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ANALYTICAL COMPARISON OF THE PHYTOCHEMICAL COMPOSITION AND ANTIOXDANT ACTVITY OF METHANOL EXTRACTS DERIVED FROM *ALCHORNIA CORDIFOLIA* AND *CORCHORUS OLITORIUS*

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ABSTRACT

Oxidative stress is an underlying pathological state in cancer, cardiovascular disease and diabetes. Medicinal plants are considered to be potential remedies for attenuating oxidative stress in these pathologies because they possess antioxidants. Thus, the quantity and activity of antioxidants in *Alchornia cordifolia and Corchorus olitorius* methanol leave extracts were evaluated in this study using phytochemical screening, 1,1 diphenyl-2-picrylhydrazyl (DPPH)free radical-scavenging ability, ferric reducing antioxidant power (FRAP) and reducing power assays. Qualitative phytochemical analysis showed that the two plant extracts had alkaloids, saponins, reducing sugar, terpenoids, flavonoids and tannins. While the quantitative phytochemical test revealed a greater presence of total flavonoids and proanthocyanidins ($p<0.05$) in *Corchorus olitorius* methanol extract compared to *A. cordifolia* methanol extract. Contrastingly, the quantities of total tannins and phenols were higher in *Alchornia cordifolia* methanol extract than in *Corhorus olitorius* extract. The capacity of *Alchornia cordifolia* to mop up DPPH (IC_{50}= 0.01µg/mL) was greater than that of ascorbic acid (IC_{50}= 0.055µg/mL) and *C. olitorius* extract (IC_{50}= 33.10µg/mL) respectively. Ferric reducing potential of *C. olitorius* (479.80µM Fe(II)/g and *A. cordifolia* methanol extracts(510 µM Fe(II)/g) were significantly lower ($p<0.05$) than what was obtained for ascorbic acid (1005µM Fe(II)/g). *A. cordifolia* methanol extract demonstrated higher reducing power than *C. olitorius* methanol extract at identical concentrations. Our findings indicated that *Alchornia cordifolia* could be used as a rich source of antioxidants to alleviate oxidative stress underlying many chronic diseases.

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INTRODUCTION

One of the inevitable consequences of aerobic metabolism is the production of reactive oxygen species (ROS) [1]. ROS include superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide H_2O_2), singlet oxygen ($^1O_2$) etc. These radicals are capable of causing oxidative cellular injury to key cellular macromolecules such as proteins, lipids and nucleic acids [2]. The destruction of these cellular constituents by the activities of free radicals is prevented by presence of endogenous and exogenous antioxidants. However, in certain disease states or due to exposure to environmental toxicants, the balance between oxidants and pro-oxidants becomes altered in favor of reactive oxygen species (ROS)leading to oxidative stress[3,4].In chronic human diseases such as cancer, cardiovascular, diabetes neurodegenerative disorders, oxidative stress is a common pathological feature[5,6].

Experimental evidence suggests that a causal relationship exists between oxidative stress and chronic human diseases [7]. The resulting implication being that ameliorating oxidative stress could lead to improved clinical outcomes in affected patients. Butylated hydroxyanisole (BHA), tert-butylated hydroxyquinone and butylated hydroxyl toluene are some of the synthetic antioxidants used in the recent past to manage oxidative stress until they were found to pose serious health risks and banned from circulation [8]. Natural antioxidants from green plants have become the focus of intense research studies to find novel therapeutics as a result of their relative safety and availability.

Alchornea cordifolia (Schum. & Thonn.) Mull.-Arg. commonly referred as Christmas bush belongs to the family of Euphorbiaceae. It is an erect bushy perennial shrub that grows up to a considerable height of 5m. It is found in Senegal, East of Kenya, Nigeria, Tanzania and Central Africa. The leave extracts are reportedly used for treatment of gonorrhea, yaws, rheumatic fever, pain and cough [9]. In some studies, it has been reported that *Alchornia cordifolia* leaves possess antibacterial, antifungal and antiulcer properties [10].

