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In-vitro evaluation of anti-oxidant activity of ageratum conyzoides linn leaves in animal models

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ABSTRACT

Antioxidants are compounds that can inhibit or prevent the oxidation of the easily oxidized substrate. One of the plants as a potential source of bioactive compounds and antioxidant activity (*Ageratum conyzoides*). This plant was commonly found in the West Africa, Australia, Colombia and India has been used by the society. This study aimed to determine the proximate compositions, bioactive compounds and antioxidant activity from large-leafed mangrove fruit which extracted by methanol.The phytochemical screening was carried on the both extracts of leaves of *Ageratum conyzoides*, revealed the presence of some active ingredients such asAlkaloid, Flavonoids, Tannins, Saponins, Phenols.The aqueous and alcoholic leaves extract were also evaluated for their antioxidant activity using FRAP assay, Metal chelating assay, DPPH radical scavenging assay, superoxide-radical scavenging assay and Hydrogen peroxide scavenging assay. The result of the present study showed that the ethanolic leaves extract of *Ageratum conyzoides*has shown the greatest anti-oxidant activity than aqueous extracts. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds. Further work is needful to isolate the exact compound which is responsible for antioxidant activity and biophysical characterization can be done in the future.

INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Theymay protect cells from damage caused by unstable molecules known as free radicals. Antioxidantsterminate these chain reactions by removing free radical intermediates, and inhibit other oxidationreactions by being oxidized themselves. Free radicals are fundamentals to any biochemical process andrepresent an essential part of aerobic life and metabolism. Majority of the diseases are mainly linked tooxidative stress due to free radicals[1,2].Our body is rich in endogenous antioxidants, the substances that have the ability to stop free radicalsformation or to limit the damage they cause[3]. The effectiveness of current used exogenous antioxidantsarises most probably from the increase of the endogenous free radical scavengers as enzymes(superoxide dismutase and selenium-dependent glutathione peroxidase), vitamins (alpha tocopherol andascorbic acid). Many plants have been also found to posses free radical scavenging activity(Polyphenols, alkaloids and terpenoids). Low levels of one or more of the essential antioxidants havebeen shown to be associated with many disorders including cancer, inflammation, atherosclerosis,coronary heart disease and diabetes.Thus, in such cases, the administration of exogenous antioxidants seems to be salutary. Nowadays, agreat deal of effort being expended to find effective antioxidants for the treatment or prevention of freeradical-mediated deleterious effects[4].

Oxidative stress is characterized as an imbalance between the production of reactive species andantioxidant defense activity, and its enhanced state has been associated with many of the chronicdiseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases[5]. Based on that,many research groups have driven efforts to assess the antioxidant properties of natural products.These properties have been investigated through either chemical (in vitro) or biological (in vivo) methods, or both [6]. The results of these researches have led some to suggest that the long-termconsumption of food rich in antioxidants can retard or

avoid the ocurrence of such diseases [7,8].According to Brewer, the effectiveness of a large number of antioxidant agents is generallyproportional to the number of hydroxyl (OH) groups present in their aromatic ring(s). Based on that,the natural compounds would seem to have better antioxidant activity than the currently used syntheticantioxidants, making them a particularly attractive ingredient for commercial foods.Despite the large number of natural products that are currently consumed as antioxidant agents, thesearch for new chemical entities with antioxidant activity still remains a burgeoning field. In thiscontext, the lichens have played an important role as a source for new antioxidant agents.Lichens are symbiotic organisms consisting of a fungus and one or more photosynthetic partners,the latter usually being either a green alga or a cyanobacterium [11,12]. They are found in a wide varietyof natural habitats or in places with low temperatures, prolonged darkness, drought and continuous light. Lichens produce characteristic and unique secondary metabolites, and most of them occurexclusively in these symbiotic organisms. The most common lichen compounds are aromaticpolyketides, particularly depsides, depsidones, depsones, dibenzofurans, and chromones.Lichens have been used in the folk medicine for numerous purposes, among them as astringents,laxatives, anticonvulsive, antiemetics, antiasthmatics, anti-inflammatories, antibiotics, and also for thetreatment of cardiovascular, respiratory, and gastric disorders. Furthermore, pharmacological andbiotechnological studies have been carried out in order to test and to develop biomaterials containinglichen-isolated natural compounds for humans use [13,14].

