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Evaluation of Antioxidant, Anti-inflammatory and Phytochemical Constituents of *Aframomum melegueta Aqueous* Leaf Extract

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Abstract
The study involved the evaluation of phytochemicals, antioxidant and anti-inflammatory properties of leaf extract of *Aframomum melegueta*.Gravimetric and spectrophotometric methods were employed in the quantifications of the phytochemicals, DPPH radical scavenging and reducing power activities, membrane stabilizing of red blood cells exposed to both heat and hypotonic induced lyse, inhibition of albumin denaturation as well as the evaluation of vitamin C content.Phytochemically,the leaf extract of *A. melegueta* contained flavonoids, phenolics, alkaloids, saponins, tannins, sterols and vitamin C. The aqueous extract of *A. melegueta* protected red blood cell exposed to heat and hypotonic induced lyses, inhibited albumin denaturation; exhibiting DPPH radical scavenging and ferric reducing activities.The results revealed that the plant is rich in vital phytoconstituentsnatural products that possess and exhibit potent and appreciable biological activities.

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1. Introduction

It has been demonstrated that biological systems possess, exhibit and effective mechanism to prevent free radicals - induced tissue cell damages. This is accomplished by a set of endogenous antioxidants (enzymatic and non-enzymatic) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) vitamins E and C.Oxidative stress arises when the generation of reactive oxygen species (ROS) in the body exceeds the ability of the anti-oxidant systems to neutralize and eliminate which through a serious of events deregulates the cellular functions leading to various pathological conditions such as cardiovascular diseases (Noemanet al., 2011; Ebeleet al., 2016).

Recently there has been up-surge in the worldwide interest in the role of medicinal plants in complementary medicine. Plants are reservoirs of secondary metabolites exploited for various pharmacological purposes (Kaur, 2017). Phytochemicals are groups of chemicals of plant origin that do perform specific function in the plants but are of immense benefits to

human play vital roles in improving the human health, and ameliorating diseases. They are preferred due to their safety and efficacy (Kaur, 2017). Some of the active constituents of the plants particularly from vegetables, fruits, spices and herbs such as triterpenoids and flavonoids have been demonstrated to possess and exhibit hepatoprotective, antioxidant and associated biological activities (Hikino and Kiso, 1988; Kang et al., 1998; Bass, 1999; Ross and Kasum, 2002).Fruits, green tea and vegetables contain polyphenols which include flavanols, flavadiols, flavonoids and phenolic acids. Studies have indicated polyphenols possess and exhibit various biological activities including antifungal, anti-inflammation, anti-mutagenic, anti-oxidative, anti-carcinogenic, anti-tumor, and anti-diabetic effects. Also, they exhibit lowering potential on plasma cholesterol and triacylglycerol levels as well as reduction of blood pressure and platelet aggregation in several systems (Ho et al., 1992; Al- Attar and Abu-Zeid, 2013; Dare et al., 2013).

A. melegueta, native to the West African region, is a plant that belongs to the ginger family, Zingiberaceae. It is obtained from the ground seeds and imparts a pungent, peppery flavour with hints of citrus. Its seeds contain 6-Gingerol, 6-Shogaol, 6-Paradol, 6-Gingeredione, {2-(5-butylfuran-2-yl) ethyl}-2-methoxyphenol, the most active beingparadol (Sugitaet al.,2013). The seed of the plant has been reported to possess and exhibit anti-inflammatory property, employed in the treatment of measles, prevent and heal asthma, body weakness and diarrhea, neuro-protective potential against monosodium glutamate – induced brain damage (Doherty et al., 2010; Oladunmoye and Kehinde, 2011; Kokou et al., 2013; Fasakinet al., 2017). Literature survey revealed the presence of several scientific literatures on the bioactivity of extracts, fractions and bioactive compounds of the seeds of *A. melegueta*. On the other hand, there are dearth of scientific information on the biological activities of extract, fractions and compounds of leaf of *A. melegueta*. As part of on-going studies in our laboratory, this study, reported the phytoconstituents, anti-oxidant as well as anti-inflammatory potentials of leaf aqueous extract of *A. melegueta* with a view to investigating the therapeutic potentials of leaf of the plant.

2.0 Materials and Methods

2.1 Reagents and Chemicals

All the reagents used were ofanalytical grade and were obtained mainly from the following Chemical Manufacturing Companies: British Drug House (BDH) Poole, U.K., Sigma Chemical Company, St. Louis, Missouri U.S.A., Fluka Chemical Company and Pharmacia Fine Chemicals, Upsalla, Sweden.

2.2 Plant Material

Fresh leaves of *A. melegueta* were collected from Babajakan Village, Ayedaade Local Government, Osun state, Nigeria. Identification and authentication were carried out at IFE Herbarium, Department of Botany, ObafemiAwolowo University, Ile-Ife, Nigeria, where specimen copy with specimen voucher number (17525) was deposited. The leaves were sun-dried over a period of 14 days and ground into powdery form using electric blender.

