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Short Communication

Antioxidant activity of *Flaveria trinervia* (Sprengel) C. Mohr

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Considering the important role of oxidative stress in the pathogenesis of several neurological diseases, and the growing evidence of the presence of compounds with antioxidant properties in plant extracts, the aim of the present study was to investigate the antioxidant capacity of *Flaveria trinervia*. Leaves of *F. trinervia* were subjected to cold extration with petroleum ether, chloroform, methanol and ethanol solvents, and chloroform extraction showed effective antioxidant property. The results showed potent antioxidant properties against nitric oxide radical with IC_{50} values of 640±1.2 µg/ml and diphenylpicrylhydrazyl (DPPH) radical with IC_{50} values of 125±4.4 µg/ml.

Key words: Chloroform extract, diphenylpicrylhydrazyl (DPPH), nitric oxide, free radical antioxidant activity.

INTRODUCTION

Plants are potential sources of natural antioxidants. The free radial scavenging activity in fruit (Kaur et al., 2006), vegetable (Conforti et al., 2006) and medicinal plant (Gulein et al., 2006) extracts has been extensively studied. In general, there are two basic categories of antioxidants: natural and synthetic. Recently, interest has increased considerably in findings naturally occurring antioxidants for use in food or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Ito et al., 1983).

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and free radicals, such as the hydroxyl radical (OH) and superoxide anion (O_2) are produced as normal products of cellular metabolism. A vast amount of evidence implicates that free radicals are able to attack lipid membranes, proteins and DNA, and lead to some detrimental effects, such as lipid peroxidation of cell membranes, alteration of lipid protein interactions, enzyme inactivation, DNA breakage (Halliwell and Gutteridge, 1998) and even result in cell death (Dean et al., 1993). So, the oxidative stress induce cell damage triggers both the physiological process of aging and many pathological progressions that eventually lead to serious health problems (Harman, 1993) such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases and premature aging (Kris-Etherton et al., 2002). Antioxidants can reduce the cellular oxidative stress inhabiting the formation of superoxide anions, and by detoxification of reactive oxygen species/reactive nitrogen species through upregulation of cellular defense mechanisms, such as superoxide dismutase, catalase or glutathione peroxidase (Violi and Cangemi, 2005).

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Flaveria trinervia (Sprengel) C. Mohr belongs to the family Asteraceae, the leaves of this plant has medicinal properties, traditionally used by the tribal people of Biligirirangan Hills, to reduce fever and to cure jaundice

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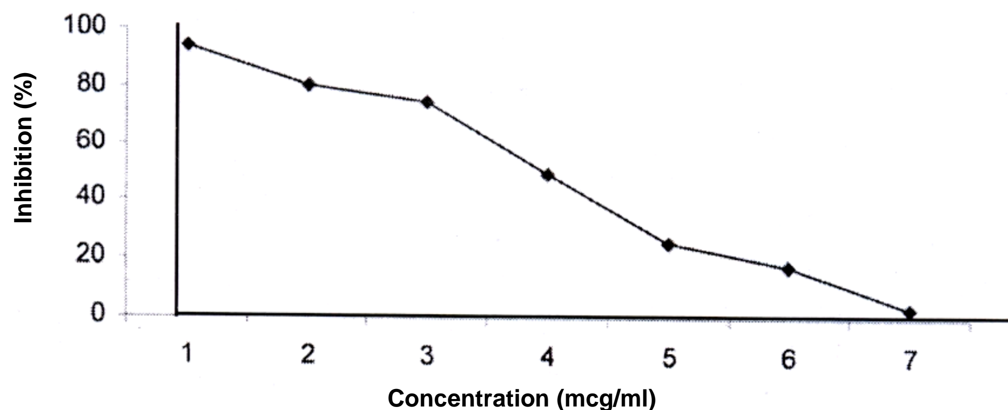

Figure 1. DPPH radical scavenging assay of chloroform extract.

(Shanthamma and Sudarshana, 1986). The aim of the present study was to evaluate free radical scavenging activity against DPPH and nitric oxide. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

MATERIALS AND METHODS

Preparation of plant extracts

F. trinervia leaves were washed, dried and powdered at room temperature. The powdered sample was suspended and extracted in 100 ml of petroleum ether, chloroform, methanol and ethanol kept for 1 day on a shaker at room temperature. The extract was filtered, and extraction of the residue was repeated twice under the same conditions. All the other extracts were first dried using a vacuum rotary evaporator in a water bath at 40°C. Dried samples were weighed and kept at 4°C until use.

