Idetification of Isolated Bioactive Compound and Antioxidant Profile of *Asparagus Racemosus* Stem Extracts

S. Prakash, A. Venkatesan, A. Kathirvel and V. Sujatha

Abstract--- *The God of nature given the gift to human being is medicinal plants to build disease free healthy lifeand from ancient time to still the modern life which is a soul to remarkable source for medicine. In this present study, the phytochemical studies reveal the antioxidant profile of Asparagus racemosus stem extracts. The various solvent extracts like hexane, chloroform, ethyl acetate, acetone, methanol and water posses numerous bioactive compounds areexhibitedbythe phytochemical screening. Determination of total phenolic (TP) and flavonoid (TF) content are evaluated and antioxidant activities of extracts are assisted by DPPH radicalscavenging and reducing power (RP) assays. The methanol extract exposed the higher contents (TP:177.75 ± 0.47, TF: 185.49±1.45 µg/mg) and higher antioxidant activities with lower IC_{50} values (DPPH: 223, RP: 234µg) than the other extracts. Therefore effective methanol extract was subjected to isolation of bioactive compounds using TLC and Column chromotography techniques. The compound isolated was tentatively identified as flavonol by using UV-Visible and FT-IR spectral analysis. From this study we recommend the Asparagus racemosus stemacts as an excellentsource for efficient antioxidant compoundsand future studies are aimed to conform the active compounds and analyzing their pharmacology applications.*

Keywords--- *Asparagus Racemosus;Antioxidant; Phytochemical; Flavonol.*

I. INTRODUCTION

HERBAL derived medicinestays still asan anchor to the modern life styleof about 75-80% population, mostly in developing countries, for major health care because, better compatibility with the human body metabolism and very less side effects [1]. Medicinal plants possessing numerous phytoconstituents have extreme antioxidant property playing a significant part in the prevention of various degenerative diseases and exposing vital benefits to humanity, the medicinal herbs have been investigated for their antioxidant properties as they are highly effective, secured and risk-free [2]. In India, thousands ofmedicinal plants are evaluated by their medicinal values for various chronic ailments and different parts of the plants has been used to recover the specific health defects since antique time[3].*Asparagus recemosus* (*Liliaceae*) is one of the Indian traditional medicinal plant, commonly called as shatavari(Satmuli in Hindi, Indian Asparagus in English), this is a shrub, subtropical plant.Ithas been used in wide range in Ayurvedic treatment like nervous disorders, neuropathy, dyspepsia, hepatopathy, inflammation, tumors and also preventing the ageing, amplify vitality and the explicitly of *Asparagus recemosus*root extract in pharmacological studies include antidiabetic, antioxidant, antiulcer, and antidiarrhoeal properties [4].The root rhizome extract is most familiar tonic remedy topromote fertility and reducing menopausal symptoms in Ayurveda [5].Steroidal saponins [6],polycyclic alkaloid [7-9],furan compound [10],carbohydrates [11],flavanoids [12],sterols [13] are the significant phytoconstituents of shatavari for responsibility of Ayurvedic and pharmacological efficacy. This survey inspired to examine the efficiency of all part of this plant and found scarce reports for its stem. Therefore in this study the different solvent fraction of *Asparagus recemosus*stem extracts using by sequential extraction were investigated for their antioxidant activity and the study guided isolation for identifying those corresponding antioxidant potent compounds.

II. MATERIALS AND METHODS

2.1 Chemical

Chemicals: Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, gallic acid, (±)-catechin, aluminium chloride, ascorbic acid,butyl hydroxy anisole (BHA) andall solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant Collection and Extraction

Asparagus recemosus stem were collected from Periyar University campus, Periyar Palkalai Nagar, Salem

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District, Tamil Nadu State, India. The plant materials were identified and confirmed by Botanical Survey of India (BSI/SRC/5/23/2013-2014/Tech.2086). The plant material washed with tap water, prior to distilled water were shade dried and powdered. The powder sample was subjected to solvent extraction using in the following order of solvents like hexane, chloroform, ethylacetate, acetone, methanol and water by soxhlet extractor. The extracts were dried in vacuum pump at 40^{o}C. The dried extract was stored in freezer at 0^{o}C for future use.

2.3. Phytochemical screening

The preliminary phytochemical screening tests were carried out to display the useful constituents by standard methods [14].