Medicinal plants with antioxidant potential

1. Rhizophora mangle is a plant from Rhizophoraceae family. The bark extract of the plant showed scavenging activity of hydroxyl radicals and the extract contained polyphenols, carbohydrates and sterols[15].
2. Diospyros malabarica is a plant from Ebenaceae family. The bark is used for the treatment offever and fruit juices for healing of wound ulcer5. The stem extract of the plant competes withoxygen to react with nitric oxide and thus, inhibits the generation of anions. The mainphytoconstituents in the extract are phenolic compounds[16].
3. Asparagus racemosus is a tree from Liliaceae family. It shows antioxidant activity through thefree radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging,nitric oxide scavenging, metal chelation, reduction power and inhibition of lipid peroxidation inrats. The main phytoconstituents are saponins, alkaloids and flavonoids[17].
4. Auricularia auricular is a tree and known as ‗tree ear or wood ear' from Auriculaceae family: Ithas shown a potent hydroxyl radical scavenging and lipid per-oxidation inhibitory activities.The main phytoconstituents are flavonoids [18].
5. Eucalyptus globules is a tree and known as ―Karpuramaram‖ from Myrtaceae family. Theantioxidant activity of Eucalyptus oil was estimated by two in vitro assays namely diphenylpicryl hydrazyl radical scavenging activity and inhibition of ascorbate induced lipid peroxidation method[19].
6. Acacia arabica is a plant from Mimosae family. The antioxidant assays were carried out invivo and in vitro experimental models. In vitro, lipid peroxidation was carried out by tertiarybutyl hydroperoxide (TBH) induced lipid peroxidation. In vivo, experiments were carried out inCCl4-induced hepatotoxicity in rats. The bark of the plant contained quercetin, (+) catechin, (-)epicatechin and gallic acid. The polyphenol rich active fraction of Acacia arabica is a potentfree radical scavenger and protects TBH induced lipid peroxidation and CCl4-induced hepaticdamage. The bark is used in the treatment of asthma, bronchitis, diabetes, dysentery and skin diseases[20].
7. Ligustrum vulgare is a plant from Oleaceae family. The leaves antioxidant activity wasevaluated using DPPH test. The main phytoconstituents are flavonoids, iridoids, coumarins andessential oil, where flavonoid aglycones are responsible for the antioxidant activity and it showsa potent free radical scavenging activity[21].
8. Terminalia chebula is a tree and known as Myrobalanuschebula. Combretaceae family. Themain phytoconstituents are tannins, chebulinic and gallic acids. The extract was tested bystudying the inhibition of radiation induced lipid peroxidation in rat liver microsomes. It showsfree radical scavenging activity due to presence of tannins and also It inhibits the developmentof duodenal ulcer and so the extract has appeared to show a cytoprotective effect on the gastric mucosa [22].
9. Lobelia nicotianaefolia is a plant from Campanulaceae family. The chemical constituents arealkaloids as lobeline and also it contains volatile oil, resin, gum and fixed oil. It is mainly usedin the treatment of asthma and as respiratory stimulant[19].
10. Citrus lemon is a tree from Rutaceae family. The antioxidant activity was estimated by two invitro assays, DPPH radical scavenging activity and inhibition of ascorbate induced lipidperoxidation (LPO) method. The main phytoconstituents are citral and limonene. Theantioxidant property is shown due to the presence of citral.

MATERIALS AND METHODS

Sodium hydroxide was gift sample from (Analytical grade, FisherChemicals Inc., Fair Lawn, NJ), citric acid was gift sample from (analytical grade), hexanes was gift sample from (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ), methanol was gift sample from (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ), ethyl acetate was gift sample from (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ), BCL3-methanol was gift sample from (Supelco Inc., Belletonte, PA), 98% 2, 2-Dimethoxypropane (Sigma-Aldrich Inc., St. Louis, MO), Anhydrous sodium sulfate was gift sample from (10-60 mesh, Fisher Chemicals Inc., Fair Lawn, NJ), cholesterol was gift sample from (Aldrich Chem. Co., Milw., WI), 5α-cholestane (Sigma-Aldrich Co., St. Louis, MO), heptadecanoic acid was gift sample (Sigma chemical Co.,

St.Louis, MO), DHA (cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid, was gift sample from Sigma-Aldrich Inc., St. Louis, MO).

