Research Article

TO EVALUATE *IN-VITRO* ANTIOXIDANT POTENTIAL OF STEM AND LEAF EXTRACTS OF *GYMNOSPORIA MONTANA* - A NATIVE OF GUJARAT

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Accepted on: 10-01-2012; **Finalized on:** 20-03-2012.

ABSTRACT

Gymnosporia montana present in the family *celeastraceae,* is commonly known as Vikalo in the Gujarat state. It is used in traditional medicine as it has been claimed to be useful in treating ulcer, gastro-intestinal disorders, toothache, dysentery antispasmodic and hepatoprotective. 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 *Gymnosporia montana.* The main objective of this study was to evaluate the free radical scavenging potential of the *G. montana* by using different antioxidant models of screening. *In-vitro* antioxidant activity of *G. montana* was performed by using different extracts such as petroleum ether, 70% methanolic and aqueous extract according to their polarity. The successive extracts of leaf and stem of *G. montana* was determined by ABTS radical cation decolorization assay, scavenging of nitric oxide radical, reduction of ferric ions and inhibition of lipid peroxide formation as per standard methodology. For the present study, ascorbic acid was used as reference standard and positive control. Based on our findings, the 70 % methanolic extract of leaf and stem of *G. montana* showed very potent free radical scavenging activity. Therefore, it was concluded that 70% methanolic extract of *G. montana* showed potent *in-vitro* antioxidant activity.

Keywords: *Gymnosporia montana*, *In-vitro* antioxidant, Free radicals.

INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, 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 glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes.[3]

It is increasingly being realized that 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 (O_2.-), 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 and perhaps dementias[8] *Gymnosporia montana*, belonging to the family *celeastraceae* commonly known as Vikalo is a shrub or tree growing wild in dry areas. The plant has been used traditionally useful in treating ulcer, gastro-intestinal disorders, toothache, dysentery[9], antispasmodic[10] and hepatoprotective[11] effects were also reported. In South Africa, leaves are used as a vermifuge for children. 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 *Gymnosporia montana.*

MATERIALS AND METHODS

Drugs and chemicals

All different organic solvents used for extraction were obtained from the S.D. Chemicals Private Limited (Mumbai, India), and were analytical grade. The other

chemicals 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), sodium nitroprusside (SN), sulphanilamide, potassium superoxide, o-phosphoric acid, napthyl ethylene diamine dihydrochloride, potassium chloride, ferric chloride, ferrous sulphate, thio barbituric acid, trichloro acetic acid (TCA), nitroblue tetrazolium (NBT), dimethyl sulphoxide (DMSO), ethylene diamine tetra acetic acid (EDTA), ortho- phenanthroline and sodium hydroxide (NaOH) used were procured from authentic standard sources.

Collection and authentification of plant material

The plant *Gymnosporia montana* were collected from the Vijapur, Gandhinagar, Gujarat, India and were authenticated by Dr. S.K.Patel, Head of The Botany Department, Government Science College, Gandhinagar. The voucher specimen PH/O8/0011 was deposited in K.B.Institute of pharmaceutical Education and Research, Gandhinagar.

Preparation of different extracts

The selected plant parts viz. leaf and stem of *G. montana* was separated and dried under sunlight. Dried powdered passed through sieve of 60 mesh (#) size and stored in airtight containers. Shade dried leaf and stem powder was extracted successively with petroleum ether (60-80), 70% methanol and water. The extraction was carried out by soxhlet assembly, for 6-8 hours. Then the solvent was filtered and repeat the process for three times in the same manner. The extracts were concentrated and dried under controlled temp of 60°C on a water bath. The dried yield of the successive extracts was weighed and used for further investigation for potential *in-vitro* antioxidant activity.

Preparation of liver homogenate[12]

Adult Wistar rats of either sex and of approximately the same age weighing about 200-250 g were used. The rats were fed with standard chow diet (Pranav Agro Industries Ltd., Sangali, and Maharashtra) and water *ad libitum.* They were housed in polypropylene cages maintained under standard conditions (12:12 hr L: D cycles; $25^0 \pm 3^0$C; 35-60 % RH). The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same (No: IAEC/ KB/08/117). Randomly selected rats were fasted overnight. They were sacrificed by cervical dislocation, dissected and the liver was removed quickly. It was further processed to get 10% homogenate in 0.15 M KCl using a Teflon homogenizer. The homogenate was filtered to get a clear solution and used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation.

***In-vitro* antioxidant activity**

ABTS radical scavenging assay by spectrophotomatery[13]

ABTS radical cation (ABTS) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium per sulfate and the mixture was allowed to stand in dark at room temperature for 12 – 16 hr before use. For the study, different concentration (100 – 1000 µg/ ml) of the different extracts (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol and double distilled water to make 1 ml. The absorbance was read at 745 nm and the percentage inhibition calculated by using the same formula as given above.

Reduction of ferric ions by ortho-phenanthroline colour method[14]

Ortho substitute phenolic compounds were found more active than unsubstituted phenol. Hence, these compounds may exert pro- oxidant effect by interacting with iron. In the presence of scavenger, reduction of ferric ions will occur which is measured at 510 nm. The reaction mixture consisting of 1 ml ortho- phenanthroline (0.005g 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 extracts (100 – 1000 µg/ml) were incubated at ambient temperature for 10 minutes. Then the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate.

