



Assessment of *in-vitro* Antioxidant/Enzymes Inhibitory Potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OTO, VOO, AOA, IOO and AO designed the materials and methods used in the course of the research work. Author OTO performed the statistical analysis and wrote the final draft of the manuscript. Author VOO collect medicinal plants used for the treatment of different infection and helps to proof read the manuscript for constrictive criticism. Author AOA has done analytical work on antimicrobial properties of medicinal plants and helps to design the materials and methods. Author IOO helps to manage the overall analyses of the study. Author AO managed the antioxidant inhibition enzymes protocol and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this research work is to evaluate the *in-vitro* antioxidant and antioxidant enzymes inhibition potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts. *Aframomum melegueta* is a spice with a similar composition as Ginger, that belongs to the same Zingiberaceae family, used in Nigeria, West Africa and it is used for the treatment of infectious diseases such as urinary tract infections, cancer and diabetes. Antioxidants neutralize the effect of free radicals through different ways and may prevent the body from various diseases. The plant extract were collected from a rain forest in Akugba Akoko, Ondo state, Nigeria. The plant sample was dried, pulverized, filtered with Whatman No 1 filtered paper and the filtrates was concentrated in-vacuum using vacuum rotary evaporator at 40°C and was later concentrated to dryness in a hot-air oven at 40°C. Assessment of antioxidant potentials were performed using Singleton method Total flavonoids (mg/g), Phenol (mg/g), Ferric reducing (FRAP) potentials (mg/g), Free radical scavenging ability (DPPH) (1, 1- diphenyl-2-picrylhydrazyl) potentials (%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging potentials (mMol/g), Fe²⁺ chelation potentials (%) and OH Radical Scavenging potentials (%) were evaluated. Antioxidant enzymes of plant were assessed using Bentler method, Superoxide Dismutase (SOD) (%), Reduced Glutathione (GSH) (%), Catalase (CAT) and Glutathione Peroxidase (GPX) were evaluated. Seed extract among other extracts of *Aframomum melegueta* has the highest quantity of flavonoids, Phenol, FRAP, DPPH, ABTS, Fe²⁺ chelation and OH radical scavenging potential. The leaf extract of *A. melegueta* has the highest percentage of Superoxide Dismutase (SOD) and Catalase (CAT) while stem bark of *Aframomum melegueta* has the highest percentage of Reduced Glutathione (GSH) and Glutathione Peroxidase (GPX). It can be deduced from this research work that all parts of the *A. melegueta* has pharmacological and therapeutic activities judging from literature that *A. melegueta* can be used to prevent oxidative damage by ROS (Reactive oxygen species) reacting with free radical chelating and catalytic metals which can be used to reduce and prevent various diseases like heart disease, cancer, DNA degeneration, pulmonary and neurological disorder. SOD, GPX, GSH help to control cytokins induced peroxidase level and mediate signal transduction in mammalian cells. It can be deduced that *Aframomum melegueta* should be the focal point in human diet, natural herbal drug supplement and even in addition to conventional drug to improve the potency and quality of natural drug system. The use of *Aframomum melegueta* should be encouraged. These results show the potential of *Aframomum melegueta* as a source of bioactive compounds to be used for pharmaceutical, nutraceutical, and cosmeceutical applications.

Keywords: *In-vitro* antioxidant potentials; antioxidant; enzymes potentials; *Aframomum melegueta* Schum.

1. INTRODUCTION

Antioxidants act as a defence mechanism that protect human against dangerous effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system [1]. Reactive oxygen species not only are produced naturally in cell following stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxin, smoking, alcohol, and psychological or emotional stress. Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neuro-degenerative disease, and aging process [2,3].

Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers [4,5]. The antioxidants in biological system can be either enzymatic or non-enzymatic. The enzymatic antioxidants include catalase, superoxide dismutase, and glutathione which catalyse neutralization of many types of free radicals [6], while the non-enzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols. There is growing evidence that antioxidants play a pivotal role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease, and neurological disorder [7]. Recently, there has been an upsurge of interest in the therapeutic potential of plants as antioxidants in reducing oxidative tissue injuries.

Plants, herbs, and spice, rich in phenolic compounds like flavonoids, have been demonstrated to have anti-inflammatory, anti-allergenic, antiviral, anti-aging, and anti-carcinogenic activities which can be attributed to their antioxidant properties [8].

Aframomum melegueta (Grains of Paradise) is a spice with a similar composition as Ginger that belongs to the same *Zingiberaceae* family [9]. It is used in Nigeria, West Africa. *Aframomum melegueta* (Grains of Paradise) has been used for the treatment of infectious diseases such as urinary tract infections such as cancer, diabetes and etc. It shows some promise in fat-mass control at doses possibly consumable in food products and medicinal concoction [10]. *Aframomum Melegueta* (Grains of Paradise) has different names such as grains of paradise, Atare (in Yoruba), chitta (Hausa), or Guinea pepper, is one seed with many healing power and its benefits to mankind seems endless [9]. *Aframomum melegueta* is an herbaceous perennial plant native to swampy habitats along the West African coast of Nigeria. Its trumpet-shaped, purple flowers develop into 5 to 7 cm long pods containing numerous small, reddish-brown seeds. melegueta pepper, and alligator pepper, *afmomum melegueta* is among the species that belong in the ginger family Zingiberaceae. It is most abundantly in the countries of Ghana, Liberia, Ivory Coast, Togo, and Nigeria [10,11].

Natural antioxidant is used for defense mechanisms in the human system, scavengers reactive species. They can be classified into different groups according to their properties. Endogenous antioxidant which include glutathione, alpha lipoic, co-enzymes Q, ferritin, Uric acid, Bilirubin, Metallothionein, Carnitine, Melatonin enzyme, Superoxide dismutase (SOD), Catalase (CAT), Glutaperooxidase (GPXs), Thioredoxin (TRX), Peroxiredoxins (PKXs). It should be noted that PRXs and GPXs are ubiquitous family of antioxidant enzymes (PRX I-V) (GPX I-V). They also control cytokine induced peroxide level and mediate signal transduction in mammalian cells. Oludare et al. [12] reported that there are other important factors or fact in natural antioxidant that co-exist in a delicate balance with oxidative inputs [9].

This plant can grow up to 1.5 m in height with orange-coloured lips and pinkish-orange upper flowers that can develop into fleshy and indehiscent pods. The size of the pods are 5-7 cm in length, are edible and contain numerous

small, reddish brown seeds (Fig. 1) with a pungent scent of ginger and cayenne pepper. The stem bark is short and covered with scars of fallen leaves. The leaves are about 30 cm long, 12 cm wide, and have close nerves underneath [13]. The leaves average 35 cm in length and 15 cm in width, with a well-structured vascular system. The flowers of the herbaceous plant are aromatic, with an orange-colored lip and rich pinkish-orange upper part. The fruits contain numerous, small, golden red-brown seeds [14].

