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Quality assessment of five herbal tea brands containing *Moringa oleifera* Lam. (Family: *Moringaceae*) sold in Port Harcourt, Nigeria

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Abstract

Addressing drawbacks issues of standardization and quality assurance of herbal products is increasingly becoming necessary. In this study, five *Moringa oleifera* herbal tea products sold in Port Harcourt- Niger-Delta region of Nigeria were sampled and assessed for quality parameters as prescribed by the World Health Organization. The DPPH standard spectrophotometric method was used for antioxidant assay. All the five *Moringa* tea brands showed quality parameters evidence of *Moringa oleifera* leaves. Two brands have values above the allowable limit for total ash and acid insoluble ash. Arsenic was detected in all the five brands above recommended limit. All the five tea brands had antioxidant activity above 50%. All the five *Moringa* tea brands had microbial contaminants. Although all the five herbal tea samples contain *Moringa oleifera*, the presence of microbial contaminant, the toxic heavy metal arsenic, and silica matter elicits quality and stability concerns with associated risk to public health.

Keywords: *Moringa oleifera*; Dosage Forms; Standardization; Contaminants; Public Health

1. Introduction

An estimated 60% of the world population patronize herbal remedies for its basic health care needs [1]. However herbal drugs are inevitably inconsistent because of their composition, and the quality of the herb with respect to concentration of potentially biologically active compounds is generally not known. Concentration of active ingredients may be lower than expected or even too high thus predisposing the consumer to certain deleterious health effects. Establishing certain standards to guide manufacturing, distribution and consumption of the herbal products becomes pertinent. *Moringa oleifera* herbal tea is a typical widely consumed herbal product. This packaged herbal tea contains powdered leaf of the medicinal plant *Moringa oleifera* commonly called “Drum stick" [2]. *Moringa oleifera* is a small graceful deciduous tree with sparse foliage growing up to 12m high. The fruit is large and distinctive, 30-120 cm long and 1.8cm wide splitting lengthwise into three parts when dry. Each fruit pod contains about 20 seeds, dark brown with papery wings [2]. In many regions of Africa it is widely consumed as food and as medication for various health conditions some of which include: as poultice to relieve soreness, headache and related inflammation, for the treatment of conjunctivitis. Obesity, indigestion, abnormal blood pressure, and as abortifacient [2]. The Leaves are an excellent source of the Sulphur – containing amino acid methionine and cysteine which are often in short supply in daily meals. The pleasantly flavoured edible oil, resembling olive oil is an excellent salad oil, the flowers can be used to make tea [3]. Since all parts of the *Moringa* tree are edible and have long been consumed by humans, in spite of the challenges facing standardization, safety and efficacy of herbal products, it becomes necessary to standardize this widely consumed herbal product.

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2. Material and methods

2.1. Materials

Microscopes, slides, water bath, heating mantle, beakers, test tubes with holders & racks, Bunsen burners, fume cupboards, oven, desiccators, porcelain crucibles (Techmel and Techimel USA) muffle furnace, pre coated TLC plates (GF_{254} Merch Chemical Company), TLC tank, UV Lamp (254 and 365nm wavelength) (Loba Chemical Pvt Ltd India), Petri dishes weighing balance. Reagents and solvents used include: N hexane, chloroform (100%) ethanol, petroleum ether (all manufactured by JHD chem gu angdong china), sulphuric acid, hydrochloric acid, Ammonia, mayers reagent, kedde reagent, Fehlings reagent (A and B) (all manufactured by sigma aldreich chemicals USA), freshly prepared Anisaldehyde spray, Nutrient ager medium, moutants. Five packaged *Moringa herbal* tea brands purchased from Pharmacy stores in Port Harcourt in the Niger-Delta region of Nigeria and coded: HLM, HMT, and MMT. PMT and SBM.

2.2. Methods

Macroscopy and microscopy parameters, extractive values determination, moisture content determination ash values determination, bitterness value determination, particle size analyses microbial contaminants, and foaming index analysis thin layer chromatographic profiling and phytochemical tests were carried out using standard methods [4-6]. The antioxidant activity of the infusion of the various tea brands prepared at 0.08mg powder/ml of distilled water was assessed using the DPPH standard spectrophotometric methods [7-8].

2.3. Statistical Analysis

Collected data were expressed as mean ± SEM. The comparison groups were achieved using one-way analysis of variance (ANOVA)and student t-test for significance at p=0.05.

