International Journal of Multidisciplinary and Current Research ISSN: 2321-3124
Research Article Available at: http://ijmcr.com

Phytochemical analysis of *Asparagus racemosus* germplasm to check presence of secondary metabolites & minerals

Mahesh Kumar[1] and Sarla Rani[2]*

[1]Depoartment of Biotechnology, Institute of Integrated & Honors Studies, Kurukshetra University Kurukshetra (Haryana)-136119
[2]Depoartment of Biotechnology, Pt.CLS Govt PG College, Karnal (Haryana)-132001

Received 15 May 2021, Accepted 12 June 2021, Available online 15 June 2021, **Vol.9 (May/June 2021 issue)**

Abstract

Asparagus racemosus (Shatavari) is an important medicinal plant in India. It has been used in various medicinal formulations because of its medicinal value which in turn is because of presence of saponins (major bioactive compound present in roots). A. racemosus are suggested in nervous disorders, dyspepsia, diarrhoea, dysentry, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases. Saponins are present in all species of Asparagus, but variations are found, in different species from different parts. Sitosterol, stigmasterol and their glucosides, sarsasapogenin and their glucosides, sarsasapogenin and two spirostanolic and two furostanolic saponins are also reported in various studies. The Asparagus germplasm in the present study was screened for the presence of various secondary metabolites like terpenoids, saponins, flavonoids, tannins, cardiac glycosides, phlobatannins and reducing sugars. This study also revealed the metal ion concentrations of Ca (17.92-37.22 mg/100 g), Mg (67.92-293.4 mg/100 g), Fe (19.92-105.72 mg/100 g), Cu (0.08-0.56 mg/100 g) and Zn (2.18-16.86 mg/100 g), Co (0.14-6.66mg/100 g), Mn (18.28-49.36 mg/100 g) in A. racemosus.

Keywords: *Asparagus racemosus, secondary metabolites, metal ion concentrations*

Introduction

About 300 species of *Asparagus* are known to occur in the world. The genus *Asparagus* has been recently moved from the subfamily *Asparagae* in the family *Liliaceae* to a newly created family *Asparagaceae*. Out of several species of '*Asparagus*' grown in India, *A. racemosus*, *A. gonaclades* and *A. adscendens* are most commonly used in indigenous medicine. *Asparagus racemosus* grows all over India in tropical areas and sub-tropical parts of India including the Andamans and ascending in the Himalayas up to an altitude of 1500 m. The common name of *Asparagus racemosus* is Shatavari. *Asparagus racemosus* has been used in India for thousands of years for its therapeutic and tonic properties. *A. racemosus* is commonly mentioned as a rasayana in the Ayurveda. Rasayanas are those plant drugs which promote general well being of an individual by increasing cellular vitality or resistance.

Root of *A. racemosus* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactogogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic (Hasan *et al.*, 2016) and as tonic.

*Corresponding author's email: sarlars@rediffmail.com
Phone: 0091(9466755369)
DOI: https://doi.org/10.14741/ijmcr.v.9.3.9

Beneficial effects of the root of *A. recemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentry, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy (Sharma *et al*., 2000) cough, bronchitis, hyperacidity and certain infectious diseases. The roots and leaves are used for medicinal purpose. The root extract of *A. racemosus* is prescribed in Ayurveda to increase milk secretion during lactation. The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant (Oketch-Rabah *et. al.*,1998). Saponins are present in all species of *Asparagus*, but variations are found, in different species from different parts. Sitosterol, stigmasterol and their glucosides, sarsasapogenin and their glucosides, sarsasapogenin and two spirostanolic and two furostanolic saponins isolated from fruits and chemically studied Chem. Four glycosides – compound a, shatavarin I, II, and IV – isolated from roots, structure of shatavarin IV elucidated (Sanafi, 2015).

The secondary metabolites present in the plant makes the plant to be used for its medicinal utility. As these secondary metabolites constitutes the bioactive components so their screening and estimation becomes essential for future uses. The present study was carried out to estimate the concentration of important minerals

and to screen for the presence of secondary metabolites in the *Asparagus racemosus* germplasm

Material and Methods

Plant material: The leaf part of the plants was being used for the present study.The plant samples were collected from Herbal garden, Yamunanagar and Herbal garden Kurukshetra. The leaves were dried at 50°C for 48 hrs. Dry powder was prepared to carry out different screening and estimation experiments.