2.3 Preparation of Aqueous Extract of A. melegueta

Powdered plant material (500 g) was suspended in 3L of distilled water for 48 hr. The suspension was stirred occasionally, filtered through muslin cloth and Whatman No 1 filter paper. The residue was re-extracted five more times until the filtrate was clear and became colorless. The filtrates were combined, followed by evaporation to reduce the volume and then freeze-dried.

2.4 Evaluation of In vitro Antioxidant Potentials of the Extract

Estimation of Saponin Concentration

The estimation of saponin content was carried out according to a procedure that was based on those earlier reported by Wagner et al. (1984) and Abdel-Gawad et al.(1999) as modified and reported by Akinpeluet al. (2012b). Aqueous extract (10 g) in triplicate was washed twice each with chloroform (50 ml x 2) and ethylacetate (50 ml x 2) respectively. The residue was dissolved in 20% (v/v) methanol and extracted three times with butanol (100 ml x 3). The butanol extract was evaporated to dryness, dissolved in 50 ml 50% (v/v) methanol followed by repeated addition and decantation of diethylether (50 ml x 5) to precipitate crude

saponins. The dissolution and precipitation processes were repeated three times until light brown residue (saponin) was obtained. The precipitate was dried in the oven (55^{o} C), the weight was noted and recorded. The yield was expressed as percentage of starting aqueous extract.

2.5 Estimation of Total Alkaloid Concentration

The extraction and quantification of alkaloid were carried out according to a procedure that was based on the method described by Iwuet al. (1990) and Djilani et al.(2006) as slightly modified (Fasanuet al., 2013). Typically, extract (1 g) in triplicate was dissolved in 3 % (v/v) HCl (30 ml x 3) and filtered. The acidic filtrate was extracted with chloroform (20 ml x 3). The chloroform fraction was collected and the aqueous fraction was basified with ammonia solution (20%) and followed by extraction with chloroform (20 ml x 4). The chloroform fractions were combined and washed with distilled water to neutral pH. The chloroform fraction was re-dissolved in 3% (v/v) (30 ml) HCl, re-extracted with chloroform and it was concentrated to dryness. The total alkaloid was expressed as percentage of the starting extract.

2.5 Estimation of Concentration of Tannins

The estimation of the concentration of tannin was carried out spectrophotometrically according to the modified method of Graham (1992). Briefly, the reaction mixture contained $K_3Fe(CN)_6$ (8mM, 1ml), $FeCl_3$ (0.2 M, 1 ml) and diluted extract (200 µl) and followed by incubation at room temperature for 15 min. The absorbance was read at 700nm against reagent blank. The total tannin concentration wasextrapolated from the standard calibration curve (0.00 – 350 µg/ml) and expressed as mg tannic acid equivalent per gram of the extract (mg TAE/g of extract).

2.6 Estimation of Concentration of Sterols

The total sterol content wasmeasured according to a procedure that was based Lieberman-Burchardreaction method as earlier reported (Burke et al., 2003; Narender et al., 2007) with slight modification. Briefly, extract, 200µl in triplicate was adjusted to 1ml with chloroform followed by the addition of 2ml of Lieberman-Burchard reagent (5 ml conc. H_2SO_4/5ml acetic anhydride in 50 ml absolute ethanol) mixed thoroughly.The reagent reacted with sterols to produce a characteristics green color whose absorbance was proportional to the concentration of the sterol. The tubes were covered with aluminium foil and kept in the dark for 15 min. The absorbance was taken at 640 nm against the reagent blank. The total sterol concentration wasextrapolated from the standard calibration curve (0.00 – 350 µg/ml) and expressed as mg/gcholesterol equivalentof the extract (mg/g CHE).

2.7 Estimation of Total Phenolics Concentration

The total phenolics content in the extract was determined spectrophotometrically (Singleton et al., 1999) using tannic acid (100µg/ml) as standard.Briefly, the reaction mixture contained 200 µl or 500 µl extract in triplicate and volume was made up to 1 ml with distilled water. Then, Folin-Ciocalteu's Phenol reagent (1:10) dilution (1.5 ml) and sodium carbonate (7.5%, 1.5ml) were added. The reaction mixtures were incubated for $1^{1}/_{2}$hr at room temperature and the absorbance was read against the blank at 725 nm. The total phenolics concentrations were extrapolated from the standard calibration curve and expressed as mg tannic acid equivalent per gram of the extract (mg/g TAE).

