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Comparative Anti-radical Activity of Five Indigenous Herbal Plants and their Polyherbal Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author IPA designed the study, wrote the protocol and supervised the work. Authors IPA and OSC carried out all laboratories work and performed the statistical analysis. Author IPA managed the analyses of the study. Author IPA wrote the first draft of the manuscript. Author AOB managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study comparatively evaluated the radical scavenging activity of the methanolic extracts of five indigenous plants, namely- *Zingiber officinale* (Zingiberaceae), *Nauclea latifolia* (Rubiaceae), *Phyllantus* spp (Euphorbiaceae), *Khaya senegalensis* (Meliaceae) and *Camellia sinensis* (Theaceae) and their polyherbal extract referred to as Gingered Polyherbal Tea (GPHT).
Study Design: Experimental.
Place and Duration of Study: Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria in July 2015.
Methodology: The extract of each plant and their polyherbal combination were studied for anti-radical activity using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), Hydroxyl ion, Nitric oxide and Super

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oxide free radical scavenging assays.
Results: The following trend was observed in the anti-radical activity of the extracts: GPHT > Ascorbic acid/Gallic acid> *Zingiber officinale*> *Camellia sinensis*> *Nauclea latifolia*>*Khaya senegalensis*>*Phyllantus* spp.
Conclusion: The extracts showed varying levels of anti-radical activity individually and contributed synergistically to the anti-radical activity of their polyherbal extract. This observation could be employed in designing new drug combinations that will provide better therapeutic options for the prevention and treatment of many oxidative stress- related diseases.

Keywords: Polyherbal extract; antioxidant; 2, 2-diphenyl-1- picrylhydrazyl; scavenging activity.

1. INTRODUCTION

Free radicals are chemical species with one or more unpaired electrons in their outermost orbital which are not contributory to bonding. The most important free radicals in the body may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules include oxygen in its singlet state (O_2), superoxide (O_2^-), hydroxyl ion (OH^-), hydrogen peroxide (H_2O_2), peroxyl radical (ROO) and alkoxy radical (RO). Nitrogen derived oxidant species are mainly nitric oxide (NO), peroxy nitrate (ONOO), nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) [1]. Other types of free radicals are Carbon-centered free radicals (CCl_3) (that arise from the attack of an oxidizing radical on an organic molecule), Hydrogen- centered free radicals (which result from the attack of the hydrogen atom) and Sulphur- centered radicals (produced from the oxidation of glutathione) [2]. Free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA. These free radicals attack the nearest stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction, resulting in the destruction of living cells [3].

Oxidative stress results when oxidant species is increased or when levels of antioxidants are diminished. The magnitude of oxidative stress depends on the size and effect of these changes, with a cell being able to overcome small perturbations and regain its original state [4]. However, severe oxidative stress can cause cell death and even trigger apoptosis and necrosis [4]. Considerable evidence has accumulated to implicate cellular damage arising from oxidative stress, at least in part, in the etiology and pathophysiology of human diseases many of which are life- threatening, such as Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, arthritis, diabetes, inflammation and neurodegenerative disorders [5]. These increasing burden placed on mankind by oxidative stress have necessitated the search for plants with the potentials of serving as natural source of antioxidants.

Antioxidant simply means 'against oxidation'. Antioxidants are substances with free radical chain breaking properties, hence reducing the effect of dangerous oxidants or free radicals by decreasing their destructive power [6]. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism [7]. Over the past two decades, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts [8]. This is due to the need for safe, economic, powerful and natural antioxidants to replace the synthetic ones which have possible activity as promoters of carcinogenesis. An expanding body of evidence from epidemiological and laboratory studies have demonstrated that some plants could actually serve as natural sources of antioxidants [9]. Thus herbal drugs are rapidly becoming popular in recent years as an alternative therapy.

