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Original Research Article

Formulation and Evaluation of Mango Leaf Tea Supplemented with Moringa and Ginger Powder

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Abstract

Herbal teas contain <u>bioactive compounds</u> which have been reported to have beneficial effects in the prevention of various metabolic diseases. Hence, the current study was conducted to assess the qualitative and quantitative phytochemical composition, functional properties and sensorial attributes of different formulations of mango leaf teas supplemented with *Moringa oleifera* and ginger powder as supporting and activating herbs using standard analytical methods of Association of Official Analytical Chemist. Data was analyzed using one way analysis of variance and results expressed as mean ±standard deviation of triplicate determinations. The qualitative phytochemical analysis indicated the presence of tannins, flavonoids, terpenoids and cardiac glycoside. While quantitative analysis revealed that flavonoids ranged from (0.17-0.52 mg/g), tannins (0.07-0.11 mg/g), cardiac glycoside (1.08-1.89 mg/g) and terpenoids (50%-70%). The results obtained for functional properties revealed that pH ranged from (5.94-6.62), reconstitution index (4-6.6 g/cm³), swelling index (0.33-1.33cm³/g), wettability (3.08-3.14 mins), bulk density (0.25-0.29 g/cm³) and water absorption capacity (27.3-31.9). Mean scores of sensory evaluations for taste ranged from: (7.2-8.9), color (7.6-8.5), flavor (7.3-8.3), consistency (7.3-8.4) and overall acceptability (7.9-8.7). Based on the results of this study, it can be concluded that mango leaf teas supplemented with *Moringa oleifera* and ginger powder are good sources of <u>bioactive compounds</u> with potential nutritional and health benefits.

Keywords: Herbal Teas, Bioactive Compounds, Functional Properties Nutrition and Health Benefits.

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Introduction

Tea (Camellia sinensis) is a widely consumed beverage. Tea is a widely consumed beverage for several thousand years since its introduction in China. This may be due to its perceived health benefits which may include anti-inflammation, analgesic and immune-modulation (Yusuf *et al.*, 2020). Tea (*Camellia sinensis*) is grown in about 30 countries but is consumed worldwide, although at greatly varying levels (Chandan, 2013). Traditionally, tea has been known to have potentially beneficial effects, but these effects were not documented by well-controlled laboratory studies until 1970s (Yusuf *et al.*, 2020). However, current studies have revealed the biological effects of tea such as anti-tumor as well as antimicrobial effects, even at a molecular level (Yusuf *et al.*, 2020).

In recent times, herbal teas are gaining popularity as consumers believe that they are natural, safe and can promote health (Akila *et al.*, 2018). Herbal

teas are produced from green and dried herbs, flowers, fruits, leaves, seeds, barks and roots of medicinal plants and sold in a loose form or packed in bags. Herbal tea formulations are consumed in many parts of the world due to their therapeutic and healing properties (De-Heer *et al.*, 2013).

Mango (Mangifera indica) is an edible stone fruit which is believed to have originated from the region between northwestern Myanmar, Bangladesh, and India. Mango is a good source of immune-boosting nutrients. One cup (165 grams) of mango juice provides 10% of daily provitamin needs. The same amount of mango provides nearly three-quarters of daily vitamin C needs. This vitamin can help the body produce more disease-fighting white blood cells, help these cells work more effectively and improve skin's defenses (Mirelle et al., 2014). Mango is packed with polyphenol compounds that function as antioxidants. It has over a dozen different types, including mangiferin, catechins,

anthocyanins, quercetin, kaempferol, rhamnetin, benzoic acid and many others. Amongst the polyphenols, mangiferin has gained the most interest and is sometimes called a "super antioxidant" (Cui *et al.*, 2012).

Moringa, native to parts of Africa and Asia, is the sole genus in the flowering plant family Moringaceae. The name is derived from murungai, the Tamil word for drumstick, and the plant is commonly referred to as the drumstick tree (Singh, 2019). It contains thirteen species from tropical and subtropical climates that range in size from tiny herbs to massive trees (Dadamouny et al., 2016). Moringa species grow quickly in many types of environments. The most widely cultivated species is Moringa oleifera, native to the foothills of the Himalayas in India, a multipurpose tree cultivated throughout the tropics and marketed as a dietary supplement (Dadamouny et al., 2016). An important factor that account for the medicinal use of Moringa oleifera is that it is a reservoir of antioxidants and antibiotics (Ahmad et al., 2014).

