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# Antioxidant properties of *Ageratum conyzoides* L. Asteraceae leaves

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**Abstract**

*In vitro* antioxidative and anti-lipid peroxidative properties of aqueous and methanol extracts of *Ageratum conyzoides* leaves were studied in controlling erectile dysfunction caused by oxidative stress. Methanol extract gave a significantly ($P < 0.05$) higher content of total phenolic (61.4 mgGAE/g), total flavonoid (42.2 mgQE/g), ascorbic (10.1 mgAAE/100g) and phosphomolybdate (45.8 mgAAE/g) than the aqueous extract. The result showed that the extracts have high antioxidant activities. However, the methanol extract showed a higher DPPH and hydrogen peroxide scavenging activities over aqueous extract but the aqueous extract had a higher reducing power. The methanol extract exhibited a greater inhibition against lipid peroxidation induced by $Fe^{2+}$ in rat pancreas and penile tissue homogenate exemplified by their least IC50 (94.21 µg/ml in pancreas) and (75.95 µg/mL in penile tissue) while in rat brain homogenate the aqueous extract exhibited a greater inhibition against lipid peroxidation induced by $Fe^{2+}$ with least IC50 of 91.74 µg/mL. Hence, these extracts can be used as a potent natural antioxidant against free radicals and as a natural source of combating erectile dysfunction caused by oxidative stress. The extracts of *Ageratum conyzoides* leaves could be useful therapeutically as erectogenic agent.

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## Introduction

Erectile dysfunction (ED) means a condition where there is consistent inability in man to get and/or maintain penile erection in order to have a satisfactory sexual performance (NIH Consensus Conference 1993). 150 million men is estimated globally to have suffered from ED and this could rise to 322 million in the year 2025 (Kassier and Veldman, 2014). Normal erection is a function of adequate balance between these factors: psychology, neurology, vascular hormone and corpus cavernous. Hence if this balance in these factors is altered it can lead to ED (Agarwal *et al.,* 2006). One important factor in penile erection is nitric oxide (NO). Erectile function depends on the relaxation of the corpus cavernous (CC) smooth muscle which the mechanism is mediated by NO (Agarwal *et al*., 2006). ED normally is described as a decrease in nitric oxide (NO) bioavailability which can be due to reduction in the function of the enzyme nitric oxide synthase present in the endothelial known as eNOS (endothelial nitric oxide synthase) and/or increase in the removal of NO (Sasatomi *et al.,* 2008). Oxidative stress is a situation where pro-oxidants activities and the antioxidant capacity in scavenging excess reactive oxygen species (ROS) is not balanced (Agarwal *et al.,* 2006). Oxidative stress can induce ED when peroxynitrite which causes reductions in NO bioavailability are produced as a result of interaction of ROS like superoxide anion with nitric oxide (NO) (Haffner *et al.,* 1998). The peroxynitrite formed and superoxide radicals may cause apoptosis in the endothelium, damage the endothelium of the corpus cavernosal CC smooth muscle and result in the reduction of NO availability (Ferreira *et al.,* 2006). The consumption of natural antioxidant rich foods like fruits and vegetables could help in fighting against this oxidative stress (Adefegha *et al.,* 2016).

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*Ageratum conyzoides* L. is a medicinal plant that belongs to the family Asteraceae. They are found close to habitation especially where waste are deposited, they thrives on any garden soil and on ruined sites. The odour of *Ageratum conyzoides* is likened to that of a male goat, therefore the name 'goat weed' or 'billy goat weed' was derived (Okunade, 2002). *Ageratum conyzoides* (L.) have been used traditionally as a medicine over a long period of time and the use varies from one region to another (Adebayo *et al.,* 2010; Okunade, 2002). In Nigeria it is used in treating skin diseases and wound healing. Also, a decoction of the plant when taken by children is used in the treatment of diarrhea and relieving the pains associated with navel (Okunade, 2002). In Central Africa, it is used as remedy for pneumonia, but used commonly in treating wounds and burns (Vaidyaratnam-Varier, 2002). In India, *A.conyzoides* is used in treating leprosy, in Brazil it is used as anti-inflammatory, analgesic and anti-diarrhoea while in Vietnam it is used for treating gynecological diseases (Okunade, 2002).