Polyherbal extracts, which are combinations of different herbal extracts/fractions, are used for the treatment of diseases. Many people believe that polyherbal extracts are just effective as drugs. Herbalists suggest that nature provide other ingredients that may act as buffers, synergists or counterbalances, working in harmony with the more powerful ingredients. Therefore, by using herbal combination in their complete form, the body's healing process utilizes a balance of ingredients provided by nature [10]. Some polyherbal extracts have been scientifically proven for efficacy in the treatment of oxidative stress related- disease while many others are yet to be investigated. One of such yet to be investigated is the polyherbal extract used by the indigenous people of Ajaka, Igalamela/ Odolu Local Government Area of Kogi State,

Nigeria. The extract is named 'Gingered Polyherbal Tea' and it consist of *Zingiber officinale* (Ginger)*, Nauclea latifolia, Phyllantus spp, Khaya senegalensis* and *Camellia sinensis*. The 'Gingered' in the name came from *Zingiber officinale* (Ginger) while the 'Tea' is from *Camellia sinensis* (tea plant).

Zingiber officinale commonly referred to as Ginger is widely used around the world as a spice. It is also widely used in traditional alternative medicine in the treatment and management of various disorders including catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes [11,12]. *Nauclea latifolia* commonly known as pin cushion tree is a straggling shrub or small tree native to tropical Africa and Asia. Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus. The plant is also used in the treatment of ailments like malaria [13-15], gastrointestinal tract disorders [16], sleeping sickness [17], prolong menstrual flow [18], hypertension [14] and as a chewing stick [19]. *Phyllantus* spp is widely cultivated in Africa. Its parts are considered to have antibiotic properties and also useful in the treatment of hemorrhage, diarrhoea, dysentery, anaemia, jaundice, diabetes, fever, dyspepsia, bronchitis and cough [20]. *Khaya senegalensis* commonly called mahogany is also known as 'madaci' and 'ago' by the Hausa and Igala ethnic groups of northern and central Nigeria, respectively. It is a large tree native to sub-Saharan savannah area from Senegal to Uganda, and one of the most popular medicinal plants in African traditional remedies. The decoction of the bark is extensively used as a febrifuge and antimalarial [21]. In northern Nigeria, the decoction of the stem bark is also used for treatment of stomach disorders, urinogenital diseases, worm infestation, and in the treatment of trypanosomiasis [22]. It is reported to be an effective agent as a gastrointestinal nematocide [23], antisickling [24], anti-microbial and as an antiprotozoal agents [25]. *Camellia sinensis* commonly known as tea plant is probably the most widely consumed beverage in the world [26]. Even though the tea plant is cultivated all over the world, it grows best in tropical and subtropical areas with adequate rainfalls, good drainage, and a slightly acidic soil [27].

The aim of this study was to compare the anti-radical activity of the extracts of the above five different plants with their polyherbal extract and explore the synergic action for designing new drug combinations that are maximally efficacious in the treatment of oxidative stress- related diseases.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The plant materials were collected from Ajaka, Igalamela/Odolu Local Government Area of Kogi State, Nigeria. The identities of the five plants were confirmed at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, as *Zingiber officinale* (Voucher No.2261), *Nauclea Latifolia* (Voucher No. 1268), *Phyllantus* spp (Voucher No. 900351), *Khaya senegalensis* (Voucher No.3755) and *Camellia sinensis* (Voucher No.217).

2.2 Preparation of Plant Extracts

The roots of *Zingiber officinale* and the leaves of *Nauclea Latifolia, Phyllantus* spp*, Khaya senegalensis* and *Camellia sinensis* were collected and sun dried to constant weight. They were pulverized separately using a mechanical grinder. One gram (1.0 g) each of pulverized dry plant was cold- macerated in methanol for 24-hours. This was followed by filtration using Whatmann filter paper (Size No1) and concentration over water bath maintained at 40℃. The extracts were transferred into clean labeled sample bottles and kept in the refrigerator at 4℃ until ready for use.

