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Research Article
Antioxidant Activities of *Asparagus adscendens* Root Ethanolic Extract and Fractions Using *in vitro* Models

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Abstract
Background and Objective: The *Asparagus adscendens* root is used in ayurvedic medicine for the treatment of various diseases. In this study, the antioxidant properties of *Asparagus adscendens* root ethanolic extract and extract fractions (n-butanol, ethyl acetate and n-hexane) were investigated using *in vitro* models. **Materials and Methods:** The antioxidant activities were determined by 2, 2-Diphenyl-1-picrylhydrazyl, hydrogen peroxide and nitric oxide radical scavenging assays. The total antioxidant capacity, total flavonoid and total phenol content of the extracts were evaluated by spectrophotometric method. **Results:** The ethanolic extract and extract fractions exerted potent radical scavenging activities. Ethyl acetate extract fraction at 62.5 µg had the highest 2, 2-Diphenyl-1-picrylhydrazyl (77.87%), hydrogen peroxide (56.63%) and nitric oxide (56.28%) radical scavenging activity when compared with the ethanolic extract and other extract fractions. Flavonoids and phenols were also present in the ethanolic extract and extract fractions. The ethyl acetate extract fraction showed a higher total flavonoid content (29.9 mg quercetin equivalent/g of extract), total phenol content (47.9 mg gallic acid equivalent/g of extract) and total antioxidant capacity (35.9 mg ascorbic acid equivalent/g of extract) when compared with the ethanolic extract and other extract fractions. **Conclusion:** The results of this research indicated that *Asparagus adscendens* root ethyl acetate extract fraction have high antioxidant potentials and could be explored as a therapeutic agent for the treatment of free radical induced diseases.

Key words: *Asparagus adscendens*, therapeutic agent, antioxidant, ethyl acetate extract, ayurvedic medicine, free radical induced diseases

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have been used since ancient times for the treatment of various diseases due to the antioxidant potentials and the presence of different phytochemicals[1]. Antioxidants are compounds found mostly in plants and they inhibit oxidative damage to body cells by scavenging free radicals that might cause degenerative disease[2,3]. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced naturally in the body during oxidative reactions and they play an important role in many normal cellular processes[4]. However, at high concentrations, free radicals can be hazardous to the body and damage all major components of cells, including proteins, cell membranes and deoxyribonucleic acid (DNA)[5]. The damage to cells caused by free radicals may lead to diseases which include neural disorders[6], aging[7], diabetes, cancer[8]. Recent studies on antioxidant from plants with free radical scavenging activities have reported that different parts of plants with antioxidant properties may be used as therapeutic agents for the treatment of diseases caused by free radicals[9,10]. *Asparagus adscendens* Roxb. has many common names viz. Safed musli, Shatawari, Shatavar and Shatamuli in India. The plant belongs to the family, Asparagaceae. *Asparagus adscendens* is usually found in all parts of India and also in forests of western Himalaya. The root of *Asparagus adscendens* is used in ayurvedic medicine as antipyretic, demulcent, nutritive tonic and for the improvement of fertility in males and females[11,12]. Studies on the *in vitro* antioxidant activities of the root aqueous and methanolic extract of *Asparagus racemosus*; a plant belonging to the genus *Asparagus* have been documented[13,14].

In this study, the radical scavenging activity, the presence of flavonoid and phenolic compounds in the root ethanolic extract and extract fractions of *Asparagus adscendens* were investigated using *in vitro* models due to its use in ayurvedic medicine.

MATERIALS AND METHODS

This research work was carried out in four months (September, 2018-January, 2019) in the School of Biotechnology, Devi Ahilya University, Indore, India.

Plant materials: The pulverized root of *Asparagus adscendens* was given as a kind gift from AMSAR Private Limited, Indore, India.

