

Antioxidant and Sensory Properties of Herbal Teas Formulated from Dried Moringa (*Moringa Stenopetala*) and Stevia (*Stevia Rebaudiana Bertoni*) Leaves

Abdela Kinki¹ Abadi Mezgebe² Tesfu Lema³

1.Researcher I (Food Science and Nutrition at Ethiopia Institute of Agricultural Research, Wondo Genet Agricultural Research Center

2.Assistant Professor (Food Science and Technology, Hawassa University)

3.Lecturer (Food Science and Technology, Hawassa University)

P.O.Box: 198, Shashamene, Ethiopia

Abstract

Herbal teas are gaining popularity and acceptance due to their sensory and health benefits. The demand for moringa tea currently increased in Ethiopia due to its nutritional and medicinal values. However, using moringa alone is difficult due to its poor sensory appeal and adding sugar to enhance the sensory has implications for health. The purpose of this study was to optimize the sensory properties (taste and aroma) of formulated herbal teas in addition to evaluating the antioxidant properties of the formulated herbal tea from dried moringa and stevia leaves. Seven moringa-based herbal teas were brewed with stevia ranging from 0 to 35% with five-level (5) and compared for their sensory and antioxidant properties. The moringa tea infusion and commercial green tea were considered as control. The results of sensory analysis showed that herbal tea brewed with 20-35% stevia in the formulation results in higher sweetness compared to 100%-moringa and green tea. Herbal tea brewed with 20-35% stevia in the formulation results in the highest in antioxidant (DPPH scavenging capacity, ferric reducing power and total antioxidant activities) values comparable to 100%-moringa. This study provides evidence that adding stevia to moringa improves the sensory and antioxidant properties without compromising its health-promoting compounds.

Keywords: Moringa, stevia, phenolic content, antioxidant activity, herbal tea, sensory, herbal infusion.

DOI: 10.7176/FSQM/102-01

Publication date: November 30th 2020

1. INTRODUCTION

Herbal teas that refer to any green or leafy part of a plant are gaining popularity among consumers (Adnan *et al.*, 2013). Herbal teas are distinguished from caffeinated beverages like the true tea (black, green, white, yellow and oolong,) or decaffeinated tea, in which the caffeine has been removed by their preparation from plants other than *Camellia* (Alakali *et al.*, 2016; Teye *et al.*, 2017). Herbal tea promotes better night sleep because it is caffeine-free and its antimicrobial activity (Omogbai & Ikenebomeh, 2013; Killedar *et al.*, 2017). Some of the popular herbal teas are chamomile, ginger, ginseng, peppermint, and cinnamon (Ravikumar, 2014). Herbal teas are made by infusion or decoction from fresh and dried leaves, seeds, grasses, nuts, barks, fruits, and flowers, or other botanical elements (Hicks, 2009; Kumar and Pandey, 2013). They have been extensively consumed because of their health benefits and sensory characteristics (Kazimierzak *et al.*, 2015; Killedar *et al.*, 2017). Antioxidants are chemical substances that inhibit the oxidation of other molecules and protect cells from the damages caused by free radicals in the human body by alleviating and prevent various diseases related to the cardiovascular system, heart, brain, liver, kidney, and cancer and it helps to retard the aging process, these are: - 2, 2-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing-antioxidant power (FRAP) and total antioxidant activity using phosphomolybdenum assay (Le *et al.*, 2007; Azadi Gonbad *et al.*, 2015). Generally, the mechanism behind the free radical scavenging activity of polyphenols is to contribute to the reduction of oxidative stress and to prevent the onset of free radicals (Huang *et al.*, 2001).

The fresh and dried leaf of moringa is widely sold and consumed in cities of Ethiopia, due to its perceived awareness of health and nutritional benefits (Habtemariam and Varghese, 2015). Moringa is becoming a very important plant rich in antioxidants, protein, calcium, and iron compared to other commonly consumed fruits and vegetables (Glarum, 2012). Stevia is recently cultivated on a large scale by entrepreneurs in Ethiopia for herbal production due to its potential uses (Kassahun *et al.*, 2012). Stevia is also grown like other vegetables in the kitchen garden (Goyal *et al.*, 2010; Kassahun *et al.*, 2012). Stevia leaves are used in food products as calory free intense natural sweetener (Kassahun *et al.*, 2012; Gupta *et al.*, 2013).

