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Ascorbic acid and mineral elements composition of powdered antimalarial (Maloff-HB) and haematinic (Haematol-B) herbal formulations from Ogbomoso, Nigeria

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Different types of powdered antimalarial and blood-enriching (haematinic) recipes are used in traditional health system of Southwestern Nigeria. Two of these from Ogbomoso (that is Maloff-HB and Haematol-B, respectively) were recently named following a quantitative definition of their botanical constituents. However, information on the physico-chemical and phytochemical properties as well as the residual constituents of both polyherbal formulations (PHFs) is lacking. The amount of ascorbic acid in them was therefore determined using ultraviolet (UV)-visible spectrophotometric method. Their elemental compositions (magnesium, calcium, manganese, iron, zinc, potassium, sodium and copper) were also quantified spectrophotometrically. Maloff-HB and haematol-B contain high quantities of ascorbic acid (mean values of 542.35 and 414.14 mg/100 g, respectively). This is the antioxidant that has been implicated in many redox reactions which promote good health. Both drugs are also rich in Mg (1319.04 and 2340.00 mg/100 g, respectively) and Ca (784.31 and 1011.67 mg/100 g, respectively), these values being comparable to the recommended dietary intakes. The values obtained for Fe/Cu and Fe/Zn ratios can promote bioavailability of these important mineral elements. The important role of ascorbic acid in enhancing iron absorption is discussed with the conclusion that the two drugs have the potential to meet some dietary requirements which promote healthy blood that prevents infections. The study recommends safety and efficacy evaluations of the two PHFs based on their residual constituents, *in vivo* activity and bioavailability of their beneficial constituents.

Key words: Antioxidants, ascorbic acid, herbal formulations, anti-malaria, mineral elements composition.

INTRODUCTION

World Health Organization defines herbal medicines as finished, labeled medicinal products that contain active ingredients (aerial or underground parts of plants), or other plant material (such as juices, gums, fatty oils and essential oils) or combinations thereof, whether in the crude state or as plant preparations (WHO, 2000). The part of plants (otherwise called medicinal herbs) or other plant material is known to contain bioactive phyto-chemicals, which are the basis for their therapeutic properties (Coker et al., 2008; Soetan and Ayelaagbe, 2009). Many of these herbs have also been acknowledged for their mineral elements and proximate compositions; they have nutritional qualities that could provide the users with additional nutrients (Abolaji et al., 2007). In fact, therapeutic and prophylactic properties of some medicinal plant parts have been attributed to their nutritional value rather than their phytochemical constituents (Akpinar- Bayizit et al., 2012; Pandey et al., 2012).

The Chiang Mai 1998 declaration at the first WHO/IUCN/WWF international consultation on conservation of medicinal plants recognized that medicinal plants were essential in primary health care, both in self-medication and in national health services (WHO, IUCN and WWF, 1993). In consonance with the provisions of this declaration, the medical and dental practitioners (Amendment) Decree number 78 of 1992 was promulgated by the Federal Government of Nigeria to place traditional and alternative medicines side by side with orthodox medicine (ABFR and Co, 1992). Ever since that time, there had been an overwhelming increase in the public awareness and usage of herbal medicines in Nigeria. Rukangira (2001) has also reported an increasing demand for herbal medicines in Africa with its growing population, but admitted that constraints and challenges existed in relation to conservation, science and technology, use of medicinal plants at local level, marketing and efficacy requirements. Similarly, Lau et al. (2003) and WHO (2003) have called the safety and efficacy of African herbal products to question on the basis of adulteration, substitution, contamination, mis-identification, lack of standardization, incorrect prepa-ration and/or dosage, and inappropriate labeling and/or advertisement.

Herbal product cannot be considered scientifically valid until the drug being tested has been authenticated and characterized so that one can ensure reproducibility in the manufacturing of the product. So, for an herbal formulation to be accorded acceptability as a therapeutic agent of disease, the issue of its standardization has to be addressed first. This is an important step for establishing a consistent biological activity and a consistent chemical profile towards putting in place a quality assurance programme for production and manu-facturing of herbal drugs (Choudhary and Sekhon, 2011). The practice in which botanical materials are converted into medicines where modern scientific techniques and traditional knowledge are properly integrated to ensure standardization and quality control is known as herbal drug technology (Patra et al., 2010).

