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Comparative DPPH (2, 2-Diphenyl-1-Pycrylhydrazyl) Radical Scavenging Activity of Five Indigenous Herbal Plants and their Polyherbal Extract

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ABSTRACT

Aims: This study compared the antioxidant activity of the extracts of five indigenous plants, namely- *Zingiber officinale* (Zingiberaceae), *Nauclea latifolia* (Rubiaceae), *Phyllantus spp* (Euphorbiaceae), *Khaya senegalensis* (Meliaceae) and *Camellia sinensis* (Theaceae) with 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 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) model was employed for the determination of the antioxidant activity of the plant extracts. Ascorbic acid was used as the standard antioxidant; hence the antioxidant activity was expressed as Ascorbic Acid Equivalent. The extracts were also evaluated for their median inhibitory concentration (IC_{50}).
Results: It was observed that the Ascorbic Acid Equivalent of GPHT (4.0mM) was much higher than any of *Zingiber officinale* (1.00 mM), *Nauclea Latifolia* (0.90 mM), *Phyllantus spp* (0.30 mM), *Khaya senegalensis* (0.50 mM) and *Camellia sinensis* (0.80 mM). From the estimated median inhibitory concentration IC_{50}, 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), *Phyllantus spp* (67.57 µL) in that other.
Conclusion: the extracts showed varying levels of antioxidant activity individually and showed synergistic anti-oxidant activity as a 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-Pycrylhydrazyl, 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,

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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 effect of oxidative stress depends on the size 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 indigenes 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 (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

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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* 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 antioxidant 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. MATERIAL 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 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°C. The extracts were transferred into clean labeled sample bottles and kept in the refrigerator at 4 ^{o}C 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 Assay for antioxidant 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.

3. RESULTS

In this study, the radical scavenging capacity of five plants, namely, *Zingiber officinale*, *Nauclea Latifolia, Phyllantus spp, Khaya senegalensis*, *Camellia sinensis* and there polyherbal extract (GPHT) were evaluated and compared using the DPPH radical

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scavenging method while utilizing ascorbic acid as the standard antioxidant. Thus, the antioxidant activity was expressed as Vitamin C equivalent. All the extracts were further evaluated for their median inhibitory concentration (IC_{50}).

Figure 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).

From the estimated median inhibitory concentration IC_{50}, 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)*, Phyllantus spp* (67.57 µL) in that other (Table 1).

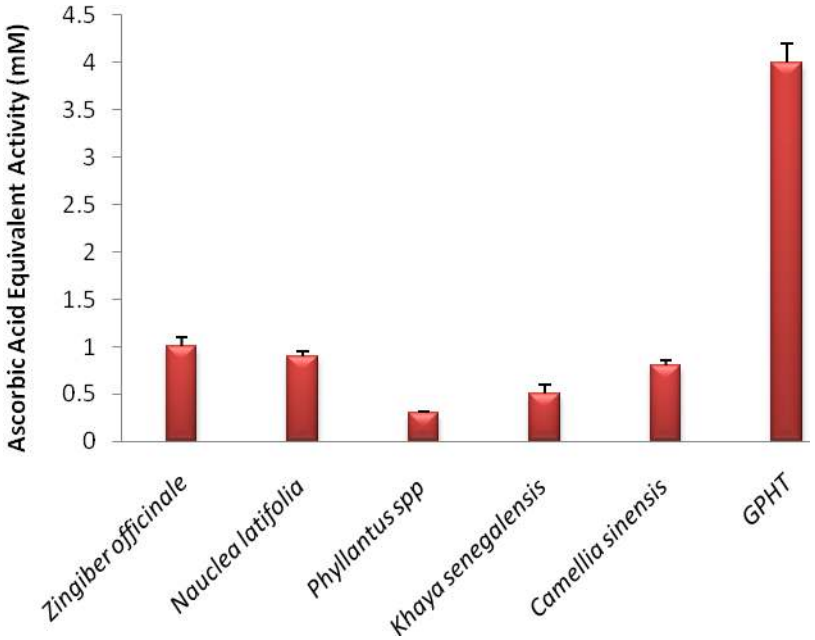

Figure 1: Comparative antioxidant activity of *Zingiber officinale, Nauclea latifolia Phyllantus spp, Khaya senegalensis, Camellia Sinensis* and GPHT. Ascorbic Acid Equivalent was calculated based on the triplicate analysis of two extracts per sample. GPHT= Gingered Poly-Herbal Tea

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

Plant	IC_{50} of Extracts (µl/3 ml)
Zingiber officinale	18.40
Nauclea latifolia	46.00

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Phyllantus spp	67.57
Khaya senegalensis	52.17
Camellia sinensis	56.83
GPHT	1.15

GPHT= Gingered Poly-Herbal Tea

4. DISCUSSION

This study compared the *in vitro* antioxidant potentials of five medicinal plants 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 antioxidant activity compared to its individual components- *Zingiber officinale, Nauclea Latifolia, Phyllantus spp, Khaya senegalensis* and *Camellia sinensis*. This is evident in the Ascorbic Acid Equivalent value of the polyherbal extract (4.0 mM) which is the highest among the extracts (Figure 1). The polyherbal extract also possesses the least IC_{50} value (1.15µl) 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 antioxidant ability is far greater than the antioxidant activities of its components put together. The observed antioxidant activity of the five plants investigated in this study is in agreement with the findings of other researchers who linked the antioxidant activities of these plants to the presence of polyphenolic compounds in them [29-31]. Thus, it could be inferred that the phenolic compounds of the individual extracts contributed synergistically to the overall antioxidant 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 [32]. 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 [33]. Polyphenols such as flavonoids, lignans and phenolic acids have the capacity to scavenge reactive oxygen species [34], thereby chemo-preventing oxidative stress- related diseases such as neurodegenerative disorders [35], asthma [36], diabetes [37, 38], cardiovascular disorders [39] and different forms of cancer [40].

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 [41].

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. More so, the bioactive

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compounds need to be sorted out and characterized as well as setting the right therapeutic doses.

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