The demand for moringa currently increased in Ethiopia due to its nutritional and medicinal values (Habtemariam and Varghese, 2015). However, using it alone is difficult as it has poor sensory appeal (De-Heer, 2011). Enhancement of sensory appeal of moringa based foods by adding cereals and sugar has been studied (Hamza & Azmach, 2017) improve the sensory properties. However, increasing consumption of sugar (sucrose) and sugar-rich products has resulted in several health problems (Gasmalla *et al.*, 2014). Hence, natural low calories

sweeteners are considered as best substitutes of cereals and sugar to enhance the sensory apples of moringa. Stevia is assumed as the main substitution of natural sugar, due to the presence of steviol glycosides that has great potential in the food industry as a strategy to reduce sugar consumption (Ahmad & Ahmad, 2018). Partial replacement of sugar with stevia and modifying the sensory appeal of moringa tea has not been studied. In this study, alternative herbal tea development from moringa and stevia leaves blend was investigated for its nutraceutical and sensory properties.

The general objective of the study was to evaluate the sensory and antioxidant properties of herbal teas formulated from dried moringa and stevia leaves to optimize the sensory appeal of moringa tea and partially replace sugar with stevia (i.e. natural low caloric sweetener).

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Fresh moringa and stevia leaves were obtained from Arba Minch and Wondo Genet Agriculture research centers (Ethiopia), respectively. The samples were packed in polyethylene (plastic) bags and transported to Wondo Genet Natural Product Research Laboratory.

The fresh leaves of uniform shape, color and size were selected and subjected to shade drying at ambient temperature for about one week according to Killedar *et al.*, (2017). The leaves were spread thinly on paper-lined wooden trays and protected from direct sunlight to prevent the loss of volatile aroma compounds and also photooxidation

The dried samples were separately milled using an electric Blender (Model BLG401, Zhejiang YiLi Tool Co., Ltd., China). The milled samples were sieved using 2 mm sieve size to separate the milled leaves. From the sieved samples, formulations were prepared and kept in an air-tight container and stored at room temperature until further analysis.

2.2. Treatments and Experimental Design

The dried moringa and stevia leaves were mixed in varying proportions to obtain 7 different formulations (Table 1) based on the preliminary test. The sensory acceptability and antioxidant activities of their tea were analyzed. The experimental design was a completely randomized design (CRD).

Table 1:-Formulations of herbal tea in the experimental design

Herbal tea formulation	Moringa leaves %	Stevia leaves (%)
TF1	100	0
TF2	95	5
TF3	90	10
TF4	85	15
TF5	80	20
TF6	75	25
TF7	70	30
TF8	65	35
TF9	0	100
GT (100%-Green tea)		

2.3. Preparation of Herbal Tea

The herbal tea was prepared according to the method developed by Horžić *et al.*, (2009); formulated samples 2 g were infused in 200 ml boiling water for 5 minutes at 97°C to mimic normal tea preparation till it becomes an appealing fragrance. Herbal tea was prepared from all formulated herbal teas and controls. The formulated herbal teas were unsweetened using sugar and considered for the analysis in this experiment.

2.4. Preparation of Extracts from Herbal Tea

The extract was prepared according to Koh *et al.*, (2009) and Mingarro *et al.*, (2003). The decoction was boiled for 5 min at 97 °C as usual for normal tea prepared. The decoction was filtered through a double-layered muslin cloth to get rid of the large particles and filtered through a filter paper (Whatman no.1). The filtered product was then allowed to concentrate at 45°C for three consecutive days by evaporate excess water and obtain the dried. The extract was weighed and its respective percentage yield was recorded. The crude extract 1 g was dissolved in 50 ml of respective solvent (methanol) to make a stock solution of 20 mg/mL. The prepared stock solution was kept at 4°C in a refrigerator, to serve as the working solution for all the phytochemicals and antioxidant tests.

2.5. Descriptive sensory analysis

The descriptive sensory analysis of formulated herbal tea infusions was conducted using trained sensory panels of 9 people. Only panelists who voluntarily accepted and signed a consent form after they have been informed about

the nature of study materials and the activity involved were considered. The sensory profiling of the products was performed using generic descriptive analysis method Einstein, (1991). The panelists were trained in two sessions of 3 hrs per day using reference samples for green tea and lexicon terms were developed Lee and Chambers, (2007). Profiles of aroma, appearance tastes, aftertaste and overall evaluation sensory properties of the formulated herbal tea infusions were generated with their definition, reference standard, and anchors (Table 3). The attributes were evaluated on 10- scale anchored with verbal descriptions at extreme ends Munoz and Cville, (1998).

2.6. Antioxidant activities

2.6.1. DPPH radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the herbal extract was determined as described by Brand-Williams *et al.*, (1995). Different concentrations (50 to 1000 µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.06%, and w/v) in methanol was added in each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards (ascorbic acid and BHT) were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 520 nm. Methanol (100%) was used as a blank. The ability to scavenge the DPPH radical was calculated using equation 1

$$\text{Radical scavenging effect (\%)} = \frac{A_c - A_s}{A_c} \times 100 \dots \dots \dots 1$$

Where A_c = Absorbance of the control; A_s = Absorbance of the sample

The antioxidant activity of the extract was expressed as EC_{50} and the EC_{50} value is the concentration in (µg/mL) of extracts that scavenges the DPPH radical by 50%.

