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Original Research Article

PHYTOCHEMICAL PROFILE AND *INVITRO* ANTIOXIDANT ACTIVITY OF *ASPARAGUS RACEMOSUS* ROOT EXTRACT, ISOPRINOSINE AND SHATAVARI SYRUP

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Abstract: A number of medicinal plants have been used in traditional system of medicine all over the world since the beginning of human civilisation, aiming to maintain health and to cure diseases. Oxidative stress has emerged as a key role player in the pathophysiology of several diseases in humans as well as in experimental animal models. Plants can represent a source of novel compounds with promising antioxidant activity since they produce a lot of antioxidants. In recent days there has been an increasing interest worldwide to identify antioxidant compounds that are pharmacologically active with less or no side effects. In the present study the phytochemical screening and *in vitro* antioxidant activity of *Asparagus racemosus* Root Extract, Isoprinosine and Shatavari Syrup was assessed. To assess the antioxidant potential, 1,1-diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging assay, 2, 2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging assay, Ferric Reducing Antioxidant Power assay and Total Antioxidant Capacity were carried out. The findings of the present study indicate that ethanolic Shatavari syrup and *Asparagus racemosus* root extract are excellent free-radical scavengers and potent natural antioxidant.

Key words: Phytochemical, antioxidant, *Asparagus racemosus*, Isoprinosine, Shatavari.

Introduction

The use of plant species in several domains including medicine, nutrition, repellents, cosmetics flavouring, and fragrances has been a practice from ancient times[1]. Most of the plant species used in traditional medicine have been recognised to impart beneficial influence on health including antioxidant activity, antiinflammatory, digestive stimulation action, antimutagenic effects, antimicrobial, hypolipidemic, and anticarcinogenic potential[2,3].In recent years, screening of medicinal plants for biologically active compounds has become an important source of drugs for various ailments with increasing recognition for the use of herbal medicine as an alternative form of health care.

Natural antioxidants and their association with health benefits have gained much importance during recent decades[4]. Plants as potential sources of natural antioxidants have been well

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documented. The balance between antioxidation and oxidation is believed to be a critical concept in maintaining a healthy biological system in modern system of medicine[5]. Therefore, *invitro* screening methods have been used for further in-depth pharmacological and chemical investigation for selecting crude plant extracts with potential useful properties[6].

Asparagus racemosus, also known by the name Shatavari is a spinous under shrub which belongs to family Asparagaceae. It is known to prevent ageing, increase longevity, impart immunity, improve mental function, and it is also used in nervous disorders, dyspepsia, tumors etc and is one of the well known drugs in Ayurveda.Pharmacological activities of *A. racemosus* root extract which include antiulcer, antidiabetic and immunomodulatory activities has been well reported[7,8].

Isoprinosine is an immunostimulator. It has been shown to enhance production of cytokines such as IFN-γ, IL-1and IL-2, increases active T-cell rosettes and induces T-cell surface markers on prothymocytes and also increases proliferation of lymphocytes in response to mitogenic or antigenic stimuli[9].

Shatavari syrup is usually prescribed for hormonal regulation and also acts as galactogogue in nursing women. Its prolonged use in traditional ayurveda to support normal digestive function has been noted[10].Based on the traditional knowledge and lack of scientific evidences for the potential pharmacological properties, the objective of the present study was to evaluate and compare the phytochemicals and *in vitro* antioxidant activity of *Asparagus racemosus* Root Extract, Isoprinosine and Shatavari Syrup which is helpful for further in depth scientific investigation.

Materials and Methods

The present study was carried out at Central Research Laboratory, K.S Hegde Medical Academy, Deralakatte, Mangalore.

Collection of Plant Material and synthetic compound: *Asparagus racemosus* roots were collected from Coorg in March 2015 and were identified.

***Asparagus racemosus* root**

Preparation of Root Extracts

Ethanolic extract: *Asparagus racemosus* (AR) roots were dried in hot air oven at 40°-50°C for a week. The dried roots were powdered using mixer grinder, further subjected to soxhlet extraction with 99% ethanol for 48 hours. The resulted mixture was evaporated to dryness using a rotary flash evaporator and the condensed extract was stored in refrigerator which was then used for preliminary phytochemical screening following standard procedures.

