COMPARATIVE STUDY OF THE *IN VITRO* ANTIOXIDANT PROPERTIES OF DIFFERENT EXTRACTS OF *ETHULIA CONYZOIDES*

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The present study was aimed to examine the *in vitro* antioxidant potential of *Ethulia conyzoides* aerial parts extracts. Different parts of *E. conyzoides* have been employed in traditional medicine for treating several diseases. Solvents of different polarity (petroleum ether, acetone and ethanol) were used to prepare extracts of the plant for determining their *in vitro* antioxidant activities using diverse *in vitro* assays. The antioxidant activity was measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, ferric thiocyanate (FTC), reducing power and thiobarbituric acid (TBA) assay methods. Here, ascorbic acid (ASA) was used as standard antioxidant. Results obtained from the study shows that all extracts of the plant have significant scavenging activity against DPPH (45.85% for acetone, 42.00% for ethanol and 37.85% for petroleum ether extracts at 2 mg/ml each). Furthermore, the three extracts of *E. conyzoides* also demonstrated significant antioxidant activity in other *in vitro* assays with the acetone extract being the most active. Hence the present antioxidant studies of *E. conyzoides*, will be beneficial in the synthesis/ preparation of new drugs of pharmaceutical importance, especially its acetone extract.

Key words: *Ethulia conyzoides*, antioxidant activity, radical scavenging activity (DPPH), reducing power activity, ferric thiocyanate (FTC), thiobarbituric acid (TBA).

INTRODUCTION

Biological combustion in living organisms involves many processes which produce harmful intermediates known as reactive oxygen species (ROS) or free radicals. When excessive amount of ROS is produced, it results to different disease conditions such as asthma, cancer, liver diseases, cardiovascular diseases and muscular degeneration (Sen *et al*., 2010), and ultimately producing oxidative stress. Oxidative stress is defined as an imbalance between oxidants and antioxidants that causes damage in biomolecules like nucleic acid, protein, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Droge, 2002). Therefore, in order to maintain good health, there must be a balance between free radicals and the antioxidants within a living system or organism.

Traditional medicine has been used for relieving symptoms of diseases from times immemorial (Maqsood *et al*., 2010). In spite of the recent advances recorded in modern medicine, medicinal plants have continued to be useful in health care. Huge number of plants with medicinal values has been scrutinized for their antioxidant potentials. Natural antioxidants are said to be very effectual in the prevention of the destructive processes triggered by oxidative stress (Zengin *et al*., 2011).

Antioxidants act like reducing agents, free radical scavengers, singlet oxygen molecule quenchers and activators for anti-oxidative enzyme to subdue the harm caused by free radicals in living systems. Several researchers have pointed out the inverse relationship between the consumption of plant products and mortality arising from age-related diseases. These properties of plants are as a result of numerous antioxidant compounds such as phenolics present in them (Gulcin, 2012; Sharma *et al*., 2012). These antioxidants help to stimulate cellular defense system against oxidative damage (Wagner *et al*., 1999).

Most of the presently used synthetic antioxidants have been criticized based on their toxicity, thereby arousing research interest into natural antioxidants that are of plant origin. These natural antioxidants might aid in novel drugs development (Jayaprakash and Rao, 2000).

Numerous antinecrotic, neuroprotective, anti-inflammatory and hepatoprotective drugs have been revealed to exhibit antioxidant and radical scavenging mechanism in their actions (Perry *et al*., 1999; Lin and Huang, 2002; Repetto and Llesuy, 2002).

Ethulia conyzoides (Asteraceae) is found in Senegal, west Cameroon and other parts of African tropics and also in Asia. The herb is used for cancer treatment in Western Nigeria and in Madagascar (Burkill, 1985; Sowemimo *et al*., 2009). In Egypt, *E. conyzoides* is used as remedy for abdominal disorder (El-Bassuony, 2009). Several Chemical constituents have been isolated from the plant which includes: methyl coumarins and related derivatives (Shukla *et al*., 1982).

The antibacterial, antihelminthic and molluscicidal activities as well as the total antioxidant capacity and free radical scavenging activity of *E. conyzoides* have been reported (Mahmoud *et al*., 1983; Kady *et al*., 1992; El-Bassuony, 2009; Aliyu *et al*., 2012).

