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Antioxidant potential of aerial part of *Asclepias curassavica.* Linn (Family – Asclepiadaceae)

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SUMMARY

Asclepias curassavica. Linn, an erect, simple (or) much branched perennial herb with a somewhat woody base, belonging to the family Asclepiadaceae. It has been reported to have multiple pharmacological effect of anti-inflammatory, cardiotonic, anticancer, anthelmintic and to treat piles and gonorrhoea. It is to be expected that several activities might be related to a possible antioxidant action from this plant. The hydro alcoholic extract of *Asclepias curassavica* was tested *in vitro* for its antioxidant activities, such as DPPH radical, nitric oxide radical, superoxide anion radical, lipid peroxidation assay, hydroxyl radical, reducing power, and total phenol content. The extract exhibited scavenging potential with IC_{50} value of 8.7 μg/ml, 198.4 μg/ml and 21.7 μg/ml for DPPH, nitric oxide and superoxide anion radicals. The values were found to higher than those of Vitamin-C, rutin, and curcumin, as standards. The extract showed 50% protection at the dose of 134.2 μg/ml and 41.4 μg/ml in lipid peroxidation as well as deoxyribose degradation, those values more to that of standard, vitamin E (IC50 values, 119.2 μg/ml and 32.5 μg/ml, respectively). The reducing power of the extract depends on the concentration and amount of extract. Since a significant amount of polyphenol could be detected by the equivalent to 0.0495 μg of pyrocatechol from 1 mg of extract. It can be concluded that hydro alcoholic extract of aerial part of *Asclepias curassavica* could be considered as potent antioxidant, which makes it suitable for the prevention of human disease.

Key words: *Asclepias curassavica;* Antioxidant; Free radical scavenging; Lipid peroxidation

INTRODUCTION

Asclepias Linn, a large genus, consisting of nearly 160 species of herbaceous plants. It is mostly American with few African and West Indian representatives. *Asclepias curassavica* has been introduced into India from U.S.A and has become naturalized. In Jamaica, it is called "blood-flower" and West Indian colonists called it "bastard (or)" wild ipecacuanha. The juice of leaves has been strongly recommended as anthelmintic, antidysentric, sudorific, arresting haemorrhages, abdominal pain, piles and gonorrhoea (Hirschmann *et al.*, 1990; Manandhar *et al.*, 1995; Kirtikar *et al.*, 1998). The latex part of plant is applied to corns, also as an emetic and bactericidal properties (Verpoorte *et al.*, 1987; Zargari *et al.*, 1992; Kirtikar *et al.*, 1998). In Mexico, the fresh (or) dried and pulverized leaf is reported to be used against cancer (Duke *et al.*, 1985). An inhibitory activity against carcinomatous

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cells of human nasopharynx has been shown by the plant extract (Neto *et al.*, 2002). The ethanolic fraction of *Asclepias curassavica* exhibited strong cardiotonic action (Bhagirath singh *et al.*, 1969). The root part of *Asclepias curassavica* has been used as an astringent, purgative, piles and gonorrhoea (Zargari *et al.*, 1992; Duke *et al.*, 1994; Caceres *et al.*, 1995; Nadkarins *et al.*, 1995). The root juice specially act upon the heart and blood vessels causing dyspnoea, vomiting and diarrrhoea (Nadkarins, 1995). The plant extract stimulated central nervous system as reflected by increased level of serotonin and noradrenalline (Rastogi *et al.*, 1970-1979). Several cardenolides (Seiber *et al.*, 1982; Abe *et al.*, 1991, 1992), alkaloids (Rothschild *et al.*, 1984), flavonols (Haribal *et al.*, 1996) and alicyclic compounds (Abe *et al.*, 2000) have been isolated from different parts of plants.