RESULTS AND DISCUSSION

Phytochemical screening of Ageratum conyzoides

The present investigation concluded that the isolated compounds from the plant *Ageratum conyzoides*are pure and the plant *Ageratum conyzoides* shows the various antibacterial effects against different bacteria and found that different phytochemical compunds. Further study is needed for the isolation of the constituents present in the plant and its individual pharmacological activity should need to consider and ultimately it should be implemented for the benefit to human beings.

Table1. Phytochemical screening of *Ageratum conyzoides*

S.No.	Phytoconstituents	Aqueous	Alcoholic
1.	Alkaloid	-	+
2.	Flavonoids	+	-
3.	Tannins	+	+
4.	Saponins	-	+
5.	Phenols	+	+

Antioxidant properties of *Ageratum Conyzoides*

Several mechanisms have been proposed to be involved in antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions and elimination of peroxides (Gordon, 1990). Antioxidant activity is system- dependent and characteristic of a particular system can influence outcome of analysis. Hence, a single assay would not be representative of antioxidant potential of plant extracts. In this present study, different models of antioxidant assays were employed, which could provide a more consistent approach to assess antioxidant activity of leaves of *Ageratum conyzoides.*

Ferric reducing ability of *Ageratum conyzoides*

FRAP assay is based on a redox-linked reaction, whereby antioxidants present in plant extracts act as reductants while ferric ions in reagents serve as oxidants. Reduction of ferric-tripyridyltriazine to ferrous complex forms an intense blue color with maximum absorption at 593 nm, which is related to amount of antioxidants in the sample. The ferric reducing ability of leaves of *Ageratum conyzoides* is shown in Table 4.6. Water and alcohol extract reduced ferric ions efficiently and had reducing activity in the range of 0.82 – 2.83 mM/g, which was greater than or comparable to synthetic antioxidant BHT (1.28 mM/g). Both extracts were less effective, when compared with reducing activity of quercetin (15.61 mM/g).

Reduction of ferric to ferrous ion is frequently used as an indicator of electron donating activity, which is considered to be an important factor dictating antioxidant activity of plant. Figure 4.5 shows dose-response curves for reducing power of different extracts from *Ageratum conyzoides* leaves. Leaves extracts showed significant ability to reduce ferric ions in a dose-dependent manner. Water and alcohol extract showed highest reducing power. Quercetin and BHT revealed potent reducing power, which were distinctly higher than that of any of *Ageratum conyzoides* extracts.
Antioxidant activity has been reported to be concomitant with reducing power of plant extract (Gordon, 1990). Significant ferric reducing ability of *Ageratum conyzoides* extracts observed in this study suggest that polyphenolics present in the extracts have the ability to donate electrons to free radicals by acting as reductones and thus could terminate free radical-mediated oxidative reactions. Catechin, sinapic acid, ferulic acid, quercetin and myricetin, which were identified in Ageratum conyzoides have been shown to possess significant ferric reducing ability in their pure form, suggesting that ferric reducing ability of *Ageratum conyzoides* could have been partly contributed by these phenolics (Pulido et al, 2000). Present findings are in line with those of other investigators, who have also reported that antioxidant properties are concomitant with development of reducing power (Chung etal, 2005).

Table 2: Ferric Reducing Ability - FRAP (expressed as mM FeSO4/g dry weight) of leaves of *Ageratum conyzoides*.

Group	Drugs	IC_{50} valueµg/ml
I	Quercitin	15.17±0.075
II	Butylated Hydroxy Toulene(BHT)	4.63±1.115
III	AQEAC	2.36±0.051
IV	ALEAC	1.43±0.090