Aqueous Extract: AR root powder was boiled in distilled water for 15-20 minutes. It was kept overnight at room temperature and filtered using whatmann filter paper. The filtrate thus obtained was evaporated to dryness using a heating mantle and the condensed extract was obtained which was stored in refrigerator. This condensed extract was then used for preliminary screening of phytochemicals following standard procedures.

Procurement of Isoprinosine and Shatavari syrup: Isoprinosine tablets were procured from Brandmedicines, European Union. Shatavari syrup was procured from a Himalaya Drug store in Mangalore.

Phytochemical analysis of different Crude extracts and Shatavari syrup: Qualitative phytochemical tests were carried out according to the method of Raman[11] (2006) and Harborne[12] (2005) to identify some phytoconstituents in the crude extracts and shatavari syrup.

Test for Steroids and Triterpenoids: Liebermann Burchard test - To the solution of crude extracts, few drops of acetic anhydride

was added (ethanolic and aqueous). It was boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and was observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Test for Glycosides: Keller Killiani Test - Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added from the sides of the test tube, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Bromine water test - Test solution was dissolved in bromine water and was observed for the formation of yellow precipitate indicating positive result for glycosides.

Test for Saponins: Foam Test - Test solution when mixed with water and shaken would result in the formation of froth, which is stable for 15 minutes indicating a positive result.

Froth test: To 5 mL of the test solution a drop of sodium bicarbonate solution was added.

The mixture was shaken vigorously and left for 3 minutes. It was observed for the formation of honey comb like froth for the positive result.

Test for Alkaloids: Hager's Test – Test solution when treated with few drops of Hager's reagent (saturated picric acid solution) would result in the formation of yellow precipitate indicating positive result for the presence of alkaloids.

Wagner's test (Iodine in Potassium iodide): The test solution was treated with few drops of Wagner's reagent. It was then observed for the formation of reddish brown precipitate which would indicate the presence of alkaloids.

Test for Flavonoids: Ferric chloride test - The test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test - Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which would become colourless on addition of few drops of dilute Hydrochloric acid, indicating the presence of flavonoids.

Lead acetate solution Test - Test solution was treated with few drops of lead acetate (10%) solution and observed for the formation of yellow precipitate which would indicate the presence of flavonoids.

Shinoda test - Test solution was treated with few fragments of magnesium ribbon and concentrated hydrochloric acid and observed for the appearance of red to pink colour after few minutes for the presence of Flavonoids.

Test for Tannins: Gelatin Test - Test solution was treated with gelatin solution to give white precipitate indicating the presence of tannins.

Test for Proteins: Biuret Test - Test solution when treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution would result in the formation of violet/pink colour indicating the presence of proteins.

Test for Free Amino Acids: Ninhydrin Test - Test solution was boiled with 0.2% solution of Ninhydrin and observed for the formation of purple colour suggesting the presence of free amino acids.

Test for Carbohydrate: Benedict's test - Few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) was added to the test solution and boiled in water bath, observed for the formation of reddish brown precipitate for a positive result.

Test for Vitamin C: DNPH Test - Dinitrophenyl hydrazine was dissolved in concentrated sulphuric acid and added to the test solution. It was observed for the formation of yellow precipitate which would indicate the presence of vitamin C.

Test for Sterols: Salkowski test: To the test solution in chloroform, few drops of concentrated sulphuric acid was added, shaken and allowed to stand, observed for the appearance of red colour in lower layer which would indicate the presence of sterols.

Test for Resins: The extract was dissolved in acetone and added to distilled water. Turbidity in the mixture would indicate the presence of resins.

Assessment of *Invitro* Antioxidant Activity: The *invitro* antioxidant potential of *Asparagus racemosus* root ethanolic and aqueous extract (ARE & ARA), Isoprinosine (IPR) and Shatavari syrup (STR) was assessed using DPPH Radical Scavenging, ABTS radical scavenging, Ferric Reducing Antioxidant Power and Total Antioxidant Capacity assays.