Corchorus olitorius L. (Family Tillaceae) is an annual herb which reaches up to 2-4 m in height. This leafy vegetable is found in India, Brazil and Nigeria. The Yoruba's in southwestern Nigeria call it "ewedu" while the Hausas in the northern part of the country refer to it as "rama". The analgesic, antitumor, antifungal, and antibacterial, antioxidant activities of *Corchorus olitorius* leaves are documented in scientific literature[11-14].Although, individual studies on the antioxidant activity of *Alchornia cordifolia* and *Corchorus olitorius* have been carried out, no comparative analysis of the antioxidant properties of the plants of interest has been reported. Thus, the authors sought to investigate the differences in free radical scavenging activities between the methanol leave extracts of *Alchornia cordifolia* and *Corchorus olitorius* and to examine the potential therapeutic implications of our findings in relation to the management of oxidative stress-linked diseases.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Alchornia cordifolia* and *Corchorus olitorius* were obtained from the premises of University of Benin and New Benin market, Benin City respectively. The plant specimens were identified by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State and were deposited at the herbarium with voucher number UBH_A325 and UBH_C542 for *Alchornia cordifolia* and *Corchorus olitorius*

Extract Preparation

The freshly collected leaves were kept under shade from sunlight in the laboratory of Medical Biochemistry, University of Benin for four weeks until they were found to be crispy in texture. The dried plant material was pulverized to powdered form using a mechanical grinder. 500 g of each powdered plant material was macerated in 2L of methanol for 72h. Whatman filter paper was used to filter the bulk liquid mixtures which were further concentrated in a rotary evaporator (BÜCHI Labortechnik AG, Switzerland) at 40^0C and finally

freeze-dried to the granular crude extract form. Aqueous and methanolic stock solutions (1mg/mL) of the freeze-dried extracts obtained from the different plants were prepared prior to phytochemical and antioxidant analyses.

Phytochemical Screening

Alkaloids, tannins, saponins, carbohydrates, anthraquinones, flavonoids and other phenolic compounds were screened qualitatively using the procedures previously described by Stahl [15], Sofowora [16], and Trease and Evans [17].

Determination of Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) was determined according to the Folin-Ciocalteaus method [18]. Serial dilutions of gallic acid in methanol, 0.01-1mg/mL were dissolved. Different concentrations of the extracts, 0.01- 1mg/ mL were prepared with methanol. A total of 0.5 mL of extract or standard 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 left undisturbed for 30 minutes at room temperature before absorbance was read at 760 nm. The total phenolic contents of the extractswere expressed in mg gallic acid equivalents (GAE). The experimental protocol was done in triplicates.

Determination of Total Flavonoids Content

The method of Miliauskas *et al.* [19] was employed to determine total flavonoids content of extracts. To 2 mL of extract or standard was added 2 mL of 2 % $AlCl_3$ in methanol. Absorbance was read at 420 nm after incubation for 1 h at room temperature. Quercetin concentrations ranging from 0.01 - 0.15 mg/mL were used to plot the calibration curve and methanol extract of a concentration of 1 mg/mL was used for determination of total flavonoids which was expressed in Quercetin Equivalents (QE).The experimental protocol was done in triplicates.

Determination of Tannin Content

Tannin contents of methanol extracts were determined according to the method of AOAC [20]. Briefly 0.5 mL of the extract (1 mg/mL) was mixed with 1.25 mL Folin Denis reagent and 2.5 mL 10 % sodium carbonate. After 30 min of incubation at room temperature, absorbance was read at 760 nm. The results were expressed as milligrams tannic acid equivalents (TA) per gram of extract (mg TA/g extract). The standard curve was prepared with tannic acid in six different concentrations (10, 25, 50, 75, 100 and 150 µg/mL). The experimental protocol was done in triplicates.

Determination of Proanthocyanidin Content

Proanthocyanidin content was determined based on the procedure described by Sun *et al.* [21]. A volume of 0.5 mL of 1.0 mg/mL of the extract preparation was mixed with 1 mL of 4 % vanillin-methanol solution and 0.75 mL concentrated hydrochloric acid. The mixture was left undisturbed for 15 min after which absorbance was read at 500nm. The extract was evaluated at a final concentration of 1.0 mg/mL. The absorbance of ascorbic acid was read under the same conditions. Standard solution of ascorbic acid (Standard solution of ascorbic acid (1 mg/mL) was prepared by dissolving 0.05g ascorbic acid in 50 mL of distilled water. Total proanthocyanidin contents (mg/g) were expressed as ascorbic acid equivalents (AE). The experiment was done in triplicates.

