

Comparative Proximate Analysis of Ethanolic and Water Extracts of *Cymbopogon citratus* (Lemon grass) and Four Tea Brands

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Abstract: The comparative nutritive values of different tea brands on sale in Nigeria were investigated and compared with natural Lemon grass extracts. The brands include Lipton tea, Top tea, Nescafe and Green tea. The percentage moisture, ash, crude fibres, lipids and protein contents and antioxidant potentials were evaluated and compared. Phytochemical screening for the various tea brands compared to lemon grass was also carried out. Results from the study showed that there were significant ($p < 0.05$) differences in the proximate composition of the various tea brands compared with Lemon grass. Top tea had the highest percentage of moisture (11.2%), crude fibre (84.35%), lipid (6.09%) and protein (0.44%) while Nescafe had the highest percentage of ash content (13.03%). Green tea contained the least ash content (4.79%) while Lemon grass had the lowest percentage lipid (0.42%). Phytochemical screening of these samples revealed the presence of flavonoids, phenolic compounds, glycosides and conjugated dienes in all the four tea brands investigated. Top tea appeared the most balanced nutritionally while the Green tea is the most susceptible to rancidity. Data of the study indicate that the indigenous tea brands including the natural extracts of lemon leaf are good sources of antioxidant compounds such as flavonoids, phenolic compounds and glycosides in addition to very few calories and only a small amount of fat with corresponding valuable minerals which they contained. They are therefore nutritionally acceptable and medicinally valuable.

Key words: Tea, moisture, antioxidant, proximate, flavonoids, *Citrus hystrix*

INTRODUCTION

Tea (*Camellia sinensis*) is the most widely consumed beverage worldwide and has become an important agricultural product (Balentine, 1992). It was originally called Kia then in 6th century AD the name evolved into Cha. On its arrival in the West, it became Te which is still the name for tea in many countries. The type and quality of tea taken varies in different countries and races (Kohlmeier *et al.*, 1997). Black (fermented) tea is popular in the West; semi-fermented oolong tea type is commonly drunk in Taiwan and parts of China. Green (non-fermented) tea is favored in the rest of China, Northern Africa and Japan (Weisbuger, 1996). Tea is known to contain some phytochemicals with antioxidant properties capable of slowing down and or prevent oxidative damage to DNA molecules (Zhao *et al.*, 1989). Some tea possess anti peroxidation property, especially that of low-density lipoprotein (Miura *et al.*, 1994). Tea antioxidants can protect against strong mutagens in animal models (Yamane *et al.*, 1991) although, lower incidence of cancer in association with high consumption of tea

has been reported in some epidemiological studies (La Vecchia *et al.*, 1992). Tea is regarded as a medicine from time immemorial due to their anti oxidant properties. They had been employed to prevent and treat diseases like heart disease and cancer in humans. The use of tea is linked with antiallergic action and can thus act as preventive agent for toxic chemicals and carcinogen. Free radicals contribute to numerous disorders in human including cancer, arthritis, ischemia, Central Nervous System (CNS) and Acquired Immune Deficiency Syndrome (AIDS) (Amie *et al.*, 2003).

This can be generated due to environmental pollutants, radiation, chemicals, toxins, physical stress and the oxidation process of drugs and food. *Cymbopogon citrates* (Lemon grass) is of Poaceae family (Burkill, 1996). It has both biological and chemotherapeutic activities which include relieve of spasms, muscle cramps, rheumatism and headache, treatment of fever, cold, cough, stomach upset and renal dysfunction (Russo, 1992). Ramirez *et al.* (1988) also reported the use of its stalks in the treatment of nervous conditions and inflammation. Tea is a brisk, refreshing

drink with mildly stimulant effects attributable to caffeine (Harbowy and Balentine, 1997; Choudhury *et al.*, 1991).

Green and black tea have cancer preventive activity such as cancer of the skin, lung, mouth, esophagus, stomach, colon, pancreas and bladder (Lambert and Yang, 2003). Green tea inhibition of lipid peroxidation in the kidneys, liver and testes of pre-treated animals as well as superoxide dismutase and catalase activities are reported by Soussi *et al.* (2006). It is associated with antidepressant property (Singal *et al.*, 2006) and maintenance of cardiovascular health (Vita, 2003). The volatile oil obtained from the fresh leaves of this plant is widely used by the perfumes and cosmetic industries. Antimicrobial (antibacterial and antifungal activities of lemon grass oil and its component had been reported (Wannissom *et al.*, 1996; Schuck *et al.*, 2001; Paranagama *et al.*, 2003).

