



**PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT
OF ROOTS OF *ASPARAGUS RACEMOSUS* (Shatavari)**

**JAYASHREE G.V, RACHITHA P, KRUPASHREE K,
HEMANTH KUMAR K AND FARHATH KHANUM***

*BIOCHEMISTRY AND NANOSCIENCES DISCIPLINE, DEFENCE FOOD RESEARCH LABORATORY,
SIDDARTHA NAGAR, MYSORE, KARNATAKA 570 011*

ABSTRACT

Asparagus racemosus (*Asparagaceae*) is one of the most frequently used herb in Indian traditional medicine. The objective of the present study was, phytochemical screening of methanolic extract of roots of *A. racemosus*. The collected roots were washed, shade dried, powdered and stored in air tight container. The dried powdered material was extracted with methanol. One kg of powder was immersed in methanol solution in a flat bottom flask. The extract was concentrated to dryness under reduced pressure and controlled temperature using rotary flash evaporator. The yield of the methanolic extract was recorded as 9%. The phytochemical screening of *A. racemosus* was performed using generally accepted laboratory techniques for qualitative determinations. The analysis shows presence of phytosterols, triterpenoids, flavonoids, lactones, glycosides, fatty acids, phenolic compounds, saponins and tannins.

KEYWORDS: *Asparagus racemosus*, methanolic extract, rhizome, shatavari, phytochemicals.



FARHATH KHANUM

**BIOCHEMISTRY AND NANOSCIENCES DISCIPLINE, DEFENCE FOOD RESEARCH
LABORATORY, SIDDARTHA NAGAR, MYSORE, KARNATAKA 570 011.**

*Corresponding author

INTRODUCTION

Asparagus racemosus locally called as Shatavari in Hindi and Majjigegade in Kannada belongs to the family Asparagaceae and genus *Asparagus*. It is widely distributed in tropical and subtropical India. *A. racemosus* is spinous under shrub with numerous succulent roots and grows at an altitude of 1500 m. The rhizome is used in traditional medical applications and also as a food supplement. Its medicinal use has been reported in Indian traditional medicine such as Ayurveda, Unani and Siddha. *A. racemosus* is widely used as antioxidant^[1, 2], antitussive^[3], gastroprotective^[4], neuroprotective^[5], and to cure depression^[6]. Pandey et al^[7] have reported the oestrogenic activity of *A. racemosus* in pregnant rats. In a previous study Singh et al^[8] reported the potential use of *A. racemosus* roots as a substrate for inulinase production which hydrolyzes inulin to form fructose. The immune-adjuvant activity of *A. racemosus* was reported by Gautam et al^[9], while Siddiq et al^[10] and Sharma et al^[11] have reported the anti-inflammatory activity. The objective of the present investigation is to identify the phytochemical constituents of methanolic extract of *A. racemosus* root by an array of qualitative methods.

MATERIALS AND METHODS

Chemicals and reagents

Sulphuric acid, acetyl chloride, zinc chloride, potassium bismuth iodide, Fehling's A and B solution, ammonia, ferric chloride, sodium picrate, and Biuret reagent were procured from Merck, Bangalore, India.

Plant material

The roots of *Asparagus racemosus* were purchased from local market of Mysore, Karnataka, India and washed with water and shade dried for eight days till the moisture completely evaporates.

Preparation of methanolic extract

The shade dried roots were powdered and extracted with methanol in 1:10 ratio. From about 2 kg of *Asparagus racemosus* root, 1kg of powder was recovered; this was immersed in methanol solution in a 1000 ml flat bottom flask and was macerated for one week. The collected extracts were filtered and concentrated to dryness under reduced pressure and controlled temperature using rotary flash evaporator. The final yield obtained was 9%.

TEST FOR PHYTOSTEROLS

Salkowski reaction

To 0.5 ml methanolic extract in a test tube 1ml of H₂SO₄ was added from the sides of the test tubes. Appearance of reddish brown color indicates the presence of phytosterols.

TEST FOR TRITERPENOIDS

Tschugajeu test

To the methanolic extract, excess of acetyl chloride was added followed by a pinch of zinc chloride. The test reaction was kept aside for the reaction to subside and finally warmed using water bath. Appearance of eosin red color indicates the presence of triterpenoids^{[12][13]}.

TEST FOR SAPONINS

Foam test

To a small quantity of water in a test tube, the methanolic extract was added and shaken vigorously. Appearance of foam and its persistence for 10 minutes indicates the presence of saponins.

TEST FOR ALKALOIDS

Dragendorff's test

The extract was dissolved in methanol and this was acidified by adding few drops of Dragendorff's reagent (Potassium bismuth iodide). Appearance of orange red precipitate indicates presence of alkaloids^[14].

TEST FOR CARBOHYDRATES

Fehling's test

The extract was heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

TEST FOR ANTHROQUINONE

Anthroquinones test

Five ml of extract was hydrolysed with H₂SO₄ and extracted with benzene, followed by addition of 1ml of diluted ammonia. Appearance of rose pink coloration indicates presence of anthroquinones.