2.8 Estimation of Total Flavonoid Concentration

The total flavonoid content in the extract was determined according to the spectrophotometric method of Sun et al. (1999) with rutin (1 mg/ml) as standard.The reaction mixture contained extract (200 µl),0.3 ml of 5% (w/v) $NaNO_2$, and 0.3 ml of 10% (w/v) $AlCl_3$and 2 ml of 4% (w/v) NaOH. The reaction mixtures were incubated for 10 min at room temperature and absorbance was taken against the blank at 500 nm. The total flavonoid concentrations were extrapolated from the standard calibration curve (0.00 – 350 µg/ml) and expressed as mg rutin equivalent per gram of the extract (mg/g RE).

2.9 Assay of DPPH Radical Scavenging Activity of A. melegueta Extract

The DPPH-radical scavenging activity of the extractwas assayed according to the procedure of Blois (1985) as reported by Padmanabhan and Jangle (2012), with ascorbic acid as standard.This assay was based on the formation of diphenylpicrylhydrazine from a blue/purple

solution. The assay mixture contained the extract (200µg) and ascorbic acid (0.00 – 50 µg) in methanol. This was followed by the addition of DPPH (1ml, 0.3mM) in methanol. The mixture was properly mixed by inversion and then incubated in a dark chamber for 30 min. The absorbance wasread at 517 nm against the reagent blank. The percentage radical scavenging activities of the standard and extract werecalculated as the percentage inhibition of DPPH using the expression:

$$I_{DPPH}\% = 100 \times \frac{\Delta Abs_{control} - \Delta Abs_{sample}}{\Delta Abs_{control}}$$

2.10 Assay of Reducing Power Activity of A. melegueta Extract

The procedure of Oyaizu (1986) as reported by Aina and Oyedapo (2013) was used to assay the reducing power of the extract. The reaction was based on the ability of the extract to reduce iron from Fe^{3+} to Fe^{2+}. Briefly, varying concentrations (0.00 -350 µg/ ml) of the extract in triplicate were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide[2.5 ml of 1 % (w/v)]. The reaction mixtures wereincubated at 50°C for 20 min, cooleddown followed by the addition of 2.5 ml trichloroacetic acid [10 % (w/v)] to each tube, vigorously shaken and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) wasmixed with 2.5 ml distilled water and $FeCl_3$ (0.1%, 0.5ml) was added. The absorbance wasreadagainst the reagent blank at 700 nm after 10 min. Standard ascorbic acid (0.00 -350 µg/ ml) was used as the standard and treated as extract. The absorbance was plotted against the different concentrations of both the extract and ascorbic acid.

2.11 Extraction and Estimation of Vitamin C

The extraction and quantification of Vitamin Cin the extract of *A. melegueta* was based on the methods of Omayeet al. (1979) and Japota and Dani (1982) as reported Fajobiet al. (2017). Typically, plant material(2.5g) was suspended in 2 % (v/v) orthophosphoric acid (5 ml), mixed thoroughly, stirred for 3 hrand centrifuged at 3000 rpm for 10 min. The supernatant (1.5 ml) in triplicate was mixed with 10% (v/v) acetic acid (1.5 ml) and followed by the addition of 0.5 ml Folin-Ciocalteu Phenol reagent (1:5 dilution). The absorbance wasread at 760 nm against the reagent blank.The vitamin C content in the extract was extrapolated from the standard calibration curve and expressed as mg/g ascorbic acid (mg AAE/g of extract).

2.12 Evaluation of In vitro Anti-inflammatory Potential of the Extract

2.12.1 Evaluation of Red Blood Cell Membrane Stability

The bovine red blood cell was prepared according to the method of Oyedapo et al. (2004). The study involved collection of fresh bovine blood into an anticoagulant (3.8% (w/v) trisodium citrate), centrifuged (1.5K rpm on Bench Centrifuge Model 90 – 2 SearchTech Instrument, England, UK.). The bovine erythrocyte, 2% (v/v) was prepared by diluting 2 ml of packed cell with normal saline to 100 ml. It was kept undisturbed at 4°C in the refrigerator.

The membrane stabilizing potentials of the aqueous extract of *A. melegueta* was carried out according to the procedure earlier reported by Bode and Oyedapo (2011). Typically, varying concentrations (0, 100, 200, 300, 400 and 500 µg/ml) of aqueous extract of *A. melegueta* was pipetted in triplicate into clean dry test tubes and adjusted to 1.5 ml with normal saline. To the reaction mixtures were added 0.5 ml hyposaline and 0.5 ml phosphate buffer (pH 7.4, 0.15 M) and 0.5 ml of 2% (v/v) erythrocyte. The blood control was prepared as above without the extract of *A. melegueta* while the drug control contained all other reagents except the erythrocytes suspension. The mixtures were then incubated at 56°C for 30 min, cooled and centrifuged at 3000 rpm for 10 min. The supernatants were carefully decanted and absorbance was read at 560 nm against the reagent blank. The percentage membrane stability was calculated as:

$$\text{Percentage Membrane Stability} = 100 - \left\{\frac{(Abs_{test} - Abs_{drug})}{Abs_{blood\ control}}\right\} \times 100$$