2.4. Determination of total phenolic content

The total phenolics in the extracts were estimated by Spectrophotometric assay [15]. One mL of sample (concentration 1 mg/mL) was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after whichthe absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (20-100 µg/mL, Y=0.012x+0.069, R^2= 0.998) and the results were expressed as µg of gallic acid equivalents/mg of extract (GAEs).

2.5. Determination of total flavanoid content

Flavonoid contents in the extracts were determined by Spectrophotometric method [15]. The (250 µL) extract (concentration 1 mg/mL) was mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of 10% $AlCl_3$ solution was added. After 6 min, 500 µL of 1 M NaOH and 275 µL of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (±)-Catechin was used to calculate the standard curve (20-120 µg/mL, Y=0.0042x+0.0164, R^2=0.999) and the results were expressed as µg of (±)-catechin equivalents (CEs) per mg of extract.

2.6.DPPH radical scavenging activity

The different concentration of *Asparagusrecemosus* stem invarious solvent extracts (0.2 mL)were mixed with 1.8 mL of methanol solution containing DPPH radicals (6 x 10^{-5} mol/L). The mixture was shaken vigorously and allowed to stand for 60 min in the dark. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm [16]. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: %RSA =[(A_{DPPH}-A_S)/A_{DPPH}] x 100, where, A_S is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The 50% of inhibition concentration (IC_{50}) of extract was calculated from the graph of RSA percentage against extract concentration. Ascorbic acid and BHA were used as standards.

2.7. Reducing power

The reducing power of *Asparagusrecemosus* stem extracts was determined [17]. Various concentrations of different solvent extract (1 mL), phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1 mL, 10 mg/mL) were mixed together and incubated at 50 °C for 20 min. Trichloroacetic acid (1 mL, 100 mg/mL) was added to the mixture and centrifuged at 8000 rpm for 5 min. The supernatant (1 mL) was mixed with distilled water (1 mL) and ferric chloride (0.1 mL, 1 mg/mL) and then the absorbance was measured at 700 nm.

2.8. Isolation and characterization

The effective fraction of *Asparagusrecemosus* stem extract were subjected to isolation part, the extract (mg/mL) dissolved in methanol solvent as stock solution to screen the number compounds present in the extract using thin layer chromatography (TLC) by the various of mobile phase (hexane : ethylacetate, hexane :chloroform etc) with increasing ratio of polarity. The TLC report revealed the suitable mobile phase to separate out individual pure compound by Column chromatography techniques. The isolated active fraction of extract were characterized by simple chemical screening tests and identified the active compound using UV-Visible, FT-IR spectral analysis.

2.9. Statistical Analysis

Statistical analysis of values was expressed as means ± standard deviations. All determinations were done at least in triplicate and all were averaged. The confident limits used in this study were based on 95% ($p<0.05$).

III. RESULTS AND DISCUSSION

3.1. Qualitative and quantitative phytochemical studies

The qualitative phytochemical studies of *Asparagusrecemosus* stem in various solvent extract revealed the presence of active phytoconstituents

(Table1). The methanol extract of stem posses numerous phytoconstituents than reported ethanolic root extract of *Asparagusrecemosus*[18].

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Table 1.Qualitative phytochemical screening of Asparagusrecemousin various solvent extracts

Phytochemical Constituents	Various solvent extracts					
	HX	CH	EA	AC	ME	WA
Alkaloids	-	-	+	+	+	+
Flavonoids	-	-	+	++	++	+
Flavonols	-	-	+	+	++	+
Glycosides	-	-	-	-	-	+
Carbohydrates	-	-	+	+	+	+
Phenolics/Tannins	+	+	++	+++	+++	++
Steroids/Terpenoids	-	-	+	++	++	+
Aminoacids/ Proteins	-		+	+	++	+
Saponins	++	++	+	-	-	-
Fats/Oils	++	++	+		-	-

HE=Hexane; CH=Chloroform; EA=Ethyl acetate; AC=Acetone ME=Methanol extract; WA=Water

+++ = Copiously present, ++ = Moderately present, + = Slightly present, - = Absent

3.2. Determination of total phenolic and flavonoid content

The total phenolic and flavonoid content of *Asparagusrecemous* stem methanol extract exposed unexpected higher amount of phenolic (177 µg GAE/mg of extract) and flavonoid (185 µg CE/mg of extract) content,when compard to other extracts in **Table 2.**The presence of phytoconstituents such as flavonoid and phenolic compounds are reason to behave as an antioxidant agent because they possess redox properties for role as reducing agents, proton donor, free radical inhibitor and chelators of metal **[**19**].**Therefore in the study, methanol extact may act as a powerful free radical scavenger is assumed for *in vitro* free radical scavenging assays.