Ginger (Zingiber officinale) is a flowering plant whose rhizome or root, is widely as spice and folk medicine (USNCCIH, 2016). Ginger originated from Southeast Asia. It is true cultigens and does not exist in its wild state (Ravindran, 2016). Ginger is a very popular spice used worldwide; whether it is used to spice up meals, or as a medicine, the demand for ginger all over the world has been consistent throughout history (Ravindran, 2016). Ginger can be used for a variety of food or medicine items such as vegetables, candy, soda, pickles, and alcoholic beverages (Nair, 2019). Ginger has a very long history of use in various forms of traditional and alternative medicine. It's been used to aid digestion, reduce nausea, and help fight the flu and common cold, to name a few of its purposes (Ravindran, 2016). Gingerol is the main bioactive compound in ginger. It's responsible for much of ginger's medicinal properties. Gingerol has powerful anti-inflammatory and antioxidant effects (Wang et al., 2014). It may help reduce oxidative stress, which is the result of having an excess amount of free radicals in the body (Wang et al., 2014).

MATERIALS AND METHODS

Procurement of Ingredients and Authentication

The Mango and Moringa leaves were obtained in Kalgo Town, Kalgo local government, Kebbi State, while the ginger was purchased from local retailers in Kalgo market, Kebbi State, Nigeria and all the materials were authenticated by the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Nigeria.

Sample Processing and Composite Blend

Mango leaves, Moringa leaves and Ginger were sorted manually to remove damage and disease ones. The leaves were washed to remove the dirt and

soaked in 1% normal saline (NaCl) for 5minutes to get rid of microbes. The ginger was peeled and cut into thin slices. The leaves were shade dried to avoid loss of nutrients. Then the sample leaves and sliced ginger were grounded and sieve using 0.5mm pore sieve and stored in air tight container.

Table-1: Percentage Proportion of Ingredients in the Formulation.

ingredients	a1	a2	a3
Mango leaf	60	40	100
Moringa leaf	30	30	-
Ginger	10	30	-

Preparation of Herbal Tea Infusion

The mango herbal tea was prepared by infusing tea bag which contained 3g of the composite blend in 150cm³ boiling water for 3 minutes (Yusuf *et al.*, 2020)

METHODS

Phytochemicals evaluation of the blends was carried out using standard procedures as described by El-Olemy *et al.*, 2004. The procedure for the evaluation of various phytoconstituents is stated below:

Test for tannins

To the sample (0.5cm³), 1cm³ of distilled water was added and stirred. Few drops of 10% ferric chloride reagent were also added. Blue color indicates presence of Gallic tannins and green black color indicates presence of catecholic tannins.

Test for saponnins

The extract was shaken with little quantity of distilled water. Foaming or frothing which persisted for about ten minutes or on warming was taken as evidence for presence of saponins.

Test for flavonoids (alkaline reagent test)

The sample (5cm³) was treated with (5cm³) 10% sodium hydroxide solution. Formation of intense or creamy yellow color indicates the presence of flavonoids.

Test for trepenoids (salkowski test)

The samples (5cm³) was mixed with 2cm³ chloroform and 3cm³ concentrated H₂SO₄ was carefully added to form a layer. A reddish brown coloration shows the presence of terpenoids.

Test for cardiac glycosides (keller-killani test)

The sample (5cm³) was treated with 2cm³ glacial acetic acid and one drop of ferric chloride solution. A brown ring appears at the interface, while in the acetic acid layer, a greenish ring may appear which confirms presence of cardiac glycosides.

Test for anthraquinones (borntragers test)

Extract (0.5g) was shaken with 10cm³ of chloroform for 5 minutes. The extract was filtered and

equal volume of ammonia was added and shaken. A bright pink color indicates the presence of free anthraquinones.

Test for alkaloids

To the sample (0.5g), 1% aqueous hydrochloric acid (3cm³) was added and stirred on a steam bath. This was filtered and filtrate (1cm³) was treated with a few drops of hager's reagent. The reaction was observed for the formation of precipitate which indicates the presence of alkaloids.

Determination of Total Flavonoids

2cm³ of 2% AlCl₃ in ethanol was mixed with 2cm³ of varying concentrations of the standard (0.1-1.0mg/cm³), in methanol. The extract at a concentration of 2cm³ of 1mg/cm³ was also mixed with the 2% AlCl₃ in ethanol. The absorbance was measured at 420 nm after one hour incubation at room temperature. Similar concentrations of quercetin, the positive control were used. The total flavonoid content was calculated as mg quercetin equivalent /g (QE) of extract (Miliauskas *et al.*, 2004).