The aim of this study was to determine the phytochemical and proximate composition of some selected tea brands and their nutritive and medicinal properties compared with two fresh plant materials namely lemon grass and lime leaf.

MATERIALS AND METHODS

Collection of samples: Tea brands were purchased from different super markets in Lagos, Nigeria in July 2010. They were kept in a dry, air tight container at room temperature prior to analysis. The brands include Top Tea (TT), Lipton Tea (LT), Green Tea (GT), Nescafe (N) and the plant material-Lemon Grass (LG).

Proximate analysis: Proximate analysis of the different tea brands was done using the method of Association of Official Analytical Chemists (AOAC, 2000) and Pearson's composition and analysis of food.

Moisture content: About 2.0 g of each sample was weighed and placed in a crucible of constant weight. This was placed in an oven at 105°C then dried; the weights were measured carefully to get a constant weight. The loss in weight indicates the moisture content.

Ash content determination: Crucibles used for ash content determination were weighed and dried in an oven at 110°C to a constant weight. About 2.0 g of each samples were weighed and placed in the crucible then the weight of the crucible and samples were taken. This was placed in a furnace and ignited for 3 h at 550°C till the samples have a cotton wool like texture; it was cooled in a desiccator and weighed using analytical balance.

Protein: About 1.0 g of the samples was weighed into the Kjeldahl flask. About 0.1 g of Ca₂SO₄ and 1.0 g K₂SO₄ were added into the flask with 20 mL of concentrated H₂SO₄. The flask was then placed in a slanting position on the kjeldahl digestion heating mantle in the fume cupboard. Digestion continued until there was a color change which was from black to bluish-green signifying that digestion has ended. It was set up against blank, the digest were removed and allowed to cool and was then diluted with water and made up to 200 mL on ice.

About 50 mL of aliquot of each digest were poured into a distillation flask. About 50 mL of NaOH were carefully layered into the solution in order to make it a strong alkaline and 50 mL of 0.1N H₂SO₄ measured and kept in a beaker with two drops of methyl red as an indicator. The H₂SO₄ acted as a receiving flask. About 150 mL was distilled over then distillation was stopped by removing the solution in the receiving flask before the heat was put off to avoid drop in pressure. The distillate (excess acid) was titrated with 0.1 M NaOH in the burette. This was done for all samples and blank and percentage nitrogen was calculated.

Lipid: About 1.0 g of the samples were weighed into a thimble of known weight. About 150 mL pet ether (60-80)°C were poured into 250 mL conical flask using the measuring cylinder. The soxhlet extractor where the sack and its content had been introduced was fitted and the solvent boiled under reflux. The extraction processed lasted for about 8 h, the sack with its content were removed, dried in an oven for 2 h and then weighed with an analytical balance.

Crude fibre: 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 %) (Kirk and Sawyer, 1998).

Phytochemical screening: The presence of the following active constituent was tested for: flavonoids, phenolic compounds, antioxidant properties, free radical scavenging activity, reducing power and conjugated diene and were done in both methanol and water extract.

About 10 g of each sample was taken from the teabags and crushed. The crushed sample were extracted with solvent of known quantity (methanol and water) using the soxhlet extractor for about 6 h until the complete extraction had occurred. The obtained extract was further

evaporated to dryness using Vacuum Rotary Evaporator machine (Olaniyi *et al.*, 1975; Trease and Evan, 1989).

Determination of total antioxidant capacity: The total antioxidant capacity of the extracts were evaluated by the Phosphomolybdenum Method as described by Prieto *et al.* (1999). The assay is based on the reduction of Molybdenum (Mo) VI to Mo V by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acid pH. About 0.3 mL of each sample solution and ascorbic acid ($100 \mu\text{g mL}^{-1}$) were combined with 3.0 mL of reagents (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 M ammonium molybdate). The blank solution contained 3 mL of reagent solution and the appropriate volume of the same solvent used for the sample. All tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled at room temperature, the absorbance of the solution of each sample was measured at 695 nm against the blank using UV-Vis spectrophotometer. The antioxidant activity was expressed as the number of equivalents of gallic acid.

