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IN VITRO ANTIOXIDANT POTENTIAL OF *ASTILBE RIVULARIS* RHIZOME

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ABSTRACT

The dried rhizomes of *Astilbe rivularis* Buch-Ham ex D.Don have been traditionally used for the treatment of inflammatory diseases, headache and infertility from the ancient period in India. The physiological mechanisms of action, especially anti-inflammatory effect of this plant extract are not yet well understood. In this study, we examined the free radical scavenging effect and anti-lipid peroxidation in successively extracted, purified solvent fractions. The fractions execute significant free radical scavenging potentiality on DPPH (95.23%) at dose of 100 µg/ml, superoxide scavenging activity (80.40%) and anti-lipid peroxidation (45.83%) at the same doses, which are comparable with reference standard. We also investigated the anti-inflammatory effect of this plant extract on rat. The oral application at the dose of 100 mg and 200 mg/kg body weight of this plant extract promoted healing action (56.20 % inhibition at the dose of 200 mg/kg after 24 hrs) on carrageenan induced rat paw inflammation; which is also comparable with standard of non steroidal anti-inflammatory drug (Ibuprofen) 20 mg/kg body weight (48.18% inhibition after 24 hours). The observations from these studies suggest the ethno medicinal use of *Astilbe rivularis* which could be commercially exploited by the pharmaceutical industry.

Keywords: *Astilbe rivularis,* solvent fractions, free radicals scavenging, antilipid peroxidation, anti-inflammatory.

INTRODUCTION

The therapeutic properties of *Astilbe rivularis* Buch.Ham.ex D.Don (AR) [local name: Buro okhati or Bhadhangoo] family Saxifragaceae have known for decades in several traditional therapeutics. It is predominant in gardens and waysides of Darjeeling Hills. High amount of polyphenols, flavonoids, coumarin berginin, triterpenoids, acetyl-β peltoboykniolic acid, peltoboykniolic acid and astilbic acid are present in AR rhizomes. Major traditional uses of its dried rhizomes are mainly for the treatment of headache and infertility by the tribal community of the hills**.** For several decades, numerous medicinal preparations of AR rhizomes have been used as potential phyto-therapeutics among different ethnic races (Chettri *et al*., 2005)**.** In addition to medicinal uses, AR rhizomes are processed into various products like antecedent (Tandon *et al*., 1996).

The increasing interest in alimentary applications of herbal components rich in antioxidant is due to possible correlation between the oxidant action of the free radicals and the onset of some important pathology. The drugs which are in presently for the management of pain and inflammatory condition are either narcotic e.g. opoid or non-narcotics e.g. Salicylates and corticosteroids e.g. hydrocortisone. All these drugs have well known side and toxic effects. Moreover synthetic drugs are very expensive to develop for the successful introduction of new products. Therefore many medicines of plant origins has been used since long time without any side effects. In the very recent consumer preference has also shifted the attention from costly synthetic to cheaply natural less toxic molecules (Dapkevicius *et al*., 1998). Several components present in plants have reported to be biologically active antioxidant and have bioregulatory properties. AR rhizomes are well known for their medicinal properties and thus have been used to treat many diseases like anti-herpes and anti-influenza (Rajbhandari *et al*., 2001). Proximate compositions and health benefit of these plants has been documented (Sastry *et al*., 1987). However no information is available on its anti-inflammatory properties, it is therefore essential that effort should be made to introduce new medicinal plants to develop cheaper phyto-products in this aspect. The lack of potent antioxidant and anti-inflammatory herbal drugs now actually in use prompted the present study, which *Astilbe rivularis* has been selected for their biological activities in successive extracts. The antioxidant potentiality and anti-inflammatory activity of AR rhizomes may guide the pharmaceutical industry to seek a large untapped source of structurally novel compounds that might serve as lead for the development of preventive phyto-pharmaceuticals.

MATERIALS AND METHODS

Materials

Description of plant

It is a perennial herb. Rhizome is erect with sparsely brown villous stem, narrowly racemose branch, alternate leaves are long and broad, leaflets ovate or elliptical, acuminate base rounded or cordate, double serrate margin, appressed brown hirsute on veins, long petiole

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bearing brown hair specially at axils of leaflets. The small flowers are arranged in terminal panicles; peduncles pale brownish pubescent, calyx divided almost two bases into lanceolate teeth, petal absent, number of stamens five, opposite sepal ovary semi-inferior. All parts of the plant are slightly aromatic in odour.

