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IN-VITRO EVALUATION OF ANTIOXIDANT POTENTIAL OF ROOT OF AERVA JAVANICA

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ABSTRACT:

Aerva Javanica belonging to family (Amaranthaceae) is used as Pasanabheda means one, which breaks the kidney stone and therefore, in Gujarati it is commonly known as Patharphod. It is used in traditional medicine as it has been claimed to be useful in treating rheumatism, swelling, toothache, inflammation, headache and kidney problems. A detailed review of literature afforded no information on the *in-vitro* antioxidant potential of the same plant. It was therefore worthwhile to investigate free radicals scavenging effect of *Aerva Javanica.* The main objective of this study was to evaluate the free radical scavenging potential of the *A. Javanica* by using different antioxidant models of screening. *In-vitro* antioxidant activity of *A. javanica* was performed by using alcoholic and aqueous extract. Both the extract of root of *A. Javanica* was determined by ABTS radical cation decolorization assay, scavenging of DPPH free radical, reduction of ferric ions, inhibition of lipid peroxide formation and total antioxidant activity as per standard methodology. For the present study, ascorbic acid was used as reference standard and positive control. Based on our findings, the alcoholic extract of root of *Aerva Javanica* showed very potent free radical scavenging activity. Therefore, it was concluded that alcoholic extract of root of *Aerva javanica* showed potent *in-vitro* antioxidant activity.

Keywords: *Aerva Javanica, In-vitro* antioxidant, Free radicals.

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INTRODUCTION

Free radicals are generated as part of the body's normal metabolic process and are usually produced in the mitochondrial respiratory chain through xanthine oxidase activity, atmospheric pollutants and from drugs and xenobiotics[1]. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders[2]. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates inactivation of enzymes and alteration in the structure and function of collagen basemen[3]. A majority of the disease of today are due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant[4]. Free radicals have been implicated in causation of ailments such as cancer, inflammation, diabetes, liver cirrhosis, nephrotoxicity etc[5]. Together with other derivatives of oxygen, they are inevitable by products of biological redox reaction[6]. Reactive oxygen species (ROS) such as superoxide anions (O2.-), hydroxyl radical (.OH), ferric ion and nitric oxide (NO) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation[7], and thus have been shown to augment collagen synthesis and fibrosis. The increased production of toxic oxygen derivatives is considered a universal feature of stress conditions. Plants and other organisms have evolved a wide range of mechanisms to contend with

this problem, with a variety of antioxidant molecules and enzymes. Antioxidants may be defined as radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, parkinson's diseases, mongolism, ageing process[8]. The plant *Aerva javanica* belonging to the family *Amaranthaceae* is a tall and woody shrub found plentiful in rainy season. This plant is used as Pasanabheda means one, which breaks the kidney stone[9]. Roots and flowers are reported to possess medicinal properties against rheumatism and kidney troubles[10]. The selected plant is also reported as anthelmintic, diuretic, demulcent[11]. It is used for the treatment of headache[12]. The decoction of the plant is administered to remove swellings[13-14]. It was applied to acne like conditions of the face[15]. It contains kaempferol, sterol, triterpenes, flavanoids, ß-sitosterol, aervanone, alkaloids, and an acylated iso-rhamnetin glycoside as phytoconstituents[16-17]. A detailed review of literature afforded no information on the *in-vitro* antioxidant potential of the plant. It was therefore worthwhile to investigate free radicals scavenging effect of *Aerva javanica*.

MATERIALS AND METHODS

Plant material: Fresh stems and leaves of *A. javanica* were collected from Bhavnagar District, Gujarat, India. The authentification of the plant was established and voucher specimen (202) deposited in the Department of Pharmacognosy and Phytochemistry, KBIPER, Gandhinagar, Gujarat, India. Identification of this plant was done by taxonomist Dr. A.S. Reddy, department of bioscience, S.P. University, V.V. Nagar, Gujarat, India.

