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RESEARCH ARTICLE

Phenolic Content, Antioxidant Activity and Nutrient Composition of Two Formulated Medicinal Teas from Moringa, Pawpaw, Tea, Soursop and Lemon grass Leaves

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Abstract

This study was conducted in order to evaluate the phenolic content, antioxidant activity and nutrient composition of two medicinal teas formulated from pawpaw, moringa, green tea, soursop and lemon grass leaves. The leaves were harvested fresh, dried in the oven to constant weight at 45 °C ground and used to formulate two different teas. A part of each formula was used for the extraction of bioactives by hot infusion in water or using ethanol or methanol as extraction solvents. The obtained extracts were characterized for their total phenolic content and antioxidant activities. The remaining parts of each tea were used for the determination of the proximate and mineral composition. Results showed that the water infusion, ethanolic and methanolic extracts from formula 1 (F1) were the best in total phenolic content compared to the same extracts from formula 2 (F1). Almost all samples exhibited good radical scavenging activities while the best ferric reducing antioxidant power was obtained with the ethanolic extracts from both formulae. The methanolic extracts of both formulae presented the best hydroxyl radical scavenging activities, followed by the ethanolic extract of formula 1. The analysis of the proximate composition showed that both F1 and F2 have interesting nutritional properties, but F2 was best in mineral composition. The present study shows that F1 can be suitably used for health related issues due to its high phenolic content while both Formulae 1 and 2 can be recommended for persons with nutritional deficiencies due to their interesting nutrient and mineral content.

Keywords: Phenolic Content; Antioxidant Activity; Nutrient Composition; Medicinal Tea

Introduction

Tea as a beverage is currently the most widely consumed in the world second to water. Because of that, it has been ranked as an important world food product [1]. Tea is generally consumed for its medicinal and boosting properties [2]. Studies have shown that medicinal plants have still been under-exploited despite the fact that several works have been carried-out to showcase their medicinal and technological properties. According to the World Health Organization (WHO), more than 80% of the world's population depends on alternative medicine to satisfy their primary health needs. In recent times, fighting for survival has been the preoccupation of many families and individuals living below the poverty line, causing them to rely on medicinal plants to cure some diseases plaguing them [3]. Medicinal plants such as moringa, pawpaw, tea, soursop and lemon plants have been demonstrated as efficient in curing some diseases such as cancer, rheumatism, urinary tract infections and others [4]. From previous reports, it has been found that oxidative stress is one of the main causes in the development of chronic and generative diseases. All beneficial effects of different teas made from the different medicinal plants have been attributed to their strong anti-oxidative activity due to phenolic compounds present, which protect the body from damages caused by free radical- induced oxidative stress [5]. Various works have shown a direct relationship between total phenolic content and antioxidant activity in various plants parts [6].

Moringa leaf is known to be beneficial for people with cardiovascular disorders. Moringa leaf juice is also known to have a stabilizing effect on blood pressure [7]. The leaves have been reported to have hypocholesterolemic [8] and anti-tumour activities [9], as well as being helpful in the treatment of cardiovascular diseases and inflammation [10]. It is also known to be useful for people with high risk factors of hypertension [11]. An infusion of leaf juice has been shown to reduce glucose levels in rabbits [9] and is known to be helpful for people with diabetes mellitus [12]. *Carica papaya* has admirable restorative properties for treatment of various diseases. The distinctive parts of this plant including the leaves, seeds, latex and natural product have been shown to have therapeutic worth. *Carica papaya* is related to the family of caricaceae and generally termed as paw-paw. *Cymbopogon citratus*, commonly known as lemon grass, is a tropical perennial herb belonging to the family Poaceae (true grasses) [13]. It is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root and it is used in folk medicine in the treatment of nervous and gastrointestinal disturbances, fever and hypertension [14] Soursop (*Annona muricata*) also called graviola or guanabana is an evergreen native tropical plant that produces a long green prickly fruit with white sub acidic pulp and is used in fresh juices, sherbets or as a desert fruit [15]. It is an accepted ethno-medicinal remedy for a range of ailments due to its anti-microbial, antifungal, anti-depressant and lowering of blood pressure properties [16]. Tea (*Camellia sinensis*) has medicinal properties that can be traced as far as 5000 years back. The chemical components of green tea chiefly include polyphenols, caffeine and amino acids. Tea also contains flavonoids, compounds reported to have anti-oxidant properties with many beneficial effects. Tea flavonoids reduce inflammation, have antimicrobial effects and prevent tooth decay [17].

