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Full Length Research Article

PREPARATION AND CHARACTERIZATION OF A POLY-HERBAL TEA WITH EFFECTIVE ANTIOXIDANT PROPERTIES

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ABSTRACT
Many herbal teas consist of poly-herbal materials that are often folk recipes with known health benefits. Poly-herbal teas containing the leaves of *Moringa oleifera, Zingiber officinale* and juice of *Citrus limon* were developed and evaluated for its physicochemical and stability properties. The herbal tea generally showed good physicochemical properties such as organoleptic properties, excellent flow, optimal ash value and moisture content. It showed stability properties that are characteristic of a good finished herbal product. The tea also showed effective free radical scavenging properties similar to that of ascorbic acid and consistent with its antioxidant benefits. Some of its physicochemical and antioxidant properties were diminished when stored in a stress environment. Formulating the poly-herbal recipe of *M. oleifera, Z. officinale* and juice of *C. limon* into herbal tea is an effective means of presenting the herbal medicine for use.

Keywords: Poly-herbal tea, tea bags, physicochemical properties, stability

INTRODUCTION

Herbal teas are indeed a presentation of herbal medicines. They consist of mono or poly-herbal materials that are brewed as decoction or infusion and drank for their therapeutic benefits (Poswal *et al*. 2019). In recent times there has been an apparent upsurge in the popularity and use of herbal teas (McKay & Blumberg 2002; Khan & Mukhtar 2013). This may be related to the acclaimed benefits in managing many chronic diseases (Builders 2019). Thus, herbal teas belong to a rapidly expanding market of wellness beverages (Byeon & Han 2004).

The herbal materials that make up the recipe are often characterized by the presence of various secondary metabolites which are responsible for their pharmacological activities and health benefits. Indeed many folk recipes that are used for managing especially chronic diseases are currently presented as herbal teas (Cohen & Ernst 2010; Park *et al*. 2014). In certain communities in Nigeria, the hot infusion of recipes containing *Moringa oleifera, Zingiber officinale* and *Citrus spp* are used for treating cold and cough.

Moringa oleifera, which is often referred to as the *miracle tree,* is believed to treat over 300 diseases. In many communities in the Northern part of the country, it is used as a vegetable in soups and salads. The leaves of *M. oleifera* are an outstanding source of nutritionally rich vegetable because of its proven high content of amino acids, fats and omega oils, potassium, calcium, phosphorous, as well as other trace minerals, antioxidants and anti-inflammatory substances. Presently, coarsely powdered *M. oleifera* leaves are packed in tea bags as herbal tea to be brewed as infusions and are commercially available in many superstores in Nigeria.

Ginger is the underground stem of *Zingiber officinale.* Nigeria produces one of the world's best *Ginger.* The herb is popularly used as medicine and as a spice in foods and beverages. It is an important constituent of many food drinks. Some nutritional/chemical components of *Ginger* include starch, omega-3 and omega-6 fatty acids, ascorbic acid, folic acid, calcium, magnesium, phosphorus, potassium and sodium (Builders 2019). Ginger is a known natural spice. Its spicy characteristic is due to the presence of an active compound known as gingerols. It has numerously acclaimed and established health benefits some of which include relief of digestive disorders such as nausea, cramping and indigestion, stress, platelet aggregation. Its blood thinning activities are known to improve circulation similar to aspirin but without the gastric irritation of aspirin. It also has cardio-protective effect which it produces via the reduction of bad cholesterol and improvement of blood circulation and strengthening of the arteries and capillaries (Mikaili *et al*. 2013).

The lemon juice is obtained from the fruit of *Citrus limon* which is found in most parts of the world. The juice of lemon has a distinctive sour taste. It is commonly used in drinks and beverages. It contains numerous phytochemicals such as citric acid, ascorbic acid, minerals, flavonoids, and essential oils, terpenoids, flavonoids, stilbenes and condensed tannins (Okwi & Emenike 2006). Some of the acclaimed health benefits of lemon juice include actions such as cardiotonic, promote weight loss, prevention of kidney stones, protection against anemia, reduction of cancer risk and improvement of digestion.

Moringa oleifera, Zingiber officinale and *Citrus limon* are commonly infused in hot water and drank as a herbal tea for their health benefits. The aim of this study was to prepare poly-herbal tea bags containing *M. oleifera, Z. officinale* and *C. limon* granules and evaluate the physicochemical and stability properties of the new poly-herbal tea.