DPPH radical scavenging assay

The radical scavenging activity of the extracts against 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was determined according to the protocol reported by Brand-Williams *et al.* [22]. The following concentrations of each extract were prepared in

methanol at concentration ranging from 0.002 to 1mg/ml. Ascorbic acid was served as standard, and the same concentrations were prepared as the test solution. To 2 mL each of the prepared concentrations in a test tube was added 0.5 mL of 1mM DPPH solution in methanol. The experiments were carried out in triplicates. The test tubes were incubated for 15 min at room temperature, and the absorbance read at 517 nm. Ascorbic acid was used as a reference. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = $(A_0 - A_1) \times 100/(A_0)$

A_0 is the absorbance of the control (DPPH radical + methanol)

A_1 is the absorbance of the test (DPPH radical + sample extract or ascorbic acid)

Ferric Reducing Antioxidant Potential Assay (FRAP)

The method of Benzie and Strain [23] was used to assess the antioxidant activity of the crude extracts being investigated. Different concentrations (0.01, 0.025, 0.05, 0.075, 0.1, 0.15mg/mL) of the extracts and the standard were serially diluted with distilled water. Then, 1mL of FRAP reagent (200 mL of 300mM sodium acetate buffer at pH 3.6, 20ml of 10.0mM TPTZ solution, 20ml of 20.0mM $FeCl_3.6H_2O$ solution and 24mL of distilled water) was added to each test tube. The resulting mixture was vigorously shaken and then incubated at 37^0C for 4min and the increase in absorbance at 593nm was measured and compared with the standard, ascorbic acid**.**

Reducing Power Assay

The reducing power of the extracts was determined using the method described by Lai *et al.* [24]. 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, pH 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. Precisely, 0.5 mL of 0.1% $FeCl_3$ was then added and the absorbance measured at 700 nm. Higher absorbance values indicate higher reducing power. Vitamin C (1mg/mL) served as a positive control.

Statistical Analysis

All determinations of antioxidant activities of plant extracts were done in triplicate. The experimental data were reported as means of treatments± standard error of means and were analyzed by One-Way Analysis of Variance (ANOVA) and Duncan multiple-wise Post- Hoc test statistics using Microsoft Excel 2000 software. p value < 0.05 was considered statistically significant.

RESULTS

Qualitative Phytochemical Screening

Alkaloids, tannins, saponins, terpenoids, flavonoids, steroidal saponins and reducing sugar were present in *Alchornia cordifolia* methanol extract while *Corchorus olitorius* methanol extract tested positive for tannins, saponins, terpenoids, flavonoids, steroidal saponins excluding alkaloids and reducing sugar (Tab. 1).

Quantitative Phytochemical Analysis

Total phenols (67.19 ± 1.56 mg/g extract) and tannins (406.67±1.34 mg/g extract) in the methanol extract of

A. cordifolia were significantly higher than in *Corchorus olitorius* methanol extract (total phenols, 20.26±1.13 mg/g extract and total tannins, 230mg/g extract) (Fig 1). In contrast, the concentrations of total flavonoids (54.31± 1.18 mg/g extract) and proanthocyanidin (17.50 ± 0.21mg/g extract) in *C. olitorius* methanol extract were significantly ($p<0.05$) those of *Alchornia cordifolia* methanol extract (total flavonoids, 20.01±2.13 mg/g extract and proanthocyanidin, 6.58± 0.29 mg/g extract) (Fig. 1).

DPPH radical scavenging activity

The results of the DPPH radical scavenging activities of *A. cordifolia* and *C. olitorius* leave extracts are presented in Figure 2 and Table 2 respectively. In Table 2, the methanol extract of *Alchornia cordifolia* (IC_{50}= 0.01µg/mL) showed a 3000 fold increase in free radical scavenging activity compared to *Corchorus olitorious* extract (IC_{50}= 33.10µg/mL) and a 5 fold increment compared to ascorbic acid(IC_{50}= 0.055µg/mL), the standard.