Several studies have reported the antioxidant activity of these medicinal plants independently. Despite the studies that have been conducted on medicinal plants for teas, there is almost no report on the antioxidant activity, phenolic content and nutrient composition of a tea from two or more of those medicinal plants. Therefore, the main aim of this study is to evaluate the antioxidant activity, phenolic content, and nutrient composition of two formulated teas from *Moringa oleifera* (moringa), *Carica papaya*(pawpaw), *Camellia sinensis* (tea), *Annona muricata (*soursop)*, and Cymbopogon citratus*(lemon grass)leaves.

Materials and Methods

Material

Pawpaw *(Carica papaya)*, moringa *(Moringa oleifera)* and citronella *(Cymbopogon citratus)* leaves were harvested in the experimental farm of the Catholic University Institute of Buea based at Wokaka, South-West Region Cameroon in March 2018. Tea *(Camellia sinensis)* and soursop *(Annona muricata)* leaves were respectively harvested at Tole Tea farm and molyko, South-West Region, Cameroon, in March 2018.

All the chemicals and reagents used were of analytical reagent grade.

Methods

Teas formulation

The fresh leaves were dried in an electric air-dried oven at 45 °C to constant mass. The dried leaves were ground in a blender machine (Moulinex) and sieved (Diameter of pore: 1mm) before being used for the formulation of different teas.The following table shows the ingredients used in the formulation of the medicinal teas as well as their proportions.

Ingredients	Formula 1	Formula 2
	Quantity (g)	
Pawpaw leaves	25	25
Moringa leaves	30	30
Tea leaves	25	25
Soursop leaves	5	0
Lemon grass leaves	15	20
TOTAL	100	100

Table 1: Ingredients used in the formulation of medicinal tea

Extraction of Antioxidants

Extraction using organic solvents

Polyphenols were extracted from different teas using the maceration method, as described by Womeni *et al.* [18]. About 20 g of each tea powder was individually extracted into 200 ml of Methanol and ethanol. The mixture was regularly subjected to shaking during the extraction. After the 48 hours of maceration, the mixture was filtered with a Wathman N°1 filter paper. The obtained filtrates were subjected to rotatory evaporation at 45 °C under reduced pressure for the removal of the solvent, and the solvent residues were removed by drying the extract at 45 °C until the extract became solid and the weight constant. The dried extracts were stored at 4 °C for further analysis.

Extraction of antioxidants by infusion in water

Antioxidants were extracted from the different tea formulae by infusion in hot water as described by Kalliopi *et al.* [19]. 20 g of tea powder was added into 200 ml of hot water (98°C) and incubated for 15 min. After filtration using the Wathman N°1 filter paper, the filtrate were subjected to evaporation in an electric air-dried oven at 50 °C for three days for the removal of the solvent. The extract was dried to constant weight. The dried extracts were stored at 4 °C for further analysis.

Determination of the total phenolic content

The total phenolic content of tea extracts was determined using the Folin-Ciocalteu colorimetric method, as described by Gao *et al.* [20]. In a test tube of 5 ml volume, 20 µl of a 2 mg/ml extract solution was added, followed by the Folin–Ciocalteu reagent (0.2 ml) and distilled water (2 ml). After 3 min incubation of the solution mixture at room temperature, 1 ml of 20% sodium carbonate solution was added and the mixture re-incubated for 20 min under the same conditions. The absorbance of the resulting blue-coloured solution was measured at 765 nm using a spectrophotometer. The total phenolic content of the extract was calculated from the gallic acid standard curve, and expressed as milligrams equivalents gallic acid per gram of extract. The analysis was done in triplicate.

Determination of the total flavonoid content

Aluminium chloride method was used for flavonoid determination using the method described by Quettier *et al.* [21]. 0.1ml of each extract was mixed with 1.9ml distilled water, then 0.1 ml 10% aluminium chloride-hexa hydrate, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415 nm. Catechin (0.2mg/ml) was used as a standard. Total flavonoid content was expressed as mg CAT/g of extract. The analysis was done in triplicate.