Free radical oxidative stress has been implicated in the pathogenesis of various diseases such as atherosclerosis, ischemic heart diseases, ageing, diabetes, neurogenerative disorders (Harmann *et al.*, 1988). The rationale for the use of antioxidants is well established in prevention and treatment of diseases where oxidative stress plays a major role. Natural extracts with proved antioxidant activity usually contain compounds with a phenolic moiety, for example coumarins, flavonoids, tocopherols and catechins (Mukherjee, 2002). Organic acids, carotenoids, protein hydrolysates and tannins can also be present and act as antioxidants (or) have a synergistic effect with phenolic compounds (Dapkevicious *et al.*, 1998). Most of its reported biological activities and active constituent of this plant may be related to its antioxidant nature. Therefore, this study was designed to investigate the *in vitro* antioxidant activities of hydro alcoholic extract of aerial part of *Asclepias curassavica* and to establish its potential therapeutic value.

MATERIALS AND METHODS

Chemicals

Rutin was obtained from Acros organics, New Jersy, USA. DPPH (1,1-diphenyl, 2-picryl hydrazyl), NBT (Nitro blue tetrazolium), NADH (Nicotinamide adenine dinucleotide phosphate reduced), PMS (Phenazine methosulphate), TCA (Trichloro acetic acid), Ferric chloride, BHT (Butyl hydroxy toluene) and Quercetin were obtained from Sigma chemical Co., USA. Ascorbic acid and Vitamin-E were obtained from SD Fine Chem. Ltd., Biosar, India. TBA (Thiobarbituric acid) and pyridine were obtained from Loba chemie, Mumbai, India. EDTA (Ethylene diamine tetra acetic acid disodium salt) and Hydrogen peroxide (H_2O_2) were obtained from Qualigens Fine chemicals, Mumbai, India. Naphthyl ethylene diamine dihydrochloride was obtained from Roch-light Ltd., Suffolk, England. Sodium nitro prusside was obtained from Ranbaxy Lab., Mohali, India. Pottassium ferric cyanide was obtained from May and Backer, Dagenham, UK. 2-deoxy-2-ribose was obtained from Fluka (Buchs, Switzerland).

Plant material

Aerial parts of *Asclepias curassavica* was collected in Nilgiri hills, Tamilnadu region and authenticated by Dr. S. Rajan, Field Botanist, Government Arts College, Ooty. Voucher specimen (SNPS-030/ 2003-2004) of this plant material has been retained in the School of Natural Product Studies, Jadavpur University, India.

Extraction

The aerial part of *Asclepias curassavica* plant was dried at room temperature and reduced to coarse powder. The powder was extracted with mixture of ethanol : water (7 : 3 ratio) for 48 hours. The solvent was completely removed by rotary evaporator and the extract was freeze dried and stored in a vacuum desiccator. Further the extract was used for the *in vitro* antioxidant studies.

Free radical scavenging activity

The free radical scavenging capacity of the extracts was determined using DPPH. A methanol DPPH

solution (0.15%) was mixed with serial dilutions (1 μg/ml to 50 μg/ml) of *Asclepias curassavica* extracts and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (Perkin-Elmer Lambda 20 UV-visible spectrophotometer). The inhibition curve was plotted and IC_{50} values obtained (Viturro *et al.*, 1999).

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garrat *et al.*, 1964). In this investigation, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Asclepias curassavica* extract (10 μg to 320 μg) or standard solution (rutin, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Rutin used as a standard.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of *Asclepias curassavica* was done based on the Nishimiki method (Nishimiki *et al.*, 1972) and slightly modified. About 1 ml of nitroblue tetrazolium (NBT) solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4) 1 ml NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of *Asclepias curassavica* (1 μg to 40 μg) in water were mixed. The reaction started by adding 100 μl of phenazine methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

Lipid peroxidation assay

The rat liver microsomal fraction was prepared by the method of Bouchet (Bouchet *et al.*, 1998). The reaction mixture contained in a final volume of 1.0 ml, 500 μl of liver microsomal fraction, 300 μl buffer containing the plant extract (50 μg-150 μg), 100 μl of $FeCl_3$ (1 mM) and 100 μl ascorbic acid (1 mM) to start peroxidation. Samples were incubated at 37°C for 1 hr, after that lipid peroxidation was measured using the reaction with thiobarbituric acid (TBA). Thio barbituric acid reactive substances were determined by the methods of Houghton and Aruoma (Aruoma *et al.*, 1989; Houghton *et al.*, 1995). The absorbance of the organic layer was measured at 532 nm. All reactions were carried out in triplicate. Vitamin E used as a standard.

