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PHYTO-CHEMICAL SCREENING AND IN VITRO ANTIOXIDANT ACTIVITY OF MERREMIA UMBELLATA (HALLIER. F.)

Science

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ABSTRACT

Antioxidants play the essential role in reducing the free radicals. Aqueous extract of Merremia umbellata (Hallier. f.) was studied for its in vitro antioxidant activity using different models of screening viz. DPPH and ABTS radical scavenging activity, reducing power ability and Superoxide anion radical scavenging. The extract showed a good dose dependent free radical scavenging property in all the models. Phytochemical analysis revealed the presence of major phyto compounds like alkaloids, flavonoids, phenol and terpenoids. Its antioxidant activity was estimated by IC50 value and the values are 86.5 µg/ml (DPPH radical scavenging), 30.1 µg/ml (ABTS radical scavenging), and 40.3 µg/ml (Superoxide anion radical scavenging). The antioxidant property may be related to the polyphenols and flavonoids present in the extract. It indicates that the aqueous extract of the plant has the potency of scavenging free radicals in vitro and may provide leads in the ongoing search for natural antioxidants to be used in treating diseases related to free radical reactions

KEYWORDS

1. INTRODUCTION

The plant Merremia umbellata belongs to Merremia genus and Convolvulaceae family. This is also called as Morning-Glory family. Merremia umbellata (Hallier.f.) is claimed to be bitter, acrid, refrigerant, deobstruant, diuretics, alterant, anthelmintic, carminative and digestive. It is also useful in nephropathy, uropathy, pneumonisis, cardiac diseases, gastropathy, metropathy, fever, anaemia, leucoderma, otalgia, cephalgia and rat bite etc.Merremia umbellata (Hallier. f.) synonym Ipomoea reniformis is prevalent throughout India, Malaysia and tropical Africa. The plant is well known as Elikadhu keerai or Paratai keerai in Tamil and Kidney leaf morning-glory in English. M. umbellata is uncultivated food crop consumed by poor people in India as green leaf vegetable . It is a creeping perennial herb rooting at the nodes. Leaves are simple, long stalked, reniform or ovate-cordate.

2. MATERIALS AND METHODS

2.1. Preparation of extract

Powdered plant material (10 g) was extracted with distilled water (250 ml; 27-30°C) on a shaker Orbitec-scigenics Biotech, India) for 48 h. The extract was filtered with Whatman No. 1 filter paper. The filtrate of aqueous extract was quickly frozen at -50°C and dried for 48 h using a vacuum freeze dryer (Christ alpha 1-2 / LD plus, Germany) to produce a yield of 8.87 % of dry extract. The resulting extract was reconstituted with distilled water to produce the desired concentrations and used for further analysis (Jayakumar et al., 2009; Gulcin et al., 2011).

2.2. Phytochemical screening

The dry extract was used for the phytochemical screening of compounds, namely flavonoids, alkaloids, saponins, steroids and reducing sugars (Harborne, 1998). Approximately 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture, followed by the addition of a few drops of concentrated hydrochloric acid. The formation of a red colour was indicative of the presence of flavonoids. Approximately 0.5 g of the extract was dissolved in 3 ml of chloroform, and a few drops of filtered concentrated sulphuric acid were carefully added to the filtrate to form a lower layer. A reddish-brown colour at the interface was a positive indicator for the presence of steroids. A 2-ml aliquot of the extract was treated with Dragendorff's reagent to test for the presence of alkaloids. Approximately 1 ml of alcoholic extract was diluted separately with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. A 1-cm layer of foam indicated the presence of saponins; 2 ml of TCA was added to 1 ml of extract, and the formation of a yellow-to-red precipitate showed the presence of terpenoids. A total of 5 ml of Benedict's solution was added to 1 ml of the extract and heated in a boiling water bath. A red, yellow or green precipitate indicated the presence of reducing sugars.