To our knowledge, there has been no previous study to directly evaluate the antioxidative, erectogenic, and anti-lipid peroxidative properties of *Ageratum conyzoides* leaf extracts. However, a lot of work has been done on *Ageratum conyzoides* L. by different researchers *in-vivo*. The crude leaf extract of *Ageratum conyzoides* has been found to have neuromuscular blocking and analgesic activities in rat. Anti inflammatory, analgesic and antipyretic activities of the essential oil of *Ageratum conyzoides* in mice and rats has been reported. The haemopoietic properties of the ethanol extract have also been reported in albino rats. The aqueous leaf extract possess hypoglycaemic or anti-diabetic and kidney protecting effects in Albino Rats have been reported. It has been reported that *A. conyzoides* L. leave extract had anti-peptic ulcer activity against ethanol induced gastric ulceration and cysteamine induced duodenal ulcerations in rats. Petroleum ether extracts of *Ageratum conyzoides* has also been found effective against the mosquito *Culex quinquefasciatus* larvae (Abena *et al.,* 1996; Ita *et al.,* 2007; Mitra, *et al.,* 2015; Agbafor, *et al.,* 2015). The present study is aimed at evaluating the antioxidative and anti-lipid peroxidative properties of aqueous and methanol extracts of *Ageratum conyzoides* leaves in combating erectile dysfunction caused by oxidative stress.

## Materials and methods

### *Sample collection*

Fresh leaves of *Ageratum conyzoides* were harvested from Ondo State University of Science and Technology (OSUSTECH) farm, Okitipupa, Ondo State, Nigeria. It was identified and authenticated at the herbarium of Biological Sciences Department, OSUSTECH.

### *Extract preparation*

The leaves collected were air dried for two weeks and it was grounded into powder. Fifty grams (50g) each of the grounded powder was soaked in 250 mL distilled water and methanol respectively for 24h and the mixture was shaken intermittently. The resulting mixture was filtered using a muslin cloth. The filtrate was concentrated using rotary evaporator at $40^0$C and concentrated samples were used for analyses immediately.

### *In vitro antioxidant assays*

### *Estimation of total phenolic content*

The total phenolic content was determined spectrophotometrically using the method of Kim *et al.* (2003). It is described thus: 1mL of the sample (1 mg/mL) was mixed with 1ml of Folin–Ciocalteu phenol reagent (1:15). After 5 min, 5 mL of a 7% $Na_2CO_3$ solution was added to the mixture then 6.5ml of distilled water was added, it was then mixed thoroughly. This was kept in the dark for 90 minutes at $25^0$C; then the absorbance was taken at 750 nm. The total phenolic content was determined from Gallic acid standard calibration curve. The estimation of the total phenolic content was in mg Gallic Acid Equivalents (GAE) per g.

### *Estimation of total flavonoids content*

Total flavonoid content was determined according to Park *et al.* (2008) method. 1 mL of sample (1 mg/mL), 3.4 mL of

30% methanol, 0.15 mL of $NaNO_2$ (0.5 M) and 0.15 mL of $AlCl_3.6H_2O$ (0.3 M) were taken mixed together and left for 5 minutes, then 1 ml of 1 M NaOH was added. The solution was thoroughly mixed; the absorbance was taken at 506 nm. The total flavonoid content was determined from Quercetin standard calibration curve. The total flavonoids were measured as mg Quercetin equivalent per g of the extracts.

*Estimation of Ascorbic acid content*

Ascorbic acid was quantified using spectrophotometric method of Rutkowski *et al.* (2007). 2 mL of the sample was mixed with 4 mL of phosphotungstin reagent. The mixture was left for 10 minutes after which it was centrifuged at 3000 x g for 10 minutes. Then the absorbance of the supernatant was read at 700 nm against the reagent blank.

Ascorbic content = (Abs of the sample /Abs of standard) × Concentration of standard

*Phosphomolybdate assay*

The total antioxidant capacity was determined by the phosphomolybdate method of Prieto *et al.* (1999). 1.0 mL (1 mg/mL) of the sample taken and mixed with 1 mL of phosphomolybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). Then it was incubated in a water bath at $95^0$C for 90 min, cooled to room temperature, the absorbance was taken at 765 nm against a blank.

*Metal chelating activity*

Metal chelating activity is measured using the method of Dinis *et al.* (1994). 0.1 mL (20-100µg/mL) of the extract was taken and 0.5 mL of 2 mM ferrous chloride was added, then 0.2 mL of 5 mM ferrozine was added and incubated for 10 min at room temperature. The absorbance was measured at 560 nm. EDTA was used as control.

Metal chelating ability = [(Abs control – Abs sample)/ (Abs control)] × 100

*Reducing power*

The reducing power was estimated using the method of Oyaizu (1986). 1.0 mL (0.25-1.0 mg/mlL) of the extract was added to 1 mL phosphate buffer of pH 6.6; then 1 mL of potassium ferricyanide (10 mg/mL) was added. The whole mixture was incubated at $50^0$C for 20 min. 1 mL of trichloroacetic acid (100 mg/mL) was then added. The mixture was centrifuged at 3000 x g for 10 min; upper layer of the solution was collected. 2 mL was taken and mixed with 1 mL distilled water and 0.2 mL of 0.1% freshly prepared ferric chloride. After 10 min, the absorbance was taken at 700 nm. Ascorbic acid was used as Standard.

