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Research Article

Assessment of *In-Vitro* Antioxidant Potential of Ethyl Acetate Fraction of Hydroalcoholic Extract of *Aerva Javanica* Linn. Flowering Tops

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A B S T R A C T

Aerva javanica (*Amaranthaceae*) is a grey coloured woolly perennial tomentose shrub. Its traditional and folklore usage motivates further investigation on its pharmacognostic parameters and pharmacological potential. Therefore, in order to establish its antioxidant potential, DPPH, SOD and superoxide scavenging and total antioxidant capacity, were determined. Hydro-alcoholic extract (CE) was prepared from flowering tops of *A. javanica*. In order to work further on activity guided fractions, ethyl acetate (AJEAF) fraction was prepared. For *in-vitro* evaluation, ascorbic acid was used as standard antioxidant compound. In DPPH assay IC_{50} was determined as 89.00 µg/ml, as compared with standard ascorbic acid with IC_{50} 21.80 µg/ml, with a concentration dependent scavenging of free radical. Superoxide scavenging potential in terms of SOD expressed as IC_{50}, was determined as 61.904 µg /ml for AJEAF in contrast to 132.413 µg /ml for standard ascorbic acid. This was equivalent to 16.154 Eq SOD units /mg (EAF) per mg of sample respectively against 7.552 Eq SOD units /mg of standard. Total antioxidant capacity was found to be 283.67 mg Ascorbic acid Eq /g. Results indicated that fraction (AJEAF had significant antioxidant potential which expressed the prospective potential of fraction against metabolic disorders.

Keywords: Aerva, SOD, superoxide, antioxidant, ascorbic acid, FRAP

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INTRODUCTION

Folklore usage of herbs in various ailments motivates research of traditional drugs in modern system. Indigenous medical system is much more explored to develop drugs from plants.[1] Traditional use of *Aerva javanica* flower tops in is the basis of present study. Chopra (1956) reported its traditional use as demulcent, diuretic, anthelmintic and also in headache. Swellings were reported to be removed by administration of plant decoction.[2,3] *Aerva javanica* Linn. (*Amaranthaceae*) also known as '*Patharphori'*, is a grey colored woolly, perennial, suffruticose, hoary-tomentose, erect to scandent dioecious conspicuous under shrub, 0.6- 1 m tall.[4,5] The present study was undertaken to evaluate the extract and its fractions for their involvement in scavenging of oxidative radicals. In this order, their superoxide scavenging and total antioxidant capacity were evaluated.

MATERIALS AND METHODS

MATERIALS:

Absolute ethanol, acetate buffer (pH 3.6) (SD finechem), ascorbic acid (Himedia), DMSO (Rankem), DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (Himedia), EDTA (SD finechem), Malondialdehyde (MDA) (Himedia), methanol, nitroblue tetrazolium (NBT) (Himedia), sodium hydroxide (Rankem), phosphate buffer pH 7.4 (Himedia), sulphuric acid (Rankem)

METHODS:

Collection and Extraction

Aerva javanica flowering tops were collected from forests of Jhalana in periphery of Jaipur, Rajasthan and authentication was done at "Department of Botany, University of Rajasthan, Jaipur" (Voucher specimen no. #RUBL2116644) (Authentication certificate ***Ref. no.: Bot/2017/5424*** dated 13/02/2017).

Hydro-alcoholic (50-50) extract was prepared from air dried plant materials using maceration method. Fractionation was carried out by first defatting and then by solvents of increasing polarity (dielectric constant). Solvents used for this purpose were petroleum ether, diethyl ether, ethyl acetate, benzene, acetone, and ethanol. Ethyl acetate (AJEAF) fraction was further used to assess antioxidant potential.

Preparation of stock solutions:

Stock solutions of extracts and standard- ascorbic acid were prepared in concentration of 1000µg/ml in methanol. From the stock solutions, serial dilutions of the samples and standard were prepared to obtain different concentration viz. 2, 4, 8, 16, 32, 64, 128, 256, 512, 1000 µg/ml were prepared in methanol and used for antioxidant studies.

