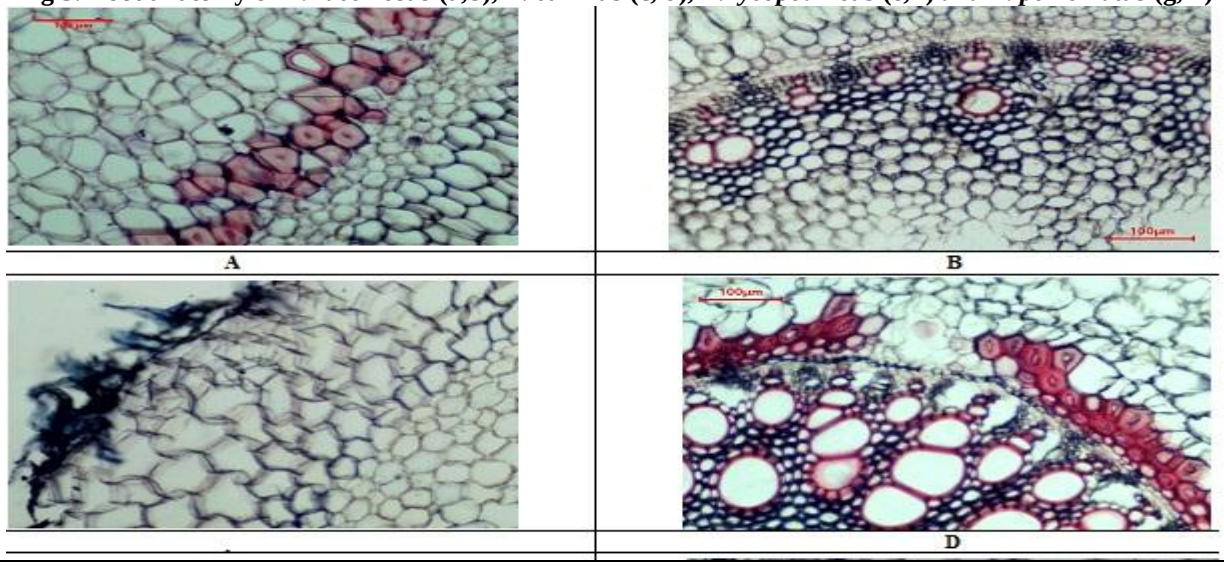
Fig 1. Habit of four studied species of Asparagus

Asparagus racemosus *Asparagus curillus* *Asparagus lycopodineus* *Asparagus penecillatus*

Fig 2. Roots of Asparagus species

Asparagus racemosus *Asparagus curillus* *Asparagus lycopodineus* *Asparagus penicillatus*

Fig 3. Root anatomy of *A. racemosus* (a,b), *A. currilus* (c, d), *A. lycopodineus* (e, f) *and A. penicillatus* (g, h)