Scavenging of nitric oxide radical

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess-Ilosvay reaction. In the present investigation, Griess Ilosvay reagent is modified by using naphthyl ethylene diamine dihydrochloride (NEDD) (0.1% w/v) instead of 1-napthylamine (5%), nitrite ions react with Griess reagent, which forms a purple azo dye. In the presence of the test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm (Garrat, 1964; Nenadis et al., 2004).

Reagents

Accurately weighed sodium nitroprusside solution of 0.2998 g was dissolved in distilled water to make up the volume to 100 ml in the volumetric flask (10 mM), and weighed NEDD of 0.1 g was dissolved in 60 ml of 50% glacial acetic acid by heating, and the volume was made up to 100 ml in a volumetric flask with distilled water, while weighed sulphanilic acid reagent of 0.33 g was dissolved in 20% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask. For the preparation of standard solutions, accurately weighed 10 mg of ascorbic acid and rutin were dissolved in 1 ml of DMSO separately. From these solutions, serial dilutions were made to obtain lower concentrations using DMSO.

Procedure

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm.

RESULTS AND DISCUSSION

In the present study, the four extracts (petroleum ether, chloroform, methanol and ethanol) were first isolated from *F. trinervia*. Free radicals scavenging activities *in vivo* indicated that chloroform extract has significant radicals scavenging abilities on DPPH, and nitric oxide radicals were powerful, which is higher than that of the other extracts. The chloroform extract also exhibited significant reducing power at high dose and it was confirmed that this extract could protect tissues against oxidative damages which includes cancer, aging, heart disease, etc., (Kris-Etherton et al., 2002). DPPH and nitric oxide stable free radical method are sensitive ways to determine the antioxidant activity of plant extract. Figure 1 and Table 1 show the amount of chloroform extract required for 50% inhibition of DPPH activity (IC_{50}). Figure 2 and Table 1 show the amount of chloroform extract required

Table 1. IC_{50} values of chloroform extract.

Sample	IC_{50} values ± SE (µg/ml)*	
	DPPH	Nitric oxide
Chloroform extract	125 ± 4.4	640 ± 1.2
Standard Rutin	3.91 ± 0.10	65.44 ± 1.56

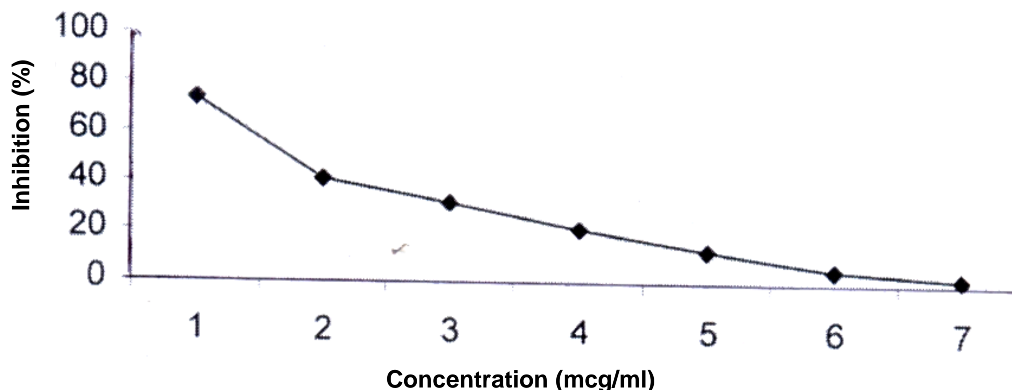

Figure 2. Nitric oxide radical scavenging assay of chloroform extract.

for 50% inhibition of nitric oxide activity. The chloroform extract shows potent antioxidant properties against DPPH radical with IC_{50} values of 125±4.4 µg/ml and nitric oxide radical with IC_{50} values of 640±1.2 µg/ml. In conclusion, the knowledge of indigenous people with laboratory assess-ments of *in vivo* antioxidant activity of all the four leaf extracts of *F. trinervia* were tested. The only chloroform plant extract was showed effective in DPPH and nitricoxide radical scavenger activity. The study revealed that herbs are effective potential source of naural antioxidants.

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