*DPPH scavenging activity*

DPPH radical scavenging activity was estimated according to method of Gyamfi *et al.* (1999). 1.0 (0.25-1.0 mg/mL) of sample extracts were added to 4 mL of DPPH (0.025 g/l prepared in methanol solution). The sample was shaken and allowed to stand in the dark for 30 min and absorbance was taken at 520 nm. The standard was Ascorbic acid and Quercetin.

DPPH scavenging ability = [(Abs control – Abs sample)/ (Abs control)] × 100

*Hydrogen peroxide scavenging activity*

Hydrogen peroxide scavenging activity was estimated using Ruch *et al.* (1989) method. 1.0 mL extracts (100-600 µg/mL) were added to 3.0 mL hydrogen peroxide (40 mM) prepared in phosphate buffer (50 mM, pH 7.4), incubated for 10 min at room temperature and the absorbance was measured at 230 nm after against a blank.

Hydrogen scavenging ability = [(Abs control – Abs sample)/ (Abs control)] × 100

*Lipid peroxidation assay*

*Preparation of tissue homogenates*

The rats (12 weeks old and weighing between 220 and 240 g) were decollated using mild diethyl ether as anaesthesia; the whole brain, pancreas and penile tissues were removed immediately, placed on ice and weighed. They were then homogenized in cold saline (1:10, w/v) with 10 up and down strokes at 1200 rpm in a Teflon glass homogenizer. The

homogenate was centrifuged at 3000 x g for 10 min, the low speed supernatant (SI) was used for the Lipid peroxidation assay (Belle, *et al.,* 2004).

*Lipid peroxidation and TBA reactions*

The lipid peroxidation assay was done according to the modified Ohkawa *et al.* (1979) method. Described thus, 100µL of the SI fraction was mixed with solution containing 30 µL of 0.1 M Tris-HCl buffer of pH 7.4, 0-100 µL extract and 30 µL of 250 µM freshly prepared $FeSO_4$. Distilled water was used to make the volume to 300 µL, it was then incubated for 1 hour at 37 °C. 300 µL of 8.1 % sodium dodecyl sulphate (SDS) was added to the mixture containing SI for colour development; 600 µL of acetic acid/HCl mixture of pH 3.4 and 600 µL of 0.8 % thiobarbituric acid (TBA) was added. The whole mixture was incubated for 1 hour at 100 °C. Absorbance was measured at 532 nm. Malondialdehyde (MDA) produced was calculated and expressed as % control (Oboh *et al.,* 2007).

*Statistical analysis*

The result was expressed as mean of triplicate determination ± standard deviation. Spread sheet soft-ware (Microsoft Excel©, version 2013) was used for the calculation of Standard deviations. Analysis of variance (ANOVA) was performed using Statistical Analysis System proprietary software (SAS, 2002). Duncan's multiple range tests was used for mean separation ($P < 0.05$). Graph pad 5.0. was used for the plotting of the graph. IC50 was calculated using a linear regression analysis.

**Results and discussion**

Total phenolics, total flavonoids and ascorbic acid of the different extracts of *Ageratum conyzoides* is presented in Table I. The total phenolic compound in the aqueous extracts is 48.9 while the methanol extract is 61.4 expressed in mg (GAE)/g. *Ageratum conyzoides* in the aqueous extracts and the methanol extract in this work exhibited a high phenolic content when compared with the phenolic contents of 56 selected Chinese medicinal plants, selected Nigerian, medicinal plants and that of traditional medicinal plant in Ecuador (Feng-Lin Song *et al.,* 2010; Agbo *et al.,* 2015; Adriana *et al.,* 2013). It is very low to the phenolic content of the leaf extract of *Asplenium platyneuron* 82.33 mg GAE/g, *Euphorbia prostrata* 97.77 mg GAE/g, *Platycerium bifurcatum* 87.62 mg GAE/g (Agbo *et al.,* 2015), *Abarema cochliacarpos* 120.39 mg GAE/g and *Stryphnodendron pulcherrimum* 86.67 mg GAE/g (Neto *et al.,* 2016). One of the secondary metabolites found in plants are the Phenolic compounds; they are derived from phenylalanine and tyrosine. They possess very different antioxidant activities and they are very effective in chain breaking (Shahidi and Wansundeara, 1992; Naczk and Shahidi, 2004). The methanol extract in this work was observed to be significantly ($P < 0.05$) greater in phenolic content in comparison with the aqueous extract.

