

A Comparative Study of *in vitro* Antioxidant Activity and Phytochemical Constituents of Methanol Extract of *Aframomum melegueta* and *Costus afer* Leaves

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Abstract

Based on local claims on the efficacy of *Aframomum melegueta* (alligator pepper) and *Costus afer* (ginger lily) in the treatment of malaria, the present study compared the antioxidant activity and phytochemical constituents of extracts of *A. melegueta* and *C. afer* leaves. Methanol extracts of the plant leaves were obtained using standard procedures. The antioxidant property of the plants extracts were evaluated using DPPH (1,1-diphenyl-1-picryl-hydrazyl) radical, Total Phenol Content (TPC), Total Flavonoid Content (TFC), Proanthocyanidin Content (PC), Ferric acid Reducing Antioxidant Potential (FRAP) and ThioBarbituric Acid Reducing Substances (TBARS) assay. The phytochemical screening test revealed the presence of alkaloids, reducing sugars, flavonoids, tannins, saponins, steroids, cardiac glycosides and terpenoids in both extracts. The antioxidant study showed that the *A. melegueta* extract had higher DPPH radical scavenging ability (IC₅₀ of 122.25ug/ml), FRAP (35.38μmol Fe(II)/g) and TBARS (% inhibition of 62.08%) than *C. afer* (IC₅₀ of 156.48ug/ml, 12.25 μmol Fe(II)/g, and 42.5%, respectively). The *C. afer* extract, however, recorded higher levels of TFC, PC and a lower TPC content when compared with the *A. melegueta* counterpart. The results suggest that *C. afer* and *A. melegueta* extracts could serve as free radical scavengers, acting as primary antioxidants. The results support local claims of their therapeutic uses in the treatment of malaria in folklore medicine.

Keywords: *Aframomum melegueta*, *Costus afer*, phytochemical, antioxidant, Medicinal plant.

1. Introduction

Medicinal plants have been used in folk medicine for generations in most of the cultures throughout the world and are still one of the primary sources of treatment in many areas today. However, among the 250,000 - 500,000 species of plants on earth, only a relatively small percentage (1 - 10%) is used for food by humans and animals (Borris, 1996), while a higher percentage may serve medicinal purposes. The medicinal values of plants have been claimed to lie in their phytochemical components including alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Anyasor, 2011). Phytochemicals have been reported to protect the cell constituents against destructive oxidative damage, inhibition of hydrolytic and oxidative enzymes including lipid peroxidation, thus limiting the risk of various degenerative diseases associated with oxidative stress (Vinay et al., 2010).

Costus afer Ker-Gawl (Costaceae) is among 150 species of stout, perennial and rhizomatous herbs of the genus *Costus* (Edeoga and Okoli, 2000). It is found in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone and Nigeria (Burkill, 1985; Edeoga and Okoli, 2000). *C. aferis* commonly called bush cane, *irekeomode* (Yoruba) and *opete* (Igbo). It bears white and yellow flowers (Stentoft, 1988). The stem, seeds and rhizomes are harvested from the wild and contain several bioactive metabolites. *C. afer* is highly valued for its anti-diabetic, anti-inflammatory and anti-arthritic properties in South-East and South-West Nigeria (Soladoye and Oyesika, 2008). It is also widely used in Nigeria for the treatment of cough, malaria, venereal diseases, skin eruption and inflammation (Okoko, 2009).

Aframomum meleguetais a West African plant, with common (local) names such as "alligator pepper," "guinea pepper" and "grain of paradise." It is a member of the family Zingiberaceae and is locally called 'atare' (Yoruba). It is widely spread across tropical rain forest regions of Africa including Nigeria, Liberia, Sierra Leone, Ghana, Cameroon, Cote D' Ivoire and Togo. The plant

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possesses both medicinal and nutritive values. The phytochemicals obtained from the seed has been used for years in the treatment of infectious diseases. The fruit pulp is chewed as a refreshing stimulant and the seeds and leaves are used for seasoning foods and in local medicine. It is also used as a remedy for a variety of ailments such as snakebite, diarrhea, smallpox, chickenpox, wounds, cough, anaemia, rheumatism, measles, malaria, toothache, cardiovascular diseases, diabetes and fertility control (Olowokudejo, 2008).

