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ORIGINAL ARTICLE

In vitro antioxidative potential of hydromethanolic rhizome extract of *Asparagus racemosus* willd.

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ABSTRACT

An imbalance between the generation and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products leads to oxidative stress. ROS can and do play several physiological roles and they are normally produced as byproducts of oxygen metabolism however environmental stressors and xenobiotics contribute to greatly increased ROS production, resulting in the imbalance that leads to cell and tissue damage. Several antioxidants have been studied in recent years for their real or alleged anti-oxidative stress properties. Therefore, present study was to carry out to evaluate the antioxidant properties of hydromethanolic extract of rhizome of Asparagus racemosus. Antioxidant properties were determined by phosphomolybdenum assay of total antioxidant activity, reducing power assay, hydrogen peroxide radical and nitric oxide radical scavenging assay.The total antioxidant capacity was found to be 146.28 mg ascorbic acid equivalents per g of extract. The reducing power was increased in dose dependent manner from 0.031 at 10µg/ml ARE up to 0.365 at 200µg/ml ARE. The IC_{50} value of ARE was found to be 54.46 µg/ml and 51.43 µg/ml when determined by hydrogen peroxide radical and nitric oxide radical scavenging assay respectively. Thus, obtained data highlighted the potential role of ARE as a source of natural antioxidant which might be useful for further studies to fight against oxidative stress related health issues.

***Keywords**: Asparagus racemosus, antioxidant potential, TAC, H_2O_2 radical scavenging assay; NO radical scavenging assay.*

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INTRODUCTION

Majority of the diseases/disorders are mainly connected to oxidative stress caused by free radicals. Free radicals are defined as free entities that include one or more unpaired electrons [1]. They can be produced as a by-product of different endogenous (such as normal cellular metabolism) or exogenous (such as irradiation) activities. Free radicals can easily combine with reactive oxygen species (ROS) to form active radicals. ROS are different activated forms of oxygen and divide into two groups, free activated oxygen radicals (such as superoxide anion radical O_2^- and hydroxyl radical OH^-) and non-free activated oxygen radicals (like hydrogen peroxide H_2O_2, singlet oxygen O_2). These activated ROS cause 10,000 oxidative impacts on human cells every second [2]. ROS have been linked to hundreds of disease, including arthritis and connective tissue disorders, as well as carcinogenesis, ageing, physical damage, infection, and acquired immunodeficiency syndrome [3, 4].

Antioxidant therapy has become extremely important in the treatment of various disorders. The current focus of study is on discovering naturally occurring antioxidants of plant origin. Antioxidants have been shown to reduce oxidative damage caused by free radicals and reactive oxygen species (ROS), and may help to prevent illness, cancer, and ageing. It can disrupt the oxidation process by interacting with free

radicals, chelating catalytic metals, and serving as oxygen scavengers[5, 6]. Natural products have long been a valuable source of therapeutic agents. Natural products account for approximately 25% to 30% of all medications available for treatment of diseases [4, 7]. Natural product research is typically based on ethnobotanical data; currently, many medications are derived from medicinal plants utilised in indigenous societies [8]. The medicinal properties of plants have been studied in recent scientific discoveries all over the world, owing to their high antioxidant activity, lack of side effects, and economic feasibility [9, 10]. Flavonoids and phenolic compounds are extensively dispersed in plants and have been shown to have a variety of biological effects, including antioxidant, free radical scavenging, anti-inflammatory, and anticarcinogenic properties [4, 11]. Novel natural antioxidants derived from plants have been actively researched for their antioxidant and radical scavenging activities in recent years. As a result, medicinal plants and natural product extracts have been proposed as an alternative therapy for a variety of ailments [12].

Asparagus racemosus (AR) is a member of the Asparagaceae family and is often known as shatavari, satawar, or satmuli in India. Its therapeutic properties have been described in Indian traditional medicine systems such as Ayurveda, Unani, and Siddha. AR is a well-known Ayurvedic rasayana that is used to treat nerve diseases, dyspepsia, tumours, inflammation, neuropathy, and hepatopathy, as well as to prevent ageing, promote lifespan, impart immunity, improve mental performanceand addvitality to the body. According to reports, AR root extract has antiulcer, antioxidant, and antidiarrheal properties, as well as antidiabetic and immunomodulatory properties, beneficial in nerve disorders, dyspepsia, diarrhoea, dysentry, tumours, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity, and some infectious illnesses [13,14, 15]. The main active elements of AR roots are steroidal saponins (Shatavarins I-IV). Shatavarin IV was found to have strong activity as an inhibitor of core Golgi enzyme transferase and, more recently, immunomodulatory action against certain T-dependent antigens in immunocompromised mice [16]. Therefore, the objective of the present study was to investigate the *in vitro* antioxidant activity of hydromethanolic rhizome extract of *Asparagus racemosus* Willd.(ARE) through estimation of total antioxidant capacity, reducing power, hydrogen peroxide scavenging activity and NO scavenging activity.