Preparation of standard solution for atomic absorption spectroscopy: The working solution of 1ppm to 9ppm was prepared from the stock of different standard mineral solutions for the standardisation of the AAS.

Minerals composition: Digestion mixture is prepared by mixing four volumes of nitric acid with one volume of perchloric acid. Five hundred mg of dried and well ground material was taken in a 250 ml conical flask to which 15 ml of digestion mixture was added .The flasks were heated gently over a hot plate till the whole material digested and solution became colourless. The digest thus obtained cooled, filtered and diluted to 100 ml with distilled water.

Biochemical analysis and Phytochemical screening test:- Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993) and Harborne (1973).

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: Two methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Reducing sugar: The crude extract of each plant was shaken with 5 ml of distilled water and filtered. The filtrate was boiled with drops Fehling's solution A and B for 2 minutes. An orange red precipitate indicates the presence of reducing sugar.

Total Phenols: 0.5 g of dried and well ground sample was taken in 50 ml conical flask and 10 ml of methanol was added. The flasks were kept overnight with intermittant shaking. The contents were filtered through whatman filter paper No.42. The residue was washed with methanol three times and volume was made upto 50 ml with methanol. One ml of methanolic extract was taken in 50 ml volumetric flask to which 30 to 40 ml of distilled water was added followed by addition of 5 ml of saturated Na_2CO_3 solution and 2.5 ml of 1N Folin reagent. The mixture was shaken throughly and volume was made to 50 ml and kept in dark for about half an hour. The absorbance was read at 725 nm against reagent blank. A standard curve was prepared with graded concentration of tannic acid. The results were expressed as g/100 g dry weight after taking in to account dilution involved.

Non-structural carbohydrates

Total soluble sugars, sucrose, total fructose, and reducing sugars were extracted by taking 100 mg of representative

dried sample which was further transferred to glass test tube and added 50 ml glass distilled water. Care was taken to ensure that dry matter was thoroughly wetted without allowing it to spread up the sides of the tube. Each tube was plugged with cotton and autoclaved at 15 Ib (121^{o}C) for 30 min. Extracts were quantitatively transferred to 50 ml volumetric flasks and made upto volume with distilled water. Aliquots of these solutions were used for analysis.

Total soluble sugars

Total soluble sugars were estimated according to the method of Yemm and Willis (1954). Five ml of 0.2 per cent anthrone reagent was pipetted in test tubes and chilled in ice cold water. The solution under test was layered on acidic reagent, cooled for 3-5 min and then thoroughly mixed, while still immersed in ice cold water. The test tubes containing reaction mixture were heated vigorously in boiling water bath for 10 min. and cooled rapidly. The intensity of green colour was measured at 625 nm. A standard curve was prepared with graded concentration of glucose. The contents of total sugar were calculated from standard curve and the results were expressed as g /100 g^{-1} of dry weight.

Total fructose

Total fructose was estimated by the method of Walte and Boyd (1953). Suitable aliquot of extract was mixed with 1 ml of resorcinol reagent. After adding 5 ml of 80 percent HCl, the contents were heated for 10 min at 80^{o}C in a temperature controlled water bath. The test tubes were cooled rapidly and the intensity of red colour was measured at 505 nm. A standard curve was prepared with graded concentration of fructose. The results were expressed as mg 100 g^{-1} dry weight in comparison to total standard curve.

Reducing sugars

Reducing sugars were obtained by followed the method of Somogyi (1952). The solution under test was transferred to a dry test tube. After adding one ml of copper reagent, the contents of the test tube were heated in a boiling water bath for 20 min. One ml of arsenomolybdate solution was added after cooling and the total volume was made to 10 ml. The intensity of blue colour was measured at 520 nm. A standard curve was prepared with graded concentration of glucose. The results were expressed as g 100 g^{-1} of dry weight in comparison to a standard curve.