Evaluation of the antioxidant activity

DPPH Radical scavenging activity

The radical scavenging activity of tea extracts was determined using the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) method, as described by Braca *et al.* [22]. 4.5 ml of 0.002% alcoholic solution of DPPH was added to 0.5 mL of different concentrations (125, 250, 500, 1000 and 2000 µg/mL) of samples and standard solutions separately, in order to have final concentrations of products of 25-200 µg/mL. The samples were kept at room temperature in the dark and after 30 min; the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control and blank were measured in comparison with methanol. Synthetic antioxidant, butylated hydroxytoluene (BHT), which is a recognized powerful radical scavenger, was used as positive control. The analysis was done in triplicate. The following formula was used for the calculation of the radical scavenging activity:

$$AA\% = [(Abs_{control} - Abs_{sample}) \times 100/Abs_{control}]$$

Ferric reducing antioxidant power

The antioxidant potential of tea extracts were also evaluated by its ability to reduce iron (III) to iron (II) following the method of Oyaizu [23]. An aliquot of 0.5 mL plant essential oil (125, 250, 500, 1000 and 2000 µg/mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% aqueous $K_3Fe(CN)_6$ solution, well shaken and incubated at 50 °C for 30 min. After incubation, 1 mL of 10% TCA solution was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 10 min. 1.5 mL of supernatant, 1.5 mL of distilled water and 0.1 ml of 0.1% $FeCl_3$ solution were mixed and incubated for 10 min and absorbance read at 700 nm on spectrophotometer. The sample blank, containing all the reagents but no essential oil was prepared under the same conditions. Catechin, a recognized powerful ferric reducer compound, was used as positive control to compare the reducing power of the extracts. Higher absorbance indicates higher reducing power. The analysis was done in triplicate.

Hydroxyl radical scavenging ability

The hydroxyl radical scavenging capacity of the leaves extracts were evaluated by the method described by Olabinri *et al.* [24]. 60µl of $FeSO_4.7H_2O$ (1 mM) was added to 90µl of aqueous 1,10 phenanthroline (1 mM), 2.4 ml of 0.2 M phosphate buffer pH 7.8 was added to the above mixture, followed by addition of 150 µl of hydrogen peroxide (0.17 mM) and 1.5ml of different concentrations of sample in sequence. The mixture was incubated for 5min at room temperature. The absorbance of the mixture was read at 560 nm against blank. All readings were taken in triplicate and Catechin was used as the standard. The percentage inhibition was calculated by the following equation.

$\%$ Hydroxyl radical scavenging capacity= $[(A_0 - A_1)/A_0] \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of the sample or standard.

Proximate composition of the different teas

Fat, ash and protein content of all the samples were determined using standard analytical methods described by AOAC [25] procedures. Moisture content was determined by drying the tea powders in oven at 103 °C to constant weight. It was achieved according to the AOAC procedures 925.40. Ash content was determined by incineration of the leave powders at 550 °C according to the AOAC procedures 942.05. Nitrogen (N) content was determined using micro-Kjeldahl method, according to AOAC procedures 984.13, the protein content was calculated as N x 6.25. Lipid content was determined using Soxhlet apparatus with hexane, following AOAC 963.15 methodology. The total percentage carbohydrate content was determined by the difference method as reported by Onyeike *et al.* [26]. This method involved adding the total values of crude protein, crude fat, moisture and ash constituents of the sample and subtracting it from 100. All samples were analyzed in triplicate.

Energy value

Energy value was calculated using the following formula:

$$\mathbf{E\ (Kcal) = (m_{Lipid} \times 9) + (m_{Carbohydrate} \times 4) + (m_{Protein} \times 4)}$$

Where,
m: mass of macronutrient

Mineral content of the different teas

For the determination of minerals, teas were ashed at 550 °C and dissolved with 10 mL of 20% HCl in a beaker and then filtered into a 100 mL standard flask to determine the mineral content. Calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and iron (Fe) were determined by atomic absorption spectrometer (Varian 220FS Spectra AA, Les Ulis, France). Phosphorus (P) was determined colorimetrically using the vanadomolybdate, according to AOAC procedure 965.17 [25] Mineral contents of the samples were determined from calibration curves of standards minerals. All samples were analyzed in triplicate.

Statistical analysis

Results (Mean ±Standard deviation) obtained in the present study were subjected to one-way analysis of variance (ANOVA) with Student-Newman- Keuls test using Graphpad-InStatversion 3.05, to evaluate the statistical significance of the data. A probability value at $p< 0.05$ was considered statistically significant.