Determination of Total Tannins

To 1cm^3 of extract (1mg/cm^3) and standard solution of tannic acid $(10\text{-}150~\mu\text{g/cm}^3)$ was made up to 7.5cm^3 with distilled water. Then 0.5cm^3 Folin-Denis reagent and 1cm^3 of 7.5~% $Na_2\text{CO}_3$ solution were added. The volume was made up to 10cm^3 with distilled water and absorbance was measured at 700nm. The total tannin content was expressed as mg of Tannic Acid equivalent /g (TAE) of extract (Polshettiwar *et al.*, 2007).

Determination of Cyanogenic Glycoside

Cyanogenic glycoside quantitative determination methodology used in this research is that by Chukwuma et al., 2016. The sample was weighed into a 250 cm³ round bottom flask and about 200 cm³ of distilled water was added to one gram of each dry wood powder sample and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250 cm³ conical flask containing 20 cm³ of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100 cm^3) , $8 \text{ cm}^3 \text{ of } 6 \text{ M} \text{ NH}_4 \text{OH}$ (ammonium hydroxide), and 2 cm³ of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02 M AgNO₃ (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the end point.

Content of cyanogenic glycoside in the sample was calculated as:

Cyanogenic glycoside
$$\left(\frac{mg}{100g}\right)$$

$$= \frac{\text{Titer Value } (cm^3) \times 1.08 \times exact \ volume}{\text{Aliquot volume } (cm^3) \times sample \ weight \ (g)} \times 100$$

Determination of Terpenoids

Dried plant extract 100mg (wi) was taken and soaked in 9cm³ of ethanol for 24 hour (Indumathi *et al.*, 2014). The extract after filtration, was extracted with 10cm³ of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula. (wi-wf/wi×100).

Water absorption capacity (WAC) determination

From the ground sample, 1g was weighed into conical graduated centrifuge tubes of known weights and mixed with 10cm³ of distilled water for one minute with a glass rod. The tubes were centrifuged at 5000 rpm for 30 min. The volume of free water (the supernatant) was discarded and each tube together with its content was reweighed as water absorbed per gram of sample. The gain in mass was the water absorption capacity of the flour sample. The volume difference represents the volume of water absorbed by 1g of the test sample. Absorption capacity is expressed in grams of water absorbed per gram of sample (Onwuka, 2005).

$$WAC = \frac{Density \ of \ water \times Volume \ absorbed}{Weight \ of \ sample}$$

Reconstitution index (RI)

From the ground sample, five grams of each sample was dissolved in 50cm³ of boiling water. The mixture was agitated for 90 seconds and then transferred into a 50cm³ graduated cylinders and the volume of the sediment was recorded after settling for 30 minutes (Onwuka, 2005).

$$RI (g/ml) = \frac{Volume \text{ of sediment}}{Weight \text{ of sample}}$$

\mathbf{P}^{H} measurement

The P^H of the samples was determined according to the method of Mathew *et al.*, 2015. The samples (10% W/V) were suspended in distilled water.

The suspension was mixed thoroughly in a 100cm³ beaker before the P^H is taken. This was repeated three times and the average was calculated (Mathew *et al.*, 2015).

Swelling index (SI) determination

The method as described by Onwuka, 2005 was used in the determination of the swelling index. Three gram portions (dry basis) of each sample were transferred into clean, dry, graduated (50cm³) cylinders. The samples were gently leveled and the volumes noted. Distilled water (30cm³) was added to each sample. The cylinder was swirled and allowed to stand for 60 min while the change in volume (swelling) was recorded every 15min. The ratio of the initial volume to the final volume was taken as the swelling index.

 $SI = \frac{Change in volume of sample}{Original weight of sample}$

Wettability

Triplicate samples were weighed and in each case, 1.00 g was introduced into a 25 cm³ measuring cylinder with a diameter of 1cm and a finger was placed over the end of the cylinder. The mixture was inverted and clamped at a height of 10cm from the surface of a 250 cm³ beaker containing 100 cm³ of distilled water. The finger was removed to allow the test material to be dumped. In this case, the wettability was taken as the time required for the sample to become completely wet (AOAC, 2006).