Results and discussion

The results of phytochemical analysis of *Asparagus racemosus* germplasm investigated are summarized in Table. The results show that the plants are rich in chemical bases such as alkaloids, flavonoids, saponins and tannins. The presence of these bases in the investigated plants was accounts for their usefulness as medicinal plants. Alkaloids are known to play some metabolic role and control development in living system. They also have a protective role in animals (Edeoga and Eriata, 2001). They are used in medicine especially the steroidal alkaloids. Saponins as sugar derivatives may be steroidal or triterpenoids. The occurrence of steroidal saponins from various studies indicate their importance and interest in pharmacy due to their relationship with such compounds such as sex hormones especially in development of the female contraceptive pill. This may be the reason why the infusion of the root extract of *A. racemosus* are given to expectant mothers and breast feeding mothers to ensure their hormonal balance since steroidal structures could serve as potent starting material in the synthesis of these hormones.

Saponin is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effect. This study revealed that total phenolic compounds concentration varies from 7.34-25.5g/100g. The presence of the phenolic compounds in these studied samples proves that they have anti-microbial and antifungal effect. Phenols and phenolic compounds had been used in disinfections and remain the standard with which other bactericides are compared. The oils there have therapeutic, anti-septic or bactericidal properties. It is believed they prevent several forms of infection. Tannins are fairly frequently encountered in food products of plant vegetable origin such as tea and many fruits.

The mineral elements contained in the plants are very important in human nutrition. This study revealed that the metal ion concentrations of Ca (17.92-37.22 mg/100 g), Mg (67.92-293.4 mg/100 g), Fe (19.92-105.72 mg/100 g), Cu (0.08-0.56 mg/100 g) and Zn (2.18-16.86 mg/100 g), Co(0.14-6.66mg/100 g), Mn (18.28-49.36 mg/100 g) (Fig. 1 to 10). Calcium, potassium, magnesium, nitrogen in the plant samples are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms. It is known that certain metal ions are essential for normal biochemical functioning and development of organs (Pier and Bang, 1980; Martins 2002). Calcium has been reported to be effective in building of skeletal structures and muscle functioning while magnesium is important in the ionic balance and enzyme co-factors.Their absence in diet might result in weak, stunted growth and poor bone development. Iron is a component of haemoglobin and is essential for vitamin B synthesis. The presence of zinc in organism also helps in the synthesis of tryptophan, an essential amino acid. Copper is the main metal component of the respiratory pigments. It has also been observed that when dietary intake of copper is inadequate in vertebrates, it leads to serious deficiency diseases such as anemia and graying of hair.

Table:- Phytochemical analysis of *Asparagus racemosus* germplasm

S. No.	Plant Name	Terpenoids	Saponins	Flavonoids	Cardiac glycosides	Tannins	Phlobaanins	Reducing sugar
1	AR1	-ve	+ve	-ve	+ve	+ve	+ve	+ve
2	AR2	-ve	+ve	-ve	+ve	+ve	+ve	+ve
3	AR3	-ve	+ve	-ve	+ve	+ve	+ve	+ve
4	AR4	-ve	+ve	-ve	+ve	+ve	+ve	+ve
5	AR5	-ve	+ve	-ve	+ve	+ve	+ve	+ve
6	AR6	-ve	+ve	-ve	+ve	+ve	+ve	+ve
7	AR7	-ve	+ve	-ve	+ve	+ve	+ve	+ve
8	AR8	-ve	+ve	-ve	+ve	+ve	+ve	+ve
9	AR9	-ve	+ve	-ve	+ve	+ve	+ve	+ve
10	AR10	-ve	+ve	-ve	+ve	+ve	+ve	+ve
11	AR11	-ve	+ve	-ve	+ve	+ve	+ve	+ve
12	AR12	-ve	+ve	-ve	+ve	+ve	+ve	+ve
13	AR13	-ve	+ve	-ve	+ve	+ve	+ve	+ve
14	AR14	-ve	+ve	-ve	+ve	+ve	+ve	+ve
15	AR15	-ve	+ve	-ve	+ve	+ve	+ve	+ve
16	AR16	-ve	+ve	-ve	+ve	+ve	+ve	+ve
17	AR17	-ve	+ve	-ve	+ve	+ve	+ve	+ve
18	AR18	-ve	+ve	-ve	+ve	+ve	+ve	+ve
19	AR19	-ve	+ve	-ve	+ve	+ve	+ve	+ve
20	AR20	-ve	+ve	-ve	+ve	+ve	+ve	+ve