2.12.2 Assay of Inhibition of Albumin Denaturation

The assay of inhibition of albumin denaturation was carried out according to the procedure of Mizushima and Kobayashi (1968) as modified Aina and Oyedapo (2013) with slight modifications. Different concentrations of the extract(0-350 µg/ml) were pipetted into clean dry test tubes in triplicate. The volumes were

adjusted to 2.5 ml with distilled water. Bovine serum albumin (0.5 ml, 1.5 mg/ml) was added to each of the reaction mixtures and incubated at 37°C for 20min, heated at 57°C for 3 min followed by the addition of phosphate buffer (pH 6.3, 2.5 ml, 0.5M).The mixtures were subjected to centrifugation at 2,500 rpm for 10 min. The amount of protein the reaction mixture was quantified before and after centrifugation using alkaline-copper reaction method as reported Scharteck and Pollack (1973). Diclofenac was used as reference drug and treated as the extract.

The % Inhibition of albumin denaturation was calculated using the formula:

$$\text{Total Protein} = \frac{absorbance\ of\ sample\ x\ concentration\ of\ BSA}{absorbance\ of\ BSA}$$

$$\text{Quantity of Protein left} = \frac{absorbance\ of\ sample - absorbance\ of\ blank}{absorbance\ of\ BSA - absorbance\ of\ blank}\ X\ 100$$

$$\%\ \text{Inhibition} = \frac{quantity\ of\ protein\ left}{total\ protein}\ X\ 100$$

2.13 Statistical Analysis

Dataare expressed as mean ± SEM. Differences between the mean values of the control and treated groups were determined by One-way Analysis of Variance with a Dunnett post hoc test using the Graph Pad Prism 5. Significant difference was considered if $p < 0.05$.

3.0 Results

3.1 Quantification of Phytochemicals

The extract weighed 19.28g which was 3.86% of starting material.The summary of the concentrations of phytochemicals are presented in Table 1.The extract contained flavonoids (91.06 ± 1.66 mg/g), phenolics (283.67 ± 4.66 mg/g), alkaloids(200.15 ± 3.45 mg/g).saponins (60.25 ± 1.25 mg/g), tannins (177.20 ± 2.50 mg/g), sterols (66.05 ± 2.00 mg/g) and vitamin C(8.70 ± 2.25 mg/g). The results implied that the plant is rich in vital phytochemicals and antioxidant potentials.

3.2 Antioxidant Potentials of the Extract
DPPH-Radical Scavenging Activity and Reducing Power Potential of the Extract

The DPPH- radical scavenging and the reducing power of the aqueous extract of *A. melegueta* are presented in Figure 1. The highest percentage DPPH-radical scavenging inhibition of the extract was 52.60 ± 3.11% while that of vitamin C was 92.68 ± 0.06 %**.** IC_{50} value of the aqueous extract of *A. Melegueta* was found to be 49.31 µg/ml while that of ascorbic acid was 26.19 µg/ml. It implied that the extract had about 57% of the capacity of ascorbic acid.The ability of theaqueous extract of *A. melegueta* to reduce Fe^{3+} to Fe^{2+} is as shown Fig. 2. The results revealed that the extract elicited a reducing power of 11% of the standard, vitamin C.

3.3 Anti-inflamatory Potentials of the Extract
Membrane Stabilizing Potential

In Figure 3 is the membrane stabilizing profiles of extract of *A. melegueta* on red blood cells exposed to both heat and hypotonic induced lyses. It was observed that the extract gave mean maximum protection of 34.72 ± 1.29%. The mode of action of the extract was monophasic which implied that the protection is concentration dependent.The reference drug, indomethacin gavemaximum stability of 21.16 ± 0.20% at 50µg/ml. It implied that the standard drug (indomethacin) a non – steroidal anti-inflammatory drug was more potent than the extract. It implied that the plant possesses and exhibits appreciable anti-inflammatory activity.

3.4 Inhibition of Albumin Denaturation

In Figure 4 is the graphical representation of inhibition of albumin denaturation by the extract and the reference drug, diclofenac sodium. Theextractof *A. melegueta*gavemean maximum percentage inhibition of albumin denaturation of 13.66 ± 0.53% at 300 µg/ml while the reference drug, diclofenac gave a maximum percentage inhibition of albumin denaturation of 17.33± 0.19% at 300 µg/ml. This implied that the extract was as effective as the standard drug in inhibiting heat induced albumin denaturation.

4.0 Discussion

Unlike the seeds of *Aframomoummeleguata*not much study has been carried out on the biological and therapeutic potentials of the leaf of the plant probably due to the economic and medicinal importance attached to the seeds. The current study reported the levels of

phytochemical constituents in the leaves of *A. meleguata* as well as its anti-inflammatory and antioxidant potentials with a view to evaluating its ethno medicinal uses and providing scientific supports for the use of the leaf of the plant as its seeds.