Nitric oxide scavenging activity[15]

Nitric oxide, generated from sodium nitropruside in aqueous solution at physiological pH, interact with oxygen to produce by Griess reaction followed by the method of Green et al, 1982. The experiment was performed in triplicate. The reaction mixture (3 ml) containing sodium nitropruside (10 mM) in phosphate buffered saline (PBS) and the compound was incubated at 25^0 C for 150 minutes. After incubation, 0.5 ml of the reaction mixture was removed and 0.5 ml of Griees reagent (1% sulphanilamide, 2% H_3PO_4 and 1% naphthylethylene diamine dihydro chloride) was added. The absorbance of the chromophore formed was observed at 546 nm.

Inhibition of lipid peroxidation assay[16]

The levels of malondialdehyde (MDA) in each sample were estimated (expressed as µmole thiobarbituric acid reactive substances [TBARS]/mg protein) was measured at 532 nm as per the method of Ohkawa *et al.,* 1979. The experiment was performed in triplicate. The reaction mixture containing rat liver homogenate (0.1ml, 25% w/v) in Tris-HCl (30 mM), ferrous ammonium sulphate (0.16 mM) and ascorbic acid (0.06 mM) and different concentration of the compound (from 100- 1000 g/ml) in a final volume of 0.5 ml was incubated for 1 hour at 37°C (15) and the resulting thiobarbituric acid reacting substance (TBARS) was, measured by the method of Ohkawa *et al.* A 0.4 ml aliquot of the reaction mixture was treated with sodium dodecyl sulphate (SDS) (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%), and acetic acid (1.5ml, 20%, pH 3.5), made to a total volume of 4 ml by adding distilled water, and kept in a water bath at 95°C for 1 hour. After cooling, distilled water (1 ml) and 5 ml of butanol/ pyridine 15.1 (v/v) were added. After shaking

and centrifugation, the organic layer was separated and the absorbance was measured at 532 nm.

The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extract as per the formula:

$$\text{Inhibition \%} = \frac{\text{(Control-Test)}}{\text{Control}} \times 100$$

Statistical analysis

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

RESULTS AND DISCUSSION

In-vitro antioxidant activity of petroleum ether, 70% methanolic and aqueous extract of leaves and stem of *Gymnosporia montana* were tested for their antioxidant activity *in-vitro* models such as ABTS radical cation decolorization assay, scavenging of nitric oxide radical, reduction of ferric ions by ortho-phenanthroline colour method, inhibition of lipid peroxide formation.

ABTS Scavenging

In figure 1, the dose response curve of ABTS Scavenging of the different extracts of stem and leaf of *Gymnosporia montana* was compared with reference standard ascorbic acid. Compared with the different extracts, 70% methanolic extract of *G.montana* leaf showed good ABTS Scavenging activity. IC_{50} value for scavenging of ABTS of methanolic extract of the leaf was 98.26±0.22 µg/ml as compared to ascorbic acid was 98.73±0.59. Since the ABTS radical assay was suitable for both polar and non-polar solvent system, it is often used for evaluating total antioxidant power of single compound and complex mixture of various plants.[17-19]

Ferric Ion

As showed in figure 2, the dose response curve of ferric ion of the extracts of stem and leaf of *Gymnosporia montana* was compared with reference standard ascorbic acid. Compared with the different extracts, 70% methanolic extract of *G.montana* leaf showed good ferric ion scavenging activity. IC_{50} value for scavenging of ferric ion of methanolic extract of the leaf was 93.54±0.34 µg/ml as compared to ascorbic acid was 94.43±0.02.The reducing power of *G.montana* was very potent and the power of the leaf methanolic extract was increased with increasing the concentration of the extract. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of ascorbic acid. Increased absorbance of the reaction indicated increased reducing power.[20]

Nitric Acid

The dose response curve of nitric acid of the different extracts of stem and leaf of *G.montana* was compared with reference standard ascorbic acid as shown in figure 3. As compared to all the extracts 70% methanolic extract of *G.montana* stem showed good nitric oxide scavenging activity. IC_{50} value for scavenging of nitric oxide for stem of 70% methanolic extract was 93.47±0.96 µg/ml as compared to ascorbic acid was 98.73±0.06 µg/ml. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological condition.[20]

Inhibition of lipid peroxidation

The dose response curve of lipid peroxidation of the different extracts of stem and leaf of *G.montana* was compared with reference standard ascorbic acid was shown in figure 4. As compared to all different extracts, 70% methanolic extract of *G.montana* leaf showed good lipid peroxidation inhibition activity. IC_{50} value for inhibition of lipid peroxidation for 70% methanolic extract of leaf was 9.22±0.11 µg/ml as compared to ascorbic acid was 20.36±0.24 µg/ml.