In Nigeria, the seeds are used as a spicy and have a wide range of folkloric uses in traditional medicine. They are used as a remedy for treating stomach ache, diarrhoea, and snakebite [15]. Previous studies have established the antiulcer, antimicrobial, anti-inflammatory, and sexual performance enhancing effects of the seed extract [9]. The seeds are very rich in the nonvolatile pungent compounds gingerol, shogaols, paradol, and related compounds [16].

The present study aimed at evaluating the *in vitro* antioxidant / enzymes inhibition potentials of the Leaf, Stem bark seed bark and seed extract of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise).

Superoxide dismutases (SODs) are a group of metalloenzymes that are found in all kingdoms of life. SODs form the front line of defense against reactive oxygen species (ROS)-mediated injury [17]. These proteins catalyze the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2). SODs can be classified into four distinct groups: Copper-Zinc-SOD (Cu, Zn-SOD), Iron SOD (Fe-SOD), Manganese SOD (Mn-SOD), and Nickel SOD [18]. The different forms of SODs are unequally distributed throughout all biological kingdoms and are located in different subcellular compartments [9].

SODs constitute a very important antioxidant defense against oxidative stress in the body [19]. Several studies have been performed that reveal the therapeutic potential and physiological importance of SOD [20]. The enzyme can serve as an anti-inflammatory agent and can also prevent precancerous cell changes. Natural SOD levels in the body drop as the body ages [19] and hence as one age, one becomes more prone to oxidative stress-related diseases. SOD is used in cosmetics and personal care products as an anti-aging ingredient and antioxidant due to its ability to reduce free radical damage to the skin, therefore preventing wrinkles, fine lines, and age

spots, and it also helps with wound healing, softens scar tissue, protects against UV rays, and reduces other signs of aging [20].

Glutathione is also employed for the detoxification of methylglyoxal and formaldehyde, toxic metabolites produced under oxidative stress. This detoxification reaction is carried out by the glyoxalase system. Glyoxalase I (EC 4.4.1.5) catalyzes the conversion of methylglyoxal and reduced glutathione to S-D-lactoyl-glutathione. Glyoxalase II (EC 3.1.2.6) catalyzes the hydrolysis of S-D-lactoyl-glutathione to glutathione and D-lactic acid [21].

In plants, glutathione is involved in stress management. It is a component of the glutathione-ascorbate cycle, a system that reduces poisonous hydrogen peroxide [22]. It is the precursor of phytochelatins, glutathione oligomers that chelate heavy metals such as cadmium [22]. Glutathione is required for efficient defence against plant pathogens such as *Pseudomonas syringae* and *Phytophthora brassicae*. Adenylyl-sulfate reductase, an enzyme of the sulfur assimilation pathway, uses glutathione as an electron donor. Other enzymes using glutathione as a substrate are glutaredoxins. These small oxidoreductases are involved in flower development, salicylic acid, and plant defence signaling, it maintains exogenous antioxidants such as vitamins C and E in their reduced (active) states [23]. GPx-1 is one of the most abundant members of the GPx family of enzymes that include an epithelial-specific enzyme that is highly expressed in intestine (GPx-2); a secreted subtype (GPx-3); and GPx-4, which is widely expressed and differs in its substrate specificity compared to the other family members. Accordingly, GPx-1 is a crucial antioxidant enzyme involved in preventing the harmful accumulation of intracellular hydrogen peroxide. It is present in all cells; found in cytosolic, mitochondrial, and, in some cells, in peroxisomal compartments [24].

2. MATERIALS AND METHODS

2.1 Collection and Identification of *Aframomum melegueta* [Roscoe] K. Schum(Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts

Aframomum melegueta, Schum (Grains of Paradise) Leaf, Stem bark, Seed bark and Seed Extracts were collected from the tropical rain forest of Akungba, Akoko, Ondo State (Nigeria) in the morning time of 6.00 am (The time varies

with the quantity of bio-active secondary metabolites in a plant organ, it is better to collect plant for ethno-botanical practice early in the morning), 21st January 2020, The plant specimen were identified and authenticated at Department of Plant Science and Biotechnology, Adekunle Ajasin University Akungba Akoko, Nigeria where the voucher specimens were kept on record. Voucher number AAU-2487 was recorded for the plant extract for future reference.

2.2 Preparation of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts

Dried and pulverized seeds of *Aframomum melegueta* were extracted by cold maceration method for 48 hours at room temperature in a Winchester bottle. The *Aframomum melegueta* extract was filtered with Whatman No. 1 filter paper. The filtrate was concentrated *in vacuo* using vacuum rotary evaporator at 40°C and was later concentrated to dryness in a hot-air oven at 40°C. The extract was stored in a refrigerator at 4°C throughout the duration of this study.

2.3 Evaluation of Antioxidant Potential *Aframomum melegueta* [Roscoe] K. Schum(Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts

2.3.1 Determination of total phenol of *Aframomum melegueta*

The total phenol content of the extract determine by the method of Singleton et al. [25]. 0.2 ml of the extract was mix with 2.5 ml of 10% Folinicalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45°C for 40 mins, and the absorbance was measure at 700 nm in the spectrophotometer, garlic acid would be used as standard phenol.

2.3.2 Determination of total flavonoid of *Aframomum melegueta*

The total flavonoid content of the extract was determined using a colourimeter assay developed by Bao et al. [26]. 0.2 ml of the extract was added to 0.3 ml of 5% NaNO₃ at zero time. After 5 min, 0.6 ml of 10% AlCl₃ was added and after 6 min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg, Rutin equivalent.

2.3.3 Determination of ferric reducing property of *Aframomum melegueta*

The reducing property of the extract will be determined by Pulido et al. [27], 0.25 ml of the extract was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25ml of 1% KFC. The mixture was incubated at 50°C for 20min, thereafter 0.25 ml of 10% TCA was also added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.1% of FeCl₃ and the absorbance was measure at 700 nm.

2.3.4 Determination of free radical scavenging ability (DPPH (1, 1- diphenyl-2-picrylhydrazyl) of *Aframomum melegueta*

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picrylhydrazyl) using [27] method. 1 ml of the extract was mixed with 1 ml of the 0.4 mM methanolic solution of the DPPH the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm [28,29,30,31].

2.3.5 Determination Fe²⁺ Chelation of *Aframomum melegueta*

The ability of the extract to chelate Fe²⁺ was determined using a method of 28 modified by 29, 30. Briefly, 150 mM FeSO₄ will be added to a reaction mixture containing 168 ml of 0.1M Tris-HCl pH 7.4, 218 ml saline and extract and the volume is made up 1 ml with distilled water. The reaction mixture will be incubated for 5min, before the additional of 13 ml of 1, 10-phenantroline the absorbance will be read at 510 nm.