A number of studies have been conducted on the phytochemistry and pharmacology of the individual plant extract, but to the best of our knowledge there have been no comparative studies on the methanol extracts of both plants. Thus, the present study comparatively evaluates the phytochemical constituents and *in vitro* antioxidant activities of methanol extracts of *Costus afer* and *Aframomum melegueta* leaves..

2. Materials and Methods

2.1. Plant Materials

The leaves of *Costus afer* and *Aframomum melegueta* were collected from a farm in Edo State, Nigeria. The leaves were identified and authenticated by a Botanist in the Department of Plant Biology and Biotechnology, University of Benin.

2.2. Preparation of Crude Extract

The leaves of both plants were rinsed thoroughly with distilled water and allowed to dry under shade. The dried samples were then pulverized and stored in an air tight container. Two hundred grams (200 g) of each sample were extracted in 1000 mL of absolute methanol at room temperature for 72 hours. After that, each of the sample were filtered using Whatman No 50 filter paper and the filtrate evaporated to dryness using a rotary evaporator to give a percentage yield of 11.43 % (*Costus afer* extract) and 20.10 % (*Aframomum melegueta* extract). The resultant yields were stored in air-tight bottles and kept in the refrigerator maintained at 4°C until subsequent use.

2.3. Phytochemical Screening

Alkaloids, tannins, saponins, carbohydrates, anthraquinones, flavonoids and other phenolic compounds were screened qualitatively using the procedures previously described by Stahl (1973), Sofowora (1982) and Evans (2002).

2.4. Determination of Total Phenol Content (TPC)

The Total Phenolic Content (TPC) was determined according to the Folin and Ciocalteu's method using gallic acid as standard (Folin and Ciocalteu, 1927). Concentrations of 0.01- 1mg/ml of gallic acid were prepared in methanol. Concentrations of 1mg/ml extracts of *Costus afer* and *Aframomum melegueta* were also prepared in distilled water. About 0.5 mL of the sample was mixed with 2.5 mL of a ten-fold diluted Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was left undisturbed for 30 minutes at room temperature before the absorbance was read at 760 nm. All the determinations were performed in triplicates. The

total phenolic content in the methanol extract was expressed in Gallic Acid Equivalents (GAE).

2.5. Determination of Total Flavonoids Content

The method of Miliauskas et al. (2004) was employed. To 2 mL of the sample was added 2 mL of 2 % $AlCl_3$ in methanol. The absorbance was read at 420 nm after incubation for 1 hour at room temperature. The concentration of 1 mg/ml of the extract in methanol was used, while quercetin concentrations ranging from 0.01 - 0.15 mg/ml were used to obtain the calibration curve. The total flavonoid content of the extract was expressed in Quercetin Equivalents (QE).

2.6. Estimation of Proanthocyanidin Content

The determination of proanthocyanidin was based on the procedure described by Sun et al. (1998). A volume of 0.5 mL of 1.0mg/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 minutes after which the absorbance was read at 500nm. The extract was evaluated at a final concentration of 1 mg/ml. The absorbance of ascorbic acid was read under the same conditions. Standard solution was prepared from 0.05g ascorbic acid. Total proanthocyanidin contents (mg/g) were expressed as ascorbic Acid Equivalents (AE).

2.7. Determination of DPPH Radical Scavenging Activity

The radical scavenging activity of the extract against 1, 1-diphenyl-1-picryl-hydrazyl radical (DPPH) was determined by a slightly modified method of Brand-Williams et al. (1995). 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 minutes 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:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract /standard.

2.8. Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

A modified ThioBarbituric Acid Reactive Species (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of poly unsaturated fatty acids, reacts with a pinkish red chromogen with an absorbance maximum at 532nm (Ruberto et al., 2000). Briefly 0.5 mL of Egg homogenate (10 %v/v) and 0.1 mL of extract were added to a test tube and made up to 1 mL with distilled water. Precisely 0.05 mL of $FeSO_4$ (0.07M) was added to induce lipid peroxidation and incubated for 30minutes. Then 1.5 mL of 20% acetic acid(pH adjusted to 3.5 with

NaOH) and 1.5ml of 0.8%(w/v) TBA in 1.1% sodium dodecyl sulphate and 0.05 mL 20% TCA were added and the resulting mixture was vortexed, and, then, heated at 95°C for 60 minutes. The generated color was measured at 523nm.

