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Comparative Physicochemical and Antioxidant Activities of Eight Commercially Available Antidiabetic Polyherbal Products

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: High cost and side effects of current drugs has led to increased use of herbal preparations for Diabetes Mellitus, which is a chronic multifactorial disease associated with several complications and more than 422 million people affected worldwide.
Objectives: To comparatively evaluate physicochemical and antioxidant properties of eight commercially available antidiabetic polyherbal products.
Material and methods: Samples were subjected to physicochemical analysis; organoleptic tests, uniformity of weight, thin layer chromatography (TLC), phytochemical screening, heavy metals, extractive values, moisture content, ash values, antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition and total phenolic content (TPC). Statistical analysis was done at $p < 0.05$.
Results: The samples which ranged from tablets, capsules to tea sachets were of varying colour, odour and taste, with obtained average weights significantly different from the labelled weight for most products The TLC analysis gave varied number of spots with the identified phytochemicals (tannins, flavonoids, phenols and proteins) confirmed by phytochemical screening. Moisture contents ranged from 5.99 to 8.84 %w/w, with water and alcohol-soluble extractives 13.0-27.7%w/w and 15.4-47.5 %w/w respectively. Lead, arsenic and cadmium contents were within WHO specification. Total ash, water-soluble ash and acid-insoluble ash ranged from 1.27 ±0.02 to 42.40 ±0.04; 0.36 ±0.01 to 37.65 ±0.05 and 0.29 ±0.02 to 7.10 ±0.03 %w/w respectively. The TPC ranged from 0.015 ±0.006 to 0.277 ±0.006 mgGAE/mL with DPPH scavenging activity (IC_{50}) ranging from 8.87 ±0.113 to 825.24 ±2.03 µg/mL.
Conclusion: Only two brands complied with all the WHO herbal products specifications, while varied antioxidant activities were observed across all the polyherbal products.

Keywords: Antidiabetic polyherbal products, Phytochemical screening, Antioxidant activity, DPPH inhibition, Total phenolic content

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder which is characterized by hyperglycemia (excessive hepatic glycogenolysis and gluconeogenesis) as a result of deficiency in insulin production by the pancreas or resistance to insulin (Hung *et al*., 2012). It is a chronic multifactorial disease that's associated with several complications. The prevalence of this disease is globally wide spread with 422 million people affected as at 2014 and a projection of 629 million by 2045 if unchecked (World Health Organization, 2019).

Diagnosis of diabetes mellitus based on their different etiology is classified into six major categories: Type 1 (insulin-dependent diabetes), Type 2 (non-insulin-dependent diabetes), Hybrid forms, other specific type, unclassified diabetes and Gestational DM (World Health Organization, 2019).

Although several classes of synthetic drugs (singly or combinations) are available for the management of DM, they are mostly accompanied by various limitations; high cost and associated side effects such as hypoglycemia, weight gain, gastrointestinal disturbance, liver toxicity etc. (Sahu *et al*, 2016). As a result of the associated side effects and other issues with the current therapies many diabetic patients resort to herbal therapies either in conjunction with their orthodox drugs as adjunct therapy or alone, most of the time without the knowledge of their physicians (Alqathama *et al*, 2020).

Plants have been the main source of medicines through the ages as they contain various phytochemicals which boost various organ functions thereby reducing occurrence of several diseases. (Bharati *et al*, 2016). Numerous traditional herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure several human diseases including DM (Bharati *et al*, 2016).

The hypoglycemic or antidiabetic activities of several plants used folklorically have been confirmed scientifically, these includes: *Allium cepa* (Onion), *Allium sativum* (Garlic), *Aloe vera, Cinnamomum cassie, Coccinia indica, Gymmema slyvestre* (Gurnar), *Momordica charantia* (Bitter Melon), *Catharanthus roseus* (Madagascar Periwinkle), *Muurrayi komingii, Ocimum sanctum, Panax ginseng, Trigonella foemum-graecum* (Fenugreek) *Pterocarpus marsupium* (Indian Kino) *and Syzigium cumini,* etc. (Chikezie *et al*, 2015; Gbolade, 2009). However, most herbal remedies including antidiabetics are usually multicomponent (Gbolade, 2009; Ogbonnia *et al*, 2010)

The presence of phytochemicals such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., has been associated with antidiabetic property of plants. Therefore, herbal preparations are gaining popularity in the management of DM in many countries, as they are frequently considered to be less toxic, free from side effects and having good antioxidant properties than synthetic compounds (Sahu *et al*., 2016).