2.2.1 Preparation of the polyherbal extract

The pulverized plants were weighed separately and mixed in equal ratio by weight. The extraction was done using the same method described above.

2.3 Chemicals

The chemicals used were of analytical grade and were purchased from the country representative of Sigma Chemical, St. Loius USA.

2.4 Assays for Radical Scavenging Activity

2.4.1 Assay for DPPH scavenging activity

The scavenging activity on DPPH free radicals by the extracts was done using the method as described by Gyamfi et al. [28]. The reaction

tubes contained 1.0 ml of 0.1 mM DPPH-ethanol solution, 1.0 ml of ethanol (98% ethanol), 0.95 ml of 0.05 M Tris-HCl buffer (PH7.4), and 50 µL of either the extract or standard (Vitamin C) solution wrapped in aluminium foil. The absorbance was measured at 517 nm exactly 30 seconds after adding each of the extracts, as loss of absorbance at this wavelength is a measure of the radical scavenging capacity of the extracts.

This antioxidant assay was based on the scavenging ability of antioxidant(s) in plant extracts towards the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH), which is deep purple in color, to form the corresponding hydrazine with accompanying color change to light purple or golden yellow. This color change is inversely proportional to increasing concentration of the antioxidant in the mixture. In order to appreciate the results from the DPPH method, the Ascorbic Acid Equivalent (AAEq) and Median Inhibitory Concentration (IC_{50}) value (the concentration of the extract that causes 50% inhibition of the DPPH activity) were established. The IC_{50} values were estimated by non- linear regression analysis of the percent (%) antioxidant activity verse concentration/volume curve of the test solutions, while Ascorbic Acid Equivalent was obtained by extrapolation from the standard curve for the ascorbic acid antioxidant activity.

2.4.2 Assay for hydroxyl radical scavenging activity

The hydroxyl radical assay is based on the qualification of the degradation product of 2 deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} - ascorbate- EDTA $–H_2O_2$ system (The Fenton reaction). The reaction mixture contained in the final volume of 1 ml 2 deoxy 2 ribose (2.8 mM) KH_2PO_4—KOH buffer (20 mM pH 7.4), $FeCl_3$ (100 µm), EDTA (100 µm), H_2O_2 (1.0 mM), Ascorbic Acid (100 µm) and various concentrations (0-250 µg/ml) of the test sample. After incubation for 1hour at 37℃, 0.5 ml of the reaction mixture was added to 1ml of 2.8% TCA, then 1 ml aqueous TDA was added and the mixture was incubated at 90℃ for 15 minutes to develop the colour. After cooling the absorbance was measured at 532 nm against an appropriate blank solution [29].

2.4.3 Assay for nitric oxide scavenging activity

The procedure for this assay is based on the fact that, sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO may lead to tissue damage. Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitro prusside (5 $mmolL^{-1}$) in phosphate buffered saline pH 7.4, was mixed with different concentration of the extract (250-2500 µg ml^{-1}) prepared in methanol and incubated at 25℃ for 30 minutes.A control without the test compound, but an equivalent amount of methanol was taken. After 30 minutes, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1- naphthyl ethylene diamine dihydrochloride).Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphathyl ethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

> % Inhibition of the NO radical= [A0 – A1]/A0 x 100

Where A0 is the absorbance before the reaction and A1 is the absorbance after reaction has taken place with Griess reagent [30].

2.4.4 Assay for super oxide free radical scavenging activity

Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan. 0.02 ml of extracts, 0.05 ml of Riboflavin solution (0.12 mM), 0.2 ml of EDTA solution [0.1 M], and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5 mM] were mixed in test tube and reaction mixture was diluted up to 2.64 ml with phosphate buffer [0.067 M]. The absorbance of solution was measured at 560 nm using DMSO as blank after illumination for 5 min and difference in OD was determined after 30 minutes incubation in fluorescent light. Absorbance was measured after illumination for 30 min. at 560 nm on UV visible spectrometer [31].