MATERIAL AND METHODS

Rhizomes of *Asparagus racemosus* Willd. were collected from MRDC, G.B.P.U.A.&T, Pantnagar, India. The plant material was cleaned with running tap water, and dried in the shade. With a motor grinder, the dried plant material was crushed to powder and stored in an airtight container at 4°C until further usage. Biological Science Department G.B.P.U.A.&T, Pantnagar, India, validated the identification of *Asparagus racemosus*Willd. The voucher specimen was also submitted to Biological Science Department G.B.P.U.A.&T, Pantnagar, India.

Extraction procedure

For preparation of ARE, the extraction procedure described by [15, 17] was adopted. 100g of this shade dried powder was mixed with 1000 ml of a double distilled water and methanol solution (1:1). For 48 hours at 37°C, the solution was homogenised in an incubator cum shaker. Muslin cloth was used to filter the mixture, which was then followed by Whatman filter paper No.1. Before being freeze dried, the extract was rotary evaporated at 45°Cto eliminate surplus solvent. Finally, after lyophilization, the extract was weighed and stored in a deep freezer at -20°Cuntil further use.

Estimation of Antioxidant Potential of ARE

Total Antioxidant Capacity estimation

The total antioxidant capacity of the extract was measured using the phospshomolybdenum procedure [18] and expressed as mg ascorbic acid equivalent/g of extract. Ascorbic acid in concentrations ranging from 2 to 20 g was used to make the calibration curve. On interaction with freshly prepared phosphomolybdate reagent solution(0.6M sulphuric acid, 28mM tri sodium phosphate, 4 mM ammonium molybdate), plant extract reduced Molybdenum (VI) and formed greenish phosphate Molybdenum (V) complex. The tubes were sealed, and the reaction mixture was incubated in a boiling water bath at 95°Cfor 90 minutes. After allowing the samples to cool to ambient temperature, absorbance was measured in a UV-visible spectrophotometer at 695 nm against a blank and the findings were recorded.

Reducing power estimation of the extract

The extracts' reducing power ability was assessed using a slightly modified approach of the ferric reducing-antioxidant power assay [19]. For 30 minutes at 50°C, the extract was incubated with 2.5 ml of phosphate buffer (0.1 mol/L pH 6.6) and 2.5 ml of 1 percent (w/v) potassium ferricyanide. To halt the reaction, 2.5 ml of 10% (w/v) trichloroacetic acid (TCA) was added to the mixture at the end of the incubation time, and the mixture was centrifuged for 10 minutes at 3000 g. The top layer (2.5 ml) was

diluted with water in equal parts and stirred with 0.5 ml of fresh ferric chloride. The absorbance at 700 nm was measured using a UV-Visible spectrophotometer against a blank. The reference solution was prepared.

Hydrogen peroxide (H_2O_2) scavenging activity

Quercetin was employed as a reference antioxidant to assess the extract's ability to scavenge H_2O_2 radicals [20]. After mixing the extract in 0.1 M phosphate buffer (pH 7.4), a 43 mM H_2O_2 solution made in the same buffer was added. The concentrations of extract and reference chemical varied from 1 to 100 g. After 10-minute incubation at room temperature, the absorbance at 230 nm was measured using a UV-Visible spectrophotometer against a blank solution containing phosphate buffer but no hydrogen peroxide. The reference compound's and extract's H_2O_2% scavenging activity was calculated and reported as IC_{50} values.