Commercial production of herbal medicines and their trade is a fast-growing industry globally due to increasing demand of medicinal plants (Yadav *et al*., 2011). There has been a recent phenomenal surge in the number of commercial advertisements, promotion, and trado-medical fairs on herb-based products globally and particularly in Nigeria. These phytomedicinal products are often promoted to the public as being "natural" and completely "safe". Thus, herbal drugs are gaining popularity in the treatment of various diseases including diabetic mellitus in many countries.

Despite the invaluable contribution of herbal therapy in healthcare, there have been many controversies with regulation, safety and standardization (Nworu *et al*., 2014). The increasing patronage of commercially promoted herbal medications has shown the need for safety and quality assessment of these products.

Major hindrance to integration of herbal medicine to modern medical practices is lack of scientific and clinical data proving their efficacy and safety. Several physicochemical analytical methods have been proposed for the assessment of quality and safety of herbal therapies. Although, therapeutic effects, cannot be fully determined by these analytical methods, total phenolic acid contents and antioxidant capacity can be linked to some therapeutic efficacy.

Etiology of some diseases such as cancer, diabetes, dementia and myocardial infection have been linked to free radicals produced as a result of various metabolic processes taking place in the body due to the interaction of the free radicals with cellular DNA (Mohamed *et al*, 2011). Thus, antioxidants have reportedly found critical application in neutralizing these free radicals thereby reversing or arresting the progression of such disease conditions. Several phytochemicals have been reported to possess significant antioxidant activities which are linked to their pharmacological action and basis for their therapeutic efficacies in diabetes mellitus, cardiovascular diseases, etc. (Asadbeigi *et al*, 2014).

Antidiabetic properties of some herbal products containing single and combination of plant extracts have been reported to support their use in herbal medicine globally with numerous mechanisms of actions proposed for these plant extracts. Some antidiabetic polyherbal drugs of Ayurvedic medicine have been reported and considered to be effective in the management of diabetes (Bera *et al*, 2010). While there have been many reports on the bioactivity of the different plants used in the treatment of diabetes (Chikezie *et al*., 2015), very limited scientific

experimental data have been reported to confirm the quality, safety and efficacy of herbal formulations available for sale in Nigeria market against their label claim till date (Nworu *et al.*, 2014). Many polyherbal antidiabetic formulations (imported and locally made) are now widely available within the country (Nigeria) with little or no scientific data supporting their claims of safety and efficacy.

Thus, there is the need to evaluate these herbal products since the verification of their claimed efficacy. These realities and a concern for public health safety informed the present study aimed at comparative quality evaluation of eight commercially available antidiabetic polyherbal products; imported and locally produced.

This study reports the comparative inequality of the physicochemical and antioxidant properties of eight commercial herbal formulations distributed within the Southwestern part of Nigeria. The herbal products were evaluated based on physicochemical parameters specified by WHO, while antioxidants properties were based on standard procedures.

METHODOLOGY

Chemicals and Reagents

Eight commercially available herbal products indicated for treatment of diabetes mellitus were purchased from herbal vendors in Southwestern part of Nigeria (Table 1). Analytical grade chemicals and reagents were used as supplied.

Profile of the herbal products

The labelled information in the various samples; coded brand name, country of manufacture, batch number, component herbs, National Agency for Food Drug Administration and Control (NAFDAC) registration status and dosage forms were noted (Table 1).

Physicochemical evaluation of the herbal products

O*rganoleptic properties*: The appearance, colour, odour, texture and taste of the herbal product were evaluated by three assessors.

Thin layer chromatography analysis (TLC): This was carried out using Silica gel GF_{254} as stationary phase and two mobile phases; M_A [Chloroform: Ethylacetate: acetic acid (9.9: 9.9: 0.2)] and M_B [Chloroform: Methanol: Water (15.2: 4.6: 0.2)]. Visualization was done using daylight, ultraviolet light (254 and 365nm), iodine vapour and vanillin-sulphuric spray reagent.

Weight uniformity determination **(International Pharmacopoeia, 2019)**

Tablet dosage form: Twenty randomly picked tablets were weighed singly and together to determine the uniformity of weight.

Capsule dosage forms: Twenty randomly picked capsules were weighed singly. The content of each was removed as completely as possible and the empty capsule shell weighed. The difference in weight between of the intact capsule and the shell gives the weight of the content. Weight of the pooled content were used to determine average weight and uniformity of weight.

Teabag dosage forms: Twenty sachets of each brand of herbal powder products picked at random were weighed singly. The contents were emptied as completely possible and the weight of individual sachet determined. The difference in weight between of the intact and empty teabag gives the weight of the content. Weight of the pooled content were used to determine average weight and uniformity of weight.

