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Phytochemical constituents and antioxidant activities of two Nigerian retailed polyherbal formulations

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ABSTRACT

Oxidative stress has been implicated in many neurodegenerative diseases, cancers and even ageing processes. This warrants that dietary antioxidants are needed to complement endogenous antioxidant defence system and prevent the development of these chronic diseases. In this study, the phytochemical constituents and antioxidant activities of two retailed Nigerian polyherbal formulations (DRHM® and GCHM®) were evaluated *in vitro* using DPPH radical scavenging activity, total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP) models. Aside for saponins, glycosides, anthraquinones and anthocyanins which were higher in DRHM® and alkaloids which were higher in GCHM®, there was no significant ($p < 0.05$) difference among the amount of other phytochemicals detected in the two polyherbal formulations. The DPPH radical scavenging effect exhibited by DRHM® (EC_{50} = 1.62x10^6 µg/ml) was significantly ($p < 0.05$) higher than GCHM® (EC_{50} = 574.5 µg/ml) but that of ascorbic acid (EC_{50} = 10.58 µg/ml) was significantly ($p < 0.05$) higher than both DRHM® and GCHM®. Similarly, GCHM® (EC_{50} = 0.005414 µg/ml) had higher FRAP than DRHM® (EC_{50} = 5.302 µg/ml). However, the TAC of GCHM® (EC_{50} = 1675 µg/ml) was significantly ($p < 0.05$) higher than that of DRHM® (EC_{50} = 6.101 µg/ml). These findings suggest that the two polyherbal formulations possess appreciable antioxidant potentials which could be attributed to the presence of phytochemicals with antioxidant potentials. The polyherbal formulations can further be explored for possible harnessing of their antioxidant effect in prevention and management of oxidative stress-related disorders and ageing process.

Keywords: polyherbal formulations, phytochemicals, antioxidants, DPPH, FRAP, TAC

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INTRODUCTION

Free radicals such as reactive oxygen species and reactive nitrogen species are endogenously generated when there are leakages of electron in the mitochondrial electron transport system, peroxisomes, xanthine oxidase activity, inflammatory processes and phagocytosis by immune cells, arachidonate pathways, and during strenuous physical activity [1]. Exogenously, free radical production is promoted by smoking, radiation, environmental pollutants, agricultural chemicals such as herbicides and pesticides, industrial chemicals such as solvents, wastes and drugs, and ozone. The detrimental effects of oxidative stress, an imbalance between the body's antioxidant defence system and pro-oxidant levels in favour of the pro-oxidants, on the cells demands the support of inherent antioxidants with dietary antioxidants. This is to prevent the development of oxidative stress-associated disorders such as cardiovascular and neurological disease, and cancers[2]. Dietary antioxidants include carotenoids, ascorbic acid, tocopherols, and phytochemicals[3].

Herbal formulations are plant-derived products applied for medicinal or nutritional purposes. They include home-made tisanes, nutritional and body-care commodities. Herbal medicine and various types of plant-based therapeutic/ prophylactic products have been available for centuries and applied in the treatments of diseases throughout history [3]. The rise in global population, inadequate supply, high cost and side effects associated with of orthodox drugs, and the development of resistance by causative agents of many infectious diseases to many of the currently-available orthodox drugs have promoted the wide use of medicinal plants for the prevention and treatment of many human diseases [4]. Medicinal plants and formulations made from them usually contain phyto-constituents with biological

activity, and hence could be useful for the therapeutic purpose.

Nigeria has rich plant diversity and many of the plant species are useful in folkloric practice for the treatment of many disease conditions. In this study, the phytochemical constituents and the antioxidant activities of two retailed Nigerian polyherbal formulations were evaluated. The idea of combining herbal products in a formulation is to harness the synergistic and additive effects of the various plant components to achieve higher bio-pharmacological effects. The first polyherbal formulation to be studied is Deep Root herbal mixture (DRHM)®. It is an oral preparation manufactured by FESCO Herbal Mixture Nigerian Limited, and is widely retailed in most parts of Nigeria and other parts of Africa. DRHM® is registered by National Agency for Food and Drug Administration and Control (NAFDAC) with registration number A7-0912L. It is indicated for malaria and typhoid fever, hepatitis, gonorrhea, fibroid, *Staphylococcus aerus*, syphilis, *Escherichia coli*, menstrual problems, low sperm count, blocked fallopian tubes, weak erection and other problems of reproductive system, poor eye sight, rheumatism and arthritis. It is also indicated for the regulation of blood pressure and blood sugar level. DRHM is an aqueous blend of *Cymbopogon citratus* (13%), *Carica papaya leaves* (12%), *Mangifera indica* bark (11%), *Moringa oleifera* leaves (11%), *Citrus limonia* (9%), *Psidium guajava* (9%), *Zingiber officinale* root (9%) and *Allium sativum* (6%). The second polyherbal formulation to be studied is Dr. Iguodo Goko Cleanser Herbal Mixture (GCHM)®, with NAFDAC registration number, A7-0804L. This formulation is a blend of *Vernonia amygdalina* (12 %), *Saccharum officinarum* (11.5%), *Allium sativum* (13%), *Cajanus cajan* (11.5%), and *Zingiber officinale* (0.5%) and caramel (1.5%) in water. Similarly to the first formulation, the indications on this polyherbal formulation suggest that it has antioxidant effect. It is therefore, pertinent that the antioxidant activities of the two polyherbal formulations are evaluated to provide scientific-based findings that will help consumers make evidence-based decisions on the potency and use of these formulations.

