European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

ANALYSIS OF *FAROE SPP.* AND *CYOMBOPOGON CITRATUS* EXTRACTS FOR THEIR BEVERAGE POTENTIAL VIS-A-VIS PROCESSED BLACK COFFEE AND BLACK TEA LEAVES

Bakari Chaka[a]*, Wyclife Olal[b], Aloys Osano[c], Martin Magu[d] and Fredrick Omondi[e]

1. Department of Mathematics and Physical sciences, Maasai mara university, P.O Box 861-20500, Narok, Kenya. Email; bichaka92@gmail.com
2. Department of Mathematics and Physical sciences, Maasai mara university, P.O Box 861-20500, Narok, Kenya. Email, olalwycky@gmail.com
3. The Centre for Innovation, New and Renewable Energy (CINRE), Maasai mara university, P.O Box 861-20500, Narok, Kenya, Email; aloysmosima@gmail.com
4. Department of Chemistry, Multimedia university of Kenya, P.O Box 30305-00100, Nairobi, Kenya. Email; magujnr@gmail.com
5. Department of Chemistry, Multimedia university of Kenya, P.O Box 30305-00100, Nairobi, Kenya. Email; odundofredrick@gmail.com
   Corresponding author email; bichaka92@gmail.com, P.O Box 861-20500, Narok, Kenya. Tel: +254-705350166, ORCID: 0000-0002-6427-0494.

ABSTRACT: *Faroe spp. and Cymbopogon citratus concoctions have continuously been used as beverages in absence of coffee or tea leaves in Kenya. This study aimed at characterizing their extracts for chemical similarities with processed black coffee and tea leaves. Wet chemistry and spectroscopic methods were used to evaluate these similarities. While the physical-chemical properties were closely related, the results indicated resemblance in functional groups, matching those of polyphenols. The Ultra-Violet (UV) spectra indicated abundance of dienes and xanthophyll pigments. Faroe spp. extracts had more antioxidants against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenger. Phytochemicals and amino acids screens of the samples closely matched those of black coffee and tea. The order of caffeine content was black coffee* ($398.990 \pm 0.000 mg/L$), *black tea* ($169.110 \pm 0.000 mg/L$), *Faroe spp.* ($116.640 \pm 0.000 mg/L$) *and Cymbopogon citratus* ($38.760 \pm 0.000 mg/L$). *The same series was observed for total alkaloids and flavonoid content. In conclusion, these extracts were found to have a lot of similarities with black tea and coffee.*

KEY WORDS: *Faroe spp., Cymbopogon citratus*, beverages, caffeine

INTRODUCTION

Alongside water, tea and coffee are the most consumed beverages in the World (Elhussein *et al.*, 2018). This is largely attributable to their wide availability and durability when processed as well as their aromatic flavor and health benefits when consumed. They are easy and fast to prepare in

a variety of forms. Caffeine is sold and distributed in several forms including brewed and espresso coffee, brewed tea, cola soft drink, energy drinks, bakers' chocolate, cocoa beverage and chocolate milk (Gray, 1998; Pray *et al*., 2014).

The first people to have reportedly used caffeine were the Chinese in 2373 B.C. who made tea using the leaves of *Camelia sinesis* plant (Bedigian, 2005). Both tea and coffee are agricultural products processed from leaves, buds, internodes and berries of plants containing caffeine or catechin (Reynertstone, 2005). There are two major kinds of tea; black and green tea both containing about 1-5% of caffeine in their dry weight (Bedigian, 2005) depending on type, brand and brewing method (Diamantini *et al.,* 2015). Catechin, a Flavan-3-ol is the primary compound present in tea leaves (*Camelia sinesis & Camelia assamica*) (Bohn *et al.,* 2015). The *assamica* variety is richer in catechins whereby the catechins are attached to a mono- or di-glycoside while in *sinesis* the catechin is attached to triglycosides (Harvey, 2012). Volatile compounds such as linalool are also found together with catechin (Zhu *et al*., 2016). There are several forms of catechin including Epicatechin (EC), Epigallocatechin (EGC), Epicatechin gallate (ECG), Epicallocatechin gallate (EGCG), Catechin (C) and Gallocatechins (GC) (Khan and Mukhtar, 2018). On the other hand, caffeine is found widely in the leaves, seeds and fruits of numerous plant species including coffee and cocoa beans, cola plant, guarana and tea leaves (Gray, 1998). Caffeine is an alkaloid belonging to the xanthine family which are naturally stimulative (Tran *et al*., 2012). Caffeine is the most powerful xanthine and is known to increase alertness, reduce sleep and increase study concentration (Halson, 2014).

The health benefits of both catechin and caffeine have been widely studied over years. Green tea have polyphenols believed to be excellent free radical scavengers (Martin and Li, 2017). Not only are polyphenols anticarcinogenic (Alam *et al*., 2018) but also anti-mutagenic exerting protective effects against cardiovascular ailments (Ahmed *et al*., 2018). No toxin effects have been proven for catechins in animals. Caffeine is a strong stimulant and antidepressor. The recommended consumption rate of caffeine is 170mg/day for adults (Dixit *et al.,* 2016) though there is no clear evidence of allegations purporting caffeine to be addictive.

Indigenous African species have not been fully exploited for presence of catechins and caffeine. *Faroe spp.* (Olesesiai) was and continues to be used as a beverage by the Maasai community of Kenya. The bark of this plant is dried and ground before boiling together with milk and sugar just as it is done for coffee and tea. Its concoction is stimulative in nature. *Faroe spp*. grows in dryland areas and is spatially distributed in Kenya and Tanzania. The bark of these plant is also associated with some medicinal properties such as curing stomach aches, tonsils, diarrhea, ulcers and snakebites. *Cymbopogon citratus* (lemongrass) water concoction is used to brew 'tea' in the Luo-Nyanza region of South-Western Kenya. The concoction is also reportedly stimulative and able to maintain alertness as well as pervert sleep. *Cymbopogon citratus* is a perennial grass plant distributed worldwide especially in tropical and subtropical regions (Cole, 1993). The shrub is affiliated to some medicinal properties such as antiseptic, antifever, anti-dyseptic and anti-inflammatory effects.

This study purposed to examine the presence and quantities of catechin and caffeine in these two traditionally used Kenyan beverages which have for long silently substituted coffee and tea amongst many Kenyan households; *Olesesiai* amongst the Maasai and Lemongrass amongst the Luo communities of Kenya.

MATERIALS AND METHODS

Design of Experiment

An independent measures design was followed in evaluating the potential of *Faroe spp.* and *Cymbopogon citratus* in substituting commercial coffee and tea leaves. Extraction was done by maceration using distilled water. All characterization and analysis studies were therefore conducted vis a vis processed black coffee and tea leaves as controls. All the samples underwent similar experimental treatments in similar conditions. The samples were characterized for physical-chemical parameters including pH, conductivity, volatile solids as well as volatile fatty acids and dissolved oxygen. Thereafter, the samples were screened for functional groups and conjugation using IR and UV-VIS spectroscopy respectively. Antioxidant levels were analyzed by UV spectroscopy using DPPH radical scavenger. Phytochemicals and amino acids were screened by wet chemistry methods. Quantitative analysis was thereafter conducted for total caffeine, total flavonoid and total alkaloids composition by titrimetric and gravimetric methods. Extraction, characterization and analysis was done at Maasai mara university, Kenya chemistry laboratory. UV-VIS analysis was conducted at Multimedia university of Kenya, Nairobi, Kenya.