Determination of total flavonoid: Total flavanoid content of the extracts was determined according to a Colorimetric Method with some modifications. About 0.5 mL of samples extract were transferred into an eppendorff tube containing 1 mL of distilled water and mixed with 75 μL of 5% sodium nitrites. After 5 min, 75 μL of 10% aluminium chloride solution was added. The mixture was allowed to stand for another 5 min and then 0.5 mL of 1 M NaOH was added. The reaction solution was mixed and kept for 15 min. The absorbance was measured at 510 nm. Total flavanoid content was calculated using a standard quereetin calibration curve. The result was expressed as the number of equivalents of gallic (Bao *et al.*, 2002).

Determination of free radical scavenging activity: The free radical scavenging activity of tea brands, Lemon grass and lime leaf extracts against DPP (1, 1 Diphenyl-1-Picrylhydrazyl) free radical was evaluated. About 1 mL of the extract was mixed with 1 mL of 0.4 M methanolic solution containing 1, 1 Diphenyl-1-Picrylhydrazyl (DPPH) radicals. These solutions were kept in the dark for 30 min and absorbance read at 516 nm.

Determination of phenolic compound: The total phenolic content of plant extract was determined using FCR (Folin-Ciocalteu Reagent). This method depends on the reducing of FCR by phenolic to a mixture of blue oxides which have a maximal absorption in the region of 750 nm. About 100 μL of plant extracts and 100 μL of gallic acid ($100 \mu\text{g mL}^{-1}$) were mixed with 500 μL of the FCR and 1.5 mL of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 μL using distilled water. The mixture was allowed to stand for 2 h and the absorbance read at 765 nm. The total phenolic content was expressed as the number of equivalent of gallic acid.

Determination of conjugated diene: About 0.02 g of the sample was weighed into test tube and made up to 20 mL with water-methanol mixture and the mixture was allowed to stand for 2 days. The absorbance was read at 240 nm.

Determination of reducing power: The reducing power of tea brands, lemon grass and lime leaf extracts was determined using the Method of Lai. The extract (1.0 mL) in phosphate buffer (2.5 mL, pH 6.6) was mixed with potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture and centrifuged for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and the absorbance read at 700 nm.

Statistical analysis: Experimental data were assessed using SPSS Version 15.0. The $p \leq 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

The results for proximate analysis of Lipton tea, Top tea, Green tea and Nescafe compared with Lemon grass are shown in Table 1. There was significant lower value ($p \leq 0.05$) in the lipid content of lemon grass compared with the branded tea while Nescafe had a significant high ($p \leq 0.05$) in ash content compared with Lemon grass and other branded tea. There were no significant differences ($p \geq 0.05$) in the moisture and crude fibres contents in three branded tea compared with Lemon

Table 1: The Proximate analysis of Lipton tea, Top tea, Green tea, Nescafe and Lemon grass tea

Proximate analysis	Top tea	Lipton	Green tea	Nescafe	Lemon grass
Ash (%)	5.11 \pm 0.01	5.72 \pm 0.03	4.79 \pm 0.01	13.03 \pm 0.14	5.01 \pm 0.02
Moisture (%)	11.20 \pm 0.29	10.00 \pm 0.49	13.85 \pm 0.78	13.08 \pm 1.15	10.12 \pm 0.11
Lipid (%)	4.50 \pm 0.09	2.66 \pm 0.03	6.09 \pm 0.01	4.35 \pm 0.09	0.98 \pm 0.04
Protein (%)	0.44 \pm 0.00	0.16 \pm 0.00	0.16 \pm 0.00	0.21 \pm 0.00	0.34 \pm 0.00
Crude fibre	78.58 \pm 0.74	78.16 \pm 0.36	84.35 \pm 0.94	79.13 \pm 0.66	78.43 \pm 0.74

grass but its value was significantly higher in Green tea compared to Lemon grass and the other 3 branded tea while protein content had significantly ($p \leq 0.05$) higher protein content in top tea compared to Lemon grass and other branded tea. The relatively lower lipid content in Lemon grass makes Lemon grass a better tea compared with the other branded tea in this study and since, other proximate contents in the branded tea are similar to that of Lemon grass, Lemon grass can be said to possess desired nutritive qualities in the branded tea. The significant differences in the comparative proximate compositions of these tea showed that each of them possess relative advantage over one another with the lemon grass being close to the ideal tea.