Deoxyribose assay

The assay was performed as described by Halliwell method (Halliwell *et al.*, 1987) with minor changes. All solutions were prepared freshly. 1.0 ml of the reaction mixture contained 100 μl of 28 mM 2-deoxy-2-ribose (dissolved in KH_2PO_4-K_2HPO_4 buffer pH 7.4), 500 μl solution of various concentrations of the *Asclepias curassavica* (10 to 100 μg), 200 μl of 200 μM $FeCl_3$ and 1.04 mM EDTA (1 : 1 v/v), 100 μl H_2O_2 (1.0 mM) and 100 μl ascorbic acid (1.0 mM). After an incubation period of 1 hr at 37°C the extent of deoxyribose degradation was measured by the TBA reaction (Aruoma *et al.*, 1989; Houghton *et al.*, 1995). Measure the absorbance at about 532 nm against the blank solution. Vitamin E was used as a positive control.

Reducing power

The reducing power of *Asclepias curassavica* was

determined according to the Oyaizu method (Oyaizu *et al.*, 1986). Different concentration of *Asclepias curassavica* extract (100 μg-1000 μg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $Fecl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of total phenolic compounds

Total soluble phenolic in the aqueous extract of *Asclepias curassavica* were determined with Folin-Ciocalteu reagent according to the standard method (Slinkard and Singleton *et al.*, 1977) using pyrocatechol as a standard. Briefly, 0.1 ml of extract solution (contains 1000 μg) in a volumetric flask diluted with distilled water (46 ml). About 1 ml of Folin-Ciocalteu reagent was added and the contents of the flask mixed thoroughly. After 3 min, 3 ml of Na_2Co_3 (2%) was added, then the mixture was allowed to stand for 2 hr with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the *Asclepias curassavica* determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph. The equation is given below:

Absorbance = 0.001 × Pyrocatechol (μg) + 0.0033

RESULTS

Free radical scavenging activity

The hydro alcoholic extract of *Asclepias curassavica* exhibited the decrease of the concentration DPPH radical due to scavenging ability. The result was mentioned in Fig. 1. The extract had a well hydrogen donating ability with an IC_{50} value of 8.7 μg/ml and the value was found to be higher than the standard vitamin C (IC_{50} value of 3.1 μg/ml).

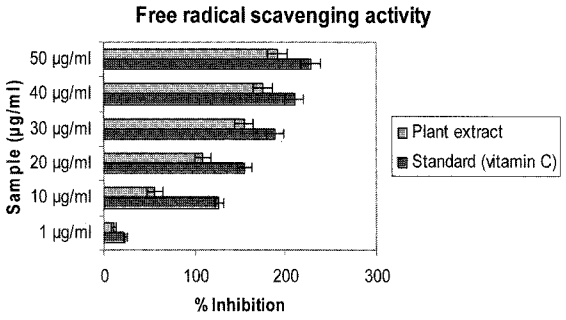

Fig. 1. Scavenging effect of *Asclepias curassavica* extract and standard Vitamin-C on 1,1'-Diphenyl-2-picryl hydrazyl (DPPH) radical.

Nitric oxide radical inhibition assay

The scavenging of nitric oxide by plant extract was increased in a dose-dependent manner as illustrated in Fig. 2. At concentration of 198.4 μg/ml of extract 50% of nitric oxide generated by incubation was scavenged. This IC_{50} value of extract found to be higher than the standard, rutin (IC_{50} 161.7 μg/ml).

Superoxide anion scavenging activity

The superoxide anion derived from dissolved oxygen by Phenazine metho sulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease the absorbance at 560 nm with the plant extract thus indicates the consumption of

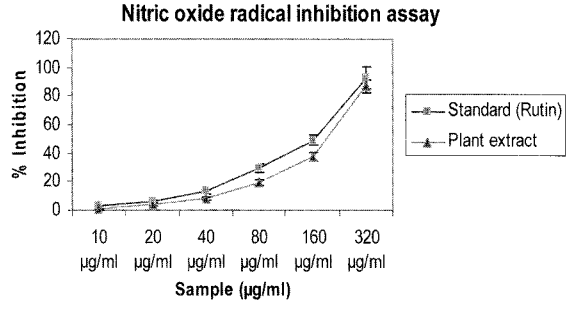

Fig. 2. Scavenging effect of *Asclepias curassavica* extract and standard rutin on nitric oxide radical.