Materials

Chemicals

All chemicals used were lab grade except for analytical grade reagents which are hereby specified. All chemicals were sourced from Sigma-Aldrich.

Sodium hydroxide pellets, methyl red indicator, ethyl acetate, ferric chloride, vanillin, hydrochloric acid, nitric acid, sulfuric acid, olive oil, ammonia solution, chloroform, Fehling's solution, ethanol, n-hexane, Mayer's reagent, acetic anhydride, α-naphthol, Molisch reagent, acetic acid, antifoaming agent, potassium iodide, silver nitrate, sodium nitrite, lead acetate, calcium hypochlorite, Millons reagent, bromine water, suphanillic acid, calcium sulfate, iodine solution, sodium thiosulfate and starch solution.

The analytical reagents used include;

Potassium bromide, methanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Equipment

Fourier transform infra-red (Shimadzu), pH meter (Hanna G-114), Ultraviolet visible spectrometer (Shimadzu 1800)

European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Methods

Characterization of samples

pH, electrical conductivity and dissolved oxygen

pH, electrical conductivity and dissolved oxygen were conducted using a pH meter, conductivity meter and oxygen meter respectively.

Volatile Solids

10.000 g of sample was weighed, M_1 using an Analytical balance and then placed in an oven conditioned at 540^0C for 1 hour before removing, cooling (in a desiccator) and reweighing. The new mass was recorded as M_2.

$$\%VS = \frac{M2}{M1} \times 100\%$$

Volatile fatty acids

A raw sample was distilled in water (1:1) and the distillate titrated against standard 0.1N NaOH solution up to pH 8.3. The volume of sample solution used was used to determine the concentration of VFAs in the sample.

IR Functional Groups analysis

The extracts will then be heated slowly at 60^0C until all the water was dried. The samples were then cast into pellets using KBr pellet before analyzing for functional groups using IR Spectrometer.

Absorption bands Analysis

Extracts were diluted serially using distilled water until a clear spectrum could be seen on the UV-VIS monitor. A scan was then run between 190-900nm wavelength.

Antioxidant analysis

2,2-Diphenyl-1-picryl hydrazyl (DPPH) solution in methanol (6×10^{-5} M) was prepared. 3ml of this solution with 100 microliters of methanolic solutions of samples were mixed. The samples were then incubated in a water bath at 37^0C for 20 minutes. The absorbance at 515nm was measured.

Test for Amino acids

a) **Lead sulphide test**
   The sample solution was boiled in 1ml sodium hydroxide solution for about 5 minutes. 3.0g of lead acetate was then added to the mixture. Formation of a black precipitate indicated presence of amino acids.
b) **Sakaguchi test**
   α-naphthol was added to 10ml of the sample in an alkaline medium followed by calcium hypochlorite solution. Formation of a red color signified positive results.
c) **Millons test**

Onto the test sample, Millons reagent was added and change in color to red indicated presence of amino acids.

d) Histidine test

Bromine water was added to the test sample in an acidic medium followed by excess ammonia solution and heated. Formation of a blue or violet color indicated presence of amino acids.

e) Pauly Diazo test

Suphanillic acid dissolved in hydrochloric acid and added onto the test sample. Sodium nitrite solution was thereafter added followed by excess ammonia solution. Change in color to red signified presence of amino acids.

f) Xanthoproteic acid test

Concentrated nitric acid was added to the test sample to form a yellow mixture. Onto the mixture, excess dilute sodium hydroxide solution was added and color change to orange monitored. This was the confirmatory test for presence of amino acids.

Extraction and quantification of caffeine levels in the test samples
Extraction

Caffeine was extracted by solvent extraction method using chloroform. 5.0g of the test sample powder were dissolved in 100.0ml distilled water and 3.0g of sodium carbonate powder added to increase the solubility of caffeine in the samples. The mixture was boiled for about 30 minutes in a water bath before leaving to cool at room temperature.

A separating funnel was well rinsed with chloroform before adding the mixture above followed by 10.0ml of chloroform solution. The mixture was gradually swirled and allowed to separate out. The lower layer was taken and this process was repeated three more times to maximize on extraction process. The chloroform mixture was put into a beaker previously rinsed with chloroform and calcium sulfate added to separate out any water particles. The mixture was shaken well to form a fluffy, cloudy effect before filtering out the calcium sulfate added. The filtrate was then heated in a water bath at about 60^0C for 20 minutes to evaporate out chloroform.

Quantification by Iodometric back-titration method

The iodometric back titration method used have been reported to be 97% efficient in caffeine analysis. Iodometric back titration using standard starch solution indicator was used.

15.0ml of the test samples above were mixed with 10.0ml of concentrated sulfuric acid in a conical flask and shaken thoroughly. 25.0ml of standardized iodine solution was then added to the mixture and swirled gently to form a red precipitate. The mixture was filtered twice and titrated against 0.15M sodium thiosulfate solution. 0.5ml of standard (0.2%) starch solution was added onto the titrand when it became brown in color and titration process proceeded until the mixture changed to dark blue.

European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Phytochemical screening
Test for polyphenols

3ml of aqueous ferric chloride solution was added to 10ml of the sample solutions, shaken and observations made. Formation of green coloration indicated presence of phenols.

Test for flavonoids

Onto the test samples, 2g of vanillin powder was added and the mixture agitated in an acidic medium.

The procedure was confirmed by adding 3ml of dilute ammonia solution to 2ml of aqueous filtrate followed by 1ml of concentrated sulfuric acid. Formation of yellow deposits confirmed presence of flavonoids.

Test for Tannins

About 0.1g of the dry samples were boiled in 4ml distilled water in a boiling tube then filtered. A few drops of 0.1% ferric chloride solution were then added and observations of change in color to brownish-green made.

Test for phlabotannins

Onto 10ml sample solution, 3ml of 1% aqueous HCl acid was added and the mixture boiled. Deposition of a red precipitate symbolized presence of phlabotannins.

Test for saponins

The sample was added to 3ml distilled water and vigorously agitated until a stable, persistent froth formed. 3 drops of olive oil were then added and shaken vigorously. Presence of emulsion indicated positive results.

Test for terpenoids (Salkowski's test)

About 3ml of the samples were mixed with 1 ml of chloroform and 1ml of concentrated sulfuric acid. Formation of intense red-brown color indicated presence of terpenoids.

Test for reducing sugars (Fehling's test)

About 0.2g of the sample in 1ml of ethanol was added to 3ml of distilled water and mixed. 1ml of Fehling's solution was thereafter added and heated to boiling then poured into the aqueous ethanolic sample extract. Change in color to red indicates presence of reducing sugars.

Test for alkaloids (Mayer's test)

About 3ml of ammonia solution was added onto the sample followed by 10ml of chloroform. The mixture was shaken well then filtered. The chloroform layer was then evaporated off and 3ml of Mayer's solution added to the remaining solution. Formation of a cream precipitate indicated positive test for alkaloids.