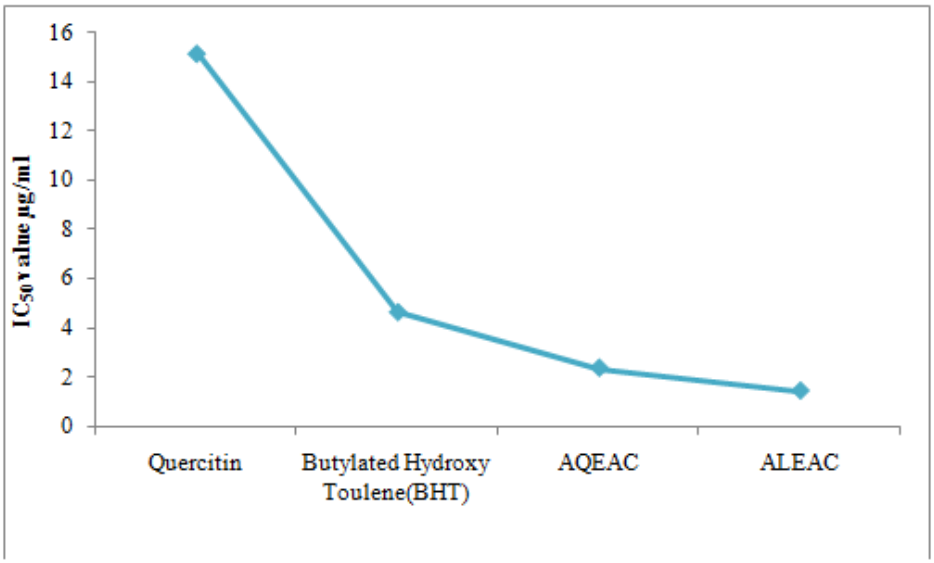

Fig 1: Reducing power of *Ageratum conyzoides*Quercetin and BHT were used as reference antioxidant Values are means ± SD (n = 3).

Metal chelating activity of *Ageratum conyzoides*

*Ageratum conyzoides*extracts were evaluated for their ability to chelate ferrous ion by competing with ferrozine in free solution. All extracts displayed an ability to chelate ferrous ion in a dose-dependent manner (Figure 4.6). However, estimated IC50 was very high (more than 2.0 mg/ml); particularly, in comparison with positive control EDTA (7.75 Jg/ml). Quercetin and BHT showed moderate metal chelating activity when compared with EDTA with an IC50 of 134Jg/ml and 86Jg/ml respectively. Water and alcohol extract showed a chelating ability of 28.54 and 20.86% respectively at 1.0 mg/ml. In case of leaves extract, metal chelating activity varied from 2.15% to 30.83%. Alcoholic extracts were the highest, followed by water extract. EDTA, quercetin and BHT exhibited 99.23%, 60.54% and 71.36% of chelating activity respectively, which were significantly higher than that of *Ageratum conyzoides* extracts.

Transition metal ions gain utmost significance in biological system due to their ability to generate reactive free radicals. They can initiate Fenton type reaction with production of hydroxyl radicals or Haber-Weiss reactions with superoxide radicals (Kehrer, 2000; Wong and Kitts, 2001). They hasten peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate chain reaction of lipid peroxidation (Halliwell and Gutteridge, 1984; Halliwell, 1991). Metal chelating capacity is imperative as it decreases concentration of catalyzing transition metal ions in Fenton type reaction and protects system from oxidative damage through inhibition of metal-dependent processes. Chelating agents that form bonds with metals are effective as secondary antioxidants because they can reduce redox potential by stabilizing oxidized form of metal ion (Gordon, 1990). Regardless of reduced activity, *Ageratum conyzoides* extracts did possess moderate iron binding capacity, suggesting their protective action against lipid peroxidation-mediated oxidative damage. This result is not surprising, as non-phenolic compounds are supposed to be better chelators of metal ions than polyphenols (Chan et al, 2007).

Table 3: Metal chelating activity of leaves of *Ageratum conyzoides*.

Group	Drugs	IC_{50} valueµg/ml
I	EDTA	7.53
II	Quercitin	132
III	Butylated Hydroxy Toulene(BHT)	85
IV	AQEAC	21.69
V	ALEAC	33.41