Qualitative phytochemical screening

Ethanol solution of the coarsely powdered sample (2 g) was prepared by maceration in 50ml ethanol (80 %v/v) in a closed flask for 24 hours; shaken frequently during 6 hours and allowed to stand for 18 hours. The sample was filtered to obtain the ethanol solution used to test for saponins, alkaloids, tannins, cardenolides, anthraquinones, flavonoids, terpenoids, phenols, proteins and carbohydrates according to conventional methods (Sofowora, 2008).

Ash value determination (World Health Organization, 2011, Ayurvedic Pharmacopoeia, 2016)

Total ash: Sample (2 g) was weighed into previously weighed tarred silica dish and incinerated at a temperature not exceeding 450 ºC for 5 hours, until it is carbon free. The residue was cooled, weighed and the percentage of total ash with reference to the air-dried drug was calculated using equation 1:

$$\%\, Total\ Ash = \frac{Wa}{Ws}\, x\, 100 \qquad -1$$

where Wa and Ws refer to the weights of ash and air-dried sample respectively

Water soluble ash: Ash obtained in total ash determination was boiled in water (25 ml) for 5 minutes, insoluble matter was collected on an ashless filter paper (Whatman 41) and washed with hot water

followed by ignition for 15 min at a temperature not exceeding 450 ºC to obtain the residue. The percentage water-soluble ash with reference to the air-dried drug was calculated using equation 2;

$$\%\ water\ soluble\ ash = \frac{W_a - W_i}{W_s} x100 \quad -2$$

Where Wa, Wi and Ws refer to the weights of ash, insoluble matter and air-dried sample respectively

Acid insoluble ash: To another sample of total ash was added drop-wisely 25 ml of dilute hydrochloric acid and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to a crucible, dried to constant weight on hot plate, cooled in a desiccator for 30 min and weighed. Acid insoluble ash with reference to the air-dried drug was calculated using equation 3;

$$\%\ Acid\ insoluble\ ash = \frac{Wai}{Ws}\ x\ 100 \qquad -3$$

Where Wai, and Ws refer to the weights of insoluble ash and air-dried sample respectively

Moisture content determination (World Health Organization, 2011): Herbal sample (3 g) was weighed onto tared evaporating dish (previously dried in the oven) and dried at 105 ºC for 1 hour and weighed. Drying and weighing continued at one-hour interval until difference between two successive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator was not more 0.01 g. Percentage moisture content was calculated using equation 4;

$$\%\ Moisture\ Content = \frac{Ws - Wd}{Ws}\ x\ 100 \qquad -4$$

Where Ws and Wd refer to the weights of sample and dried sample respectively

Extractive values

This was determined based on Ayurvedic Pharmacopoeia, 2016 as follows;

Water soluble extractive (WSE) values: coarsely powdered sample (2 g) was macerated with 100 ml of chloroform water (0.25 %w/v) in a closed flask for 24 hours; shaken frequently for first 6 hours, allowed to stand for 18 hours and filtered. The filtrate (25 ml) was evaporated to dryness on a petri dish, dried at 105 ºC and weighed. Percentage water-soluble extractive was calculated using equation 5 with reference to the powdered sample;

$$\%\ WSE\ value = \frac{(Wad - Wpt)}{Ws\ x\ Vf}\ x\ 100\ x\ 100 \quad -5$$

Where Wad, Wpt, Ws and Vf refer to weight after drying, weight of petri dish, weight of sample and volume of filtrate respectively.

Alcohol-soluble Extractive (ASE) value: coarsely powdered sample (2 g) was macerated with 50 ml of *ethanol* (80 %v/v) in a closed flask for 24 hours; shaken frequently during first 6 hours, allowed to stand for 18 hours and filtered rapidly, taking precautions against loss of solvent. The filtrate (25 ml) was evaporated to dryness on a petri dish, dried at 105 ºC and weighed. Percentage alcohol-soluble extractive was calculated using equation 6 with reference to the powdered sample;

$$\%\ ASE\ value = \frac{(Wad - Wpt)}{Ws\ x\ Vf}\ x\ 100 \qquad -6$$

Where Wad, Wpt, Ws and Vf refer to weight after drying, weight of petri dish, weight of sample and volume of filtrate respectively.

Heavy metal determination: The samples were analyzed for lead, mercury, arsenic and cadmium using atomic absorption spectrometry (Ayurvedic Pharmacopoiea, 2016).

Total phenolic content determination

This was determined using Folin-Ciocalteau method (Adegbolagun et al, 2017).

Ethanol solution (50 ml) of the sample as prepared in phytochemical screening was used for this determination.