Ferric Reducing Potential Assay

The ferric reducing potential of *C. olitorius* and *A. cordifolia* methanol extracts was determined (Figure 3).*A. cordifolia* methanol extract had a FRAP value of 510 µM Fe(II)/g which was significantly($p<0.05$) higher than the FRAP value of 479.80µM Fe(II)/g of *C. olitorius* methanol extract. The FRAP result of ascorbic acid (1005µM Fe(II)/g was significantly higher than ($p<0.05$) than the values obtained for the two extracts.

Reducing Power Assay

Within the 100 – 1000µg/mL range, *A. cordifolia* methanol extract consistently demonstrated higher reducing power than *C. olitorius* methanol extract (Fig 4). Between 400 -800 µg/mL, *A. cordifolia* extract had higher reducing power than ascorbic acid. However, ascorbic acid as the standard showed more potency in reducing activity than *A. cordifolia* methanol extract at 100- 200 µg/mL range and 1000 µg/mL respectively.

DISCUSSION

Nigerians of various ethnic extractions consume ample amounts of green vegetable in their diets many of which also offer medicinal benefits. The therapeutic importance of herbal plants arises from bioactive principles present in them which are called phytochemicals [25]. Phytochemicals include saponins, alkaloids, tannins and the polyphenolics. Polyphenolics of the flavonoids, proanthocyanidin and phenol classes are the most abundant dietary antioxidants (26). Therefore, studies on the antioxidant properties of these compounds have gained the attention of researchers in recent years [27]. The present study seeks to provide scientific information on the qualitative phytochemical composition, quantitative amounts of polyphenolic phytochemicals and antioxidant activities of *Alchorna cordifolia* and *Corchorus olitorius* methanol leave extracts. For the purpose of this study, we employed methanol for plant extraction because it has the distinctive quality of being able to extract a large variety of phenolic compounds from plants [28]. After qualitative analyses for these phytochemical components in the plant extracts, alkaloids, saponins, terpenoids, flavonoids and reducing sugars were detected. One potential implication of the preceding observation is that medicinal compounds for treatment of diverse

diseases could be developed from parts of the plants of interest. Furthermore, the presence of total tannins and phenols in *A. cordifolia* extract in comparatively higher amounts indicated that the plant itself could serve as a rich resource of the aforementioned secondary metabolites. *C. olitorius* extract had relatively higher amounts of flavonoids and proanthocyanidin. Dietary intake of *C. olitorius* may help to decrease susceptibility to atherosclerosis [29], viral infections [30], neurodegenerative diseases [31], leukaemia [32] because of the protective effects conferred by flavonoids constituents. The mechanisms of action to explain the antioxidant activity of plant extracts are varied. Thus, antioxidant activity cannot be comprehensively explained using a single assay model [33]. Consequently, three complementary *in vitro* tests were used in this study to assess the antioxidant activity of *A. cordifolia* and *C. olitorius* extracts namely: DPPH free radical-scavenging activity, Ferric reducing antioxidant potential (FRAP) and reducing power assays. The DPPH scavenging assay assesses the free radical quenching activity of biological sample [34] while the FRAP assay measures the reducing potential [35]. In this study, the methanol leave extract of *A. cordifolia* showed a higher potency in scavenging DPPH free radicals than the *C. olitorius* extract, an indication that *A. cordifolia* possessed a stronger antioxidant activity. This finding is consistent with the report of Osadebe *et al.* [9]. The remarkable efficiency with which *A. cordifolia* extract was able to quench DPPH radicals *in vitro* could be a consequence of the significant predominance of phenolics and tannins in *A. cordifolia*. Piluzza and Bullitta [36] reported that the phenolic contents of plant extracts examined in their study showed significant correlation with DPPH scavenging activities of those extracts suggesting that phenolic content could be used as an index of the antioxidant potential of plants. Phenolics are potentially good reducing agents because the presence of multiple hydroxyl groups in their chemical structure implies they could act as spontaneous donors of electrons to free radicals in biological systems. In ethno pharmacological research, reducing power assay is often used to assess the electron donating capacity of plant extracts [37]. And there have been reports which assert that a linear correlation exists between the antioxidant potential and reducing power of plant extracts (38). We observed an increase in the reducing power of *A. cordifolia* methanol extract when measured in comparison to the reducing power of the second extract (*C. olitorius* extract). This experimental outcome reaches a similar inference with the DPPH radical scavenging assay that the antioxidant effect of *A. cordifolia* is relatively more potent. Although, in a previous study [39] FRAP assay showed a strong correlation with DPPH assay, our findings indicated otherwise. There was no statistically significant difference ($p>0.05$) between *A. cordifolia* and *C. olitorius* methanol extracts in their FRAP results. The observed variance between the research outcomes of the FRAP and DPPH radical scavenging assays might be due to differences that exist in the mechanisms of action of these tests. It is possible that the ambient pH and the stable DPPH radical present in the DPPH radical inhibition assay created a micro-environment which favoured phenols and tannins in the plant extracts to act as better electron donors than the corresponding flavonoids and proanthocyanidins constituents. In contrast, the acidic environmental condition during FRAP experiment may have diminished the electron-donating ability of the phenols, tannins, flavonoids

