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Phytochemical, Toxicological, Antioxidant, Proximate and Mineral Evaluation of Locally Mixed and Black Tea

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Abstract:
Background: *Tea is one of the oldest medicine which has been used for the prevention and treatment of various diseases and was consumed in China for its ability to detoxify, stimulate, reduce joint pain, improve urine and blood flow, and improve the immune system. The aim of the study was to evaluate the phytochemistry, elemental analysis, and antioxidant effects of Black and mixed tea in a comparative study.*
Materials and Methods: *The phytochemicals, proximate composition, elemental and 1,1 diphenyl-2-picrylhydrazyl (DPPH)free radical scavenging activity were all analyzed using the standard protocol.*
Results: *The results of the phytochemical analysis indicate the presence of flavonoids, Glycoside, Alkaloids, Saponins, Steroids, Tannins, Phenols, Terpenes, and Reducing sugar in mixed tea at varying degrees. While, Glycoside, Alkaloids, and Tannins were absent in black tea with a significant ($p<0.05$) increase of flavonoids in mixed tea as compared to black tea. Meanwhile, there was a highly significant ($p<0.001$) increase in phosphate and a significant ($p<0.05$) decrease in chromium in mixed tea as compared to black tea. The moisture content increased significantly ($p<0.001$) in mixed tea as compared to black tea. Consumption of the mixed tea will be beneficial in maintenance of good health.*
Conclusion: *Analysis of mixed tea yielded impressive results when compared to black tea analysis.*
Key Words: *Tea; Phytochemicals; Medicine; Diseases; Plants.*

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I. Introduction

Tea, a traditional beverage originally from China, is the oldest, most popular, non-alcoholic caffeine containing beverage in the world, and its infusion is prepared by brewing of processed leaves of the tea plant, *Camellia sinensis* (Kumar, 2014). Tea is the second most widely consumed beverage in the world following water. The most commonly consumed teas are black, green, and oolong which are all derived from the plant *Camellia sinensis*, a member of the Theaceae family. Approximately 3.0 million metric tons of dried tea is produced annually, 20% of which is green tea, 2% is oolong, and the remainder is black tea. Green tea and oolong tea are predominantly consumed in Asian countries, whereas black tea is widely consumed in India and Western countries (Jolvis, Sanjib and Santanu, 2019).

In the early 1820s, the British East India Company began large-scale production of tea in Assam. In 1837, the first English tea garden was established at Chabua in Upper Assam; in 1840, the Assam Tea Company began the commercial production of tea in the region and thereafter, the tea industry rapidly extended to other parts of the country. Today, India is one of the largest tea producers in the world and about 70% of tea produced is consumed within India itself. By the turn of the century, Assam became the leading tea producing region in the world. However, due to certain specific soil and climatic requirements its cultivation was confined to only certain parts of the country. (vanDriem, 2020). Ultimately tea cultivation was commissioned in many districts in India wherever there was some hope of a success. Within a few months, India along with Sri Lanka dominated the world tea trade/market (Karmakar, 2005). Commercial cultivation of tea gradually expanded to Indonesia, and Sri Lanka until the middle of the 19th century. The first record of cultivation of tea in Africa was in 1850; Commercial production in Africa started in former British colonies such as Kenya, Tanzania and Malawi. However, the tea industry developed until the middle of the 20th century. Now, tea plants are distributed worldwide and grown commercially in tropical, subtropical and temperate climatic regions of Asia, Africa and South America, and in limited areas in North America, Europe and Australia (van-Driem, 2020).

Nigeria since its introduction into the country around 1952, commercial tea planting started in 1982. Nigeria produces black tea with the CTC method, labeled 'Highland tea'. The total land area planted to tea is 1,200 ha. The average annual national production is 1,640 tones, which meets only 10% of domestic need. Opportunities thus exist for further local and foreign investments in the Nigerian tea industry. Tea improvement

started in 1982 with the acquisition of 33 clones by the Cocoa Research Institute of Nigeria. Since then moderate achievements have been recorded (Omolaja and Iremiren, 2012).