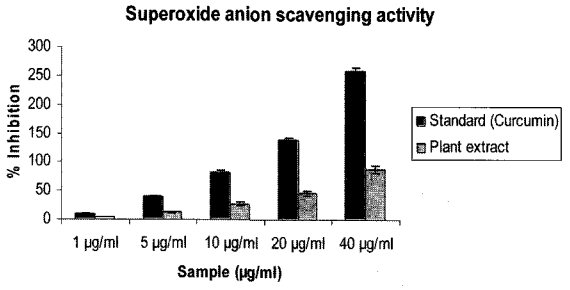

Fig. 3. Effect of *Asclepias curassavica* extract and curcumin on scavenging of superoxide anion radical formation.

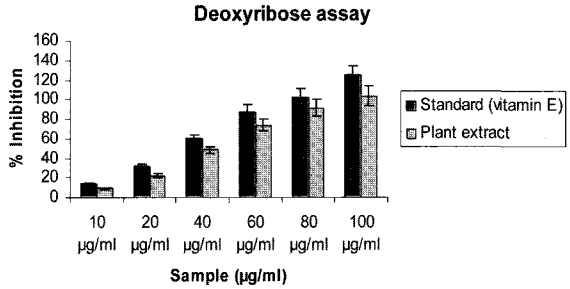

Fig. 5. Effect of *Asclepias curassavica* extract and vitamin E on deoxyribose degradation assay.

superoxide anion in the reaction mixture. As mentioned in Fig. 3, the plant extract as well as curcumin showed the scavenging activity; IC_{50} values, 21.7 µg/ml and 5.84 µg/ml, respectively.

Lipid peroxidation assay

The extract showed inhibition of lipid peroxidation effect in homogenated microsomal content from rat liver. The extract exhibited inhibition of peroxidation effect in all concentrations, which showed 50% inhibition effect at 134.2 µg/ml. The extract effect depends on concentration in the reaction mixture and has higher inhibition value when compared to reference compound of vitamin-E (119.2 µg/ml).

Deoxyribose assay

To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of Ferric-EDTA together with H_2O_2 and ascorbic acid. When the plant extract were incubated with the above reaction mixture, it could prevent the damage against sugar. The results are shown in Fig. 5, the concentrations of 50% inhibition were found to be 41.4 µg/ml and 32.5 µg/ml for the extract and standard of vitamin-E, respectively. The extract inhibition value was found to be higher than the standard.

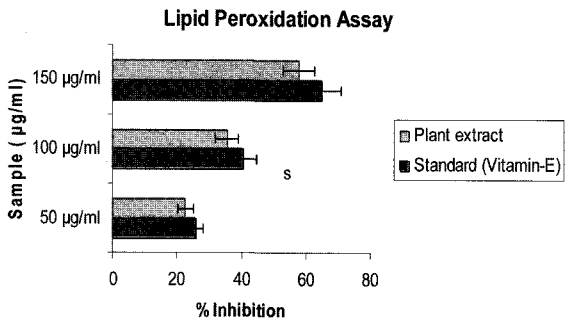

Fig. 4. Effect of *Asclepias curassavica* extract and Vitamin E on lipid peroxidation of liver microsome induced by Fe^{2+}/ascorbate.

Reducing power

The reducing power of extract of *Asclepias curassavica* was very potent and the power of the extract was increased with quantity of sample. The absorbance value of extract at different concentration showed less than the reference compound (butyl hydroxy toluene).

