
Sensory Performance, Proximate and Antioxidant Activity of Tea from Composite Formulation of *Cymbopogon citratus*, *Lippia multiflora* and *Ganoderma lucidum*

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To cite this article:

Courage Sedem Dzah. Sensory Performance, Proximate and Antioxidant Activity of Tea from Composite Formulation of *Cymbopogon citratus*, *Lippia multiflora* and *Ganoderma lucidum*. *Journal of Food and Nutrition Sciences*. Vol. 3, No. 3, 2015, pp. 131-138.

doi: 10.11648/j.jfns.20150303.19

Abstract: Commercially produced herbal green teas consumed in Ghana are mostly those imported into the country. However, there are many plants such as “Srenunum” (*Lippia multiflora*) and lemongrass (*Cymbopogon citratus*), that are used by Ghanaians to brew herbal tea. The objectives of this study were to develop a tea product from the aforementioned herbs and “reishi” (*Ganoderma lucidum*), a medicinal mushroom. Three formulations (A, B and C) were produced according to the respective percentages (50:30:20, 30:20:50 and 20:50:30 for *G. lucidum*: *L. multiflora*: *C. citratus*). Formulation A came out as the most liked tea through an affective test (7 point hedonic scale) which was then compared to a known control (Lipton-yellow label) by chemical, proximate, antioxidant activity determination and sensory evaluation. The two tea samples (Tea A and Lipton) differed significantly with respect to aroma, colour, after taste and overall acceptability ($p < 0.05$). Tea A had significantly higher values for proteins, crude fibre and ash contents than Lipton in terms of their proximate compositions whereas Lipton contained higher levels of moisture and lipids ($P < 0.05$). For increasing concentrations of tea samples (10, 15, 20, 30, 50 μ L) used in antioxidant activity determination, tea A recorded between 59.07 and 88.91 mgTE/g, significantly higher than for Lipton (between 22.81 and 34.45 mgTE/g) ($P < 0.05$). Given the high performance of Tea A, *G. lucidum*, *L. multiflora* and *C. citratus* can be successfully used to produce tea of equally good quality to encourage local consumption of indigenous herbs as well as reduce tea imports.

Keywords: *Cymbopogon citratus*, Herbal Tea, *Lippia multiflora*, *Ganoderma lucidum*

1. Introduction

Tea is a common beverage and most consumed after water worldwide and can be classified into three types known as green (unfermented), oolong (partially fermented) and black (fermented) teas [1]. There are many types of tea products, one of such is the common brand, Lipton among others which are imported and marketed in Ghana. As a developing country with a food security and economic growth agenda, Ghana's indigenous herbs which are already being used for medicinal and refreshment purposes and which form part of Ghanaian culture should be utilized in tea production [2-4]. Also, in order to reduce the nation's expenditure on tea importation, other herbs such as the lemon grass (*Cymbopogon citratus*) and an indigenous plant called “Srenunum” in the Ghanaian Twi dialect (*Lippia multiflora*) should be valorized to enhance their use as herbal teas in Ghana.

Beside economic benefits earlier mentioned, tea from *C. citratus* leaves is used for its analgesic, antispasmodic, anti-inflammatory, antipyretic, diuretic and sedative medical effects and as well in the treatment of digestive disorders, fevers, menstrual disorder, rheumatism and other joint pains [5-8]. A meta-analysis of a number of population studies suggests that consumption of green and black tea beverages may bring positive health effects due to the high levels of flavonoids and antioxidants [9]. Another study showed that substantial number of studies with green and black tea demonstrated a significant increase in plasma antioxidant capacity in humans approximately 1 hour after consumption of moderate amounts of tea (1-6 cups/day) leading to reduced oxidative damage [10].

Lippia multiflora is one of the about 240 plant *Lippia* species which are extremely plentiful, aromatic and occur mostly in the tropics of America and Africa [11,12]. Many

species are widely used for their medicinal properties and to season foods. *Lippia* species are also used traditionally for the treatment of a variety of ailments such as gastrointestinal, respiratory complaints and for relaxation and sedation [3,12]. Other studies also linked the use of *Lippia multiflora* to the treatment of blood pressure, malaria, diarrhea and mild hypertension [2,3,13].