2.3.6 Determination of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging ability of *Aframomum melegueta*

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging ability. The ABTS scavenging ability of the extract was determined according to the method describe by Re et al. [32]. The ABTS was generated by reacting an (7 mM). ABTS aqueous solution with K₂S₂O₈ (2.45 mM/l, final conc.) in the dark for 16hours and adjusting the absorbance at 734 nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0 ml of ABTS solution and the absorbance was read at 732 nm after 15 mins. The TROLOX equivalent

antioxidant capacity was subsequently calculated (548.88 g).

2.4 Determination of OH Radical Scavenging Ability of *Aframomum melegueta*

The ability of the extract to prevent Fe²⁺/H₂O₂ induced decomposition of deoxyribose will be carried out using the method of Halliwell and Gutteridge [33]. Briefly, freshly prepared extract (0-100 µl) will be added to a reaction mixture containing 120 µl, 20 mM deoxyribose, 400 µl, 0.1 M phosphate buffer pH 7.4, 40 µl, 20 mM hydrogen peroxide and 40 µl, 500 µM FeSO₄, and the volume will be made to 800 µl with distilled water. The reaction mixture will be incubated at 37°C for 30 min and the reaction will be stopped by the addition of 0.5 ml of 2.8% TCA, this will be followed by the addition of 0.4 ml of 0.6% TBA solution. The tubes will subsequently be incubated in boiling water for 20 min. The absorbance will be measured at 532 nm in spectrophotometer.

2.5 Estimation of Antioxidant Enzymes Inhibition Potentials *Aframomum melegueta* [Roscoe] K. Schum(Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts

2.5.1 Estimation of Reduced Glutathione (GSH) levels of *Aframomum melegueta*

The method of Beutler et al. [34] was adopted in the estimation of the level of reduced glutathione (GSH) in the testis and brain supernatants. Sample homogenate (0.2 ml) was added to 1.8 ml of distilled water and 3 ml of the precipitating agent, sulphosalicylic acid was mixed with the sample. This was centrifuged at 3,000 g for 4 minutes. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman reagent. A blank was prepared with 0.5 ml of the diluted precipitating agent and 4 ml of phosphate buffer and 0.5 ml of Ellman's reagent. The absorbance of the reaction mixture was taken within 30 mins of colour development at 412 nm against a reagent blank. The concentration of the GSH was extrapolated from the GSH standard curve.

2.5.2 Assay of Glutathione Peroxidase (GPx) activity of *Aframomum melegueta*

GPx activity in the sample was determined according to the method adopted by Rotruck et al. [35]. The reaction mixture containing 500 µl phosphate buffer, 100 µl of sodium azide, 200 µl

GSH, 100 μ l H_2O_2 were added to 500 μ l of the sample, after which 600 μ l of distilled water was added and mixed thoroughly. The whole reaction mixture was incubated at 37°C for 3 minutes after which 0.5 ml of TCA was added and thereafter centrifuged at 3000 rpm for 5 minutes. To 1 ml of each of the supernatants, 2 ml of K_2HPO_4 and 1 ml of DNTB was added and the absorbance was read at 412nm against a blank.

2.5.3 Assay of Superoxide Dismutase (SOD) activity of *Aframomum melegueta*

The activity of SOD in the homogenates was determined according to the method described by Misra and Fridovich [36]. A dilution of 1 ml of the sample was made with 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectro-photometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

2.5.4 Assay of Catalase activity of *Aframomum melegueta*

Catalase activity was determined according to the method described by Aebi [37]. Sample (70 μ l) was mixed with 920 μ l Na- PO_4 buffer pH 7 containing 0.1 mM EDTA. The reaction started by adding 10 μ l of H_2O_2 . The decrease in H_2O_2 concentration was taken by reading the absorbance at 240 nm (10 seconds intervals) for 180 seconds.

H_2O_2 extinction coefficient (E) = 39.4 $mM^{-1}cm^{-1}$ at 240 nm and 1 mmol

H_2O_2 $ml^{-1}min^{-1}$ was defined as 1 unit of CAT.

3. RESULTS

Figs. 1-8 shows each of parameters of Antioxidant potentials of *Aframomum melegueta* [Roscoe] K. Schum Grains of Paradise) leaf, stem Bark, seed bark and seed extracts.

Fig. 1 Antioxidant potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts. Fig. 1 shows the graphical representation of antioxidant potentials of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract inclusive, it was observed that DPPH (%) and OH (%) has the overall highest percentage in the tested plant sample while flavonoids (mg/g), ABTS (mMol/g) has the lowest quantities composition by mass .it was observed that FRAP (mg/g), Phenol and Fe^{2+} chelate has an appreciable quantity of *Aframomum melegueta* Schum. The following graphical representation will show the percentages of antioxidant present in each *Aframomum melegueta* Schum parts from 2-8.

Fig. 2 shows the graphical representation of antioxidant potentials of tested plant sample, Leaf, Stem bark ,Seed bark and Seed extract inclusive, it was observed that seed extracts has the highest quantity of flavonoid (mg/g) antioxidant potentials compared to seed bark, leaf and stem bark. The quantity decreases in geometrical order (Seed<Seed bark<Leaf<Stem bark) from seed to stem bark respectively.

Fig. 3 shows graphical representation of antioxidant potentials of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract respectively. The quantity of antioxidant

Table 1. Antioxidant potentials of *Aframomum melegueta* [Roscoe] K. Schum (grains of paradise) leaf, stem bark, seed bark and seed extracts. Table 1 shows the overall antioxidant potentials of leaf, stem bark, seed bark and seed of *Aframomum melegueta* extracts

Parameter	Seed	Seed bark	Leaf	Stem bark
DPPH %	43.58	22.04	20.79	9.51
FRAP mg/g	16.80	11.08	8.04	5.75
FLAVONOID mg/g	0.31	0.24	0.21	0.08
PHENOL mg/g	21.40	15.06	17.87	12.68
ABTS mMol/g	0.02	0.01	0.01	0.01
Fe chelation %	34.41	32.79	10.33	9.04
OH radical scavenging potential %	67.18	37.50	20.31	14.06