Plants are regarded as reservoirs of secondary metabolites known as phytochemicals that are synthesized generally by plants to assist them to thrive or thwart competitors, predators or pathogens. Some phytochemicals are employed as "poisons" and others as "traditional medicines", and exhibit varying biological activities in the host plants (Molyneuxet al., 2007). Quantitative analyses of the aqueous extract of *A. meleguata*revealed the presence of vital and important constituents such as phenolic acid, flavonoids, alkaloids, sterols, tannins, terpenoids and saponins in varying proportions (Table 1).Phenolic compounds (tannins and flavonoids) are very important plant constituents because of their free radical scavenging, metal chelating, reducing, and hydrogen donating and single oxygen quenching abilities. They are ubiquitous groups of biomoleculesthat possess diverse biological properties ranging from anti-apoptosis, anti-aging, anti-carcinogen, antioxidant to anti-inflammatory (Ghasemzadeh and Ghasemzadeh 2011; Hossain et al., 2011; Saradha and Paulsamy, 2012; Akinpelu et al., 2012b). Studies have indicated polyphenols to possess and exhibit various biological activities including antifungal, anti-inflammatory, anti-mutagenic, anti-oxidative, anti-carcinogenic, anti-tumor as well as anti-diabetic effects. Also, the plasma lowering potentials of cholesterol and triacylglycerol levels as well as reduction of blood pressure and platelet aggregation in several systems have been reported (Ho et al., 1992; Al-Attar and Abu-Zeid 2013; Dare et al., 2013. Scientific evidences have shown that diets and herbs rich in polyphenolic compounds play prominent role against oxidative stress related disorders owing to their anti-oxidative activities (Saradha and Paulsamy, 2012; Ekpoet al., 2013).

Sterols are a class of secondary metabolites that are quite important in medical field. Several steroid compounds are employed in the world of drugs which include estrogen (sex hormone) used for contraception that inhibit ovulation, progestin (a synthetic steroid) that prevents miscarriage and pregnancy, anti-inflammatory, glucocorticoids, allergies, hay-fever, leukemia and hypertension as well as cardiac glycosides (kardenolic) which is employed as diuretic and cardiac amplifier (Surgelita 2000). Some steroids have been demonstrated to possess and exhibit anti-bacterial activities (Awad and Frink, 2000).Some of the active chemical constituents of the plants such as saponins, flavonoids and triterpenoids have been noted for their anti-obesity effect in various plants (Yun, 2010). Steroids have been reported to possess and exhibit hepatoprotective, antioxidant and associated biological activities (Kang et al., 1998). Flavonoids are the most common group of polyphenolic compounds in the human diets, fruits, vegetables and some herbs where they exhibit antioxidant activities (Bass 1999; Ross and Kasum, 2002). Tannins are involved in antioxidant activities, wound healing and numerous biological activities. Alkaloids are organic compounds that contain nitrogen, and are physiologically active with sedative and analgesic properties for relieving pains, anxiety and depression (Chaudhuri et al., 2014). Quite a number of biological activities have been associated with alkaloids which include allelopathic (Fasanu et al., 2013), antiplasmodial as well as antimicrobial activities. Saponinson the other hand have been shown to boost the immune system, lower cholesterol concentration by competing against it for absorption in the body, hypoglycemic, anti-inflammatory, hypocholesteromic as well as hypotriacylglyceromic in experimental animals(Ostlund et al., 2003;Poongothai et al., 2011). Ascorbic acid (Vitamin C) a water soluble and naturally occurring antioxidant has been shown to modulate adipocyte lipolysis (Garcia-Diaz et al., 2009; Rahman et al., 2014), inhibit inflammatory response (Mohammed et al., 2013) and inhibitsleptin concentration

(Garcia-Diaz et al., 2010). Supplementing rats with vitamin C reduced the circulating levels of leptin and decreased body weight and adiposity in rats (Garcia-Diaz et al., 2010).

Antioxidants are biological molecules that possess and exhibit ability to protect, directly scavenge reactive oxygen species or indirectly act to up regulate antioxidant defenses and or inhibit reactive oxygen species production (Doss and Pugalenthi, 2012, Jaiswal et al., 2014). Synthetic antioxidants have been demonstrated to elicit negative health effects, attention is shifted to the development, isolation and activity of naturally occurring anti-oxidants (polyphenols, phenolic acids, tannins and saponins) with little or no toxic potentials (Hodzic et al., 2009). In this study, the antioxidant potentials of aqueous leaf extract of *A. melegueta*were studied using DPPH-radical scavenging and ferric reducing assays. The free radical-scavenging activity of aqueous extract of *A. melegueta* was determined by the DPPH test (Fig. 1). This test aimed atmeasuring the capacity of the extract to scavenge the stableradical, 2, 2-diphenyl-1-picrylhydrazil formed in solution by donation of a hydrogen atom or an electron (Fkiet al., 2005). The extract exhibited free radical-scavenging activity with an IC_{50} (49.31 µg/ml). This compared favorably with the standard (ascorbic acid) with IC_{50} value of 26.19 µg/ ml. This result is in agreement with that of Onoja*et al*. (2014) who reported that *A. melegueta* seed has potent antioxidant activities when compared to ascorbic acid.