The mushroom, *Ganoderma lucidum*, commonly referred to as “Lingzhi” or “reishi” has been used in Asia, especially in China for health promotion and longevity for centuries. This mushroom has recently been studied and known to have numerous pharmacological effects including immunomodulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidative and radical scavenging, and anti-aging effects among others [14-17]. Another study conducted earlier also confirmed that *G. lucidum* extracts possess anti-proliferative effects on many cancers, such as acute myelogenous leukaemia [18].

Considering literature support on the benefits of *L. multiflora*, *C. citratus* and *G. lucidum*, and the fact that they have all been used traditionally for quite long, it was not out of place to consider combining the three to develop a unique tea product. This study therefore sought to develop a unique tea product from these three healthful ingredients and compare with a top, known and common brand, Lipton for acceptability, chemical, proximate composition and antioxidant activity.

2. Material and Methods

2.1. Materials

L. multiflora and *C. citratus* were collected from Juapong in the Volta region of Ghana while *G. lucidum* was obtained from the botanical garden of Kwame Nkrumah University of Science and Technology, Ghana and identified at the Center for Scientific and Industrial Research-Food Research Institute (CSIR-FRI), Accra. *L. multiflora* and *C. citratus* were cleaned and dried in the solar dryer for 48 hours and *G. lucidum* for 72 hours. *G. lucidum* was dried for 72 hours because of its hard, woody basidiocarp which slowed the rate of drying. The materials were then separately milled into powder and three (3) out of nine (9) formulations selected (A=50:30:20, B=30:20:50 and C=20:50:30 for %*G. Lucidum*: *L. multiflora*: *C. citratus*). The various formulations were packed in air tight plastic containers and stored at -20°C for further analyses.

2.2. Methods

2.2.1. Proximate Analyses

Proximate analyses of the tea formulation A (selected through an affective test as most accepted) and Lipton were done using the method of Association of Official Analytical Chemists [19].

2.2.2. Moisture Content Determination

Petri dishes were selected, cleaned and labeled. The dishes

were dried in a hot air oven at 105°C for about 30 minutes and cooled in an air-tight desiccator. The dishes were then weighed after being removed from the desiccator using a calibrated digital analytical balance and their various weights were recorded. 2g of each sample was weighed into the labeled pre-weighed Petri dishes and weighed again. They were immediately transferred into an air oven pre-set at 105°C to dry. Samples in crucibles had their weights measured severally until they obtained constant weights as final values. The difference in initial and final weights indicated moisture contents of tea samples.

2.2.3. Lipids Content Determination

1.0g each of the samples was weighed into a pre-weighed thimble. 150ml pet ether was measured into a 250ml conical flask using the measuring cylinder. The samples were then extracted under reflux using a soxhlet extractor fitted with thimble containing samples. The extraction was stopped after eight (8) hours and the thimbles with contents were removed, dried in an oven at 105°C for two hours and weighed with an analytical balance.

2.2.4. Ash Content Determination

Porcelain crucibles were selected (3 for each sample), cleaned and labeled. The crucibles were dried in the hot air oven at 105°C for about 30 minutes and placed in a desiccator to cool. The initial weights of the cooled crucibles were recorded. 2.0g of each sample was weighed into each of the crucibles and placed in a muffle furnace (Gallenkamp model) to carbonize at 600°C for 2 hours till the samples had a cotton wool like texture. The crucibles with the ash were removed and kept in a desiccator to cool. They were reweighed and the percentage ash was calculated.

2.2.5. Protein Content Determination

One gram (1.0g) each of the samples (tea formulation A and Lipton) was weighed into the kjeldahl flask. 1.0g of K₂SO₄ and 0.1g Ca₂SO₄ were added into the flask and mixed with 20ml of H₂SO₄ after which the flask was placed on the kjeldahl digestion heating mantle in a slanting position in a fume chamber. The process was monitored until a color change which was from black to bluish-green was observed. The reaction was then stopped and digests removed and cooled. They were then topped with water and made up to 200ml mark on ice. 50ml of aliquot of each digest was poured into a distillation flask and carefully layered with 50ml of NaOH. The solution was then distilled into a receiving flask containing 50ml of 0.1N H₂SO₄ with two drops of methyl red as an indicator. Distillation was stopped by removing the solution in the receiving flask immediately before putting of hot mantle to avoid drop in pressure. The distillate was titrated with 0.1M NaOH and percentage nitrogen calculated as protein content.