Collection and treatment of rhizome

A. rivularis (Saxifragaceae) rhizomes are collected from the Kurseong local area, Darjeeling, West Bengal in December 2006. Taxonomic position was authenticated by the Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal under accession number 9481 dated 05-02-2008. The collected rhizomes were then dried in shade at temperature between 21º to 30º C for 30 days after which 500 g of this part of plants were chopped and ground. Finally successive extractions were carried out in different solvents.

Preparation of rhizome extracts and purification

The ground plant materials approximately 100g were soaked in 100 ml of methanol and boiled for 8 hours on a hot plate with glass beads. The flask containing methanol extracts were kept overnight for cold percolations. The extracts were filtered through double layer of muslin cloth and volume was reduced by evaporation under reduced pressure in rotary evaporator. Percentage of methanol yield was obtained as 53.1. The residual part were treated with chloroform and filtered through the same. Residue was washed three times and extracts were concentrated. Finally condensed filtrate was used for purification of components from hydrophobic to hydrophilic in the silica gel column chromatography (Volume of the column 47.1 cc.). Different solvent were used for elution one after another in three different ratios 3:1, 1:1 and 1:3 respectively. All the solvent fractions were evaporated to dryness under reduced pressure. After removal of solvent, extracts were reconstituted in methanol, and then used for evaluation of DPPH free radical and superoxide scavenging and anti-lipid peroxidation assay at a dose of 100 µg/ml. The fractions showing considerable (>90% inhibition) total free radical scavenging activities were mixed and evaporated to dryness, then after reconstitution with normal saline (0.9% w/v) was only used for the evaluation of anti-inflammatory effects.

Study animals

Male albino rats for anti-inflammatory studies were used in the present study. Weights of the rats ranged from 150 to 200 g respectively. All animals were maintained in groups of three at 25º ±1ºC with light/dark cycle of 12:12 h. They were starved overnight but allowed fresh water before administration of the extracts.

Fresh goat liver used for anti-lipid peroxidation assay was purchased from slaughter house immediately after slay.

Chemicals

Solvent used for column chromatography were of analytical grades. Silica gel G-60; 2,2-diphenyl-1-picrylhydrazyl (DPPH); nitroblue tetrazolium (NBT); reduced nicotinamide adenine dinucleotide phosphate sodium salt monohydrate (NADPH); phenazine methosulphate (PMS); trichloro acetic acid (TCA); thiobarbituric acid (TBA); $FeSO_4$, 7 H_2O; KOH; KH_2PO_4; quercetine were either of Sigma or Hi Media analytical grades.

Drugs

All the drugs used in this study were of pharmaceutical grade. Carrageenan was purchased by Sigma Chemical Company and Ibuprofen; a propionic acid derivative was purchased from local retail medicine shop (Marketed by Albert- David, brand name Alfan).

Antioxidant assay

DPPH based free radical scavenging activity

The free radical scavenging activities of each reconstituted fraction were assayed using stable DPPH, according to the method of Blois (1958). Percentage of free radical scavenging activity was expressed as percent inhibition from the given formula.

$$\% \text{ inhibition} = \frac{\text{Abs. of cont. abs. of sample}}{\text{Abs. of cont.}} \times 100$$

Superoxide anion scavenging activity

The superoxide anion scavenging activity was performed using method of Nishikimi *et al*. (1972) followed by slight modification. Percentage inhibition was calculated using the formula given above.

Anti-lipid peroxidation (ALP) assay

The anti-lipid peroxidation assay in the goat liver homogenate was measured by the method of Ohkawa *et al.* (1979), followed by slight modification. ALP % was calculated using the following formula.

$$\text{ALP}\% = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation - abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation - abs. of control}} \times 100$$

Procedure for testing anti inflammatory activity

Hind paw edema method

In the present study anti inflammatory activity was determined in male Albino rats; following the method of Winter *et al*. (1962) using three animals in each group. Carrageenan (1% w/v suspension in normal saline) was injected to animals, in the right hind foot under the plantar aponeurosis (Goudgaon *et al*., 2003). The test groups (1 and 2) of rats were given orally 100 and 200 mg/kg of normal saline reconstituted mixture of extract of AR one hour before the carrageenan injection. The controls were treated with the same volume of saline as in test group. Another group of rats were treated with 18 mg/kg (dose of

the drug were calculated as per body weight of the rat) of Ibuprofen (NSAID) orally one hour before carrageenan injection. The inflammation was quantitated by using vernier caliper immediately before carrageenan injection and then 1, 2 and 24 hours after carrageenan injection. The percentage inhibition of edema was calculated for each group with that of vehicle treated control group by using the following formula:

$$\frac{V_d V_p}{V_c V_p} \times 100$$

Where $V_d - V_p$ = Difference in paw volume after carrageenan injection and initial paw volume for drug treated animal, $V_c - V_p$ = Difference in paw volume after carrageenan injection and initial paw volume for control animals.