Extraction procedure: The dried pulverized root of *Asparagus adscendens* was macerated in 70% ethanol for 72 h and the suspension was then filtered using whatman no. 1 filter paper[15]. The filtrate (ethanolic extract) was concentrated to a solid form at 45°C and stored in a refrigerator at 4-8°C temperature prior to use.

Fractionation of ethanolic extract: A liquid paste of the ethanolic extract of *A. adscendens* was prepared in distilled water and thereafter was partitioned using various solvents of different polarities in the following order: n-hexane, ethyl acetate and n-butanol (one solvent at a time) to obtain the respective fraction[16]. Each of the partitioned fractions was air dried and stored in a refrigerator at 4-8°C temperature prior to use.

Qualitative antioxidant assays: The ethanolic extract at 62.5, 125, 250, 500 and 1000 μg extract mL^{-1}; each extract fractions at 62.5 μg mL^{-1} and standard (acarbose) at 100 μg mL^{-1} distilled water was used for the free radical scavenging assays.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: A volume of 1.0 mL of 0.3 mM DPPH in methanol was added to 1.0 mL of samples in different test tubes. The contents were mixed and incubated in the dark for 30 min and thereafter; absorbance was read at 517 nm against a reagent blank[9]. The percentage radical scavenging activity (RSA) of the sample was calculated using the Eq. 1:

$$\text{RSA (\%)} = \frac{\text{Absorbance of reagent blank-(Absorbance of test-Absorbance of test blank)}}{\text{Absorbance of reagent blank}} \times 100 \quad (1)$$

Nitric oxide (NO) radical scavenging assay: A volume of 0.5 mL 10 mM Sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was added to 1.0 mL of samples in different test tubes and then incubated for 180 min at 25°C. After incubation, 1.5 mL of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid) was added and absorbance was measured[17,18] at 546 nm. The percentage radical scavenging activity (RSA) of the sample was calculated using Eq. 1.

Hydrogen peroxide (H_2O_2) assay: A solution of hydrogen peroxide (43 mM) was prepared in 1.0 M phosphate buffer, pH 7.4. A 1.0 mL of samples in different test tubes was added to 0.6 mL hydrogen peroxide and absorbance was measured[10] at 230 nm after 10 min. The percentages of radical scavenging activity (RSA) of the extracts and standard were calculated using Eq. 1.

Quantitative antioxidant assay: The concentration of the ethanolic extract and each of the extract fractions used for the quantitative antioxidant assays was 1.0 mg mL^{-1}.

Total antioxidant capacity (TAC) assay: A volume of 1.0 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to 1.0 mL of the samples in different test tubes and then incubated in a water bath at 95°C for 90 min. Thereafter, the mixture was cooled to room temperature and the absorbance was measured at 695 nm against a reagent blank[10]. The total antioxidant capacity of the extracts expressed as mg standard (ascorbic acid) equivalent per gram of the plant extract was calculated using the Eq. 2:

$$C = \frac{c \cdot v}{m} \quad (2)$$

Where:

C = Total antioxidant in mg standard
c = Concentration of standard established from the calibration curve (mg mL^{-1})
v = Volume of the extract (mL)
m = Weight of the extract (g)

Total flavonoid content assay: To a volume of 0.5 mL of the samples in different test tubes, 0.3 mL of 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminum chloride was added and thereafter 6 min, 2.0 mL of 1 M sodium hydroxide was added and the volume was made up to 5.0 mL with distilled water. The absorbance was measured at 510 nm against a reagent blank[10]. The total flavonoid contents expressed as mg standard (Quercetin) equivalents per gram of the plant extract was calculated using Eq. 2.

Total phenol content assay: A volume of 0.2 mL of the samples in different test tubes was added in 8.0 mL of 1 M Folin-Ciocalteu's phenol reagent. The mixture was vortex and after 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added and the volume was made up to 3 mL with distilled water. The reaction was kept in the dark for 30 min and the absorbance was measured at 765 nm against a reagent[19]. The total phenolic content of the extracts expressed as mg standard (gallic acid) equivalent per gram of extract was calculated using Eq. 2.