Results and Discussion

Total phenolic content

The total phenolic content of tea formulae extracts obtained from different extraction solvents is presented in Figure 1. The extracts obtained from the infusion of both formulae (F1 and F2 infusions) exhibited a significantly higher total phenolic content compared to the other extracts. These samples were followed by F1 Ethanol, F1 Methanol, F2 Ethanol and F2 Methanol. The ethanolic and methanolic extracts of formula 1 presented a significantly higher total phenolic content compared to the same extracts from formula 2. The phenolic antioxidant extracted from plants have been deeply investigated for their biological functions amongst which their antioxidant activity. In many reports, plants extracts have been demonstrated to be rich in phenolic compound with antioxidant activity [27, 28]. The significant difference observed amongst the samples can be attributed to the nature of the ingredient used in the formulation, the classes of antioxidants present and the extraction solvents [29]. Reports on the antioxidant activity of formulated tea have previously been made by Barreira *et al.* [30].

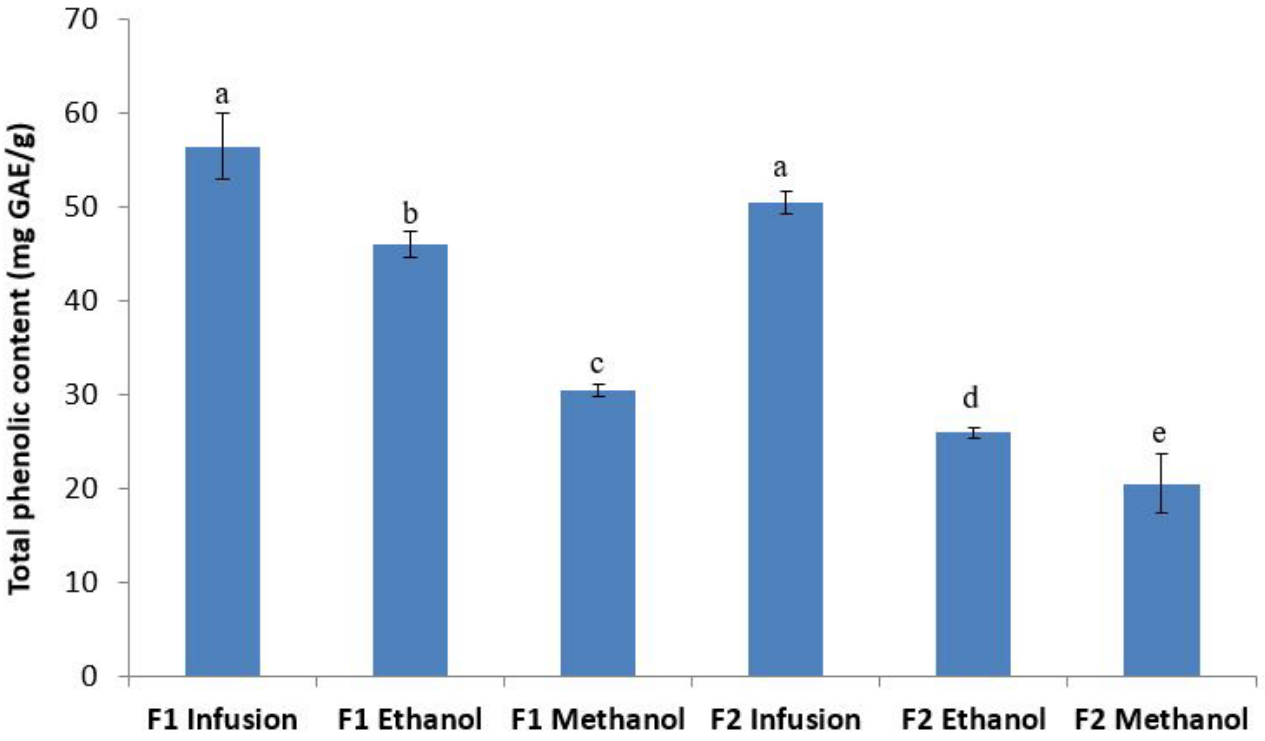

Figure 1: Total phenolic content of different extracts obtained from Tea formula

Data are presented as mean ± SD (n = 3). [a-e]Means with different superscripts are significantly different ($p < 0.05$)

DPPH Radical Scavenging activity

Figure 2 shows the radical scavenging activity of different formula extracts. It can be observed that this parameter significantly increased with the concentration. The highest activity was recorded in F2 Methanol followed by F2 infusion, F2 Ethanol, F1 Ethanol, F1 infusion and F1 Methanol. From these results, it is clear that the extracts from formula No 2 have greater antioxidant activity compared to extracts from formula No 1. The phenolic antioxidant present in this extract might be responsible for the activities observed. Similar results showing that different tea formula have free radical scavenging properties; have been reported by Barreira *et al.* [30] while evaluating the insights on the formulation of herbal beverages with medicinal claims. In the same line, Selvakumar *et al.* [31] show that teas formulated from ginger and green tea have very good antioxidant properties.