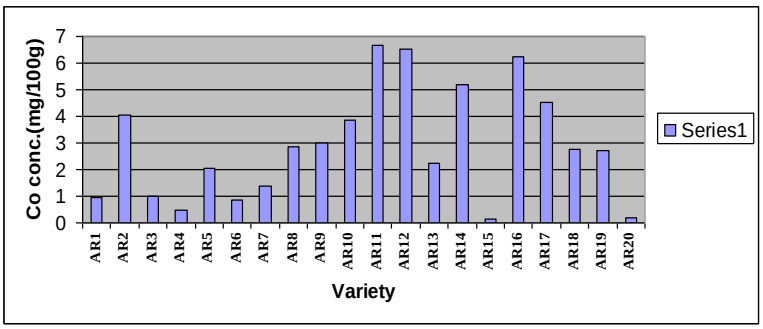

Fig. 1

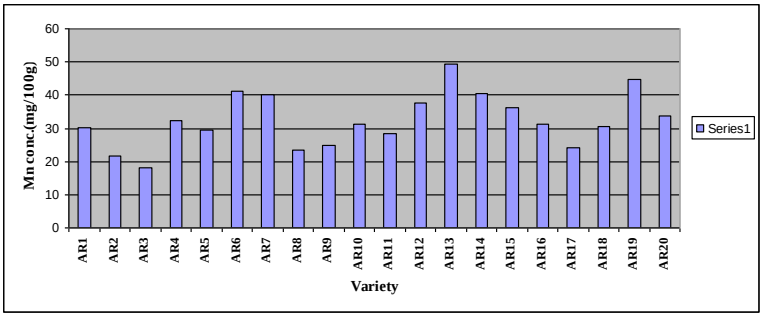

Fig. 2

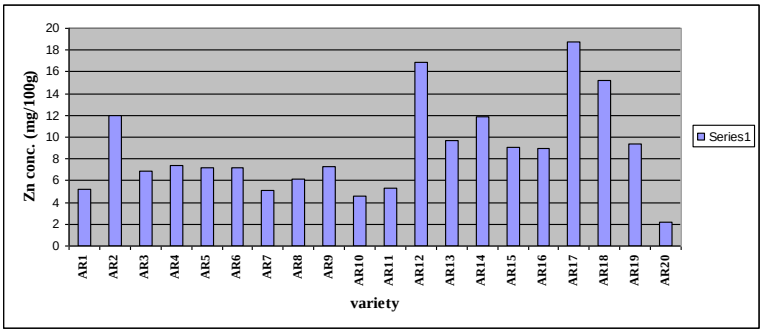

Fig. 3

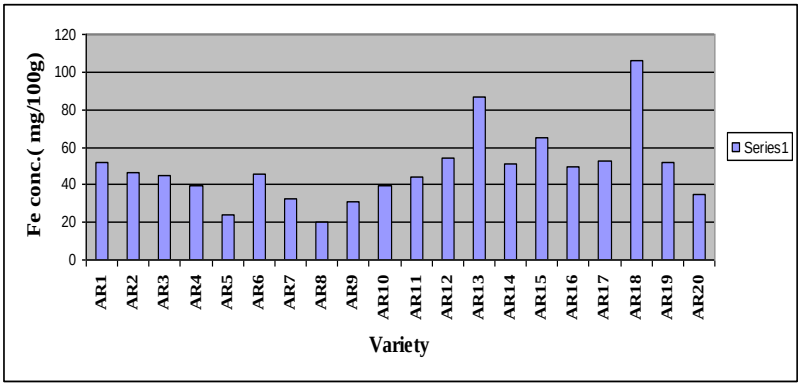

Fig. 4

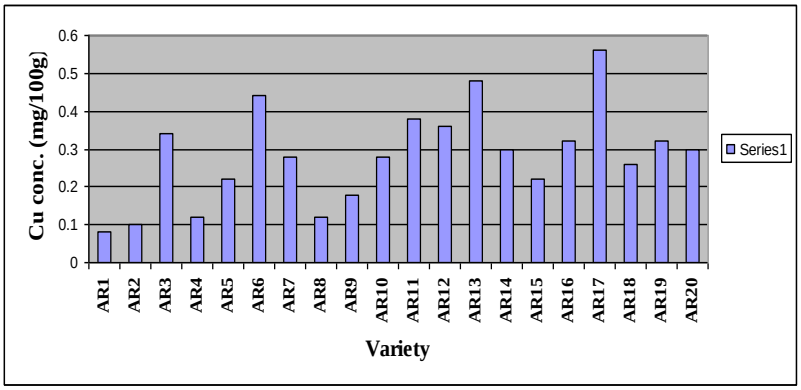

Fig. 5

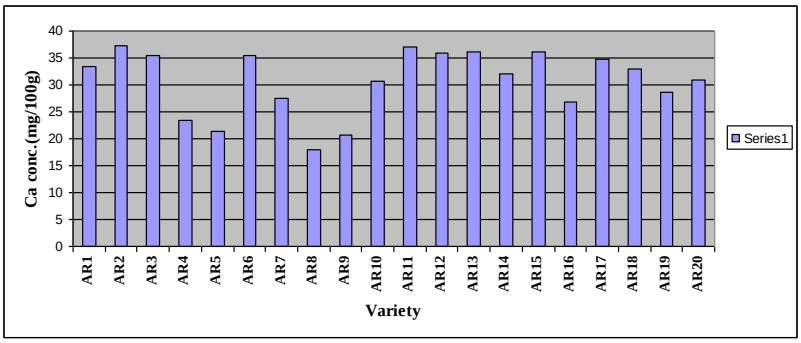

Fig. 6

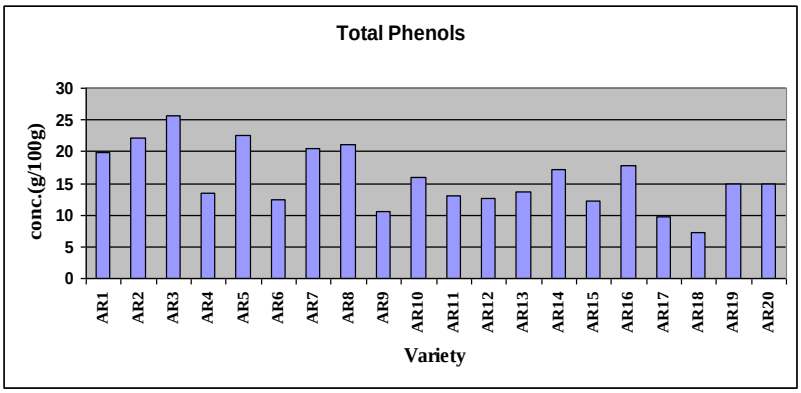

Fig. 7

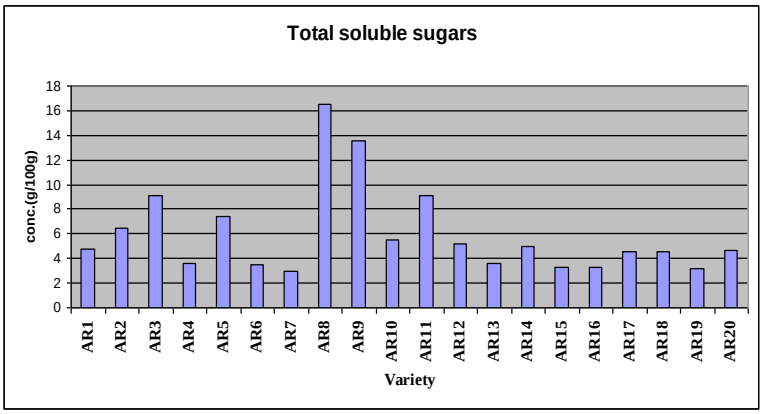

Fig. 8

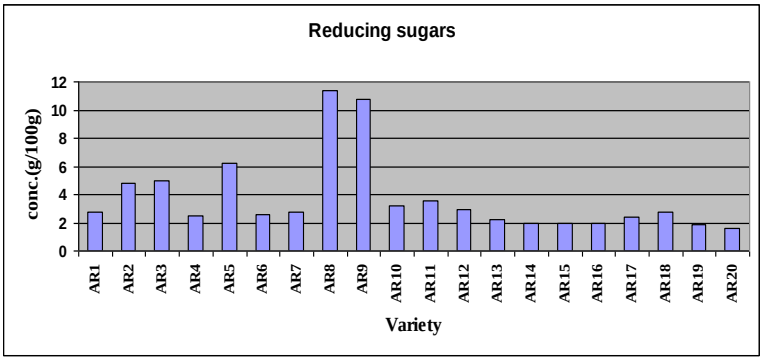

Fig. 9

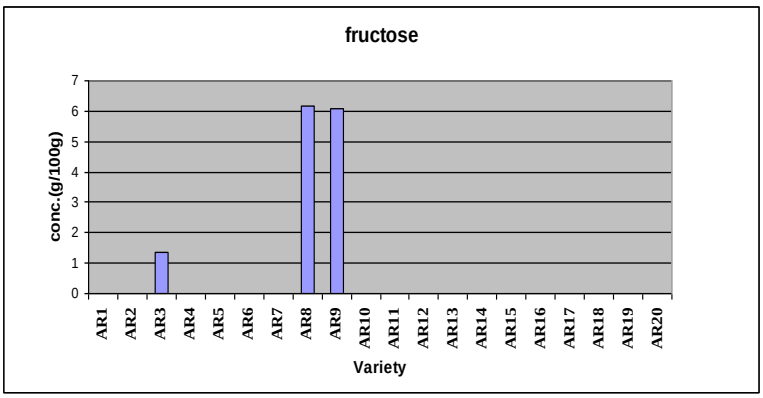

Fig. 10

Conclusion

The twenty *A. racemosus* germplasm were chemically screened for their chemical constituents including alkaloids, tannins, saponins, flavonoids and phenols, terpenoids, cardiac glycosides, phlobatanins and metal ion concentrations. The extract of the leaves of the samples were found to contain the required major elements and other nutritive compounds needed by the pharmaceutical companies as well as in food supplements.The significance of the plants in traditional medicine and the importance of the distribution of these chemical constituents