Nitric oxide (NO) scavenging activity

The antioxidant activity of the extract was measured on the basis of the scavenging activity of free radical according to the method described by [21]. 10mM sodium nitroprusside in phosphate buffer saline was prepared. 200 mg of Griess reagent was dissolved in 5 ml of autoclaved double distilled water. Plant extract at various concentrations ranging from 1-100µg/ml was mixed with sodium nitroprusside to make final volume of 100µl. The mixture was incubated for 150 minutes at 25°C in dark. The extract was mixed with an equal volume of freshly prepared Griess reagent and absorbance was measured at 546 nm using UV-Visible spectrophotometer. Ascorbic acid was used as a positive control and Griess solution along with sodium nitroprusside in the absence of test sample was used as a negative control. The percent NO scavenging activity of the reference compound and extract was computed and the expressed in IC_{50} values.

RESULTS AND DISCUSSION

Asparagus racemosus has long been recommended to treat a variety of disorders whose pathophysiology is connected to oxidative stress. According to the findings of this study, ARE is a good free radical scavenger that may minimise or reverse free radical damage in the human body.

The total antioxidant capacity of ARE was evaluated through reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate complex at acid pH. The total antioxidant capacity of ARE was calculated as ascorbic acid equivalents (AAE) per gram.AREdemonstrated a strong antioxidant capability (146.28 mg ascorbic acid equivalents g^{-1} extract) (Table 1). Several investigations have revealed that various flavonoids and related polyphenols contribute considerably to medicinal plants' phosphomolybdate scavenging action [13, 22]. The total antioxidant capacity of *Asparagus racemosus* root extract, Isoprinosine, and Shatavari Syrup was evaluated and concluded that *Asparagus racemosus* root extract has high total antioxidant activity[17]. The redox characteristics of phytoconstituents found in the extracts, act as reducing agents, hydrogen donors, free radical scavengers, singlet oxygen quenchers, metal chelators and might explain a large variance in antioxidant activity [24].

For the measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transition was examined in the presence of ARE. A compound's reducing capacity might be a good predictor of its potential antioxidant action. However, antioxidant activity has been attributed to a number of pathways, including chain initiation prevention, transition-metal ion catalyst binding, peroxide breakdown, continuing hydrogen abstraction prevention, reductive capacity, and radical scavenging [25]. Figure 1and Table 2 depicted the reductive effect of ARE. The reducing power of ARE was 0.031 at 20 µg/ml and increased to 0.365 at 200 µg/ml. At 200 µg/ml concentration, the standard drug ascorbic acid had a reducing power of 0.544. it was found that the reducing power of *Asparagus racemosus* root extract increased in a dose-dependent manner, with absorption peak at 700 nm[23]. This was also confirmed by other researchers as well [22, 26].

ARE demonstrated concentration dependent H_2O_2 scavenging effect with the values ranging from 9.30% to 74.58% at concentrations ranging from 1 to 100 µg/ml (Figure 2 and Table 3). The methanolic extract of *Asparagus racemosus* demonstrated significant H_2O_2 scavenging activity, ranging from 47.62 % to 64.83 % at doses ranging from 10 to 500 µg/ml [22]. H_2O_2 decomposes fast into oxygen (O_2) and water (H_2O), and it can produce hydroxyl radicals (OH), which can cause lipid peroxidation and DNA damage. ARE was capable of scavenging hydrogen peroxide, which may be due to the presence of phenolic groups, which might transfer electrons to hydrogen peroxide, neutralising it into H_2O.

Nitric oxide (NO) is a powerful pleiotropic regulator of physiological processes such as smooth muscle relaxation, neuronal signaling, platelet aggregation inhibition, and prevention of cell mediated toxicity. It is a diffusible free radical that functions as an effector molecule in a variety of biological processes, including neuronal messenger, vasodilation, and antibacterial and anticancer activity [27]. When nitric oxide combines with superoxide radicals, it creates extremely reactive oxidantn-peroxy nitrite anions

($ONOO^-$), which decompose and release OH and NO, causing oxidative damage. ARE inhibited nitric oxide in a dose-dependent way (Figure 3 and Table 4), with an IC_{50} of 51.43 µg/ml. *Asparagus racemosus*extract displayed significant antioxidant properties by inhibiting nitric oxide in a dose-dependent manner[3].
Thus, our *in vitro* experiments revealed that ARE exhibited outstanding antioxidant activity as measured by total antioxidant capacity and reducing power, as well as scavenging of free radicals such as hydrogen peroxide and nitric oxide, which have roles in the pathophysiology of illnesses such as ageing. The presence of flavonoids and polyphenols in ARE can be linked to its antioxidant capabilities [28].

