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In vitro antioxidant activity of *Ageratina adenophora* (King & Rob) and *Ipomoea cairica* (L) Sweet

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Abstract

In this study, the in vitro antioxidant activity of the methanolic extracts of leaves and flowers of *Ipomoea cairica* and leaves of *Ageratina adenophora* were determined by spectrophotometric method. Antioxidant activity of extracts were expressed as percentage of DPPH radicals inhibition and IC_{50} values (µg/ml). Values in percentage ranged from 2.70 % for 0.0005 mg/ml concentration of *I. cairica* leaves to 93.24% for 0.05 mg/ml concentration of *I.cairica* flowers. The largest capacity to neutralize DPPH radicals was found for methanolic extract of *I.cairica* flowers which neutralized 50% of free radicals at the concentration of 1.00 µg/ml. *I. cairica* can be regarded as promising candidates for natural plant sources of antioxidants with high value.

Key words: *Ipomoea cairica*, *Ageratina adenophora*, DPPH scavenging activity, IC_{50}.

Introduction

Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential.[1] Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference.

Many disorders in human organism such as atherosclerosis, arthritis, Alzheimer disease, cancer etc., may be the result of increased concentrations of free radicals in an organism. Reactive oxygen species (ROS) and nitrogen (RNS) species, as the most frequent pro-oxidants, either originate from normal metabolism or are induced by UV radiation and different pollutants. Harmful effects of disturbed antioxidant-prooxidant balance can be largely prevented by intake of antioxidant substances.[2,3] Antioxidants have already been found in plant materials and supplements. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones.[4,5] The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect.[6,7] There-

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fore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous

Ageratina adenophora (Fig. 1) is a a perennial herbaceous shrub from *Asteraceae* family which may grow to 1 or 2 metres (3.3 or 6.6 ft) high with trailing purplish to chocolate-brown branches that strike roots upon contact with soil, resulting in dense thickets[8]. The base of the plant is woody and densely clothed with stalked glandular hairs. Leaves are dark green, opposite, deltoid-ovate, serrate, and purple underneath, and each grows to about 10 cm in length. Flowers are borne terminally in compound clusters during spring and summer. The seed is an achene, varying from elliptic to oblanceolate, often gibbous, 1.5–2 mm long, 0.3–0.5 mm wide; with five prominent ribs and five to 40 pappi with slender scabrous bristles[9]. Each flowerhead is upto 0.5 cm in the diameter and creamy white in colour. They are followed by a small brown seed with a white feathery 'parachute'[10].

Ipomoea cairica (Fig. 2) is a vining perennial herb from the Convolvulaceae family growing from a tuberous footstock. It is a prostrate creeper or twining into other vegetation and has large palmate leaves with 5 – 7 lobes with showy white to lavender colour flowers. Each fruit matures at about 1 cm across and contain hairy seeds**.** The genus *Ipomoea* occurs in the tropics of the world although some species also reach temperate zones[11]. Convolvulaceae are found throughout tropical and subtropical regions of the world.

Figure 1. *Ageratina adenophora*

Materials and Methods

Materials

All the solvents used for extraction and isolations were distilled prior to their used and were obtained from commercial source and were of analytical grade. All the chemicals were purchased from Merck Specialities Pvt. Ltd., Mumbai and Sigma Aldrich, Bangalore.

Preparation of plant extract

Leaves and flowers of *Ipomoea cairica* and leaves of *Ageratina adenophora* were collected along the road sides of Mizoram University, Aizawl, Mizoram and was identified by Botanical Survey of India, Shillong, Meghalaya (No.BSI/ERC/2012/Plant identification/ dated 28-8-2013/coll no.1&2). The collected leaves and flowers were washed under tap water, air dried under shade with occasional shifting, and then they are ground to powder by an electrical blender and stored in air tight containers.

The dried powder materials of the leaves and flowers were defatted with petroleum ether (60 –

Figure 2. *Ipomoea cairica*

80 °C), it was further extracted with chloroform and then finally with methanol.[12] Each extraction was carried out exhaustively by maceration, followed by soxhlet extraction. Rotary evaporator (Yamato RE 1000) was used to recover solvents by distillation under reduced pressure. The recovered solvents were distilled twice prior to their reuse.

Determination of DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging was carried out according to the Blois method with slight modification[12]. A standard stock solution of the plant extracts were made by dissolving 10 mg of plant extract in 10 ml of distilled water. 0.0005, 0.001, 0.005, 0.01, 0.025 & 0.05 mg/ml concentrations of each plant extracts were made. 3 ml of plant extract was then mixed with 1 ml of 0.1 mM DPPH solution (in MeOH) and the solution was made 6 ml with distilled water in a test tube. It was then vortexed and incubated at 370C for 30 minutes. Absorbance of the solution was then measured at 517 nm using Thermo Scientific EVOLUTION 201 UV-Visible spectrophotometer. The percentage inhibition was calculated by comparing the absorbance values of the test samples with those of the controls (not treated with extract). The inhibition percentage (I) was calculated as radical scavenging activity as follows:

I (%) = (Control absorbance - sample absorbance)/Control absorbance X 100

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

Results and Discussions

The antioxidant activities of MeOH extracts of *A. adenophora* leaves, *I. cairica* flowers as well as leaves were determined using a methanol solution of DPPH reagent. DPPH is very stable organic molecular free radical. The unpaired electron of DPPH is predominantly situated on one of the hydrogen of hydrazine moiety and it is extensively delocalised over phenyl rings that are attached with the nitrogens of hydrazine moiety. Unlike free radicals generated *in vitro* such as the hydroxyl radical and superoxide anion radical ($O^{2-\bullet}$), DPPH has a unique advantage of not being perturbed by unwanted side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour in general, fades when antioxidant molecules quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band[13].