To 100 µL of the sample was added Folin-Ciocalteau reagent (100 µL, 500 mg/L), mixed properly and incubated in the dark for 2 minutes. This was followed by the addition of Na_2CO_3 solution (2 ml, 0.2 % w/v), the mixture was allowed to stand in the dark for 30 minutes at 25 ºC after which the absorbance was determined at 750 nm using UV-VIS spectrophotometer (Perkin Elmer, Lambda 25, Singapore) against a reagent blank. The total phenolic acid (TPA) content was determined using the standard Gallic acid calibration curve (0.025 – 0.250 mg/ml) and the result expressed as mg GAE/mL. The procedure was repeated in the absence of the sample to obtain the blank reading. The determinations were done in triplicates.

Determination of antioxidant activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay was used to determine the antioxidant capacity (Mensor *et al*, 2001). Solutions of the sample in ethanol as prepared for phytochemical screening was used for this determination. One milliliter of 0.3 Mm DPPH reagent in methanol was added to 2.5 ml of the different concentrations (0, 5, 10, 15, 20, 25, 30, 35 µg/ml) of the sample solutions and mixed properly. This was followed by incubation at room temperature for 30 minutes after which the absorbance of the mixtures was determined at 520 nm using UV/Visible spectrophotometer (Perkin Elmer, Lambda 25, Singapore). Mixture of methanol (2.5 ml) and 1 ml of DPPH subjected to the same condition was used as negative control. The procedure was repeated with gallic acid (standard) at similar concentrations. The determinations were done in triplicate for each sample preparation.

The DPPH radical-scavenging activity was calculated using the following equation;

% DPPH scavenging = [Abs (control) – Abs (sample) /Abs (control)] x 100

Where Abs (control) is the absorbance of the negative control reaction; containing all the reagents except the test compound, while Abs (sample) is the absorbance of the sample.

Statistical analysis

Results were presented as mean ±standard deviation (SD). Statistical differences between test and control treatments were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The increasing demand in the consumption of herbal products as result of perceived safety, high cost and adverse effects of orthodox medicines, has contributed immensely to making commercial production of herbal medicines a fast-growing industry (Yadav *et al*., 2011). Furthermore, the recent surge in commercial advertisements, promotion, and trado-medical fairs on herb-based products with various sometimes spurious claims globally and particularly in Nigeria calls for appropriate post market quality evaluation and control. Hence, this report on the physicochemical and antioxidant assessment of eight brands of commercially available herbal products indicated for the management of diabetes mellitus in the Southwestern area of Nigeria.

The eight antidiabetic herbal products were within their indicated shelf life as at the time of the study, with their profile showing that only four brands (NCH, GSC, PHF and PHD) were registered with NAFDAC (Table 1). Of all the samples, three brands each were tablets (NCH, PHF and DT) and teabags (PHD, ADT and NBS), while two brands (GSC and SN) were capsule dosage forms. The obtained average weight per brand for tablets and capsules ranged from 0.300 ±0.035 to 0.561 ±0.053, while the three teabags were 1.291 ±0.047 to 1.992 ±0.190 g. However, there was significant ($p < 0.05$) differences between the observed average weights and labelled weights across all the samples (Table 2), this indicates inconsistences in the herbal contents.

Herbs are specific types of plants that are known for their scent, taste and colour. There are numerous varieties of herbs, each with distinct attributes that can be useful for identification purpose, although there are also characteristics that are shared by all herbs (Anuj *e al*., 2014). Thus various organoleptic properties obtained for the herbal products under study (Table 2) are characteristic of herbal products which maybe useful for identification of the individual products. The samples had varied characteristic odour, colour (greyish white to greenish brown) and degree of bitterness except GSC which was tasteless (Table 2). The obtained variation in the organoleptic properties; taste, colour and odour is an indication of the different herbal extracts combined in the formulation of the herbal products which is expected to be characteristic for each product (Table 1).

Table 1: ***Profile of eight brands of antidiabetic polyherbal products investigated***