Determination of total phenolic compounds

Phenols are very important plant constituents because of their scavenging ability due to their

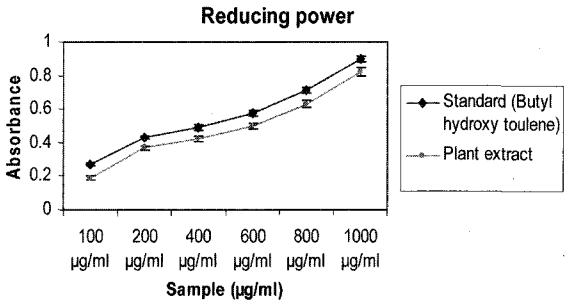

Fig. 6. Reducing power of *Asclepias curassavica* and butyl hydroxy toulene extract.

hydroxyl groups (Hatano *et al.*, 1989). The total phenolic contents of hydro alcoholic extract of *Asclepias curassavica* was 0.0495 μg pyrocatechol equivalent/mg.

DISCUSSION

Free radicals, species with one (or) more unpaired electrons, are produced in normal (or) pathological cell metabolism from xenobiotics, (or) through ionizing radiation. Electron acceptors such as molecular oxygen react easily with free radicals to become radicals themselves ROS (Reactive oxygen species).The primary derivatives of oxygen (O_2, H_2O_2, $^{\cdot}OH$, $^1O_2$) play an important role in mediating ROS related effects. Short-lived reactive species generated in situation can react with non radicals and produce chain reaction. (Halliwell *et al.*, 1984). Thus, antioxidant defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. The free radical scavengers such as β-carotene, α-tocopherol, ascorbic acid, and α-lipoic acid were shown to protect the cell against cytotoxic ROS. There is increasing evidence suggest that consumption of natural antioxidant contained in vegetables, fruits and medicinal herbs useful in preventing the deleterious consequences of oxidative stress (Rice-Evans *et al.*, 1995).

Hydrogen donating ability is an index of primary chain breaking antioxidants. These antioxidants serve hydrogen to free radicals. This conversion lead to non radical species and thus to inhibition of the propagation phase of lipid oxidation. The Fig. 1 illustrates a decrease of the DPPH radical due to the scavenging ability of hydro alcoholic extract of *Asclepias curassavica.* Nitric oxide radical inhibition study demonstrates that, aerial part of the extract is a potent scavenger of nitric oxide. This nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The extract of *Asclepias curassavica* inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Lucia Marcocci *et al.*, 1994). In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Addition of various concentration of extract in above coupling reaction showed a decrease of the absorbance and inhibited the superoxide anion radicals formation.

In the lipid peroxidation assay, peroxidation of rat liver homogenated microsomes is induced by Fe(iii)Cl_3 and ascorbic acid as reducing agent. Hydroxyl radicals were generated by mixing Fe^{3+} and ascorbate and they attack the biological material (Aruoma *et al.*, 1996). This leads to the formation of MDA (malonodialdehyde) and other aldehydes, which form a pink chromogen with TBA, absorbing at 532 nm (Kosugi *et al.*, 1987). The extract inhibits lipid damage caused by hydroxyl radicals and the inhibition values mentioned in Fig. 5. The antioxidant effect of the extract may be due to its flavonol content (Haribal *et al.*, 1996) and the plant might contribute to the positive effects in the treatment of certain inflammatory diseases (Bork *et al.*, 1997). The extract was examined for their ability to act as $^{\cdot}OH$ radical scavenging agents. Ferric EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4; hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA at low pH form a pink chromogen (Halliwell *et al.*, 1987; Aruoma *et al.*, 1989; Mukherjee, 2003). When *Asclepias curassavica* plant extract were added to the reaction mixture they removed hydroxyl radicals and prevented the degradation of 2-deoxy-2-ribose. For the measurements of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of hydro alcoholic extract using the method of Oyaizu (Oyaizu *et al.*,

1986). The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al.*, 1995). The reducing power increased with increasing the amount of extract. The phenolic compounds may contribute directly to anti-oxidative action (Duh *et al.*, 1999). This result indicates that polyphenol present in aerial part and its extract could be partly responsible for the beneficial effects.

CONCLUSION

This study suggested that the *Asclepias curassavica* Linn plant extract had significant antioxidant activity, which might be helpful in preventing (or) slowing the progress of various oxidative stress-related diseases. Further investigation could be done on the isolation and identification of antioxidative components in *Asclepias curassavica*.

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