> % scavenging/Inhibition = [Absorbance of control] – [Absorbance of test sample/Absorbance of control] X 100

3. RESULTS

3.1 DPPH Scavenging Activity

Fig. 1 shows the ascorbic acid equivalent of the extracts studied. The Ascorbic Acid Equivalent of the GPHT (4.1 mM) was much higher than that of any of the extracts- *Nauclea latifolia* (0.90 mM), *Phyllantus* spp (0.30 mM), *Khaya senegalensis* (0.50 mM) and *Camellia sinensis* (0.80 mM).

GPHT had the least value of 1.15 µL followed by *Zingiber officinale* (18.0 µL), *Nauclea latifolia* (46.0), *Khaya senegalensis* (52.17 µL), *Camellia sinensis* (56.83 µL), and *Phyllantus* spp (67.57 µL) from the estimated median inhibitory concentration IC_{50} (Table 1).

Table 1. The median inhibitory concentration (IC_{50}) of the test samples

Plant	IC_{50} of extracts (µl/ml)
Zingiber officinale	18.40
Nauclea latifolia	46.00
Phyllantus spp	67.5
Khaya senegalensis	52.17
Camellia sinensis	56.83
GPHT	1.15

GPHT= Gingered Poly-Herbal Tea

3.2 Hydroxyl Radical Scavenging Activity

The inhibition of hydroxyl radicals by the different samples is presented in Fig. 2. The percentage inhibition of hydroxyl radical by GPHT (at 200 µg/ ml) was found to be 98% which is highest among the extracts. This value was higher than that of the standard- ascorbic acid which was 80.00% at the same concentration. This high scavenging activity of the polyherbal extract was evident in its IC_{50} value (80.97 µg/ ml) which was the least among the extracts. *Nauclea latifolia* had the highest IC_{50} value 1826.00 µg/ml while ascorbic acid had a value of136.90 µg/ml (Table 2).

3.3 Nitric Oxide Scavenging Assay

The polyherbal combination, *Zingiber officinale* and *Camellia sinensis* showed high scavenging activity of 99%, 64% and 57% respectively at 200 µg /ml, where as Gallic acid at the same concentration exhibited 79% inhibition (Fig. 3). GPHT has the least IC_{50} value of 32.98 µg/ ml while *Phyllantus* spp showed the least nitric oxide scavenging activity (IC_{50} value of 255.50 µg/ ml) (Table 3).

Table 2. IC_{50} values of different samples in hydroxyl radical scavenging assay

Plant	IC_{50} of extracts (µg/ml)
Zingiber officinale	104.40
Nauclea latifolia	212.30
Phyllantus spp	691.20
Khaya senegalensis	363.50
Camellia sinensis	184.40
GPHT	27.97
Ascorbic acid	59.71

Table 3. IC_{50} values of different samples in nitric oxide scavenging assay

Plant	IC_{50} of extracts (µg/ml)
Zingiber officinale	83.72
Nauclea latifolia	220.20
Phyllantus spp	255.50
Khaya senegalensis	233.50
Camellia sinensis	182.80
GPHT	32.98
Gallic acid	53.10

3.4 Superoxide Free Radical Scavenging Activity

GPHT has potent superoxide scavenging activity (IC_{50} value 16.81 µg/ml) while the *Zingiber officinale* showed the least superoxide scavenging activity (IC_{50} value 359.70 µg/ml) (Table 4). GPHT showed maximum activity of 99%, at 200 µg /ml which was greater than that of ascorbic acid standard which at the same concentration exhibited 63% inhibition (Fig. 4).

