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Full Length Research Article

Comparative Study of Phenolic Content and Antioxidant Activity of Leaf Extracts of *Alstonia boonei* and *Eupatorium odoratum*

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ABSTRACT: This study investigated the *in vitro* antioxidant activity and phenolic content of aqueous and ethanol extracts of *Alstonia boonei* and *Eupatorium odoratum* leaves. Total phenol, flavonoid and flavonol content of the extracts were estimated to determine the levels of phenolics in the extracts. The antioxidant capacity of the extracts were assessed through diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and reducing power (RP). The results revealed that the DPPH radical scavenging ability of aqueous extract of *E. odoratum* (with IC_{50} = 0.07± 0.003mg/ml) compares favourably with that of the standard ascorbic acid (IC_{50} = 0.06 ± 0.01 mg/ml). There was a dose-dependent increase in FRAP and RP capacity in all the extracts. The ethanol extract of *E. odoratum* had the highest FRAP while the aqueous extract of *A. boonei* showed the highest RP ability when compared with the other extracts. However, flavonol content was highest in the aqueous extract of *E. odoratum* followed by ethanol extract of *A. boonei*. The phenolic and flavonoid contents of extracts of *E. odoratum* were higher than that of *A. boonei* extracts. This study, therefore, reveals that aqueous extract of *A. boonei* and ethanol extract of *E. odoratum* leaf are more effective free radical scavengers and antioxidants relative to the other extract forms. These findings support the use of these extracts as potential sources of natural antioxidants.
Keywords: *Alstonia boonei, Eupatorium odoratum,* phenolics, antioxidant

INRODUCTION

Free radicals are inevitably produced in biological systems and also encountered exogenously. When in excess, they have damaging effects on cells. These effects include various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (Kedare and Singh, 2011; Das *et al*., 2014). Antioxidants combat free radicals by intervening at any one of the three major steps of the free radical mediated oxidative processes, viz., initiation, propagation and termination (Kedare and Singh, 2011). Antioxidants from plant sources currently receive increasing attention especially due to their potential health benefits, availability, and affordability and in many cases, reduced toxicity (Omoregie and Osagie, 2012).

Alstonia boonei, a member of Apocynaceae family, is a deciduous plant found abundantly around Africa, from the rain forest regions of Senegal to Western Cameroon extending to Egypt in the North and Uganda and Zaire in the East. It is known by several common names in different localities, *awun* (Nigeria), *sinupo* (Ghana), *botuk* (Cameroon) and *emien* (Ivory Coast) (Amole and Ilori, 2010). *A. boonei* is reported to have diverse uses. In Ghana, it is given to ameliorate toothache and after child delivery, to aid in expelling the placenta. In Cote d'Ivoire and Burkina Faso, it is applied topically to reduce oedema and to clear suppurant sores and exposed fractures. In Nigeria, it is used for ulcers and in Cameroon and Liberia as remedy for snake bite and arrow poison (Akinmoladun *et al*., 2007). Traditional African medicine has also reported the use of *A. boonei* for treatment of chronic diarrhoea, dysentery, fever, pain and intestinal disorders (Amole and Ilori, 2010). The stem bark of *A. boonei* has been reported to possess anti-inflammatory, anti-malarial, analgesic and antipyretic activities (Akinloye *et al*., 2013).

Eupatorium odoratum, also known as *Chromolaena odorata* (L.), is a wildly growing free standing shrub from the family of Asteraceae. It is one of the world's most successful invasive plants, occurring all over the world (Codilla and Metillo, 2011). Its common names are as diverse as its locations, variously referred to as

siam weed, devil weed, communist weed, French weed, etc (Rak *et al*., 2012). In some parts of Nigeria it is locally known as *Awolowo* leaf (*Ewuro Awolowo*). Despite its seemingly irritant nature as an invasive plant, *Eupatorium odoratum* is known to be exploited for several uses. Its anti-microbial properties have made *E. odoratum* a local agent for disinfecting and treating wounds. It has been used as an effective therapy against diarrhoea, malaria fever, tooth ache, diabetes, skin diseases, dysentery and has been demonstrated to have anti-inflammatory activity (Owoleye *et al*., 2005; Anyasor *et al*., 2011; Vaisakh and Pandey, 2012). Ibegbulam *et al* (2011) revealed that *E. odoratum* may be used as an inhibitor of corrosion in aluminium alloy products while its ability to absorb oil from oily waste water was shown by Rak *et al* (2012) who also demonstrated that *E. odoratum* in concert with selected bacterial strains can effectively degrade the absorbed oil. *E. odoratum* may thus find use in remediation of contaminated soils.