MATERIALS AND METHOD

MATERIALS

Buffer tablets 4, 7 and 9 and silica gel, were obtained from Sigma Aldrich, Germany. Empty tea bags were purchased from Jumia online trading company. *Moringer oleifera* leaf was collected from a farm in Barnawa area of Kaduna town*, Zingiber officianale rhyzome,* and *Citrus limon* fruits were bought from Kaduna Central Market and identified at the Department of Biological Sciences, Kaduna State University.

METHODS

Processing of herbal materials

Zingiber officinale: Fresh rhizomes of *Zingiber officinale* was bought from the open market in Kaduna. The rhizome was scrapped to remove sand clumps, rotten and fibrous sections. The ginger was washed several times with a copious amount of potable water. This was then cut into smaller sizes of about 2 mm length with a kitchen knife (Aoshima *et al*. 2007; Serafini *et al*. 2011). The cut pieces of rhizome were then pulverized with a kitchen blender (Kenwood, Japan). The wet mass of the rhizome pulp was dried in an oven set at 50 °C and screened through an appropriate sieve. The dry powder was packed in a waterproof bag and stored in a cool dry place until used

The fresh leaves of *Moringa oleifera* were collected from the farm, washed and then pulverized while still wet with a kitchen blender (Kenwood, Japan). The wet mass was then dried and the dry mass was screened through an appropriate sieve and further dried in an oven set at 50 °C. The powder was then packed in a waterproof bag and stored in a cool dark place until used.

Citrus limon: The fruits of *Citrus limon* were bought in Kaduna central market, the fruits were washed with copious quantities of water, then the juice was extracted using a manual hand extractor and used freshly prepared.

Formulation of herbal teas

Appropriate quantities of previously processed dry powders of *M. oleifera* and *Z. officinale* required to prepare 50 tea bags were weighed and granulated, such that each tea bag corresponded to 5 g of the herbal material mixture and 4 ml of the lemon juice as shown in Table 1. The dry granules were then packed in a polythene bag, stored in a cool dry place until used for further analysis, and some dispensed into the tea bags and appropriately sealed.

Table 1: Ratio of ingredients used for the preparation of the poly-herbal tea

Ingredient	Ratio
Moringa oleifera	2
Zinger officinalis	1
Citrus limon	3 mL/5 g

Phytochemical screening

The secondary metabolites present in the herbal tea blend were evaluated by phytochemical analysis using standard procedures (Harborne 1998; Jack & Okorosaye-Orubite 2008).

Organoleptic properties

A 5 g quantity of the dry herbal tea product was placed in a Petri dish and the color, odor, taste and texture evaluated by a panel of six assessors as described by Juszczak *et al*. (2015).

Determination of ash value

The International Organization for Standardization (ISO) 1575, methods for determination of total ash in tea reviewed and confirmed in 2015 was used (ISO-1575 2015). Five tea bags were randomly selected from each formulation and the content emptied in a large Petri dish. A 2 g quantity of the herbal tea was weighed and transferred into a crucible which has initially been heated to 105 °C for 5 min and placed in a desiccator until use. The crucible containing the herbal material was then incinerated. The heating temperature was 525 ± 25 °C. The crucible was allowed to cool before the weight was determined. Then it was again heated for 30 min and reweighed. This was repeated twice until a constant weight was obtained. The percentage of ash value was determined using Equation 1.

$$\%\ ash\ value\ = \frac{W3-W1}{W2-W1}\ X\ 100 \qquad \ldots.1$$

where W_1= weight of ash and crucible; W_2= weight of granule and crucible; W_3= weight of crucible.

Determination of moisture content

The World Health Organizational (WHO) method indicated for quality control of medicinal plant materials was adopted (WHO 1998). Five tea bags were randomly selected from each formulation and the content emptied into a large Petri dish. A 3 g quantity of the herbal material was weighed and transferred into an evaporating dish crucible which has initially been heated to 105 °C for 5 min and placed in a desiccator until use.

The evaporating dish containing the granules was placed into an oven maintained at 105 °C, removed every 30 min and weighed until no change in weight was obtained over two consecutive readings. This was repeated three times. The percentage moisture content was determined using Equation 2.

$$\%\ moisture\ content\ = \frac{C2-C3\ X\ 100}{C2-C1} \qquad \ldots\ldots2$$

where: C1 =weight of empty evaporating dish
C2 = weight of crucible + sample before heating
C3 = weight of crucible = sample after heating.