Bulk density

Bulk density was determined as described by Mathew *et al.*, 2015. The bulk density (g/cm³) was calculated as weight of sample (g) divided by volume (cm³) of sample.

Bulk density
$$(\frac{g}{cm^3}) = \frac{\text{Weight of sample}}{\text{Volume of sample}}$$

SENSORY EVALUATION

The mango leaf tea supplemented with moringa and ginger powder samples were presented to 15 semi-trained panelists using the method described by Mathew *et al.*, 2015. The panelists was asked to indicate their observations using a 9 point hedonic scale for attributes like colour, texture, aroma, mouth feel, taste, flavor and overall acceptability. Mean scores of sensory evaluation were expressed as: 9- Like extremely, 8- Like very much, 7- Like, 6-Like slightly, 5- Neither like nor dislike, 4- Dislike slightly, 3- Dislike moderately, 2- Dislike and 1- Dislike extremely respectively.

STATISTICAL TOOL

Values were analyzed statistically using Instant statistical software (Sandiago USA). Values were presented as mean \pm standard deviation. Data obtained was one-way analysis of variance (ANOVA). Significant difference was established at P < 0.05.

RESULTS AND DISCUSSION

Table-2: Phytoconstituents of the Formulated Teas

Phytochemicals	A1	A2	A3
Tannins	+	+	+
Saponins	-	-	-
Flavonoids	+	+	+
Trepenoids	+	+	+
Cardiac Glysocides	+	+	+
Anthraquinones	-	-	-
Alkaloids	1	1	-

(+ = Detected; - = Not detected).

Table-3: Quantitative Value of Phytoconstituents of the Formulated Teas

Phytochemicals	A1	A2	A3
Flavonoids	$0.17^{a}\pm0.01$	$0.37^{a}\pm0.15$	$0.52^{a}\pm0.10$
Tannins	$0.09^{a}\pm0.01$	$0.07^{a}\pm0.01$	$0.11^{a}\pm0.02$
Glycosides	$1.89^{b} \pm 0.1$	$1.86^{\text{b}} \pm 0.15$	$1.08^{b} \pm 0.20$
Terpenoids	$70^{\circ}\pm2.00$	50°±1.00	$60^{\circ}\pm2.00$

superscript in a row are not significantly different at P>0.05.

Table-4: Functional Properties of the Formulated Teas

Functional Property	A1	A2	A3
pН	$6.49^{a}+0.1$	$6.62^{a}+0.1$	$5.91^{a}+0.15$
Reconstitution Index	$4.00^{b}+1.0$	$6.00^{b}+1.0$	$6.60^{b} + 0.2$
Swelling Index	$0.33^{c}+0.1$	$0.66^{c}+0.2$	$1.33^{c}+0.1$
Wettability	$3.11^{a}+0.15$	$3.52^{a}+0.2$	$3.14^{a}+0.1$
Bulk Density	$0.29^{b}+0.1$	$0.25^{b}+0.1$	$0.25^{b}+0.1$
Water Absorption Capacity	29.80 ^a +0.2	27.27 ^a +0.25	31.90°a+0.1

Values are mean ± standard deviation of triplicate determinations, values bearing same

superscript in a row are not significantly different at P>0.05.

Table-5: Sensory attributes of the formulated Teas

Sensory Test	A1	A2	A3
Color	$8.40^{a}\pm0.1$	$8.70^{a}\pm0.1$	$7.60^{a}\pm0.1$
Flavor	$8.30^{a}\pm0.2$	$8.30^{a}\pm0.2$	$7.30^{a}\pm0.2$
Taste	$8.90^{b} \pm 0.1$	$8.70^{\text{b}} \pm 0.1$	$7.20^{b} \pm 0.1$
Consistency	$8.40^{a}\pm0.2$	$8.70^{a}\pm0.2$	$7.30^{a}\pm0.2$
Overall Acceptability	$8.70^{a}\pm0.1$	$8.70^{a}\pm0.1$	$7.90^{a}\pm0.1$

Values are mean ± standard deviation of triplicate determinations, values bearing same

superscript in a row are not significantly different at P>0.05.