2.2.6. Crude Fibre Content Determination

This is organic residue which remains after the materials have been treated with standardized conditions with light petroleum, boiling dilute H₂SO₄, boiling dilute NaOH

solution, dilute HCl, alcohol and ether. The crude fibre consists largely of cellulose together with a little lignin and it can be extrapolated as 100-(% Moisture+% Ash+% Lipid+% Protein).

2.3. Chemical Analysis

2.3.1. pH Determination

The pH of tea samples (tea A and Lipton) were read using a digital pH meter (model HI 2211, Hanna instruments, USA) in triplicates by dipping electrode in tea samples and allowing readings to stabilise.

2.3.2. Water Extractives Determination

Beside proximate analysis, the amount of solids that can be extracted from tea samples when brewed (water extractives) was determined for both tea samples. 2.0g of each sample was weighed into round bottom flasks and fitted to reflux condensers. 100ml distilled water was added to each of them and were boiled for one hour and filtered using Whatman filter paper. The filtrates were poured into beakers and evaporated over steam on a water bath until the contents on the beakers reduced by about 95%. At this time, the beakers were removed into a hot air oven to dry. The beakers containing the dry sample solids were removed after drying and placed in a desiccator to cool. They were then reweighed and their respective weights recorded. The percentage total solids were calculated for the two tea samples.

2.3.3. Sensory Evaluation

The most accepted of the three tea formulations (A) was sensorially evaluated against Lipton tea brand. A total of thirty five (35) untrained panellists were randomly recruited from among students in The Kwame Nkrumah University of Science and Technology, Kumasi. The criteria with which sensory panellists were selected were their familiarity with tea quality parameters, were in good health and willing to participate in the exercise.

The sensory room was well lit and air conditioned to ensure comfort of panellists as well as prevent exterior distractions. Two sets of sensory evaluation were organized; the first for the three tea formulations A, B and C in order to select the most preferred, and the second to evaluate the acceptance of the most preferred against a known brand (Lipton). In each case, the same approach was used. For the second part of the sensory evaluation, panellists were then asked to evaluate the 2 randomly coded samples (tea A and Lipton) on a 7 – point hedonic scale (1 dislike very much, 2 dislike moderately, 3 dislike slightly, 4 neither like nor dislike, 5 like slightly, 6 like moderately, 7 like very much) in an affective sensory evaluation of parameters such as aroma, colour, taste, after taste, astringency and overall acceptability. Panellists had no previous information about samples tested in order to avoid bias.

2.4. Antioxidant Activity Determination

Prior to antioxidant activity determinations for tea samples (formulation A and Lipton), hydrophilic extraction of

antioxidants was done for each tea sample [20]. The Trolox Equivalent Antioxidant Capacity (TEAC) assay was used to determine antioxidant activity. Buffer solutions were prepared from 0.1 M NaOH₂PO₄·2H₂O (N = 2.9, MW= 120), 0.1M Na₂HPO₄ (N = 2.6, MW = 177.99) and NaCl. Reaction solution was prepared from 0.5335g 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 0.0339g 2,2'-azobis(2-amidopropane) (ABAP) each in 50mL of buffer solution. Reaction mixture was incubated at 65°C for 30 minutes to enhance the formation of ABTS radicals (ABTS*) which gave a greenish solution of a ±0.4 absorbance at 734nm against a blank buffer solution. The reaction solution was then centrifuged at 20000 rpm for 15 minutes at 20°C and supernatants used immediately. Hydrophilic antioxidant extracts were then diluted by a factor of 5 (200 µL sample: 800 µL buffer solution) before use. 10, 15, 20, 30 and 50µL samples were respectively added to 290, 285, 280, 270 and 250µL reaction solutions in spectrophotometry plate wells in triplicates and incubated at 37°C for 6 minutes and absorbance read at 734nm against blank (buffer solution). Reduction in greenish colour of reaction mixture represents radical inhibition by antioxidants in samples analyzed.