Inhibitions of lipid peroxidation (%) by concentrates were calculated with the formula:

$$(C-E) / C \times 100 \%$$

where C is the absorbance value of the fully oxidized control and E is (Abs523+Abs523-TBA).

2.9. Ferric Reducing Antioxidant Power (FRAP) Assay

The method employed was that of Benzie and Strain (1996) with a slight modification. 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 (200ml of 300mM sodium acetate buffer at pH 3.6, 20ml of 10.0mM TPTZ solution, 20ml of 20.0mM FeCl₃.6H₂O solution and 24ml of distilled water) was added to each test tube. The resulting mixture was vigorously shaken and then incubated at 37°C for 4mins and the increase in absorbance at 593nm was measured and compared with the standard ascorbic acid.

2.10. Statistical Analysis

All values were expressed as mean ± S.E.M. One way analysis of variance (ANOVA) was employed to assess the difference in mean between the groups. Turkey's multiple range post-hoc test was used to check the level of significance at p values less than 0.05

3. Results and Discussion

The therapeutic effects, derived from several medicinal plants, have been attributed to the presence of phenolic compounds such as flavonoids, phenolic acid, proanthocyanidins and tannins (Pourmorad et al., 2006). Phenolics exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (Jimoh et al., 2008). These potential mechanisms make the diverse group of phenolic compounds an interesting target in the search for health beneficial phytochemicals (Akinpelu et al., 2010). In this study the phytochemical constituents and free radical scavenging activity of medicinal plants, were evaluated.

The result of phytochemical screening test of methanol extracts of *Aframomum melegueta* and *Costus afer* revealed the presence of medically active compounds including terpenoids, reducing sugars, flavonoids, saponins, alkaloids and cardiac glycosides in both leaves extracts. However, steroids and tannins were below the detectable levels in *Aframomum melegueta* and *Costus afer*, respectively (Table 1). Steroids have been reported with antibacterial properties and tannins have been found to have healing effects. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants where they exhibit antioxidant activities. Alkaloids are organic compounds that contain nitrogen, and are physiologically active with sedative and analgesic properties for relieving pains, anxiety and depression (Jisika et al., 1992). Therefore, the

presence of these substances in the methanol extracts of *Costus afer* and *Aframomum melegueta* may be responsible for their therapeutic claims.

Figure 1 shows the total phenolic, flavonoid and proanthocyanidin contents of methanol extracts of *C. afer* and *A. melegueta*. Total phenolic content was expressed as mg gallic acid equivalent/g of extract by reference to a standard curve ($y = 0.001x + 0.033$; $R^2 = 0.975$). The total flavonoid and proanthocyanidin content were expressed as mg quercetin equivalent/g of extract by reference to a standard curve ($y = 0.006x + 0.025$; $R^2 = 0.996$) and mg ascorbic acid equivalent/g of extract by reference to standard curve ($y = 0.002x + 0.09$; $R^2 = 0.915$), respectively. The results showed that *A. melegueta* extract had significantly higher ($p < 0.05$) concentration of total phenolic compounds than the *C. afer* extract, while the amounts of flavonoid and proanthocyanidin in *C. afer* were considerably high compared to *A. melegueta*. The different levels of antioxidant activities in these extracts may be due not only to differences in their phenolic contents, but also to their phenolic acid components (Horax et al., 2005). The presence of hydroxyl groups in the phenolic compounds may directly contribute to the antioxidant activity and is a key determinant of their radical scavenging and metal chelating activity (Amarowicz, 2004; Elmastas, 2007). Phenolic compounds could be a major determinant of antioxidant potentials of foods (Parr and Bolwell, 2000), and could therefore be a natural source of antioxidants (Aberoumand and Deokule, 2008). This finding is in agreement with Ukpabi et al. (2012) who state that extracts of *A. melegueta* and *C. afer* have considerably high amounts of polyphenols.