This result agrees with the report of other researchers where the methanol extract exhibited a higher phenolic content than other solvents used in the extraction of phenols (Ghasemzadeh *et al.,* 2011; Ho *et al.,* 2012; Miguel, *et al.,* 2014; Do *et al.,* 2014). That the methanol extract was higher in phenolic content than the aqueous extract could be because there are more non-phenolic compounds in aqueous extracts than in methanol extracts since phenol is frequently found connected to other biomolecules like polysaccharides, chlorophyll, proteins, terpenes, inorganic compounds and so on. There could be possible complex formation of some of these phenolic compounds in the extract which dissolve in methanol (Do *et al.,* 2014).

The total flavonoid is expressed in mg/g quercetin equivalents (QE). The flavonoid in *Ageratum conyzoides* leaves in the two extracts are higher than the flavonoids reported for some plants from Brazilian flora (Neto *et al.,* 2016). It is very low when compared to the flavonoids of *Asplenium platyneuron*, *Allamanda cathartica, Euphorbia prostrata*, *Platycerium bifurcatum* (Agbo *et al.,* 2015).The flavonoid content of *Ageratum conyzoides* is similar to that of total phenol with the methanol extract having the highest flavonoid content of 42.2 mg QE/g which was significantly ($P < 0.05$) higher than that of aqueous extract. It has been observed by Ghasemzadeh *et al.* (2011), Miguel *et al.* (2014)

**Table I. Total phenolics, flavonoid, ascorbic acid and phosphomolybdate of aqueous and methanol extracts of *Ageratum conyzoides* leaves**

| | Total Phenolics mg GAE/ g | Total Flavonoids mg QE/ g | Total Non Flavonoids mg QE/ g | Ascorbic acid mg/100g | Phosphomolybdate mg AAE/ g |
|---|---|---|---|---|---|
| Aqueous | 48.9± 2.95b | 18.7±0.91b | 30.2±1.11a | 2.6±0.06b | 33.5±1.86b |
| Methanol | 61.4± 8.64a | 42.2±6.25a | 19.2±0.87b | 10.1±0.10a | 45.8±2.02a |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($p < 0.05$). GAE – Gallic Acid Equivalent, AAE – Ascorbic Acid Equivalent, QE – Quercetin Equivalent

and Do *et al.* (2014) that methanol extract has a higher flavonoid in comparison with other solvent used for extraction. Due to the phenolic nature of flavonoids, they are quite polar alright but very poor when dissolving in water (Miguel *et al.,* 2014); this can explain the higher concentrations of flavonoids in methanol extracts than in the aqueous extracts. The total non-flavonoid content of *Ageratum conyzoides* (Table I) showed that the aqueous extract has significantly ($P < 0.05$) higher non-flavonoids content than the methanol extract. This showed that there are several classes of phenolic phytochemicals still present in the aqueous extract. Flavonoids are naturally present in plants and are thought to have positive impact on human health. There have been several reports on its high effectiveness in scavenging most oxidizing molecules that includes singlet oxygen, and other free radicals that have been implicated in several diseases (Bravo, 1998).

The ascorbic acid of the aqueous extract of *Ageratum conyzoides* leaves was found to be 2.6 mg/100g and the methanol extract 10.1 mg/100g. *Ageratum conyzoides* leaves extract contain a very low ascorbic acid when compared with the ascorbic acid of some medicinal plants in Nigeria, Manipur and Pakistan (Okerie and Alonge, 2006; Okram *et al.,* 2016; Iqbal *et al.,* 2011). Ascorbic acid has been observed to be a powerful antioxidant because it can donate a hydrogen atom thereby forming a stable ascorbyl free radical. It scavenges reactive oxygen and nitrogen oxide species, also very effective in combating superoxide radical ion, hydrogen peroxide and hydroxyl radicals (Weber *et al.,* 1996). Ascorbic acid should be more soluble in water than methanol, though methanol is a polar solvent. This result is contrary, the ascorbic acid in this work was observed to be higher in methanol extract of *Ageratum conyzoides* than the aqueous extract. The reason for this result cannot be categorically explained.

One electron transfer (ET) and hydrogen atom transfer

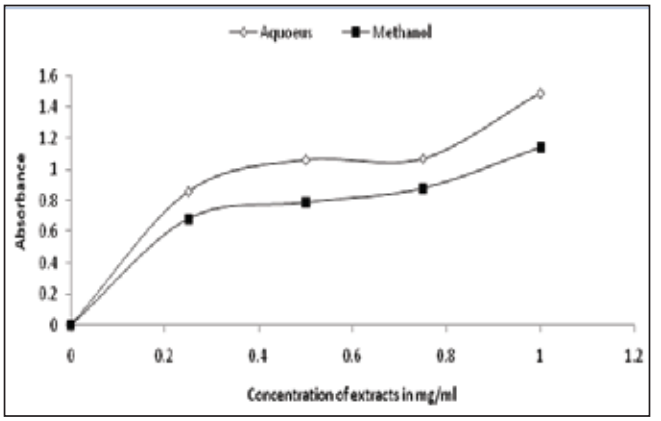


**Fig. 1. Reducing ability of the aqueous and methanol extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

(HAT) made up the antioxidant capacity assays; HAT and ET assays measures the radical (or oxidant) scavenging capacity of samples and they do not look into the preventive antioxidant capacity of samples.ET assays are much more easier to evaluate than HAT assays because it involved colour development as the oxidant is reduced (Phatak and Hendre, 2014; Huang *et al.,* 2005). ET assays of reducing power,