2.5. Data Analysis

Data were analysed by analyses of variance (ANOVA) using SPSS (version 22.0) with a 95% confidence interval. Significance was set at P < 0.05. Results were presented in tables and figures and interpreted accordingly.

3. Results and Discussion

3.1. Chemical Analysis

Tea A was slightly more acidic than the control (Lipton) (Table 1). Concerning the amount of dissolved solids in infused tea samples, Tea A recorded significantly higher value for water extractives (36.53) than Lipton (19.77) (Table 1). This difference was partly due to the smaller granular size of tea contents as Tea A contained more tiny granules than Lipton, which gave it a larger surface area, enabling faster and easier dissolution in hot water [21]. This difference was also explained by the inherent differences in total soluble solids contents of the sample components. *G. lucidum* was found to contain high levels (82%) of water-soluble polysaccharides [22]. Similar assertion can be made for *L. multiflora* with high soluble solids [23].

3.2. Proximate Analyses

Tea A had significantly higher values than Lipton in terms of their proximate compositions with respect to proteins, crude fibre and ash (P < 0.05) whereas Lipton contained significantly higher levels of moisture and lipids (Table 2). An earlier study showed proximate results for *C. citratus* to be 4.56, 64.00 and 20.00% for crude protein, crude fibre and ash respectively [6]. Similar analysis in other studies showed results for crude protein, crude fibre and ash contents for *G. lucidum* (26.40, 51.50 and 6.50) and *L. multiflora* (12.95,

80.00 and 10.25 %) which did not differ much from those obtained in this study (Table 2) [24,25]. These findings compare fairly with values obtained for protein, crude fibre and ash contents of tea A. Although protein content was relatively high for Tea A (9.02%) (Table 2), results only showed protein contents of raw tea but did not determine how much protein is available in infused tea. This gap will be addressed in future studies as a study by the American Heart Association showed that high protein drinks may pose health risks to certain individuals beside the fact that they are helpful in weight loss and muscle building [26]. The high protein content was also explained by the 30% *G. lucidum* (mushroom) in the formulation.

3.3. Sensory Evaluation of Tea Samples

The need for sensory evaluation of formulated tea sample is crucial and more so when compared with a known brand like Lipton. And this is a commonly accepted approach adopted when developing new food products [27]. A 7-point hedonic scale (1 dislike very much - 4 neither like nor dislike - 7 like very much) was used to assess sensory attributes such as aroma, colour, taste, after taste, astringency and overall acceptability by means of an affective test. Mean scores were calculated for each attribute and a summary presented (Tables 3). Table 3 depicts results for the three different tea formulations (A, B and C) with sample A being ranked best with a mean overall score of 5.44 (like slightly) higher than samples B (4.98) and C (4.04). There were significant differences observed between tea formulations regarding all sensory attributes (Table 3) ($P < 0.05$). Best tea sample (Tea A) was compared sensorially with a known control, Lipton and results tabulated (Table 4).

Mean scores for aroma, colour, after taste and overall acceptability showed significant differences between tea A and the known control brand, Lipton ($P < 0.05$) (Table 4). No significant differences were however observed between samples for taste and astringency. Tea A which consists of 50% *C. citratus*, 30% *G. lucidum* and 20% *L. multiflora* significantly out-performed the control (Lipton) regarding the attributes aroma and overall acceptability ($P < 0.05$) (Table 4). Nonetheless, panellists rated the control (Lipton) significantly higher in colour and aftertaste than tea A ($P < 0.05$).

4. Effects of Formulation Components on Sensory Attributes of Tea A

4.1. Aroma

The mean scores for aroma of tea samples were 5.63 (like slightly) and 4.00 (neither like nor dislike) respectively for tea A and Lipton (Table 4). Given that the infused tea samples contained no additives, it was believed that their aroma were coming only from the samples themselves. Serving temperature was held constant to avoid bias as temperature is known to influence aroma perception in foods

[28]. Tea A which contained 50% *C. citratus*, 30 % *G. lucidum* and 20% *L. multiflora* had a significantly higher acceptability for aroma than Lipton. It was realized from preliminary sensory test for three (3) tea formulations that those with higher percentages of *C. citratus* had higher scores for aroma (Table 3). Other studies also support this point that pure lemongrass tea's acceptability was strongly linked to its citral aroma [29,30].