European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Test for anthraquinones

About 2ml of the sample was boiled in a similar volume of concentrated sulfuric acid. 3ml of chloroform was then added to the mixture and the chloroform layer pipetted out into another test tube containing 1ml of dilute ammonia solution (1:1). Change in color indicated presence of anthraquinones.

Test for steroids

The sample solution was dissolved in 10ml of chloroform followed by 3ml of concentrated sulfuric acid. Formation of red precipitates indicated presence of steroids.

Test for carbohydrates

About 2 drops of α-naphthol solution was added to the sample followed by concentrated sulfuric acid and Molisch reagent. Formation of a violet color indicated presence of carbohydrates.

Test for glycosides

Onto 5ml of the sample solution, 2ml of glacial acetic acid, 1 drop of 5% ferric chloride and concentrated sulfuric acid was added. Formation of a brown ring indicated presence of glycosides.

Quantitative test for crucial beverage phytochemicals
Total Alkaloids

This method is according to Harborne, 1973.

2.5g of the samples were added onto 200ml of 10% acetic acid in ethanol solution. The mixture was allowed to stand for 5 hours before transferring to a round bottomed flask and gradually concentrating it to about 50ml. The mixture was thereafter filtered then about 10ml of concentrated ammonia solution was added to form a precipitate. The mixture was allowed to sediment and the supernatant discarded. The precipitate was washed with about 20ml of 0.1M ammonia solution and filtered. The residue was dried in an oven for about 1 hour and reweighed again.

% alkaloids = residue mass/ weight of sample x 100

Total Flavonoids

This method is according to Close and Mc Author, 2002.

3g of the sample was added 50ml distilled water, 2ml of hydrochloric acid and 3ml ethyl acetate solution. The mixture was boiled for about 30 minutes then allowed to cool and filtered using Whatman filter paper (# 42). About 10ml of ethyl acetate extract was recovered while the aqueous layer was discarded. The mass of an empty filter paper was taken and the filter paper used to filter the ethyl acetate layer. The residue was then dried in an oven for about 1 hour at 60^0C before cooling and reweighing again.

European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Statistical Analysis

Several statistical tools including the average, median, standard deviation and variance were used during the research. Correlation analysis and f-tests were also used to prove whether values were of similar populations. A 95% confidence level and n=11 degrees of freedom were maintained. The data was analyzed using Ms Excel and Originlab (version 6.5) statistical packages.

RESULTS AND DISCUSSIONS

Physical-chemical and bio-chemical characterization

The average pH values of coffee (4.560 ± 0.220) was found to be quite lower than that of tea leaves (6.500 ± 1.003). Most of the sample pH values were in tandem with those of the commercial coffee and largely deviated from the tea leaves properties. The pH of beverages is controlled by quantity of volatile acids present. There are very many volatile compounds in tea and coffee grains with tannins and polyphenols forming the most common ones (Bizuayehu, 2016). Polyphenols are slightly basic owing to presence of hydroxide ions thus tend to increase the pH values. Tea leaves have abundant polyphenols thus had the highest pH values. The *Faroe spp*. sample indicated the lowest pH values of 3.930 ± 0.956 citing more concentrations of volatile acids or amino acids. Table 1 below summarizes the physical-chemical properties of the samples analyzed against commercial coffee and tea leaves.

Table 1: Physical-chemical and bio-chemical properties of samples analyzed

Samples	Parameters				
	pH	Conductivity (mS)	Dissolved oxygen (%)	Volatile solids (g/L)	Volatile fatty acids (mg/L)
Coffee	4.560 ± 0.220	0.315 ± 0.894	7.721 ± 0.217	19.802 ± 0.231	1.101 ± 0.164
Tea leaves	6.500 ± 1.003	0.548 ± 0.669	7.303 ± 0.984	21.120 ± 0.456	1.212 ± 0.133
Faroe spp.	3.930 ± 0.956	0.603 ± 0.982	9.606 ± 0.165	18.899 ± 1.120	1.498 ± 0.121
Cymbopogon citratus	5.880 ± 1.010	0.101 ± 0.124	5.882 ± 0.445	13.650 ± 0.998	0.456 ± 0.909

All electrical conductivity values were significantly similar except for the *Cymbopogon citratus* sample ($p \geq 0.05$, $n = 11$). Conductivity of plant extracts is a function of solubility and total solids, more so the more ionic ones (Pardo *et al*., 2016). Singh, 2016 found out that the conductivity of coffee solutions is dependent on many other physical-chemical parameters, temperature being the key factor. Beverages are known to inhibit appreciable concentrations of minerals including potassium and fluoride ions which directly increase conductivity (Derman *et al*., 2011). *Faroe spp.* sample recorded the highest conductivity values ($0.603 \pm 0.982 mS$) citing more abundance in these compounds. The dissolved oxygen content of beverages has been proven to be dependent on the precursor oxidation processes done (Nemecz, 2005). Most of the caffeine and tea leaves are products of oxidation (Nemecz, 2005). Oxidation occur during growth, after plucking during withering and during processing (Pluskal, 2018). The amount of dissolved oxygen in samples is

crucial in several metabolic processes common to tea and coffee (Nemecz, 2005). The volatile solids content of *Faroe spp.* ($18.899 \pm 1.120 g/L$) was found to be significantly belong to the same population as that of the two controls (processed black coffee and black tea leaves). However, *Cymbopogon citratus* was found to have outlier volatile solids ($13.650 \pm 0.998 g/L$) ($p \geq 0.05$, n = 11). Various tea and coffee samples from different backgrounds have been proven to have adequate volatile compounds (Baqueta *et al.*, 2019). Most of the volatile matter in tea and coffee are acidic (Bizuayehu, 2016). Processed tea leaves can have up to 30% volatile matter per dry weight sample (Oellermann, 1964). The overall volatile fatty acids in the samples thus increase with increment in volatile matter. *Faroe spp.* extracts were found to have more volatile fatty acid concentration ($1.498 \pm 0.121 mg/L$) compared to both coffee ($1.101 \pm 0.164 mg/L$) and tea leaves ($1.212 \pm 0.133 mg/L$) citing presence of more organic acids. (Xiong *et al.*, 2014) found out that the average volatile acids in coffee was between 0.5-1.0% while (Amarowicz *et al*., 2009) showed that most tea leaves had about 1% organic acids concentration. The volatile acids concentration of both test samples (*Faroe spp*. and *Cymbopogon citratus*) were way below these values.

Functional group analysis

There were high levels of similarity on the spectra of *Cymbopogon citratus* and processed black tea leaves just as was the case with *Faroe spp.* and processed black coffee. All four spectra had identical peaks after 3500cm^{-1} and before 1000cm^{-1} with concise trends in peaks in between the two regions. Figure 1 below illustrates the FTIR spectra of these samples.