Table 4. IC_{50} values of different samples in superoxide scavenging assay

Plant	IC_{50} of extracts (µg/ml)
Zingiber officinale	359.70
Nauclea latifolia	190.70
Phyllantus spp	212.70
Khaya senegalensis	167.20
Camellia sinensis	123.80
GPHT	16.81
Ascorbic acid	54.65

4. DISCUSSION

In a normal cell, there is appropriate oxidant: antioxidant balance. However, this balance can be shifted, when oxidant species is increased or

when levels of antioxidants are diminished. This stage is called oxidative stress. Oxidative stress causes serious cell damage leading to a variety of human diseases like Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc. Numerous medicinal plants and polyherbal formulations, which are combinations of different herbal extracts/fractions, are used for the treatment of

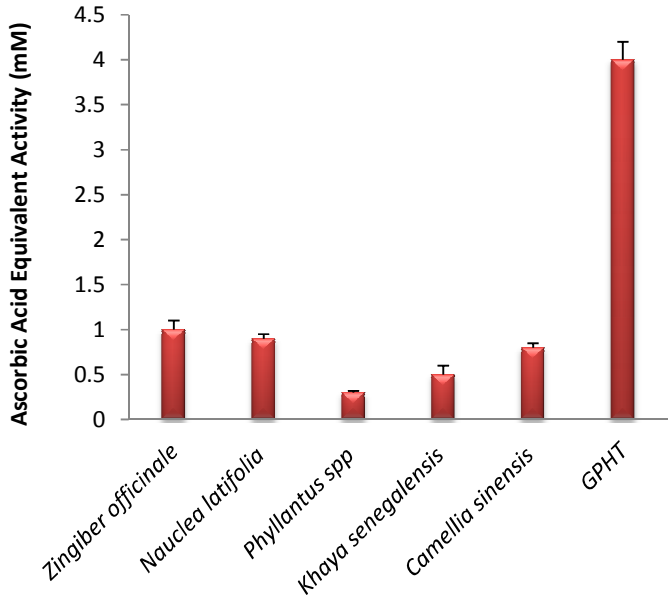

Fig. 1. Comparative antioxidant activity of *Zingiber officinale, Nauclea latifolia, Phyllantus* spp, *Khaya senegalensis, Camellia sinensis* and GPHT

GPHT= Gingered Poly-Herbal Tea

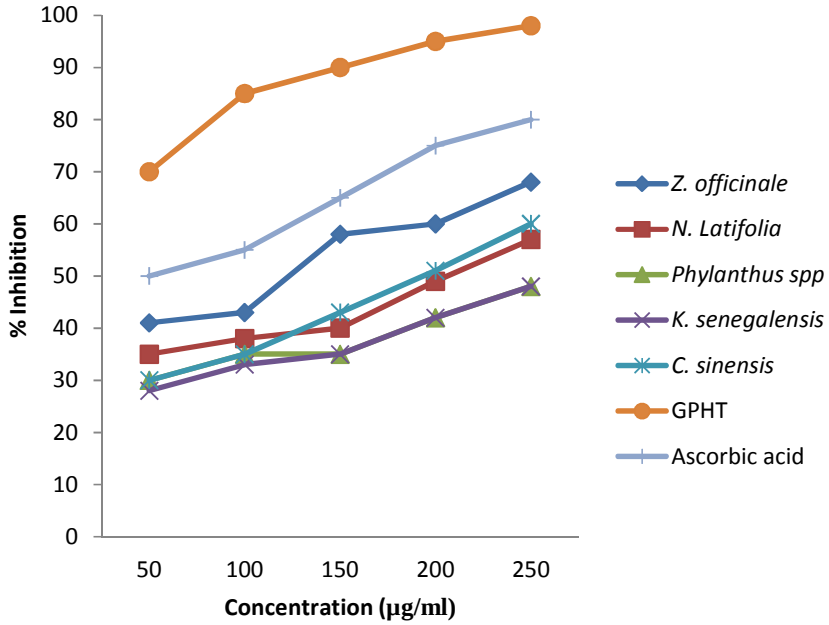

Fig. 2. Comparative Hydroxyl radical scavenging activity of *Zingiber officinale, Nauclea latifolia, Phyllantus* spp, *Khaya senegalensis, Camellia sinensis* GPHT and ascorbic acid