3. Results and discussion

The result of the phytochemical screening of the five *Moringa* tea brands are as shown in Table 1. Whereas alkaloids, phenolic compounds such as flavonoids, reducing sugars, saponins and triterpenoids are present in all the five selected brands of *Moringa* tea, secondary metabolites such as anthraquinones and phlobatannins are not detected. The observed trend in the phytochemical constituents agree with that reported in the literature for *Moringa oleifera* [2,9]. The presence of these constituents offers a rationale for the health benefits for the investigated *Moringa oleifera* tea products as claimed by the various manufacturers and also as documented in reports of scientific investigation validating same. The bactericidal principle pterygospermin, β-carotene. β-sitosterol a steroidal triterpene alcohol, alkaloids such as: moringine and moringinine; acetylated glycosides such as: niaziminin A, niaziminin B, niazirin, and niazirinin and some isothiocyanate glycosides have been reported as bioactive compounds present in *Moringa oleifera* [2, 10-12].

Table 2 clearly shows similar macroscopic features occurring between all five tea brands studied with the chemo-microscopic analysis report in Table 3, giving a confirmatory presence for phenolics and starch with anthraquinone pigments absent as evidenced from the phytochemical screening result in Table 1. The presence of cutin (the waxy component of plant leaves) and very obvious lignified fibres gives a clear indication of the presence of leaf fragments as a content of the tea bags in each manufactured product. Also, the structural microscopy analysis (see Table 4) confirmed scattered on tea fragments for all five brands, the characteristic presence of long slender and uniseriate trichomes, prismatic calcium oxalate crystals and anomocytic stomata which are diagnostic for *Moringa oleifera* leaves.

The total Ash, acid insoluble ash and water soluble ash values for the five *Moringa* tea brand investigated in this study are as shown in Table 5. The rank order trend in the total cash value: PMT < HLM < SBM < HMT < MMT, acid insoluble ash value: SBM < HLM < PMT < MMT < HMT and water soluble ash value: PMT < SBM < HLM <HMT < MMT were observed. These were significantly different ($p < 0.05$). Except for two brands HMT and MMT, the other three brands have total ash and acid insoluble ash values below the allowable limit of not more than 10 and 1.5 % respectively for *Moringa* leaf [2]. Total ash content is an indication of the metallic components and silica matter in any given herbal sample. These could be essential mineral elements such as Na, K, Ca, Mg, Fe, Cr, Zn, Se etc and toxic heavy metals like As, Hg, Pb, Cd etc. The alkali and alkali-earth metals will constitute the water soluble ash while the silica matter constitute the acid insoluble ash. Thus an unusually high total ash with correspondingly high acid insoluble matter as observed for the two *Moringa* tea brands: MMT and PMT, is an indication of toxic heavy metal pollutant and silica matter. Of the two toxic heavy metals arsenic (As) and lead (Pb) investigated using flame atomic absorption spectrophotometry, Pb was not detected while As was detected in all the five *Moringa* tea brands. The rank order observed for As was: HLM <HMT

<PMT <MMT < SBM with all the values higher than the recommended limit of 10µ/day by the American Herbal Products Association (APHA) [13] or 0.01ppm. Arsenic poisoning is known to elicit vomiting, abdominal pain, diarrhoea, numbness, muscle cramps, skin pigmentation and cancer as well as cancer of the lungs and bladder and eventually death . This, coupled with the observed high acid insoluble ash- a pointer to sand and silica matter well above the recommended limit, is a quality assurance issue that is of concern to public health and safety.

Table 1 Phytochemical screening profile of the five selected *Moringa oleifera* Tea Brands

	HLM	**HMT**	**MMT**	**PMT**	**SBM**
Saponins: Frothing test	+	+	+	+	+
Reducing sugars: Fehlings test	+	+	+	+	+
Phenolic compounds: $FeCl_3$ test	+	+	+	+	+
Shinoda test for Flavonoids	+	+	+	+	+
Phlobatanins	-	-	-	-	-
Alkaloids: Dragendorff's test	+	+	+	+	+
Meyer's test	+	+	+	+	+
Hager's test	+	+	+	+	+
Triterpenoids: Lieberman test	+	+	+	+	+
Salkowski test	+	+	+	+	+
Anthraquinone: Borntrager's test	-	-	-	-	-

"+" means present, "-" means absent

Table 2 Organoleptic and Macroscopic profile of the Five selected Brands of *Moringa oleifera* Herbal Tea

Macroscopic features assessed	**Colour**	**Odour**	**Taste**	**Texture**
HLM	Greenish-grey	Slightly pungent	Tasteless	Coarse
HMT	Grey	Slightly pungent	Tasteless	Coarse
MMT	Greenish-grey	Odourless	Tasteless	Coarse
PMT	Black	Slightly pungent	Tasteless	Coarse
SBM	Greenish-grey	Pungent	Tasteless	Coarse

Table 3 Chemo microscopic profile of the five selected brands of *Moringa oleifera* Herbal Tea