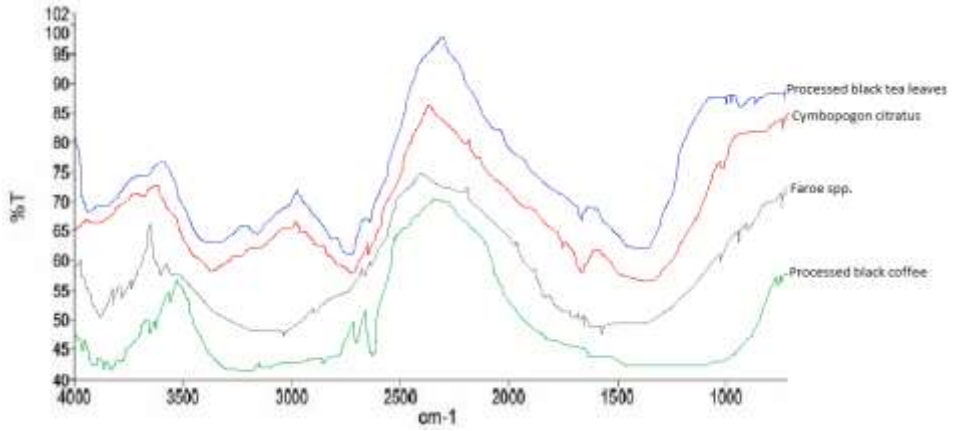

Figure 1; The functional group peaks of the test samples

Black tea and black coffee are all known to contain multiple compounds, both organic and inorganic which largely influenced the spectra in figure 1 above. All samples exhibited broad peaks after 2700cm^{-1} citing presence of O-H_{RCOOH} and consequently weak carboxylic acids. These findings are further justified by C-$OH_{stretch}$ peaks at 1035cm^{-1} though the C=$O_{stretch}$ peaks at 1680-1720cm^{-1} were suppressed by a descending peak towards the fingerprint region. Tea and coffee are all known to contain varying amounts of organic acids (Christian and Brent, 2001). The region after 2700cm^{-1} was characterized by multiple spectra indicating abundance of alcohols (rift at

3300cm^{-1}), amines (narrow rifts at 3500cm^{-1}), alkenes and olefins (sp^2 C-H shifts at 3200cm^{-1}) and alkyne compounds (sp C-H shifts at 3500cm^{-1}). This translates to abundance of conjugation in compounds present as well as myriad of organic compounds. The fingerprint region further justified these claims with numerous peaks in the region. For *Faroe spp*. and processed black coffee, the region between 1400-900cm^{-1} was vibrant with peaks indicating presence of double bonds and mixed isomers in the compounds (Suhm and Kollipost, 2013). Yuan *et al.*, 2018; indicated that the FTIR spectra of pure catechins and their products had a lot of activity in this region. Presence of narrow peaks between 700-500cm^{-1} indicated presence of organohalides, organometallic and ligands in the compounds (Kubis *et al*., 2016). These peaks were more pronounced in the *Faroe spp.* spectra.

Conjugation analysis of the samples

The abundance of multiple double bonds in FTIR analysis were further confirmed in the UV-VIS scans of the samples. Figure 2 below illustrates UV VIS spectra of the test samples analyzed between 200 and 800nm.

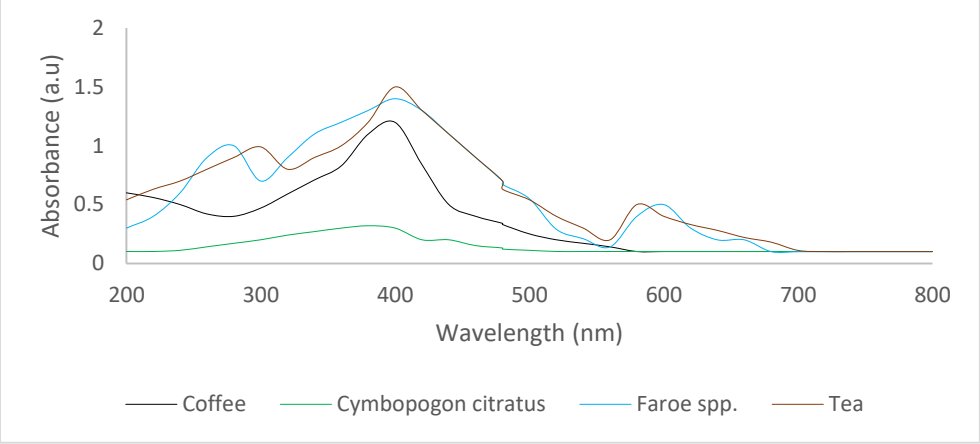

Figure 2; UV VIS spectra of the beverage samples

Faroe spp. and processed black tea leaves had the most conjugation with 3 peaks for each of the two. All samples except *Cymbopogon citratus* had sharp peaks at around 400nm. Several chromophores that have been found to absorb at this wavelength including ligands (such as sulphates, sulphites), dienes and other complex ions (Kumari *et al.,* 2010). Processed tea and coffee are products of several organic and inorganic compounds containing this ligands, complexes and dienes (Jeszka-Skowron *et al.,* 2015). (Ye *et al.,* 2017) found similar UV scans when analyzing the conjugation of various types of tea, i.e green, black, white, oolong and pu-uerh tea. Similar UV scans were also found by (Hong *et al.*, 2017) for processed black coffee. *Faroe spp*. and processed black coffee also indicated mild peaks at around 600nm. Carotenoids and xanthophyll pigments are crucial ingredients of tea and coffee known to absorb UV radiations at this wavelength (Hazarinka and Mahanta, 1983). *Cymbopogon citratus* UV spectra was quite inactive citing less conjugation compared to the other samples.

European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Antioxidant analysis

The antioxidant behavior of the test samples was found to vary as analyzed using DPPH antioxidant radical. Though a quantitative analysis was not done, *Faroe spp*. sample had the highest inhibition of the radicals, judging from the peak heights. The UV spectra of the test samples are given in figure 3 below.

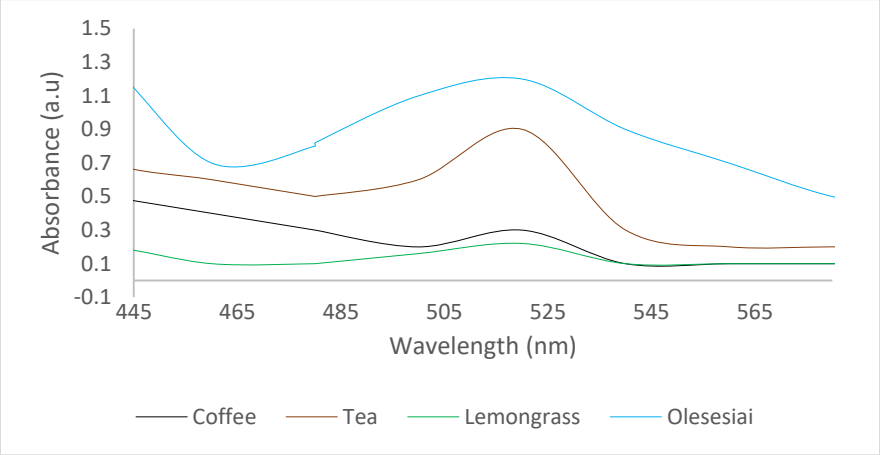

Figure 3; UV-VIS spectra for antioxidant analysis of the beverage samples

The inhibition rates of antioxidant according to peak heights were in the order of *Faroe spp.*, processed black tea, processed black coffee and *Cymbopogon citratus* extracts. The findings are however contrary to those of (Elsalamouny *et al*., 2009) who found out that black coffee contains more antioxidants than processed black tea. This is because processed black coffee has about twice the number of polyphenol compounds as found in black tea (Louwrens *et al*., 2009). Processed tea leaves have appreciable loads of antioxidant compounds such as catechins and theaflavins. Actually, all flavonoid groups present in tea and coffee are antioxidants (Nikoo *et al*., 2018). (Klempner and Bubly, 2012) confirmed that most phenolic groups present in tea and coffee have antioxidant potential. Carotenoid and xanthophyll pigments observed in the UV spectra of *Faroe spp.* and processed black tea are also rich sources of antioxidants (Verhoeven *et al.*, 2005; Havaux and Kloppstech, 2001).