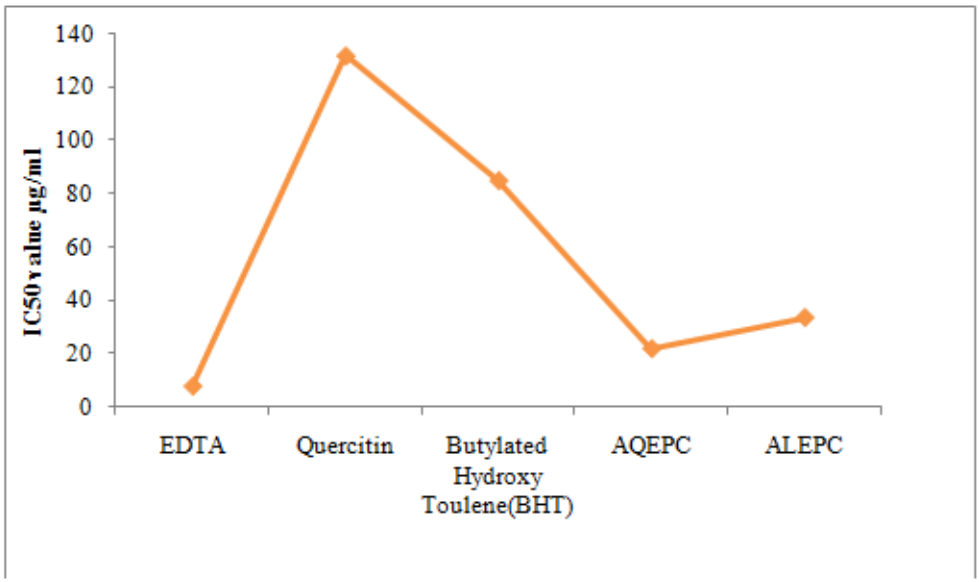

Fig-2 Metal chelating activity of *Ageratum conyzoides*. EDTA was used as positive control. Quercetin and BHT were used as reference antioxidants. Values are means ± SD (n = 3).

DPPH radical scavenging activity of *Ageratum conyzoides*

Basic information on efficacy of compounds in *Ageratum conyzoides*extracts to quench free radicals can be deduced from DPPH assay. The DPPH is a stable free radical, which is recognized as a tool for evaluating radical scavenging ability of compounds and antioxidant activity of foods (Sanchez-Moreno, 2002). It accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH is determined by decrease in its absorbance at 517 nm, induced by antioxidants. It has also been used to quantify antioxidants in complex biological systems, because of its ease and convenience. Even though, DPPH radicals may not be biologically pertinent, it presents an indication of hydrogen/ electron-donating capacity of plants and provides a useful means to measure in vitro antioxidant activity. *Ageratum conyzoides*extracts revealed a concentration-dependent scavenging of DPPH radicals, with leaves presenting strongest effect followed by leaves (Figure 4.8). of aqueous and alcoholic extracts showed strongest effect (IC50 at 31 Jg/ml for leaves), followed by water and alcohol extracts (Table 4.8.1). Comparison of DPPH radical scavenging activity with standard antioxidants showed that the most potent *Ageratum conyzoides*extracts had scavenging ability higher than BHT (IC50 at 493 Jg/ml), but lower than quercetin (IC50 at 11 Jg/ml).

Effective DPPH radical scavenging activity exhibited by *Ageratum conyzoides*extracts could be explained by the presence of polyphenolics in them, whose radical scavenging properties were reported previously in various model systems (Fukumoto and Mazza, 2000). Radical scavenging ability of polyphenolics is attributed to their ability to donate a hydrogen atom from a phenol to give DPPH-H and a phenoxyl radical. Alcoholic extracts contained more amounts of ferulic acid and sinapic acid, which could partially explain higher ability to scavenge DPPH (Kim et al, 2008), in comparison with water and alcohol extracts. Catechin, the major component of water extracts was found to be moderately active as an antioxidant in DPPH assay (Hwang et al, 2001). A comparison between DPPH radical scavenging activities of *Piper Cubeba*. *Ageratum conyzoides* extracts were more potent in terms of radical scavenging activity whereby their IC50 values were comparatively much lower than these BHT (Lee et al, 2008; Koksal and Gulcin, 2008; Borowski et al, 2007), thus further demonstrating effectiveness of *Ageratum conyzoides* leaves as natural antioxidants.

Table -3 Scavenging ability of root, stem and leaves of *Ageratum conyzoides* and standard antioxidants on DPPH• as determined by their IC50, expressed as mg/ml.

