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Research Article

In Vitro Antioxidant Activity on Leaves of *Ageratum Conyzoides* Extract

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Abstract: ***Objective:*** The present study investigated the antioxidant activity of ethanol extract of leaves of *Ageratum conyzoides*. ***Methods:*** leaves of ethanol extract of *Ageratum conyzoides* were tested for *in vitro* free radical scavenging assays, nitric oxide, such as hydroxyl radical, inhibition of superoxide anion radical, 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), and hydroxyl radical. ***Results:*** *leaves* extract effectively scavenged free radicals at all different concentrations and showed its potent antioxidant activity. Further, these effects were in a dose dependent manner. Results were compared to standard antioxidants ***Conclusion:*** leaves of *Ageratum conyzoides* showed strong antioxidant potential.

Keywords: Vitro Antioxidant *Ageratum Conyzoides.*

INTRODUCTION

Over production of reactive oxygen species (ROS) in human beings, by endogenous or external sources, e.g. Tobacco smoke, certain pollutants, organic solvents or pesticides, leads to oxidative stress [1]. Oxygen is an essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer [2]. In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favoring such diseases states [3]. Though synthetic antioxidants, butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) and radio protector, war far in are being used widely, however, due to their potential health hazards, they are under strict regulation [4]. Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits [5].

Ageratum conyzoides plant also referred to as *Billy Goat Weed* is widely utilized in traditional medicine. Traditional communities in India use this plant as a bacteriocide, antidysenteric, and antilithic. And in Asia, South America and Africa, aqueous extract of this plant is used as a bacteriocide. In Central Africa it is used to treat pneumonia; however the most common use is to cure wounds and burns [6, 7]. *Ageratum conyzoides* is among such medicinal plants that are effective against diseases and may contain biologically active compounds, which are effective against ill health. *Ageratum conyzoides* has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments, such as burns and wounds, headaches, pneumonia, anlogestic, inflammation, asthma, spasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases [8].

The objective of the present study was to investigate the antioxidant activity of the ethanol extract of leaves of *Ageratum conyzoides* by using *in-vitro* models.

MATERIALS AND METHODS

Chemicals

Methanol, ethanol, DPPH (2, 2-diphenyl-1-picrylhydrazyl), naphthyl ethylene diamine dihydrochloride, sulfanilic acid, EDTA, deoxyribose, hydrogen peroxide were obtained from Sigma-Aldrich (St Louis, MO, USA). All other chemicals used were of analytical grade.

Collection of plant material

The leaves of *Ageratum conyzoides* were collected from Niger Delta University Botanical Farm and the plant material was taxonomically identified and authenticated by the Department of Botany Niger Delta University

Extraction of plant material

The leaves of *Ageratum conyzoides* were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol in soxhlet apparatus at 60 ^{o}C. The solvent was completely removed by rotary vacuum evaporator and concentrated. The extract was freeze dried and stored in a vacuum desiccators for further antioxidant studies.

In-vitro antioxidant assay

DPPH radical scavenging assay

The free radical scavenging capacity of the ethanol extract of leaves of *Ageratum conyzoides* was determined using DPPH assay. A methanol DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (0.15%) was mixed with serial dilutions (100 µg/ml to 800 µg/ml) of the ethanol extract of leaves of *Ageratum conyzoides* and after 10 min; the absorbance was read at 515 nm using a spectrophotometer. Quercetin used as a standard. The percentage inhibition of DPPH was recorded [9].

Nitric oxide radical scavenging assay

Nitric oxide radical inhibition by Griess Illosvoy chemical agent was changed by victimization naphthyl ethylene diamine dihydrochloride (0.1% w/v) rather than 1-napthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and therefore the ethanol extract of leaves of *Ageratum conyzoides* (100 µg to 800 µg/ml) was incubated at 25 ^{0}C for a 150 min. After incubation, 0.5 mL of the reaction mixture mixed with 1 mL of sulfanilic acid chemical agent (0.33% in 20% glacial carboxylic acid) and allowed to face for five minutes for finishing diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to face for thirty minutes at 25 ^{0}C. The absorbance of those solutions was measured at 540 nm against the corresponding blank solutions [10].