Sample	Country	Batch no	NAFDAC No.	Component herbs (As indicated on the package)	Dosage form
NCH	Nigeria	MFG/0018	A7-2358L	*Azadirachta indica, Vernonia amygdalina, Aloe bitters*	Tablet
GSC	Nigeria	KDFMA1GS	A7-0466L	*Radix rehmannia preparata, Cortex montan, Fructus comi, Rhizome dioscoraea, Poria and Rhizomia alismatis*	Capsule
PHF	Nigeria	FMB0028GRW	A7-0201L	*Viscum album &* Flax seeds and extract of natural *Kaolin*	Tablet
DT	Nigeria	2016.10.0101	-	*Ginseng radix rubrie, Rehmannia glutinosa, Cortex phellodendri and Fructus lycil*	Tablet
SN	Nigeria	-	-	*Piper nigrum*	Capsule
PHD	Nigeria	-	A7-0196L	*Mangifera indica, Tridax procumbens, Viscum album, and Zingiber officinale*	Teabag
ADT	China	-	-	Not stated	Teabags
NBS	China	-	-	*Trichosanthis Radix, Lobed Kudzuvine Root, Stevia rebaudiana (Bertoni) Hemsl, Common Yam Rhizome, etc*.	Teabags

Thin layer chromatographic (TLC) profiling of all the samples in the different mobile phases showed a wide variation in the number of spots identified which cannot be directly linked with the number of component herbs on the product label. Chloroform: Ethyl acetate: Acetic acid (9.9:9.9:0.2) and Chloroform: Methanol: Water (15.2:4.6:0.2) mobile phases showed the highest number of components for most of the samples; SN, NBS, ADT and PHD samples gave the highest number of spots ranging from six to Nine (Table 2). The different resolved compounds detected using varying spray reagents confirmed the presence of various phytochemicals such as alkaloids, terpenoids, phenolic compound, tannins and flavonoids in the herbal products.

Table 2: Organoleptic properties, average weights and TLC spots of the eight antidiabetic polyherbal products

Sample	Labelled weight (g)*	Average weight (g ± SD) *	Organoleptic properties			No of TLC Spots	
			Colour	Taste	Texture	M_A	M_B
NCH	0.50	0.561 ± 0.053	Brown	Slightly bitter	Hard	3	3
GSC	0.25	0.394 ± 0.011	Grey white	None	Soft	2	2
PHF	0.30	0.35 ± 0.026	Ash	Bitter	Hard coarse	7	5
DT	0.30	0.345 ± 0.003	Yellowish-green	Bitter	Hard	2	6
SN	0.38	0.300 ± 0.035	Amber-yellow	Bitter	Soft and rough	10	9
PHD	2.20	2.032 ± 0.19	Dark-brown	Bitter	Soft and rough	8	6
ADT	2.00	1.623 ± 0.048	Greenish-brown	Slightly bitter	Soft and rough	9	7
NBS	2.00	1.291 ± 0.047	Greenish-brown	Bitter	Soft and rough	9	8

M_A – Mobile phase A - Chloroform: Ethylacetate: acetic acid [9.9: 9.9: 0.2]; M_B – Mobile phase B - Chloroform: Methanol: Water [15.2: 4.6: 0.2]; * - $p < 0.05$

Phytochemical screening showed the presence of tannins, flavonoids, saponins, steroid, alkaloids, terpenoids and phenolic compounds at various concentrations in the different samples (Table 3).

Table 3: Qualitative phytochemical content of eight antidiabetic polyherbal products

Phytochemicals	Samples							
	NCH	GSC	PHF	DT	SN	PHD	ADT	NBS
Proteins	-	+	-	++	-	-	++	++
Carbohydrates	+	++	+	+	+	+	+	+
Tannins	++	+	+	+	+	+++	+++	+++
Flavonoids	+	+	+	+	+	+	+	+
Saponins	++	-	+	-	++	+	++	++
Cardiac Glycosides	-	-	-	-	-	-	-	-
Steroids	+	-	-	-	+	-	+++	++
Terpenoids	+++	+	-	+	+	+	++	++
Alkaloids	-	+	+	++	+++	+	-	-
Anthraquinones	-	-	-	-	-	-	-	-
Phenols	+++	+++	+	++	++	+++	+++	+++

Key: + = *slight;* ++ = *moderately;* +++ = *intense*, - = *absent*

Herbs are rich in phytochemicals which have been linked to antioxidant properties and associated with various health benefits imparted by the herbs in general (Nobuji, 2000). Some of the previously reported phytochemicals with strong antioxidant activities such as alkaloids, glycosides, flavonoids, carelessness in preparing the drug or drug combinations for marketing (Anuj *et al*, 2014; Chandel *et al*, 2014). Total ash value for these polyherbal formulations investigated at 1.27 to 13.29 %w/w were within the WHO specification (< 14 saponins, polysaccharides, glycolipids, peptidoglycans, amino acids could be responsible for any antidiabetic claims reported for the investigated products (Gaikwad et al, 2014).

Ash value is aimed at identifying possible contamination, substitution, adulteration or

%w/w) except PHF (42.40 %w/w) (Figure 1). The high total ash obtained for PHF could be as a result of incorrect processing or contamination as one of the labelled constituent is an extract of kaolin; an inorganic silicate mineral found in the earth crust.