Whereas a lot of researches had been done on the plant (*E. conyzoides*), there is paucity of information relating to its antioxidant properties. Therefore, in this study, a comparative evaluation of its antioxidant activity was done by employing several *in vitro* antioxidant assays and different solvents extracts of acetone, ethanol and petroleum ether were used in the study.

Plant materials

The aerial parts of *E. conyzoides* were obtained from Markudi, in Benue State, Nigeria following leads provided by traditional healers. The plant was identified at the Department of Biological Science, Ahmadu Bello University, Zaria.

Treatment and extraction of plant samples

The plant was collected and washed with distilled water. It was then air-dried and pulverized using pestle and mortar. The powdered sample obtained was extracted with different solvents (petroleum ether, acetone and ethanol) based on their differences in polarity using the method of Okoro *et al*. (2014). The powdered sample (50 g) was soaked in 200 ml of the solvents each in an airtight conical flask for 24 h at room temperature. This was firstly filtered through a doubled layered cloth and was re-filtered through Whatman No 1 filter paper. The resultant filtrate was collected into clean bottles and the solvents used for extraction were each separated from the filtrate by the use of rotary evaporator which was done at low temperature under reduced pressure. This was thereafter concentrated at 50°C in a water bathe.

Determination of *in vitro* antioxidant activities

The antioxidant activities of the extracts were evaluated employing different assay methods. The radical scavenging (DPPH) assay was done by the method of Liyana-Pathiranan and Shahidi (2005) while the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) assays were carried out according to the methods of Osawa and Namiki (1981) and Ottolenghi (1959) respectively. Similarly, the method of Oyaizu (1986) was used for the reducing power assessment.

Statistical analysis

The results were analysed using ANOVA followed by Tukey kramer multiple comparison test on Graphpad Instant Software, version 6.0 (Graph Pad Software, San Diego, CA, USA). Values of $P< 0.05$ were considered significant which are presented as mean ± SD.

RESULTS

In the present study, the percentage of scavenging effect on the DPPH radical was concomitantly increased with increase in the concentration of all extracts from 0.125 to 2 mg/ml. The percentage of inhibition was from 25.79 at 0.12 5 mg/ml to 45.85 at 2 mg/ml for acetone extract which showed the strongest DPPH radical scavenging activity and the lowest DPPH radical scavenging activity was shown by the petroleum ether extract from 18.15 at 0.125 mg/ml to 37.85 at 2 mg/ml (Figure 1).

The reducing power activity of all extracts of *E. conyzoides* increased consistently with increase in the volume of extract from 0.125 to 2 mg/ml. When the extracts were compared, acetone extract (1.49 mg/100 g AAE at 2 mg/ml) showed the highest reducing power (Figure 2).