CONCLUSION

Antioxidant capabilities of medicinal plants are currently of great attention owing to their potential usage as natural additions to replace synthetic ones. Thus, the current study's findings revealed that the hydromethanolic rhizome extract of *Asparagus racemosus*Willd. had potent antioxidant potential which can scavenge different reactive oxygen species (ROS) and free radicals under *in vitro* conditions. The present study suggested that ARE can be used as a good source of natural antioxidants for health benefits and pharmacological properties.

Table 1: Total antioxidant capacity of ARE in terms of ascorbic acid equivalents. Each value represents mean ± SD (n=3)

Plant extract	Total Antioxidant Capacity (AAE mg/g of dry matter)			Mean Total Antioxidant Capacity (AAE mg/g of dry matter)
	I	II	III	
ARE	144.061	146.636	148.151	146.28 ± 2.068

Table 2: Reducing Power of ARE. Each value represents mean ± SD (n=3)

S.No.	Concentration (µg/ml)	Mean OD of ARE ± SD	Mean OD of Ascorbic acid ± SD
1	20	0.031±0.006	0.077±0.005
2	40	0.084±0.006	0.116±0.014
3	60	0.118±0.01	0.139±0.004
4	80	0.198±0.014	0.198±0.005
5	100	0.223±0.014	0.233±0.005
6	120	0.259±0.009	0.276±0.022
7	140	0.277±0.01	0.331±0.006
8	160	0.297±0.012	0.36±0.014
9	180	0.32±0.014	0.43±0.006
10	200	0.365±0.005	0.544±0.019

Table 3: Hydrogen peroxide scavenging (H_2O_2) activity for ARE and Quercetin.Each value represents mean ± SD (n=3)

S.No.	Concentration (µg/ml)	Mean Percent Scavenging of ARE ± SD	Mean Percent Scavenging ofQuercetin ± SD
1	1	9.31±2.142	22.73±1.604
2	5	18.3±0.76	34.69±1.574
3	10	23.27±2.025	45.89±2.457
4	20	30.39±2.014	56.36±0.813
5	40	48.8±1.811	68.23±1.925
6	60	56.23±0.802	80.74±2.632
7	80	64.31±0.723	87.14±1.124
8	100	74.58±0.546	91.59±0.935

Table 4: Nitric oxide scavenging (NO) activity for ARE and Quercetin. Each value represents mean ± SD (n=3)

S.No	Concentration (µg/ml)	Mean Percent Scavenging of ARE ± SD	Mean Percent Scavenging ofQuercetin ± SD
1	1	7.27±0.913	36.36±2.463
2	5	17.4±2.317	52.22±2.384
3	10	27.69±2.13	61.71±1.157
4	20	36.86±2.175	78.78±2.899
5	40	47.45±3.123	86.95±2.402
6	60	55.54±2.193	90.48±1.613
7	80	67.26±2.703	94.7±0.547
8	100	78.76±1.956	96.62±0.529

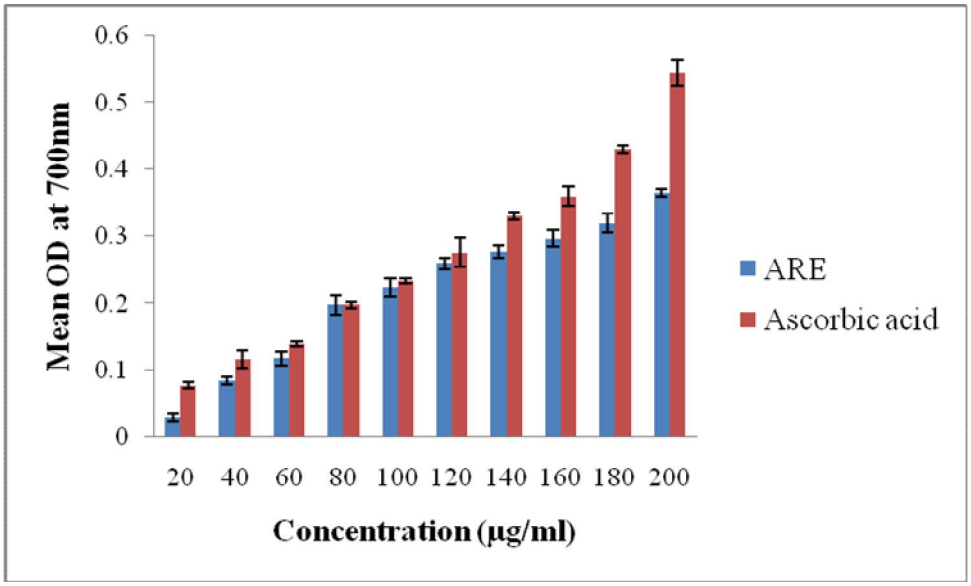

Fig 1: Reducing power for ARE and Ascorbic acid. Each value represents mean ± SD (n=3)