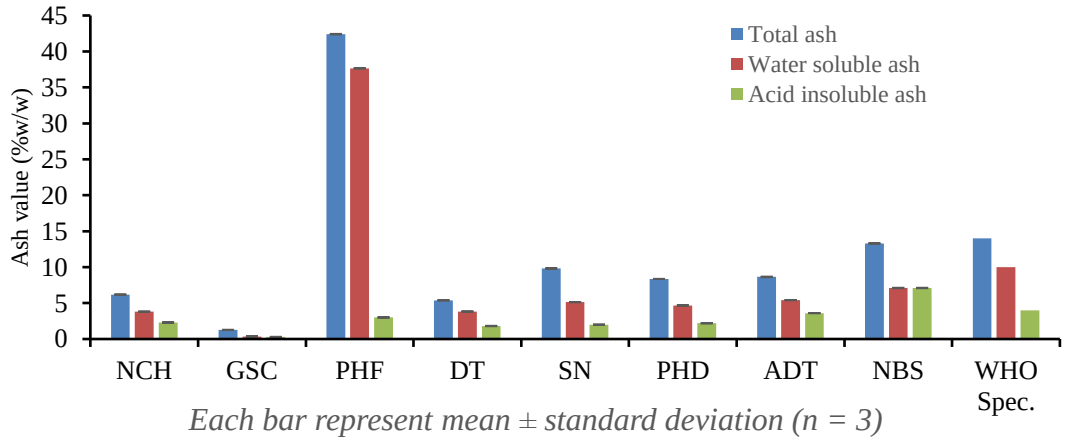


Figure 1: Total ash, water-soluble ash and acid-insoluble ash valuesof the eight polyherbal antidiabetic products

Water-soluble ash is the part of the total ash content, which is soluble in water and is an indicator water-soluble salts in the drug or incorrect preparation. As obtained with the total ash, the water-soluble ash values for all the brands varied from 0.36 ± 0.01 to 5.41 ± 0.02 %w/w, and were within the acceptable limit set by WHO (< 10 %w/w), except for the significantly high value ($p < 0.001$) obtained with PHF (37.65 ± 0.05 %w/w) (Figure 1) which could be explained by the high total ash obtained for the product. Furthermore, acid-insoluble ash which measures the amount of silicate present (sand and siliceous earth) is indicative of contamination, substitution, adulteration, or carelessness in preparation of drug combinations for marketing (Bele and Khale, 2011). The acid-insoluble ash values of the different formulations which ranged from 0.29 ± 0.02 to 3.60 ± 0.01 %w/w compiled with WHO limit (< 4 %w/w) except NBS with 7.10 ± 0.03 %w/w (Figure 1). The high acid insoluble ash obtained for NBS raises a concern in the processing method because though the total ash and water soluble values were within the specification, the obtained values were actually very high relative to the other brands except PHF.

Moisture contents of all the polyherbal formulations assessed ranged from 5.99 ± 0.03 to 8.84 ± 0.04 % w/w which were within the specification set by WHO (< 10 %w/w) (Figure 2). Moisture is one of the major factors responsible for the deterioration of the drugs and formulations, with low moisture content always desirable for stability of drug compounds including herbs and herbal products. Thus, all these herbal product brands are expected to be stable to deterioration due to microbial contamination growth.