The results for the anti oxidant potentials (reducing power and free radical activity (DPPH) are shown in Fig. 1-13. At $25 \mu\text{g mL}^{-1}$, there was a significant difference for water and methanol extracts for all the teas. Lipton tea had the highest value for methanol extract when compared to lemon grass while Top tea had the highest value for

water extract when compared to Lemon grass and other teas. Lemon grass had the lowest value for methanol extract when compared to Top tea which had the highest value for both methanol and water extract. The percentage inhibition value methanol extract of Nescafe was significantly high when compared to Lemon grass and other teas while for water extract Green tea had the highest value.

When compared to Lemon grass, Green tea had the highest value of methanol extract although Lemon grass had the highest value of water extract when compared to other teas. Lemon grass tea had the lowest value for both water and methanol extract while Lipton had the highest for methanol and Nescafe had the highest for water extract. Lemon grass tea had a low value for methanol and for water extract when compared to Nescafe which had the highest value for both water and methanol extract. For Lemon grass tea the total flavonoid was moderately low for methanol and water extracts when

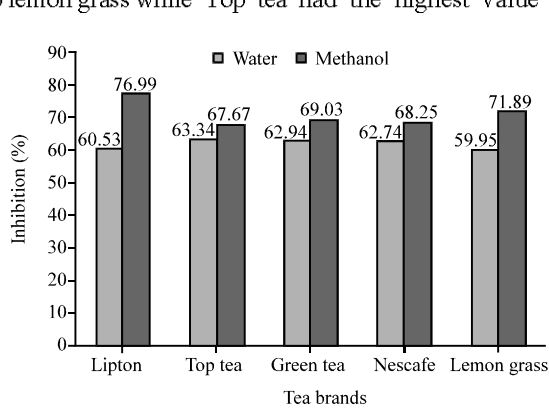


Fig. 1: DPPH of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea at $25 \mu\text{g mL}^{-1}$

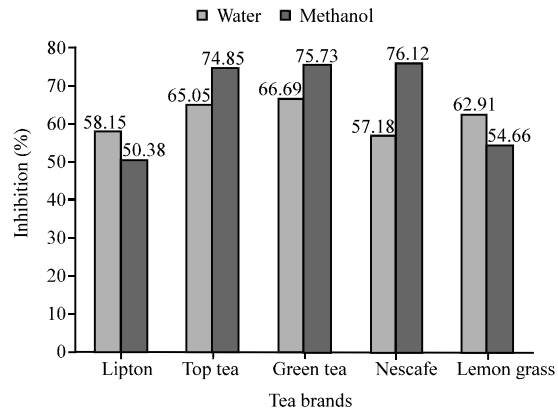


Fig. 3: DPPH of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea at $75 \mu\text{g mL}^{-1}$

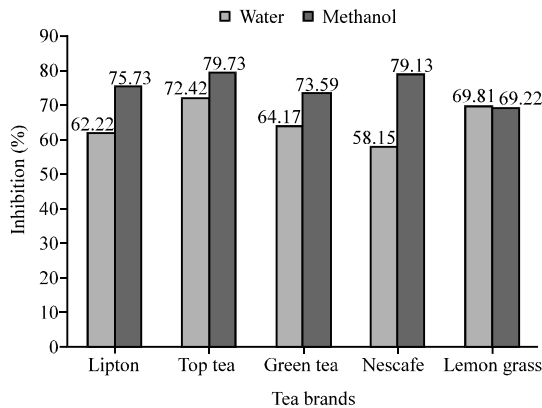


Fig. 2: DPPH of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea at $50 \mu\text{g mL}^{-1}$

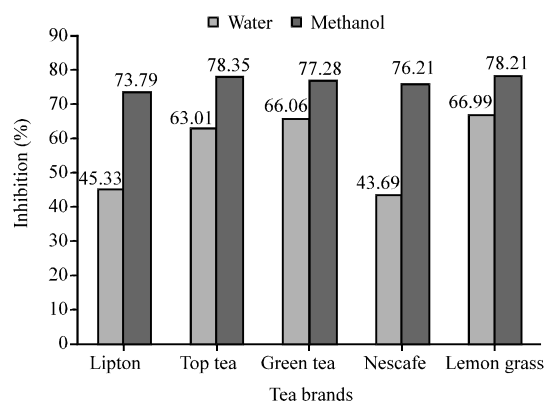


Fig. 4: DPPH of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea at $100 \mu\text{g mL}^{-1}$