Drugs and chemicals: All organic solvents used for extraction were obtained from the S.D. Chemicals Private Limited (Mumbai, India), and were analytical grade. The other chemicals 1, 1- diphenyl-2-picryl hydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), ortho-phenanthroline, sodium nitroprusside (SN), sulphanilamide, potassium superoxide, o-phosphoric acid, napthyl-ethylene diamine dihydrochloride, potassium chloride, ferric chloride, ferrous sulphate, thio barbituric acid (TBA), trichloro acetic acid (TCA), nitroblue tetrazolium (NBT), dimethyl sulphoxide (DMSO), ethylene diamine tetra acetic acid (EDTA) and sodium hydroxide (NaOH) used were procured from authentic standard sources.

Preparation of extracts:

- **Preparation of alcoholic extract:** The plant powder was exhaustively extracted by Soxhlet apparatus (6 hr) with alcohol. The total alcoholic extract was then concentrated *in vacuo* to a syrupy consistency.
- **Preparation of aqueous extract:** The powdered plant material was macerated with chloroform water (1:99) for seven days. The extract was filtered and concentrated in *vacuo* to syrupy consistency. After completion of extraction, the solvent was removed by distillation and concentrated[18-19].

In-Vitro antioxidant studies of alcoholic and aqueous extract of root of *Aerva javanica*:

1. Reduction of DPPH free radical[20]: To the 1 ml of various concentrations of alcoholic and aqueous extract, 1 ml of solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added. An equal amount of ethanol and DPPH was added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes, incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate. Percentage scavenging was calculated by the formula mention below.

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

2. ABTS scavenging activity[21]: To 0.5 ml of various concentrations of extract, 0.3 ml of ABTS radical cation and 1.7 ml of phosphate buffer, (pH 7.4) was added. Methanol and water was taken as control for alcoholic extract and aqueous extract respectively. The absorbance was measured at 734 nm. The experiment was performed in triplicate. Percentage scavenging was calculated by the formula mention in above method.

3. Lipid peroxidation[22]

Procedure for preparation of rat brain homogenate: Wistar albino rats (180-200 g) of either sex were used for the study. After decapitation, the brain was removed carefully. The tissue was immediately weighed and homogenated with cold 1.15% KCl to make 10% homogenate and centrifuged for 10 min. The supernatant was immediately used for the *in vitro* lipid peroxidation study.

Method: 0.5 ml of rat brain homogenate was added to the 1 ml of various concentrations of the drug. Then the mixture was incubated for 30 min. The peroxidation was terminated by the addition of 2 ml of TBA-TCA–HCl reagent. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm. The experiment was performed in triplicate. Percentage scavenging was calculated by the formula mention in above method.

4. Reduction of ferric ions by ortho-Phenanthroline method[20]: The reaction mixture consisting of 1ml ortho-Phenanthroline (0.005 g in 10 ml methanol), 2 ml ferric

chloride 200 µM (3.24 mg in 100 ml distilled water) and 2 ml of various concentrations of the extract was incubated at ambient temperature for 10 min. Then the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate. Percentage scavenging was calculated by the formula mention below.

$$\% \text{ Scavenging} = \frac{\text{Test - Control}}{\text{Test}} \times 100$$

5. Total antioxidant activity[23]: 0.1 ml of extract (2, 4, 6, 8 and 10 mg/ml) was dissolved in water. Added to 1 ml of the reagent and incubated at 95 ºC for 90 min, cooled to room temperature. The absorbance was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml).

Statistical analysis: Data obtained were analyzed by statistically. Values at $p<0.001$ were considered as significant.

RESULTS AND DISCUSSION

1. Reduction of DPPH free radical

In fig. 1, At the concentration of 1024 µg/ml, alcoholic extract of root of *Aerva javanica* showed more % scavenging (86.34 %) of –OH radical as compare to the aqueous extract of root of *Aerva javanica* (79.36 %) with reference to the ascorbic acid as standard drug. IC_{50} value for scavenging of DPPH of alcoholic extract of root of *A. javanica* was 190 µg/ml as compared to aqueous extract that was 275 µg/ml. The IC_{50} value for ascorbic acid was found 5.40 µg/ml.