Plants have been the primary source of most medicines in the world, and they still continue to provide mankind with new remedies. Natural products and their derivatives represent more than 50% of all drugs in clinical use, of which higher plants contribute more than 25% (Miller, 2001). Herbal Medicines in African Traditional Medicine are no doubt more important in developing countries but quite relevant in industrialized world in the sense that pharmaceutical industries have come to consider them as a source or lead in the chemical synthesis of modern pharmaceuticals. A number of African plants have found their way in modern medicine. These plants which had been used traditionally for ages have through improved scientific expertise been the sources of important drugs (Salmerón-Manzano, Garrido-Cardenas and Manzano-Agugliaro, 2020).

Teas apart from their phytochemicals also contain minerals and heavy metals that are found to be anti-microbial, anti-inflammatory, anti-carcinogenic, anti-oxidant, etc., which can be used as an effective preventive agent against infections. This review provides an insight on the multitude of actions of tea as a preventive and anti-infectious agent besides production and consumption. Herbal teas or tisanes, like all foods of plant origin, start to take an important consideration in new product development in the recent years. This based on the increased awareness of their health benefits. It's widely known that large number of medicinal plants exist world-wide, research in product development of herb teas is limited (Mathivha, Msagati, Thibane and Mudau, 2020).

Black Tea

Black tea liquor has a dark brown color and a sweet aroma. Relatively, it has more flavor than green and oolong tea and is obtained from a plant called *Camellia sinensis*. Black tea contains negligible amounts of calories, fat, protein, and sodium. Taking or consuming tea contributes to fluid balance in body which is vital for physical and mental health. Black tea consumption is now an excellent option if you are looking for an alternative of coffee or energy drinks (Ho, Zheng and Li, 2015). Until about 20 years ago, the information on the working mechanisms of several constituents of black tea was limited although it has been considered in several countries for centuries that it has numerous health benefits. Black tea provides rich dietary sources of flavan-3-ols, flavonols, and other flavonoids, however, databases of values for some of these constituents are not available (Bond and Derbyshire, 2020).

The antioxidant activities in black tea are attributed to polyphenolic components, TF, TR, and unoxidizedcatechins. These anti-oxidative properties of black tea are due to its capability to scavenge free radicals, chelate transition metal ions, and inhibit the generation of free radicals (Łuczaj and Skrzydlewska, 2005; Someswararao and Srivastav, 2012). Also, other compounds which have potential to decrease inflammation and to reduce the risk for the onset of complex conditions are greatly inhibited by the antioxidants in the black tea (Enloe, 2018). Research indicates that black tea has the potential to prevent cigarette smoke-induced oxidative damage of protein in guinea pigs (Misra et al., 2003). If these outcomes were extrapolated to human being, consumption of black tea may protect from oxidative damage induced by cigarette smoke and subsequent degenerative diseases. Excess generation of free radicals has the ability either directly or indirectly to damage our biomolecules including the damage of nucleic acids or proteins, which has now considered being one of the key sources responsible for the induction of a number of chronic disorders (Al-Shobaili, 2015). It is now well documented that black tea polyphenols have potential to perform an antioxidant function to neutralize the harmful effects of elevated levels of free radicals, and thus drinking black tea reduced the chances of onset of numerous chronic disorders (Łuczaj andSkrzydlewska, 2005).

Studies have shown the anticancer activity of black tea in various types of cancers such as breast, oral, prostrate, intestinal, esophageal and gastric, urinary tract, skin, liver, and lung (Luo and Jiang, 2021). Black tea can relieve the glucose and lipid metabolic rate disorders linked with type 2 diabetes. Black tea has the ability to improve oral health by preventing the growth of bacteria and decreasing the occurrence of dental cavities. Therefore, black tea can be used as a natural treatment for periodontal diseases (Ben Lagha and Grenier, 2017). Research also indicated that black tea consumption can reduce cholesterol levels. This effect may be attributed to the actions of TFs, which have the potential to reduce the absorption of intestinal cholesterol (Zhao, Asimi, Wu, Zheng and Li, 2015).

Black tea, despite its huge benefits from consumption is also reported to be associated with some unprecedented adverse effects that commonly arise especially when the black tea plant of unregulated mixture is consumed at a greater quantity across some period of time. These side effects of some black teas are widely associated due to unprecedented presence of some quantities of heavy metals as well as caffeine (Bohn *et al*., 2014). Ideally, tea should be free from contaminants such as heavy metals, which are toxic and harmful to the human body because of their non-biodegradable nature, long biological half-lives and persistent accumulation in different body parts (Sharma,Agrawal and Marshall, 2007).