4.2. Colour

For colour, the control sample, Lipton scored significantly higher than tea A as shown in Table 4. Although *C. citratus* had excellent colour attribute due to its light and clear green colour, combining it with *L. multiflora* and *G. lucidum* impacted overall colour of the formulation [29]. When brewed separately, *L. multiflora* and *C. citratus* had light green colour with the exception of *G. lucidum* which had a dark brown colour. A mixture of the three components resulted in an infusion with a hazy light brown colour against a clear, bright brown colour of Lipton. It was not surprising that the control tea sample (Lipton) scored higher on colour as clearness of infusions is a more preferred attribute that enhances colour brightness than haziness in tea [4].

4.3. Taste

Mean scores showed no significant difference between tea samples for taste (Table 4). It was observed that the control tea (Lipton) scored slightly higher (5.03) than tea A (4.89) for taste and this was best explained by the bitter taste contributed by *G. lucidum* [31,32]. This bitter taste from *G. lucidum* masked the pleasant citral tastes of *L. multiflora* and *C. citratus* significantly thereby influencing overall taste of tea A.

4.4. After Taste

The aftertaste of foods including tea describes the lasting impression of the food after consumption and seems to mask acceptable attributes such as taste since it is most often perceived last. As the taste of new products and functional foods such as teas is a crucial determinant of acceptance, aftertaste plays a similar role [33]. Unlike *C. citratus* and *L. multiflora*, *G. lucidum* has a bitter aftertaste which however does not linger for long. This was the best explanation for the low score for tea A regarding aftertaste. It is therefore understandable that while *C. citratus* and *L. multiflora* are patronized for their medicinal and refreshing attributes, *G. lucidum* is mostly used as medicinal and not for refreshment [6,7,12,14].

4.5. Astringency

Lemongrass (*C. citratus*) has been found to be astringent [34,35]. Another study also confirmed that the young leaves of *L. multiflora* are especially astringent [36]. However, neither tea A nor Lipton was preferred for astringency as they both had scores around 4 (neither liked nor disliked) as shown in Table 4.

4.6. Overall Acceptability

For overall acceptability, tea A scored significantly higher than the control (Lipton). Regardless of the fact that tea A scored lower for taste and aftertaste, its strong citrus aroma due to the high percentage of lemongrass (*C. citratus*) (50%) in the formulation explained its preference to Lipton (Table 3).

5. Antioxidant Activity Determination

Antioxidant capacity assessment of tea samples by TEAC method showed that all the sample extracts were able to

quench the ABTS* radical (Figure 2). However, tea A recorded significantly higher TEAC values ranging between 59.07 and 88.91 mgTE/g whereas control tea sample (Lipton) had values ranging from 22.81 and 34.45 mgTE/g of sample (Figure 2). The relatively higher antioxidant activity of tea A can be justified by its high content (50%) of *C. citratus* which was confirmed to have high antioxidant activity [6]. Other studies also demonstrated that the antioxidant activity of 1g of *L. multiflora* leaf is equivalent to 2.7-12.4g of Trolox, indicating its potential as a strong antioxidant [37]. Similar comments could be passed about *G. lucidum* as results clearly indicated that it is effective as an antioxidant against free radical mediated diseases [38].