The acetone extract showed the highest

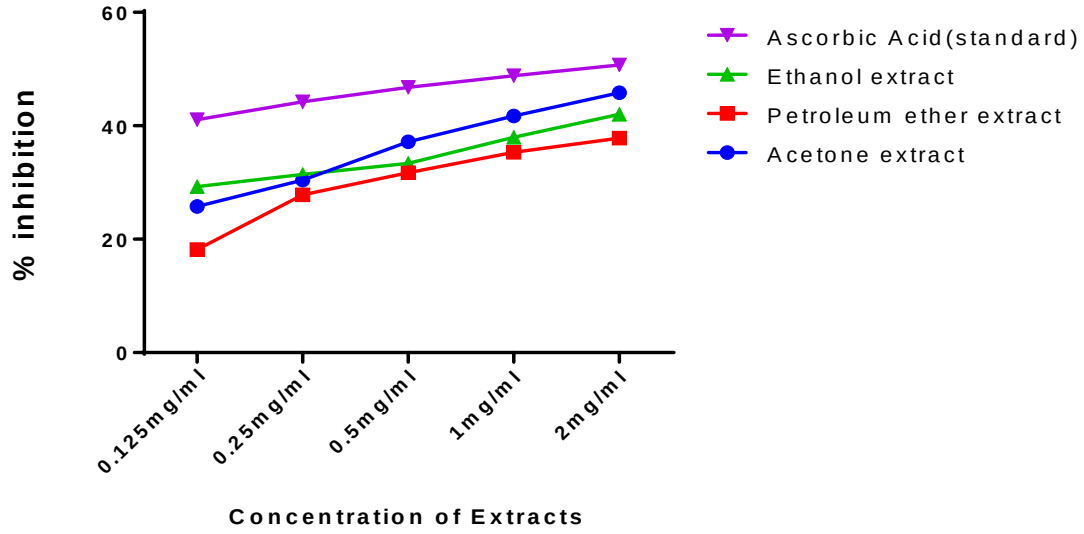

Figure 1. *In vitro* DPPH radical scavenging activities of ***E. conyzoides*** extracts.

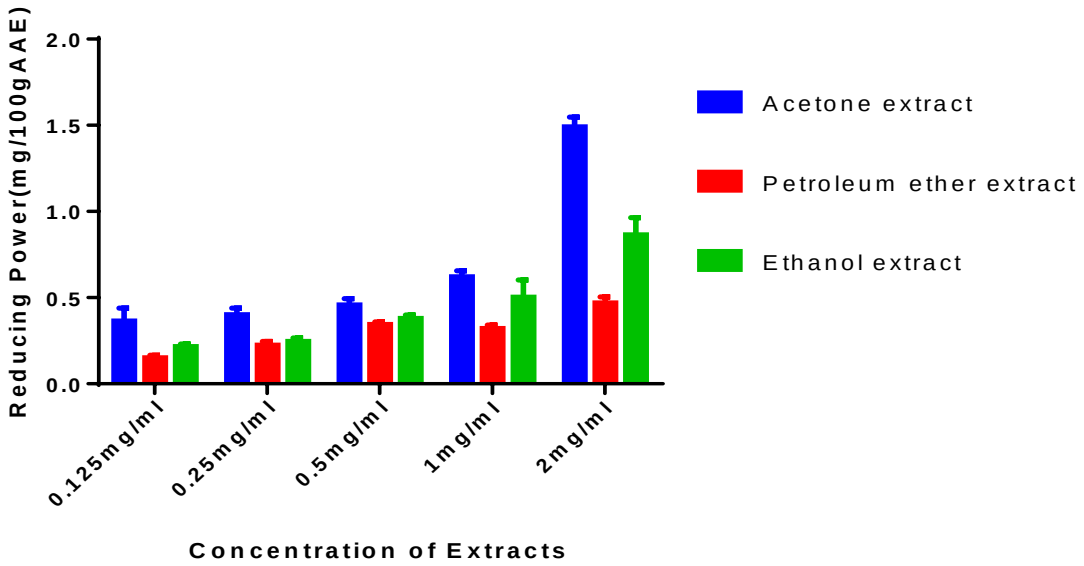

Figure 2. *In vitro* reducing power of *E. conyzoides* extracts.

FTC value of 66.93% while the lowest percentage was exhibited by ethanol extract (38.00%). Similarly, in TBA assay, the extracts showed percentage inhibition in the order: acetone > ethanol > petroleum ether at 40.70, 22.65 and 15.85% respectively, with the acetone extract (2 mg/ml) showing even higher percentage inhibition than the standard (2 mg/ml ascorbic acid) as shown in Figures 3 and 4.

DISCUSSION

Various techniques have been employed for the determination of *in vitro* antioxidant activity and for speedy screening of constituents in plants since it has been observed that substances with low *in vitro* antioxidant activity, will most likely display small activity *in vivo* (Nunes *et al.*, 2012).

A free radical is said to be a molecule having an unpaired electron. Free radicals have been implicated in lung damages, reperfusion injury, inflammation, cardiovascular disorders, aging, atherosclerosis, rheumatoid arthritis, parasitic infections and neoplastic diseases (Roy, 1994; Rao *et al.*, 2004).