Mixed Tea combination

Today through the African traditional medicine, the use of whole herbs and extractives has remained the main approach of folk medicine practitioners in the treatment of ailments and debilitating diseases. They usually claimed that such whole herbs and extractives are efficacious against several ailments and diseases without recourse to scientific proofs. Increased cases of opportunistic diseases emanating from side effects associated with synthetic drugs continue to necessitate incremental efforts in searching for effective biological substitutes with little or no side effects. Therefore, efforts are being directed towards elucidating potential sources such as ethno-medicinal plants (Patil, 2010). New, robust and less cumbersome extraction techniques assisted by recent developments in biotechnology have enhanced investigation of natural compounds faster with more precision than before leading to isolation of bioactive compounds with intense health benefits (Wang and Weller, 2006). According to folk medicine, several plants possess ethno medicinal benefits with Lemon grass, Mango leaves, Ginger, Soursop grass and Guava leaves all considered as options.

II. Materials and Methods

The plant samples were collected from the surroundings of Keffi, Nasarawa State, Nigeria. The authentication of the specimen was done at the Laboratory of the Department of Biochemistry, Nasarawa state university and later deposited at the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD) Jos, Plateau State, Nigeria.

Preparation of Plant Extract

Black Tea Extract: 200g of dried tea leaves was soaked in 2000 mls of boiled de-ionised water in a stainless pot for thirty (30) minutes, the tea infusion was allowed to cool to room temperature and thereafter filtered to remove the tea leaves. The aqueous filtrate obtained was subjected to heat to dryness at 50^0C to 60^0C, the dried extract was weighed and the percentage (%) yield was calculated as follows:

$$\% \text{ yield} = \frac{\text{Weight of dried extract} * 100\%}{\text{Weight of raw tea leaves}}$$

$$\% \text{ yield} = \frac{70 * 100}{200} = 35\%$$

Preparation of Mix Tea Extract: Mix tea is made up of varying percentages of different plants which include Lemon grass leaves, mango leaves, soursop leaves, guava leaves and ginger rhizomes. The plant parts were suitably identified and collected from Keffi environment of Nasarawa State and extracted at the African Center of Excellence in Phytomedicine Research and Development (ACEPRD) University of Jos, thereafter, dried under a shade and pulverized individually. The powders were triturated in ascending order of quantities until all were mixed uniformly to obtain a homogenous mixture. An infusion of this homogenous mixture was then made and treated similarly as in black tea above. The percentage yield was also calculated thus:

$$\% \text{ yield} = \frac{111.2 * 100}{200} = 55.6\ \%$$

Phytochemical Screening: Qualitative phytochemical screening of the extracts was done using the methods of Trease and Evans (2004) for flavonoid, tannin and glycosides, while the methods of Sofowora (1993) was used for phenol, reducing sugar, steroids, terpene, saponin and alkaloid. The methods of Harborne (1998) were used for quantitative phytochemical screening of the extracts.

Mineral (Heavy metal) Analysis: Mineral analyses were carried out after acid digestion of 5g of the grounded sample with 10ml of nitric acid until a clear solution was obtained. The digest was allowed to cool and then filtered into a 100ml standard flask and make up to the mark with deionized water. The mineral elements were analyzed with atomic absorption spectrophotometer.

DPPH Scavenging activity: The antioxidant activity (free radical scavenging activity) of the tea sample on the stable radical 1,1 diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method described in (Brand-Williams et al., 1995).

The following concentrations of extract were prepared in methanol; 500,250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg/mL. 2mL of each concentration was mixed with 4mL of 50µM DPPH solution in methanol in triplicate. The mixture was vortexed for ten seconds to homogenize the mixture and test tubes were incubated for 30min at room temperature in the dark and the absorbance was measured at 515 nm using UV-vis spectrophotometer (Shimadzu, 1620 Japan). Lower absorbance readings of the reaction mixture indicate higher

free radical scavenging activity. Gallic acid, ascorbic acid and rutin were used as standards at the following concentrations; 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391, & 0.195 µM.
Blank solutions were prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH solution in methanol. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability of scavenge the DPPH radical was calculated by using the following equation;

% inhibition = *100 x (Abs control – Abs sample)/ Abs control*
Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/Ml).
Proximate Analysis: Preparation of plant materials for proximate analysis: Dried sample extracts for both the black and mixed teas were ground into fine (100-mesh screen) powder. The ground samples were then examined for moisture content, protein, crude fat, crude fiber, carbohydrate and ash using the methods described by AOAC (1990).