DPPH Radical Scavenging Assay: The free radical scavenging capacity of ARE, ARA, IPR and STR was determined using DPPH assay according to the method of **Blois** (1958)[13] which was slightly modified. Freshly prepared DPPH solution (0.004% w/v) in 99% ethanol was added to test solutions (0-1000µg/ml) and then incubated at room temperature in dark for 20 minutes. Following incubation, the mixture was vortexed and the optical density was measured at 517nm using a spectrophotometer. 99% ethanol was used as blank. Control sample was prepared containing the same volume without any extract. Ellagic acid (EA) was used as a reference standard. All tests were performed in triplicates. Percentage scavenging of the DPPH free radical was calculated using the following equation, DPPH radical scavenging activity (%) = (Acontrol-ATest)/Acontrol X 100. Where 'A' control indicated the absorbance of the control reaction and 'A' test indicated the absorbance in the presence of the extracts or standard. Lower absorbance of the reaction mixture suggested higher free radical scavenging activity.

ABTS Radical Scavenging Assay: The 2, 2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) - commonly called as ABTS cation scavenging activity was carried out. The $ABTS^{\bullet+}$ reagent was prepared by reacting 7 mM $ABTS^{\bullet+}$ stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hours prior to use. The resulting mixture was diluted with deionised water and 95% ethanol (1:1) to obtain an absorbance of 0.70(±0.02) at 734 nm. Different concentrations of the sample solutions, ARE, ARA, IPR and STR (0-250 µg/ml) was mixed with 3 ml of diluted $ABTS^{\bullet+}$ solution. The absorbance was recorded at 735 nm after 20 minutes incubation in dark at room temperature. Control sample was prepared containing the same volume of the reagent without any extract. Water: 95% ethanol (1:1) was used as blank. All tests were performed in triplicates. Ellagic acid was used as a reference standard. Percentage scavenging of the $ABTS^{+}$ free radical was calculated using the equation, $ABTS^{\bullet+}$ radical scavenging activity (%) = (Acontrol-ATest)/Acontrol X 100. Where 'A' control indicated the absorbance of the control reaction and 'A' test indicated the absorbance in the presence of the extracts or standard.

Ferric Reducing Antioxidant Power Assay: To determine Fe^{3+}reducing power of the extracts the method of **Oyaizu** [15] with a slight modification was followed. Different concentration of the sample solutions ARE, ARA, IPR and STR (0-250 µg/ml) were taken and the volume of each is made upto 1 ml with distilled water. 0.5 ml of 1% potassium hexacyanoferrate (potassium ferricyanide) was added and incubated at 50°C in a water bath for 20 minutes. Control tube is maintained without adding the extract. After incubation, 0.5 ml of TCA (10%) was added to terminate the reaction. The upper portion of the solution was mixed with 1 ml distilled water, and 0.1 ml $FeCl_3$ solution (0.1%) was added. The reaction mixture was incubated for 10 minutes at room temperature and the Optical density was measured at 700 nm against an appropriate blank solution. Ellagic acid was used as reference standard. All tests were performed in triplicates.

Total Antioxidant Capacity: The total antioxidant capacity of ARE, ARA, IPR and STR was determined using Phosphomolybdenum method of **Prieto et al.** (1999) [16]. Different concentration of the sample solutions ARE, ARA, IPR and STR (0-250 µg/ml) were taken and the volume of each is made upto 2 ml with distilled water. Trichloroacetic acid (0.5 ml) was added to the

sample solutions to precipitate out the proteins if any. The mixture was then allowed to stand for about five minutes and centrifuged. 100µL of the clear supernatant was transferred into a clean test tube to which 1ml of Total Antioxidant Capacity (TAC) reagent was added and the mixture was incubated in water bath at 90^{o}C for 90 minutes. After incubation, the reaction mixture was cooled and the optical density of the greenish to bluish colour formed was read at 695nm against appropriate blank. Ellagic acid was used as reference standard. All tests were performed in triplicates. The total antioxidant activity was expressed as the number of microgram equivalents of Ellagic acid.

Statistical Analysis: The obtained experimental data were expressed as Mean±SD of three measurements.

Results and Discussion

Phytochemical Analysis: The ethanol and aqueous extracts of *Asparagus racemosus* root revealed the presence of Saponins, Flavonoids, Tannins, free amino acids, Carbohydrate, Vitamin C and sterols. Tannins was present only in the ethanol root extract. Shatavari syrup showed positive test for saponins and carbohydrates. The results of preliminary phytochemical screening are shown in Table 1.