In the present study, the *in vitro* antioxidant activities of three different solvent extracts (petroleum ether, acetone and ethanol)

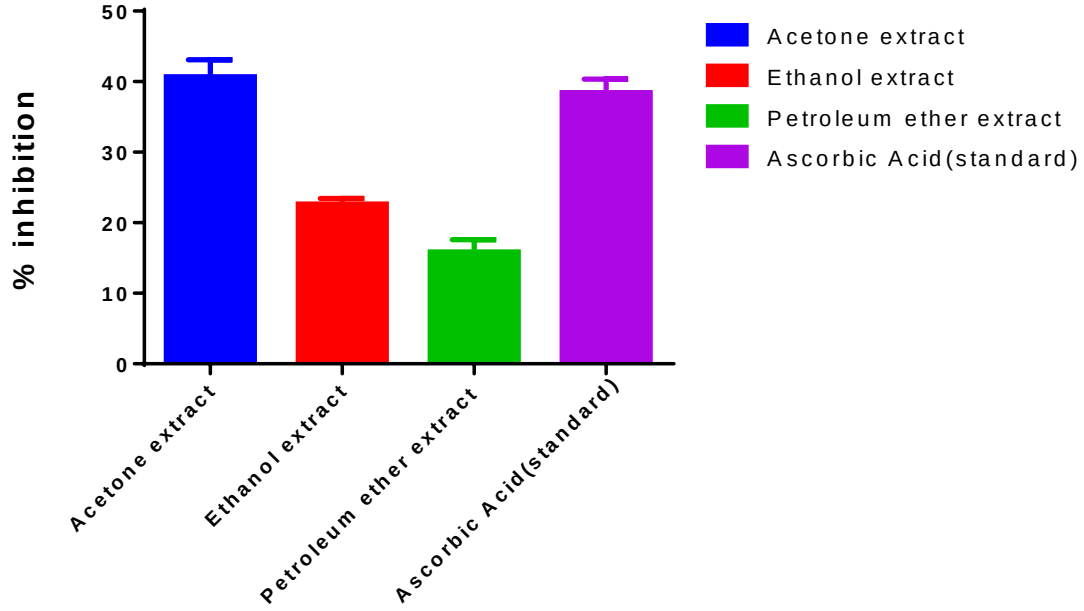

Figure 3. *In vitro* antioxidant activity of *E. conyzoides* extracts using the TBA method.

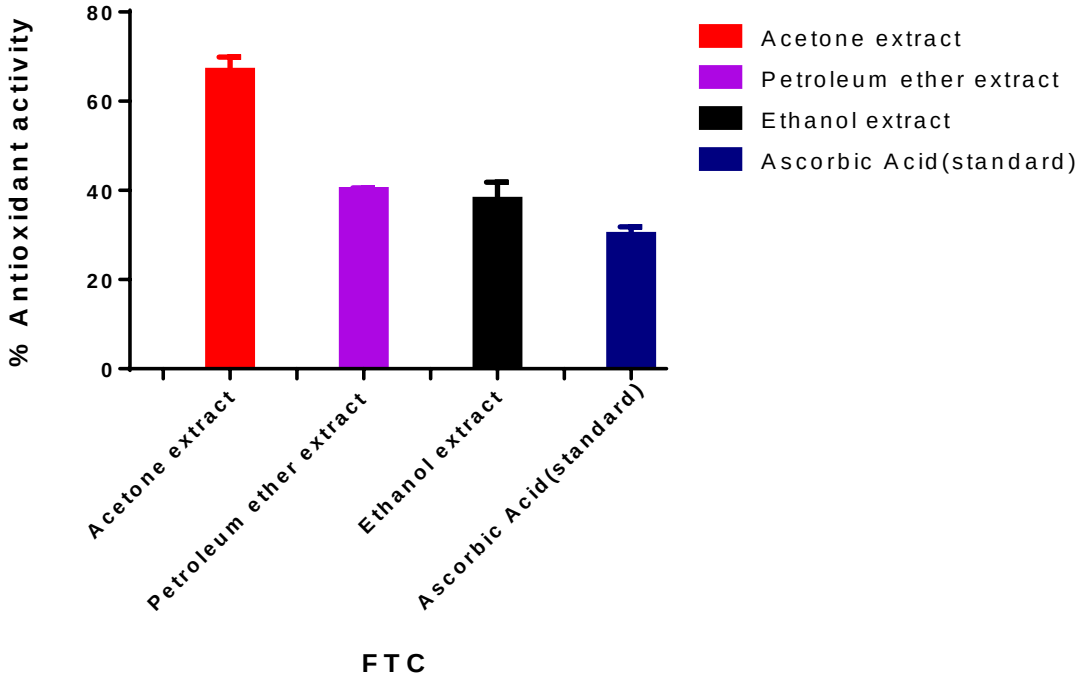

Figure 4. *In vitro* antioxidant activity of *E. conyzoides* extracts using the FTC Method.