Annexure I of the guidelines published by the World Health Organization (WHO, 2000) on evaluation of traditional medicines provide, among others, quantitative list of active ingredients to accompany each herbal product for the information of the consumer. This aspect of the guidelines is being violated with impunity, especially by many traditional healers, believing that such pieces of information constitute a trade secret that must be jealously guarded. This practice has effectively hindered the development of phyto-medicine in Africa. United States Embassy in Nigeria (2011) has listed malaria fever as a major public health problem in Nigeria. This disease is said to be responsible for over 70% of outpatient hospital visitation with its attendant toll on productivity and major source of discomfort and complications in children and pregnant women (Oyibo et al., 2008). Much as in orthodox medical practice, Fabeku and Akinsulire (2008) have pointed out the care of the blood as paramount to preventive and curative health care with respect to herbalism in southwestern Nigeria.

In recognition of the aforementioned facts on Nigerian health requirements and with regard to the guidelines provided by WHO on herbal drug standardization, Ogunkunle et al. (2014) have qualitatively and quantitatively defined the botanical constituents of the commonly used antimalarial and haematinic powdered herbal formulations in Ogbomoso southwestern Nigeria which they named Maloff-HB and Haematol-B, respectively (Table 1). However, the physico-chemical and phytochemical properties as well as the residual constituents of these drugs are yet to be documented. Therefore, the objective of the present study was to elucidate the ascorbic acid and mineral elements composition of Maloff-HB and Haematol-B as a contribution to the standardization of these herbal formulations with a view to examining them for general acceptability. Even though the direct link between the essential elemental composition of herbal drugs and their curative efficacy has not been established, the results of this study will be of immense importance in defining the nutritional status of Maloff-HB and Haematol-B. In addition, since the proportions of the various ‘active ingredients’ in the two herbal formulations are known, the outcome of the present study may be useful in deciding and managing the dose of each of the two drugs.

MATERIALS AND METHODS

Preparation of herbal formulations

Dried herbal materials representing nine plant species for Maloff-HB and 10 species for Haematol-B were procured from traditional

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Table 1. Botanical characterization and percentage composition (wt/wt) of antimalarial (Maloff-HB) and haematinic (Haematol-B) powdered herbal formulations from Ogbomoso Nigeria*.

Plant species	Family	Part used	Parts per 100 (wt/wt)	
			Maloff-HB	Haematol-B
Enantia chlorantha Oliv	Annonaceae	Stem bark	30.0	-
Sorghum bicolor Moench.	Poaceae	Leaf sheath	-	30.0
Alstonia boonei De Wild	Apocynaceae	Stem bark	20.0	-
Hibiscus sabradiffa L.(red variety).	Malvaceae	Fruit calyx	-	20.0
Calliandra haematocephala Hassk	Fabaceae	Root	10.0	-
Mangifera indica L.	Anacardiaceae	Stem bark	10.0	5.5
Theobroma cacao L.	Sterculiaceae	Stem bark	-	10.0
Okoubaka aubreviilei Phelleg et Nomand	Santalaceae	Stem bark	8.0	-
Sarcocephalus latifolius (J.E.Smith) E. A.Bruce	Rubiaceae	Root bark	8.0	5.5
Aristolochia ringens Vahl.	Aristolochiaceae	Roots	-	7.0
Parquetina nigrescens (Afz.) Bullock	Periplocaceae	Root bark	6.0	-
Garcinia kola Heckel	Guttiferae	Seed	-	5.5
Khaya senegalensis (Desr.) A. Juss.	Meliaceae	Stem bark	-	5.5
Uvaria chamae P. Beauv.	Annonaceae	Root bark	-	5.5
Zanthoxylum zanthoxyloides (Lam.) Zepern & TimLer	Rutaceae	Root bark	-	5.5
Cassytha filiformis L.	Lauraceae	Vines	4.0	-
Pterocarpus osun Craib.	Papilionaceae	Stem bark	4.0	-
Total			100.0	100.0

*Adapted from Ogunkunle et al. (2014); - = not applicable.

herbal practitioners in Ogbomoso, Nigeria in March 2012 (Table 1). The plant materials were chipped, cleaned and re-dried in the sun for five days and then, milled separately by passing them several times through a fabricated milling machine head (with sterile interiors) attached to a 2 HP Lister engine. Sieving of each fraction was carried out separately using a 450 micrometer sieve. 200 g of each of the two herbal remedies were formulated by measuring out a double portion of the quantity of each of the herbal constituents in Table 1 which were mixed for about 2 min in a sterilized electricity-operated kitchen blender (Figure 1). The powdered herbal formulations were stored as drug samples in two labeled bottles for subsequent analyses.