Statistical analysis

All the experiments were repeated three times and data were represented as mean ± S.D and were analyzed by SPSS (version 11.0 SPSS, Inc.). Analysis of variance (ANOVA) was used to determine the difference among the fractions. The Duncan's multiple range test (DMRT) was used for making comparison (Gomez and Gomez, 1984) $P < 0.01$ was regarded as significant.

RESULTS

DPPH Assay

Among the 33 extracts tested for antioxidant activity using DPPH method the successive diethyl ether in ethyl acetate and ethyl acetate in acetone extracts exhibited strong antioxidant activity as evidenced by their higher percentage of inhibition (Table 1). The values were comparable to that of standard quercetine. When considering the organic fractions of *A. rivularis*, the DPPH radical scavenging capacities increased with increasing the polarity of the solvent.

Inhibitory effect of different fractions on superoxide anion formation

Scavenging effect of different concentration of all extracts

Table 1. DPPH free radical scavenging activity in the different solvent fractions of AR rhizomes.

Solvent fractions v/v	100%	75%	50%	25%
Hexane in benzene	91.67 ± 0.58^{D}	27.17 ± 0.46^{L}	10.51 ± 0.50^{O}	8.06 ± 0.24^{P}
Benzene in chloroform	5.05 ± 0.05^{RS}	3.01 ± 0.04^{T}	12.98 ± 0.13^{M}	3.00 ± 0.05^{T}
Chloroform in diethyl ether	6.97 ± 0.15^{Q}	5.07 ± 0.21^{RS}	2.13 ± 0.15^{U}	44.10 ± 0.10^{J}
Diethyl ether in ethyl acetate	93.10 ± 0.36^{C}	95.23 ± 0.49^{A}	55.50 ± 0.44^{I}	60.60 ± 0.53^{H}
Ethyl acetate in acetone	35.03 ± 0.15^{K}	$93.78 \pm O.46^{BC}$	94.18 ± 0.18^{B}	93.20 ± 0.35^{C}
Acetone in ethanol	90.73 ± 0.31^{E}	84.37 ± 0.32^{F}	3.10 ± 0.10^{T}	70.70 ± 0.62^{G}
Ethanol in methanol	93.00 ± 0.10^{C}	93.01 ± 0.31^{C}	27.10 ± 0.36^{L}	4.30 ± 0.30^{S}
Methanol in water	1.17 ± 0.15^{V}	2.13 ± 0.15^{U}	15.22 ± 0.20^{M}	1.07 ± 0.21^{V}
Water	5.43 ± 0.15^{R}			
Quercetine(standard)	$44.67 \pm 0.1.53^{J}$			

$CD_{0.01}$ = O.8187

Values indicate % inhibition of DPPH free radical over control ± S.D (n = 3), figures symbolized by the same alphabet/s (in superscript) are not significantly different at $P < 0.01$

Table 2. Superoxide radical scavenging activity in the different solvent fractions of AR rhizomes.

Solvent fractions v/v	100%	75%	50%	25%
Hexane in benzene	26.03 ± 0.55^{E}	12.03 ± 0.15^{J}	2.33 ± 0.15^{N}	1.63 ± 0.21^{O}
Benzene in chloroform	0.96 ± 0.02^{PQR}	0.77 ± 0.15^{QR}	1.47 ± 0.25^{OP}	1.37 ± 0.21^{OPQ}
Chloroform in diethyl ether	$1.07\ 0.15^{OPQR}$	1.33 ± 0.15^{OPQR}	0.70 ± 0.10^{R}	18.80 ± 0.20^{H}
Diethyl ether in ethyl acetate	2.53 ± 0.15^{N}	2.47 ± 0.25^{N}	22.43 ± 0.25^{G}	11.47 ± 0.35^{J}
Ethyl acetate in acetone	24.83 ± 0.21^{F}	42.77 ± 0.25^{D}	48.77 ± 0.15^{B}	48.83 ± 0.15^{D}
Acetone in ethanol	46.33 ± 0.38^{C}	80.40 ± 0.36^{A}	2.50 ± 0.20^{N}	1.57 ± 0.15^{OP}
Ethanol in methanol	5.40 ± 0.20^{K}	16.87 ± 0.25^{I}	3.37 ± 0.25^{M}	2.47 ± 0.15^{N}
Methanol in water	2.67 ± 0.15^{N}	1.47 ± 0.35^{OP}	1.57 ± 0.15^{OP}	1.67 ± 0.25^{O}
Water	4.50 ± 0.20^{L}			
Quercetine(standard)	46.01 ± 0.79^{C}			