Table 1.Qualitative Phytochemical Screening

Chemical Tests	***Asparagus racemosus* root extract**		**Shatavari**
	Ethanol	**Aqueous**	
I. Test for Triterpenoids & Steroids			
Liebermann Burchard Test	-	-	-
II. Test for Glycosides			
Keller Killiani Test	-	-	-
Bromine water	-	-	-
III. Test for Saponins			
Foam test	+	+	+
Froth test	+	+	+
IV. Test for Alkaloids			
Hager's Test	-	-	-
Wagner's test	-	-	-
V. Test for Flavonoids			
Ferric Chloride test	+	+	-
Alkaline reagent test	+	+	-
Lead Acetate Solution test	+	+	-
VI. Test for Tannins			
Gelatin Test	+	-	-
VII. Test for Proteins			
Biuret test	-	-	-
Xanthoproteic test			
VIII. Test for Free amino acids			
Ninhydrin Test	+	+	-
IX. Test for Carbohydrates			
Benedict's Test	+	+	+
X. Test for Vitamin C			
DNPH test	+	-	-
XI. Test for sterols			
Salkowski test	+	-	-
XII. Test for Resins	-	-	-

'+' and '-'indicates the presence and absence of phytochemicals respectively.

Phytochemical constituents which occur naturally in plants contribute to defense mechanism and protect from various diseases. The phenolic compounds in plants have been shown to exert multiple biological activities which include antioxidant, free radical scavenging, antiinflammatory, anti carcinogenic etc [17].

The phenolic and flavonoids are widely distributed secondary metabolites in plants responsible for a wide range of biological activities such as anti-oxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities[18,19]. Recent studies have shown that most dietary polyphenolic constituents derived from plants are more effective antioxidants *In-vitro*, and thus might contribute significantly to the protective effects *in vivo* [20].

The obtained results of phytochemical analysis indicate that *Asparagus racemosus* root extract and Shatavari syrup hold promises as source of pharmaceutically important phytochemicals.

Antioxidant Studies: *In-vitro* antioxidant studies are widely carried out to screen the antioxidant potential of phytoconstituents. Plant derived antioxidant compounds have received considerable attention because of their physiological effect like antioxidant, anti-inflammatory, antitumour activities and low toxicity compared with those of synthetic phenolics [21,22].

DPPH Radical Scavenging Assay: DPPH assay is widely used to evaluate the free radical scavenging activity. DPPH is a free radical stable at room temperature, which produces a violet solution in ethanol and shows a strong absorption band in visible spectrum at 517 nmUsing this assay, ethanolic and aqueous *asparagus* root extracts, Isoprinosine and shatavari syrup were evaluated for their free radical scavenging potential using Ellagic acid as standard. The DPPH radical scavenging effect of ARE, ARA, IPR, STR and EA at different concentrations is summarized in Fig 1. At various concentrations tested, 200,400,600,800 and 1000µg/ml, the percentage scavenging activity of ARE, ARA, IPR, STR and EA were found to be56.34±0.266, 64.82±0.177, 73.11±3.19, 78.95±0.62, 83.54±0.53; 42.82±0.86, 47.85±0.69, 50.24±0.95, 54.84±1.73, 56.93±0.69; 37.54±1.56, 39.07±0.26, 40.92±0.60, 40.73±0.34, 41.28±0.26; 84.78±0.34, 90.36±0.08, 89.38±0.08, 89.63±0.78, 87.36±1.21 and 68.90±1.33, 75.75±0.35, 80.77±0.17, 83.54±0.35, 84.92±0.17 respectively. The increase in the scavenging activity was observed with increase in concentration of extracts. From the results of DPPH assay, we found that the highest antioxidant activity was exhibited by Shatavari syrup and ethanolic *Asparagus racemosus* root extract.

According to our observations, the strong activity of the ethanolic *Asparagus racemosus* root extract and shatavari syrup might be due to the available hydroxyl group present in the substance [23].