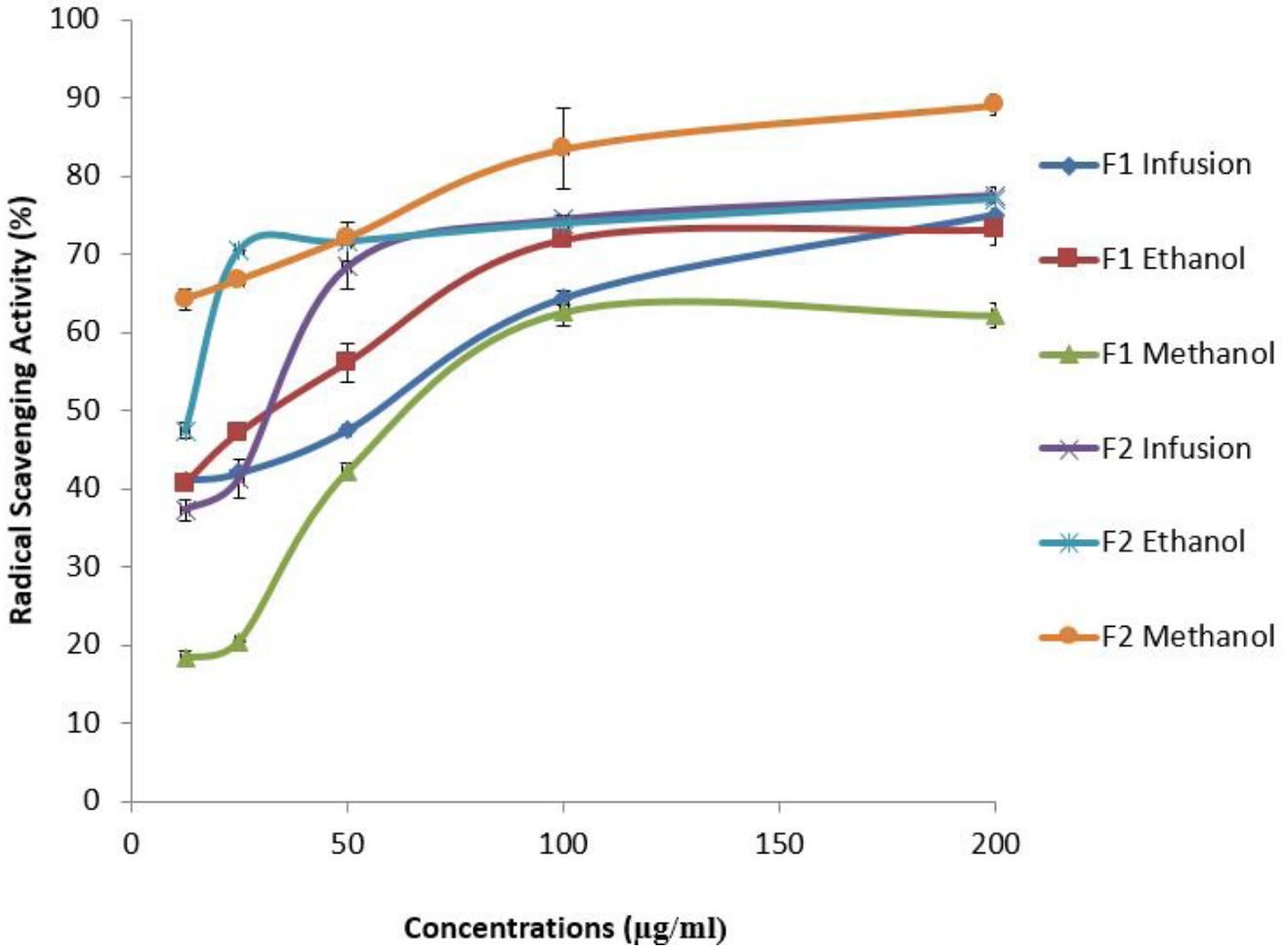

Figure 2: Radical scavenging activity of different extracts obtained from Tea formula

Data are presented as mean ± SD (n = 3). [a-e]Means with different superscripts are significantly different ($p < 0.05$)

Ferric Reducing Antioxidant Power

The efficacy of a molecule or substance to reduce Fe^{3+} into Fe^{2+} by donation of electrons is a good indicator of its antioxidant activity [32]. This parameter was evaluated in both formulae extracts and the results are presented in Figure 3. As previously observed with the DPPH test, the ferric reducing power of all extracts was significantly increasing with the concentration. The highest activity was registered in F2 ethanol followed by F1 ethanol, F1 methanol, F2 methanol, F2 infusion and F1 infusion. Results showed that organic solvents were more efficient in extracting antioxidant molecules with ferric reducing abilities. Ethanol was the best, and this can be attributed to their capacity of extract polar and some apolar molecules at the same time. These results are in accordance with the findings of Igbal and Bhanger [33], which show that organic polar solvents have good antioxidants activities. They are also in line with the findings of Array *et al.* [34] who show that the ethanolic extract of *Curcuma longa* has very good ferric reducing power followed by the methanolic extracts and the aqueous extracts.