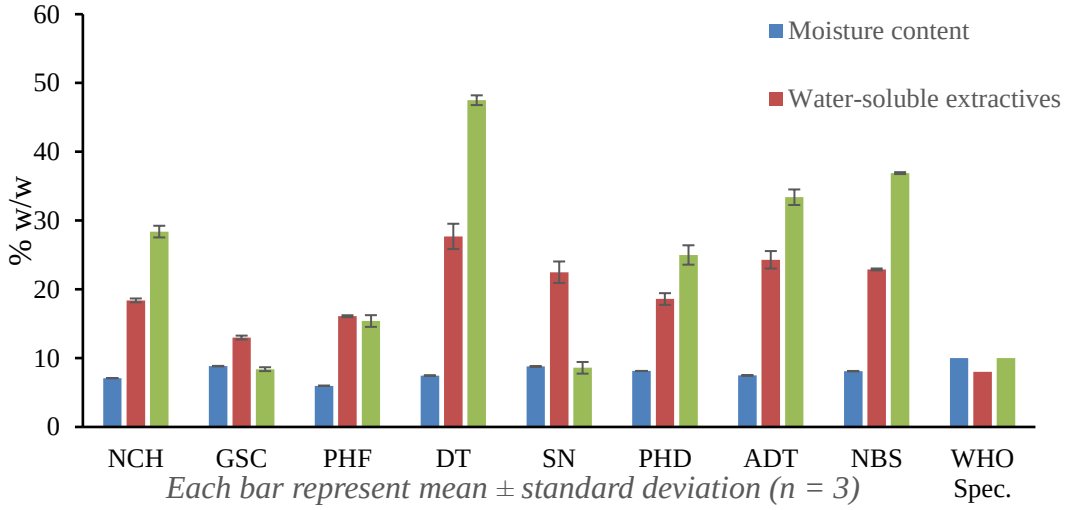


Figure 2: **Moisture content, water-soluble and alcohol-soluble extractive values of eight antidiabetic polyherbal products**

Water-soluble extractive value plays an important role in evaluation and quality control of crude drugs. Water-soluble and alcohol-soluble extractive for the different samples which were in the range of 13.00 ± 0.28 to 27.70 ± 1.84 %w/w and 8.40 ± 0.28 to 47.50 ± 0.71 %w/w respectively (Figure 2) complied with the WHO limit of not less than 8.0 %w/w and 10.0 %w/w respectively. Generally, alcohol-soluble extractive values of NCH, DT, PHD, ADT and NBS were higher than their water soluble extractive values, indicating more alcohol-soluble phytochemicals constituents, while water-soluble extractive values were the reverse with samples GSC, SN, and PHF. The alcohol soluble extractive value indicates the presence of polar constituents like phenols alkaloids steroids glycosides flavonoids (Junejo *et al*, 2014).

Medicinal plant materials used in herbal formulation are susceptible to contamination due to environmental pollution and traces of pesticides (Bele and Khale, 2011); which are considered dangerous to human health. It therefore becomes necessary to ensure their concentration do not exceed the limit set by the regulatory authority. The heavy metals; lead, cadmium and arsenic levels investigated were below the permissible limit allowed for heavy metals, while mercury was totally absent (Table 4), (World Health Organization, 2007; Ayurvedic Pharmacopoeia, 2016).

Table 4: Heavy metal content of the eight antidiabetic polyherbal products

Samples	**Trace metal (mg/Kg)**		
	Lead	**Arsenic**	**Cadmium**
NCH	1.05	1.50	0.000
GSC	5.75	0.75	0.000
PHF	3.50	1.50	0.001
DT	2.85	2.33	0.000
SN	3.25	1.83	0.004
PHD	6.20	1.67	0.004
ADT	2.05	2.50	0.006
NBS	3.05	2.11	0.000
WHO Spec.	10.00	3.0	0.300

Mercury was absent in all the samples

Free radicals produced as a result of various metabolic processes taking place in the body are important in the etiology of diseases like cancer, diabetes, dementia and myocardial infection as they interact with cellular DNA and cause its mutation (Mohamed *et al*, 2011). Hyperglycaemia has been reported to increase free radical production and impairs endogenous antioxidant defense mechanism (Nasri and Rafieian-Kopaei, 2014). Antioxidants which are highly rich in phytochemicals are responsible for the defense mechanism of organisms against the pathologies associated with the attack of free radicals, neutralizing these free radicals by donating required number of electrons to stabilize them. Once the free radicals are stabilized after the acceptance of electrons, they become non-reactive to cellular DNA (Enrique and Davies, 2000). Thus the intake of plant derived antioxidants has been linked to the prevention of degenerative diseases caused by oxidative stress, such as diabetes, cancer, Parkinson, Alzheimer or atherosclerosis (Shirazi *et al*, 2014). The percentage inhibition of DPPH (IC_{50}) is widely used as an indicator of the antioxidant and free radical scavenging power. The lower the IC_{50} value of an antioxidant the higher would be its free radical scavenging power (Shirazi *et al*, 2014). Although significant ($p < 0.001$) variation in free radical scavenging properties was observed within the herbal products; very low IC_{50} were observed in NBS, ADT and NCH indicating good antioxidant activity (Figure 3, Table 5).