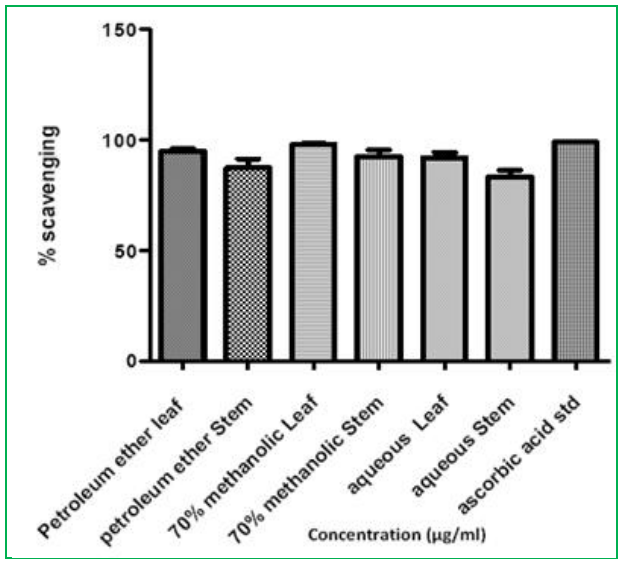

Figure 1: ABTS scavenging activity of leaf and stem extracts of *G. montana*

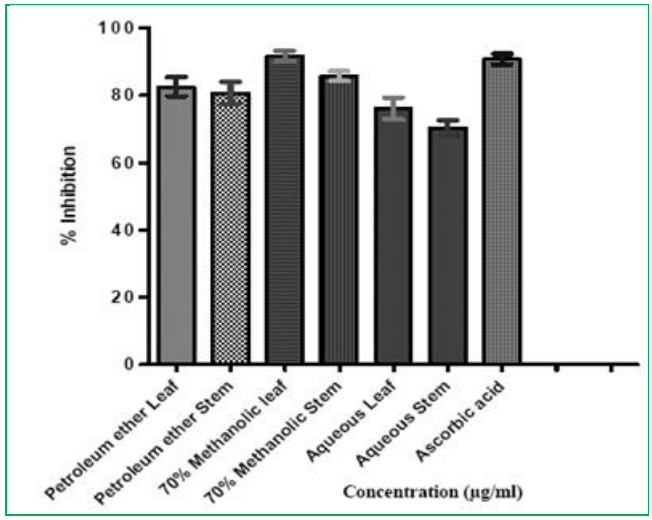

Figure 2: Ferric ions scavenging activity of leaf and stem extracts of *G. montana*

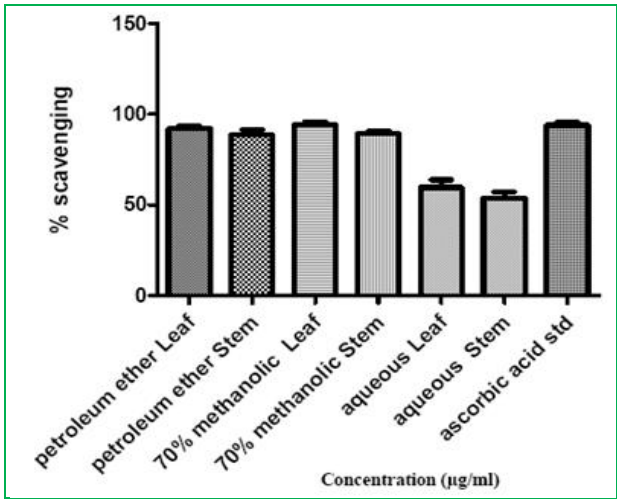

Figure 3: Nitric oxide scavenging activity of leaf and stem extracts of *G. montana*

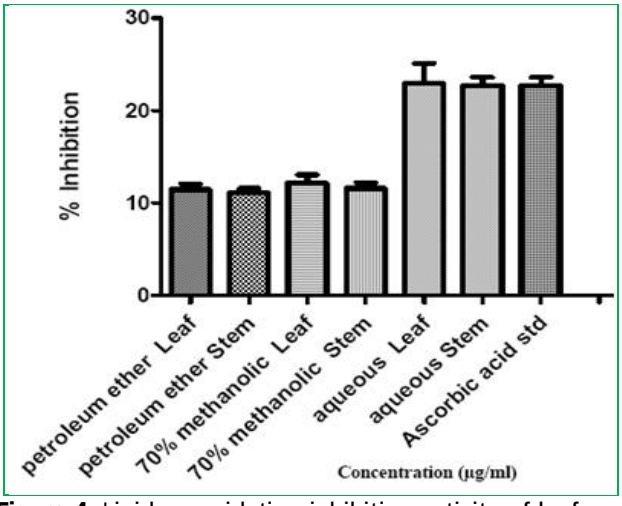

Figure 4: Lipid peroxidation inhibition activity of leaf and stem extracts of *G. montana*

CONCLUSION

The results obtained in present study were indicated that extracts of *G. montana* inhibits free radical scavenging activity. 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 each 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 phytoconstitutes presents in it.

It can be observed that 70% methanolic extract of leaf and stem of *Gymnosporia montana* showed very significant scavenging activity as compared to the reference standard ascorbic acid. Therefore, it was concluded that 70% methanolic extract of *G. montana* showed potent antioxidant activity.

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