**Table II. IC50 of reducing power, iron chelating ability, hydrogen peroxide ($H_2O_2$) scavenging ability and inhibition of $Fe^{2+}$ induced MDA Production of Aqueous and Methanol extracts of *Ageratum conyzoides* leaves**

| | Reducing power mg/ml | Iron chelating ability mg/ml | $H_2O_2$ scavenging ability µg/ml | MDA pancreas µg/ml | MDA Brain µg/ml | MDA penile tissue µg/ml |
|---|---|---|---|---|---|---|
| Aqueous | 32.86± 1.95a | 1.7±0.01b | 1052.40±37.29b | 105.32±4.2b | 91.74±3.1a | 115.9±4.9b |
| Methanol | 42.59± 2.72b | 1.23±0.01a | 946.40±23.12a | 94.21±3.1a | 108.69±4.6b | 75.95±2.2b |

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($p < 0.05$). MDA- malondialdehyde

metal chelating ability, hydrogen peroxide scavenging ability, 1,1-diphenyl 2 picrylhydrazyl (DPPH) radical scavenging ability and the phosphomolybdate method were selected for the measurement of total antioxidant capacity in *Ageratum conyzoides* leaves extracts.

The reducing power of *Ageratum conyzoides* leaves extracts was based on the capacity of the extract to reduce the transition metal $Fe^{3+}$ by electron transfer to $Fe^{2+}$. A higher absorbance at 700 nm is an indication of a higher activity (Zarena and Sankar, 2009). The reducing power of *Ageratum conyzoides* leave extracts in Figure 1 shows to be dose dependent with the aqueous extract exhibiting the highest reducing power as the concentration of extracts increases from 0.25 mg/mL to 1.0 mg/mL. However, the reducing power of *A. conyzoides* of aqueous extract was high but statistically not significantly ($P < 0.05$) higher than the reducing power of methanol extract. The IC50 value for the reducing power (Table II) of aqueous extract of *A. conyzoides* is 32.86 mg/mL which was significantly ($P < 0.05$) lower than 42.59 mg/mL for the methanol extract. A lower IC50 value is an indication of a stronger antioxidant activity. The reducing power capacity showed that the antioxidant compounds can donate electron which can cause reduction in the oxidized intermediates produced during the process of lipid peroxidation (Tachakittirungrod *et al.,* 2007).

However, the reducing power assay is disadvantaged in that any electron-donor which has a redox potential that are lower than the redox pair $Fe^{3+}/Fe^{2+}$ can contribute to the reducing power value and this will give a falsely high values even if they do not have antioxidant properties (Nilsson *et al.,* 2005).

Bivalent transition metal ion $Fe^{2+}$serves as catalysts of oxidative processes, that give rise to the formation of hydroxyl OH• radicals through Fenton chemistry (Halliwell, 1997). This reaction can be prevented by iron chelation and deactivation. The ability of the extracts of *A. conyzoides*to chelate $Fe^{2+}$ ions was evaluated and the result is presented in Fig. 2. The methanol extracts of *Ageratum conyzoides* leaves showed the best iron chelating ability as the concentration of extracts increases from 0.2 mg/mL to 0.6 mg/mL which was significantly ($P < 0.05$) higher than that of the aqueous extract. The IC50 value for the chelating ability (Table II) of methanol

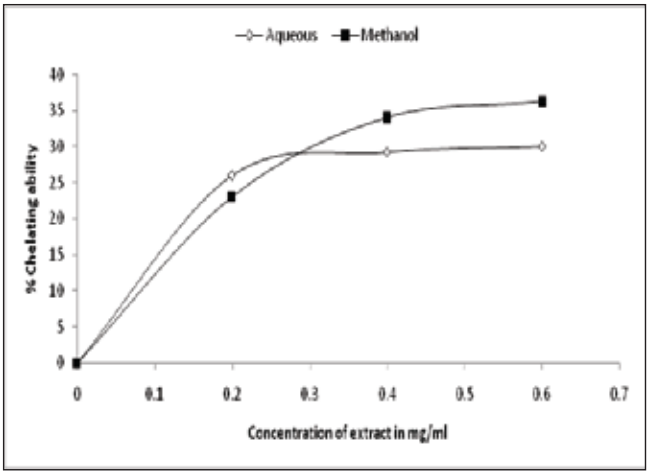


**Fig 2. Metal Chelating ability of aqueous and methanol extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

extract of *A. conyzoides* is 1.23 mg/mL which was significantly ($P < 0.05$) lower than 1.7 mg/mL for the aqueous extract. It is expected that methanol extracts would have a high chelating ability because of its high phenolic and flavonoid content which has been positively correlated to the iron chelating activity (Al-Farga *et al*., 2014; Hinneburg *et al*., 2006).