Free radical scavengers of synthetic nature such as butylhydroxyanisole (BHA) and butylhydroxytoulene (BHT) exist but concerns over side effects necessitate the continued screening of natural plant parts with strong antioxidant activities. Thus, this study compared the phenolic content, antioxidant and free radical scavenging activities of ethanol and aqueous extracts of *E. odoratum* and *A. boonei*.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh leaves of *A. boonei* and *E. odoratum* were collected from a private farm in Benin City in April, 2014. They were identified and authenticated by a Botanist in the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria.

Preparation of Plant Extracts

A. boonei and *E. odoratum* leaves were collected from private farms at different locations in Benin City, Nigeria. The leaves were washed then air dried under shade. The dry leaves were crushed and then extracted with either ethanol and distilled water at room temperature for 48 hours with stirring at interval. The extracts (ethanol and aqueous extracts) obtained were concentrated to dryness at 40^{o}C using a rotary evaporator under reduced pressure (Ayoola *et al*., 2008). The dried extracts were weighed and then stored at 4^{o}C for subsequent analysis.

Estimation of Diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the plant extracts against 1, 1–diphenyl–2–picrylhydrazyl (DPPH) free radical was determined by a slightly modified method of Brand-Williams *et al*. (1995). Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2ml of various concentrations (0.2 - 1.0 mg/ml) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark, and the absorbance read at 517 nm. All tests were performed in triplicate. Vitamin C was used as reference control, with similar concentrations as the test samples prepared. A blank containing 0.5ml of 0.3 mM DPPH and 2ml methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%)

$$= [(A_0 - A_1)/ (A_0)] \times 100,$$

Where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

Estimation of Ferric Reducing Antioxidant Power (FRAP)

A modified method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay. This depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II) - TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593 nm. Briefly, 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10mM 2,4,6-tripyridyls-triazine (TPTZ) in 40mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution) was mixed with 1mL of the extracts at various concentrations (0.2 - 1.0 mg/ml). The reaction mixtures were incubated at 37^{o}C for 30 min and increase in absorbance at 593 nm measured. $FeSO_4$ was used for calibration and values expressed as mmol $FeSO_4$ equivalents per gram of sample. Ascorbic acid served as the reference control.

Determination of Reducing Power (RP)
The reducing power of extract was determined according to the method described by Lai *et al.* (2001). Briefly, 1ml of different concentrations of extracts (0.1-1.0 mg/mL) in water was mixed with 2.5 mL of 0.2 M phosphate buffer, P^H 6.6 and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10%) was added to the mixture to stop the reaction. 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were then added and the absorbance measured at 700 nm. Higher absorbance values indicated higher reducing power. Vitamin C served as the reference control.

Estimation of Total Phenolic Content (TPC)
Total phenolic content was determined according to the Folin and Ciocalteau's method (1927). Concentrations (0.2 - 1 mg/mL) of gallic acid were prepared in methanol. Then, 0.5 mL of the sample (1 mg/mL) was mixed with 2.5 mL of a ten-fold diluted Folin- Ciocalteau reagent and 2 mL of 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature then absorbance read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the reference control.

Determination of total flavonoid content (TFC)
Total flavonoid content was determined using the method described by Ayoola *et al*., (2008). Briefly, 2 mL of 2% $AlCl_3$ in ethanol was added to 2 mL extracts. A concentration of 1 mg/mL of the extract prepared in methanol was used. Similar concentrations of quercetin, the standard control were used. The absorbance was measured at 420 nm after 1 hr incubation at room temperature.

Determination of Total Flavonol Content
The flavonol content was determined by the Yermakov method (1987). The quercetin calibration curve was prepared by mixing 2 ml of varying concentrations of standard quercetin (0.2 - 1.0 mg/ml) quercetin - methanol solutions with 2ml of 2g % aluminium trichloride and 6 ml of 5g % sodium acetate. The absorbance was read at 440 nm after 2.5hr incubation at 20^o C.

Statistical Analysis
All analyses were carried out in triplicate and results expressed as mean ± SEM. The IC_{50} values were calculated after extrapolation from linear regression graphs. The data were subjected to one-way analysis of variance (ANOVA), where applicable. Differences between means were determined by Duncan's multiple range tests using Graph Pad Prism statistical package version 6. P values < 0.05 were regarded as significant.