Evaluation of extractive matter

A tea bag corresponding to 5 g of the herbal tea was brewed using 250 mL of boiling potable water (100 **±** 2 °C). The water containing the tea bag was allowed to stand for 24 h with intermittent stirring. A 20 mL quantity of the brew was transferred into a pre-weighed porcelain dish and the water evaporated on a water bath set at 100 °C. The porcelain dish was then transferred into a hot air oven set at 50 °C and allowed to stay for 1 h. The percentage of the soluble extractive matter was calculated with reference to the dry weight of the sample without the empty bag (WHO 1999).

pH of brew solution

A tea bag containing the herbal tea was brewed using 250 mL of boiling water (100 **±** 2 °C) in a 250-mL conical flask. The brew was allowed to cool to room temperature (27 **±** 2 °C). The pH of the brew solution was determined using a digital pH meter (SPER Scientific, China) (Builders *et al*. 2010). The brew was stored in a refrigerator at approximately 5 °C and the pH was determined weekly for four weeks.

Dust leak

Two tea bags of the herbal tea were selected at random, weighed and placed in a friability tester. The machine was operated for 4 min to undergo the abrasive fall. After the expiration of the 4 min, the tea bags were dusted and weighed.

The percentage leak was evaluated using Equation 3:

$$\frac{W1-W2}{W1}\ X100 \qquad \ldots\ldots3$$

where: W_1 is the initial weight before the leak test
W_2 is the weight after the test.

Particle size analysis of herbal tea granules
A 50 g quantity of the granules of the herbal tea was transferred to a set of sieves mounted on a sieve shaker (Jin-Ling Shang, China). The sieves were arranged in descending order from top to bottom: 0.9 mm, 0.45 mm, 0.28 mm, 0.18 mm, 0.15 mm and pan collector. The vibrations were set for 10 min after which the granules retained in each sieve and the collecting pan were weighed and recorded. This evaluation was done three times. The results obtained were presented as particle oversize (Well 2003).

Flow properties of granules
Angle of repose: The static angle of repose was measured according to the fixed funnel and free-standing cone method (Well 2003). A 50 g quantity of powder was transferred into a funnel of 1 cm orifice. The funnel was clamped 10 cm from the base (such that the funnel neck tip was 10 cm from the flat surface). The tip of the funnel neck was closed with a finger until all the granules have been transferred. The granules were then allowed to flow through to the surface. When all the granules had completely drained, the height (H) and the diameter (D) of the granule mound on the surface was measured and recorded. This was repeated three times.
The angle of repose was calculated using Equation 4:

$$\tan \Theta^{o} = 2H/D \qquad \ldots\ldots 4$$

where: Θ^{o} = angle of repose, H= height and D= diameter.

Carr's compressibility index:
Bulk and tapped densities: The volume occupied by 50 g of the granules was determined using a 200-mL graduated measuring cylinder. The tapped volume which corresponds to the final volume of consolidation after tapping with an automated tapping machine (Stampfvolumeter, STAV 2003JEF, Germany) was determined. The bulk (V_o) and tapped (V_T) volumes were evaluated (Well 2003). The bulk and tapped densities were calculated as the ratio of weight to volume (V_o and V_T, respectively) as presented by Equation 5:

$$\text{Mass (g)/Volume } (V_o \text{ or } V_T) \qquad \ldots\ldots 5.$$

Compressibility index: The Compressibility index (CI %) was extrapolated from the bulk and tapped densities using Equation 6:

$$Compressibility\ index\ (\%) \ = \frac{PT-Po}{PT}\ X\ 100 \qquad \ldots\ldots 6$$

Stability studies
The effect of storage on the pH of tea brew: storage in the refrigerator at 0 °C
A tea bag corresponding to 5 g of the herbal blend was brewed by infusion using 250 mL of hot freshly boiled water (≈ 100 °C). The brew was allowed to cool to room temperature (27 ± 2 °C). The pH of the brew solution was determined using a digital pH meter (SPER Scientific, China). The brew was stored in a refrigerator (≈ 5 °C) and the pH determined weekly for four weeks.