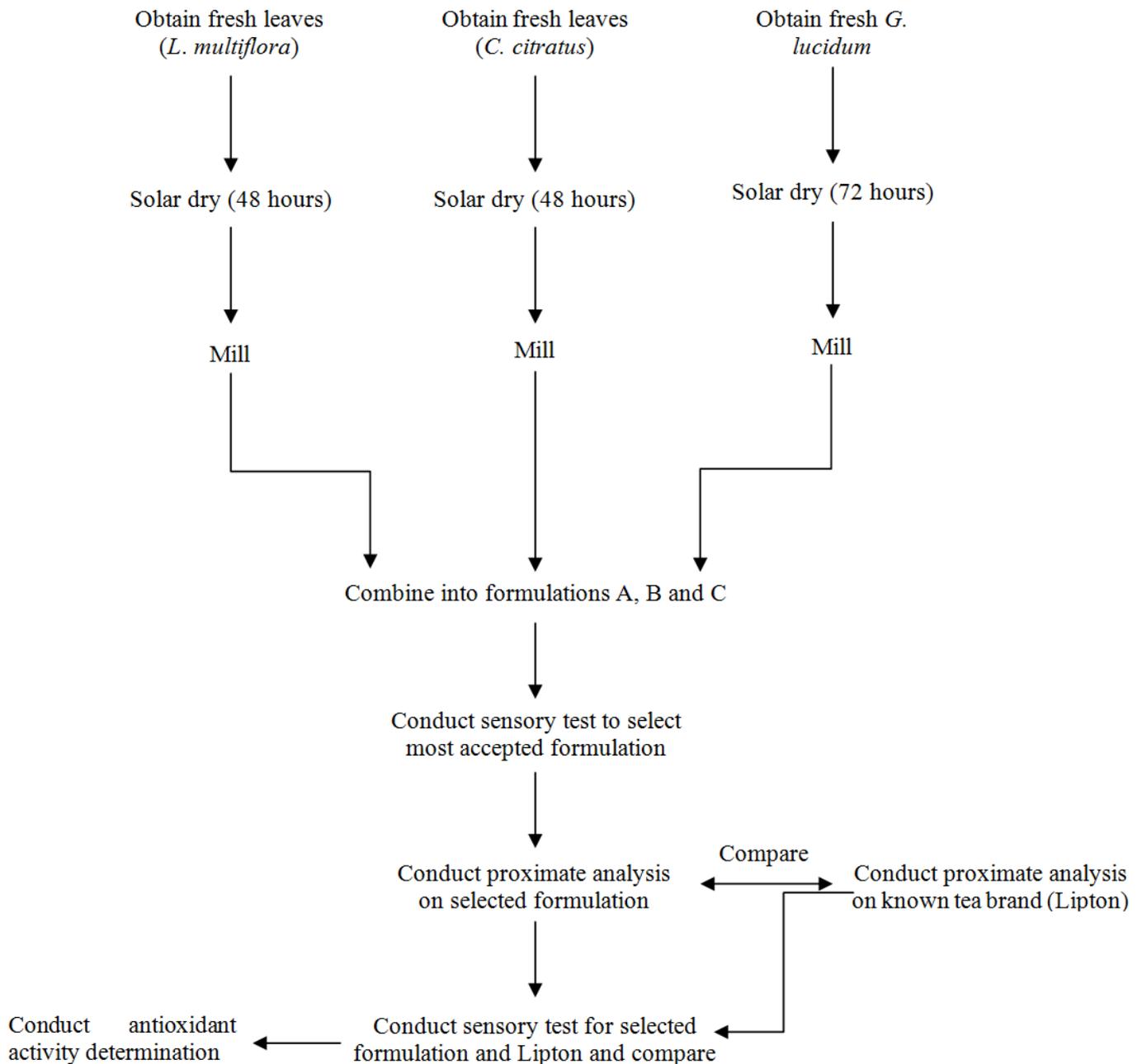


Figure 1. Method summarized in flow diagram.