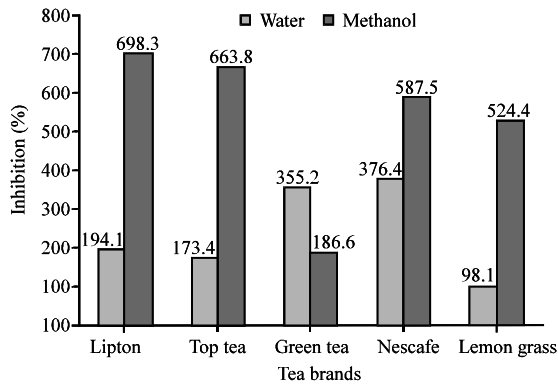


Fig. 5: Total antioxidant of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea

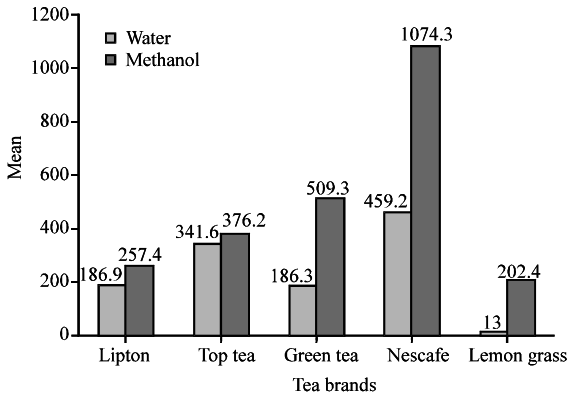


Fig. 6: Total phenolic compounds of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea

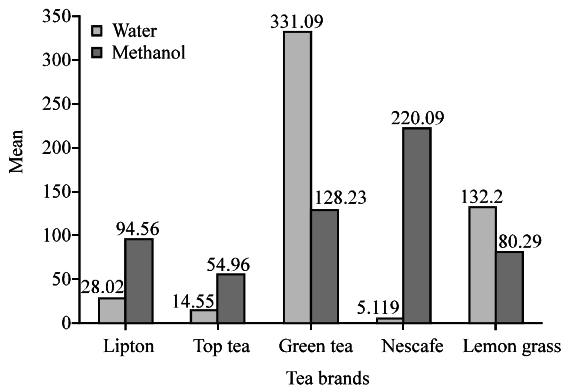


Fig. 7: Total flavonoid of Top tea, Lipton tea, Nescafe, Green tea and Lemon grass tea

compared to others although, Green tea produced a high content of water extract while Nescafe had the highest methanol compared to the others.

In the proximate analysis, it was observed that the ash content in Lemon grass has favourable range of values with the other tea brands except Nescafe which