The effect of storage on the pH of tea brews
Six tea bags of each formula were divided into two groups (each containing three tea bags). The first group was stored in a photostability chamber while the second group was stored at ambient condition (cool dark home kitchen cupboard) for 12 weeks. At the end of this period, the tea bags were brewed using 250 mL of hot freshly boiled water (≈ 100 °C) in a 250-mL conical flask. The brew was allowed to cool to room temperature (27 ± 2 °C) . The pH of the brew solution was determined using a digital pH meter (SPER Scientific, China).

The effect of storage on antioxidant properties
Three tea bag of each of the samples were selected and stored at ambient temperature in a cool dark place and in a photostability chamber for 12 weeks. At the end of this period, each sample of the teas was emptied into a 250-mL beaker and mixed, after which a 1 g quantity was weighed into a sample bottle and 20 mL of ethanol added. The mixture was allowed to stand with continuous stirring on a shaker for 24 h.
Radical scavenging activity of the ethanol extract of the herbal tea samples against stable 2, 2 diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams *et al*. (1995). A 0.1 mL of the stock solution (equivalent to 0.1 %) was transferred into a screw-capped test tube with a micropipette, 1.0 mL quantity of freshly made 1.0 mM of DPPH ethanol solution was added and made up to 10.0 mL with ethanol. The mixture was shaken and allowed to stand for 30 min in the dark. The absorbance of the mixture was then determined at 517 nm using a UV spectrophotometer. This was also done for the same concentration of Ascorbic acid.
The experiment was carried out in triplicate. The radical scavenging activity was calculated using Equation 7:

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B] \times 100 \qquad \ldots\ldots 7$$

where: A_B = absorption of blank sample and A_A = absorption of test extract solution.

Statistical Analysis
The results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA).

RESULTS
The phytochemical screening of the herbal tea blend indicated the presence of secondary metabolites as shown in Table 2. The poly-herbal tea granules exhibited a mottled dark green and gray colour, characteristic spicy taste and aroma, with a coarse granular texture (Table 3). The granules had a pH, Carr's index and angle of repose of 4.3, 11.3% and 29.8°, respectively. It also gave a moisture content, extractive matter and ash value of 9.0, 17.0 and 2.5, respectively. The leakage of the herbal material from the tea bag was 0.12%. The particle size distribution of the poly-herbal tea granules obtained by sieve analysis is presented in Figure 1. The herbal tea brew gave a slightly acidic pH. Table 4 shows the effect of storage on the pH of the herbal tea brews while the effect of storage on the antioxidant properties is presented in Figure 2.

Table 2: Phytochemical constituents

Phyto-constituents	Presence (+)
Alkaloids	+
Anthraquinones	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	+
Triterpenes	+
Tannins	+

Table 3: Organoleptic properties of the poly-herbal tea

Organoleptic properties	Character
Colour/Appearance	Mottled dark green and gray colour.
(tea granules)	Yellowish-brown
Colour of brew	Characteristic spicy.
Taste	Characteristic aromatic
Smell	Coarse granular powder
Texture	

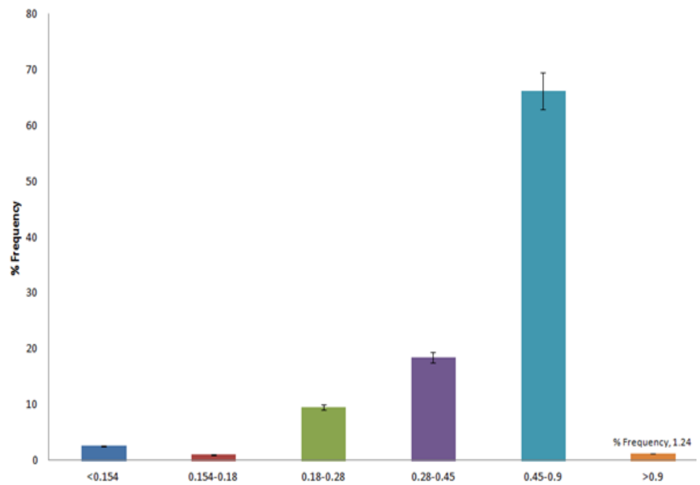

Figure 1: Particle size distribution of the poly-herbal tea granules

Table 4: pH of the tea brews stored in a refrigerator at approximately 5 °C for 28 days

Time (Days)	pH
1	6.31 ± 0.22
7	6.28 ± 0.12
14	6.30 ± 0.20
21	6.31 ± 0.16
28	6.21 ± 0.42