were determined using DPPH, FTC, TBA and reducing power assays. All extracts of the plant demonstrated significant scavenging activity against DPPH (45.85% for acetone, 42.00% for ethanol and 37.85% for petroleum ether extracts at 2 mg/ml each). Results obtained from the TBA and FTC methods were also similar to that of the DPPH and this is in conformity with earlier studies on the plant by Aliyu *et al.* (2012). However, it should be noted that whereas, Aliyu *et al.* (2012) used only methanol extract in their study, the present study was a comparative one using three different solvents where the acetone extract was found to possess the highest antioxidant activity.

The method of ferric thiocyanate was used to assess peroxide amount at the early phase of peroxidation, while the thiobarbituric acid

procedure was for measurement of the free radicals concentration at the concluding phase of peroxide oxidation (Aiyegoro and Okoh, 2010).

DPPH is a free radical commonly used for assessing the radical scavenging activity of plant extracts (Bhuiyan *et al*., 2009). It is frequently employed as a substance to estimate the antioxidant activities of plants (Tara Chand *et al*., 2012).

The ability of natural products to donate electron can be determined by 2,2-diphenyl-1- picrylhydrazyl radical (DPPH), which is based on the bleaching of purple-coloured solution (Nunes *et al*., 2012). The method is on the basis of DPPH scavenging whereby the presence of radical species (or antioxidant) causes decolourization of the DPPH solution. The extent of colour change is dependent on the strength and concentration of the antioxidants. In this case, a huge increase in the absorbance of reaction mixture is an indication of non-significant free radical scavenging action of the tested compound (Krishnaiah *et al*., 2011).

From the results, it is revealed that the plant, *E. conyzoides* possess hydrogen donating abilities for all three extracts and they scavenge free radicals. Besides, it was observed that the acetone extract has a distinct and significant scavenging activity than that of the other two extracts.

Moreover, the results also revealed that the extracts showed different behaviors in the four *in vitro* assays, which may most probably be as a result of differences in mechanisms involved in the oxidation process steps.

Reducing power assay is commonly used to estimate the capability of antioxidants of natural sources to donate electrons (Yildirim *et al*., 2000; Dorman *et al*., 2003). Numerous reports have shown a direct association between the antioxidant activities of certain plant extracts and their reducing power (Duh, 1998; Duh *et al*., 1999; Yildirim *et al*., 2000).

In reducing power assessment, there is a change of colour of the test solution from yellow to green and this is dependent on the test sample and its reducing power. In this process there is reduction of the Fe^{3+}/ferricyanide complex to its ferrous form by the presence of the reductants in the test solution. Thus, Fe^{2+} can be examined by measurement of absorbance at 700 nm (Gordon, 1990). In this study, there was a steady increase observed in the reducing power activity of all extracts with increase in their volume. The results assert the *in vitro* antioxidant ability of the acetone, ethanol and petroleum ether extracts of the aerial parts of *E. conyzoides*.

Consequently, extracts of the plant demonstrated high antioxidant activity as seen in the DPPH, reducing power, FTC, and TBA assays. Comparatively, the acetone extract of the plant, *E. conyzoides* exhibited the highest antioxidant activity as revealed in the different *in vitro* assays.

CONCLUSION

The substitution of synthetic antioxidants with natural ones because of their adverse effects on health may obviously be advantageous. An *in vitro* antioxidant study normally offers scientific proof to the traditional claims for the use of *E. conyzoides*. This study has shown that the plant extracts possess modest to significant free radical scavenging and antioxidant activities. Thus, the findings of this study indicate that *E. conyzoides* can be useful as a source of antioxidants that may be applied for pharmacological preparations, especially its acetone extract.

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