Determination of ascorbic acid content

One gram of each sample was accurately weighed into a 25 mL conical flask, 10 mL of oxalic acid (0.05 M) solution and the sample was placed under shade for 24 h for extraction of vitamin C contents. After 24 h, the samples were filtered through a 0.45 µm filter paper. Then 2.5 mL of each sample was transferred to a separate 25 mL volumetric brown flask containing 2.5 mL of oxalic acid (0.05 M) solution. 0.5 mL of metaphosphoric acid mixed with 0.5 mL of acetic acid, 1 mL of (5%v/v) sulphuric acid solution and 2 mL ammonium molybdate solution was added to each volumetric brown flask and made up to 25 mL mark with distilled water. Each sample was then analyzed for Vitamin C at a wavelength of 760 nm and compared with the standard (Anon, 1995-1996). For the sake of accuracy and precision, analysis of each herbal drug for its ascorbic acid content was replicated three times and the data were analyzed by means of descriptive statistics (means and standard deviations). The means of ascorbic acid contents in the two herbal formulations were also compared at α = 0.05 using independent sample t-statistic.

Determination of mineral elements composition in herbal formulations

The formulations were dried in oven at 70°C for 24 h until the dry weight was constant. The dried formulations were then ground and passed through a 0.2 mm plastic sieve. Then, 0.5 g of each sample was wet digested with an Ultra-pure nitric acid (HNO_3 (10 mL) in a polyethylene test tube using a heating block digestion unit at 120°C. The final solution was filtered into a 50 mL volumetric flask through a 45-µm filter paper and diluted to the mark with deionized water. All reagents used in this study were of analytical grade. Mineral contents of the two herbal formulations were analyzed using atomic absorption-spectrophotometer (Perkin Elmer Model 3300). In order to ensure some level of precision, the two samples were analyzed for magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn), potassium (K) sodium (Na) and copper (Cu) in triplicate and each result obtained was analyzed by means of descriptive statistics. In order to ensure some level of precision, each result obtained was analyzed by means of descriptive statistics as in the ascorbic acid content. Also, variations in the ratios of some minerals (Na/K, Ca/K, Zn/Cu, Na/Mg, Ca/Mg, Fe/Cu and Fe/Zn) were computed in replicates of three and their means and standard deviations were determined. The mean values for all the mineral contents and ratios in the two herbal drugs were then compared at α = 0.05 using independent sample t-statistic.

RESULTS AND DISCUSSION

Antioxidant potentials of the herbal formulations

The presence of free radicals or highly reactive oxygen species has been linked to many diseased conditions in humans because they are capable of inducing oxidative

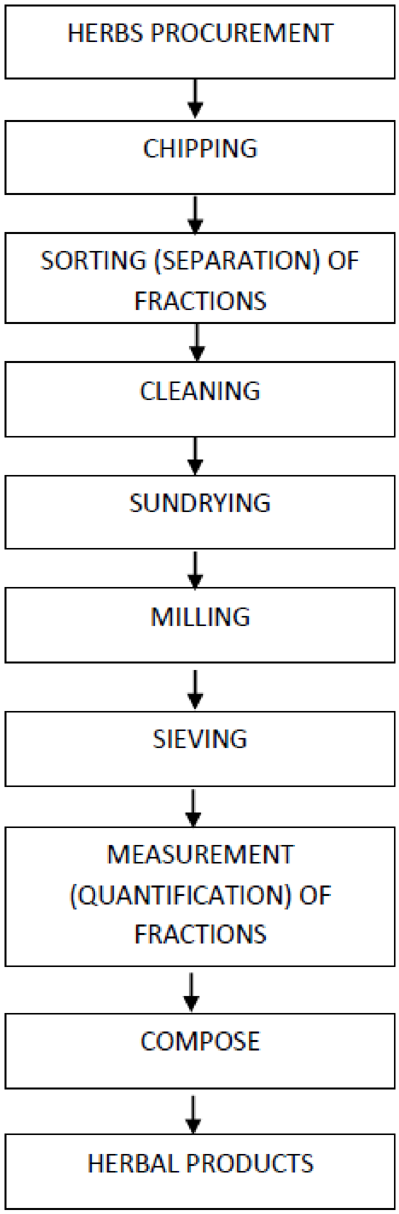

Figure 1. Flow chart of the steps followed in the manufacture of powdered antimalarial and haematinic herbal formulations used for the study (Option A of the manufacturing protocols reported in Ogunkunle et al., 2014).