A number of methods are available for the determination of antioxidant capacity but the assay involving the stable 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) has received the maximum attention due to its ease of use and its convenience (Sanchez-Moreno et al., 1998). The DPPH radical scavenging ability of the extract of *A. melegueta* showed maximum activity ($p < 0.05$) with IC_{50} of 122.25 µg/ml when compared with the *C. afer* counterpart (IC_{50} of 156.48 µg/ml). However, the standard reference compound, ascorbic acid gave a better DPPH scavenging ability than the two extracts with a lower IC_{50} of 118.55µg/ml. DPPH is a relatively stable free radical (Table 2). From the present result, it may be postulated that *A. melegueta* reduces the radical when it reacts with hydrogen donors in antioxidant principles. Also, when DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up (Ou, 2005). This is in agreement with Onoja et al. (2014) who state that *A. melegueta* seed has potent antioxidant activities when compared to standard ascorbic acid.

Our results in the present study showed that the ferric reducing antioxidant potential (FRAP) of methanol extract of *A. melegueta* (35.38µmole Fe (II)/g) was significantly higher ($p < 0.05$) than that of *C. afer* (12.25µmole Fe (II)/g). However, the FRAP values of both extracts were significantly ($p < 0.05$) lower than that recorded for standard ascorbic acid (140.5 µmole Fe(II)/g)

(Figure 3). Shiddhuraju et al. (2002) suggested that ferric reducing power of bioactive compounds was associated with antioxidant activity. It is a measure of the reductive ability of antioxidants and it is evaluated by the transformation of Fe^{2+} to Fe^{3+} in the presence of sample extracts (Huda-Faujan et al., 2009).

The methanol extracts of *C. afer* and *A. melegueta* leaves showed a varied degree of inhibition of lipid peroxidation induced by ferrous sulfate in egg yolk homogenates (Figure 4). The percentage inhibition of lipid peroxides by the methanol extract of *A. melegueta* (62.08 %) was significantly higher than that of *C. afer* (42.5 %). Lipid peroxidation contains a series of free radical mediated chain reaction processes and is also associated with several types of biological damages (Perry et al., 2000). These findings corroborate with Anyasor et al. (2014) who posited that the aqueous fractions of the leaves and stem bark of *C. afer* exhibited high inhibition of lipid peroxidation.

Our findings suggest that *C. afer* and *A. melegueta* extracts could serve as free radical scavengers, acting possibly as primary antioxidants which could be used in the treatment/management of disease caused as a result of oxidative damaged.

Table 1. Qualitative Phytochemical Screening of Methanol Extracts of *Costus afer* and *Aframomum melegueta* leaves

Phytoconstituents	<i>Aframomum melegueta</i>	<i>Costus afer</i>
Cardiac glycosides	+	+++
Steroids	-	+
Terpenoids	++	+++
Reducing sugars	+	+
Flavonoids	+	+
Tannins	+	-
Saponins	+	+
Alkaloids	++	+

Keys: - = Absent;+ = positive; ++ = moderately positive; +++ = highly positive

Table 2. IC_{50} values of *Costusafer* and *Aframomummelegueta* crude methanol extracts

Extract	IC_{50} value (μ g/ml)
Ascorbic acid	118.55 \pm 0.06 ^a
<i>C.afer</i>	156.48 \pm 0.17 ^b
<i>A.melegueta</i>	122.25 \pm 2.08 ^c

Data represent mean \pm Standard Deviation of triplicate analysis. Different lowercase letters within column indicate significant difference at $p < 0.05$.