were discussed. A total of seven minerals are reported in this study. The minerals like Fe, Ca & Mg are present in great concentrations where as other like Co, Mn & Zn are present in considerable amount and Cu is reported in very less amount. The quantitative analysis of the trace elements of the plants will be an interesting area for further study.

References

[1]. Ali Esmail Al-Snafi. (2015). The pharmacological importance of Asparagus officinalis- a review. J. Pharma. Biol, 5(2): 93-98.

[2]. Edeoga, H.O. and Eriata,D.O. (2001).Alkaloid, tannin and saponin contents of some Nigeria medicinal plants. J. Med. Aromatic Plant Sci. 23:344 – 349.

[3]. Harborne,J.B. (1973).Phytochemical methods, London. Chapman and Hall, Ltd. pp. 49-188.
[4]. Hasan, N., Ahmad, N., Zohrameena, S., Khalid, M and Akhtar, J. (2016). Asparagus racemosus: For medicinal uses & pharmacological actions. International Journal of Advanced Research, 4(3): 259-267.
[5]. Martins,A.E. (2002). Coincise Medical Dictionary, Institute of health education, 6th edition, Oxford University Press, UK. pp. 144.
[6]. Oketch-Rabah HA. (1998).Phytochemical Constituents of the Genus Asparagus and their biological activities. Hamdard;41:33-43.
[7]. Pier, M.S. and Bang, M.K. (1980).The role of heavy metals in human health, Environment and Health, Ann Arbor Science Publisher Inc, The Butterworth group, New York, pp. 370-377.
[8]. Sharma, P.C., Yelne, M.B. and Dennis, T.J.(2000). Data base on medicinal plants used in Ayurveda. Delhi: Documentation & publication Division, Central Council for Research in Ayurveda & Siddha; Vol I. pp. 418-30.
[9]. Sofowara, A. (1993).Medicinal plants and Traditional Medicine in Africa 2nd edn. Spectrum Book Ltd. Ibadan.
[10]. Somogyi, AI. (1952). Notes on sugar determination. J. biol. Chem., 195, 19.
[11]. Waite, R. and Boyd, J. (1953). The water-soluble carbohydrates of grasses. I.—Changes occurring during the normal life-cycle. J Sci. Food and Agricul. https://doi.org/10. 1002/ jsfa.2740040408
[12]. Yemm, E. W. and Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochem J. 57(3): 508–514.