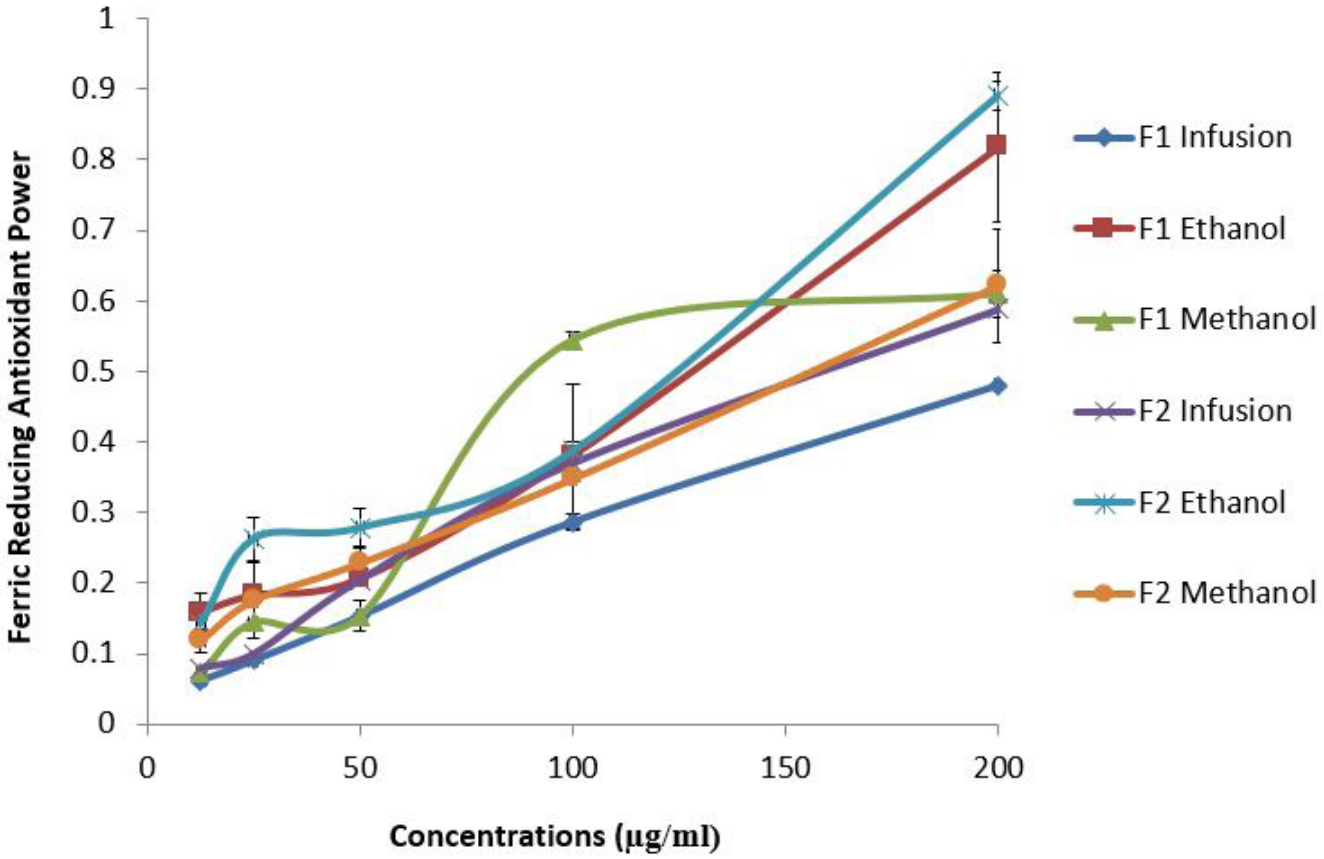

Figure 3: Ferric reducing antioxidant power of different extracts obtained from Tea formula