$CD_{0.01}$= 0.5843

Values indicate % inhibition of super oxide radical over control ± S.D (n = 3), figures symbolized by the same alphabet/s (in superscript) are not significantly different at $P < 0.01$

Table 3. Percentage of anti-lipid peroxidation in the different solvent fractions of AR rhizome.

Solvent fractions v/v	100%	75%	50%	25%
Hexane in benzene	15.87 ± 0.15^{L}	10.77 ± 0.15^{O}	5.63 ± 0.31^{Q}	4.67 ± 0.32^{R}
Benzene in chloroform	3.33 ± 0.31^{S}	2.67 ± 0.25^{T}	1.60 ± 0.10^{U}	1.63 ± 0.21^{U}
Chloroform in diethyl ether	2.43 ± 0.25^{T}	1.63 ± 0.21^{U}	1.73 ± 0.21^{U}	11.63 ± 0.06^{N}
Diethyl ether in ethyl acetate	30.30 ± 0.20^{H}	35.70 ± 0.20^{E}	18.63 ± 0.31^{K}	14.80 ± 0.26^{M}
Ethyl acetate in acetone	7.57 ± 0.35^{P}	34.57 ± 0.32^{F}	33.67 ± 0.21^{G}	28.60 ± 0.30^{I}
Acetone in ethanol	44.67 ± 0.17^{C}	42.50 ± 0.30^{D}	1.63 ± 0.23^{U}	19.47 ± 0.06^{J}
Ethanol in methanol	45.83 ± 0.15^{B}	35.67 ± 0.49^{E}	5.73 ± 0.15^{Q}	1.42 ± 0.27^{UV}
Methanol in water	0.73 ± 0.26^{W}	1.07 ± 0.02^{UVW}	2.60 ± 0.10^{T}	0.93 ± 0.05^{UW}
Water	1.29 ± 0.18^{UVW}			
Curcumin(standard)	46.83 ± 0.76^{A}			

$CD_{0.01}$= 0.5854

Values indicate ALP % over control ± S.D (n = 3), figures symbolized by the same alphabet/s (in superscript) are not significantly different at $P < 0.01$

on superoxide anions are shown in table 2. The superoxide radical scavenging activity of different organic extracts was measured by reduction of NBT. DMRT classification shows that the radical scavenging action was mostly concentrated in between ethyl acetate in acetone and acetone in ethanol solvent fractions. Polar solvent fractions like ethanol in methanol and methanol in water showed lesser superoxide anion scavenging property and the scavenging rates were in the range of 1.47 and 16.87 percent. Highly non-polar solvents like benzene in chloroform were inactive toward chemically generated superoxide radicals. The results showed that the acetone in ethanol fraction of *Astilbe rivularis* rhizome was found to be most potent antioxidant and was followed by ethyl acetate in acetone fraction. So these fractions are active against superoxide anion at only 5 fold higher concentration as that of the well known superoxide anion scavenger quercetine which was used as standard for positive control. The anti- lipid peroxidation effect of rhizome extract was observed in Fe^{2+} ion induced lipid peroxidation in goat liver homogenate against control. These effects in four fractions of AR rhizomes were significant as shown in table 3.

The mixture of four fractions possessed varying degree of anti-inflammatory activity which is comparable with the standard drug shown in table 4. The plant extract showed quicker anti-inflammatory response.

DISCUSSION

It has been observed that herbal antioxidants can prevent stress induced diseases in human beings. These activities of rhizome extract may be due to its polyphenolic contents. The free radicals scavenging activity is generally associated with the presence of reductions. Gordon (1990) reported that these activities of reducton's are believed to break radical chain by donation of a hydrogen atom, indicating the antioxidative properties concomitant with the development of reducing power. DPPH accept an electron or hydrogen radical to become a diamagnetic molecule (Blois, 1958). It can react with specific antioxidant molecules which involves reduction of DPPH, therefore the marked free radical scavenging properties in different fractions may be related with its higher reducing power.