Statistical analysis: The results were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett *post hoc* multiple comparisons tests at 95% ($p<0.05$) level of significance using Primer (version 3.01). All results were expressed as mean±standard error of mean (SEM). The 50% inhibition (IC_{50}) of the plant extract was calculated from graph plotted using excel.

RESULTS

Radical scavenging activity of *A. adscendens* root ethanolic extract and extract fractions: The ethanolic extract significantly ($p<0.05$) exerted free radical scavenging activity in a dose dependent manner (Table 1). The ethyl acetate extract fraction at 62.5 µg exerted a significant ($p<0.05$) increase in free radical scavenging activities when compared with the ethanolic extract, other extract fractions but less than standard (ascorbic acid) (Table 2). The standard (ascorbic acid) had a significant ($p<0.05$) higher percentage radical scavenging activity when compared with the ethanolic extract and the other extract fractions (Table 1, 2).

Antioxidant activity of *A. adscendens* root ethanolic extract and extract fractions: The result of this study (Table 3) shows that the ethanolic extract and extract fractions (n-butanol, ethyl acetate and n-hexane extract fractions) had moderate amount of flavonoids and phenolic compounds. However, the ethyl acetate extract fraction had a significant ($p<0.05$) higher total antioxidant capacity, total flavonoids content and total phenolic content when compared with the ethanolic extract and other extract fractions (Table 3).

Table 1: Radical scavenging activity of *A. adscendens* root ethanolic extract

Extract concentration (mg)	DPPH	Hydrogen peroxide	Nitric oxide
62.5 µg	14.61±0.27^{bcdef}	11.53±0.29^{bcdef}	18.52±0.58^{bcdef}
125 µg	22.78±0.31^{acdef}	24.29±0.34^{acdef}	21.90±0.58^{acdef}
250 µg	31.14±0.51^{abdef}	30.65±0.40^{abdef}	28.70±0.33^{abdef}
500 µg	39.07±0.58^{abcef}	48.87±0.47^{abcef}	43.50±0.40^{abcef}
1000 µg	57.19±2.24^{abcdf}	56.14±0.09^{abcd}	54.47±0.58^{abcdf}
Ascorbic acid (100 µg)	67.85±0.25^{abcde}	61.30±0.35^{abcd}	58.50±0.29^{abcde}
IC_{50} of extracts	793.33 µg	761.40 µg	831.84 µg

Mean±SEM, n = 3, a: 62.5 µg, b: 125 µg, c: 250 µg, d: 500 µg, e: 1000 µg and f: 100 µg ascorbic acid (standard), DPPH: 2, 2-Diphenyl-1-picrylhydrazyl. ^{abcdef}: Significantly from a, b, c, d, e and f at $p<0.05$

Table 2: Radical scavenging activity of *A. adscendens* root ethanolic extract fractions at 62.5 μg

Ethanolic extract and extract fractions (62.5 μg)	DPPH	Hydrogen peroxide	Nitric oxide
Ethanolic extract	14.61±0.27*#	11.53±0.29*#	18.52±0.58*#
n-butanol fraction	60.07±0.64*#	29.79±0.31*#	43.72±0.59*#
Ethyl acetate fraction	77.87±0.30 #	56.63±0.32 #	56.28±0.64 #
n-hexane fraction	46.13±0.19*#	18.93±0.58*#	16.23±0.31*#
Ascorbic acid	79.76±0.34*	91.0±0.58*	98.94±0.03*

Mean±SEM, n = 3, DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, BF: n-butanol fraction, EF: Ethyl acetate fraction and HF: n-hexane fraction, *Significantly different from ethyl acetate extract fraction at $p<0.05$ and #Significantly different from ascorbic acid at $p<0.05$

Table 3: Antioxidant activity of *A. adscendens* root ethanolic extract and fractions