damage to human body that invariably suppresses the body immune system (Lobo et al., 2010). Reduction in the risk of diseases is however ensured to some extent with the help of enzymes such as super-oxide dismutase and catalase; and through the activities of antioxidant compounds such as ascorbic acid (or vitamin C), tocopherol, phenolic acids, polyphenols, flavonoids and glutathione (Meena et al., 2012). Dietary antioxidants (in the form of antioxidant supplements) have been in use for a long time against the damaging effects of free radicals but more attentions have continued to be focused on the use of antioxidants from natural sources as spices and herbs (Khalaf et al., 2008).

The mean values of ascorbic acid and mineral elements content of the two polyherbal formulations (PHFs) studied are shown in Table 2. In general, the standard deviation for the nine parameters studied were less than 10% of the means. The results show that the two formulations are rich in ascorbic acid, a demonstration that they might have good antioxidant activity and could be a good source of natural antioxidants. Ascorbic acid, being an important precursor of redox reactions is known to promote a healthy immune system. It is therefore an integral part of many drugs, for it enhances the quality and efficacy of the products (Joshi, 2012). The presence of high concentrations of ascorbic acid in the two PHFs (542.35 mg/100 g and 414.14 mg/100 g in Maloff-HB and Haematol-B respectively) might be responsible for their therapeutic effects and uses in the traditional system of medicine.

Mineral element composition of the herbal formulations

The herbal drugs studied are also rich in Mg and Ca (Table 2) and could be said to be potential sources of these two essential elements for proper functioning of the body. Although the direct link between the essential elements of herbal drugs and their curative capacity is yet to be established, awareness is on the increase, as regards the importance of dietary minerals in the prevention as well as in the cure of several diseases (Prasad, 1993). Minerals are also globally acknowledged to be of critical importance in human diets. Major minerals are those required in amounts greater than 100 mg per day (mpd) and they include calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), potassium (K), chlorine (Cl) and sodium (Na). Trace minerals on the other hand are required in much smaller amounts (less than 100 mpd) and they include zinc (Zn), iron (Fe), silicon (Si), manganese (Mn), copper (Cu), fluorine (F), iodine (I) and chromium (Cr) (Imelouane et al., 2011).

Many secondary metabolites found in herbal drugs are known to make them to be medicinally potent for therapeutic purposes, In addition, it is now well established that many of the mineral elements earlier enumerated play vital roles in general well-being of humans who consume the plant material either as diet or as herbal product. These minerals are known to work in synergy with vitamins and a deficiency in some of them can lead to poor health and serious illnesses (Miller, 2012; Chandler, 2014; WebMD, 2005-2014). Using the entries in Table 3 as a reference, it can be observed that the composition of ascorbic acid (vitamin C), Ca and Mg

Table 2. Mean ascorbic acid content and mineral compositions in the powdered antimalarial (Maloff-HB) and haematinic (Haematol-B) herbal formulations from Ogbomoso, Nigeria.

Parameter	Mean (mg/100 g) ±SD	
	Maloff-HB	**Haematol-B**
Ascorbic acid	542.350^{b} ± 0.845	414.140^{a} ± 2.498
Mg	1319.040^{a} ± 4.401	2340.000^{b} ± 2.205
Ca	784.310^{a} ± 4.942	1011.670^{b} ± 2.797
Mn	0.026^{a} ± 0.002	0.052^{b} ± 0.004
Fe	0.613^{a} ± 0.004	0.977^{b} ± 0.006
Zn	$<0.001^{a}$ ± 0.000	0.282^{b} ± 0.004
K	2.459^{a} ± 0.046	4.483^{b} ± 0.003
Na	0.265^{a} ± 0.008	0.608^{b} ± 0.002
Cu	0.471^{a} ± 0.001	0.486^{b} ± 0.005

n, number of replicates of each parameter) = 3; mean values in a row with the same superscript are not significantly different (P>=0.05) while means in a row with different superscripts are significantly different (P<0.05).