Table 2.Determination of total phenolic and flavonoid content of Asparagusrecemousstem in various solvent extracts

Content (µg/mg of extract)	Various solvent extracts					
	HX	CH	EA	AC	ME	WA
Phenolic	12.58 ± 0.41	21.70 ± 0.62	42.40 ± 0.40	71.50 ± 0.23	177.75 ± 0.47	67.41 ± 0.52
Flavonoid	16.60 ± 0.77	24.14 ± 0.71	37.08 ± 1.53	90.57 ± 1.90	185.49 ± 1.45	84.22 ± 1.91

The three replicates values are mean with standard deviations (mean ±S.D; n =3), $p<0.05$.

3.3.In vitro Antioxidant studies

3.3.1. DPPH radical scavenging activity

The antioxidant capacity of the*Asparagusrecemosus* stem extracts were displayed by *in vitro* DPPH radical scavenging study. The percentage of radical scavenging activity increases with increasing concentrations. The lowest IC_{50} value (73µg/mL) of methanol extract is evidenced to its antioxidant property and compared to the standard (BHA and ascorbic acid). The 100% scavenging of DPPH radical scavenging activity was revealed that methanol (93%), acetone (83%) >water (72%)> ethyl acetate (71%) > chloroform (69%) > hexane (64%) shown in **Figure 1.**From this *in vitro* DPPH assay indicating the methanol extract of *Asparagusrecemous* stem act as potent antioxidant agent due to having higher content of secondary metabolites.

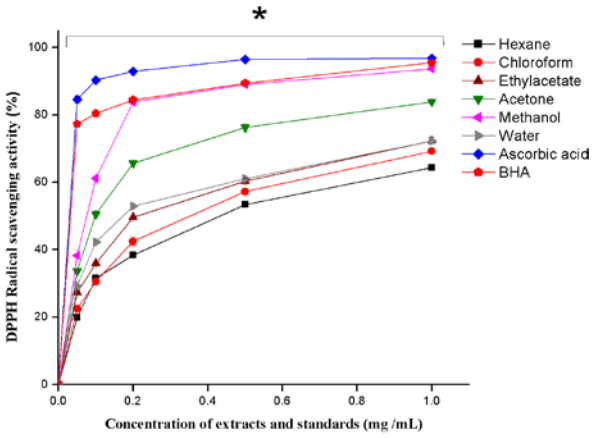

Figure 1. DPPH radical scavenging activities of Asparagusrecemosus stem extracts in different concentrations. Each value represents amean ± S.D. Symbols represent statistical significance from control (*p <0.05).

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3.3.2. Reducing power activity

The antioxidant potential of *Asparagusrecemosus* stem extracts was also tested with reducing power activity. The reduction of Fe^{+3} ion to Fe^{+2} ion is shows the reducing ability of plant extracts. However the methanol extract revealed the significant EC_{50} value40 µg followed by acetone 53 µg, water 73 µg and the reducing power of all extracts is shown in **Figure 2.**

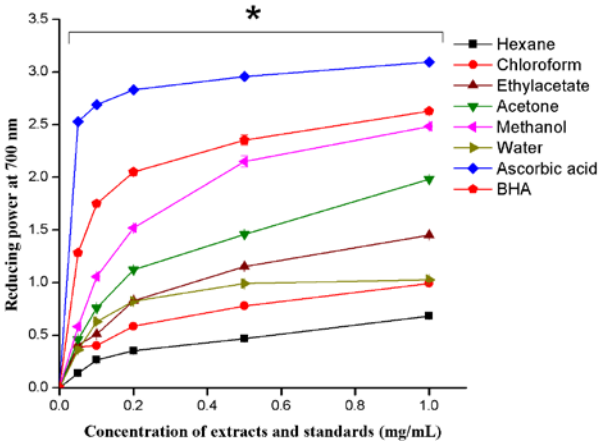

Figure 2. Reducing power activity of Asparagusrecemosus stem extracts in different concentrations. Each value represents a mean ± S.D. Symbols represent statistical significance from control (*p <0.05).