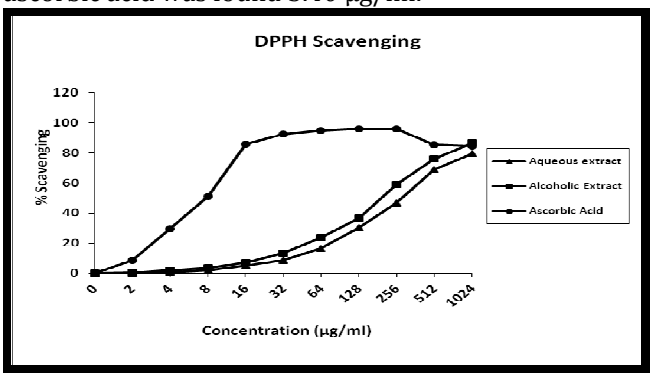

Fig. 1: Effect of alcoholic and aqueous extracts of root of *Aerva javanica* on DPPH Scavenging

2. ABTS scavenging activity

ABTS radical assay is often used for evaluating total antioxidant power of single compound and complex mixture of various plants. In addition, it is proven significant model to evaluate antioxidant activity[24]. In fig. 2, At the concentration of 128 µg/ml, alcoholic extract of root of *Aerva javanica* showed more % scavenging (98.24 %) of ABTS radical as compare to the aqueous extract of root of *Aerva javanica* (95.15 %) with reference to the ascorbic acid as standard drug. IC_{50} value for scavenging of ABTS of alcoholic extract of root of *A. javanica* was 13.06 µg/ml as compared to aqueous extract that was 20.66 µg/ml. The IC_{50} value for ascorbic acid was found 8.48 µg/ml.

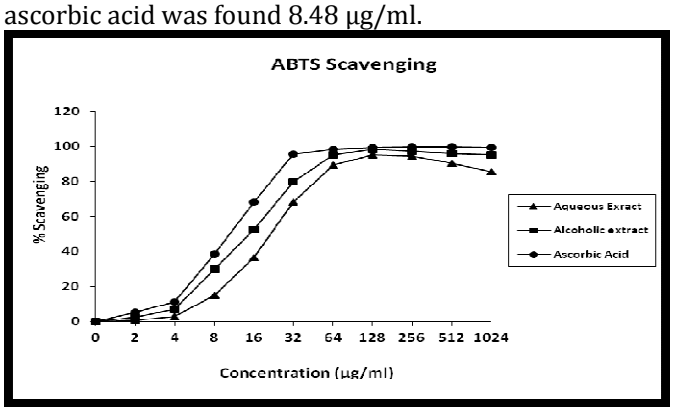

Fig. 2: Effect of alcoholic and aqueous extracts of root of *Aerva javanica* on ABTS Scavenging

3. Lipid peroxidation

In fig 3, Alcoholic extract of root of *Aerva javanica* showed more % scavenging (57.48 %) of TBARS radical as compare to the aqueous extract of root of *Aerva javanica* (30.76 %) at the concentration of 256 µg/ml and 1024 µg/ml respectively. IC_{50} value for inhibition of lipid peroxidation for alcoholic extract of root of *A. javanica* was 272 µg/ml as compared to aqueous extract that was 14539 µg/ml.