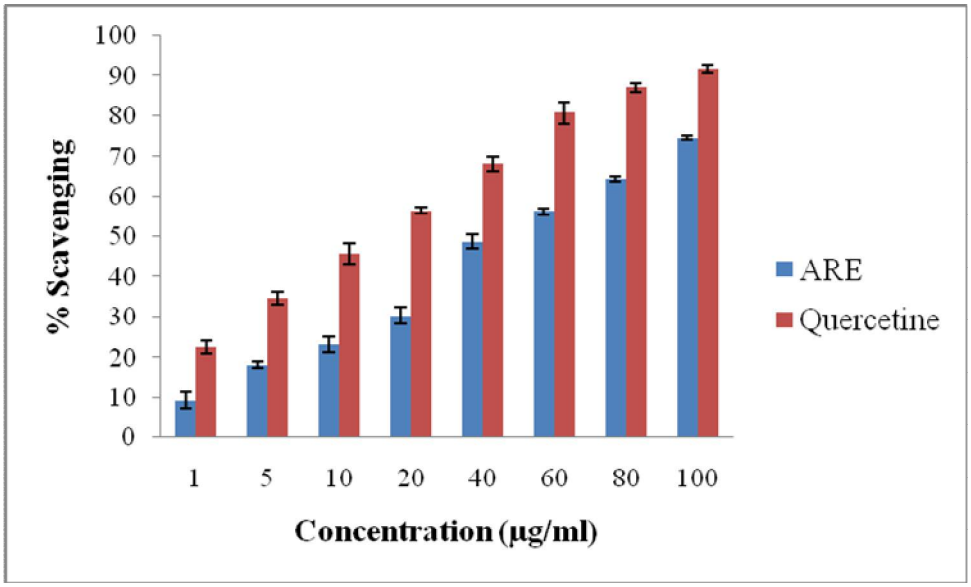

Fig 2: Hydrogen peroxide scavenging (H_2O_2) activity for ARE and Quercetin. Each value represents mean ± SD (n=3)

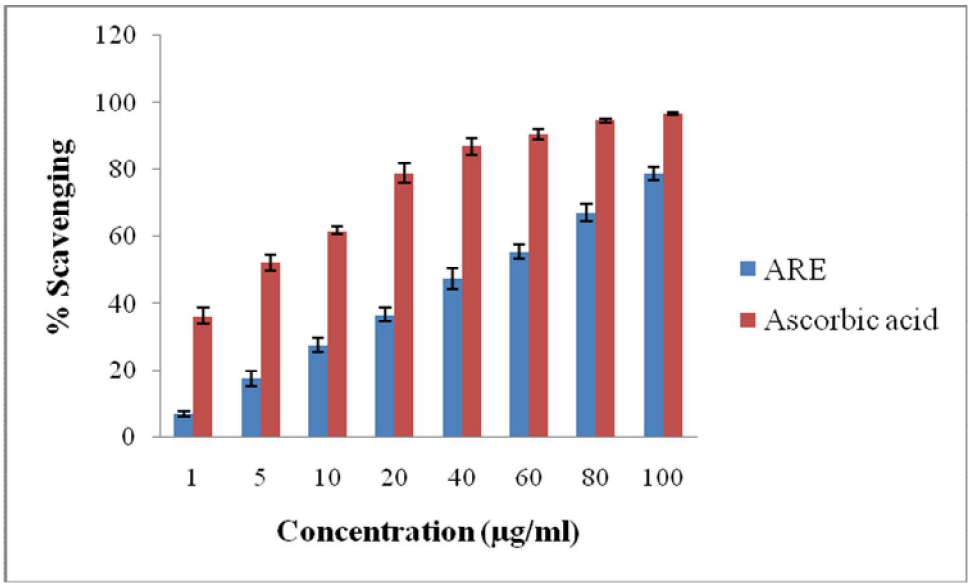

Fig 3: Nitric oxide scavenging (NO) activity for ARE and Quercetin. Each value represents mean ± SD (n=3)

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