GPHT= Gingered Poly-Herbal Tea

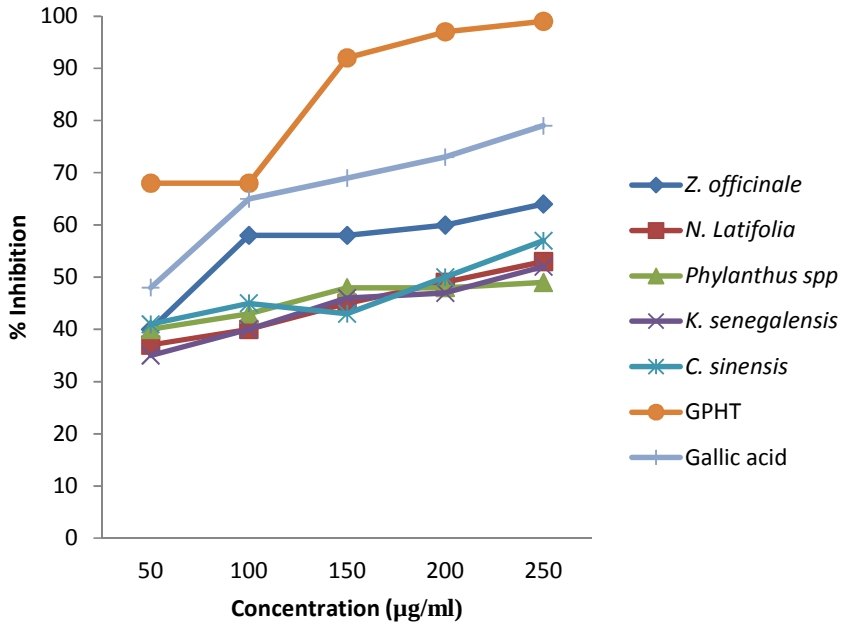

Fig. 3. Comparative nitric oxide radical scavenging activity of *Zingiber officinale, Nauclea latifolia, Phyllantus* spp, *Khaya senegalensis, Camellia sinensis* GPHT and ascorbic acid

GPHT= Gingered Poly-Herbal Tea

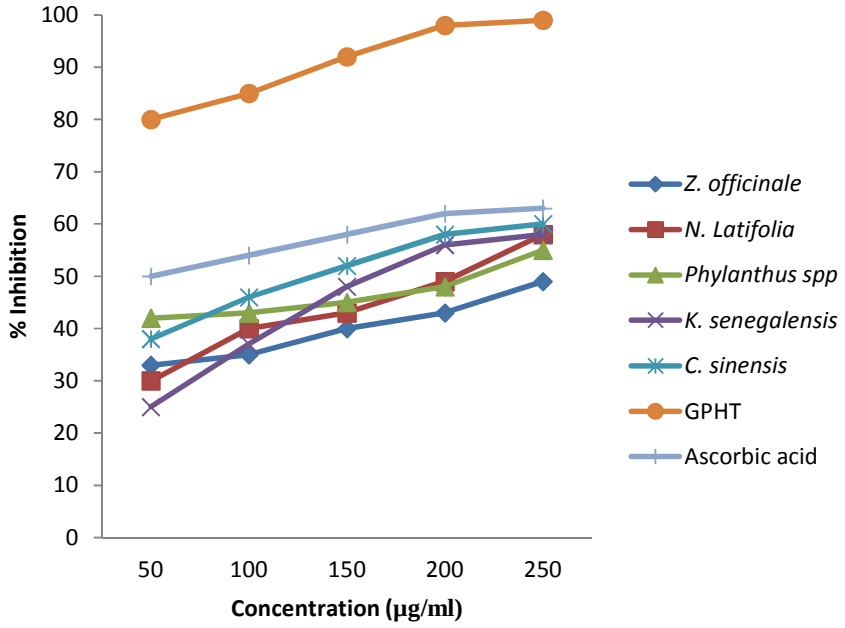

Fig. 4. Comparative superoxide radical scavenging activity of *Zingiber officinale, Nauclea latifolia, Phyllantus* spp, *Khaya senegalensis, Camellia sinensis* GPHT and ascorbic acid