The reducing power activity of *Asparagusrecemosus* stem extracts is an evidence and support to methanol extract was being an effective antioxidant than other extracts. The qualitative and quantitative phytochemical studies and *in vitro* DPPH radical scavenging activity and reducing power activity also obviously exhibited the methanol extract of stem being potent antioxidant and still no reports for isolated bioactive compounds from *Asparagusrecemosus* stem extract, is guided to methanol extract was subjected to isolation for identifying responsible bioactive compounds.

3.4. Isolation and identification of bioactive compound

The different solvent systems such as (HX:CH), (HX:EA), (HX:ME), (CH:ME) and (EA:ME) in the following ratio (0:100 start up to 100:0) were tried for optimization the TLC systems to identify the constituents present in the extract. Clear spots are separately obtained only HX:EA solvent system when compared with other solvent systems. Therefore HX:EA solvent system is repeated several times in different ratio (90:10 to 0:100). Three spots were obtained closely in (80:20) ratio and when ratio of ethylacetate increasing the spots are migrating into top of the plate. In other solvent systems, all the spots moved very fast in these mobile phase and individual spots did not appear in these solvent systems. Therefore from TLC reports revealed HX:EA solvent system is suitable mobile phase to isolate the compounds from stem methanol extract of *Asparagusracemosus.*

The isolation of compounds from methanol extract of *Asparagus recemosus*stem was carried out by glass column having the length and diameter of 55 cm and 2 cm and silica gel (60-120 mesh) as adsorbent. HX:EA mixture respectively (90:10, 80:20, 70:30, 60:40, 50:50) ratios were collected. In fraction-I (90:10) ratio, the rosy red color band appeared and was collected by continuous eluting. In next fraction II-(80:20) ratio, yellow color substance came out. On increasing the polarity of the solvent mixture then compounds were eluted quickly. In fraction III-(70:30) ratio, the reddish brown color compound was eluted and fraction-IV ratio (60:40) the large scale of dark brown color band eluted with higher quantity when compared with other fractions. Then (50:50) ratio fraction-V light green color band appeared and it was collected in very small scale of quantity therefore it was not satisfactory. The solvent fractions-III and IV revealed above 200 mg quantity then other fractions were in very lower quantity.The each fraction was dissolved in methanol for phytoconstituent screening and compound analysis. The fraction-IV displayed positive results for all phenolic, flavonoid and flavonol tests held by standard phytochemical screening tests [14]. Therefore this effective fraction-IV was selected to spectroscopy characterization and physical properties were observed, Yellowish brown powder, melting point 305-310^{o}C, molecular weight is expected in 290-310 range, soluble in ethylacetate, acetone, methanol and DMSO.

3.5. UV-Visible spectroscopy analysis

Presence of phenolic compounds was further confirmed by UV- Visible spectroscopic analysis. Phenolic compounds exhibit two major absorption bands in the ultraviolet/visible region, a band-I in the range between 320 and 380 nm and a band-II in the 250 to 285 nm range [20]. The UV- Visible spectrum (200-600 nm) of leaves of *Achyranthes aspera*methanol and aqueous extracts is reported[21]. The absorption peaks at 230 (aqueous extract), 420 and 470 nm (methanol extract), characteristic of flavone/flavonol derivatives, carotenoids and b-cryptoxanthin epoxide respectively

The isolated fraction exhibited two absorption bands in UV spectrum **Figure 3**. The maximum absorption band observed at 203nm (band-I) is corresponding to $\pi \rightarrow \pi^{*}$ transition for C=C and C=O group and band-II absorption at 290nm is corresponding to $n \rightarrow \pi^{*}$ transition represented α,β-unsaturated C=O group respectively. From the UV results showed the isolated fraction is flavonol in nature.