Analysis of amino acids

Several tests conducted revealed abundance of amino groups in the test samples. These findings are summarized in table 2 below.

European Journal of Food Science and Technology
Vol.8, No.1, pp.34-54, February 2020
Published by ECRTD UK
Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 2: Characterization of amino acids in the beverage samples

Amino acid test	Samples			
	Tea	Coffee	*Faroe spp.*	*Cymbopogon citratus*
Lead sulphide test	-	-	++	-
Sakaguchi test	++	++	-	++
Millons test	+	+	+	+
Histidine test	-	-	-	-
Pauly Diazo test	++	++	++	++
Xanthoproteic acid test	+	++	++	+

Faroe spp. samples tested positive for lead sulphide test used to test for sulfur containing amino acids such as cysteine and cystine (Yablokov *et al.,* 2009). All the other samples tested negative but instead were found to have monosubstituted guanidine compounds such as aginine. This was according to Sakaguchi test. These amino acids are heavily laden with nitrogen (Kijima and Ueno, 1986). Bernd *et al.,* (2000); reports that guanine and adenine result during processing of caffeine at elevated temperatures. The Sakaguchi test is thus a good indicator for presence of caffeine in the samples. All samples showed traces of tyrosine compounds as seen in Millons test. Tyrosine is a phenolic based amino acid thus expected to be present in coffee and tea beverages (Comert and Gokmen, 2017). Histidine was found absent in all the test samples. Pauly diazo test signified high abundance of aromatic tryptophan in all the samples. These findings were confirmed by xanthoproteic acid test which also portrayed traces of phenylalanine and tyrosine. Processed black coffee and *Faroe spp.* samples had more concentrations of these amino acids. Both phenylalanine and tyrosine have been found present in many types of coffee and tea (Cathie *et al.,* 2017).

Caffeine concentration analysis

All samples tested appeared to have appreciable levels of caffeine except *Cymbopogon citratus* extracts. These findings are illustrated in figure 4 below.

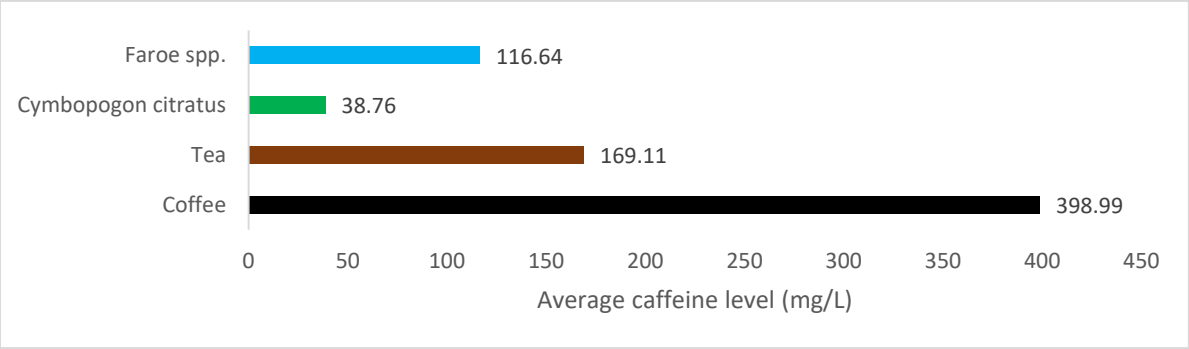

Figure 4; Average caffeine levels in the beverage samples analyzed

Both *Faroe spp*. sample had adequate caffeine levels to fit as a commercial source of caffeine. The hot report: hot beverages, 2014; indicates that the average caffeine levels for processed black tea is 20-60mg per cup. This translates to 100-240mg/L. Thus, *Faroe spp.* had 116.640 ± 0.000mg/L making it a prime substitute of black tea in rural domestic households. Combrink *et al*., 2011; also confirms that most commercial beverage brands produce black tea with caffeine levels between 80-300mg/L. This sample had similar caffeine content to oolong tea and more caffeine content than green tea and white tea (Boros *et al.*, 2016). These findings justify the common use of these extracts in local households as an alternative to tea and coffee. *Cymbopogon citratus* extracts were however quite low in caffeine levels ($38.760 \pm 0.000 mg/L$) and actually significantly outlying to the population as far as caffeine content was concerned ($p \geq 0.05$, $n = 11$). It was normal for processed black coffee to have 3-4 more caffeine levels compared to black tea (Nawrot *et al*., 2003; Choi and Curhan, 2007).

Phytochemical and carbohydrates analysis

The beverage samples were found to contain most of the crucial phytochemicals present in processed tea and coffee. *Faroe spp*. sample had identical phytochemical compounds compared to processed tea and coffee, while *Cymbopogon citratus* exhibited traces of most of the phytochemicals. All samples tested positive for polyphenols. Current literature in mass spectrometry, 2009; reports that tea can harbor up to 40% of polyphenols in its total composition by dry weight. Most of the phytochemicals present in tea and coffee are polyphenols (Karas *et al*., 2017). Fukushima *et al.*, 2009; found out that the total polyphenols in coffee and tea amongst consumers was 200 and 96mg/100ml of sample respectively. Tannins and flavonoids, both subsets of polyphenolic compounds were also found present in the samples. These samples are common in majority of tea and beverage types. Table 3 below illustrates the registry of phytochemicals and carbohydrates in these beverage samples.

Table 3: Phytochemical and carbohydrate characterization in the beverage samples

Test	Samples			
	Coffee	Tea	*Faroe spp.*	*Cymbopogon citratus*
Polyphenols	++	++	+	++
Flavonoids	++	++	++	+
Tannins	++	++	++	++
Phlobatannins	-	-	+	-
Terpenoids	-	-	-	-
Alkaloids	++	+	+	+
Saponins	+	+	+	-
Anthraquinones	++	+	++	-
Glycosides	++	++	++	+
Carbohydrates	++	++	+	+
Reducing sugars	+	++	+	-
Steroids	+	+	+	-