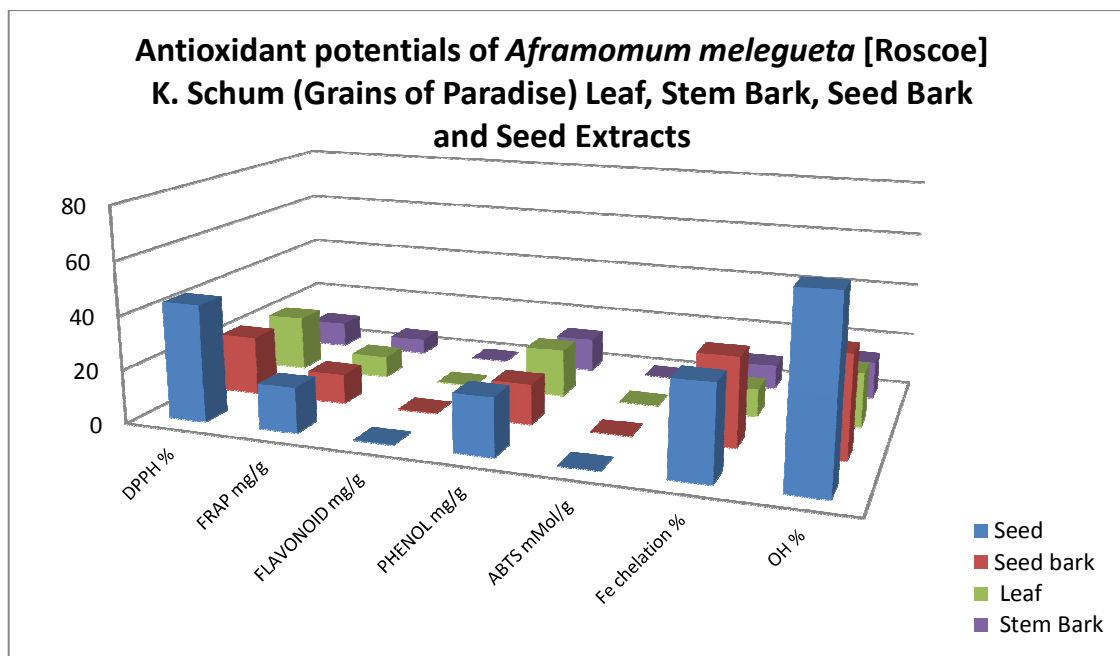


Fig. 1. Shows the overall Antioxidant potentials of leaf, stem bark, Seed bark and Seed of *Aframomum melegueta* extracts

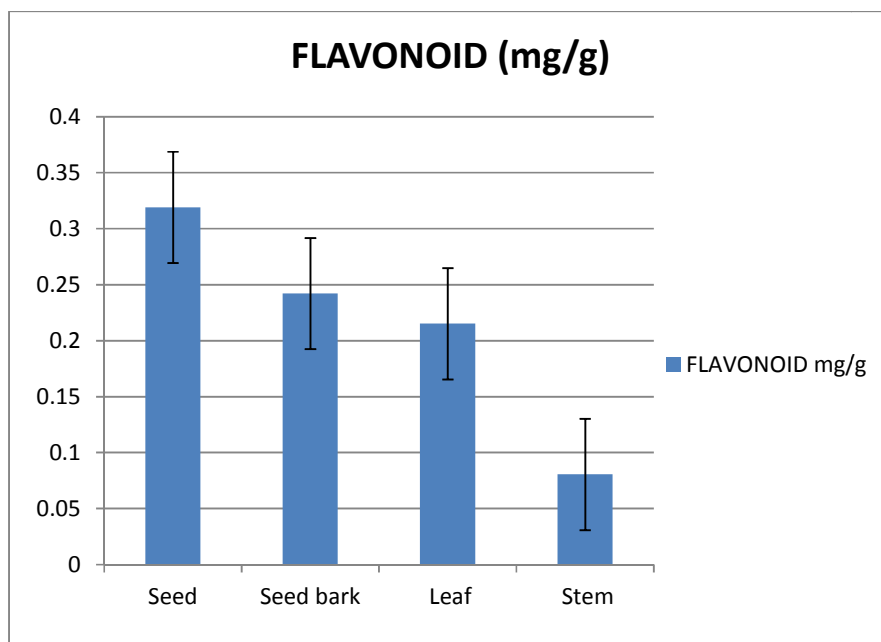


Fig. 2. Flavonoids potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) leaf, stem bark, seed bark and seed extracts

phenol (mg/g) shows appreciable quantity in the seed extract, leaf, seed bark and stem bark in this order(Seed< Leaf < Seed bark< Stem bark),

Stem bark has the lowest quantity. It shows that seed extract has the highest quantity of phenols of *Aframomum melegueta* Schum.

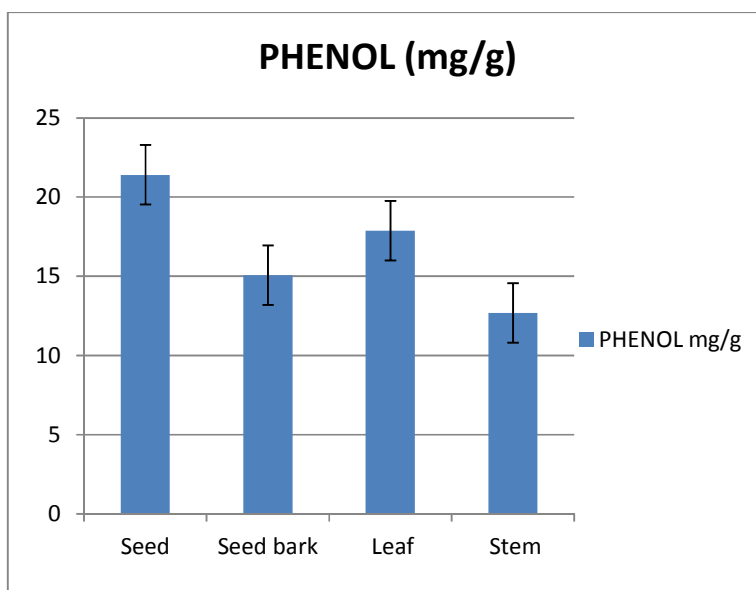


Fig. 3. Phenol potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

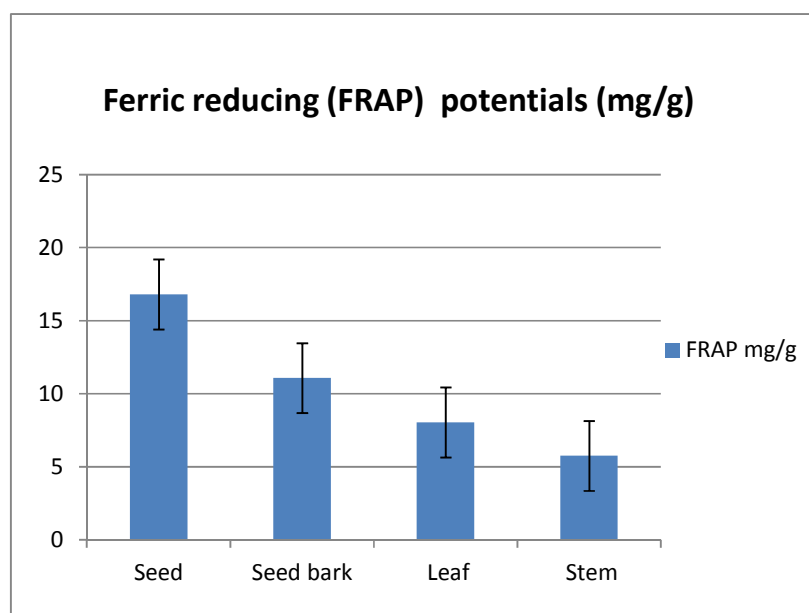


Fig. 4. Ferric reducing (FRAP) of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

Fig. 4 shows the graphical representation of antioxidant potentials of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract inclusive, it was observed that seed extracts has the highest quantity of Ferric reducing (FRAP) potentials (mg/g) antioxidant potentials compared to seed bark, leaf and stem bark. The quantity decreases in geometrical order (Seed < Seed

bark < Leaf < Stem bark) from seed to stem bark respectively.