Chemo-microscopic parameters	**Staining Reagent Used**	**Selected brands of *Moringa* tea**				
		HLM	**HMT**	**MMT**	**PMT**	**SBM**
Starch	Iodine	+	+	+	+	+
phenolics	FeCl3 solution	-	-	-	-	-
Anthraquinone	10% ammonia solution	-	-	-	-	-
Cutin	Sudan III reagent	+	+	+	+	+
Lignified fibres	Phloroglucinol reagent + conc HCl	+	-	+	+	+

"+" means present, "-" means absent

Moisture content analysis result in Table 5 for all 5 Moring tea studied showed values less than the recommended upper limit of 10% for *Moringa* [2] with the rank order observed for the moisture content being: PMT <HMT <SBM<MMT<HLM. The moisture content gives information about the amount of water (or moisture) still remaining in a given sample or a finished product after all the manufacturing processes. Elevated moisture content makes the product more vulnerable to microbial attack leading to spoilage under storage. This is a stability concern.

Table 4 Structural and cellular microscopic profile of the Five selected brands of *Moringa oleifera* Herbal Tea

	Structural and cellular microscopic features		
Samples	**Trichomes**	**Calcium Oxalate Crystals**	**Stomata type**
HLM	Thin walled long slender uniseriate trichomes	Prismatic calcium oxalate crystals present	anomocytic type seen
HMT	Thin walled long slender uniseriate trichomes	Prismatic calcium oxalate crystals present	anomocytic type seen
PMT	Thin walled long slender uniseriate trichomes	Calcium oxalate crystals not detected	anomocytic type seen
SBM	Thin walled long slender uniseriate trichomes	Calcium oxalate crystals not detected	anomocytic type seen
Freshly collected *Moringa* leaf (Reference sample)	Thin walled long slender uniseriate trichomes	Calcium oxalate crystals of cluster type (prismatic)	anomocytic type seen

Results of extractive values for all five Moring tea brands from also in Table 5 show that all the five tea brands have their water and alcohol soluble extractive values within permissible limits (not less than 3% for dilute alcohol soluble extractive values; not less than 7% for water soluble extractive values [2] with the trend in rank order of: (water extractive: HMT < SBM < MMT < HLM < PMT; alcohol extractive: HLM < SBM < PMT < MMT < HMT; chloroform extractive: HMT < HLM < MMT < PMT < SBM).Extractive value describes the amount of active constituents extracted with the given solvent from a given amount of herbal material [4]. For materials with no established suitable chemical or biological assay method, this serves as a pointer to the likely amount of content of actives and by extension the potency.

Bitterness value assessment for all five *Moringa* tea brands (see Table 5) show all five *Maringa* tea samples having a bitterness value above 0.5, with SBM and MMT tea brands showing the highest value of 1. The palatability of the product and hence compliance and acceptability is greatly influenced by the bitterness value. The higher the bitterness value, the lower the palatability and hence consumption of the tea product.

Foaming index for all the five *Moringa* tea brands (see Table 5) show values between 100 (i.e. froth height of not more than 1 cm). These results is a direct confirmation test for the presence of saponins in each tea infusion sample. High foaming index values raise concerns as to the nature and type of saponins present in the herbal material and its safety profile for consumption. High foaming ability may also influence the quantity (or dose) of the product that is to be consumed in order to obtain the desired effect.

The result of the particle size analysis also in Table 5 showed that the MMT, HLM and SBM tea brands can be said to having higher proportion of fines. This is because although all their powder particles just like the other two brands HMT and PMT virtually passed un-retained by 2000 micron or size 10 mesh, however not more than 40 % pass through un-retained by the 250 micron or size 60 mesh unlike for HMT and PMT where a similar trend was observed at a more larger 500 micron or size 35 mesh. Further analysis revealed that whereas at least 90 % of the particles were cumulatively retained by the 250 micron or size 60 mesh for the HMT and PMT, this was not the case for MMT, HLM and SBM where the similar trend of at least 90 % of particles cumulative retained was at sizes 400 (or 37 micron), 230 (or 63 micron) and 120 (or 125 micron) mesh respectively. Thus the rank order in terms reducing fines is: MMT > HLM > SBM > HMT≈PMT.

The results of antioxidant activity for the five *Moringa* tea brands (see Table 5) show that all the five *Moringa* tea brands are replete with antioxidants at the 0.08mg powder /ml of aqueous solution used for the infusion. This supports claims on the packaged tea as regards their recommendation for use as anti-aging and immune boosting effects. The

antioxidant activity of HLM, HMT and MMT tea brands were not significantly different (p=0.261 >0.05) but significant higher (p <0.05) compared to the other two. Thus the tea brands: HLM, HMT and MMT can be said to be bio-equivalent. PMT tea brand however showed the least antioxidant activity. The observed trend in antioxidant activity being: HLM>HMT>MMT>SBM>PMT.