2.3. 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

The antioxidant activity of the plant extract was measured as described previously Kikuzaki and Nakatani, (1993). A total of 1 ml of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of aqueous extract ranging from 20 to 100 µg/ml. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was calculated using the following equation:

$$\text{DPPH Scavenging activity (\%)} = [(Ac - As)] / (Ac)] \times 100 \quad \text{----------(1)}$$

Where Ac is the absorbance of DPPH + methanol; As is the absorbance of DPPH radical + sample (i.e. standard or plant extract).

2.4. ABTS scavenging activity

The working solution was prepared by mixing stock solutions of 7 mM ABTS and 2.4 mM potassium per sulphate in equal amounts and allowing them to react for 12 h at room temperature in the dark. The resulting solution was later diluted with distilled water, and the absorbance read at 734 nm using a UV-visible spectrophotometer. A total of 1 ml of freshly prepared ABTS solution was added to 1 ml of the plant extract, the reaction mixture was vortexed for 10 seconds and the absorbance was measured at 734 nm after 6 min. The above protocol was used for the standard BHT of various concentrations (20 to 100 µg/ml). The percentage of the extract's ABTS scavenging inhibition activity was calculated and compared with that of BHT. The percentage of the ABTS scavenging inhibition was calculated from the equation in section (1).

2.5. Scavenging activity of superoxide anion

The reaction mixture consists of 1 ml Nitroblue tetrazolium (NBT) and 1 ml of plant extract (20 to 100 µg/ml), 1 ml of 60 µM potassium metabisulphite (PMS) (prepared in phosphate buffer 0.1 M, pH 7.4) and 1 ml of NADH (in phosphate buffer) was incubated at 25° C for 5 min, the absorbance was read at 560 nm. The percent of scavenging inhibition of superoxide radical was calculated from the above mentioned same equation .

2.6. Determination of inhibitory activity on lipid peroxidation

A total of 0.1 ml of Swiss Albino rat liver homogenate (100 mg of rat liver tissue in 1 ml of 50 mM phosphate buffer, pH 7.0) was homogenised and centrifuged at 10,000 rpm for 15 min at 4 °C, and the supernatant was used for analysis. Then, 100 µl of 0.16 mM ferrous ammonium sulphate, 100 µl of 30 mM KCl, and different concentrations of extract (100 to1000 µg/ml) were incubated for 1 h at 37 °C. The lipid peroxide formed was estimated by measuring thiobarbituric acid-reacting substances (TBARS) (Ohkawa et al., 1979). A total of 0.4 ml of the incubation mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% TBA and 1.5 ml of 20% glacial acetic acid, pH 3.5. The volume of the mixture was

brought up to 4 ml with distilled water, and the reaction mixture was kept in a water bath at 100 °C for 1 h. After cooling, 1 ml of distilled water and 5 ml of an n-butanol and pyridine mixture (15:1, v/v) were added, and the solution was stirred vigorously. After centrifugation, the absorbance of the organic layer was measured at 532 nm. The percentage of lipid peroxidation inhibition was determined by comparing the results of the test compounds (treated with the M. umbellata extract) with those of the control (not treated with the M. umbellata extract). BHT was used as a standard. The percentage of the lipid peroxide scavenging ability of the extract was calculated by the formula in section (1).

3. RESULTS AND DISCUSSION

3.1. Phytochemicals

The phytochemical screening of the aqueous extract of M. umbellata indicated the presence of six main classes of compounds: flavonoids, steroids, alkaloids, saponins, terpenoids and reducing sugars . While some of the paper suggests alkaloids are absent in M. umbellata (Bhatt Mehul et al., 2010), but our qualitative assay for alkaloids shows the presence of alkaloids in M. Umbellata.

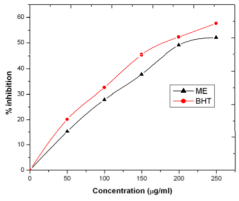
3.2. 2,2-diphenyl-1-Picrylhydrazyl (DPPH) assay

M. umbellata extract was treated with DPPH, which is reacted with methanol or absolute ethanol to yield purple DPPH radicals. Antioxidants, such as polyphenolics and flavonoids, in the sample might have scavenged DPPH radicals, resulting in a decrease in the observed intensity of purple (Blois, 1958). Table 2.2 shows the inhibition of the DPPH radical-scavenging activity of the ME extract (IC50 =86.5µg/ml) compared with the control BHT (IC50=23.4 µg/ml). The ME extract had a high DPPH radical inhibitory capacity compared with those previously reported for other Indian leafy vegetables, namely Asteracantha longifolia Nees (Dasgupta and De, 2007).