TEST FOR FLAVANOIDS

Ferric chloride test

Appearance of green coloration on addition of a few drops of neutral ferric chloride solution to the alcoholic solution of the plant extract, indicates presence of flavanoids.

TEST FOR LACTONES AND GLYCOSIDES

Baljets test

The extract was treated with sodium picrate solution. Appearance of yellow to orange color indicates presence of glycosides with lactone ring.

TEST FOR PHENOLIC COMPOUNDS AND TANNINS

Ferric chloride test

Ferric chloride solution was added drop by drop to 2 ml of extract in a test tube. Appearance of bluish black precipitate indicates presence of tannins and phenolic compounds.

TEST FOR STEROIDS

Steroids test

One ml of extract was dissolved in 10 ml of methanol, to this equal volume of concentrated H₂SO₄ was added. Red coloration of the upper layer and yellow with green fluorescence of the sulphuric acid layer indicates presence of steroids.

TEST FOR PROTEINS

Biuret test

Two ml of extract dissolved in methanol was added to the Biuret reagent (2 ml). The contents were shaken well and warmed on water bath. Appearance of red or violet color indicates presence of proteins.

FIXED OILS AND FATTY ACID

Spot test

A spot was prepared on the filter paper with the plant extract dissolved in methanol. Appearance of oil staining on the filter paper indicates the presence of fixed oil and fats.

RESULTS AND DISCUSSION

Phytochemical analysis of methanolic extract of *Asparagus racemosus* roots

In this study, we found that of methanolic extract of *Asparagus racemosus* roots, contains phytosterols, triterpenoids, saponins, alkaloids, glycosides, phenolic compounds, flavonoids, lactones, tannins, carbohydrates, proteins, fixed oils and fatty acids as listed in Table 1.

Table 1

Phytochemicals	Methanolic extract of roots of <i>Asparagus racemosus</i> : + / -
Phytosterols	+
Triterpenoids	+
Saponins	+
Alkaloids	+
Anthroquinones	-
Carbohydrates	+
Flavanoids	+
Lactones	+
Steroids	-
Tannins and phenolic compounds	+
Proteins	+
Glycosides	+
Fixed oils and fatty acids	+

(+) Presence, (-) Absence.

Ayurvedic drugs play important role in Indian system of medicine and have been used from ancient times and also followed widely across the world. The phytochemical constituents such as phytosterols, triterpenoids, saponins, alkaloids, glycosides, phenolic compounds, flavonoids, lactones, tannins, carbohydrates, proteins and fatty acids possess a wide array of biological activities. In an earlier study Awad and Fink et al ^[15] have demonstrated the anticarcinogenic activity of phytosterols and their dietary applications. In an elegant study Wang et al ^[16] have shown that trillin, a steroidal saponin isolated from *Dioscorea nipponica* exhibits anti-hyperlipidemic and anti-oxidative effects. In another study, Ramadan et al ^[17] have demonstrated that *Celastrus paniculatus* fatty acids possess radical scavenging activities. In a previous report Mullen et al ^[18] have reported that polyphenols, flavonoids, tannins in red raspberries exhibits vasorelaxation and antioxidant effects. The neuro-protective effect of glycosides of *Cornus officinalis* fruits was recently reported by Jeong et al ^[19] against glutamate induced toxicity of HT22 hippocampal cells. In a recent study Harmatha et al ^[20] have determined the immunomodulatory active lactones of *Laser trilobum*. These reports

demonstrate the wide array of bioactivities of phytochemicals constituents of plants, which have various applications to cure several ailments. The wide distribution *A. racemosus* and presence of wide array of phytochemicals demonstrates that further study is necessary to determine the various compounds present in *Asparagus racemosus* and their biological activities.

CONCLUSION

The present study demonstrates the phytochemical constituents of methanolic extract of *Asparagus racemosus* roots. Further study is needed to identify and study different class of compounds of *A. racemosus* and their biological activity.

ACKNOWLEDGEMENT

The authors are grateful to Dr. HV Batra, Director, Defence Food Research Laboratory, Mysore, for providing all the necessary facilities, constant guidance and encouragement during this investigation.