in the two PHFs (Table 2) can adequately be compared with the recommended dietary requirements for all age groups. Table 2 also indicates that Maloff-HB contained a significantly higher amount of ascorbic acid (542.35 mg/100 mg) than Haematol-B (414.14 mg/100 mg) while the amount of all the eight mineral elements studied was significantly higher in Haematol-B. The active ingredients in the two PHFs are yet to be identified, but within the context of the findings of this study, the therapeutic property of Maloff-HB as claimed by the traditional medical practitioners could be attributed mainly to its comparatively high ascorbic acid content while the blood-enriching function of Haematol-B could be due to its comparatively higher amount of mineral elements, some of which have been acknowledged as blood enhancers (Herber and Stoeppler, 1994; Miller, 2012; Chandler, 2014; WebMD, 2005-2014). Although the mean values of Mn, Fe, Zn, K, Na and Cu in Haematol-B (Table 2) did not come any close to the recommended values in Table 3, the results obtained in the present study are in fair conformity with those obtained by Lokhande et al. (2010) from the roots of *Withonia somnifera* and *Hemidesmus indicum* which are largely used as blood purifiers in India. However, the percentage of Fe, Zn, Na and K in Haematol-B are comparatively lower than those reported by Abolaji et al. (2007) in *Xylopia aethiopica* (fruit), *Parinari polyandra* (fruit) and *Blighia sapida* (root) which are traditional herbs used by pregnant women in some parts of Southwestern Nigeria as blood purifiers and fertility enhancers.

The mineral ratios in a diet or an herb are frequently considered more important in defining its nutritional and/or possible therapeutic status than the individual mineral concentrations themselves. According to Watts (2010), these ratios, not only reveal the important balance between these elements, but also provide information regarding the many possible factors that may be represented by disruption of their relationships. A proper interpretation of the relationships between minerals within the body can therefore provide information on the state of the health as well as enable a specific and targeted approach to therapy. On the strength of the above argument, the relationships between several elements in a medicinal herb can be said to be suggestive of synergistic or antagonistic effects, with the resultant provision of various elements to the body in bioavailable forms, and in a balanced manner with negligible harmful effects except for some environmental contaminants (Lokhande et al., 2010).

Inter-elemental correlations

Based on the foregoing, and the information provided in Table 4, it is arguable that the ratios of the mineral elements are an important factor for consideration in determining possible extent of bioavailability of the nutrients to the body. In recognition of this factor, it can be said that only Fe/Cu ratio in both Maloff-HB and Haematol-B and Fe/Zn ratio in Haematol-B appear to meet the acceptable dietary requirements (Table 4). By implication therefore, dietary iron availability appears to be guaranteed with the use of these herbal formulations even though the absolute quantities of Fe, Zn and Cu were insignificant (Table 2). The entries for Na/K , Ca/K, Na/Mg, Ca/Mg and Zn/Cu in Table 4, which fell short of the stated dietary requirements should however, not be interpreted to mean that the two herbal formulations are invalid with respect to the enumerated mineral nutrients. The fact is that, the reservoir of the various elements required in the human body at a given period is as a result of an additive process, such that an herb consumed or a meal taken can always add its piece of mineral constituents.

Bioavailability of a mineral nutrient is a condition that has a direct relationship with the quantity absorbed into the body of the consumer. The rate of mineral absorption

Table 3. Nutritional goals for age gender groups based on dietary reference intakes and dietary guidelines recommendations*.

Parameter	Source of goal	Amount of daily recommended dietary intakes												
		1-3 years	4-8 years		9-13 years		14-18 years		19-30 years		31-50 years		51 years and above	
		Child	F	M	F	M	F	M	F	M	F	M	F	M
Ascorbic acid (mg)	RDA	15	25	25	45	45	65	75	75	90	75	90	75	90
Calcium (mg)	RDA	700	1000	1000	1300	1300	1300	1300	1000	1000	1000	1000	1200	1200
Iron (mg)	RDA	7	10	10	8	8	15	11	18	8	18	8	8	8
Magnesium (mg)	RDA	80	130	130	240	240	360	410	310	400	320	420	320	420
Phosphorous (mg)	RDA	460	500	500	1250	1250	1250	1250	700	700	700	700	700	700
Potassium (mg)	AI	3000	3800	3800	4500	4500	4700	4700	4700	4700	4700	4700	4700	4700
Sodium (mg)	UL	<1500	<1900	<1900	<2200	<2200	<2300	<2300	<2300	<2300	<2300	<2300	<2300	<2300
Zinc (mg)	RDA	3	5	5	8	8	9	11	8	11	8	11	8	11
Copper (mcg)	RDA	340	440	440	700	700	890	890	900	900	900	900	900	900

*Source: USDA and HHS (2010); F, female; M, male; RDA, recommended dietary allowance; AI, adequate intake; UL, upper limit.