Presence of tannins and flavonoids in *Faroe spp*. and *Cymbopogon citratus* extracts indicated that these samples indeed had beverage potential. Various forms of flavonoids, alongside caffeine; have been reported as the key ingredients in categorizing extracts as being tea, coffee, cocoa or non-beverages. Tea is known to contain important flavonoids such as catechin and epigallocatechin (Alhafez *et al.*, 2014). Only *Faroe spp*. sample tested positive for phlobatannins while no sample had detectable terpenoids. Alkaloids were found present in all the test samples. Gad *et al.*, 2013; reported that tea samples analyzed had adequate alkaloids with trace amounts of saponins. Alkaloids are crucial beverage requirements since caffeine and amino acids are all alkaloids. The coffee sample had more alkaloids since coffee is known to have more caffeine content compared to tea (Oba *et al*., 2010). Both coffee and *Faroe spp*. had appreciable anthraquinones while tea leaves had traces of this compounds. *Cymbopogon citratus* extracts lacked these phytochemicals. Wang *et al.,* 2016; reports that most types of tea have anthraquinones. Anthraquinones are also subsets of polyphenols, abundant in coffee and tea samples (Renouf *et al.,* 2014). All samples tested positive for presence of glycosides. Soluble glycosides have been reported to constitute of up to 3% by weight of black tea samples (Vrba, 1985). IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Coffee, Tea, Mate, Methylxanthines and Methylglyoxal. Lyon (FR): International Agency for Research on Cancer; 1991; analyzed glycosides to constitute 0.2-1.2mg/cup of black coffee. All samples had carbohydrates in significant amounts except for the *Cymbopogon citratus* extract which only showed traces of the compound. Chen, 2006; analyzed different carbohydrate types in both tea and coffee but found no significant differences in their concentrations. However, from this study, black tea samples indicated more reducing sugars present compared to coffee. *Faroe spp.* and *Cymbopogon citratus* also showed traces of reducing sugars. All samples except *Cymbopogon citratus* indicated traces of steroids.

Quantitative analysis of alkaloids and flavonoids

All samples were found to contain significant beverage samples. The average concentrations of total alkaloids and total flavonoids are summarized in table 4 below.

Table 4: Concentrations alkaloids and flavonoids in beverage samples

Phytochemicals	Samples			
	Coffee	Tea	*Faroe spp.*	*Cymbopogon citratus*
Total alkaloids (mg/L)	421.120±0.000	181.004±0.000	120.789±0.000	69.075±0.000
Total flavonoids (mg/L)	44.150±0.000	60.100±0.000	31.210±0.000	30.150±0.000

The coffee sample had the highest amounts of total alkaloids ($421.120 \pm 0.000 mg/L$) indicating high levels of caffeine. Caffeine is the most pronounced alkaloid in coffee samples (Shearer, 2014). The order of total alkaloid content actually echoed that of total caffeine (as discussed above).

Khanum *et al.,* 2017; reported higher values of up to 200ppm of distilled water in different parts of raw black tea. These findings were way much higher than those obtained in this study. Ousmane *et al.,* 2017; estimated the total alkaloid concentration in green tea (*Camellia sinensis*) to be up to 6.96 µg/mg. The researcher also estimated total flavonoids in the same plants to be 97.70 µg/mg. Both of these values were higher than those obtained in this study. Total flavonoid concentration were in the order of tea ($60.100 \pm 0.000 mg/L$), coffee ($44.120 \pm 0.000 mg/L$), *Faroe spp.* ($31.210 \pm 0.000 mg/L$) and *Cymbopogon citratus* ($30.150 \pm 0.000 mg/L$). The black tea sample unexpectedly had the highest flavonoid content owing to more phenolic compounds in tea. There was little difference in flavonoid concentration between the two test samples (*Faroe spp*. and *Cymbopogon citratus*).

CONCLUSION

Physical-chemical analyses showed *Faroe spp*. sample to be more acidic (3.930 ± 0.956) compared to the other test samples. However, the conductivity, volatile solids and volatile fatty acids value were similar to those of processed black coffee and black tea unlike those of *Cymbopogon citratus*. The functional group peaks were concisely similar in all the test samples whereas *Faroe spp.* and processed black tea showed more conjugation in their UV-VIS profiles. Similarly, *Faroe spp*. had the highest antioxidant levels when analyzed using DPPH radical scavenger while *Cymbopogon citratus* exhibited very little antioxidant capacity. All samples portrayed abundance in amino acids especially tyrosine and phenylalanine.

The order of caffeine content in the samples was black coffee ($398.990 \pm 0.000 mg/L$), black tea ($169.110 \pm 0.000 mg/L$), *Faroe spp.* (116.640 ± 0.000mg/L) and *Cymbopogon citratus* ($38.760 \pm 0.000 mg/L$). The levels of caffeine in *Faroe spp.* were significant enough to be classified as black or green tea. The crucial phytochemical and carbohydrate scans in *Faroe spp*. and *Cymbopogon citratus* were similar to those of black tea and coffee. *Cymbopogon citratus* extracts however lacked vital reducing sugars and sterols. The order of total alkaloid content was similar to that of caffeine with black coffee having the highest concentrations. Black tea however had more total flavonoid concentration.

Faroe spp. was characterized and analyzed to have very high correlation to the black tea sample. *Cymbopogon citratus* extracts were found to differ with the rest of the beverage analytes in most of the test carried out.

References

[1] Elhussein, E. A. A., Kurtulbaş, E., Bilgin, M., Birteksöz, T. A. S., Hacıoğlu, M., & Şahin, S. (2018). Screening of the most consumed beverages and spices for their bioactive non-nutrient contents. *Journal of Food Measurement and Characterization, 12,* 4, 2289-2301.

[2] Gray, J. (1998). Caffeine, coffee and health. *Nutrition & Food Science, 98,* 6, 314-319.

[3] Pray, L. A., Yaktine, A. L., Pankevich, D. E., Institute of Medicine (U.S.)., & Caffeine in Food and Dietary Supplements: Examining Safety (Workshop). (2014). *Caffeine in food and dietary supplements: Examining safety: workshop summary*.

[4] Bedigian, D. (2005). Tea. Bioactivity and Therapeutic Potential. Medicinal and Aromatic Plants & ndash; Industrial Profiles Vol. 17. *Economic Botany, 59,* 1, 89.

[5] Reynertson, K. A. (2005). Taxus. The Genus Taxus. Medicinal and Aromatic Plants & ndash; Industrial Profiles, Vol. 32. *Economic Botany, 59,* 1, 88-89.

[6] Diamantini, G., Pignotti, S., Antonini, E., Chiarabini, A., Angelino, D., & Ninfali, P. (2015). Assessment of antioxidant capacity of energy drinks, energy gels and sport drinks in comparison with coffee and tea. *International Journal of Food Science & Technology, 50,* 1, 240-248.

[7] Bohn, T., McDougall, G. J., Alegría, A., Alminger, M., Arrigoni, E., Aura, A.-M., Brito, C., ... Santos, C. N. (2015). Mind the gap—deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites—a position paper focusing on carotenoids and polyphenols. *Molecular Nutrition & Food Research, 59,* 7, 1307-1323.

[8] Harvey, D. J. (2012). Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: An update for 2007-2008. *Mass Spectrometry Reviews, 31,* 2, 183-311.

[9] Zhu, X.-J., Zhao, Z., Xin, H.-H., Wang, M.-L., Wang, W.-D., Chen, X., & Li, X.-H. (2016). Isolation and dynamic expression of four genes involving in shikimic acid pathway in *Camellia sinensis* 'Baicha 1' during periodic albinism. *Molecular Biology Reports: An International Journal on Molecular and Cellular Biology, 43,* 10, 1119-1127.