The antioxidant activities of methanol extracts from *A. adenophora* leaves, *I. cairica* flowers and leaves were expressed in terms of percentage of inhibition [Table 1 & Figure 3] and IC_{50} values in µg/ml [Table 1 & Figure 4]. BHA (Butylated hydroxyanisole) was used as the standard compound, parallel to examination of the antioxidant activity of plant extracts, the values

Table 1. DPPH free radical scavenging activities of MeOH extracts of *I.cairica* leaves, *A.adenophora* leaves and *I.cairica* flowers expressed in terms of IC_{50} and IC_{99} values in µg/ml

Plant part/species	IC_{50} µg/ml	95% confidence limits		IC_{99} µg/ml	95% confidence limits	
		Lower Limit	Upper Limit		Lower Limit	Upper Limit
I. cairica leaves	**5.0**	3.0	13.0	**5050.0**	760.0	10790.7
A.adenophora leaves	**3.0**	1.0	6.0	**2327.0**	301.0	5478.3
I. cairica flowers	**1.0**	1.0	3.0	**153.0**	44.0	278.8

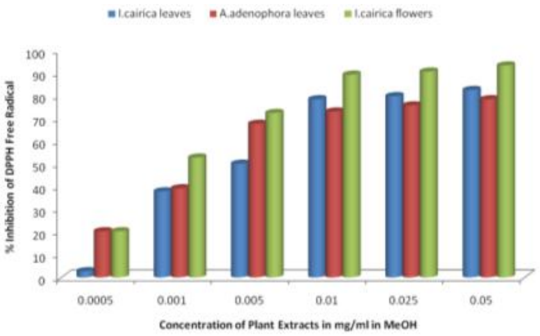

Figure 3. Bar graph showing percentage inhibition of DPPH free radical by the MeOH extracts of the plants at various concentrations

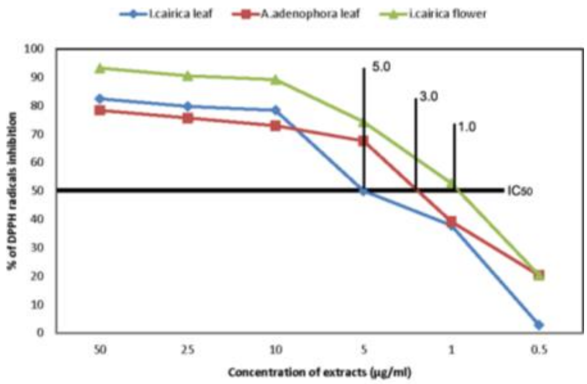

Figure 4. Antioxidant (DPPH scavenging) activity of investigated plant extracts presented as percentage of DPPH radicals inhibition and IC_{50} values (µg/ml).

for the standard compound were obtained and compared to the values of the antioxidant activity.

The examination of antioxidant activities of methanol extracts from *A. adenophora* leaves, *I. cairica* flowers and leaves showed different values. Several concentrations ranging from 0.0005 - 0.05 mg/mL of the plant extracts were tested for their antioxidant activity. It was observed that the DPPH free radicals were scavenged by the test extracts in a concentration dependent manner. The obtained values were in a range from 2.70 % inhibition for 0.0005 mg/ml concentration of *I. cairica* leaves to 93.24% inhibition for 0.05 mg/ml concentration of *I.cairica* flowers. The largest capacity to neutralize DPPH radicals was found for methanolic extract of *I.cairica* flowers which neutralized 50% of free radicals at the concentration of 1.00 µg/ml. A moderate activity was found for methanol extract of *A. adenophora* leaves which neutralized 50% of free radicals at the concentration of 3.00 µg/ml. The lowest capacity to inhibit DPPH radicals was determined for MeOH extract of *I.cairica* leaves for which the IC_{50} was calculated to be 4.00 µg/ml. In comparison to IC_{50} values of BHA, MeOH extract from *I.cairica* flowers manifested the strongest capacity for neutralization of DPPH radicals.

The antioxidant activity may be due to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, quinones and other structural motifs[14,15]. The commercially available synthetic antioxidants have been suspected of causing or instigating negative health effects, so strong restrictions imposed over their application and there is an urgent trend to substitute them with naturally occurring antioxidants[16,17]. It is important to substitute synthetic antioxidants with naturally occurring safer antioxidants as the synthetics have been suspected of causing or provoking unfavourable side effects, while stronger restrictions are encountered on their application[17].

Conclusion

In our study, leaves of *A. adenophora* and leaves and flowers of *I. cairica* were extracted with solvents of varying polarity viz; petroleum ether, chloroform and methanol to gain a preliminary knowledge on the exact nature and amount of metabolites present in the biomass. Furthermore, the determination of DPPH radical scavenging activities and subsequently the IC_{50} of methanolic extract (polar extract) of the two selected plants showed that these plants can be one of the potential substitute to synthetic antioxidants that have been suspected of causing or instigating negative health effects. It has been reported that the intake of natural antioxidants has been associated with the concomitant reduced risks of cancer, cardiovascular disease, diabetes, and other diseases related with age as they have the advantage of being almost devoid of side effects[18,19]. Thus, replacement of synthetic antioxidants with secondary metabolites from plant sources may be advantageous.

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