Hydrogen peroxide $H_2O_2$ occasionally can be toxic to cell

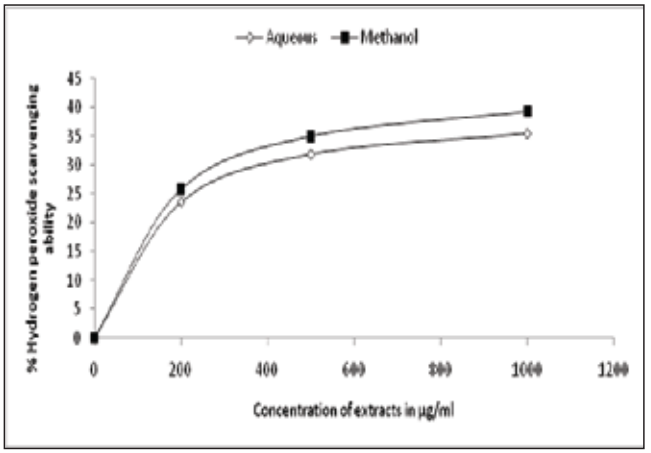


**Fig 3. Hydrogen peroxide scavenging ability of aqueous and methanol extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

because it may lead to the formation of hydroxyl OH• radical in the cells that can cause initiation of lipid peroxidation and result in DNA damage (Halliwell, 1991; Sahreen *et al.,* 2011), the removal of this $H_2O_2$ before it causes damage is an important step in antioxidant defence of cell or food systems. As depicted in Fig. 3, the extracts of *Ageratum conyzoides* leaves exerted a concentration-dependent scavenging of hydrogen peroxide ($H_2O_2$). The methanol extracts of *Ageratum conyzoides* leaves showed the best hydrogen peroxide ($H_2O_2$) scavenging ability as the concentration of extracts increases from 200µg/mL to 1000 µg/mL which was significantly ($P < 0.05$) higher than that of the aqueous extract. The IC50 value (Table II) of methanol extract of *Ageratum conyzoides* is 946.4µg/mL which was significantly ($P < 0.05$) lower when compared to 1052.4 µg/mL for the aqueous extract. The best activity found in the methanol extract of *Ageratum conyzoides* leaves may be related to their higher content of phenols and flavonoids which has the ability to donate electrons to $H_2O_2$ and thus converting it to water (Shahriar *et al.,* 2012).

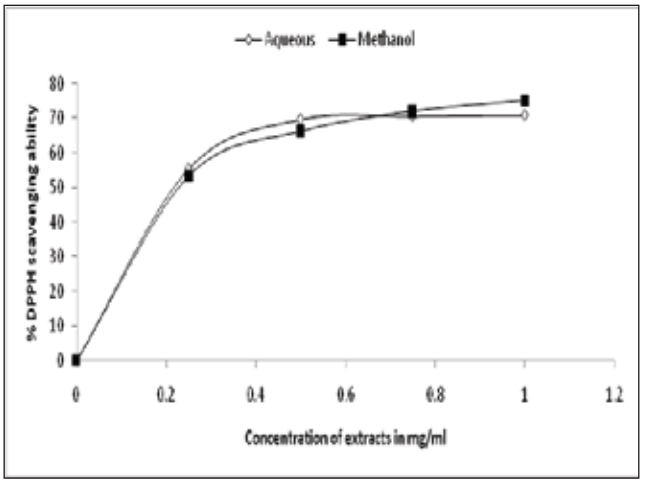


**Fig 4. DPPH radical scavenging ability of aqueous and methanol extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

The DPPH results are very important in confirming the results obtained in total phenolic content (Palafox Carlos *et al.,* 2012). The DPPH radical- scavenging ability of *A. conyzoides* leave extract at 0.25–1.0 mg/mL concentrations was measured and the results are presented in Fig. 4. A dose-response relationship was found in the DPPH radical-scavenging ability of *A. conyzoides* leave extracts; the ability increased with an increase in the concentration of the *A. conyzoides* leave extracts. The methanol extracts of *Ageratum conyzoides* leaves recorded the highest DPPH radical-scavenging ability which was significantly ($P < 0.05$) higher than the aqueous extract.