[10] Khan, N., & Mukhtar, H. (2018). Tea Polyphenols in Promotion of Human Health. *Nutrients, 11,* 1.)

[11] Tran, H. T. M., Furtado, A., Vargas, C. A. C., Smyth, H., Slade, L. L., & Henry, R. (2018). SNP in the *Coffea arabica* genome associated with coffee quality. *Tree Genetics & Genomes, 14,* 5, 1-15.

[12] Halson, S. L. (2014). Sleep in Elite Athletes and Nutritional Interventions to Enhance Sleep. *Sports Medicine, 44,* 1, 13-23.

[13] Martin, C., & Li, J. (2017). Medicine is not health care, food is health care: plant metabolic engineering, diet and human health. *New Phytologist, 216,* 3, 699-719.

[14] Alam, M. N., Almoyad, M., & Huq, F. (2018). Polyphenols in Colorectal Cancer: Current State of Knowledge including Clinical Trials and Molecular Mechanism of Action. *Biomed Research International, 2018.*

[15] Ahmed, S., Hasan, M. M., Khan, H., Mahmood, Z. A., & Patel, S. (2018). The mechanistic insight of polyphenols in calcium oxalate urolithiasis mitigation. *Biomedicine & Pharmacotherapy, 106*, 1292-1299.

[16] Dixit, S., Stein, P. K., Dewland, T. A., Dukes, J. W., Vittinghoff, E., Heckbert, S. R., & Marcus, G. M. (2016). Consumption of Caffeinated Products and Cardiac Ectopy. *Journal of the American Heart Association, 5,* 1.)

[17] Cole, D. L. M. C. (1993). Proceedings of the thirty first Congress. South African Society for Plant Pathology. *Phytophylactica, 25*, 3, 167.

[18] Harborne, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK.

[17] Close, D.C. and Mc Arthur, C., (2002) Rethinking the role of many plants phenolics protection from photo damage Oikos, 99; 166-172.

[18] Bizuayehu, D., Atlabachew, M., & Ali, M. T. (2016). Determination of some selected secondary metabolites and their invitro antioxidant activity in commercially available Ethiopian tea (*Camellia sinensis*). *Springerplus, 5,* 1, 1-9.

[19] Pardo, T., Bes, C., Bernal, M. P., & Clemente, R. (2016). Alleviation of environmental risks associated with severely contaminated mine tailings using amendments: Modeling of trace element speciation, solubility, and plant accumulation. *Environmental Toxicology and Chemistry, 35*, 11, 2874-2884.

[20] Singh, P. (2016). Effect of chitosans and chitooligosaccharides on the processing and storage quality of foods of animal and aquatic origin. *Nutrition & Food Science, 46*, 1, 51-81.

[21] Derman, E. W., Schwellnus, M. P., Micklesfield, L., Nossel, C., Whitesman, S., Patel, D. N., & Dreyer, M. (2011). Healthy lifestyle interventions in general practice part 13: lifestyle and osteoporosis: CPD article. *South African Family Practice, 53,* 1.)

[22] Nemecz, G. (2005). The health benefits of dark chocolate: nutrition. *Journal of Modern Pharmacy, 12,* 6, 43-46.

[23] Pluskal, T., & Weng, J.-K. (2018). Natural product modulators of human sensations and mood: molecular mechanisms and therapeutic potential. *Chemical Society Reviews, 47,* 5, 1592-1637.

[24] Baqueta, M. R., Coqueiro, A., & Valderrama, P. (2019). Brazilian Coffee Blends: A Simple and Fast Method by Near-Infrared Spectroscopy for the Determination of the Sensory Attributes Elicited in Professional Coffee Cupping. *Journal of Food Science, 84,* 6, 1247-1255.

[25] Oellermann, R. A. (1964). Nutritive value of Themeda triandra I. The influence of variables on the determination of the different protein fractions in forages and on the in vitro fermentation procedure employed. *South African Journal of Agricultural Science, 7,* 4, 633-648.

[26] Xiong, Z., Dong, Y., Zhou, H., Wang, H., & Zhao, Y. (2014). Simultaneous Determination of 16 Organic Acids in Food by Online Enrichment Ion Chromatography−Mass Spectrometry. *Food Analytical Methods, 7,* 9, 1908-1916.

[27] Amarowicz, R., Carle, R., Dongowski, G., Durazzo, A., Galensa, R., Kammerer, D., Maiani, G., ... Piskula, M. K. (2009). Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. *Molecular Nutrition & Food Research, 53.*

[28] Christian, M. S., & Brent, R. L. (2001). Teratogen update: Evaluation of the reproductive and developmental risks of caffeine. *Teratology, 64,* 1, 51-78.

[29] Suhm, M. A., & Kollipost, F. (2013). Femtisecond single-mole infrared spectroscopy of molecular clusters This work is dedicated to M. Quack on the occasion of his 65th birthday. *Physical Chemistry Chemical Physics, 15,* 26, 10702-10721.

[30] Yuan, X., He, Y., Zhou, G., Li, X., Feng, A., & Zheng, W. (2018). Target challenging-cancer drug delivery to gastric cancer tissues with a fucose graft epigallocatechin-3-gallate-gold particles nanocomposite approach. *Journal of Photochemistry and Photobiology. B, Biology, 183,* 147-153.

[31] Kubis, C., Profir, I., Fleischer, I., Baumann, W., Selent, D., Fischer, C., Spannenberg, A., ... Börner. A. (2016). In Situ FTIR and NMR Spectroscopic Investigations on Ruthenium-Based Catalysts for Alkene Hydroformylation. *Chemistry - a European Journal, 22,* 8, 2746-2757.

[32] Kumari, L., Li, W. Z., Kulkarni, S., Wu, K. H., Chen, W., Wang, C., Vannoy, C. H., ... Leblanc, R. M. (2010). Effect of Surfactants on the Structure and Morphology of Magnesium Borate Hydroxide Nanowhiskers Synthesized by Hydrothermal Route. *Nanoscale Research Letters, 5,* 1, 149-157.

[33] Jeszka-Skowron, M., Zgoła-Grześkowiak, A., & Grześkowiak, T. (2015). Analytical methods applied for the characterization and the determination of bioactive compounds in coffee. *European Food Research and Technology: 240,* 1, 19-31.

[34] Ye, Y., Sun-Waterhouse, D., You, L., & Abbasi, A. M. (2017). Harnessing food-based bioactive compounds to reduce the effects of ultraviolet radiation: a review exploring the link between food and human health. *International Journal of Food Science & Technology, 52,* 3, 595-607.

[35] Hong, E., Lee, S. Y., Jeong, J. Y., Park, J. M., Kim, B. H., Kwon, K., & Chun, H. S. (2017). Modern analytical methods for the detection of food fraud and adulteration by food category. *Journal of the Science of Food and Agriculture, 97,* 12, 3877-3896.

[36] Hazarika, M., & Mahanta, P. K. (1983). Some studies on carotenoids and their degradation in black tea manufacture. *Journal of the Science of Food and Agriculture, 34,* 12, 1390-1396.