Data are presented as mean ± SD (n = 3). [a-e]Means with different superscripts are significantly different ($p < 0.05$)

Hydroxyl Radical Scavenging Activity

The antioxidant activity of a substance can also be tested by evaluating its ability to inhibit hydroxyl radicals. This is a dangerous radical in the human body obtained from the Fenton's reaction, with the ability to create several damages in the body Seladji *et al.* [35]. The ability of different formula extracts infusion inhibiting the hydroxyl radical is presented in Figure 4. As previously recorded with the other tests, the activity of the extracts was significantly increasing in the concentration. The best activity was obtained with the methanolic extracts followed by ethanolic extracts and water. The molecules with the ability to trap these radicals are therefore more extracted by methanol. The nature of the extraction solvent, the ingredient used in the formula as well as the classes of antioxidants extracted can explain these results. The data obtained in this study are in accordance with those reported by Selvakumar *et al.* [31] who show that, different tea extracts formulated from ginger and green tea have good hydroxyl radical scavenging activity.

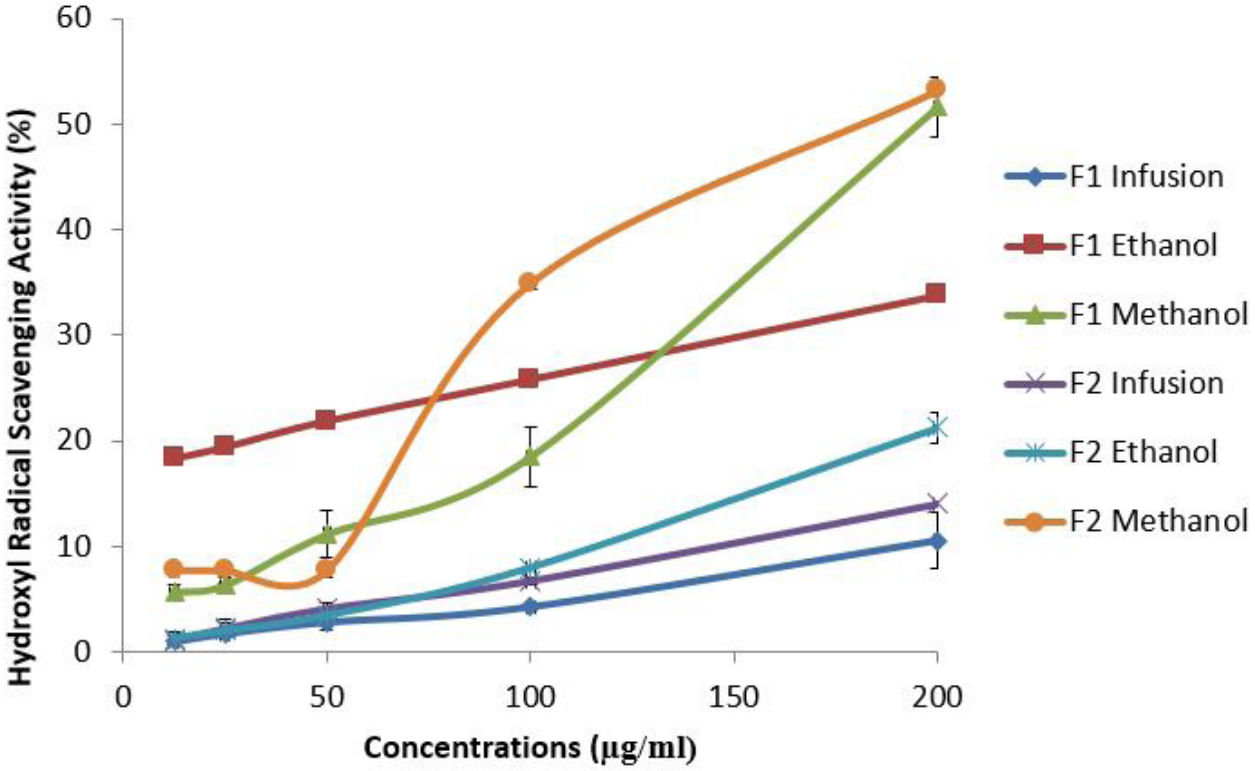

Figure 4: Hydroxyl radical scavenging activity of different extracts obtained from Tea formula

Data are presented as mean ± SD (n = 3). [a-e]Means with different superscripts are significantly different (p < 0.05)