Statistical Analysis

All results were analyzed and presented as mean ± SD and analysis was done using Statistical Package for the Social Sciences (SPSS) Version 23. One-way analysis of variance (ANOVA) was used. Differences in mean were considered to be statistically significant at 5% confidence level.

III. Results

Table no 1 shows qualitative phytochemical screening results of aqueous extract of the Mixed Tea and Black Tea. The table revealed the presence of flavonoids, Glycoside, Alkaloids, Saponins, Steroids, Tannins, Phenols, Terpenes, and Reducing sugar in mixed tea, while, flavonoids, Saponins, Steroids, Phenols, Terpenes, and reducing sugar were present in black tea.

Table no 1: shows qualitative phytochemical screening results of aqueous extract of the Mixed Tea and Black Tea.

Phytochemicals	Samples for Mixed Tea	Samples for Black Tea
Flavonoid	+	+
Glycoside	+	-
Alkaloids	+	-
Saponins	+	+
Steroids	+	+
Tannins	+	-
Phenols	+	+
Terpenes	+	+
Reducing sugar	+	+

+ = present, - = absent

The results of quantitative phytochemical analysis for aqueous extract of the Mixed and Black Tea as presented in figure 1. The various phytochemical quantities were; Total Phenol (66.25 ± 2.78),
Phenol without Tannin (14.13 ± 3.18), Tannin (52.12 ± 2.48), Flavonoid (183.20 ± 4.92), PH (2.58 ± 0.65), and Relative Density (1.00 ± 0.01) in Mixed Tea, respectively. While, Total Phenol (58.55 ± 3.23), Phenol without Tannin (41.38 ± 2.98), Tannin (15.09 ± 2.34), Flavonoid (121.67 ± 5.42),
PH (4.75 ± 0.95), and Relative Density (1.00 ± 0.02).