DPPH Radical Scavenging

1 ml of methanolic extract of various concentrations was taken in test tube with 1ml of DPPH solution 0.1 mM (0.39 mg in 10ml methanol). Control was prepared with an equal amount of methanol and DPPH. Ascorbic acid was used as the standard to compare. All samples were incubated in dark for 20 minutes and absorbance was recorded at 517 nm in UV spectrophotometer. Experiment was performed in triplicate. [6,8]

Superoxide Scavenging

Different concentrations of extracts were prepared. Alkaline DMSO (1 ml DMSO containing 5 mM NaOH in 0.1 ml water) and nitro blue tetrazolium (NBT) 20 mM (50 mg NBT in 10ml phosphate buffer pH 7.4) solutions were prepared. 1.5 ml of sample of different concentrations was taken and 2 ml alkaline DMSO was mixed and vortexed with it. To this mixture 0.6ml NBT reagent solution was added and vortexed. Final mixture was measured for absorbance at 560 nm under UV spectrophotometer. (9–11)

Scavenging of superoxide free radicals by extracts and fractions was calculated using following formula as % scavenging:

$$\%\ Scavenging = 100 - \left(\frac{Abs.of\ control - Abs\ of\ sample}{Abs\ of\ control}\right) x100$$

Total Antioxidant

10mg/ml stock solutions of extracts were prepared in water. 0.1ml of extract solution was mixed with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Test tubes were covered from top and incubated at 95^{o}C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm in UV spectrophotometer (Shimadzu). Ascorbic acid was used as standard and calibration curve was prepared which was used to calculate total antioxidant activity in terms of number of equivalents of ascorbic acid per gram extract. [6,8]

Statistical Analysis

All results are expressed as mean ± S.E.M. Linear regression analysis was used to calculate the IC_{50} values when required.

RESULTS AND DISCUSSIONS

DPPH Radical Scavenging

Table 1: Inhibition (%) and inhibitory concentration (IC50) values of DPPH free radical by extracts

Sample type	Conc. (µg/ml)					Linear equation	Correlation co-efficient (R^2)	IC_{50} (µg/ml)
	20	40	60	80	100			
	% Inhibition*							
AJEAF	31.26 ± 0.12	35.23 ± 0.13	41.51 ± 0.18	48.77 ± 0.11	52.63 ± 0.25	y = 0.281x + 24.99	0.989	89.00
Asc. acid	48.57 ± 1.98	57.76 ± 1.81	65.94 ± 0.55	72.53 ± 0.55	80.63 ± 1.51	y = 0.394x + 41.41	0.997	21.80

*Inhibition % represented as mean ±SD of triplicate values.

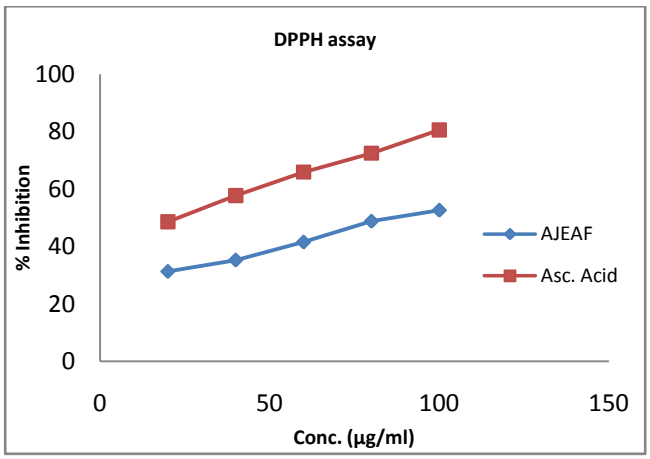

Figure 1: DPPH assay of various extracts and fractions

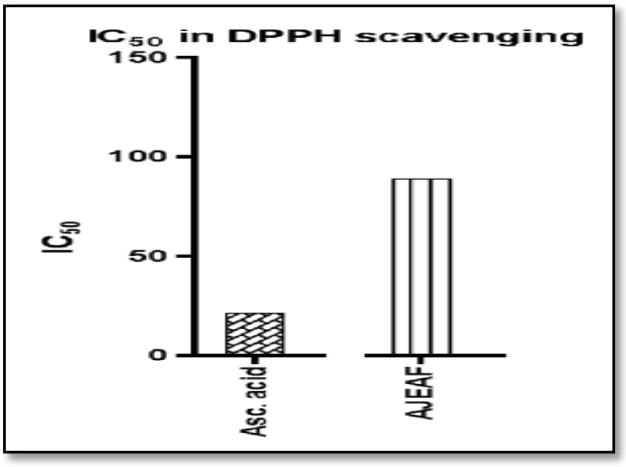

Figure 2: IC_{50} of AJEAF and ascorbic acid in DPPH scavenging

DPPH scavenging was determined as IC50 comparing with standard ascorbic acid. Ethyl acetate fraction (AJEAF) show 89 µg/ml comparing to ascorbic acid at 21.80 µg/ml. Fair DPPH radical scavenging capacity suggests its use as antioxidant scavenger.