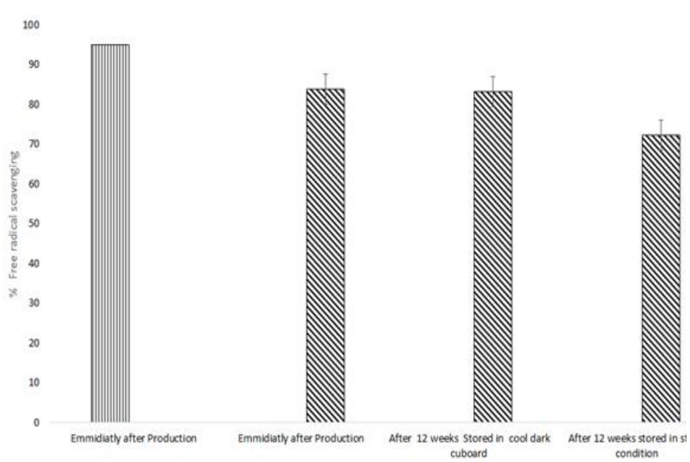

Figure 2: Effect of storage on the antioxidant properties of the poly-herbal tea

DISCUSSION

The pre-formulation processing which included washing, milling, sieving, packaging and labeling of the herbal matareials was done to ensure the production of a good final product. It was ensured that the leaves of *M. oleifera* and the ginger rhizomes were washed separately to allow for the peculiar cleaning needs of the different herbal materials. The drying and control of moisture content in the various raw materials was to ensure that accurate and reproducible quantities of herbal active materials were used always. Packaging the dry material in sealable poly-bags helps to prevent moisture uptake from the atmosphere and labeling with indelible ink was to prevent any mix-up. Storage in a cool dark place was done to prevent degradation due to light or heat, to ensure that the herbal materials were maintained in good condition for use (Alamgir 2017).

The herbal tea recipe is presented as leachable tea bags which are easy to brew by infusion to produce a palatable tea with an acceptable aroma and colour. The granules particle sizes were controlled by the length of time and speed of pulverization as well as sieving. Also, the particle size distribution was controlled to ensure that particles are small enough to support leaching while ensuring that the amount of fines is limited to prevent leakage through the pores of the tea bags. Formulation evaluations showed good throughput and acceptable products.

Organoleptic evaluation is a low cost and first line means of identification and assessment of the quality of the product using the human perceptible senses. Though the assessment and judgment may appear to be subjective, they are usually the simplest and rather most accurate means of identification and quality assessment (Munoz & Civille 1998; Ahirwal *et al*. 2006; Alam & Saqib 2015). The colour, smell, feel and taste of this herbal tea blends reflect the characteristics of the various components that make up the poly-herbal recipe.

Ash value is the residue that remains after the incineration of the herbal tea. This parameter is characteristic and may help determine the authenticity and purity of the herbal product (Alam & Saqib 2015). The ash of most plant materials contains calcium carbonate as its major component. Calcium carbonate constitutes 25 to 45 % of the ash (Alam & Saqib 2015). Other components include potash (≈10 %), iron, manganese, zinc, copper and heavy metals. Unusually high ash value may reflect contamination or adulteration.

Moisture content is a quality factor in relation to stability during storage. For many solid herbal materials, a moisture content of less than 10 %, is recommended for effective stability during storage to prevent microbial, enzymatic and chemical degradations (European Pharmacopoeia 2006; Müller & Heindl 2006). The moisture content of fresh ginger is up to 89%, while that of *M. oleifera* is about 86 % (Ali *et al*. 2015). Low moisture content in herbal tea will ensure its effective storage until when it will be used.

The water extractive matter relates especially to compounds that can be leached out when the tea is brewed. Some likely compounds that can be leached include sugar, acids and various inorganic compounds which may include minerals and salts. The high extractive matter may be related to the high solubility of the biochemical constituents of the herbal tea in water. pH is an important parameter in the quality and production of beverages. Small changes in pH may affect the stability of the brew and may cause changes in especially the taste, colour and smell of beverages. The brew pH of 4.3 is characteristic and is related to the relative phytochemical constituents of the herbs.