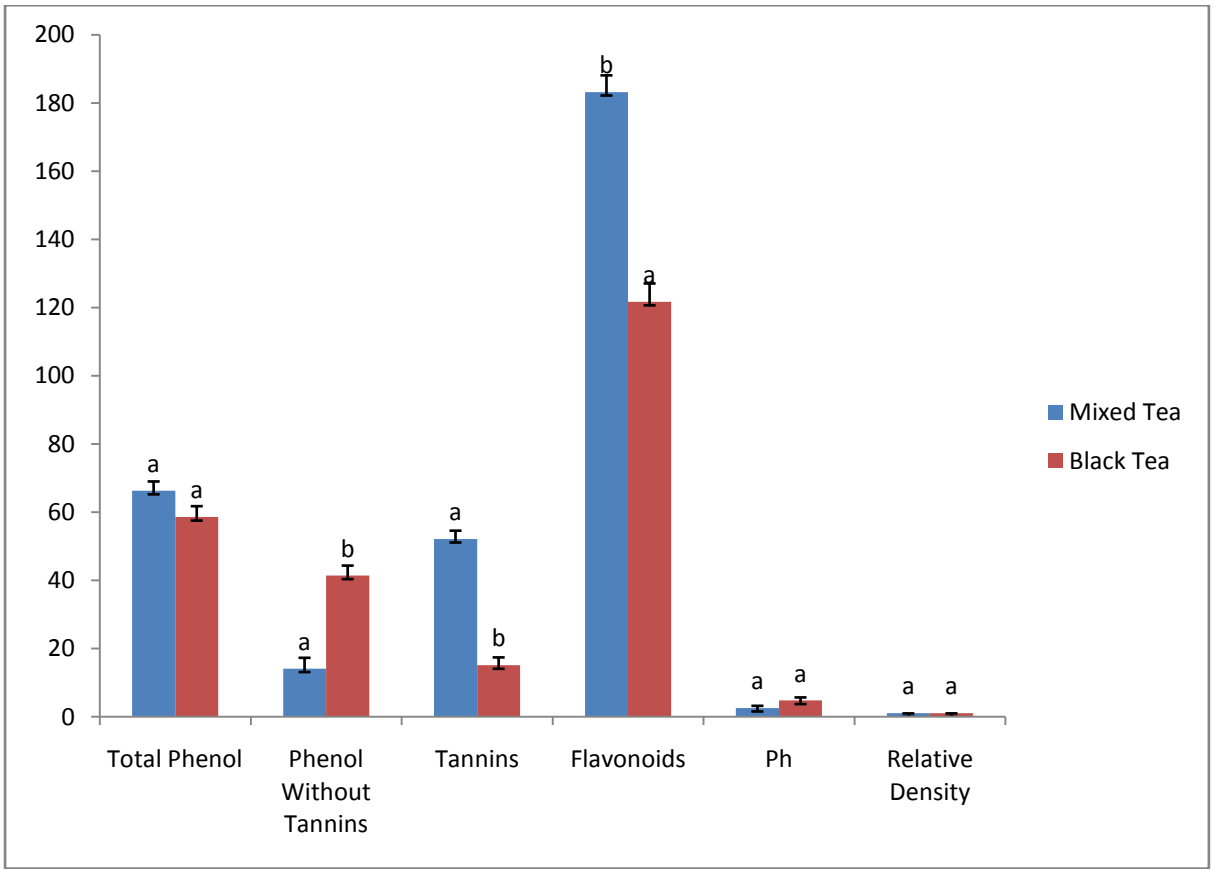

Figure 1: Quantitative Phytochemical Analysis for Mixed and Black Tea extract

The results of Elemental Analysis for aqueous extract of the Mixed and Black Tea were presented in Figure 2 below. The Results are expressed in Means ± SD (n= 3) where Mean values with different superscripts down the column are considered significantly different (P< 0.05).