Moreover, the result of this present study showed that the ferric reducing potential of aqueous extract of *A. melegueta* was concentration dependent with the highest activityat 350 µg/ml (Fig. 2). Shiddhurajuet al. (2002) reported that ferric reducing power of bioactive compounds was associated with antioxidant activity and is a measure of the reductive ability of antioxidants. It is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of sample extracts (Huda-Faujanet al., 2009).

Blood is a sensitive index of the physiological changes of an animal to any environmental pollutants and toxic stress of any nature exhibits or elicits conspicuous and significant changes in the hematological parameters (Elzoghbyet al., 2014). Earlier studies have demonstrated that anti-inflammatory drugs also possess and exhibit antioxidant and/or radical scavenging activities (Pallares et al., 2013, Fajobi et al., 2017, Fasakin et al., 2017). Inflammation is a complex series of events and functionally protective response that develop when an organism is injured either by mechanical or chemical agents by a self-destructive process (Sadique et al., 1989, Oyedapo and Famurewa 1995). The mechanism of inflammatory injury involves oxidative stress induced by reactive oxygen species (ROS) from activated neutrophils and macrophages. Also reactive oxygen species extend inflammation by promoting release of cytokines which also stimulate additional production of free- radicals. In many inflammatory disorders, there is an excessive activation of phagocytes and production of free radicals which increase vascular permeability, protein denaturation and membrane alteration (Umapathy et al., 2010, Sakat et al., 2010, Muragana and Parimelazhagan 2014).

Table 1: Concentrations of Phytochemicals in the Aqueous Extract of the Leaves of *A. melegueta*

Phytoconstituent	Concentration
Total Flavonoids	91.06 ± 1.66 mg/g (RE)
Total Phenolics	283.67 ± 4.66 mg/g (TAE)
Saponins	60.25 ± 1.25 mg/g extract
Alkaloids	200.15 ± 3.45 mg/g of extract
Tannins	177.20 ± 2.50 mg/g (TAE)
Sterols	66.05 ± 2.00 mg/g (CHE)
Vitamin C	8.70 ± 2.25 mg/g

Values are expressed as mean ± SEM; n = 3;
RE (Rutin Equivalent); TAE T(annic Acid Equivalent); and CHE (Cholesterol Equivalent)

Figure 1: DPPH-radical Scavenging Activity of *A. melegueta* and Ascorbic acid
Each values represented mean ± SEM of n = 3 readings

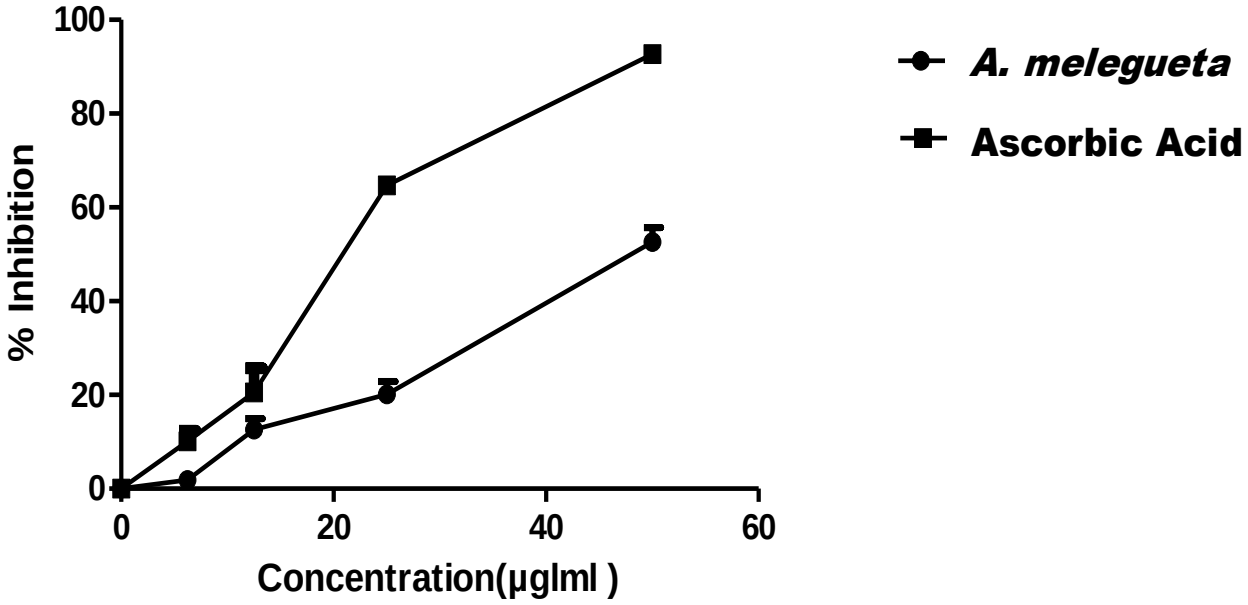


Figure 2: Reducing Antioxidant Potential of *A. melegueta* and Ascorbic acid
Each value represented the mean ± SEM of n = 3 readings