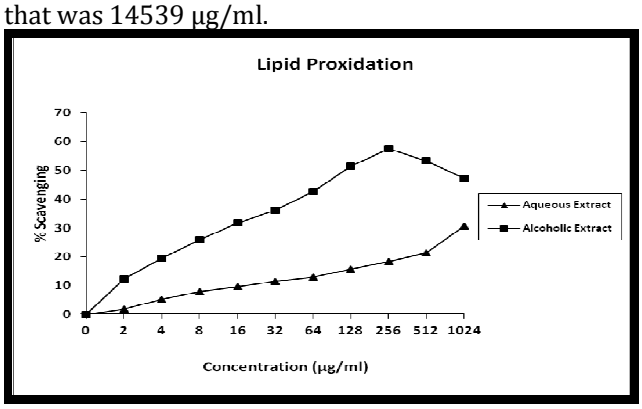

Fig. 3: Effect of alcoholic and aqueous extracts of root of *Aerva javanica* on TBARS Scavenging

4. Reduction of ferric ions by ortho-Phenanthroline method

In fig 4, Alcoholic extract of root of *Aerva javanica* showed more % scavenging (95.21 %) of ferric ion at 256 µg/ml as compare to the aqueous extract of root of *Aerva javanica* (87.24 %) at 1024 µg/ml with reference to the ascorbic acid as standard drug. IC_{50} value for scavenging of ABTS of alcoholic extract of root of *A. javanica* was 6.66 µg/ml as compared to aqueous

extract that was 9.08 µg/ml. The IC_{50} value for ascorbic acid was found 1.10 µg/ml.

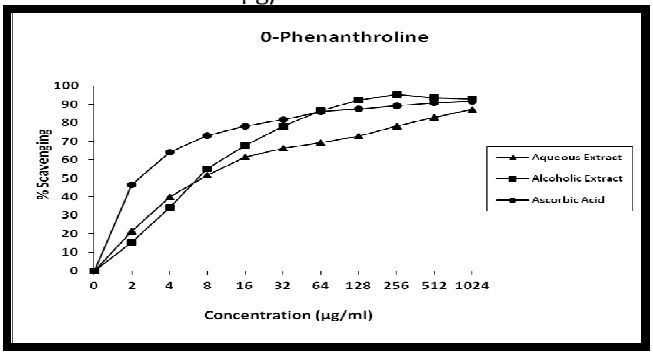

Fig. 4: Effect of alcoholic and aqueous extracts of root of *Aerva javanica* on Ferric ion reduction

5. Total antioxidant activity

Alcoholic extract (10 mg/ml) of root of *Aerva javanica* was showing activity equivalent to 0.368 mg/ml of Ascorbic acid. Aqueous extract (10 mg/ml) of root of *Aerva javanica* was showing activity equivalent to 0.176 mg/ml of Ascorbic acid.

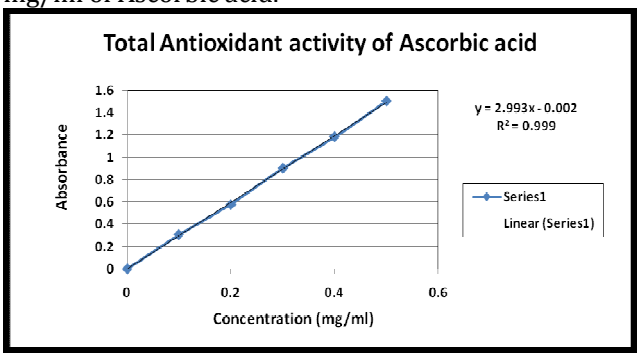

Fig. 5: Standard curve of total antioxidant activity of ascorbic acid

CONCLUSION

The results obtained in present study were indicated that alcoholic extract of root of *A.* javanica inhibits free radical scavenging activity significantly. The overall antioxidant activity depends on its triterpenoid and polyphenolic content and other phytochemical constituents were present. It could be a source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. However, the efficacy of alcoholic extract differed against varies free radicals depending on the specific assay methodology, reflecting the complexity of the mechanisms and diversity of the chemical nature of the phytoconstituents presents in it. It can be observed that alcoholic extract of root of *Aerva javanica* showed very significant scavenging activity as compared to the reference standard ascorbic acid. Therefore, it was concluded that alcoholic extract of root of *Aerva javanica* showed potent antioxidant activity.

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