RESULTS
The 1, 1–diphenyl–2–picrylhydrazyl (DPPH) radical scavenging ability of the extracts is presented in Figure 1 and IC_{50} values shown in Table 1. The DPPH scavenging capacities of all the extracts showed a dose-dependent increase with IC_{50} values of 0.07± 0.003 mg/ml and 0.42 ± 0.02 mg/ml for aqueous and ethanol extracts of *E. odoratum*, respectively and 0.12 ± 0.01 mg/ml and 0.15 ± 0.01 mg/ml for aqueous and ethanol extracts of *A. boonei* when compared with the reference control (ascorbic acid) with IC_{50} value of 0.06 ± 0.01mg/ml.

The ferric reducing antioxidant power (FRAP) and reducing power (RP) both show a dose-dependent increase in the reducing ability of the extracts as concentration of the extracts increased when compared with the reference control ascorbic acid (Figures 2 and 3). In all the extracts, the ethanol extract of *E. odoratum* had the best FRAP with the ethanol extract of *A. boonei* having the least (Figure 2). The reducing potential of the extracts was however lower than that of the standard ascorbic acid at all concentrations studied. On the other hand, the aqueous extract of *A. boonei* show the best RP, while the ethanol counterpart show the least activity but was still lower than that of the control (Figure 3).

Table 2: Polyphenolics in aqueous and ethanol extracts of *E. odoratum* and *A. boonei* leaf. Total phenolic content was highest in the ethanol extract of *E. odoratum.* The result reveals that the total flavonoid content was higher in both *E. odoratum* extracts compared to the *A. boonei* extracts. The aqueous extract of *E. odoratum* was observed to contain the highest levels of flavonols followed by the ethanol extract of *A. boonei*.

DISCUSSION
Reactive oxygen species play important roles in the pathogenesis of several diseases. These highly reactive species are generated *in vivo* during several physiological processes. Antioxidants help prevent tissue damage by neutralizing the released free

radicals. They therefore act as scavengers. Antioxidants of nutritional origin thus play important roles in complementing *in vivo* antioxidants in "fighting" free radicals encountered by the body's cells.

Scavenging of the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of samples (Lee *et al*., 2003). The DPPH method is rapid, sensitive, and reproducible and requires simple conventional laboratory equipment for accessing antioxidant activity of samples (Du *et al*., 2009). The DPPH radical is a stable free radical that can accept an electron or hydrogen radical to become a stable molecule. The reduction of DPPH radical by antioxidants can be measured by a decrease in absorbance at 517nm.

At concentrations higher than 0.2 mg/ml, DPPH radical scavenging activity of the *E. odoratum* ethanolic extract decreases consistently. The reason for this is not well understood. However, the result suggests that this extract may lose its DPPH radical scavenging activity at higher concentrations.

The IC_{50} value is the amount of antioxidant required to reduce the DPPH radical concentration by 50%. The IC_{50} value is inversely proportional to the antioxidant activity and hence a lower IC_{50} value corresponds to higher antioxidant activity (Chanda *et al*., 2011). The low IC_{50} value obtained for the ethanol extract of *E. odoratum* suggests that it may possess strong antioxidant capacity. This IC_{50} value compares favourably with that of the standard, ascorbic acid. *E. odoratum* may thus be a good source of natural antioxidant. The antioxidative property of *E. odoratum* extract may be as a result of its phytochemical constituents. Omoregie and Okugbo, (2013) have previously shown that the ethanol extract of *E. odoratum* (syn. *Chromolaena odorata*) leaf contained some important phytochemicals such as alkaloids, terpenoids, saponins, tannins and flavonoids.

Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been profusely linked to antioxidant activities (Ayoola *et al*., 2008; Padmanabhan and Jangle, 2012). Phenolic compounds are antioxidant agents which act as free radical terminators. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group(s) (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells (Uyoh *et al*., 2013). Several studies have demonstrated a direct relationship between total phenol content and antioxidant activity in different plants. High phenolic content-containing plant materials have high radical scavenging abilities (Ayoola *et al*., 2008; Ghasemi *et al*., 2009; Hegazy and Ibrahim, 2012). Our results are in agreement with this phenomemon as demonstrated by the extracts at the concentrations studied. This therefore suggests that phenolics present in these extracts may play a role in the free radical scavenging activities of the plants.

In this study, both extract forms of *E. odoratum* show the highest flavonoid contents. Flavonoids represent the most common and widely distributed groups of plant phenolics. They are potent water-soluble super antioxidants that function in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Flora, 2009; Oseni and Okoye, 2013). On the other hand, the flavonol content of the extracts revealed a different pattern. The ethanol extract of *A. boonei* had higher flavonol content than the aqueous counterpart, while the aqueous extract of *E. odoratum* recorded the highest flavonol level. This finding suggests that the major flavonoid group in these extracts may be of flavonol origin, whereas, the high amounts of flavonoids present in the other extracts may be of different origin. Also, the extracting solvent (ethanol) might have selectively extracted mostly the alcohol-containing flavonols.