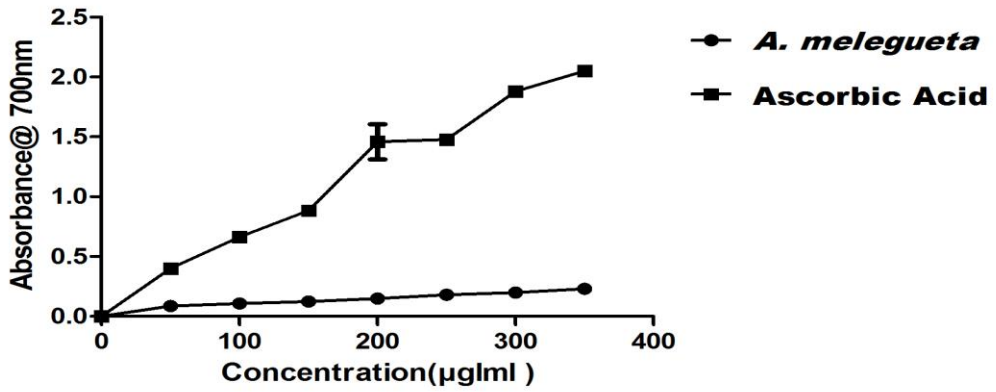


Figure3: Membrane Stabilizing Potential of *A. melegueta* and Indomethacin
Each value represented the mean ± SEM of n = 3 readings

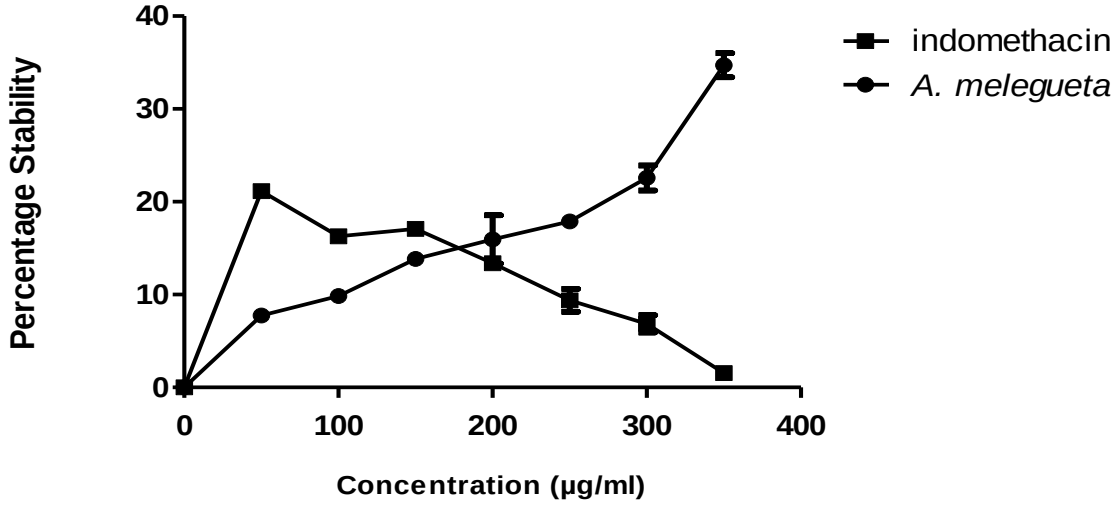
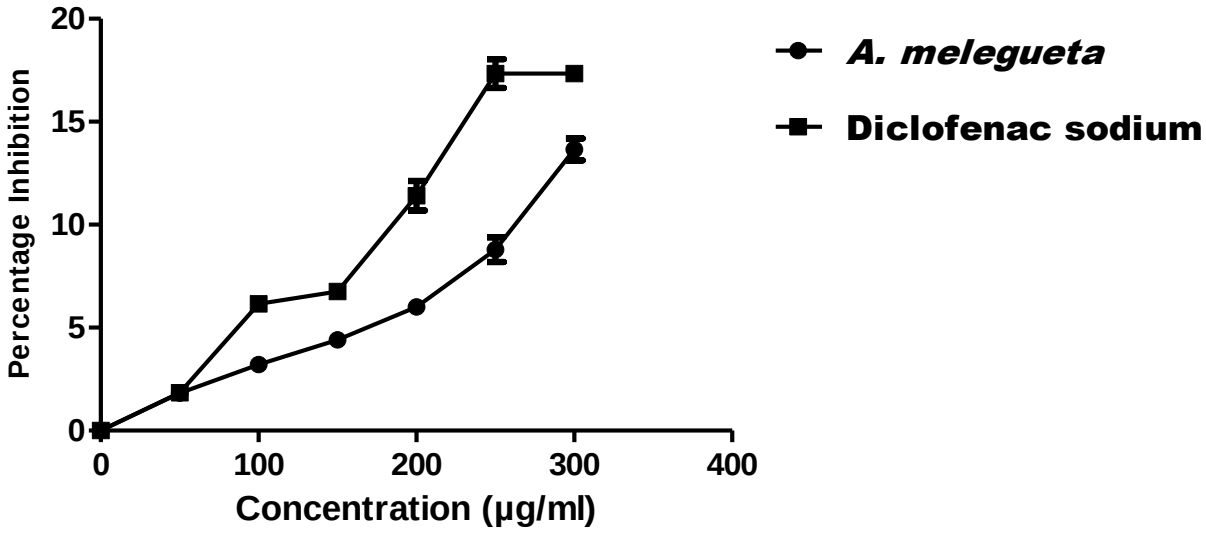