Group	Drugs	IC_{50} valueµg/ml
I	Quercitin	5.1±0.097
II	Butylated Hydroxy Toulene(BHT)	3.63±0.081
III	AQEAC	1.82±0.057
IV	ALEAC	1.8±0.031

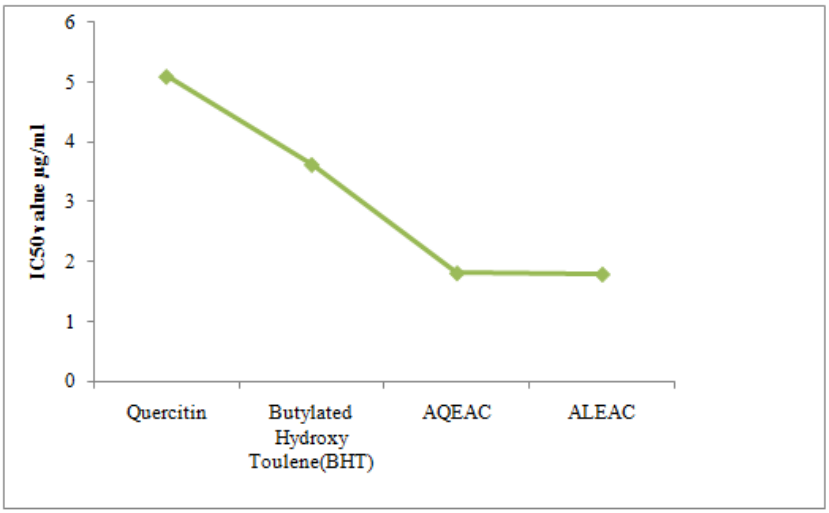

Fig-3 DPPH radical scavenging activity of *Ageratum conyzoides*. Quercetin and BHT were used as reference antioxidant.Values are means ± SD (n = 3).

Superoxide radical scavenging activity of *Ageratum conyzoides*

Superoxide anion is a reduced form of molecular oxygen that is generated during normal metabolic processes. It is known to be destructive to cellular components as a precursor of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen (Stief, 2003), contributing to tissue damages and various chronic diseases (Halliwall, 1991). The scavenging activity of *Ageratum conyzoides*extracts on superoxide radicals is shown in Figure 4.9. Extracts from different parts of *Ageratum conyzoides*displayed concentration dependent protective activity against superoxide radicals Of which, leaves were the most effective. Alcoholic extracts of leaves (IC50 at 23 Jg/ml) showed potent scavenging activity. Aqueous extracts exhibited moderate activity with IC50 in the range of 131 – 841 Jg/ml. When radical scavenging activity of *Ageratum conyzoides* extracts compared to IC50 values calculated for reference antioxidants BHT (IC50 at 19 Jg/ml), but less effective than quercetin (IC50 at 10 Jg/ml).

Table-5 Scavenging ability of root, stem and leaves of *Ageratum conyzoides* and standard antioxidants on superoxide radical (O2•) as determined by their IC50, expressed as mg/ml.

Group	Drugs	IC_{50} valueµg/ml
I	Quercitin	0.018±0.005
II	Butylated Hydroxy Toulene(BHT)	0.012±0.001
III	AQEAC	0.28±0.006
IV	ALEAC	0.305±0.003

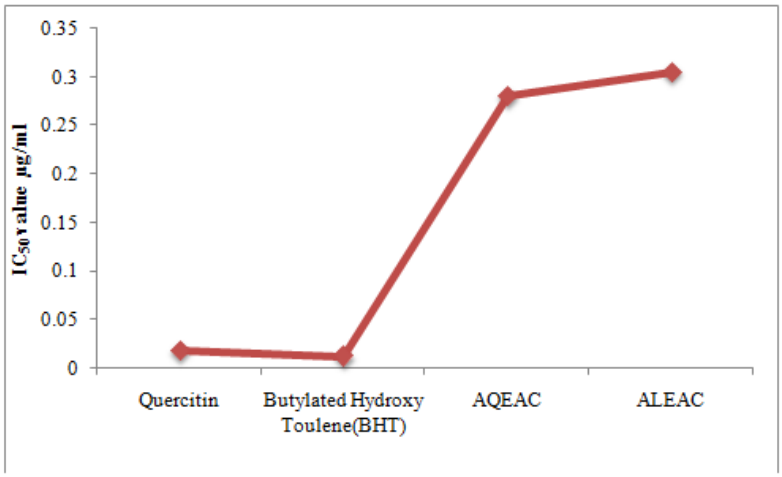

Fig-4 Superoxide radical scavenging activity of *Ageratum conyzoides*. Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