GPHT= Gingered Poly-Herbal Tea

these diseases, as herbal drugs are a rich source of natural antioxidants. The aim of this study was to compare the antioxidant activity of the extracts of five different plants viz *Zingiber officinale,*

Nauclea Latifolia, Phyllantus spp, Khaya senegalensis and *Camellia sinensis* with their polyherbal extract. The polyherbal extract called 'Gingered Polyherbal Tea' (GPHT) is used locally in the north- central part of Nigeria for the treatment of several ailments believed to have oxidative damage as the underlying etiology.

Results showed that the polyherbal extract exhibited a very high anti-radical activity compared to its individual components. This is evident in all the radical scavenging assays employed in this study. In the DPPH assay, the Ascorbic Acid Equivalent value of the polyherbal extract (4.0 mM) was the highest among all the extracts (Fig. 1). The polyherbal extract also possesses the least IC_{50} value (1.15 µl) among the extracts (Table I). It should be noted that the lower the IC_{50} value, the stronger the antioxidant potential. The Ascorbic Acid Equivalent and the IC_{50} value of the polyherbal extract indicate that its anti-radical activity is far greater than the anti-radical activities of its components put together.

The polyherbal extract also showed the greatest hydroxyl radical scavenging activity than any of the extracts and even Ascorbic Acid at the same concentration. Hydroxyl radical is one of the most potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. GPHT therefore, could exert its protective effects on cellular membranes. This study also examined the scavenging effect of the extracts on Nitric oxide (NO). NO has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects of NO, its contribution to oxidative damage is also reported. This is due to the fact that NO can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH and NO. Results showed that the extracts showed varying degrees of NO scavenging activity but the polyherbal formulation showed the highest scavenging activity. At the same concentration, the scavenging activity of the polyherbal extract was found to be greater than gallic acid; therefore it is safe to say that GPHT is also a potent scavenger of nitric oxide. Results also showed that all the extracts at all concentrations used, inhibited superoxide radicals but to varying degrees. Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. GPHT could therefore be helpful in preventing such consequences.

The observed anti-radical activity of the five plants investigated in this study is in agreement with the findings of other researchers who linked the anti- radical activities of these plants to the presence of polyphenolic compounds in them [32-34]. Thus, it could be inferred that the phenolic compounds of the individual extracts contributed synergistically to the overall anti-radical activity of the polyherbal extract. Polyphenols are secondary metabolites of plants believed to be generally involved in their defense against ultraviolet radiation and aggression by pathogens [35]. The term 'polyphenol' in chemical terms, is a substance which possess an aromatic ring bearing one or more hydroxyl substituent and a functional derivative such as esters, methyl ethers or glycosides [36]. Polyphenols such as flavonoids, lignans and phenolic acids have the capacity to scavenge reactive oxygen species [37], thereby chemo-preventing oxidative stress- related diseases such as neurodegenerative disorders [38], asthma [39], diabetes [40,41], cardiovascular disorders [42] and different forms of cancer [43].

On the basis of the results obtained in this study, the polyherbal extract has been shown to be a potent scavenger of free radicals. Thus, GPHT might be helpful in combating the progression of various diseases with oxidative stress components such as atherosclerosis, diabetes mellitus among others. Although, polyherbal extracts are less likely than most conventional medicines to cause adverse effects, they may interact negatively with prescribed medications; it is therefore wise to use polyherbal extracts appropriately and not indiscriminately [44].

5. CONCLUSION

This study was carried out using the crude extracts, it is therefore, considered a preliminary study. More advanced research is required to ascertain the observations made in this study and to further demonstrate that this polyherbal extract is an excellent candidate for further bio-guided investigation to develop a polyherbal formulation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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