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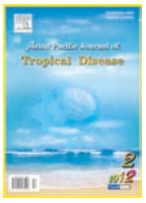

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In vitro antioxidant activity of *Ageratum houstonianum* Mill. (Asteraceae)

Samuel Tennyson[1*], K. Balaraju[2], Kyungseok Park[2], K. John Ravindran[3], Alex Eapen[3], S. John William[4]

[1]*Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India.*
[2]*National Academy of Agricultural Science, RDA, Suwon 441 707, South Korea.*
[3]*National Institute of Malaria Research (ICMR) Field Unit, Chennai 600 077, Tamil Nadu, India.*
[4]*Department of Advanced Zoology and Biotechnology, Loyola College,Chennai 600 034, Tamil Nadu, India.*

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ABSTRACT

Objective: To determine the antioxidant property of *Ageratum houstonianum* leaves. **Method:** The present study was conducted in three different solvent extracts of leaves of *Ageratum houstonianum* Mill. (Asteraceae) to evaluate the antioxidant properties such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals which were carried out at various concentrations under *in vitro* condition. **Results:** It was found that ethyl acetate extract could scavenge both the oxidants at 500 μg/mL with high percentage inhibition (88.26 ±0.35) of DPPH, and in the case of hydroxyl radicals the maximum percentage inhibition was 75.81 ±0.39, which were found to be greater in ethyl acetate extract than in positive controls such as Butylated hydroxytoluene (BHT) and ascorbic acid. The next higher inhibitory extract was found to be methanol. **Conclusion:** This shows that the plant *Ageratum houstonianum* may be a potent source of natural antioxidant.

1. Introduction

Ageratum houstonianum is native to Mexico and Central America, with many escapes becoming established as weeds in all parts of the world. Currently, *Ageratum* is an important seed-propagated, annual bedding plant. In addition to its use as an ornamental, *Ageratum houstonianum* and the closely related species *Ageratum conyzoides* have been used in folk medicine [1], and as a source of a potential insecticide that acts as an anti-juvenile hormone [2]. Recently it has been screened for its antimicrobial property [3] and mosquitocidal activity [4].

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by the Reactive oxygen species (ROS). ROS are either free radicals, reactive anions containing oxygen atoms or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them [5]. Some synthetic antioxidants, such as butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BTH), exhibit potent free radical scavenging effects, which have been demonstrated to exert toxicological effects as compared with natural toxicants [6]. Free radicals play an important role in the pathogenesis of several human diseases, such as cancer, rheumatoid arthritis and cardiovascular diseases [7]. Natural antioxidants present in food of plant origin protect against these radicals and are therefore important tools in obtaining and preserving good health [8, 9].

DPPH is a stable, free radical used to determine the antioxidant activities of various compounds [10] and is one of the ROS which plays an important role in oxidative stress related to the pathogenesis of various important diseases [11]. Antioxidants have been reported to prevent oxidative damage by free radical and ROS and may prevent the occurrence of disease, and there is considerable evidence that antioxidants could help to prevent these diseases because they have the capacity to quench free radicals [12]. In the present study, an attempt has been made to study the antioxidant properties of the different solvent crude extracts of *Ageratum houstonianum* leaves using DPPH and Hydroxyl scavenging assays.

2. Materials and methods

2.1. Chemicals and reagents

0.2 mM DPPH (1, 1-diphenyl-2-picrylhydrazyl), 99 per cent ethanol, ascorbic acid, Butylated hydroxytoluene (BHT), hexane, ethyl acetate and methanol. All the chemicals and reagents used in this study were purchased from Himedia,

*Corresponding author: Dr. Samuel Tennyson, Assistant Professor, Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India
E-mail: samtennyson@gmail.com

Mumbai, India, which are of analytical grade.

2.2. Plant collection and extraction

Healthy, disease free plants of *Ageratum houstonianum* (leaves) were collected from Javadhu hills, Tiruvannamalai district, Tamilnadu, India. The species was identified, authenticated and voucher specimen deposited at Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamilnadu, India. Freshly collected leaf material was washed thoroughly, shade dried in open air and grounded into powder. The leaf powder (3 kg) was sequentially soaked in (6 L) hexane, ethyl acetate and methanol for 72 h each with intermittent shaking. After 72 h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate was then air dried to yield 16.8, 57.9 and 24.1g of hexane, ethyl acetate and methanol crude extracts respectively. All the crude extracts obtained were stored at 4^0C in air tight containers until assay.

2.3. DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of different crude extracts of *Ageratum houstonianum* were performed in accordance with the method proposed by Sanchez et al. [13]. The extracts and standard reference compounds were prepared with 99 per cent ethanol at various concentrations (50, 100, 200, 300, 400 and 500 μg/mL). One mL of various concentrations (50-500 μg/mL) of the extracts and standard reference compounds were dissolved in 1 mL of 0.2 mM DPPH separately and made up using 99 per cent ethanol in 10 mL test tube to achieve a final volume of 3 mL. The mixture was vortexed and incubated for 90 minutes at room temperature. The optical density was measured at 517 nm.