MATERIAL AND METHODS

Polyherbal formulations

The Nigerian polyherbal formulations used in this study include Goko Cleanser Herbal Mixtures (GCHM®) and Deep Root Herbal Mixture (DRHM®). They were purchased from reputable drug stores in Nsukka, Enugu State, Nigeria.

Phytochemical Analyses

The method of Harborne [5] and Trease and Evans [6] were adopted for the phytochemical constituents of the polyherbal formulations.

In vitro Antioxidant Assays

Assay of total antioxidant capacity: The total antioxidant capacities (TACs) of the polyherbal formulations were assessed using the phosphomolybdate method previously reported [7]. Aliquot (0.1 ml) of various concentration of the polyherbal formulations (100, 80, 60, 40, and 20 m*g*/L) was mixed with 1 ml of reagent solution which is composed of 600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in the ratio of 1:1:1 v/v) in well labelled test tubes. The test tubes containing the mixtures were covered with aluminium foil and incubated at 95°C for 90 min in a water bath. They were thereafter cooled to room temperature and the absorbances of the mixtures were read at a wavelength of 765 nm against a blank containing 1 mL of the reagent solution only. Ascorbic acid was used as standard and test was done in triplicate. The total antioxidant capacity (TAC) was expressed as mg equivalents of ascorbic acid per gram (EAA/g). The antioxidant capacity of each polyherbal formulation was calculated as follows:

Total antioxidant capacity (%)

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2,2–diphenyl–picrylhydrazyl (DPPH) radical scavenging assay: The free radical scavenging activities of the polyherbal formulations were determined using the DPPH model as previously reported [8,9]. One hundred milligram of each of the polyherbal formulations was mixed with 100 ml of methanol to form stock solutions (1 mg/ml or 1000 µ*g*/ml). Serial dilutions (10, 25, 50, 100, 250 and 500 m*g*/L) of the polyherbal formulations was made from the stock solution. DPPH solution (3 ml of 4.5 mg/100 ml of methanol) was added to 1.0 ml of the serially dilutions/ concentrations of polyherbal formulations and the mixtures were incubated in the dark at room temperature for 30 min. The absorbance of each mixture was read at a wavelength of 517 nm against a blank. Ascorbic acid was used as a standard and the test was done in triplicate. The percentage inhibition was determined using the formula:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Assay of the ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power of the polyherbal formulations were determined according to the method described by Sahreen *et al.* [10]. Briefly, 2 ml of the herbal drugs was separately mixed with 2 ml of 0.2 M phosphate buffer (pH 6.6) and 2 ml of 10 mg/l potassium ferricyanide (0.1% (w/v) solution. The mixture was incubated in a water bath at 50°C for 20 min. Two milliliters of 100 mg/l trichloroacetic acid solution (10% (w/v) was then added and an aliquot of 2 ml from the mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1 % (w/v) ferric chloride ($FeCl_3.6H_2O$) solution. A set of standard solutions of garlic acid (20, 40, 60, 80 and 100 mg/L) were prepared similar to that for in like manner as described for the polyherbal formulations. The absorbance of the mixture was read against a reagent blank at a wavelength of 700 nm (UV/VIS-1800 spectrophotometer, Shimadzu, Japan) after 10 mins of incubation. This test was done in triplicate and expressed as milligrams of garlic acid equivalent (GAE) per gram of the polyherbal formulations.

Statistical analysis

Data were analyzed using Graph Pad Prism and student T-test was used to compare the results of the two polyherbal formulations. Values with $p < 0.05$ were considered statistically significant.