The total antioxidant capacity of *Ageratum conyzoides* leaves extracts is presented in Table I and expressed as mg/g ascorbic acid equivalents (AAE). The phosphomolybdenum method was used for the total antioxidant capacity evaluation; this is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound of the extract forming a green phosphomolybdate (V) complex and then evaluated spectrophotometrically (Prieto *et al.,* 1999). This reduction can be caused by natural products like phenols and flavonoids. The total antioxidant capacity (TAC) of the extracts was higher in methanol extracts of *Ageratum*

*conyzoides* leaves (45.8 mg AAE/ g) than the aqueous extract (33.5 mg AAE/ g). In the same trend of hydrogen peroxide scavenging ability and DPPH assays, the methanol extracts of *Ageratum conyzoides* leaves had a higher TAC; in addition they also have a higher total phenolic and flavonoid content. The high phenolic content of the methanol extracts of *Ageratum conyzoides* leaves is an indication of high antioxidant capacity since the phenolic compound reacts with radicals like hydroxyl radical, superoxide anion radical and lipid peroxide radical. It has been revealed that there is high correlation between antioxidant capacity and phenolic content of samples (Aliyu *et al.,* 2011). Recent study showed that many flavonoid compounds and polyphenols contribute significantly to the phosphomolybdate reduction activity of medicinal plants (Khan *et al.,* 2012). It is to be noted that the possible antioxidant qualities of plant extracts does not only depend on its phytochemical composition but also on the extraction systems which may include methods, duration and polarity of organic solvents (Wong *et al.,* 2006).

Parkinson's as well as Alzheimer's disease is a neurological disorder that often be the cause of erectile dysfunction ED since they cause decrease in libido or inability to kick start the erectile process (Eleazua *et al.,* 2017). The polyunsaturated fatty acids (PUFAs) in the brain membranes are very rich in phospholipids and they are subject to free radicals attacks easily, leading to oxidative damage in the brain phospholipids which could lead to the development of Alzhemier's disease (Axelsen *et al.,* 2011). Vasculogenic erectile dysfunction ED is caused by diabetes mellitus. In diabetes, an increased level of ROS causes the activation of Rho-kinase 2 which increases the contraction of the corpus cavernosum (CC) smooth muscles. This ROS under diabetes also inhibit the endothelial nitric oxide synthase NOS, thereby causing a decrease in the CC smooth muscle relaxation. (Eleazua *et al.,* 2017).

The accumulation of $Fe^{2+}$, in the islets of Langerhan of the pancreas, causes oxidative destruction of the β-cells of the pancreas thereby impairing the pancreas from its functions and leading to diabetic melitus (Shah and Fonseca, 2011). The ability of *Ageratum conyzoides* leaves extracts to protect

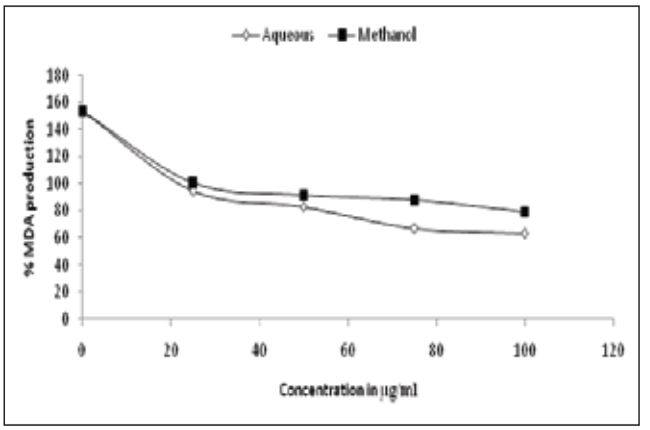


**Fig. 5. Inhibition of $Fe^{2+}$ induced MDA production in rat brain by the aqueous and methanol extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

cultured rat brain and pancreas against $Fe^{2+}$ induced lipid peroxidation is shown in Fig. 5 and 6. Incubation of rat's brain and pancreas in the presence Sof 250 µM iron (II) has caused a significant increase in the MDA content of these two organs: 153.41 % in the brain and 130.39 % in the pancreas. The formation of reactive oxygen species (ROS) facilitates lipid peroxidation through the Fenton reaction and it could have accounted for the increase in MDA contents of these organs after incubation in the presence of $Fe^{2+}$. The addition of the *Ageratum conyzoides* leaves extracts (aqueous and methanol) significantly ($P < 0.05$) inhibited MDA production in the brain and pancreas in a dose dependent manner. The methanol extract exhibited a higher inhibitory effect on the $Fe^{2+}$ induced lipid peroxidation in rat pancreas with IC50 of 94.21 µg/mL against 111.65 µg/mL for aqueous extract (Table II) while the aqueous extract showed a higher inhibitory effect on $Fe^{2+}$ induced lipid peroxidation in rat brain with IC50 of 91.74µg/mL against 110.82 µg/mL for methanol extract (Table II). The possible way by which these extracts protect the organs could be by $Fe^{2+}$ chelation and the scavenging of the hydroxyl radical (Ademosun and Oboh, 2014). The ability of the *Ageratum conyzoides* leaves extracts, both aqueous and methanol, to inhibit $Fe^{2+}$ induced lipid peroxidation in the brain and pancreas homogenate implied that the extract could prevent the oxidative damage of the brain and pancreas cells thereby preventing the development of Alzheimer's diseases and diabetic mellitus