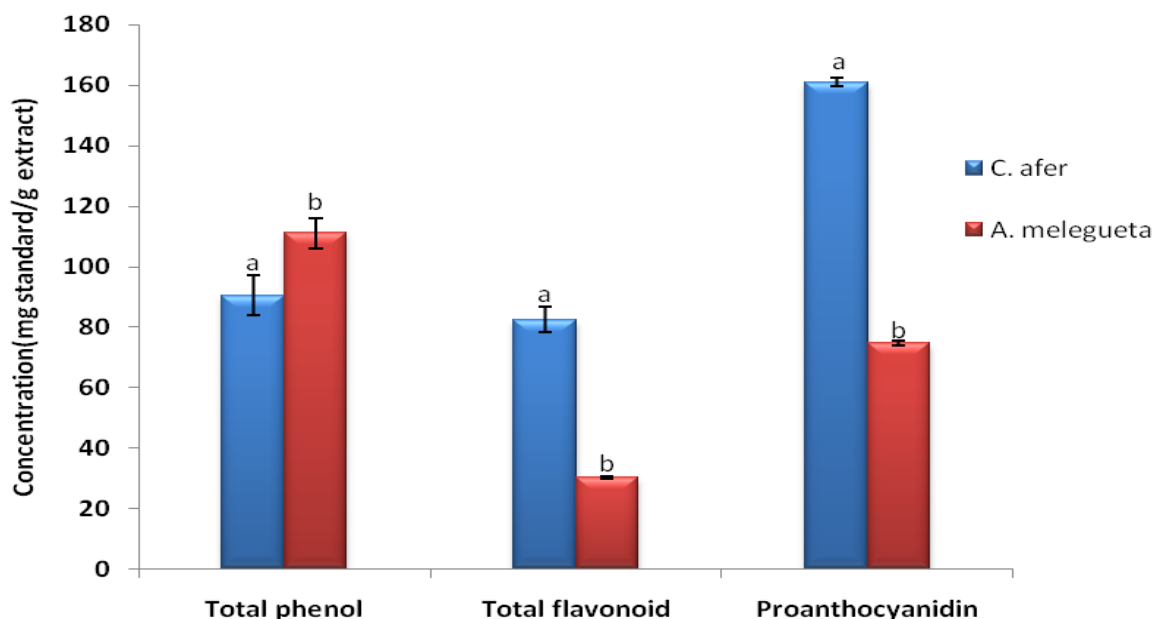


Figure 1. Polyphenolic contents of *Costus afer* and *Aframomum melegueta*

Values are expressed as mean \pm SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $p > 0.05$.

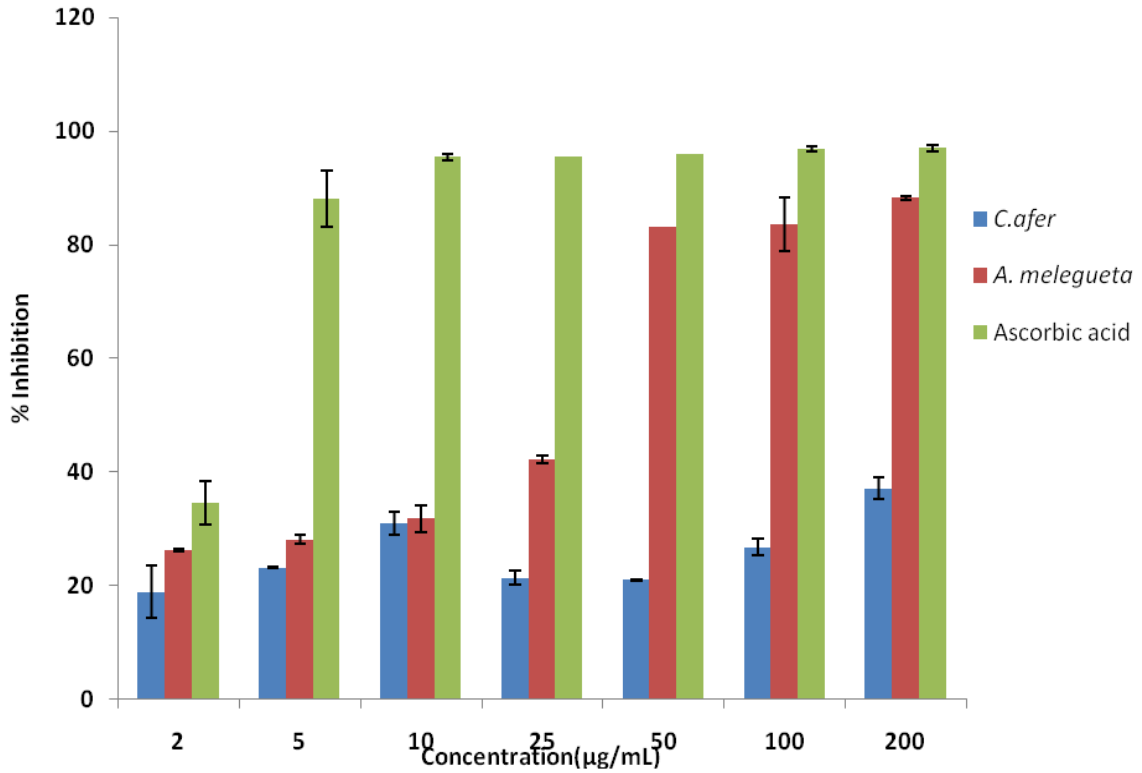


Figure 2. DPPH radical scavenging activity of *Costusafer* and *Aframomummelegueta* crude methanol extract compared with standard (ascorbic acid) Data represent mean \pm Standard Error of mean of triplicate analysis.