Table 5 Physico-chemical, selected toxic heavy metals and antioxidant profile of the five selected brands of *Moringa* Herbal Tea

Evaluated parameters	HLM	HMT	MMT	PMT	SBM
Total Ash (% w/w)	8.346±0.110	10.393±0.491	12.091±0.264	4.407±0.268	8.862±0.259
Acid insoluble ash (% w/w)	0.727± 0.033	3.086 ± 0.192	1.848±0.112	1.081±0.072	0.678±0.128
Water soluble ash (% w/w)	3.503± 0.080	5.167±0.377	5.912±0.249	2.311±0.239	2.445±0.204
Arsenic (As) in ppm	0.671±0.191	1.015±0.015	1.083±0.157	1.046±0.118	1.135±0.107
Lead (Pb) in ppm	BDL	BDL	BDL	BDL	BDL
Moisture content (% Loss on drying)	5.150±0.076	1.700±0.229	3.533±0.559	1.267±0.176	1.800±0.236
Water extractive (% w/w)	9.067±2.067	7.867±2.226	8.933±2.491	17.667±2.333	8.667±1.764
Alcohol extractive (% w/w)	6.367±1.472	9.100±1.274	8.933±1.020	8.733±3.737	6.450±1.050
Chloroform extractive (% w/w)	3.233±1.697	0.633±0.033	1.433±0.884	7.500±0.681	8.100±0.651
Bitterness value	0.866	0.644	0.866	1.000	1.000
Foaming index*	100	100	100	100	100
Sieve analysis#	2000/250 (-10 +230mesh)	2000/500 (-10+60mesh)	2000/250 (-10+400mesh)	2000/500 (-10+60mesh)	2000/250 (-10+120mesh)
Antioxidant capacity (% inhibition of DPPH activity)	81.660±0.000†	81.087±2.136†	75.437± 3.326†	63.090±0.308	69.897±2.745

***** Foaming index is taken as 100 if the height of foam in every tube is less than 1cm (WHO, 2011. p49). # values above implies virtually all powder particles passed unretained by mesh indicated as numerator while not more than 40 % passes through unretained by the mesh indicated as denominator. For the convention in parenthesis - sign before a mesh indicates that 90% of particles passes through the specified mesh unretained while the + sign before the second mesh implies at least 90 % of the particles were retained by the specified mesh such that at least 90 % of the particles are retained within the specified range of the meshes. †No significance difference (p> 0.05)

Results of microbial contaminant (Table 6) show the presence of microbial contaminants (moulds) in all the five *Moringa* tea brands. This result raises concerns as to the storage stability and safety of these packaged products on consumption.

Table 6 Assay of mould contamination of five *Moringa* tea brands

***Moringa* tea Brand**	**Antimicrobial Assay result**
HLM	Presence of confluence growth seen scattered on surface of SDA
SBM	Presence of confluence colonies seen scattered on surface of SDA
HMT	Confluence colonies seen on surface of SDA
MMT	Confluence colonies seen on SDA surface
PMT	Confluence colonies seen on SDA surface

According to the West African Herbal Pharmacopeia, the TLC profile for *Moringa oleifera* should show the presence of characteristic spots with pf values of 0.9, 0.8, 0.5 and 0.3 and colour bands of pink, grey and dark grey [2]. Results obtained from the study as shown in Table 7 gives similar information as pharmacopeia standards. This confirms that the packaged product contains *Moringa oleifera* as stated in labels claims.

Table 7 Thin layer Chromatography Profile for five brands of *Moringa oleifera* tea

Tea brand	No of bands seen	Colour of band seen under daylight	Retardation Factor
HLM	4	dark grey	0.36
		grey	0.56
		pink	0.86
		pink	0.98
HMT	4	grey	0.72
		grey	0.79
		dark grey	0.87
		dark grey	0.98
MMT	4	dark grey	0.36
		grey	0.52
		pink	0.85
		pink	0.97
PMT	4	grey	0.67
		dark grey	0.75
		dark grey	0.73
		dark grey	0.93
SBM	3	grey	0.85
		dark grey	0.89
		dark grey	0.92

4. Conclusion

Quality control parameters assessed aided confirmation of the identity, contaminants and purity of the packaged herbal tea samples as containing *Moringa oleifera.* The findings exposed safety and stability concerns as microbial and heavy metal contaminants were detected in all the five packaged *Moring* tea products investigated in this report. Further work can however be done on the assessment of quality control parameters for other commonly consumed herbal medicinal products available in Nigeria markets to assure of their safety and efficacy on consumption.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

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