Proximate Composition

The proximate composition of the formulated tea is presented in Table 2. Formula No 1 has exhibited a significantly higher protein content compared to formula 2. However, the lipids, ash, crude fibers contents and energy values of both formulae were similar. As far as carbohydrates are concerned, the highest concentration was recorded in formula 2. From these observations, formula 1 and formula 2 have good nutritional values and can provide almost the same energy value. Similar results was reported by Christine *et al.* [36], which showed that herbal tea, green tea B and green tea YL have interesting biochemical composition (ash, lipid, protein and total sugar). The ash content obtained in our study was significantly lower to those reported by this author who obtained a value ranging between 7 and 13.94. However, the protein, lipids, carbohydrates content where significantly higher compared to those obtained by this same author. The high protein and lipid contents obtained in this study can be attributed to the presence of *Moringa oleifera* leaves which were proven to have protein and lipid contents of 25 and 10.42% respectively by Gonzalez-Burgos *et al.* [37]. Similar lipid (4.03-9.51%) and protein (22.99-29.36%) contents were also obtained by Sultana [36] with the leaves of this same plant.

Parameter	Formula 1	Formula 2
Protein (%)	22.00 ± 0.44[a]	10.00 ± 0.57[b]
Lipid (%)	12.15±0.39[a]	11.97±0.04[a]
Carbohydrates (%)	5.82 ± 0.74[a]	18.00 ± 0.21[b]
Ash (%)	4.60 ± 0.05[a]	3.40 ± 0.00[a]
Crude Fibers (%)	55.43 ± 2.12[a]	56.63 ± 1.43[a]
Energy (Kcal)	220.63[a]	219.73

Table 5: Proximate composition (dry basis) of tea formula
Data are presented as mean ± SD (n = 3). [a-b]Means within each row with different superscripts are significantly different (p < 0.05)

Mineral Content

The mineral composition of both tea formulae is presented in table 3. The analyzed minerals were present in both formulae at different levels. Formula 2 exhibited significantly higher Fe, Ca, Mg and K contents compared to Formula 1. The Na content was similar ($p>0.05$) in both formulae. From these observations, Formula 2 had richer mineral composition than Formula 1. Similar observations were made by Christine *et al.* [36] during the determination of minerals of the herbal and green tea from *Lippia multiflora.* The presence of these minerals in these teas shows their potential importance in the good functioning of the human body as they are implicated in many enzymatic reactions, energy production, transmission of nerves impulses and other biological reactions [39]. A mineral like Fe is an essential part of the respiratory pigment myoglobin, hemoglobin and many other enzymes. Its deficiency leads to anemia which is one of the most severe nutritional disorders in the world [40]. K and Ca play a very important role in bones mineralization [41]. Na and K help against hypertension and Mg facilitates a good functioning of the brain [42].

Mineral	Formula 1	Formula 2
Fe (mg/100g)	12.70 ± 0.00^{b}	14.10 ± 1.00^{b}
P (mg/100g)	192.00 ± 12.13^{a}	174.00 ± 4.56^{b}
Ca (mg/100g)	936.00 ± 8.74^{a}	1176.00 ± 2.64^{b}
Mg (mg/100g)	228.00 ± 3.55^{a}	277.00 ± 1.01^{b}
K (mg/100g)	1302.00 ± 13.22^{a}	1367.00 ± 10.11^{b}
Na (mg/100g)	111.00 ± 7.22^{a}	118.00 ± 5.32^{a}

Table 3: Mineral composition of tea formulae

Data are presented as mean ± SD (n = 3). $^{a\text{-}b}$Means within each row with different superscripts are significantly different ($p < 0.05$)

Conclusion

The objective of this study was to assess the phytochemical and nutritional properties of two formulated medicinal teas from paw-paw (*Carica papaya)*, moringa *(Moringa oleifera)*, green tea *(Camellia sinensis)*, soursop *(Annona muricata)* and lemon grass *(Cymbopogon citratus)* leaves. Results showed that the water infusion, ethanolic and methanolic extracts from formula 1 were the best in total phenolic content compared to the same extracts from formula 2. Almost all samples exhibited good radical scavenging activities while the best ferric reducing antioxidant power was obtained with the ethanolic extracts from both formulae. The methanolic extracts of both formulae presented the best hydroxyl radical scavenging activities, followed by the ethanolic extract of formula 1. The analysis of the proximate composition showed that both F1 and F2 have interesting nutritional properties, but F2 was best in mineral composition. The present study shows that F1 can be suitably used for health related issues due to its high phenolic content while both Formulae 1 and 2 can be recommended for persons with nutritional deficiencies due to their interesting nutrient and mineral content.

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