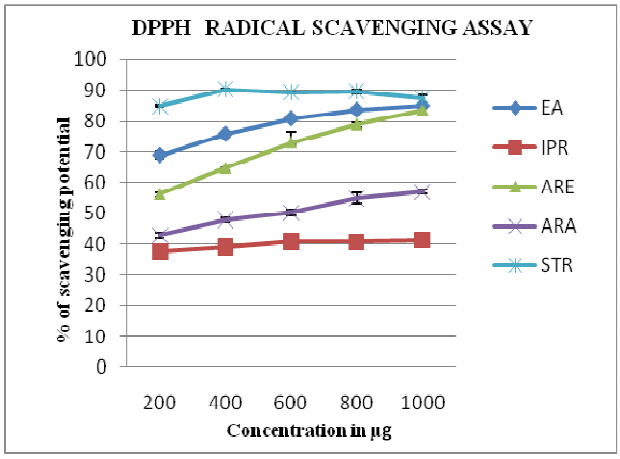

Figure 1: DPPH Radical Scavenging activity of EA, IPR, ARE, ARA and STR.

ABTS Radical Scavenging Assay

The basic principle of the $ABTS^{\bullet+}$ decolorization assay is that $ABTS^{\bullet+}$, on reaction with $K_2S_2O_8$, results in the formation of a greenish blue radical cation. Standard and sample antioxidants are able to transfer an electron to ABTS radical cation and scavenge the color of the solution proportionate to their amount. The scavenging potential depends both upon the concentration of antioxidant and time duration for the reaction under analysis[24] .The $ABTS^{\bullet+}$ radical scavenging

potential of ARE, ARA, IPR and STR at different concentrations is shown in Fig 2. Standard ABTS assay with slight modification was used which showed absorption maximum 734 nm. In this assay, ethanolic and aqueous *asparagus* root extracts, Isoprinosine and shatavari syrup were evaluated for their free radical scavenging potential using Ellagic acid as standard. At different concentrations tested, 50,100,150,200 and 250µg/ml, the percentage scavenging activity of ARE, ARA, IPR, STR and EA were found to be55.25±0.09,67.92±0.19,72.40±0.89,72.89±0.39,76.75±0.59;28.78±0.49,31.58±1.58,34.24±0.49,40.68±0.49,43.83±1.98;20.23±3.26,23.17±1.88,24.92±6.33,26.12±2.07,26.61±4.35;68.41±2.67,68.48±2.97,75.77±3.46,83.33±0.39,91.87±0.79and84.31±0.39,88.79±0.39.92.99±0.39,96.35±0.59,98.03±0.39. The scavenging potential of the extracts increased in a dose dependent manner.According to the obtained results, the highest free radical scavenging potential of ethanolic *Asparagus racemosus* root extract and shatavari syrup might be due to their antioxidant capability to inhibit the oxidation of ABTS to $ABTS^{\bullet+}$ radical cation[25].

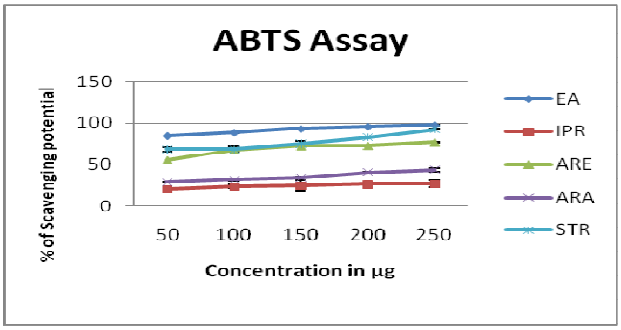

Figure 2: ABTS Radical Scavenging activity of EA, IPR, ARE, ARA and STR.

Ferric Reducing Antioxidant Power Assay

The Fe^{3+}reducing power of ARE, ARA, IPR and STR at different concentrations is shown in fig 3. At different concentrations tested, 50,100,150,200 and 250µg/ml, the scavenging activity of ARE, ARA, IPR, STR and EA were found to be 0.026±0.002, 0.073±0.002, 0.163±0.002, 0.282±0.004,0.507±0.002;0.023±0.004,0.015±0.008,0.050±0.016,0.082±0.022,0.155±0.010;0.074±0.004, 0.075±0.009, 0.091±0.003, 0.094±0.011, 0.10±0.009; 0.47±0.144, 0.79±0.002, 1.19±0.055, 1.30±0.035, 1.51±0.089 and 0.89±0.038, 0.98±0.036, 1.09±0.025, 1.10±0.025, 1.55±0.057 respectively. The reducing power of the extracts increased in a dose dependent manner with increase in concentration which showed absorption maximum at 700 nm. In this assay EA and STR exhibited highest reducing potential followed by ARE, ARA and IPR. The reducing power of the extracts is based on the hydrogen donating ability. The reducing property of a compound serves as a significant indicator of its potential antioxidant activity[26]. The increased reducing power in STR and ARE indicated that some components in the syrup and extract were strong electron donors that could react with the free radicals to convert them into more stable products to terminate radical chain reaction.