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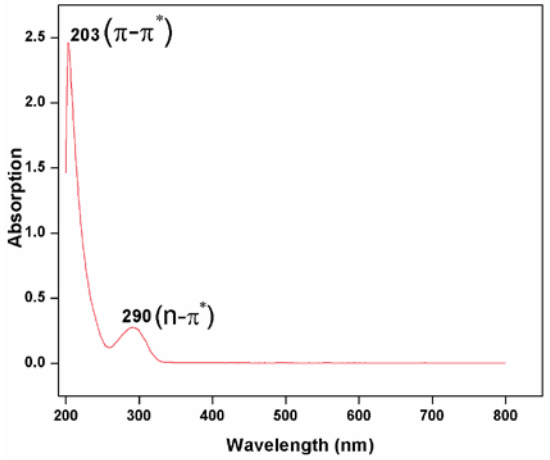


Figure 3. UV-Visible spectrum of Asparagusrecemosus stem isolated compound

3.5. FT-IR spectroscopy analysis

FT-IR fingerprinting provides functional group of isolated compound and the chemical characterization. The spectral analysis of rutin and ethanolic extract of *Polyalthia longifolia* leaves showed wave numbers with functional groups: 1450/1449 cm^{-1} indicating C-H bend stretching alkanes, 3366/3350 cm^{-1} indicating OH stretched phenols, 2833/2834 cm^{-1}indicating H-C=0 stretched aldehydes, 2941/2943 cm^{-1}indicates C-H stretched alkanes, 2230/2326 cm^{-1}indicates C(triple bond) N stretched nitriles, 1652/ 1650 cm^{-1} indicating conjugated dye and 679/657 indicating =C−H out of plane (loop) bending or aromatic mono substituted benzene (C-H) bond, 1030/ 1026 cm^{-1} indicating C-N stretched aliphatic amines. The fingerprint also proved the presence of OH stretched phenol, conjugated dye and monosubstituted benzene, which are common functional groups for all the flavonoid reported in plant world. Recently, FT-IR associated KBr pellet method [22].

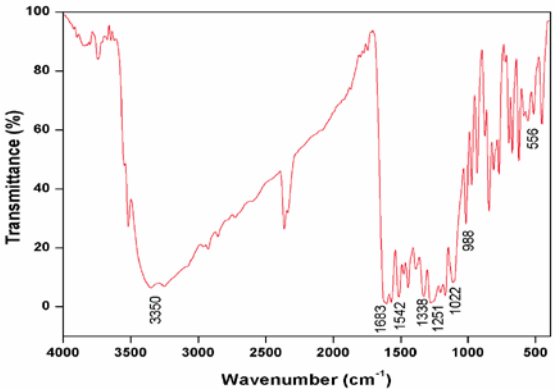


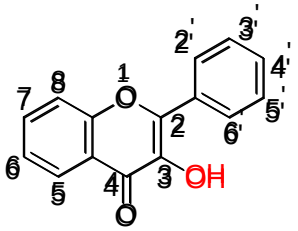
Figure4. FT-IR spectrum of isolated fraction of Asparagusrecemosus stem isolated compound

FT-IR spectrum exhibited absorption in the range from 3350 cm^{-1} to 556 cm^{-1}in **Figure 4** for isolated fraction-IV of stem methanol extract of *Asparagusrecemosus*. The spectrum exhibited broad peak in the range of 3350 cm^{-1} for aromatic hydroxyl group. A sharp peak at 1683 cm^{-1} indicated the presence of (C=O) carbonyl group in the isolated compound. The sharp peaks in the range 1542 cm^{-1} to 1449 cm^{-1} indicated the aromatic -C=C- bond. Sharp peaks at 1338 cm^{-1} to 1251 cm^{-1} for bending mode of -C-O- and 1121 to 1022 cm^{-1} for stretching mode of -C-O-, further the presence of peaks at 988 to 556 cm^{-1} represents the -C-H group out of the plane bending for aromatic compound. The IR spectrum results is also correlated with UV results so isolated compound is tentativelyidentified as flavonol.

IV. CONCLUSION

The present studies revealed the *Asparagus recemosus*stem has valuable phytochemical profile. The bioactive compounds such as phenolic, flavonoids, alkaloids, steroid, terepenoids and saponins etc were detected for stem and had significant content of phenolic and flavonid constituents. Therefore we assure that*Asparagus recemosus* stem willalso provide versatile pharmacology benefits like its root. The stem methanol extract had excellent radical scavenging activity against the DPPH radicals and reducing capacity exposed its potent antioxidant properties. Hence the methanol extract can apply to healing free radical mediateddiseases. The isolated fraction is identified in flavonol nature consequently being effective antioxidant agent for alternate of synthetic agent with risk-free to benefit of medical society. This worth full studies are continued for future studies focused of purification, structual elucidation of biologically active compounds and to examine their pharmacuatical profile.

Flavonol



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