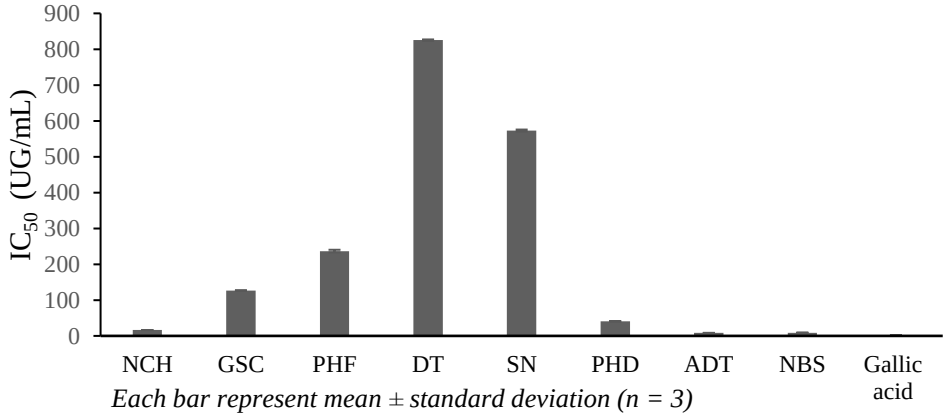


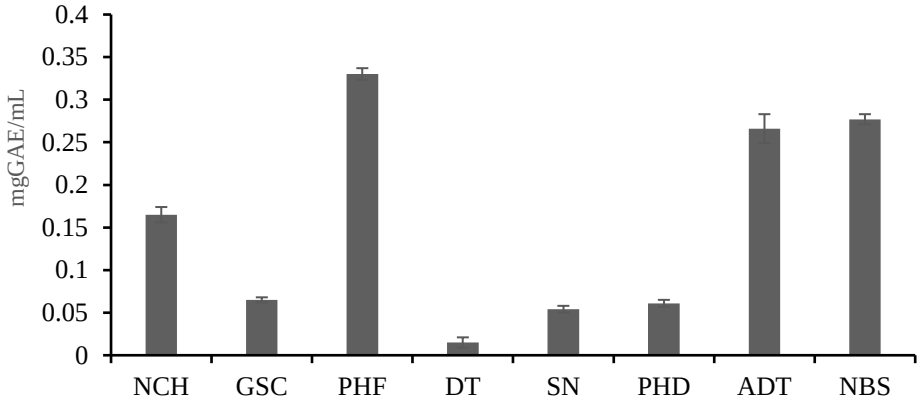
Table 2: Free radical scavenging activities of eight antidiabetic polyherbal

Table 5: Free radical scavenging properties (IC_{50}) and total phenolic content (TPC) of the eight antidiabetic polyherbal products

Sample	**Total phenolic acid (mg GAE/ml, ± S.D)**	**Free radical scavenging properties (IC_{50} µg/ml, ± SD)**
NCH	0.165 ± 0.009	16.214 ± 0.115
GSC	0.065 ± 0.003	126.476 ± 0.305
PHF	0.033 ± 0.007	263.583 ± 3.776
DT	0.015 ± 0.006	825.241 ± 2.025
SN	0.054 ± 0.004	573.044 ± 2.785
PHD	0.061 ± 0.004	40.558 ± 0.705
ADT	0.266 ± 0.017	8.303 ± 0.016
NBS	0.277 ± 0.006	8.870 ± 0.113
Gallic acid standard	-	1.329 ± 0.015

None of the samples with good antioxidant activities were comparable with the gallic acid standard (IC_{50}; 1.329 ±0.015µg/mL). This is in agreement with the high TPC observed in these samples, which is generally indirectly related with IC_{50}.

The obtained total phenolic acid content (TPC) from the Gallic acid calibration curve (y = 1.7512x + 0.0936, R^2 = 0.9963) ranged from 0.015 ± 0.006 to 0.277 ± 0.004 mg GAE/mL (Figure 4).



Each bar represent mean ± standard deviation (n = 3)

Figure 4: **Total phenolic acid content of eight polyherbal antidiabetic products**

Similar variation was observed with the antioxidant properties (IC_{50}) ranging from 8.303 ± 0.011 to 825.241 ± 1.432µg/ml respectively (Figure 4, Table 5). There seems to be a direct correlation between the antioxidant activities and total phenolic acid contents of the herbal products samples; NBS, NCH and ADT with high TPC had moderate antioxidant activities when compared with Gallic acid, while samples (DT, PHF, GSC and SN) with low TPC content showed weak activities. Established relationship between antioxidant activity and antidiabetic activity has been previously reported for plants and herbal products. Antioxidant activity have been reportedly linked with hypoglycaemic activity of many plants (Rahimi-Madiseh, *et al*, 2016).

The observed variations in the physicochemical and antioxidant activities among the different herbal products can be linked to the differences in the herbal extract components.

CONCLUSION

Of the eight antidiabetic herbal products investigated in this study, two (DT and ADT) complied with all the WHO specifications for herbal products, while the other samples defaulted in one or more specifications.

Also, the observed variations in the antioxidant activities propose potential differences in the antidiabetic activity of the herbal products.

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