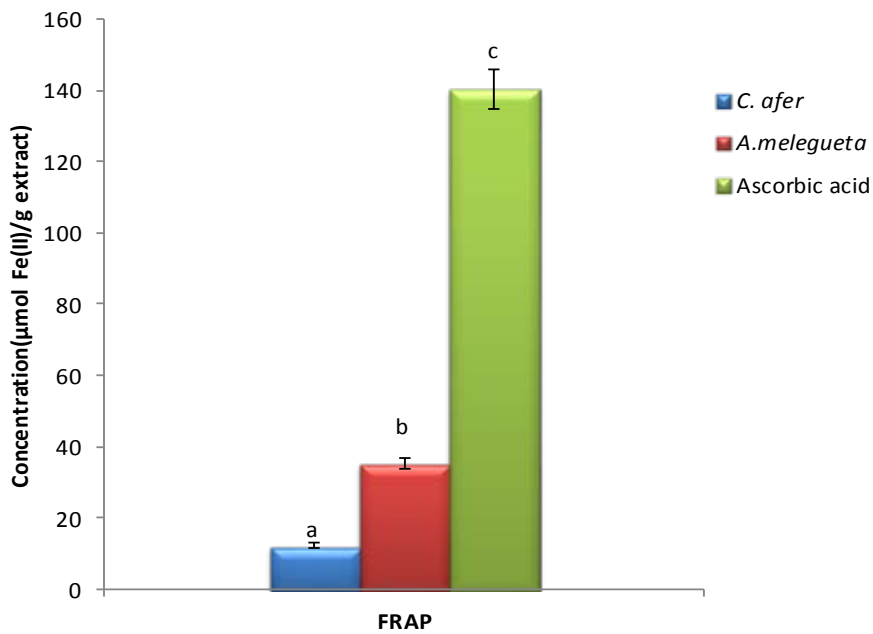


Figure 3. Ferric Reducing Antioxidant Potential (FRAP) of *Costusafer* and *Aframomummelegueta* crude methanol extracts Values are expressed as mean \pm SEM, $n = 3$ /group. Different lowercase letters represent significant difference between means at $P < 0.05$.

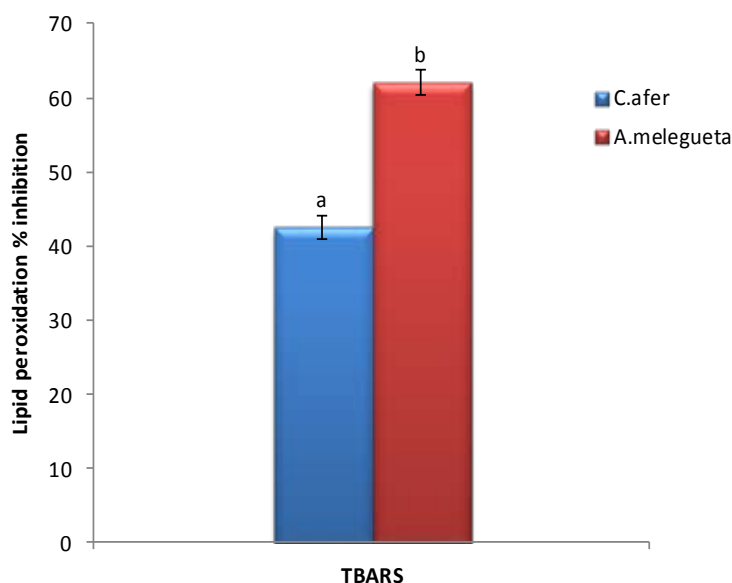


Figure 4. Thiobarbituric acid reactive substances (TBARS) of *Costus afer* and *Aframomum melegueta* crude methanol extracts. Values are expressed as mean ± SEM, $n = 3/\text{group}$. Different lowercase letters represent significant difference between means at $P < 0.05$.

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