Fig. 5 shows the graphical representation of antioxidant potentials of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract respectively. It was observed that the seed extract has the highest percentage of free radical

scavenging ability (DPPH), Seed bark and leaf almost has the same percentages and stem has the lowest quantity of free radical scavenging ability of *Aframomum melegueta* Schum.

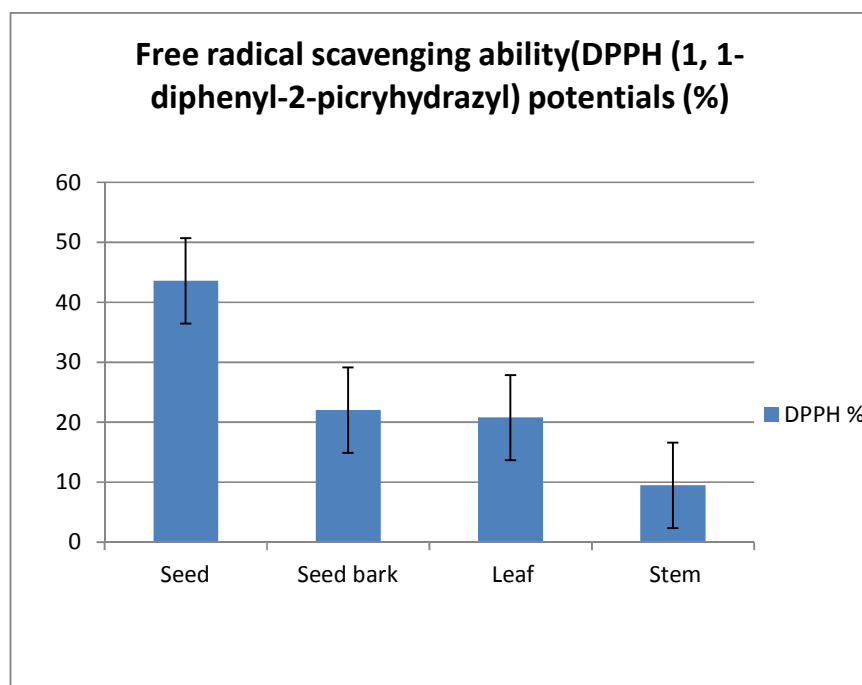


Fig. 5. Free radical scavenging ability (DPPH (1, 1- diphenyl-2-picrylhydrazyl) potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise leaf, stem bark, seed bark and seed extracts

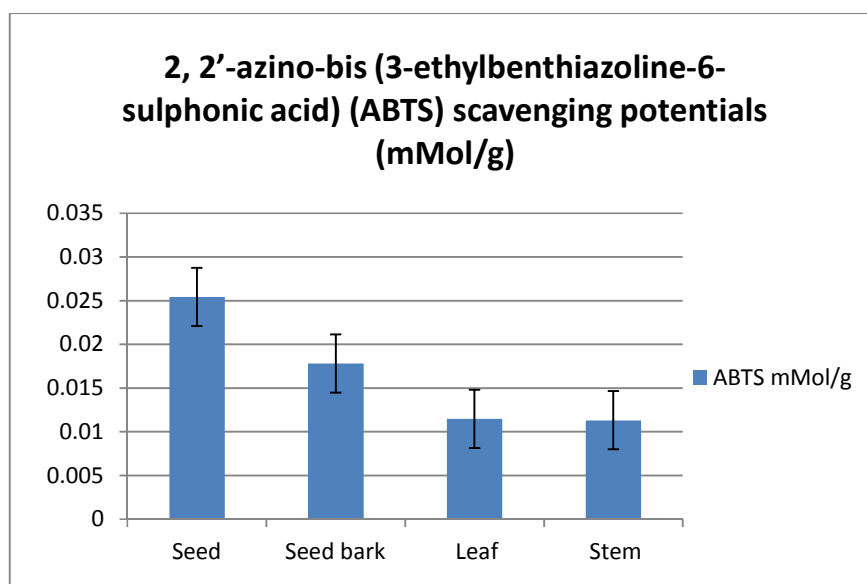


Fig. 6. 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging potentials of *Aframomum melegueta* Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