Superoxide Scavenging

Table 2: Superoxide scavenging with SOD values for AJEAF and ascorbic acid at 560 nm

Conc. (µg/ml)	Ascorbic acid		AJEAF	
	Absorbance	% inhibition	Absorbance	% inhibition
31.125	0.055	8.112	0.218	32.153
62.500	0.131	19.322	0.334	49.263
125.000	0.309	45.575	0.600	88.496
150.000	0.412	60.767	--	--
250.000	0.671	98.968	--	--
500.000	--	--	--	--
Linear Equation	y = 0.421x - 5.746		y = 0.604x + 12.61	
Correlation co-efficient (R^2)	0.988		0.998	
IC_{50} (µg/ml) ≡ 1 unit of SOD*	132.413		61.904	
Eq SOD units/mg ext or std.	7.552		16.154	
***IC_{50} value is equivalent to 1 unit of SOD**				

Scavenging of superoxide free radicals by extracts and fractions was calculated using following formula as % scavenging:

$$\%\ Scavenging = 100 - \left(\frac{Abs.of\ control - Abs\ of\ sample}{Abs\ of\ control}\right) x100$$

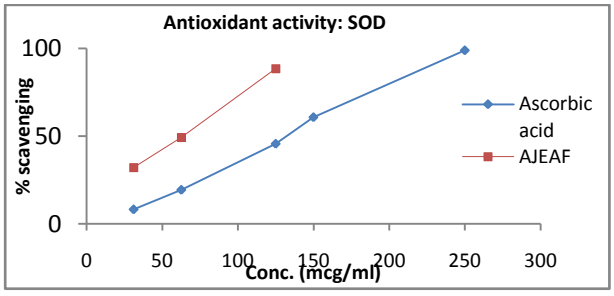

Figure 3: Superoxide scavenging activity as % inhibition

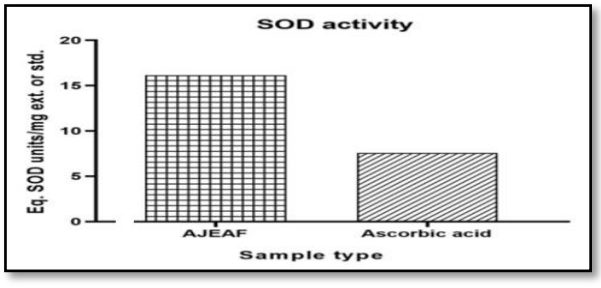

Figure 4: Superoxide scavenging activity as % inhibition

SOD activity (superoxide scavenging capacity) was found to be 16.15 equivalent SOD unit/mg of ethyl acetate fraction of *Aerva javanica*. The activity of AJEAF was found even better than standard ascorbic acid (7.55 eq. SOD unit/mg of extract) used. This suggests the very high concentrations of polyphenolics and in particular, flavonoids in the fraction and, was therefore of particular interest for further pharmacological investigation.

Total Antioxidant

Table 3: Total antioxidant- calibration curve data for standard ascorbic acid

Ascorbic acid Conc.(µg/ml)	**31.25**	**62.5**	**125**	**2500**	**500**
Mean Abs.	0.045	0.178	0.274	0.479	0.892
SD	0.010	0.015	0.019	0.018	0.019
Equation	y = 0.001x + 0.039				
R^2	0.991				

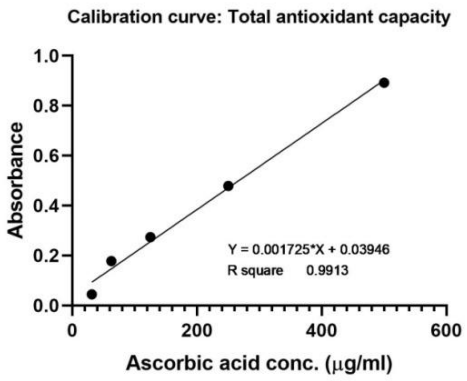

Figure 5: Total antioxidant- calibration curve for standard ascorbic acid

Total antioxidant capacity of ethyl acetate fraction (AJEAF) was calculated as 283.67 mg Ascorbic acid Eq/g using the calibration curve of standard ascorbic acid. High value of total antioxidant capacity was due to the fact that the fraction have high concentrations of polyphenloics which are, by nature, powerful antioxidant.

CONCLUSIONS

From the results of present study, this can be concluded that extract in study possess powerful antioxidants which are more firmly distributed to ethyl acetate fraction. The possibility of counteracting oxidative stress by a pool of proper antioxidants plus an appropriate diet, mainly in patients whose blood antioxidant deficiencies can be easily rebalanced, may have real health benefit and represent a promising way of inhibiting the progression of disease.

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