RESULTS

Phytochemical constituents of the polyherbal formulations

Result of the phytochemical constituents of polyherbal formulations (DRHM® and GCHM®) are shown in Table 1. The presence of alkaloids, steroids and terpenoids, glycosides, anthocyanins, anthraquinones, saponins, flavonoids, tannins, phenols and carotenoids were detected in both polyherbal formulations. Similarly, aside for saponins, glycosides, anthraquinones and anthocyanins which were higher in DRHM® and alkaloids which was higher in GCHM®, the two polyherbal formulations appear

to have no significant ($p > 0.05$) difference in the amount of other phytochemicals detected. In addition, alkaloids, steroids and terpenoids are the most abundant phytochemicals in the two polyherbal formulations.

Table 1: Phytochemical constituents of the polyherbal formulations

Phytochemicals	Amount (%) in DRHM®	Amount (%) in GCHM®
Saponins	0.40 ± 0.06[b]	0.10 ± 0.02[a]
Tannins	0.03 ± 0.01[a]	0.04 ± 0.01[a]
Alkaloids	3.50 ± 0.82[a]	5.00 ± 0.77[b]
Flavonoids	0.18 ± 0.03[b]	0.10 ± 0.08[a]
Glycosides	0.50 ± 0.08[b]	0.32 ± 0.05[a]
Terpenoids	1.00 ± 0.03[a]	1.00 ± 0.04[a]
Phenols	0.22 ± 0.06[a]	0.24 ± 0.05[a]
Steroids	1.04 ± 0.03[a]	1.03 ± 0.07[a]
Carotenoids	0.11 ± 0.01[a]	0.12 ± 0.02[a]
Anthraquinones	0.43 ± 0.05[b]	0.33 ± 0.07[a]
Anthocyanins	0.46 ± 0.08[b]	0.22 ± 0.03[a]

Data represent mean ± standard deviation of triplicate determinations. Values with different superscripts in a row are significant at $p < 0.05$.

DPPH radical scavenging effects of the polyherbal formulations

Table 2 represents the results of DPPH radical scavenging assay of the polyherbal formulations. The effective concentration that can inhibit 50% (EC_{50}) of DPPH radical was calculated to be $1.62x10^6$ µg/ml and 574.5 µg/ml for DRHM® and GCHM® respectively. These values are significantly ($p < 0.05$) higher than that of standard antioxidant, ascorbic acid (with EC_{50} = 10.58 µg/ml). Meanwhile, the DPPH radical scavenging effect exhibited by DRHM® is significantly ($p < 0.05$) higher than that by GCHM® but lower than that exhibited by ascorbic acid.

Table 3: DPPH radical scavenging effects of the polyherbal formulations

Concentration (µg/ml)	% inhibition by DRHM®	% inhibition by GCHM®
31.25	97.25[b]	79.57[a]
62.5	95.36[b]	89.28[a]
125	93.91[b]	86.09[a]
250	91.16[b]	75.65[a]
500	84.64[b]	48.84[a]
	$EC_{50} = 1.62x10^6$ (µg/ml)	EC_{50} = 574.5 (µg/ml)
EC_{50} of standard (Ascorbic acid) = 10.58 µg/ml		

Values with different superscripts in a row are significant at $p < 0.05$.

Total antioxidant capacity (TAC) of the polyherbal formulations

The effective concentration that can inhibit 50% (EC_{50}) of the radical was calculated to be 6.101 µg/ml and 1675 µg/ml for DRHM® and GCHM® respectively. The EC_{50} value for GCHM® is significantly ($p < 0.05$) higher than that of standard antioxidant (ascorbic acid) with EC_{50} value of 10.58 µg/ml while ascorbic acid has higher EC_{50} than DRHM®. Similarly, the TAC of GCHM® is significantly ($p < 0.05$) higher than that by DRHM® (Table 3).

Table 3: Total antioxidant capacity of the polyherbal formulations

Concentration (µg/ml)	TAC in AAE for DRHM®	TAC in AAE for GCHM®
31.25	6.929[a]	10.357[b]
62.5	6.107[a]	9.964[b]
125	4.857[a]	9.893[b]
500	5.098[a]	12.884[b]
1000	4.732[a]	7.29[b]
	EC_{50} = 6.101 (µg/ml)	EC_{50} = 1675 (µg/ml)
EC_{50} of standard (Ascorbic acid) = 10.58 µg/ml		

Values with different superscripts in a row are significant at $p < 0.05$.

Ferric reducing antioxidant power (FRAP) of the polyherbal formulations

The result of the FRAP assay (in Gallic acid equivalent - GAE) for the polyherbal formulations is shown in Table 4. The effective concentration that can inhibit 50% (EC_{50}) of the radical was calculated to be 5.302 µg/ml and 0.005414 µg/ml for DRHM® and GCHM® respectively.