A B D

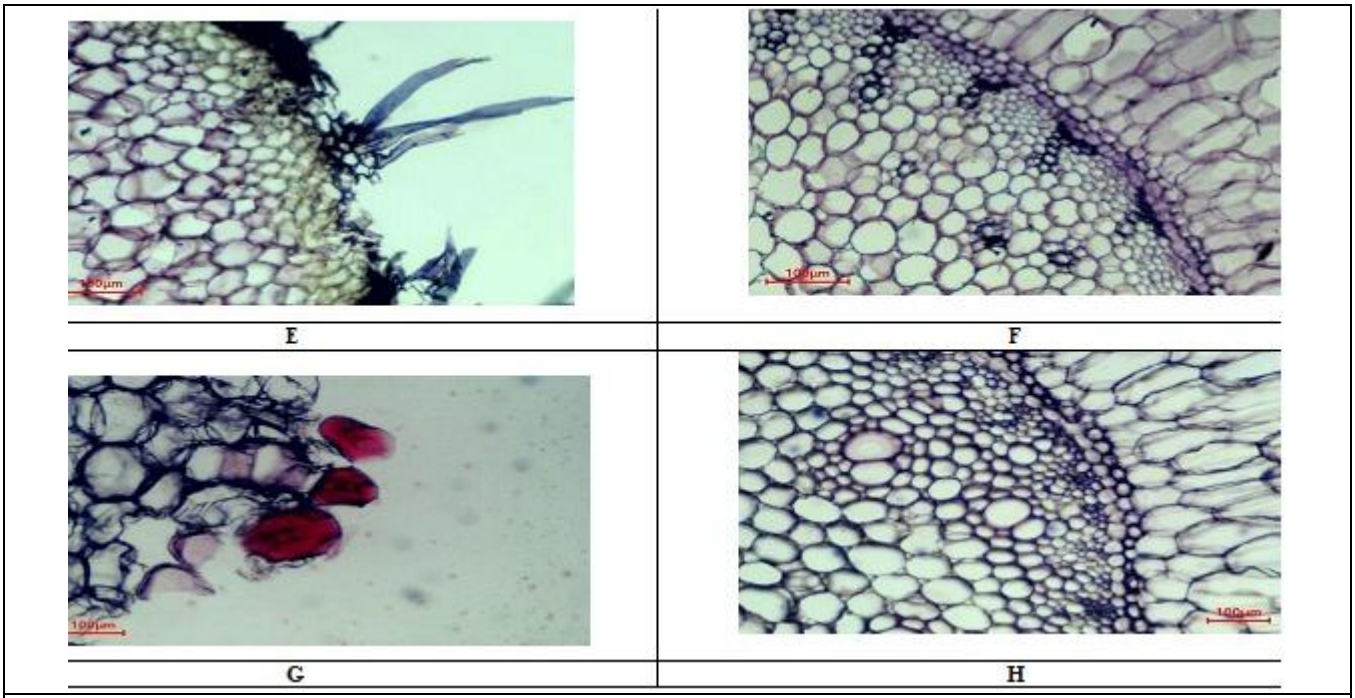
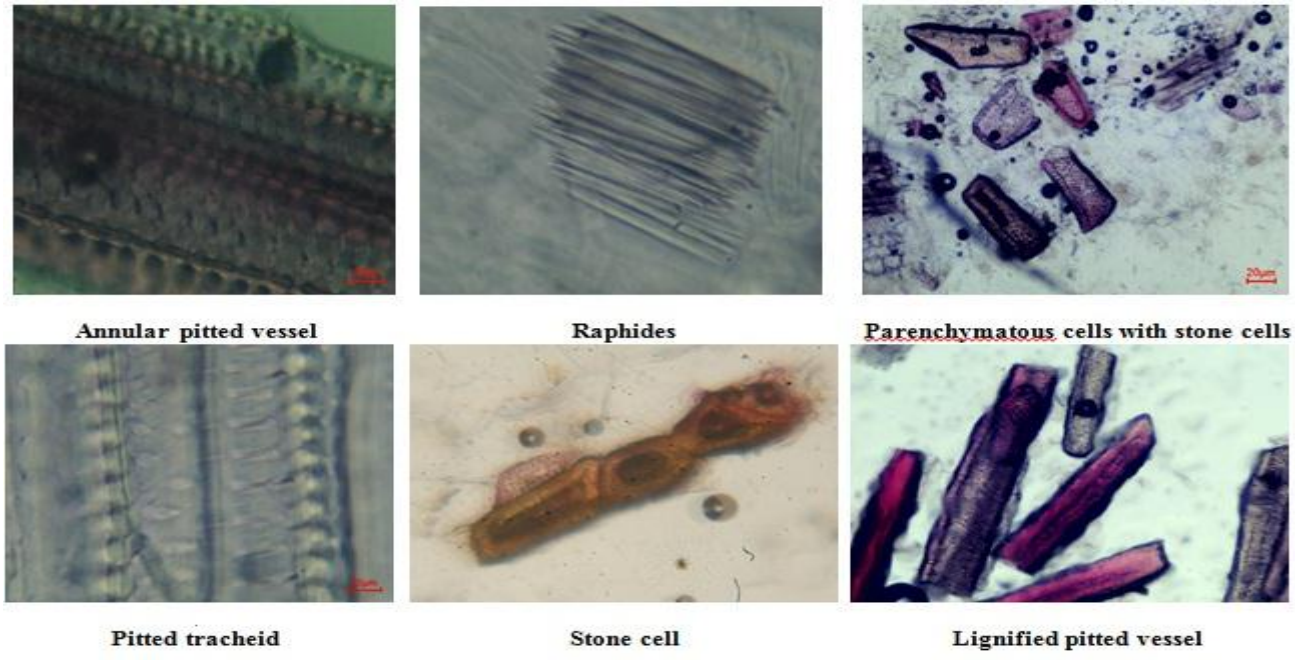