The *E. odoratum* extracts also show higher ferric reducing antioxidant power (FRAP) than the *A boonei* extracts. The aqueous *A. boonei* extract was also observed to contain a much higher ferric reducing antioxidant potential than the ethanol extract. The FRAP test is based on the ability of antioxidants present in the test extracts to reduce Fe^{3+} to Fe^{2+}. This ability may be hinged on the donation of hydrogen from phenolic compounds. The number and position of hydroxyl groups may thus be a factor in the antioxidant activity of a plant extract (Chanda *et al*., 2011).

CONCLUSION

The results from this study reveal that the aqueous and ethanol extracts of *A. boonei* and *E. odoratum* leaf possess varied degree of free radical scavenging and antioxidant activities in a dose-dependent manner. The aqueous extract of *A. boonei* and ethanol extract of *E. odoratum* leaf were observed to be more effective free radical scavengers and antioxidants in contrast to the other extract forms. These findings support the ethno-

medicinal uses and may provide effective intervention for free radical mediated diseases.

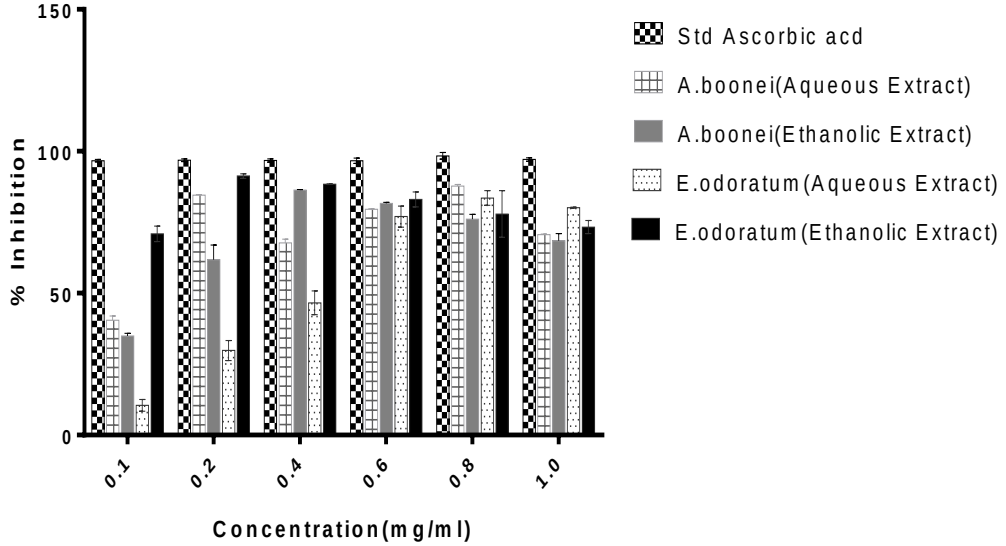

Figure 1: % Inhibition of DPPH Free Radical Scavenging Ability of Aqueous and Ethanolic Leaf Extracts of *Alstonia boonei* and *Eupatorium odoratum (*Values are expressed as mean ± SEM, n = 3)

Table 1: *IC_{50} Values of Aqueous and Ethanolic Leaf Extracts of A. boonei and E. odoratum*

Sample	IC_{50} values (mg/ml)
Ascorbic acid	0.06±0.01[a]
Eupatorium odoratum (Ethanol Extract)	0.07±0.003[a]
Eupatorium odoratum (Aqueous Extract)	0.42±0.02[b]
Astonia boonei (Aqueous Extract)	0.12±0.01[c]
Astonia boonei (Ethanol Extract)	0.15±0.01[d]

Values are presented as mean ± SEM for standard ascorbic acid and extracts (n=3).
Values with different superscript in same column are significantly different (P < 0.05).