Table 4. Mean values of some mineral ratios in Maloff-Hb and Haematol-B, in comparison with recommended dietary ratios and ranges.

Mineral ratio	Mean ± SD		Ideal ratio	Acceptable range of ratio
	Maloff-HB	Haematol-B		
Na/K	$0.108^{a} \pm 0.005$	$0.135^{b} \pm 0.001$	[2.4:1]	[1.4 - 3.4]
Ca/K	$319.013^{b} \pm 4.892$	$225.667^{a} \pm 3.861$	[4.2:1]	[2.2 - 6.2]
Na/Mg	$<0.001^{a} \pm 0.000$	$<0.001^{a} \pm 0.000$	[4:1]	[2 - 6]
Ca/Mg	$0.594^{b} \pm 0.004$	$0.432^{a} \pm 0.001$	[7:1]	[3 - 11]
Zn/Cu	$0.002^{a} \pm 0.000$	$0.580^{b} \pm 0.007$	[8:1]	[4 - 12]
Fe/Cu	$1.301^{a} \pm 0.011$	$2.009^{b} \pm 0.014$	[0.9:1]	[0.2 - 1.6]
Fe/Zn	$613^{b} \pm 3.605$	$3.464^{a} \pm 0.055$	{< 2:1}	{0.67 - 3.33}

n, number of replicates of each computed ratio) = 3; mean values in a row with the same superscript are not significantly different (P>=0.05) while means in a row with different superscripts are significantly different (P<0.05); [ ] source = Watts (2010) and { } source = Solomons and Jacob (1981).

in turn, has been found to be dependent on additional factors other than the ratios of the mineral elements as earlier highlighted. A discussion of iron absorption is of particular interest in evaluating the qualities of Maloff-HB and Haematol-B with the understanding that both drugs should have a direct bearing to blood enhancement and body immunity boosting. Iron absorption refers to the amount of dietary iron that the body obtains and uses from food or herb. Healthy adults absorb only about 20% of dietary iron (NIH, 2007) but absorption by individuals is influenced by several factors which can be grouped into three: dietary status of the body (form and storage level of iron); amount of iron absorption enhancers; and quantity of iron absorption inhibitors (phytic acid) (NIH, 2007; Dewar, 2009).

Potential for iron absorption enhancement

Ascorbic acid has long been recognized as a versatile enhancer of dietary iron absorption in the body system (Hallberg, 1981) and, using a multiple regression analysis, this antioxidant has been reported as a biochemical predictor of iron absorption (P = 0.0441) along with the content of animal tissue (P = 0.0001) and phytic acid (P = 0.0001) by Reddy et al. (2000). With this background information, it can be said that the two herbal drugs studied are potential sources of essential mineral elements with the compositions that fairly agree with the recommended dietary intake of all ages (Table 3). Even though the observed quantities and or derived ratios of the elements in the drugs did not agree perfectly with the recommended values, the high amount of ascorbic acid confers on them the potential to enhance iron absorption in the body, even from other sources. In a related study, Hussain et al. (2011) reported high amounts of this iron absorption enhancer in some selected medicinal plants.

Conclusion and recommendations

Based on the results of this study, it can be concluded that Maloff-HB and Haematol-B are good reservoirs of ascorbic acid. This antioxidant has been found in many redox reactions as promoters of good health. The primary functions of these polyherbal formulations as drugs can therefore be traced to the good supply of this antioxidant. The two herbal formulations are also rich in Ca and Mg and their Fe/Cu and Fe/Zn ratios are such that can promote bioavailability of these important mineral elements. Hence, these drugs have the potential to meet some dietary requirements which will promote good quality blood that prevents infections. Further explanations of the basis for using the formulations can be sought through a study on their biochemistry. Their consumption rate should however be under some caution because there is lack of empirical information on their safety and efficacy, more so, the present study did not evaluate the drugs for their residual constituents (heavy metals, pesticides, toxins, etc). In order to confirm the beneficial effects of the two herbal drugs, it is important to carry out studies on the *in vivo* activity and bioavailability of their constituents.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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