Hydroxyl radical scavenging assay

The assay was performed as represented by Halliwell et al., 1987 with minor changes. All solutions were ready freshly. 1.0 ml of the reaction mixture contained one hundred µL of twenty eight mM 2-deoxy-2-ribose (dissolved in phosphate buffer pH 7.4), 500 µL resolution of assorted concentrations of the ethanol extract of leaves of *Ageratum conyzoides* (100 µg to 800 µg/ml), 200 µL of 200 µM FeCl3 and 1.04 mM ethylenediamine tetracetic acid (EDTA, 1:1 v/v), 100 µL H_2O_2 (1.0 mM) and 100 µL ascorbic acid (1.0 mM). At the end of 1 hr at 37 ^{o}C, the extent of deoxyribose degradation was measured by the TBA reaction. The absorbance was measured at 532 nm against the blank solution.

Reducing power activity

The reducing power of ethanol extract of leaves of *Ageratum conyzoides* (100 µg to 800 µg/ml) was determined according to the method of Oliveira *et al*., [15]. Various concentrations of the extract in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50 ^{0}C for 20 min; aliquots of trichloroacetic acid (2.5 mL, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

RESULTS

Table-1: Percentage effect of ethanolic extract of leaves of *Ageratum conyzoides* against DPPH radical, Nitric oxide and reducing power

µg/ml)	% Inhibition DPPH	% Inhibition NO•	% Inhibition Reducing power	% Inhibition hydroxyl radical
100	50.31 ± 0.16	8.13 ± 0.66	20.15 ± 3.15	33.22 ± 0.55
300	70.21 ± 3.42	21.69 ± 1.45	35.86 ± 1.87	40.87 ± 1.55
500	84.31 ± 2.61	33.65 ± 2.98	57.11 ± 0.22	60.34 ± 0.77
800	93.22 ± 0.22	49.31 ± 1.10	77.53 ± 0.98	80.03 ± 0.33

Results are mean ± S.D of five measurements

DISCUSSION

Free radicals, species with one (or) additional unpaired electrons, are a unit made in traditional (or) pathological cell metabolism from xenobiotics, (or) through radiation. Electron acceptors like molecular chemical element react simply with free radicals to become radicals themselves ROS (Reactive chemical element species). The first derivatives of chemical element (O_2, H_2O_2, .NO•, O_2) play a vital role in mediating ROS effects [11].

The present investigation demonstrated that, DPPH, a radical, stable at temperature that produces a purple color solution in methanol. DPPH is reduced within the presence of an antioxidant or reducing agent. Ethanol extract of leaves of *Ageratum conyzoides* (100 µg to 800 µg/ml) decreases the purple colouraton of DPPH radical as a result of its radical scavenging ability of ethanol extract of *Ageratum conyzoides*, which is comparable to the reported values of Raja and Ravindranadh [16].

Nitric oxide radical inhibition study proved that ethanol extract of *Ageratum conyzoides* (100 µg to 800 µg/ml) was a potent scavenger of Nitric oxide. This nitric oxide generated from sodium nitroprusside reacts with oxygen to nitrate group. The extract of plant inhibits nitrate formation by competitively inhibiting oxygen to react with nitric oxide directly and additionally to inhibit its synthesis. Scavengers of nitric oxide contend with oxygen resulting in reduced production of nitric oxide [12]. The present study showed *Ageratum conyzoides* inhibit nitric oxide at 100 µg to 800 µg/ml.

Ageratum conyzoides (100 µg to 800 µg/ml) extract was examined for its ability to act as .OH radical scavenging agent. Ferric EDTA was incubated with H_2O_2 and ascorbic acid at pH -7.4; hydroxyl group radicals were shaped in free solution and were detected by their ability to degrade 2-deoxy-2-sugar into fragments that on heating with TBA at low hydrogen ion concentration form a pink compound [13]. When ethanol extract of *Ageratum conyzoides* was added to the reaction mixture, they removed hydroxyl group radicals and prevented the degradation of 2-deoxy-2-sugar as mentioned earlier; values of each plant extract were analogous to the reportable values of Sen *et al*., [14].

Table 1 shows the reductive capability of ethanol extract *Ageratum conyzoides.* Like the antioxidant activity, the reducing power increased with increasing concentration of *Celosia trigyna.* The ethanol extract of *Ageratum conyzoides* (800 µg/ml) showed the highest reducing ability than all the other concentration tested.

CONCLUSION

The present study proved promising antioxidant potential of ethanol extract of *Ageratum conyzoides* against a variety of free radicals. It is reported that phenolics and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms. Thus the antioxidant activity of *Ageratum conyzoides* may be attributed to the presence of these compounds. Hence these results support the view that some traditionally used Nigerian medicinal plants are promising source of potential antioxidants.

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