Ethanolic extract and extract fractions (62.5 μg)	TAC (mg AAE g^{-1})	TF (mg QE g^{-1})	TP (mg GAE g^{-1})
Ethanolic extract	28.3±0.34*	20.2±0.12*	36.7±0.03*
n-butanol fraction	22.4±0.31*	21.5±0.01*	39.9±0.01*
Ethyl acetate fraction	35.9±0.30	29.9±0.01	47.9±0.01
n-hexane fraction	10.9±0.31*	11.2±0.19*	26.5±0.01*

Mean±SEM, n = 3, TAC: Total antioxidant capacity, TF: Total flavonoid, TP: Total phenol, mg AAE g^{-1}: mg ascorbic acid equivalent g^{-1} of extract, mg QE g^{-1}: mg quercetin equivalent g^{-1} of extract, mg GAE g^{-1}: mg gallic acid equivalent g^{-1} of extract, EE: Ethanolic extract, BF: n-butanol fraction, EF: Ethyl acetate fraction and HF: n-hexane fraction. *Significantly from ethyl acetate extract fraction at $p<0.05$

DISCUSSION

Asparagus adscendens root ethanolic extract and extract fractions exerted potent radical scavenging activities (Table 1, 2) and the extracts also contained moderate amount of flavonoid and phenolic compounds (Table 3). The free radical scavenging activities of *Asparagus adscendens* root ethanolic extract and extract fractions were similar to previous studies carried out on one of the genus of *Asparagus* (*Asparagus racemosus*)[13,14]. It was reported that *Asparagus racemosus* root methanolic extract scavenged 2, 2-Diphenyl-1-picrylhydrazyl free radical[13], whereas another study investigated that the root ethanolic of *Asparagus racemosus* scavenged 2, 2-Diphenyl-1-picrylhydrazyl, nitric oxide and hydroxyl free radicals[14]. In this study, *Asparagus adscendens* root ethyl acetate extract fraction had a significant ($p<0.05$) higher free radical scavenging activity, total antioxidant capacity, total flavonoid and total phenol content more than the ethanolic extract and other extract fractions (Table 2, 3). Previous studies reported that *Asparagus adscendens* root extract exerted antifilarial effect against *Setaria cervi*, antiprotozoal activity on *Plasmodium berghe*, analgesic activity and antiviral effect on ranikhet and vaccinia disease virus[20]. It was also studied that *Asparagus adscendens* root inhibited carcinogenesis process by enhancing the activity of catalase and superoxide dismutase enzymes involved in antioxidant function[21] whereas researchers reported that *Asparagus adscendens* root ethanolic extract exerted reproductive, anabolic and sexual behavioural activities in adult Sprague-Dawley rats[22]. Previous studies carried out on antioxidant activities of plants have investigated that plants are rich sources of antioxidants and activity guided studies may lead to new therapeutic agents for the treatment of diseases[9,10,18]. The antioxidant activities of *Asparagus adscendens* root ethanolic extract and extract fractions (Table 2, 3) justified the report from previous studies on the pharmacological activities of *Asparagus adscendens* root[20-22]. Studies on the potential analgesic and anti-inflammatory effect of some plants showed that plants exert these effects due to their antioxidant activities[23,24]. Also, recent research work carried out on the treatment of diseases such as cancer[25-27], diabetes[28,29] and cardiovascular diseases[30,31] with medicinal plants have reported that plants exert these effects due to their antioxidant potentials.

Further studies directed at the use of *Asparagus adscendens* root for the treatment of free radical induced diseases may reveal new therapeutic agents.

CONCLUSION

The results of this study showed that *Asparagus adscendens* root ethanolic extract and extract fractions especially have potent antioxidant activity and this justified the use of the plant root in ayurvedic medicine for the treatment of some diseases. Besides, the study showed that *A. adscendens* root ethyl acetate extract fraction posses more potent antioxidant activity than the ethanolic extract and other extract fractions.