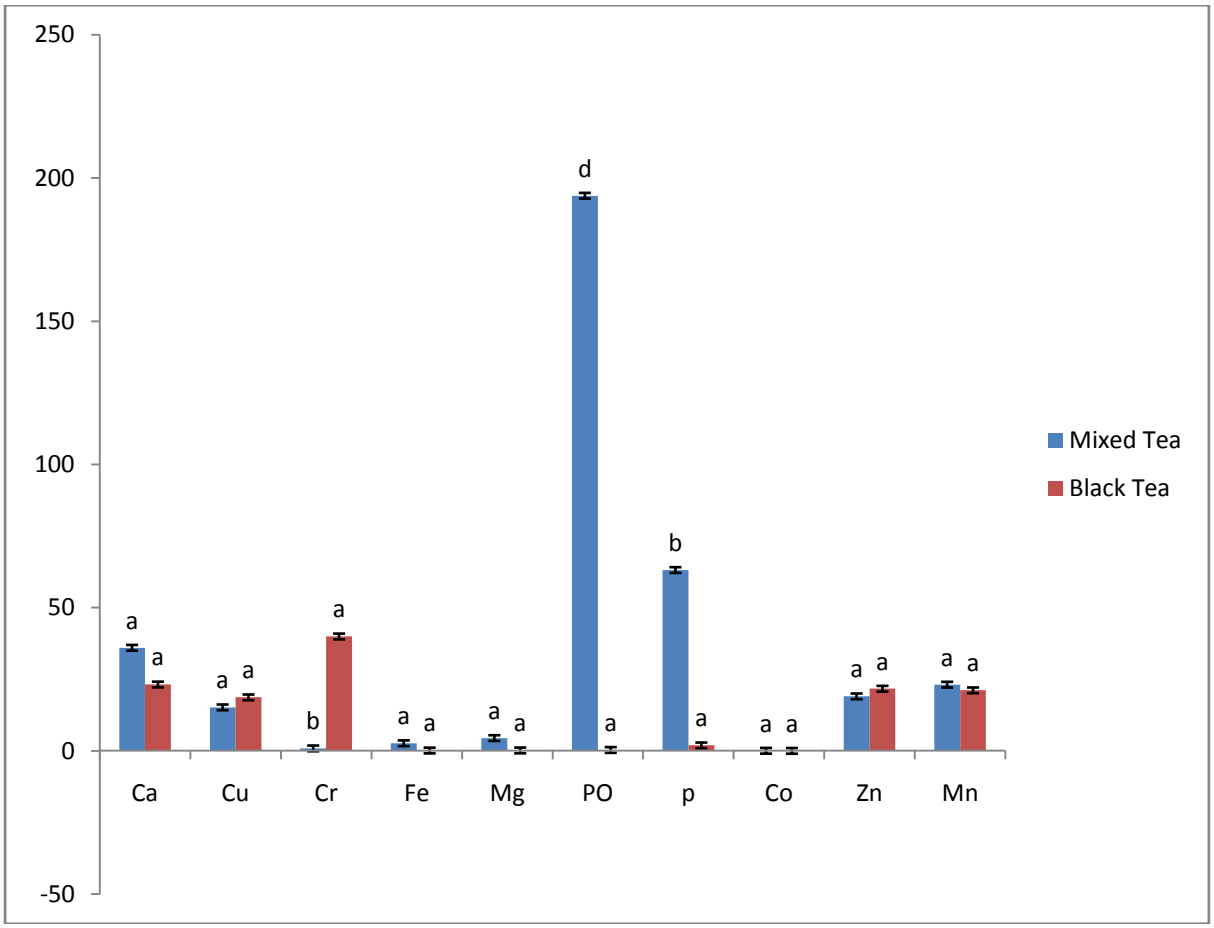

Figure 2: Elemental Analysis for Mixed and Black Tea extract

The results of free radical scavenging activities (*in vitro)* for aqueous extract of the Mixed and Black Tea is presented in figure 3. At concentration of 200, 150, 100, 75, 50, 25, 10, 5, and 0, the DPPH radical scavenging activity of mixed tea was 92, 87, 77, 60, 40, 17, 13, 13, and 0. While, black tea was 91, 89, 74, 54, 41, 10, 8, 6, and 0 as compared to vitamin C.