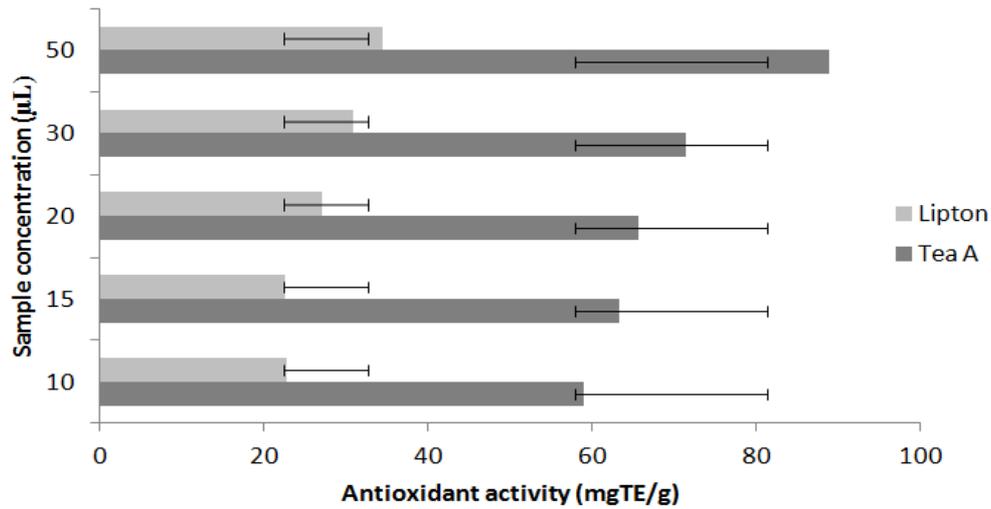


Figure 2. Antioxidant activity for tea samples. Tea A contained 50% *C. citratus*, 30 % *G. lucidum* and 20% *L. multiflora*. Lipton was control sample. Error bars represent standard deviations. Values are means of three readings (triplicates).

Table 1. pH and water extractives values for tea samples.

Sample	pH (±SD)	Water extractives (±SD)
Tea A*	4.65 (±0.03)	36.53 (±0.13)
Lipton	5.07 (±0.00)	19.77 (±0.06)

* depicts formulated tea with 50% *C. citratus*: 30% *G. lucidum*: 20% *L. multiflora*. Values are means of triplicate readings.

Table 2. Proximate analysis of formulated tea (A) and Lipton.

Sample	Moisture(%±SD)	Proteins(%±SD)	Crude fibre(%±SD)	Lipids(%±SD)	Ash(%±SD)
Tea A*	7.15 (±0.17)a	9.02 (±0.00)a	77.06 (±0.33)a	0.07 (±0.02)a	5.83 (±0.36)a
Lipton	12.05 (±0.04)b	0.08 (±0.00)b	50.11 (±0.27)b	2.37 (±0.04)b	4.99 (±0.40)b

* depicts formulated tea with percentages of 50% *C. citratus*: 30% *G. lucidum*: 20% *L. multiflora*

Table 3. Sensory evaluation for tea formulations.

Sample	Mean score for sensory attributes					
	Aroma	Colour	Taste	After taste	Astringency	Overall acceptability
A	6.32(±2.00) ^a	5.91(±1.03) ^a	5.00(±0.74) ^a	4.93(±2.22) ^a	4.47(±0.08) ^a	6.01(±1.87) ^a
B	5.11(±1.87) ^b	5.77(±1.29) ^a	4.28(±1.07) ^b	5.15(±1.92) ^a	3.92(±1.01) ^b	5.66(±2.02) ^b
C	4.28(±1.03) ^c	3.06(±2.13) ^b	4.90(±2.19) ^c	3.26(±1.33) ^b	4.75(±1.16) ^a	4.00(±1.18) ^c

A (50% *C. citratus* :30% *G. lucidum*: 20% *L. multiflora*); B (30% *C. citratus* :20% *G. lucidum*: 50% *L. multiflora*); C (20% *C. citratus* :50% *G. lucidum*: 30% *L. multiflora*).

Table 4. Sensory evaluation of tea samples.

Sample	Mean scores for sensory attributes					
	Aroma	Colour	Taste	After taste	Astringency	Overall acceptability
Tea A	5.63(±1.92) ^a	5.43(±1.86) ^a	4.89(±2.00) ^a	4.15(±1.80) ^a	4.02(±1.91) ^a	5.31(±1.52) ^a
Lipton	4.00(±1.02) ^b	6.25(±1.91) ^b	5.03(±2.21) ^a	5.20(±1.94) ^b	4.33(±2.26) ^a	4.74(±1.78) ^b

A (50% *C. citratus* :30% *G. lucidum*: 20% *L. multiflora*). Mean scores with same letters in the same column do not differ significantly (P > 0.05). Lipton was the control sample.

6. Conclusion

For the development of herbal tea, the formulation consisting of 50% *C. citratus*, 30 % *G. lucidum* and 20% *L.*

multiflora was used to produce tea that was better accepted than a known control brand, Lipton-yellow label sensorially. Tea A as well performed better with significantly higher antioxidant activity than the control tea beside its higher

protein, ash and fibre contents. Consequently *C. citratus*, *G. lucidum* and *L. multiflora* can be used as composite tea of better quality to enhance healthful benefits from their high contents of antioxidants. This will also encourage the consumption of indigenous products and generate employment to many, culminating in economic development as tea imports may also reduce.

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