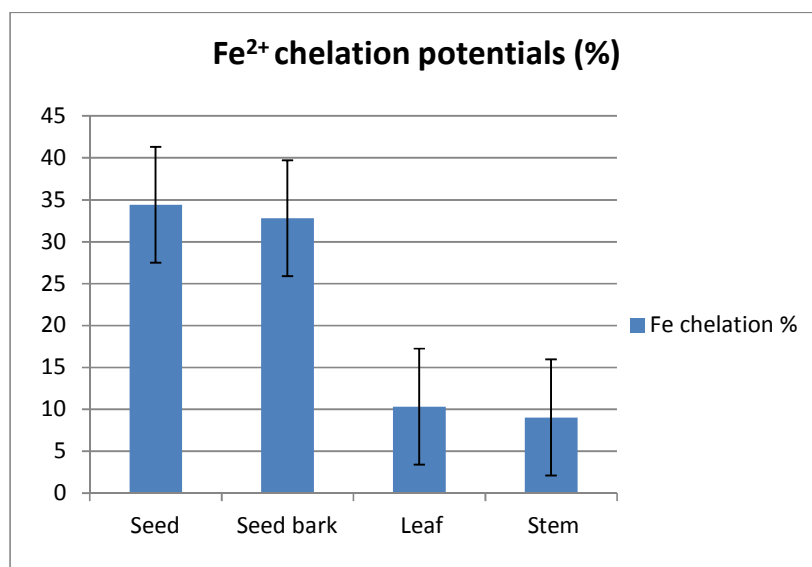


Fig. 7. Fe²⁺ chelation potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

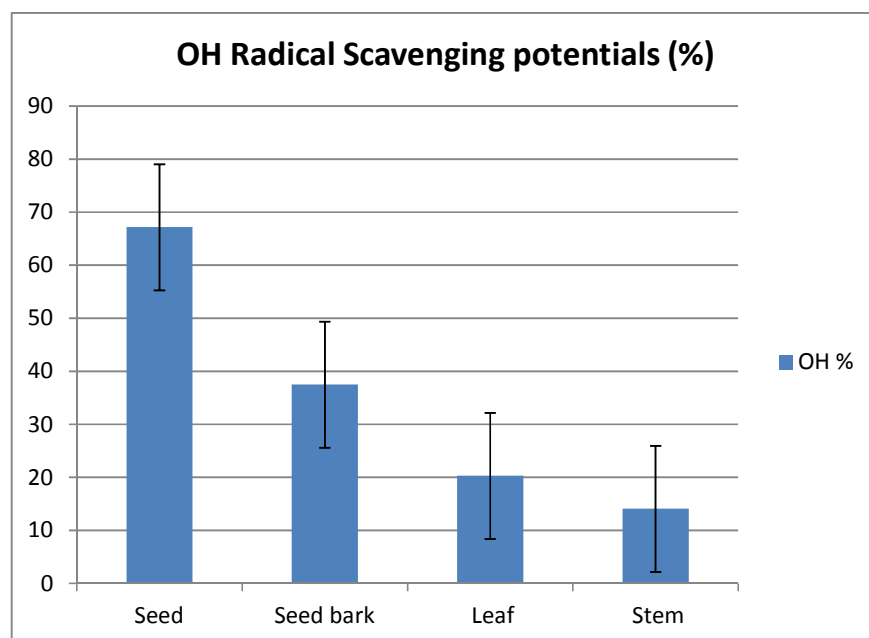


Fig. 8. OH radical scavenging potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

Fig. 6 shows the graphical representation of antioxidant potentials of tested plant sample. Leaf, Stem bark, Seed bark and Seed extract respectively. It was observed that seed has the highest quantity of 2, 2'-azino-bis (3-ethyl benothiazoline-6-sulphonic acid) (ABTS) scavenging (mMol/g) followed by Seed bark. The leaf and stem bark has the same quantity inclusive.

Fig. 7 shows the graphical representation of antioxidant potentials of tested plant sample. Leaf, Stem bark, Seed bark and Seed extract respectively. The seed and seed bark extracts has the highest percentage of Fe chelation potentials, while the stem bark and leaf has the lowest percentage of Fe²⁺ chelate of *Aframomum melegueta* Schum.

Fig. 8 shows the graphical representation of antioxidant potentials of tested plant sample. Leaf, Stem bark, Seed bark and Seed extract respectively. The seed and the seed bark extract has the highest percentage by mass of OH Radical Scavenging potentials while the leaf and the stem bark extract has the lowest percentages of OH Radical Scavenging ability of *Aframomum melegueta* Schum.

Fig. 9 shows the overall parameters of Antioxidant Enzymes inhibitory potentials of *Aframomum melegueta* Leaf, Stem Bark, Seed Bark and Seed extracts.

Figs. 10-13 shows each of parameters of Antioxidant Enzymes inhibitory potentials of *Aframomum melegueta* Leaf, Stem Bark, Seed Bark and Seed extracts.

Fig. 9 shows the graphical representation of antioxidant enzymes of tested plant sample,

Leaf, Stem bark, Seed bark and Seed extract of *Aframomum melegueta* [Roscoe] K. Schum. It was observed Glutathione Peroxidase (GPX) and Reduced Glutathione (GSH) has the highest percentage of antioxidant enzymes of *Aframomum melegueta* Schum. The following graphical representation will show the percentages of antioxidant emzymes present in each *Aframomum melegueta* Schum parts from 11-13.

Fig. 10 shows the graphical representation of antioxidant enzymes of tested plant sample, Leaf, Stem bark ,Seed bark and Seed extract of *Aframomum melegueta* Schum. In graph 10, it was observed that Superoxide Dismutase (SOD) enzymes has the highest percentage in leaf extract followed by seed extract. The seed bark and stem bark has almost the same graphical data of quantity. The leaf has great percentage of Superoxide Dismutase (SOD) enzymes.

Table 2. Shows the overall antioxidant enzymes inhibitory potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

Paramter	Seed	Seed bark	Leaf	Stem bark
SOD %	55.00	25.00	70.00	20.00
CATALASE	0.01	0.01	0.02	0.00
GSH	123.19	39.36	67.65	13.82

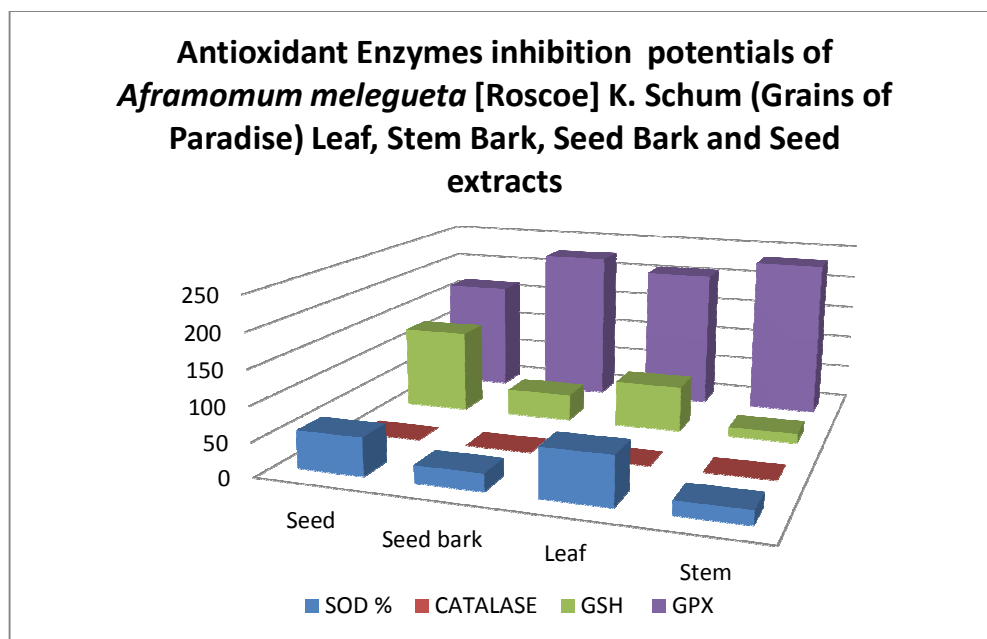


Fig. 9. Antioxidant enzymes potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise leaf, stem bark, seed bark and seed extracts