[37] El-Salamouny, S., Ranwala, D., Shapiro, M., Shepard, B. M., & Farrar, R. R. (2009). Tea, Coffee, and Cocoa as Ultraviolet Radiation Protectants for the Beet Armyworm Nucleopolyhedrovirus. *Journal of Economic Entomology, 102,* 5, 1767-1773.

[38] Louwrens, H., Venter, I., & Rautenbach, F. (2009). South African dietary total antioxidant capacity based on secondary intake data in relation to dietary recommendations: original research. *South African Journal of Clinical Nutrition, 22,* 4, 195-202.

[39] Nikoo, M., Regenstein, J. M., & Ahmadi, G. H. (2018). Antioxidant and Antimicrobial Activities of (-)-Epigallocatechin-3-gallate (EGCG) and its Potential to Preserve the Quality and Safety of Foods. *Comprehensive Reviews in Food Science and Food Safety, 17,* 3, 732-753.

[40] Klempner, S. J., & Bubley, G. (2012). Complementary and Alternative Medicines in Prostate Cancer: From Bench to Bedside? *The Oncologist, 17,* 6, 830-837.

[41] Verhoeven, A. S., Swanberg, A., Thao, M., & Whiteman, J. (2005). Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in *Taxus x media* growing in sun and shade environments. *Physiologia Plantarum, 123,* 4, 428-434.

[41] Havaux, M., & Kloppstech, K. (2001). The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and tt mutants. *Planta, 213,* 6, 953-966.

[42] Yablokov, V. A., Vasina, Y. A., Zelyaev, I. A., & Mitrofanova, S. V. (2009). Kinetics of thermal decomposition of sulfur-containing amino acids. *Russian Journal of General Chemistry, 79,* 6, 1141-1145.

[43] Kijima, T., & Ueno, S. (1986). ChemInform Abstract: Uptake of Amino-acids by Zirconium Phosphate. Part 3. Intercalation of L-Histidine, L-Lysine, and L-Aginine by γ-Zirconium Phosphate. *Chemischer Informationsdienst, 17,* 19.)

[44] Bernd Gillissen, Lukas Bürkle, Bruno André, Christina Kühn, Doris Rentsch, Birgit Brandl, Wolf B. Frommer (2000). A New Family of High-Affinity Transporters for Adenine, Cytosine, and Purine Derivatives in Arabidopsis *Plant Cell. 2000 Feb; 12(2):* 291–300.

[45] Cömert, E. D., & Gökmen, V. (2017). Antioxidants Bound to an Insoluble Food Matrix: Their Analysis, Regeneration Behavior, and Physiological Importance. *Comprehensive Reviews in Food Science and Food Safety, 16,* 3, 382-399.

[46] The hot report: hot beverages. (2014). *South African Food Review, 41,* 5, 33-34.

[47] Combrink, M. J., De, L. T., Toerien, A. S., Bedeker, M., & Maree, J. E. (2011). Incidence, severity and management of cancer chemotherapy related oral mucositis in Eastern Cape and Western Cape: original research. *Health Sa Gesondheid, 17,* 1, 1-7.

[48] Boros, K., Jedlinszki, N., & Csupor, D. (2016). Theanine and Caffeine Content of Infusions Prepared from Commercial Tea Samples. *Pharmacognosy Magazine, 12,* 45.)

[49] Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A., & Feeley, M. (2003). Effects of caffeine on human health. *Food Additives & Contaminants, 20,* 1, 1-30.

[50] Choi, H. K., & Curhan, G. (2007). Coffee, tea, and caffeine consumption and serum uric acid level: The third national health and nutrition examination survey. *Arthritis Care & Research, 57,* 5, 816-821.

[51] Current literature in mass spectrometry. (2009). *Journal of Mass Spectrometry, 44,* 4, 566-577.

[52] Fukushima Y, Ohie T, Yonekawa Y, Yonemoto K, Aizawa H, Mori Y, Watanabe M, Takeuchi M, Hasegawa M, Taguchi C, Kondo K. (2009). Coffee and green tea as a large source of antioxidant polyphenols in the Japanese population. *J Agric Food Chem.57(4):*1253-9. doi: 10.1021/jf802418j.

[53] AlHafez, M., Kheder, F., & AlJoubbeh, M. (2014). Polyphenols, flavonoids and (-)-epigallocatechin gallate in tea leaves and in their infusions under various conditions. *Nutrition & Food Science, 44,* 5, 455-463.

[54] Gad, H. A., El-Ahmady, S. H., Abou-Shoer, M. I., & Al-Azizi, M. M. (2013). Application of Chemometrics in Authentication of Herbal Medicines: A Review. *Phytochemical Analysis, 24,* 1, 1-24.

[55] Oba, S., Nagata, C., Nakamura, K., Fujii, K., Kawachi, T., Takatsuka, N., & Shimizu, H. (2010). Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes in Japanese men and women. *The British Journal of Nutrition, 103,* 3, 453-9.

[56] Wang, N., Su, M., Liang, S., & Sun, H. (2016). Investigation of six bioactive anthraquinones in slimming tea by accelerated solvent extraction and high-performance capillary electrophoresis with diode-array detection. *Food Chemistry, 199,* 1-7.

[57] Renouf, M., Marmet, C., Giuffrida, F., Lepage, M., Barron, D., Beaumont, M., Williamson, G., ... Dionisi, F. (2014). Dose-response plasma appearance of coffee chlorogenic and phenolic acids in adults. *Molecular Nutrition & Food Research, 58*, 2, 301-309.

[58] Vrba, E. S. (1985). Species and speciation. Development and speciation. Faunal case histories. *Transvaal Museum Monographs, 4*, 1.)

[59] IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Coffee, Tea, Mate, Methylxanthines and Methylglyoxal. Lyon (FR): International Agency for Research on Cancer; 1991. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 51.) COFFEE. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507020/

[60] Chen, Ê M. Robert F., Meghan L., and Michelle M. (2006). Differences in the Protein, Carbohydrate, and Caffeine content of Black Tea and Coffee. LBS 145.

[61] Shearer, J. (2014). Methodological and metabolic considerations in the study of caffeine-containing energy drinks. *Nutrition Reviews*, *72*, 137-145.

[62] Khanum, H., Faiza, S., Sulochanamma, G., & Borse, B. B. (2017). Quality, antioxidant activity and composition of Indian black teas. Journal of food science and technology, 54(5), 1266–1272. doi:10.1007/s13197-017-2506-y

[63] Ousmane N. Diop, A. Mariko, M. Géye, R. Thiam, K. Omar S., Serigne N., Bara D. (2017). Comparative Study of the Composition of Aqueous Extracts of Green Tea (*Camellia Sinensis*) in Total Alkaloids, Total Flavonoids, Total Polyphenols and Antioxidant Activity with the Leaves of Combretum Glutinosum, Combretum Micranthum and the Red Pulps of Hibiscus Sabdariffa. 5. 71-75.

Conflicts of Interest
The authors declare to have no conflicts of interest whatsoever.

Source of Funding
No source of funding was received for this work.

Data Availability Statement
All data used in this research is enclosed within the manuscript and any supplementary sheets attached.

Acknowledgement
The authors wish to express the sincere gratitude to Maasai mara university for provision of lab facilities to extract, characterize and analyze the samples. Multimedia university was also helpful with conjugation and antioxidant analysis using UV-VIS spectroscopy.