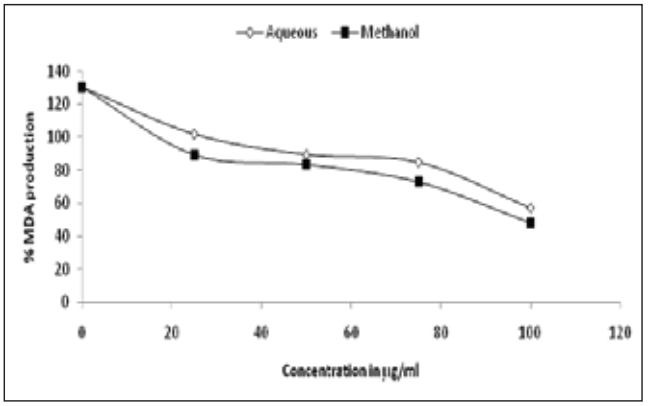


**Fig. 6. Inhibition of $Fe^{2+}$ induced MDA production in rat pancreas by the aqueous and methanolic extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

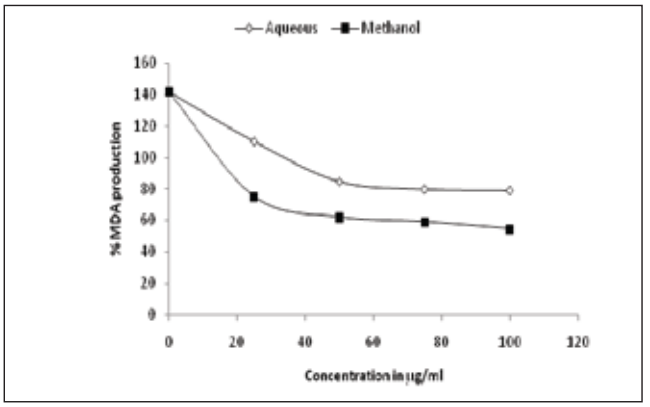


**Fig. 7. Inhibition of $Fe^{2+}$ induced MDA production in rat penile tissue by the aqueous and methanolic extracts of *Ageratum conyzoides* leaves. Values represent mean ± standard deviation of triplicate determination**

that could cause neurogenic and Vasculogenic erectile dysfunction ED. The MDA level in the penile tissue was observed to have been elevated to 141.46% after the rat penile tissue homogenate was incubated in the presence of 250 µM $Fe^{2+}$ (figure 3). This is an indication of oxidative damage in the penile tissue caused by free radicals generated by $Fe^{2+}$ which could be the breakdown of $H_2O_2$ to generate OH radical that can cause injuries to the penile tissues (Oboh *et al.,* 2017). The aqueous and methanol extracts of *Ageratum conyzoides* leaves caused a significant ($P < 0.05$) decrease in the MDA level in a manner that is dose dependent. The methanol extracts showed a higher inhibition of $Fe^{2+}$ -induced lipid peroxidation in the penile tissue of rat with IC50 of 75.95µg/mL (Table II) than did the aqueous extracts with IC50 of 115.9µg/mL (Table II). MDA produced as a result of oxidative stress in the penile tissue causes decrease in the bioavailability of NO and low levels of NO result in impairment of the guanyl cyclase/cGMP pathway which is an important pathway in penile erection by interacting with ROS (superoxides) to form toxic peroxynitrites (Akomolafe *et al.,* 2017). The inhibition of $Fe^{2+}$ -induced lipid peroxidation in penile tissue homogenates by the extracts of *Ageratum conyzoides* indicates that the extract could prevent peroxynitrite-induced MDA production and by implication NO will be more bio-available to mediate in the erectile process thereby preventing ED.

Conclusively, in the present study, the aqueous and methanol extracts of *Ageratum conyzoides* leaves showed a considerable antioxidant and anti-lipid peroxidation activities but methanol extract has a higher activity. Hence, these extracts of *Ageratum conyzoides* leaves can be used as potent natural antioxidant against free radicals and lipid peroxidation of organs and prevent erectile dysfunction ED.

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