SIGNIFICANCE STATEMENT

This study discovered that *Asparagus adscendens* root have antioxidant activities and it contains moderate amount of flavonoid and phenols that can be beneficial for the treatment of free radical induced diseases which include

cancer, diabetes and cardiovascular diseases. This study will help researchers to uncover the critical areas in the use of the plant root for the treatment of various diseases caused by oxidative stress that many researchers were not able to explore. Thus a new theory in the treatment of free radical induced diseases may be arrived at.

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REFERENCES

1. Sabli, F., M. Mohamed, A. Rahmat, H. Ibrahim and M.F. Abu-Bakar, 2012. Antioxidant properties of selected etlingera and zingiber species (Zingiberaceae) from Borneo Island. Int. J. Biol. Chem., 6: 1-9.
2. Yamagishi, S.I. and T. Matsui, 2011. Nitric oxide, a Janus-faced therapeutic target for diabetic microangiopathy-friend or foe? Pharmacol. Res., 64: 187-194.
3. Wu, Y.Y., W. Li, Y. Xu, E.H. Jin and Y.Y. Tu, 2011. Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses. J. Zhejiang Univ. Sci. B, Vol. 12. 10.1631/jzus.B1100041.
4. Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.
5. Valko, M., M. Izakovic, M. Mazur, C.J. Rhodes and J. Telser, 2004. Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem., 266: 37-56.
6. Sas, K., H. Robotka, J. Toldi and L. Vecsei, 2007. Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorders. J. Neurol. Sci., 257: 221-239.
7. Hyun, D.H., J.O. Hernandez, M.P. Mattson and R. de Cabo, 2006. The plasma membrane redox system in aging. Ageing Res. Rev., 5: 209-220.
8. Kinnula, V.L. and J.D. Crapo, 2004. Superoxide dismutases in malignant cells and human tumors. Free Radical Biol. Med., 36: 718-744.
9. Huang, B., H. Ke, J. He, X. Ban, H. Zeng and Y. Wang, 2011. Extracts of *Halenia elliptica* exhibit antioxidant properties *in vitro* and *in vivo*. Food Chem. Toxicol., 49: 185-190.
10. Sasikumar, V. and P. Kalaisezhiyen, 2014. Evaluation of free radical scavenging activity of various leaf extracts from *Kedrostis foetidissima* (Jacq.) Cogn. Biochem. Anal. Biochem., Vol. 3. 10.4172/2161-1009.1000150.
11. Umashanker, M. and S. Shruti, 2011. Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer: A review. Int. J. Res. Pharm. Chem., 1: 1152-1159.
12. Thakur, S. and D.R. Sharma, 2015. Review on medicinal plant: *Asparagus adscendens* Roxb. Int. J. Pharm. Sci. Health Care, 5: 82-97.
13. Dohare, S., M. Shuaib and K.J. Naquvi, 2011. *in vitro* antioxidant activity of *Asparagus racemosus* roots. Int. J. Biomed. Res., 4: 228-235.
14. Karuna, D.S., P. Dey, S. Das, A. Kundu and T. Bhakta, 2018. *In vitro* antioxidant activities of root extract of *Asparagus racemosus* linn. J. Trad. Complement. Med., 8: 60-65.
15. Oluwakanyinsola, S.S.A., T.Y. Adeniyi, J.A. Akingbasote and O.E. Florence, 2010. Acute and subacute toxicity study of ethanolic extract of the stem bark of *Faidherbia albida* (Del) A. chev (Mimosoidae) in rats. Afr. J. Biotechnol., 9: 1218-1224.
16. Jahan, N., M.S. Parvin, N. Das, M.S. Islam and M.E. Islam, 2014. Studies on the antioxidant activity of ethanol extract and its fractions from *Pterygota alata* leaves. J. Acute Med., 4: 103-108.
17. Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum, 1982. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. Anal. Biochem., 126: 131-138.
18. Boora, F., E. Chirisa and S. Mukanganyama, 2014. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. J. Food Process. 10.1155/2014/918018.
19. Chandra, S., S. Khan, B. Avula, H. Lata, M.H. Yang, M.A. ElSohly and I.A. Khan, 2014. Assessment of total phenolic and flavonoid content, antioxidant properties and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evidence-Based Complement. Alternat. Med., Vol. 2014. 10.1155/2014/253875.
20. Aswal, B.S., A.K. Goel, D.K. Kulshrestha, B.N. Mehrotra and G.K. Patnaik, 1996. Screening of Indian plants for biological activity: Part XV. Indian J Exp Biol., 34: 444-467.
21. Singh, M., S. Singh and R.K. Kale, 2011. Chemomodulatory potential of *Asparagus adscendens* against murine skin and forestomach papillomagenesis. Eur. J. Cancer Preven., 20: 240-247.
22. Bansode, F.W., K.R. Arya, R.K. Singh and T. Narender, 2015. Dose-dependent effects of *Asparagus adscendens* root (AARR) extract on the anabolic, reproductive and sexual behavioral activity in rats. Pharm. Biol., 53: 192-200.