Flow is an important parameter in the production of especially bulk powders and granules because it affects the critical processes of production such as mixing and transfers into primary containers/packaging materials (Höhne 2011). Based on the Carr's scale, the herbal tea granules had an excellent flow; this is because CI values below 15% represent good flow, 15% to 25% represents fair, while values above 25% are indicative of poor flow (Well 2003). The flow of powders as assessed by the angle of repose is based on the inter-particulate cohesion: values less than 25° is indicative of "very-good flow", whereas values equal and greater than 25° but less than 50° indicate "good flow" while values greater than 50° indicate "poor flow". The values obtained for CI corroborates that for angle of repose as excellent flow since the latter for all the batches were less than 25° (Höhne *et al*. 2011).

The particle size of the herbal tea granules is very important as this affects other important parameters such as flow, handling and mixing during production, leaching time, leakage of tea powder from the teabags and the weight of the filled teabags. Particles with sizes less than 0.15 mm were responsible for the fine particles that quickly initiate leaching out of the tea bag during brewing. They were also responsible for the leaking of fine particle from the tea bags (Poswal *et al*. 2019). Large particles (> 0.9 mm) are more likely to delay the brewing time. The results of the particle size analysis corroborate the results obtained for the dust leak from the various herbal tea bags. The particle size properties of the herbal teas are not intrinsic to the herbal materials but resulted from the techniques used for processing such as grinding and granulation.

Dust leak relates to the loss of material through the pores of the tea bags as fines. The test assesses the quantity of the herbal tea material that is lost and the ability of the tea bags to withstand handling due to stress conditions such as agitation and vibration. These conditions are physically related to conditions encountered during use, such as handling during packaging, transportation and other events involving vibration and movements. Leakage occurs when very fine particles migrate out through the pores of the tea bags. The leakage was less than 1 %. This can be acceptable in relation to the limit for the friability test which is 1%. The low leak values obtained for the various herbal tea bags could be attributed to the stable granules and low quantities of fines in the herbal teas' granule formulations. The low leak value will prevent the tea dust from accumulating on the tea bags and secondary packaging. The low leak value showed that the formulation technique was good.

The stability of the herbal tea relates to the quality of the product in relation to the effect of time and environmental factors such as temperature, humidity and light. The test is most appropriately carried out in the primary containers or packaging material. The maintenance of the various physical, chemical and functional properties of a product are important to preserve the effectiveness and safety of the product. The properties of the herbal tea, such as physical appearance, color of the brew and pH are affected by the storage environment.

The colour of the brew of teas is often characteristic of the herbal material that constitutes the ingredients. The colour also constitutes one of the major attractions to the patronage and consumption of the product (Kumamoto & Sonda 1998; Delgado-Vargas *et al*. 2000). The samples stored at ambient condition retained the initial colour and appearance of the various teas as compared to those stored under stress conditions. The pH of the poly-herbal teas was determined as one of the intrinsic properties of the products (Choi *et al*. 2000; Friedman & Jürgens 2000). The pH of the brewed tea stored in the refrigerator at a temperature of ≈ 5 °C did not show any significant ($p > 0.05$) changes in pH while that stored under stress conditions in a photo stability chamber at 40 °C showed a reduction in pH values which corresponds to instability attributable to chemical changes (Chan *et al*. 2009). This thus gives the condition for storage of the brewed herbal tea.

Many herbal medicines have important health benefits some of which have been tracked to their antioxidant properties (Lobo *et al*. 2010; Rodrigues *et al*. 2017). The free radical scavenging effect of the herbal teas on DPPH indicates that the herbal tea has high antioxidant activity in relation to that of ascorbic acid. This thus, means an effective free radical mopping up properties that are only slightly ($p > 0.005$) lower than that of ascorbic acid. Storing the herbal tea under stressful environmental condition in a photostability testing machine reduced the antioxidant activity remarkably. However, there were no significant ($p > 0.005$) differences between the anti-oxidant effect of the initial product and that stored in ambient condition for 12 weeks in a dark, cool cupboard.

Conclusion

A herbal tea containing *M. oleifera, Z. officinale* and *C. limon* was successfully formulated. The herbal tea showed good physicochemical properties that were characteristic of good products. The products also showed discrepancies in stability that was related to the storage condition. The herbal tea showed physicochemical properties that were intrinsic and non-intrinsic as well as effective free radical scavenging properties that were comparable to that of ascorbic acid. However, some of its physicochemical properties and antioxidant activity were affected by the storage environment. Storage under stressful environmental conditions resulted in degradation of the herbal tea as shown by the significant ($p < 0.05$) differences between that which was obtained immediately after production and that obtained after exposure to a stress environment. Thus, it is recommended that the products should be stored in a cool dry place away from light.

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