Table-6: Ranking of the Formulated Herbal Teas based on Selected Parameters

Parameters	A1	A2	A3
Flavonoid	3	2	1
Tannin	2	3	1
Trepenoid	1	3	2
Cardiac Glycoside	1	2	3
Swelling Index	1	2	3
Bulk Density	2	1	1
Water Absorption Capacity	2	1	3
Colour	2	1	3
Flavour	1	1	2
Taste	1	2	3
Consistency	2	1	3
Overall Acceptability	1	1	2
Total	19	20	27

Most desirable (1), desirable (2) and least desirable (3)

DISCUSSION

Phytochemical constituents are non-nutritive plant constituents that are considered beneficial to human health. The current study revealed that the tea under investigation contain some phytochemicals which are; tannins, flavonoids, terpenoids and cardiac glycosides. The quantitative determination of the phytochemicals revealed similar result to the investigation carried out by Orimadegun et al., 2018. The quantity of flavonoids and tannins were higher in sample A3 than sample A1 and sample A2, this is due to higher percentage composition of the mango leaf in sample A3 while sample A1 has the highest quantity of cardiac glycosides and trepenoids which is as a result of the percentage composition of moringa leaves in sample A1. The presence of Terpenoids, Tannins and Flavonoids suggests the composite blend is a reservoir of antioxidants (Okoli et al., 2006).

The flavonoids exhibit the properties of hormone such as oestrogen, a hormone that might affect the risk of breast cancer. The growth of breast depends on estrogen. When an estrogen like substance replaces the body's natural oestrogens position, the substance can act as anti-estrogen. By acting in this way, flavonoids might help work against breast cancer that depends on estrogen for its growth (Ndamitso *et al.*, 2013). Cardiac glycoside have several uses among these uses are the increment of the output force of the heart and increase its rate of contraction by acting on the cellular sodium-potassium ATPase pump. Other

beneficial medical uses are as treatment for congestive heart failure and cardiac arrhythmias; however their relative toxicity prevents them from being widely used. These secondary metabolites also have a wide range of biochemical effects regarding cardiac cell function and have been suggested for use in cancer treatment (Ndamitso *et al.*, 2013).

Tannins were found to be present in all teas samples with the results of the qualitative evaluation. According to Shiavone *et al.* (2008), diets containing tannins at low dosages (0.15-0.20%) improve wellbeing. Tannins also have therapeutic effect on gastric and intestinal illness (Shiavone *et al.*, 2008). They also aid digestion, however, if tannins are ingested in excessive quantities, they inhibit absorption of minerals such as iron; this may lead to anemia over a long period of time (Bajaj, 2002). Tannins can cause regression of tumors but if used excessively over time they can also cause tumors in healthy tissues (Bajaj, 2002).

The result of the functional properties indicated that A3 recorded the highest value for reconstitution index, swelling index and water absorption capacity while A2 recorded the highest value for pH and wettability. However, there was no significant difference (p>0.05) in the listed parameters among all the samples. All samples recorded low swelling index. This may due to the ingredients in the composite blends. It is pertinent to note that high swelling index implies that high amount of water would

be needed to prepare the tea infusion. A1 recorded the highest bulk density. Bulk density is a measure of heaviness of flour or powdered food sample. High bulk density reduces the caloric intake, most especially in children which can result in growth faltering. Nutritionally, low bulk density promotes digestibility, particularly among children with immature digestive system (Afam-Anene and Ahiarakwem, 2014).

The results for the sensorial attributes of the formulations indicated that the composite blends differ in some sensorial attributes evaluated. The results indicated that A1 was rated highest among the three composite blends in all the attributes evaluated. The scores for overall acceptability indicated that the products were highly accepted by the panelist.

CONCLUSION

According to the recent research in last 30 years, tea has been identified as a Nature's reward for promoting human health. There is a continuous increase in the amount of experimental evidence filing the properties of tea and its constituents. The current study established that tea is a source of a large variety of phytochemicals that can be digested, absorbed and metabolized by the body. These phytochemicals have beneficial effect in the prevention of various metabolic diseases in humans. The functional properties of the composite blend formulated suggest that, the samples could be prepared using a small amount of water yet giving the desirable energy density in order to achieve optimum color and taste. The result of the sensory evaluation as revealed by the panelists point to acceptability of the tea prepared. Equally, the study revealed that the herbal tea brewed from A1 is most preferable in color, flavor and overall acceptability. Hence, a blend of mango leaf, moringa leaf and ginger powder can produce herbal tea with appealing sensory attributes. Furthermore, it can be inferred that mango leaf tea supplemented with moringa and ginger is a reservoir of phytochemicals. It can be consumed and commercially distributed to household and community as herbal tea.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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