Fig 4. Microscopic features of Root powder Asparagus



CONCLUSION

Morphological variation of roots was found significant while microscopic features are more or less similar among the 3 species studied. Only slight structural variation detected in anatomical characters. Phytochemical constituents are also found similar. Steroidal saponins, coumarin, flavonoids, are major compounds detected. Physicochemical parameters and extractive values are in the range of standard value coated by Indian Pharmacopeia (Anonymous 1996). However, the results are found to be significant in proper identification of different Asparagus species. *Asparagus racemosus* is a rich source of steroidal saponin, and also contains alkaloids, proteins, starch, tannin, mucilage and diosgenin (Parveen AK *et al*., 2007). Nepalese Satavar (Pili Satavar) is considered as best quality drug in the Indian market in relation to its efficacy. Roots of *A. curillus* is generally used as substitutes/adulterants for *A.*

racemosus. *Asparagus lycopodineaus* is insignificant commercially as well as productivity point of view. *Asparagus penicillatus* is endemic to Nepal, and it is also found to be used as substitute for *A. racemosus* and gaining popularity for its vigorous root tubers. Further detail study of this species is needed to exploit its potentialities.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

Alok S, Jain SK, Verma A, Kumar M, Mahor A and Sabharwal M. Plant profile, phytochemistry and pharmacology of Asparagus racemosus (Shatavari): A review. *Asian Pacific Journal of Tropical Disease*, 3(3), 2013, 242–251.

Anonymous. Indian Pharmacopoeia. Government of India, Ministry of Health, New Delhi, India, 1996, 140-191.

Battu G and Kumar B. Phytochemical and Antimicrobial Activity of Leaf Extract of Asparagus racemosus Willd. *Pharmacognosy Journal*, *2*(12), 2010, 456–463.

Bhattarai NK, Tandon V and Ved DK. Highlights and Outcomes of the Conservation Assessment and Management Plan (CAMP) Workshop. In: Bhattarai NK and Karki M. Sharing Local and National Experience in Conservation of Medicinal and Aromatic Plants in South Asia; Proceedings of the Regional Workshop at Pokhara, Nepal. IDRC/MAPPA, New Delhi, India and Ministry of Forests and Soil Conservation, Kathmandu, Nepal, 2002.

Ciulei I. Practical Manuals on the industrial Utilization of Chemical and Aromatic Plants. Methodology for analysis of Vegetable Drugs. 1st ed., Ministry of Chemical Industry, Bucharest, 1982, 67.

Go N. *Nepalko Aarthik Bikaskalagi Prathamikata Prapta Jadibutiharu (Nepali*). Department of Plant Resources, Ministry of Forest and Soil Conservation, Kathmandu, Nepal, 2006.

Gupta AK, Tandon N and Sharma M, Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, India, 2003.

IUCN Nepal. National Register of Medicinal and Aromatic Plants. The World Conservation Union Nepal, 2004, 202.

Janani SR and Singaravadivel S. Screening of Phytochemical & GC-MS Analysis of some Bioactive Constituents of *Asparagus racemosus* Willd. *IJPRIF*, 6 (2), 2015, 428-432.

Mandal SC, Mukherjee PK, Nandy A, Pal M & Saha BP. Some pharmacognostical characteristics of *Asparagus racemosus* willd , roots. *Ancient Science of Life*, 15(4), 1996, 282–5. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3331220&tool=pmcentrez&rendertype=abstract

Nagmani, Suresh J, Ahuja J & Reddy V. Comparative phytochemical screening of Vatashunga, Shatavari and Shatapushpa claimed for Prajasthapana activity. *Annals of Biological Research*, 3(3), 2012, 1294-1304.

Nawaz T, Hameed M, U-Nisa W, Ahmad MSA, Younis A and Kanwal H. Comparative Anatomy of Root and Stem of Some Native and Exotic Asparagus L. Species. *Pak. J. Bot.*, 44, 2012, 153-158.

Negi JS, Sing P, Joshi GP, Rawat MS &Bisht VK. Chemical constituents of Asparagus. *Phcog. Rev*., 4(8), 2010, 215-220.

Parveen AK, Ginwal HS Barthwal S and Brajeshwar**.** Studies on Genetic Variability in *Asparagus racemosus* Willd. for Different Traits of Economic Value. *Dehradoon*, 7(2), 2007, 45.

Rajbhandary TK, Joshi NR, Shrestha T, Joshi SKG and Acharya B (eds.). Medicinal Plants of Nepal for Ayurvedic Drugs, Department of Plant Resources, Kathmandu, Nepal, 1995, 387.

Raycheva T and Stojanov K. Comparative anatomical study of five species of genus *Asparagus* in Bulgaria. *Trakia Journal of Sciences*, 2, 2013, 104-109.

Tiwari NN, Poudel RC and Uprety Y, Study on Domestic Market of Medicinal and Aromatic Plants (MAPs) in Kathmandu Valley, Winrock International, BDS/MAPs, Bakhundole, Lalitpur, Nepal, 2004.

Tuszyńska M. Pharmacological and therapeutic application of Asparagus racemosus Willd. *Herba Polonica*, 56(2), 2010, 92-103.

WHO. Macroscopic and Microscopic Examination, Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, 1998.