Table 4: Ferric reducing antioxidant power of the polyherbal formulations

Concentration (µg/ml)	FRAP (GAE) for DRHM®	FRAP (GAE) for GCHM®
15.63	2.06[a]	2.03[a]
31.25	1.07[a]	1.02[a]
62.5	0.60[a]	0.57[a]
125	0.38[b]	0.30[a]
250	0.25[b]	0.18[a]
500	0.17[b]	0.11[a]
1000	0.28[b]	0.07[a]
	EC_{50} = 5.302 (µg/ml)	EC_{50} = 0.005414 (µg/ml)

DISCUSSION

In the present study, generally DRHM® exhibited higher DPPH radical scavenging effect than GCHM®. The DPPH radical scavenging assay provides information on the radical-reducing capacity of test compounds to a stable free radical, DPPH. Due to the presence of unpaired electrons, DPPH radical absorbs maximally at a wavelength of 517 nm in visible spectroscopy (with a deep violet colour). In the presence of free radical scavenger (an antioxidant with reducing effect – ability to donate an electron and remain stable), the unpaired electron in DPPH® becomes paired causing the disappearance of absorption in that wavelength. The decolorization is proportional to the number of electrons taken up by DPPH radical. Thus, the ability of antioxidants to scavenge stable and highly reactive free radicals is a measure of their potencies [11]. Oxidative stress generated by free radicals has been linked with the development of diseases such as cancers, cardiovascular, cerebrovascular and neurodegenerative disorders, and diabetes mellitus [12]. Antioxidant compounds like polyphenolics and other phytochemicals help in scavenging free radicals and in inhibiting lipid peroxidation they induce [13]. It was observed in this study that the DPPH radical scavenging activities of the two polyherbal formulations are lower compared to that of standard antioxidant (ascorbic acid) used. This lower antioxidant activity might be attributed to low amount of polyphenolic compounds in the polyherbal preparations. The antioxidant activity exhibited by these polyherbal formulations could be attributed to the presence of some of the herbal constituents such as *M. oleifera* [14], *Z. officinale*[15] and *V. amygdalina* whose antioxidant activities are established. The result of the DPPH radical scavenging activities of these polyherbal formulations is in line with the reports of Vyas *et al.*[16], Wrigh *et al*. [17] and Bamidele *et al*. [18], and Omoregie *et al.*[13] who reported antioxidant activity and DPPH radical scavenging activity of *M. oleifera*, and *V. amygdalina* respectively.

Total antioxidant capacity (TAC) assay involves single electron transfer; this was done to evaluate the reducing capacity of the polyherbal formulations. It was observed that DRHM has higher % TAC compared with GCHM® and ascorbic acid (standard antioxidant used for this study. The variation in the % TAC could be attributed to differences between the plant components and percentage composition in the two polyherbal formulations. The antioxidant capacity observed in the two polyherbal formulations could be linked with the existence of antioxidant secondary plant metabolites such as tannins, flavonoids, phenols, carotenoids and anthocyanins detected. Scientific report has shown that there is a strong correlation between antioxidant capacity and phenolic contents [19]. This study showed that the polyherbal formulations contain low contents of phenolics and other antioxidant compounds which may explain why they have lower antioxidant capacity when compared to ascorbic acid (the standard antioxidant used in this study). *In vivo* studies on *Allium sativum* by Rahman *et al*. [20] and *Z. officinale* by Anosike *et al*. [21] and Tohma *et al*. [22] revealed the antioxidant activity of these plants. These plants are components of the two polyherbal formulations and may be responsible for the observed antioxidant effects.

Iron (Fe) accumulation enhances iron-mediated free radical production that might result in oxidative stress, which is associated with neurodegenerative diseases and ageing processes [23]. The principle of determination of the reducing powers of the two polyherbal formulations by FRAP is based on their capacity to reduce ferric ion (Fe(III)) to ferrous ion (Fe(II)) by their ferric reducing antioxidant power (FRAP). This result follows the same trend as the DPPH radical scavenging activity as GCHM® had higher FRAP than DRHM. The risks to oxidative stress and associated diseases could be prevented or reduced by use of iron chelators [23]. Metal-chelating antioxidants prevent transition metals from participating in the initiation and propagation of lipid peroxidation and hence, inhibit the development of oxidative stress that is detrimental to cells and tissues of the body [24].

CONCLUSION

The findings of this study demonstrated that the two polyherbal formulations (GCHM® and DRHM®) possess appreciable antioxidant potentials which could be attributed to the presence of phytochemicals that are previously reported to have antioxidant activities in the two polyherbal formulations. Further study is warranted to establish the antioxidant effects of these polyherbal formulations *in vivo*. In addition, the possibility of heavy metal and persistent organic pollutants existence should be evaluated while long term safety of the polyherbal formulations should be assessed.

Conflict of interest: The authors declare none.

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