REFERENCES

1. Kamat, J.P., Bloor, K.K., Devasagayam, T.P.A., Venkatachalam, S.R.,. Antioxidant properties of *Asparagus racemosus* against damage induced by γ -radiation in rat liver mitochondria. Journal of Ethnopharmacology, 71:425-435, (2000).
2. Parihar, M.S., Hemnani, T., Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of *Asparagus racemosus*. Journal of Neural Transmission, 111: 1-12, (2004).
3. Mandal, S.C., Kumar, C.K.A., Mohana Lakshmi, S., Sinha, S., Murugesan, T., Saha, B.P., Pal M., Antitussive effect of *Asparagus racemosus* root against sulphur dioxide-induced cough in mice. Fitoterapia, 71: 686-689, (2000).
4. Sairam, K., Priyambada, S., Aryya, N.C., Goel. R.K., Gastroduodenal ulcer protective activity of *Asparagus racemosus* an experimental, biochemical and histological study. Journal of Ethnopharmacology, 86:1-10, (2003).
5. Jairam Meena ., Rakesh Ojha., A.V. Muruganandam., Sairam Krishnamurthy ., *Asparagus racemosus* competitively inhibits in vitro the acetylcholine and monoamine metabolizing enzymes. Neuroscience letters, 503:6-9, (2011) .
6. Singh, R.S., Rajesh Dhaliwal., Munish Puri., Production of inulinase from *Kluyveromyces marxianus* YS-1 using root extract of

- Asparagus racemosus*. Process Biochemistry, 41:1703-1707, (2006).
7. Pandey, S.K., Sahay, A., Pandey, R.S., Tripathi, Y.B., Effect of *Asparagus racemosus* rhizome (Shatavari) on mammary gland and genital organs of pregnant rat. Phytotherapy Research, 19: 721-724, (2005).
 8. Singh k. Gireesh., Garabadu Debapriya., Muruganandam, A.V ., k, Vinod., Joshi., Krishnamurthy Sairam., Antidepressant activity of *Asparagus racemosus* in rodent models. Pharmacology Biochemistry and Behavior, 91: 283-290, (2009).
 9. M. Gautam., S. Saha., S. Bani., A. Kaul., S. Mishra., D. Patil., N.K. Satti., K.A. Suri., S. Gairola., K. Suresh., S. Jadhav., G.N. Qazi., B. Patwardhan., Immunomodulatory activity of *Asparagus racemosus* on systemic Th1/Th2 immunity: Implications for immunoadjuvant potential. Journal of Ethnopharmacology, 121: 241-247, (2009).
 10. Sidiq, T., Khajuria, A., Suden, P., Singh, S., Satti, N.K., Suri, K.A., Srinivas, V.K., Krishna, E., Johri, R.K., A novel sarsasapogenin glycoside from *Asparagus racemosus* elicits protective immune responses against HBsAg. Immunology Letters, 135:129-135, (2011).
 11. Sharma, P., Chauhan, P.S., Dutt, P., Amina, M., A. Suri K.A., Gupta, B.D., Suri, O.P., Dhar, K.L., Sharma, D., Gupta, V., Satti, N.K., A unique immuno-stimulant steroidal sapogenin acid from the roots of *Asparagus racemosus*. Steroids, 76: 358-364, (2011).
 12. Fransworth, N.R., Biological and phytochemical screening of plants. Journal of Pharmaceutical Science. 55: 225-227, (1996).
 13. Rangari, V.D., Pharmacognosy and Phytochemistry Volume I & II 18) Wagner, and Phytochemistry", Part-1, 1st Edn, Career Publications, Nasik, 132, (2002).
 14. DE, S., DEY, N., Ghosh, A.K., Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphophallus paeoniifolius* (araceae). International Journal on Pharmaceutical and Biomedical Research. 1:150-157, (2010).
 15. Awad, A.B., Fink, C.S., Phytosterols as anticancer dietary components : evidence and mechanism of action. Nutrition Journal. 130:2127-2130, (2000).
 16. Wang, T., Choi, R.C., Li, J., Bi, C.W., Ran, W., Chen, X., Dong, T.T., Bi, K., Tsim, K.W., Trillin, a steroidal saponin isolated from the rhizomes of *Dioscorea nipponica*, exerts protective effects against hyperlipidemia and oxidative stress. Journal of Ethnopharmacology. 139:214-220, (2012).
 17. Ramadan, M.F., Kinni, S.G., Rajanna, L.N., Seetharam, Y.N., Seshagiri, M., Morsel J.T., Fatty acids, bioactive lipids and radical scavenging activity of *Celastrus paniculatus* Willd. seed oil. Scientia Horticulturae. 123: 104-109, (2009).
 18. Mullen, W., McGinn, J., Lean, M.E., MacLean, M.R., Gardner, P., Duthie, G.G., Yokota, T., Crozier, A., Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. Journal of Agricultural and Food Chemistry. 50: 5191-5196, (2002).
 19. Jeong, E.J., Kim, T.B., Yang, H., Kang, S.Y., Kim, S.Y., Sang, S.H., Kim, Y.C., Neuroprotective iridoid glycosides from *Cornus officinalis* fruits against glutamate-induced toxicity in HT22 hippocampal cells. Phytomedicine. 19: 317-321, (2012).
 20. Harmatha, J., Buděšínský, M., Vokáč, K., Kostecká, P., Kmoníčková, E., Zídek, Z., Trilobolide and related sesquiterpene lactones from *Laser trilobum* possessing immunobiological properties. Fitoterapia. 89: 157-166, (2013).