and proanthocyanidins in essence levelling the antioxidant activity of *A. cordifolia* and *Corchorus olitorius* extracts in this assay. It is also pertinent to state that the capacity of the plant extracts to mop up free radicals is not the exclusive preserve of phenolics, flavonoids and tannins rather it also involves the input of other plant nutrients such as vitamins, ascorbic acid and reducing sugars [40].It therefore appears that the DPPH free radical inhibition assay gave a better measure of total antioxidant capacity of plant extracts than the FRAP assay.

CONCLUSION

Our findings indicated that the methanol extract of *A. cordifolia* scavenges free radicals more efficiently than the corresponding extract of *C. olitorius* based on the final considerations reached from the different antioxidant assays. Accordingly, we recommend *A.cordifolia* leaves as a rich source for natural antioxidants.

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TABLE 1: Qualitative Phytochemical Analysis

PHYTOCHEMICALS	*Alchornia cordifolia*	*Corchorus olitorius*
Alkaloids	+++	_
Flavonoids	+++	+++
Tannins	+++	++
Saponins	+++	++
Terpenoids	+++	++
Phenols	+++	+++
Reducing sugar	++++	-
Carbohydrate	++	++
Steroid saponins	++	+++

+ indicates presence of constituent, -Indicates absence of constituents

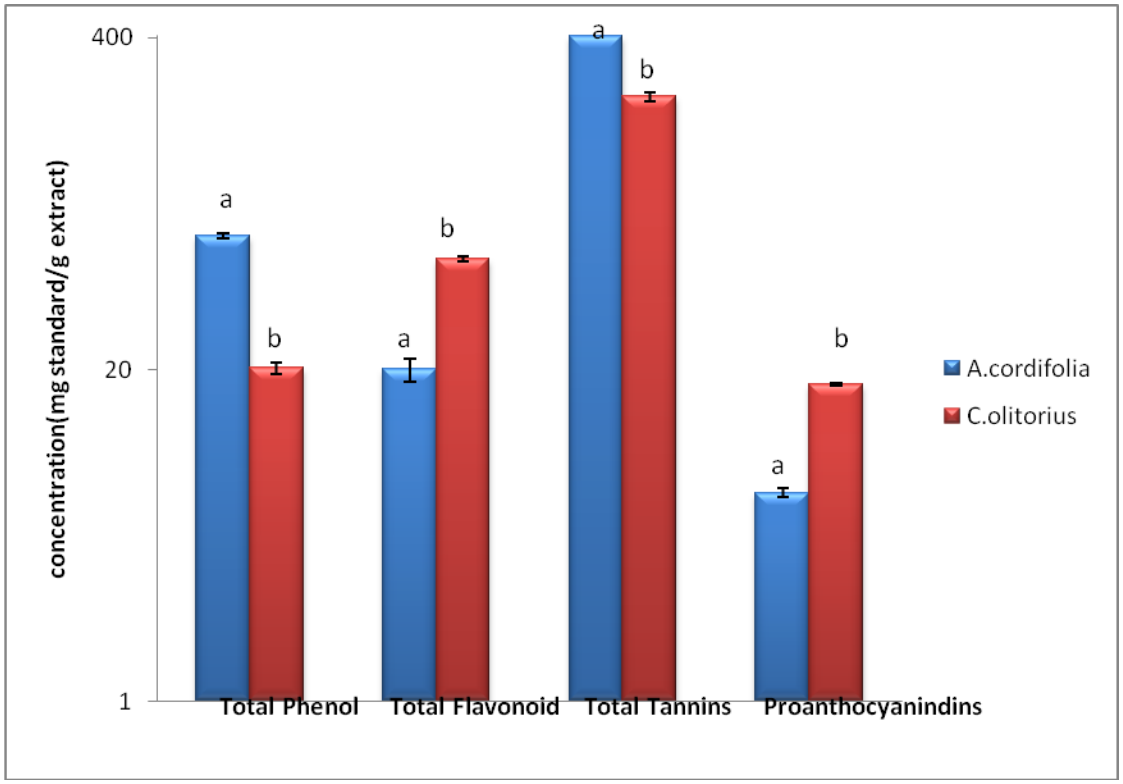

Figure1:Polyphenolic contents of ofA. *cordifolia and C. olitorious*methanol extracts .Values are expressed as mean ±SEM, n = 3/group.

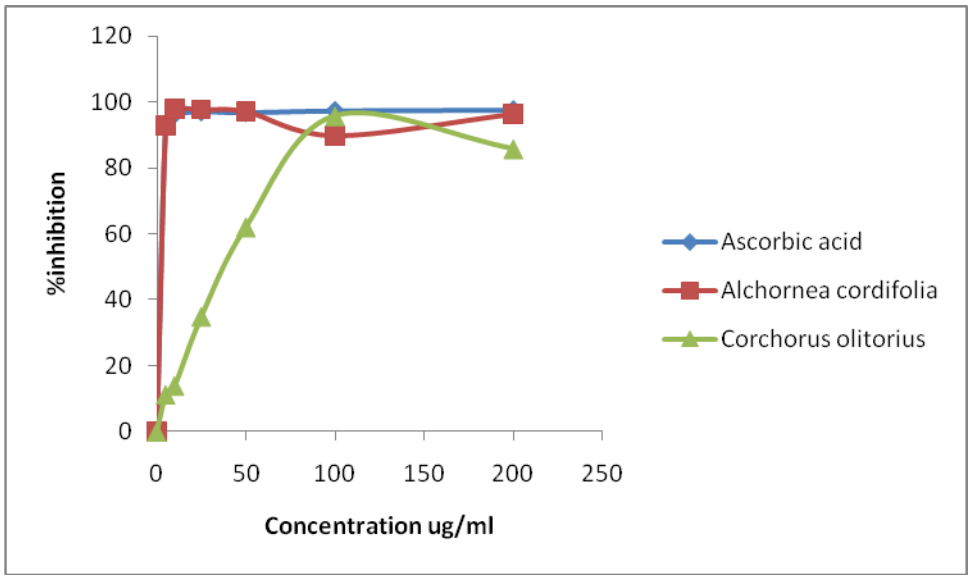

Figure 2: Percentage inhibition of DPPH radical versus concentration of extract

Table 2:DPPH free radical scavenging activity of extracts

Plant extract	**IC_{50}(µg/ml)**
Ascorbic acid	0.055
A .cordifolia extract	0.01*
C. olitorious extract	33.10*

Values in a column with asterisk (*) are significantly different from the standard ($p < 0.05$)