Hydrogen peroxide scavenging activity of *Ageratum conyzoides*

Though hydrogen peroxide (H2O2) itself is not ver8y reactive, it can occasionally be toxic to cells, since it may give rise to potentially reactive hydroxyl radicals (Halliwell, 1991). The scavenging activity of *Ageratum conyzoides*extracts on H_2O_2 is shown in Figure and compared with quercetin and BHT as standard antioxidants. *Ageratum conyzoides*extracts were capable of scavenging H_2O_2 in a concentration-dependent manner. Of different extracts, alcoholic group showed strongest H_2O_2 scavenging activity. The aqueous extract of leaves displayed the most potent activity with IC50 at 67 Jg/ml, which was comparable to quercetin (IC50 at 34 Jg/ml) and more effective than BHT (IC50 at 89 Jg/ml).

Table-6 Scavenging ability of leaves of *Ageratum conyzoides* and standard antioxidants on hydrogen peroxide (H_2O_2) as determined by their IC50, expressed as mg/ml

Group	Drugs	IC_{50} valueµg/ml
I	Quercitin	0.038±0.001
II	Butylated Hydroxy Toulene(BHT)	0.058±0.006
III	AQEAC	0.070±0.017
IV	ALEAC	0.486±0.031

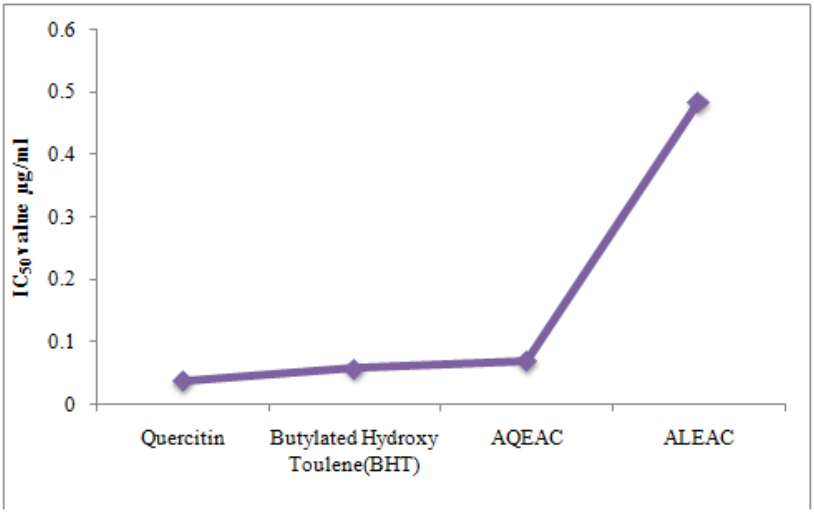

Fig-5 Hydrogen peroxide scavenging activity of *Ageratum conyzoides*. Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3)

CONCLUSION

The result of the present study showed that the aqueous and alcoholic extract of *ageratum conyzoides*plant, which contains phenolic and flavonoidal compounds, exhibited the great antioxidant activity. The high scavenging property of methanolic extract of *ageratum conyzoides*plant may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. Aqueous and alcoholic extracts of *ageratum conyzoides*plant in this research exhibited antioxidant. The antioxidant potential may be attributed to the presence of polyphenolic compounds.

In this study, all antioxidant methods (FRAP assay, Metal Chelating assay, DPPH radical-scavenging assay, Superoxide radical scavenging assay and Hydrogen peroxide scavenging assay) showed that the both aqueous and alcoholic extracts of *Ageratum conyzoides*contain more antioxidant activities. More- over, this study demonstrated the important source of phenol compounds, which are a good source of antioxidant activity. The phenol component has a high inhibitory effect that prevents lipid peroxidation. However, the solvent type has an important role in detecting phenol compounds and antioxidant factors. Thus, we concluded that *Ageratum conyzoides*act via its free radical scavenging to preventlipidperoxi-dation. Therefore, natural antioxidants and phenol compounds in *Ageratum conyzoides*have the capability to be used medically and in food systems to preserve food quality.

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