23. Sen, S., R. Chakraborty, B. Rekha, D. Revathi and S.C. Ayyanna *et al*., 2013. Anti-inflammatory, analgesic and antioxidant activities of *Pisonia aculeata*: Folk medicinal use to scientific approach. Pharm. Biol., 51: 426-432.
24. Ibrahim, B., A. Sowemimo, A. van Rooyen and M. Van de Venter, 2012. Antiinflammatory, analgesic and antioxidant activities of *Cyathula prostrate* (Linn.) Blume (Amaranthaceae). J. Ethnopharmacol., 141: 282-289.
25. Ghagane, S.C., S.I. Puranik, V.M. Kumbar, R.B. Nerli and S.S. Jalalpure *et al*., 2017. *In vitro* antioxidant and anticancer activity of *Leea indica* leaf extracts on human prostate cancer cell lines. Integrat. Med. Res., 6: 79-87.
26. Turkez, H., O.O. Tozlu, T.C. Lima, A.E.M. de Brito and D.P. de Sousa, 2018. A comparative evaluation of the cytotoxic and antioxidant activity of *Mentha crispa* essential oil, its major constituent rotundifolone and analogues on human glioblastoma. Oxidat. Med. Cellul. Longevity, Vol. 2018. 10.1155/2018/2083923.
27. Das, A., K. Harshadha, S.K.D. Kannan, K.H. Raj and B. Jayaprakash, 2018. Evaluation of therapeutic potential of eugenol-a natural derivative of *Syzygium aromaticum* on cervical cancer. Asian Pac. J. Cancer Preven., 19: 1977-1985.
28. Rehman, G., M. Hamayun, A. Iqbal, S. Ul Islam and S. Arshad *et al*., 2018. *In vitro* antidiabetic effects and antioxidant potential of *Cassia nemophila* pods. BioMed Res. Int., Vol. 2018. 10.1155/2018/1824790.
29. Agnihotri, A. and V. Singh, 2013. Effect of *Tamarindus indica* Linn. and *Cassia fistula* Linn. stem bark extracts on oxidative stress and diabetic conditions. Acta Pol. Pharm., 70: 1011-1019.
30. Wattanapitayakul, S.K., L. Chularojmontri, A. Herunsalee, S. Charuchongkolwongse, S. Niumsakul and J.A. Bauer, 2005. Screening of antioxidants from medicinal plants for cardioprotective effect against doxorubicin toxicity. Basic Clin. Pharmacol. Toxicol., 96: 80-87.
31. Jahan, N., K.U. Rahman and S. Ali, 2012. Cardioprotective and antilipidemic potential of *Cyperus rotundus* in chemically induced cardiotoxicity. Int. J. Agric. Biol., 14: 989-992.