Table 1. The scavenging activity of aqueous extract of *Merremia umbellata*

Name of antioxidant assay	IC 50	
	Plants extract(µg/ml)	BHT (µg/ml)
DPPH radical scavenging	86.5	23.4
ABTS radical scavenging	30.1	27.2
Superoxide anion radical scavenging	40.3	18.1

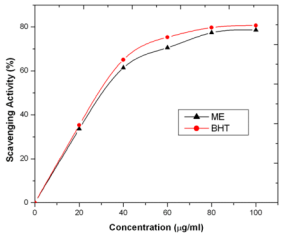
Figure 1. Inhibitory effect of Merremia umbellata aqueous extract on DPPH radicals compared with butylated hydroxytoluene (BHT)



3.3. ABTS scavenging activity

The aqueous extract (100 µg/ml) inhibited the blue colour by 70.5%. Table 2.2 depicts the ABTS radical-scavenging activity of the ME extract (IC50 = 30.1 µg/ml) compared with that of the BHT control (IC50 =27.2 µg/ml) .This was carried out by measuring the ability of methanol extracts of these plants.

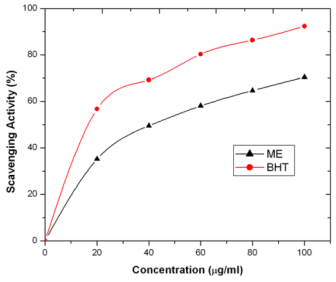
Figure 2. Inhibitory effect of Merremia umbellata aqueous extract on ABTS radicals compared with butylated hydroxytoluene (BHT)



3.4. Scavenging activity of superoxide anion

The plant extract was found to be a prominent scavenger of superoxide radicals. BHT is a commercial antioxidant, which was used as a positive control for comparative study .The IC50 values were found to be 40.3 µg/ml and 18.1 µg/ml for ME plant aqueous extract and BHT respectively .

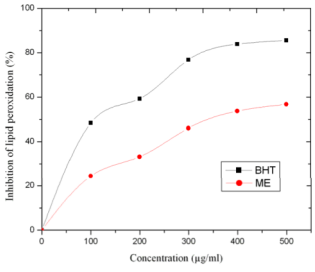
Figure 3. Scavenging effect of Merremia umbellata aqueous extract on superoxide radicals compared with butylated hydroxytoluene (BHT)



3.6. Determination of inhibitory activity on lipid peroxidation

M. umbellata plant extract was evaluated for inhibitory activity of lipid peroxidation. Both M. umbellata extract and BHT standard inhibited lipid peroxidation in a concentration dependent manner. The IC 50 value for M. umbellata extract and BHT are 325 µg/ml and 100 µg/ml respectively, against lipid peroxidation. These in vitro antioxidant assay results obtained show a fairly constant high percentage inhibition of M. umbellata.

Figure 4. Inhibitory effect of Merremia umbellata aqueous extract on lipid peroxidation radicals compared to butylated hydroxytoluene (BHT). Each value is expressed as mean ± standard deviation (n=3)



4. CONCLUSION

The results and discussion of present study conclude that the aqueous extract of M. umbellata consists of considerable quantity of total phenolics, flavonoids compounds and the phytochemicals screening indicated presence alkaloids, steroids, saponin, phenolics, flavonoids, reducing sugars and terpenoids. It exhibited high antioxidant and free radical scavenging activities. The in vitro assays like DPPH, ABTS, superoxide anion scavenging activity and inhibitory activity of lipid peroxidation indicates that the M. Umbellata plant extract is a significant source of natural antioxidant

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