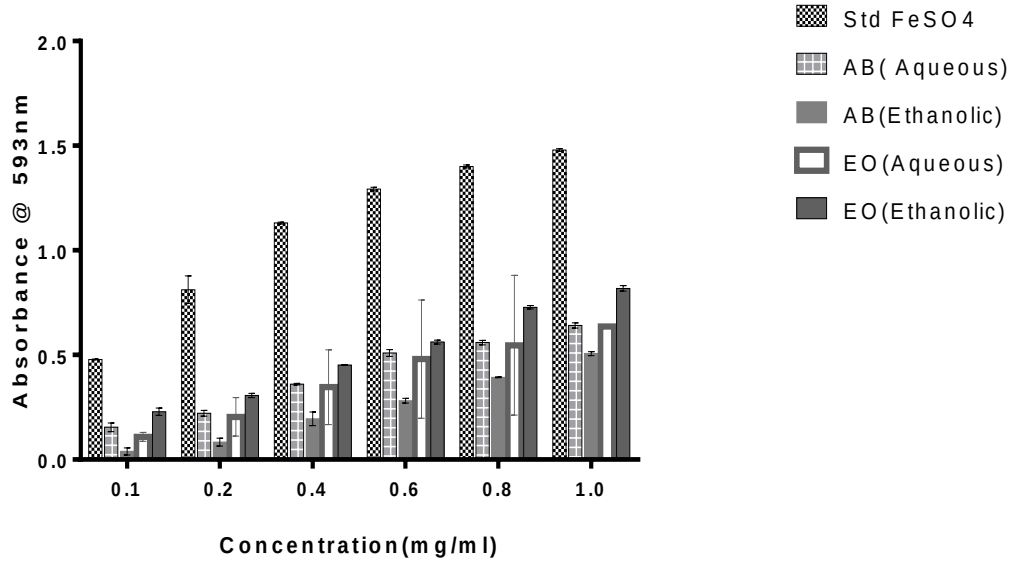

Figure 2: Ferric Reducing Antioxidant Power (FRAP) of *A. boonei* and *E. odoratum* Leaf Extracts

(Values are expressed as mean ± SEM, n = 3)

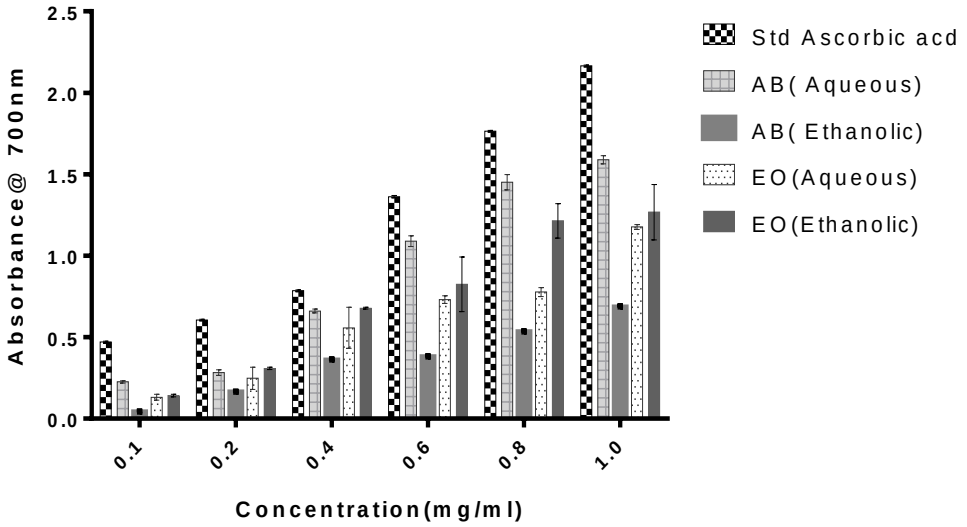

Figure 3: Reducing Power (RP) of Aqueous and Ethanolic Extracts of *A. boonei* and *E. odoratum* Leaf (Values are expressed as mean ± SEM, n = 3)

Table 2: Polyphenolic Content of Aqueous and Ethanol Leaf extracts of *A. boonei* and *E. odoratum*

	Total Phenol (mg GAE/g of extract)	Total flavonoid (mg QE/g of extract)	Total flavonol (µg QE/g of extract)
Eupatorium odoratum (Aqueous Extract)	379.0 ± 7.00^{a}	263.33 ± 1.00^{a}	689.0 ± 12.0^{a}
Eupatorium odoratum (Ethanol Extract)	536.3 ± 9.17^{b}	268.75 ± 0.08^{a}	237.0 ± 2.0^{b}
Astonia boonei (Aqueous Extract)	320.5 ± 7.17^{c}	241.84 ± 0.84^{b}	218.0 ± 1.0^{c}
Astonia boonei (Ethanol Extract)	308.0 ±16.33^{c}	89.83 ± 5.50^{c}	444.0 ± 20.0^{d}

Values are presented as mean ± SEM for standard ascorbic acid and extracts (n=3).
Values with different superscript in same column are significantly different (P < 0.05).

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