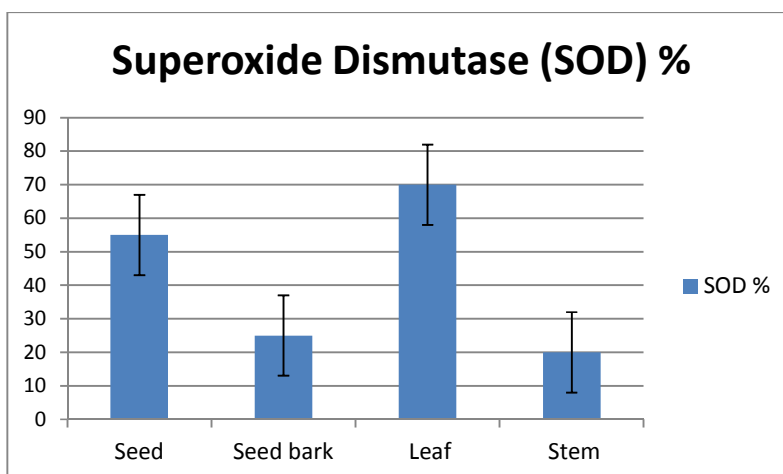


Fig. 10. Superoxide Dismutase enzymes inhibition potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

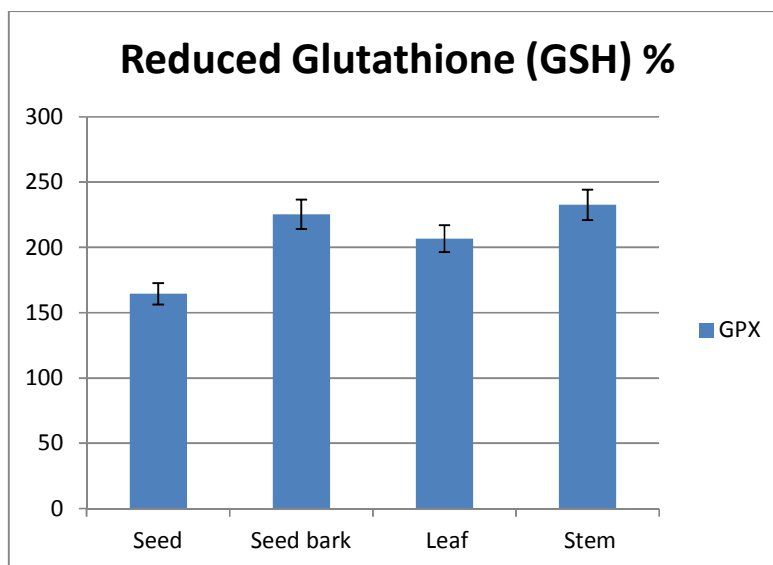


Fig. 11. Reduced Glutathione (GSH) enzymes inhibition potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

Fig. 11 shows the graphical representation of antioxidant enzymes of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract of *Aframomum melegueta* Schum. The seed bark and stem bark has the highest percentage of Reduced Glutathione (GSH) enzymes compared with leaf and seed. The percentage of Reduced Glutathione (GSH) leaf enzymes is greater than seed of *Aframomum melegueta* Schum representaed in Fig. 11.

Fig. 12 shows the graphical representation of antioxidant enzymes of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract of *Aframomum melegueta* Schum. The

percentage of Catalase (CAT) enzymes is greater in the leaf extract compared to seed bark while the percentage is lower in stem bark and seed extract of *Aframomum melegueta* Schum.

Fig. 13 shows the graphical representation of antioxidant enzymes of tested plant sample, Leaf, Stem bark, Seed bark and Seed extract of *Aframomum melegueta* Schum. Glutathione Peroxidase (GPX) enzymes has the greatest percentages in seed bark and stem bark while the percentage is lower in stem bark and leaf extract of *Aframomum melegueta* Schum.

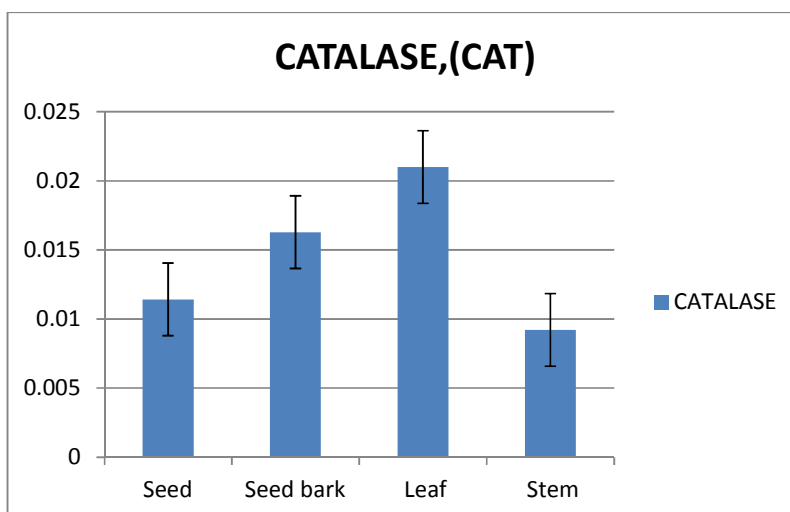


Fig. 12. Catalase enzymes inhibition potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

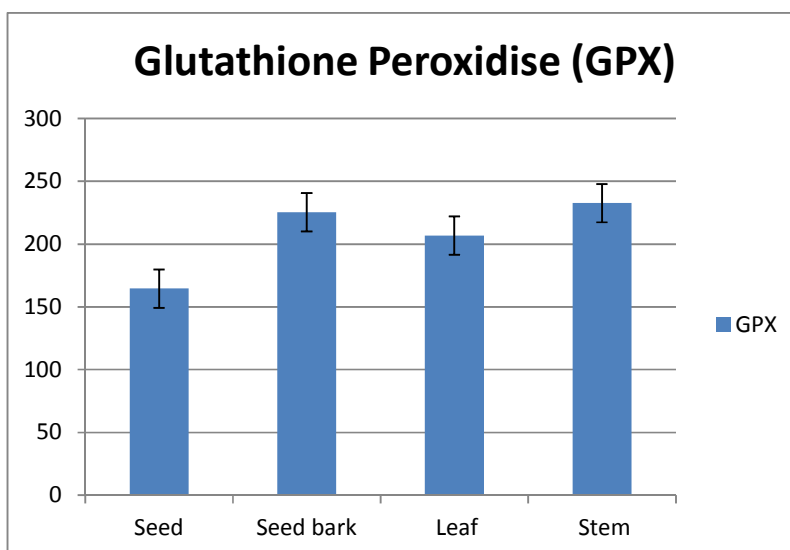


Fig. 13. Glutathione Peroxidise (GPX) enzymes inhibition potentials of *Aframomum Aframomum melegueta* [Roscoe] K. Schum (Grains of paradise) leaf, stem bark, seed bark and seed extracts

4. DISCUSSION

The purpose of this research work is to evaluate the in-vitro antioxidant potentials and antioxidant enzymes inhibition potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts. Antioxidants (free radical scavengers) are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system [38]. Antioxidants may be synthetic or natural.