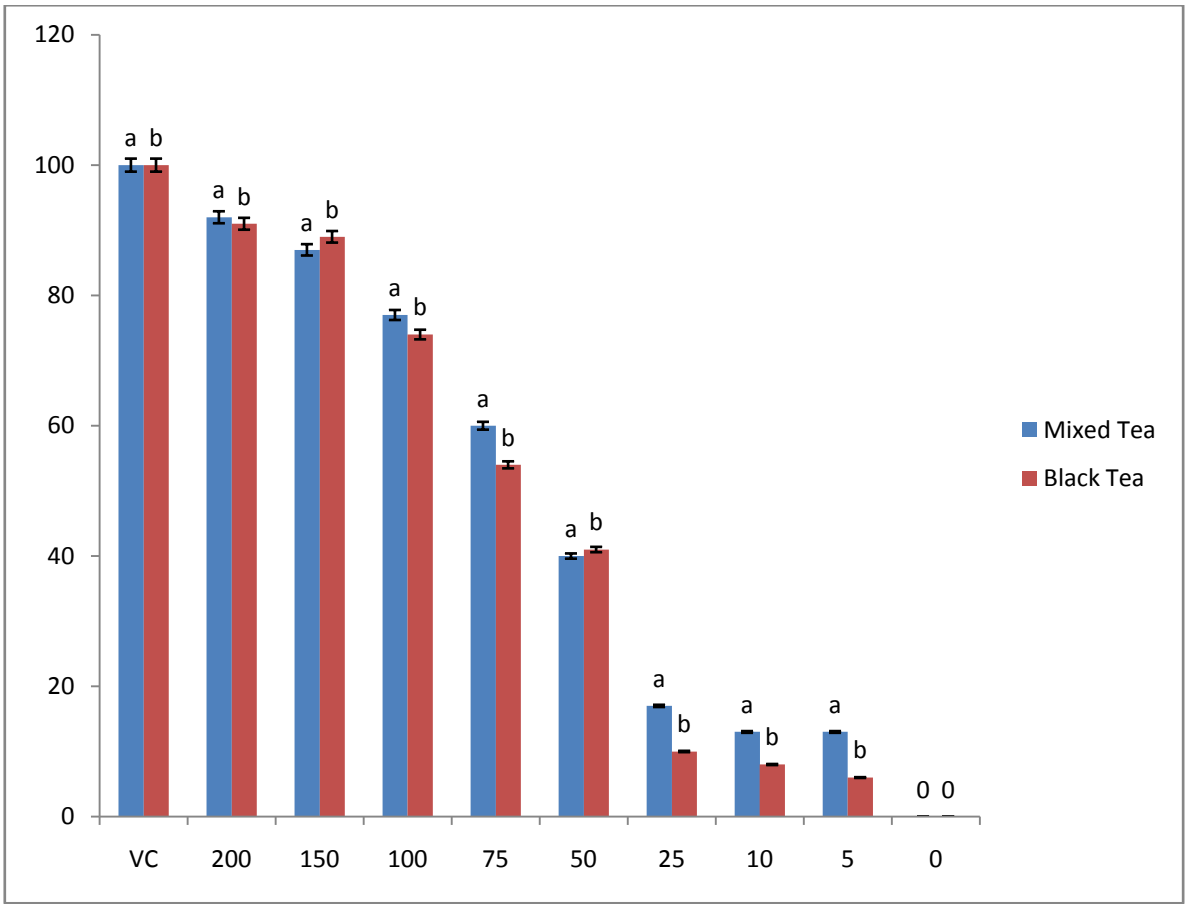

Figure 3: Free radical scavenging activities (*in vitro*) for Mixed Tea and Black Tea

The results of proximate composition for aqueous extract of the Mixed and Black Tea were presented in Figure 4.1.8 below. The Results are expressed in Means ± SD (n= 3) where Mean values with different superscripts down the column are considered significantly different (P< 0.05).

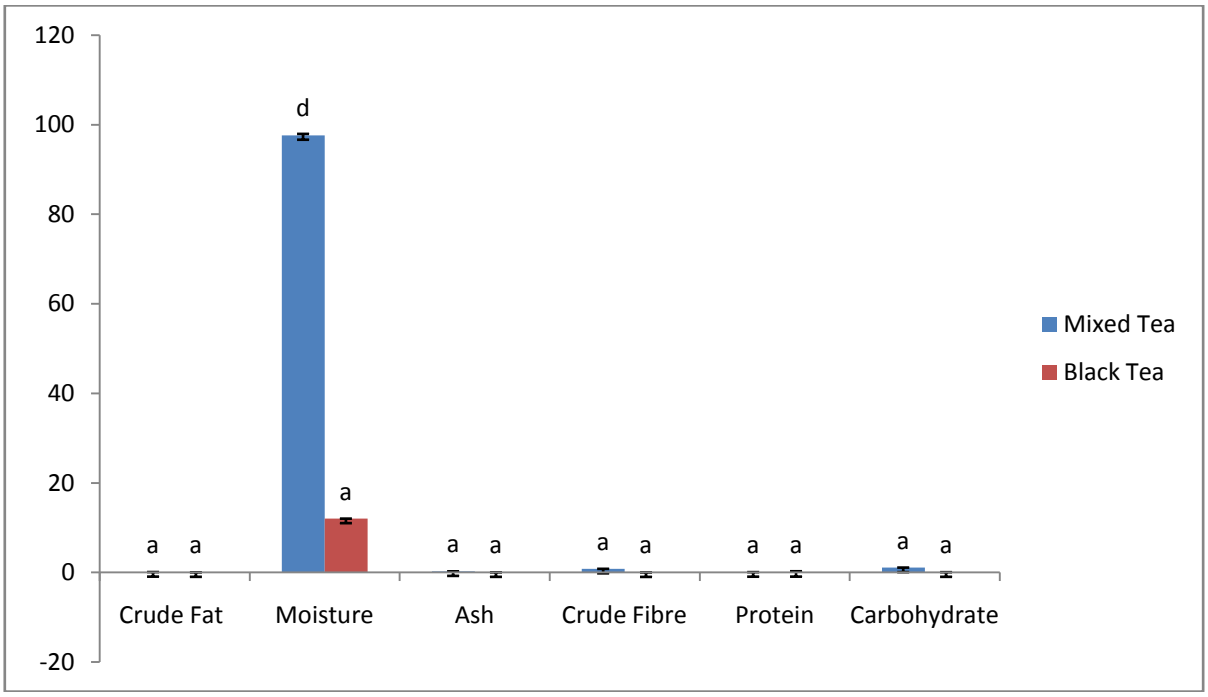

Figure 4: Proximate Composition for Mixed and Black Tea

IV. Discussion

Drinking tea is today a culture amongst several elites in the entire country of Nigeria (Giesinger*et al.,* 2015). Hence, the introduction of different brands of tea other than from the original plants (*C. sinesis and T. sinesis*) known can be seen to have evolved to solve specific problems in the immediate communities which are mostly health related; such as Fever, Cough, Flu etc. In certain cases, special teas like the mixed teas are brewed and steams from therein, inhaled in a Sauna or similar contraption thereby exploiting essential oils from the plants and later drinking the brew (Zaruwa*et al*., 2008).