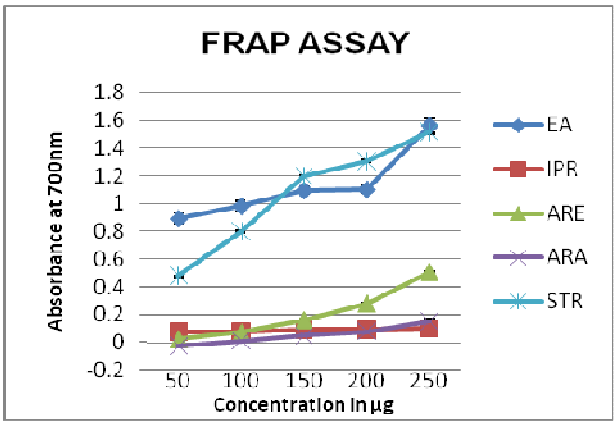

Figure 3: Ferric Reducing Antioxidant power of EA, IPR, ARE, ARA and STR.

Total Antioxidant Capacity

The Total Antioxidant capacity of ARE, ARA and STR at different concentrations is shown in figure 4. The Phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex which has a maximal absorption at 695 nm. In this assay, the total antioxidant capacity of ARE, ARA and STR in Ellagic acid equivalents [EAE (µg/ml)] at different concentrations tested, 50, 100, 150, 200 and 250µg/ml, were found to be 4.28±0.20, 10.92±1.51, 14.85±0.20, 21.28±0.20, 26.0±1.41, 0.92±1.51, 0.42±0.80, 1.85±0.20, 2.07±0.30,

2.92±0.90 and 123.71±0.21, 170.92±0.11, 202±0.01, 92.24±0.11, 261.28±0.12 respectively. Total Antioxidant Capacity (TAC) of Isoprinosine was very less whereas the total antioxidant capacity of the extracts increased linearly in a dose dependent manner with shatavari syrup showing the highest antioxidant capacity. A significant variation of antioxidant activity could be attributed to the redox properties of phytoconstituents present in the extracts and shatavari which acts as reducing agents, hydrogen donors, free radical scavenger, singlet oxygen quenchers and metal chelators[27]. The results of TAC indicate that the saponins and phytoconstituents present in STR, ARE and ARA are major contributors to antioxidant activity.

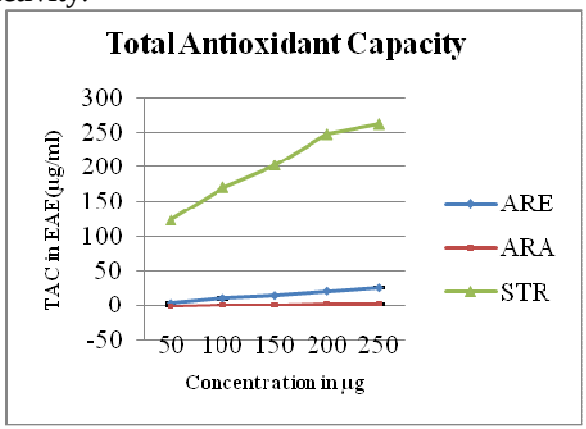

Figure 4: Total Antioxidant Capacity of ARE, ARA and STR.

Conclusion: Through our systematically comparative study, the ethanolic *Asparagus racemosus* root extract and Shatavari syrup were found to be excellent free-radical scavengers and potent natural antioxidant. The present study revealed the presence of various important phytochemicals in the ethanolic *Asparagus racemosus* root extract and proves that the root extract can be of medicinal importance. These results may contribute for further standardisation and research in *Asparagus racemosus* root based drugs which are used in traditional and modern plant based medicines.

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