Other plants have proved their activities against superoxide radical scavenging viz. *Cissus quadrangularis* L. (Jainu *et al*., 2005), *Rosa damascene* Mill. (Achuthan *et al*., 2003). McCune *et al*. (2003) reported superoxide scavenging property of 35 different medicinal plant species. Superoxide anion scavenging activity has also been observed in the different fractions of AR rhizomes. Higher percentage of superoxide radical inhibition is the indication of the presence of bioactive compounds in the fractions of AR rhizomes. The superoxide can be formed enzymatically or non-enzymatically in the biological system. $O_2^{\cdot}$ may decrease the activity of other antioxidant defense, like catalase and catalase type enzymes (Halliwell *et al*., 1999). $O_2^{\cdot}$ radical requires a slight input of energy that is often provided by NADPH in biological system.

It is known that cleavage products of lipid peroxidation accumulate in the central nervous system and in cardiac muscle fibers (Nohl, 1993). AR extracts has been found reduce lipid peroxidation of microsomal tissues in goat liver. Generally Malonaldehyde formed during the lipid peroxidation, may serve as active antioxidant molecules in the AR extract inhibiting this type of reaction. This is why AR rhizome extract may be considered as a corroboration of Ayurveda claims for the plant.

NSAIDs are widely used for the treatment of inflammation and pain and they act via inhibition of synthesis of prostaglandins (Vane, 1971). The selective COX-2 inhibitors are as efficacious as NSAIDs in preventing inflammation and pain. This study showed that the mixture of nine best fractions with antioxidant activity inhibited carrageenan induced rat paw edema, which is a valuable test for predicting the anti-

Table 4. Anti-inflammatory effects of plant extract on carrageenan induced rat paw edema.

Treatment	Group	Dose/kg BW	Initial paw volume (cm)	After carrageenan treated paw volume (cm)		
				1 Hr.	3 Hr.	24 Hrs.
Normal saline (0.9% w/v, Nacl)	Control	0.00	2.19	3.74	3.88	2.68
			2.31	3.81	3.97	2.71
			2.11	3.85	3.99	2.59
AR extract (Experimental)	Test-1	100 mg	1.97	2.73	2.75	2.27
			1.85	2.67	2.77	2.18
			2.13	2.82	2.86	2.32
AR extract (Experimental)	Test-2	200 mg	1.83	2.47	2.58	2.08
			1.91	2.59	2.63	2.01
			1.89	2.45	2.53	2.14
Ibuprofen	Standard	18 mg	2.08	2.55	2.63	2.26
			2.01	2.61	2.72	2.31
			2.16	2.51	259	2.39
Mean values and standard deviation from difference calculated as compared to 0 hr reading						
Normal saline (0.9% w/v, Nacl	Control	0.00		1.60± 0.13	1.74 ±0.12	0.46 ± 0.05
AR extract (Experimental	Test-1	100 mg		0.76 ± 0.07	0.81 ± 0.10	0.27 ± 0.07
AR extract (Experimental	Test-2	200 mg		0.63 ± 0.06	0.70 ± 0.06	0.20 ± 0.09
Ibuprofen	Standard	18 mg		0.47 ± 0.13	0.56 ± 0.14	0.24 ± 0.06
Percentage inhibition						
Normal saline (0.9% w/v, Nacl	Control	0.00		0.00	0.00	0.00
AR extract (Experimental	Test-1	100 mg		52.61	53.54	40.15
AR extract (Experimental	Test-2	200 mg		60.75	59.66	56.20
Ibuprofen	Standard	18 mg		70.35	67.69	48.18

inflammatory action of this plant extracts, acting by inhibiting the mediator induced inflammation and also comparable with standard NSAIDs. It appeared to inhibit induced prostaglandin in inflamed tissue possibly due to the presence of Berginin and astilbic acid present in the extracts mixture. Other plants of the same genera like *Astilbe thunbergii* Miq. (Kimura *et al*., 2007), *Astilbe chinensis* Maxim. ex Knoll (Taechul *et al*., 2005) and *Astilbe koreana* Nakai (Na *et al*., 2004) has also been observed to show the anti-inflammatory activity due to the presence of the same kinds of active constituents. Quick anti-inflammatory action might be due to the rapid absorption of the bioactive component of this plant (Ahmad *et al*., 1992).

Phytochemical investigation will also be needed in order to isolate the active fraction of pure compounds and in the same fraction pharmacodynamic studies should also be under taken to evaluate the mechanism of action.

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