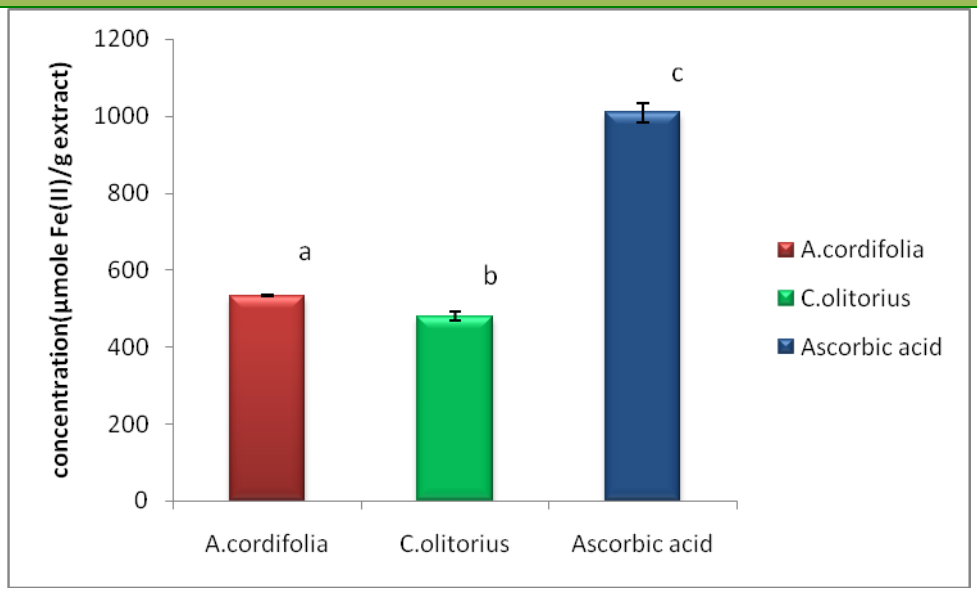

Figure3: Ferric reducing antioxidant potential (FRAP) of extracts. Different letters represent statistical significant difference between means at $p < 0.05$.

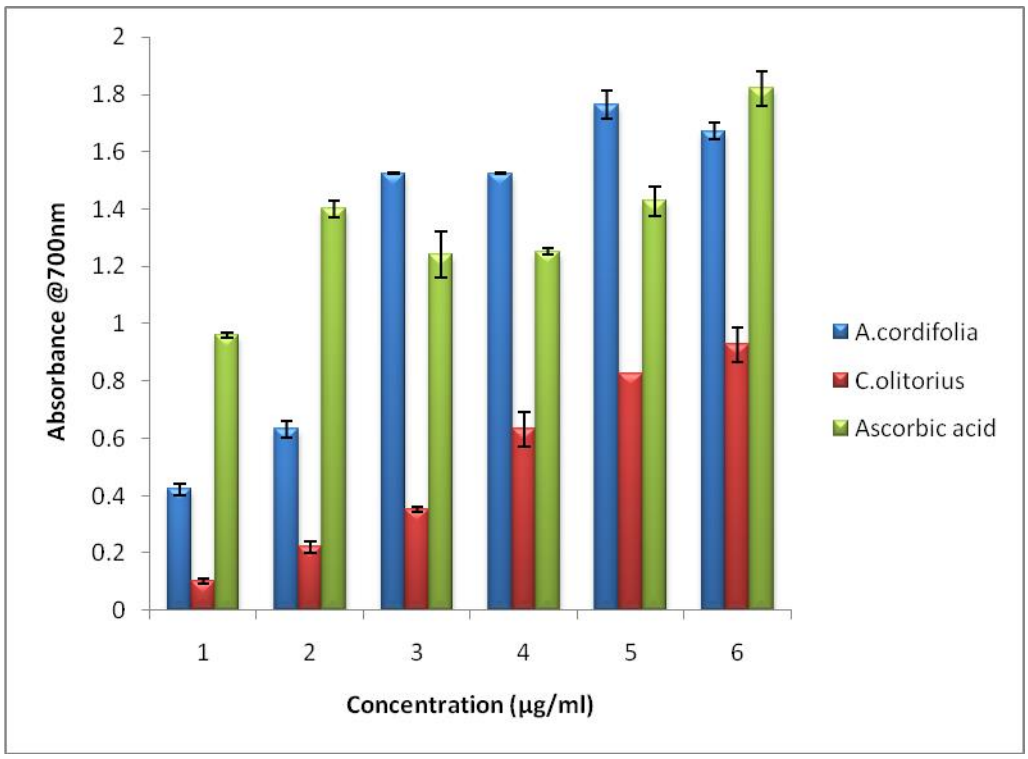

Figure 4: Reducing power of extracts of. Values are expressed as mean ±SEM, (n = 3/group).