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been reported to be dangerous for human health. Thus, the search for effective, non-toxic natural compounds with antioxidative activity has been intensified in recent years. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body also relies on external (exogenous) sources, primarily the diet and some essential herbal drug

like *Aframomum melegueta*, to obtain the rest of the antioxidants it needs [39].

These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants [40]. The antioxidant compounds neutralize the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH [41]. During this research work it was observed that, it was observed that DPPH(%) and OH(%) has the overall highest percentage in the tested plant sample while flavonoids (mg/g), ABTS (mMol/g) has the lowest quantities composition by mass. it was observed that FRAP (mg/g), Phenol and Fe²⁺ chelate has an appreciable quantity of *Aframomum melegueta* Schum.

Flavonoids are known to be anti-microbial, anti-ulcer, anti-virus, tumors, and affecting platelet aggregation [42]. Flavonoids are known to inhibit specific enzymes as well. for example, flavonoids block the angiotensin-converting enzyme (ACE) that raises blood pressure [43]. By blocking the "suicide" enzyme cyclooxygenase (that breaks down prostaglandins).it is also reported by Ramage et al. [44] that flavonoids prevent platelet stickiness and platelet aggregation and flavonoids are also known to protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells. Numerous studies have reported that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals [45].

Kohen and Nyska [46] attributed the pharmacological activities (anti-inflammatory, anti viral, antibacterial, antiulcer, antiosteoporotic, antiallergic, and antihepatotoxic actions) of flavonoids to their potent antioxidant activity [47].

Polyphenols exert antioxidant properties, acting as therapeutic agents involved in the prevention of disorders in which oxidative stress and inflammation and the ability to block specific enzymes that cause inflammation [48]. It has been reported that phenols demonstrated well the ability to block the uptake of cholesterol and facilitate its excretion from the body. Cholesterol has long been implicated as a significant risk factor in cardiovascular disease, thus justifying the traditional medicinal claims [49]. It was observed that the Seed of *Aframomum melegueta* Schum has the largest concentration of Ferric reducing potentials (FRAP),2,2'-azino-bis(3-ethylbenthiiazoline-6-sulphonicacid) (ABTS)

scavenging ability, Fe²⁺ chelation potentials and OH Radical Scavenging ability compared to the seed coat stem bark and leaf. The other parts composed of varying degree of antioxidant potential which may be used for different pharmacological and therapeutic purpose [49].

This is an increasing evidence that antioxidant potential derived from indigenous plant such as *Aframomum melegueta* Schum. Sources may be useful in preventing the deleterious consequences of oxidative stress [50]. Therefore, there is increasing interest in the protective biochemical functions with natural antioxidants present in medicinal plants like *Aframomum melegueta* Schum. Should not be neglected rather it should be a big plus to the herbal and pharmaceutical industries in the developing country like Nigeria and Africa as a whole. It can be a big challenge to the international world because of its availability but developing country can earn a lot of foreign earning and a collaborative research partnership to the developing world such like Africa, this will once again popularized this medicinal plant and beam more search light to its efficacy and usefulness [51].

Phenolic compounds have been widely studied and found in abundant quantity in *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise), as they are considered to be crucial for the prevention of certain diseases, such as metabolic disorders, in particular diabetes and obesity. The beneficial properties of phenols could be in relation to the modulation or inhibition of certain physiologically relevant targets, such as lipase, alpha-glucosidase, or other receptors by polyphenols or triterpenes. For example, the dihydrochalcone phloridzin is a competitive inhibitor of the sodium glucose co-transporter types 1 and 2, thus exerting an antidiabetic action [52]. It has also been reported that catechin and epicatechin reduce hyperglycemia and hepatic glucose output, while quercetin improves insulin-dependent glucose uptake [53]. Some catechin-like flavan-3-ols have been shown to have inhibitory activity against enzymes such as - alpha glucosidase and lipase.

Luisa et al. [54] found that Superoxide dismutases (SODs) constitute a very important antioxidant defense against oxidative stress in the body. The enzyme acts as a good therapeutic agent against reactive oxygen species-mediated diseases. *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise), Glutathione Peroxidase (GPX) and

Reduced Glutathione (GSH) has the highest percentage of antioxidant enzymes inhibition. The leaf has the highest level of Superoxide Dismutase (SOD) and Catalase (CAT) and the stem bark has the lowest level of SOD, the stem bark has the highest level of Reduced Glutathione (GSH) while the seed has the lowest level of enzyme inhibition, the main function of As the generation of free radicals exceeds the body's ability to neutralize and eliminate them, oxidative stress occurs. A primary function of glutathione is to alleviate this oxidative stress.

Kangralkar et al. [55] reported that Reduced glutathione (GSH) is a linear tripeptide of L-glutamine, L-cysteine, and glycine. Maritim et al. [56] reported that Glutathione Peroxidase (GPx) is a cytosolic enzyme that catalyzes the reduction of hydrogen peroxide to water and oxygen as well as catalyzing the reduction of peroxide radicals to alcohols and oxygen. The *in vivo* antioxidant assay showed that the extract increased the activity of serum superoxide dismutase (SOD) and Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic [57] and ROS production [58].

Pastore et al. [21] reported SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite [59]. The increased serum activities of catalase and SOD as observed in this study suggest that the *Aframomum melegueta* [Roscoe] K. Schum extract has an *in vivo* antioxidant activity and is capable of ameliorating the effect of ROS in biologic system [59].

Some of the phytochemical constituents of the extract may be responsible for the antioxidant activity as demonstrated in our study. Flavonoids or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers [47]. Numerous studies have shown that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals [60,61].

5. CONCLUSION

In conclusion, *Aframomum melegueta* should be the focal point in human diet, natural herbal drug

supplement and even in addition to conventional drug to improve the potency and quality of natural drug system. It is a fact through this research work that contains a lot of limitless ability in the control of ROS in human system and physical well being and also help neutralize the effect of free radicals through different ways and may prevent the body from various diseases. These research show the potentials of *Aframomum melegueta* as a source of bioactive compounds to be used for pharmaceutical, nutraceutical, and cosmeceutical applications. The potentials of *Aframomum melegueta* [Roscoe] K. Schum (Grains of Paradise) is overwhelming and its uses should be encouraged especially during the outbreak of viral infection like Ebola, COVID-19 and laser fever. It is unique plant which has a limitless therapeutic uses which should be encouraged. Furthermore, more research should be welcome, to elucidate its medicinal uses and isolation of the bioactive compound in *Aframomum melegueta*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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