Figure4: Percentage Inhibition of Albumin Denaturation by*A. melegueta* and Diclofenac
Each value represented the mean ± SEM of n = 3 readings



In this study, anti-inflammatory potentials of water extract of *A. melegueta* were investigated using membrane stabilizing and inhibition of protein denaturation methods (Oyedapoet al., 1999, Sakatet al., 2010).The red blood cell membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity of the extract because the erythrocyte membrane is analogous to the lysosomal membrane (Shenoyet al., 2010; Leelaprakash and Dass, 2011) and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosome is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release (Shenoyet al., 2010). Study of the membrane stabilizing potentials of the aqueous extract of *A. melegueta* revealed that the maximum red blood cell membrane stabilizing activity was 34.72 ± 1.29 % at 350 µg/ml while the maximum red blood cell membrane stabilizing activity of diclofenac was 21.16 ± 0.20 % at concentration of 50 µg/ml(Fig. 3). The percentage red blood cell membrane stability of the extract increased with increase in concentration while that of the diclofenac increased at certain concentration and then decreased (i. e biphasic mode of protection). The activity of the extract was comparable to that of diclofenac, a non-steroidal anti-inflammatory drug. The response of the red blood cells to the extract was monophasic and concentration dependent. These activities might be due to the presence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. Studies have revealed that saponins and flavonoids elicit potent, significant and appreciable membrane stabilizing effect both *in vivo* and *in vitro*(Oyedapoet al., 2010; Akinpeluet al., 2012b). The reactive oxygen species are important mediators that lead and maintain inflammatory processes while their neutralization by antioxidants and radical scavengers attenuate inflammation.

Denaturation of proteins is a well-documented consequence of inflammation. It is one of the causes of rheumatoid arthritis. The production of auto antigen in certain arthritic diseases could be due to denaturation of protein. The mechanism of denaturation probably involves alteration of electrostatic, hydrogen, hydrophobic and disulfide bonds/interactions (Umapathyet al., 2010). As part of the

investigation on the mechanism of the anti-inflammatory activity, the ability of the extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. *A. melegueta* showed maximum inhibition at 300 µg/ml with percentage inhibition of 13.70 ± 0.55% while the reference drug, diclofenac showed a maximum inhibition at 300 µg/ml with percentage inhibition of 18.09 ± 0.24% (Fig. 4). One of the causes of rheumatoid arthritis is the denaturation of protein, as such any agent (drug or extract) that possess and exhibit the inhibition of protein could be regarded as a potent and appreciable anti-inflammatory agent. The aqueous extract of *A. melegueta* possesses and exhibited the ability to protect the protein membrane from heat and hypotonic induced disruption. The results are comparable to the protective ability of diclofenac a non-steroidal anti-inflammatory drug (NSAID). Earlier observations, (Oyedapoetal., 2015, Murugan and Parimelazhagan, 2014) have shown that phenols, flavonoids, tannins and saponins possess and exhibit ability to bind peripheral proteins and cations, as such able to protect the membrane proteins from denaturation and stabilize the erythrocyte membranesagainst both heat, hypotonic and alkaline induced lyses and denaturation.

Conclusion

The results of this study revealed that the aqueous leaf extract of *A. melegueta* possessed and exhibited anti-inflammatory, anti-oxidant and vital phytochemicals. Hence, the plant could be employed in the treatment and management of these disorders.

Declaration

There was no conflict of interest among the authors.

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