2.4. Hydroxyl radical scavenging assay

The ability of different crude extracts of *Ageratum houstonianum* to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to the modified method of Chung et al. [14]. The Fenton reaction mixture containing 200 μL of 10 mM $FeSO_4 \bullet 7H_2O$, 200 μL of 10 mM EDTA and 200 μL of 10 mM 2-deoxyribose was mixed with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing 200 μL of leaf extracts of *Ageratum houstonianum*. Thereafter, 200 μL of 10 mM H_2O_2 was added to the mixture and incubated for 4 hours at 37°C. After incubation, 1ml of 2.8 per cent TCA and 1mL of one per cent TBA were added and placed in a boiling water bath for 10 minutes. The resultant mixture was then allowed to cool to room temperature and centrifuged at 395×g for 5 minutes. Absorbance was recorded at 532 nm in a UV-VIS spectrophotometer. Radical scavenging (%) = Absorbance control − Absorbance test sample/Absorbance control × 100

2.5. Statistical analysis

All the experiments were carried out at different concentrations, each with three replicates and the entire experiment was repeated thrice and the data were analyzed (Mean ±S.E.) from three experiments.

3. Results

The present study was undertaken to evaluate the free radical scavenging activity of hexane, ethyl acetate and methanolic leaf extracts of *Ageratum houstonianum* against DPPH and hydroxyl radicals. In DPPH radical scavenging activity, ethyl acetate extract was found to be higher (88.26 ±0.35) followed by (82.62 ±0.89) methanol extracts at 500 μg/mL, when compared to hexane extract (72.82 ±0.41). BHT and ascorbic acid were used as positive controls. The IC50 values for ethyl acetate and methanol were 90.3 and 198.7 μg/mL respectively (Table 1).

Table 1.
DPPH free radical scavenging activity of crude extracts of *Ageratum houstonianum* leaves

Concentration(μg/mL)	% Inhibition of DPPH free radical				
	Hexane	Ethyl acetate	Methanol	BHT	Ascorbic acid
50	25.23 ±0.25	36.19 ±0.54	31.12 ±0.55	35.19 ±1.13	40.22 ±0.22
100	33.24 ±1.12	55.52 ±0.23	48.26 ±0.69	46.19 ±0.89	51.19 ±0.41
200	48.21 ±0.91	62.76 ±1.09	53.76 ±1.22	54.21 ±0.79	60.11 ±1.32
300	52.17 ±1.02	71.26 ±0.82	66.81 ±1.00	62.91 ±0.91	68.26 ±0.43
400	60.24 ±0.29	80.27 ±1.12	76.19 ±0.38	70.19 ±1.04	78.33 ±0.98
500	72.82 ±0.41	88.26 ±0.35	82.62 ±0.89	74.49 ±0.39	85.12 ±1.08

All values represent Mean ±S.E from three experiments, each with 3 replicates per dose

Table 2.
Hydroxyl free radical scavenging activity of crude extracts of *Ageratum houstonianum* leaves

Concentration(μg/mL)	% Inhibition of Hydroxyl free radical				
	Hexane	Ethyl acetate	Methanol	BHT	Ascorbic acid
50	22.25 ±0.38	26.89 ±0.11	25.23 ±0.34	28.41 ±0.19	33.91 ±0.39
100	32.55 ±0.65	32.29 ±0.43	30.14 ±0.65	34.51 ±1.18	40.12 ±0.82
200	40.09 ±0.98	53.91 ±0.61	44.13 ±0.29	45.24 ±0.29	49.89 ±0.55
300	47.31 ±0.11	60.44 ±1.02	56.23 ±0.19	57.89 ±0.49	58.29 ±0.88
400	55.33 ±0.14	68.53 ±0.76	60.08 ±0.55	68.15 ±0.92	66.81 ±0.83
500	64.61 ±0.52	75.81 ±0.39	68.29 ±0.91	72.12 ±0.54	70.21 ±0.19

All values represent Mean ±S.E. from three experiments, each with 3 replicates per dose

4. Discussion

The DPPH test is the oldest indirect method for determining the antioxidant activity based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols [15]. DPPH radical scavenging of the acetone extracts were significantly higher ($P<0.05$) than those of methanol extracts except for *Adansonia digitata* and *Vitellaria paradoxa*. The effect of the free radical scavenging activity of hydro alcoholic extract on DPPH radicals is thought to be due to the hydrogen donation ability of polyphenols from *Ichnocarpus frutescens* [16].

Even in the case of hydroxyl radical scavenging activity, the ethyl acetate extract was found to be high activity (71.99 $\pm$1.10) at 500 μg/mL, whereas in hexane and methanol extracts of *Ageratum houstonianum* the highest activity was found to be 64.61 $\pm$0.52 and 68.29 $\pm$0.91 percentage of inhibition respectively at 500 μg/mL (Table 2). The IC50 values for ethyl acetate and methanol were 231.25 and 285.70 μg/mL respectively. IC50 values of the extracts of *Millettia pulchra* and *Pittosporum moluccanum* displayed hydroxyl-scavenging activities as 0.16-0.67 μg/mL [17]. Certain biologically active agents containing phenolic rings have the capacity to scavenge hydroxyl radicals by virtue of aromatic hydroxylation at the orthoposition, and it is shown to have a protective effect on ischemia-induced cerebral neuron damage [18]. The extract of *Eclipta alba* exhibited dose dependent inhibition of oxidation of DMSO indicating hydroxyl radical scavenging activity up to 22.11 per cent at the concentration of 100 μg/mL [19].

These results are in agreement with the statement of Opoku *et al.* [20], Mahakunakorn *et al.* [21] and Katalinic *et al.* [22], in the way that many phenolic compounds widely distributed in the plant kingdom behave as reducing agents with antioxidant and free radical scavenging activities. In conclusion, the free radical scavenging activities for three solvent extracts of our test plant were evaluated by using DPPH and hydroxyl methods, providing to be an effective for the selection of the species with strong antioxidant activities with potential use in cosmetics and medicinal preparations. Further study on the test plant is required to identify and isolate the secondary metabolites responsible for their antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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