In this research, results from the phytochemical analysis (Table1) showed the presence of flavonoids, phenols, terpene and reducing sugar in the mixed tea. This is because the mixed tea is a blend of several medicinal plants (Guava leaf, Lemon grass, Ginger, Soursop leaf and Mango leaf) which are also used individually for several therapeutic purposes as mentioned previously. The black tea on the other hand was devoid of Glycosides, Alkaloids and Tannins, this gives mixed tea added advantage over a black tea. Clinically therefore, the mixed tea may have more therapeutic advantage, the presence of antioxidants are known to mop-up free radicals in the system, play roles of anti-inflamatory, anti-cancer, diabetes mellitus management,anti-toxicity etc. (Bagchi, 2000).

The absence of caffeine in the mixed tea is also an added advantage, since the risk of psychostimulation attributed cum addition to the substance is mixed out (Ferre, 2016). Its absence is equally good for consumption by pregnant women, as it is known to be hazardous to the human fetus and neonate (James, 2021) since it is proven to cross the placenta and can concentrate in the breast milk.

The quantitative phytochemical analysis (Figure 1) showed copiously high concentration of flavonoids and total phenols followed by tannins, these substances are known potent anti-oxidants, hence, may aid relieve of symptoms of flu including antimicrobial and anti-viral roles (Hossain, 2011).

The elemental constituents of these teas showed higher concentration in the mixed tea obviously because of the blend of several plants over the black tea which is a singular plant. The inorganic constituents play vital role in the biological and pharmacological activities in the medicinal plant extracts (Pavel*et al*., 2021). The Figure 2 showed higher concentration of Phosphate, Phosphorous and Calcium in the mixed tea, while copper and chromium were higher in the black tea. It is expected that high concentration of Calcium, Phosphate, Phosphorous and some Magnesium would enhance muscular contraction, whole site activation, bones and teeth agility, blood glucose and blood pressure regulation; as well as muscle recovery after exercise, facilitate kidney function, enhance blood clotting capabilities, promote healthy nerve transmission through the body and in protein, DNA and RNA synthesis and very importantly regulate heart beats and fluid balance within the cells (Ravina*et al*., 2015). The Phosphate-Phosphorous contents maybe for most of the attributes obtained from the use of the mixed tea, because several people interviewed attested to the efficiency of the mixed tea against cold, fever, malaria and pains after child birth, some stomach infirmities among others (Personal contact 2021). It is known that Phosphorous containing drugs are parts of important therapeutic agents wildly used in daily clinical practices. They are mostly designed as pro-drugs with enhanced selectivity and bio availability, reduced toxicity and sometimes made as analogue with endogenous biochemical substances and antagonistic endozymes supplements (Yu *et al.,* 2020). To a larger extent the benefit of using mixed tea may not have been fully understood and probably under estimated, in actual sense possessed advantages over the black tea, due to the synergism of the mixed medicinal plants especially the Phoshate-Phosphorous compounds. The black tea showed Chromium, Calcium and Copper to be the dominant inorganic elements which also appeared in high concentration in the mixed tea. It connotes therefore that, the mixed tea is indeed a beverage with the properties of black tea within. These elements are mostly enzyme enhancers thereby acting as coenzymes or cofactors (Soetan,Ol 2010).

A comparative study *in vitro* when the mixed tea and black tea for a free radical scavenging activities showed percentage inhibition of DPPH to be almost at pal with a very negligible superiority by the mixed tea over the black tea probably due to synergetic actions of the phytochemicals contained therein.

V. Conclusion

The consumption of the mixed tea compared favorably with the black tea though locally brewed evidence showed that it possesses a lot of health benefit because of the availability of the raw materials which forms its components the mixed tea can comfortably be commercialized and may eventually be exported because of the raw materials are of natural origin and not treated with hazardous pesticides, herbicides or preservatives.

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