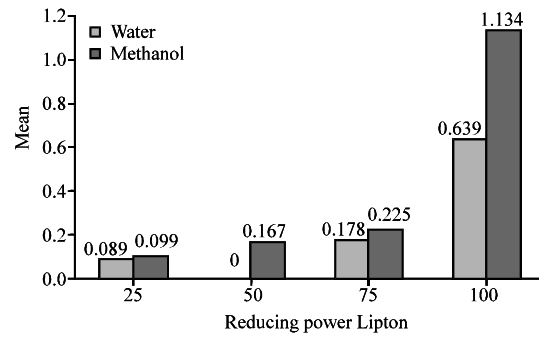


Fig. 8: Reducing power of Lipton tea

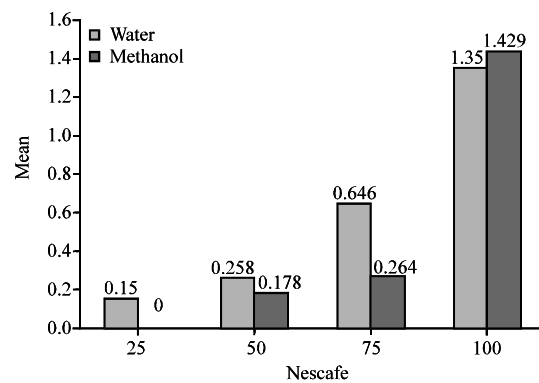


Fig. 9: Reducing power of Nescafe

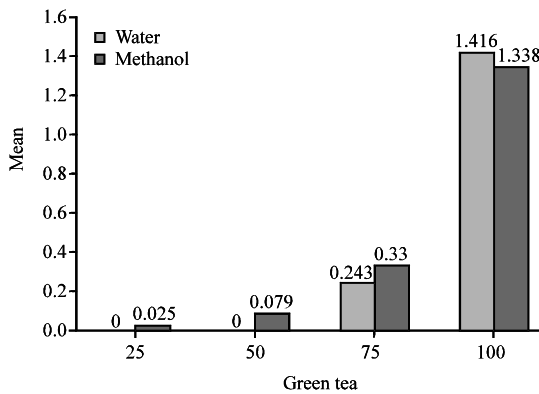


Fig. 10: Reducing power of Green tea

had a higher value. The moisture content in these teas did not have significant differences with one another while Green tea had a higher value compared to the rest; Lemon grass also had a fairly high value of moisture content which indicates that it is susceptible to microbial growth and in variance to Asaolu *et al.* (2010). Lemon grass had a moderate high value of protein required for balanced diet and can be considered a good source of plant protein. Lemon grass has a low level of lipid an indication that it

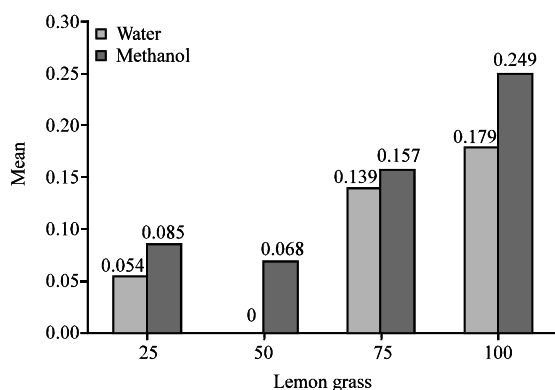


Fig. 11: Reducing power Lemon grass tea

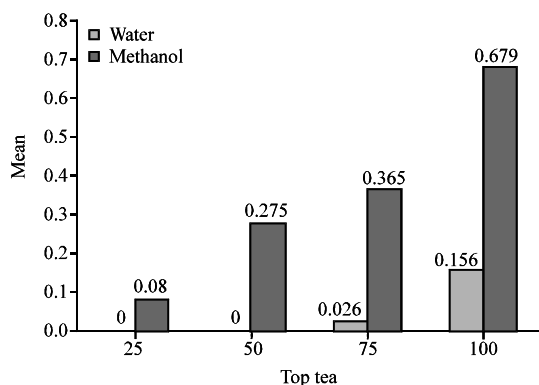


Fig. 12: Reducing power of Top tea

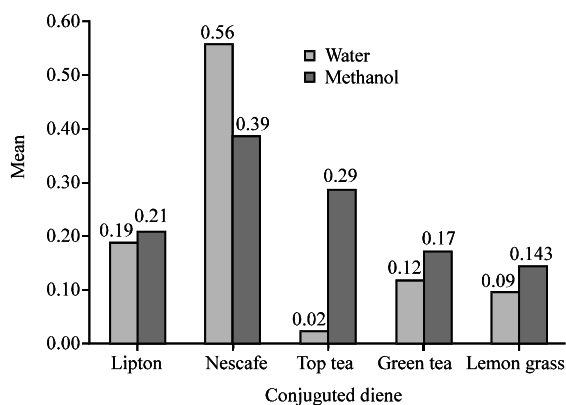


Fig. 13: Conjugated diene of Lemon grass tea

would have little or no cholesterol. The crude fiber was moderately high which makes it a good source of crude fibres.

Phytochemical screening carried out showed that phenolic content in Lemon grass was low both in methanol and water extract and since it was present it is known for being an erythrocyte modifier and beneficial for

human health (Ozcan *et al.*, 2009). The total antioxidant potentials were high but not comparable to branded teas indicating the presence of the antioxidant property which can help boost the immune system. All the branded tea and Lemon grass had high reducing power and free radical activity (DPPH) at increased concentrations. For conjugated diene, Lemon grass had the lowest value for methanol and water extract, respectively. The total flavonoid was highest in methanol extract for Lemon grass.

CONCLUSION

Researchers conclude that all the four tea brands evaluated in this study are nutritionally and medicinally valuable including the natural extract of Lemon grass tea which has potentials for protective and therapeutic effects on the immune system and the body in general. The contents of these tea brands and Lemon grass are also safe; Lomon grass however, seems to have an edge over the four branded tea in terms of nutritional qualities and medicinal potentials on the basis of its phenolic compounds and proximate contents. Researchers therefore recommend them for regular consumption.

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