2.6.2. Ferric reducing antioxidant power

This assay was carried out according to Safdar *et al.*, (2016). One milliliter of the herbal extract with a concentration of 1 mg/mL was mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Then the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%) and the absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 700 nm. The reducing power was expressed as mg of ascorbic acid equivalents/g of dried extract (mg AAE/g) using the calibration curve ($y=0.0063x+0.148$, $R^2=0.99$ ($p<0.01$)).

2.6.3. Total antioxidant activity using phosphomolybdenum assay

The total antioxidant activity of formulated herbal tea was determined by phosphomolybdenum assay according to Prieto *et al.*, (1999). Sample of 0.3 mL of extract (1 mg/ mL) in the solution was mixed with 3 mL phosphomolybdenum reagent (28mM sodium phosphate and 4mM ammonium molybdate in 0.6 M sulphuric acid) in capped test tubes. The samples were incubated for 90 min in a water bath at 95°C. After cooling to room temperature, the absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 695 nm against a blank (3 mL methanol without plant extract). The total antioxidant activity was expressed as milligram butylated hydroxytoluene equivalent/gram of dried extract (mgBHTE/g) using a calibration curve ($y=0.0094x+0.112$, $R^2=0.99$ ($p<0.001$)).

Evaluation of formulated herbal tea infusions was performed on single products in replicated sessions. The actual product evaluation was done in the Wondo Genet Research center hall room. The formulated herbal tea infusions were presented in a transparent teacup with random three-digit codes. A glass of drinking water was provided for rinsing between tastes.

2.7. Statistical Analysis

All data were analyzed using one-way ANOVA with traits as an independent variable. The means were separated using Tukey's HSD test at $p<0.05$. Principal Component Analysis (PCA) for all numerical results was performed using XLSTAT version 2016.03.30882 (Addinsoft, New York).

3. RESULTS AND DISCUSSIONS

3.1. Antioxidant Activities of the Formulated Herbal Teas

The antioxidant activities g for formulated herbal tea were determined using DPPH Scavenging activity, Ferric reducing power (FRAP) and total antioxidant activity assay. DPPH Scavenging activity, FRAP and total antioxidant activity measured the hydrogen, electron-donating abilities of primary antioxidants and reduction of Mo (Molybdenum) (VI) to Mo (Molybdenum) (V) in the presence of antioxidant compound and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH and at higher temperature were studied, respectively according to Lim *et al.*, (2007) and presented in Figure 1 and Table 2.

3.2. DPPH scavenging activity

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a

parameter widely used to measure antioxidant activity (Sánchez-Moreno *et al.* 1998). The lower the IC₅₀ the higher is the antioxidant activity (Brand-Williams *et al.*, 1995). All the formulated herbal teas had considerable variation and ranged from 0.05 to 0.73 g/ml (Table 2). The IC₅₀ of herbal teas of TF6, TF7 and TF8 were lower ($p < 0.05$) compared to the infusions of 100% moringa (TF1) (Table 2). These samples had comparable ($p > 0.05$) IC₅₀ to the references ascorbic acid (AA). All other formulations had higher ($p < 0.05$) IC₅₀ compared to samples of TF6, TF7, and TF8. This is indicative of the samples with a higher level of stevia (25-35%) improved the DPPH Scavenging Activity of the herbal tea formulations. This can be attributed to the high level of phytochemicals in the dried stevia leaves (Table 2).

As shown from the dose dependence curve for the DPPH radical scavenging activity, the concentration of the sample increased, the percent inhibition of DPPH radical scavenging activity also increased (Figure 1). At a concentration of 1 mg/ml, the scavenging effect of ascorbic acid and formulated herbal tea from dried moringa and stevia, the DPPH radical scavenging decreased in the order of the ascorbic acid, stevia and formulated herbal tea. The DPPH radical scavenging activity (IC₅₀) of formulated herbal tea infusions was higher than the moringa leaves tea infusion. The variations in the results of the present study could be due to the difference in addition to stevia to the moringa.

3.3. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) of all the formulated herbal tea ranged between 1.01 and 4.24 mg AAE/g with a considerable variation (Table 2). Herbal tea samples with a relatively high level of stevia (TF7 and TF8) had higher ($p < 0.05$) FRAP compared to the 100% moringa (TF1). Whereas the samples with a lower level of stevia (TF2 and TF3) had lower FRAP compared to the 100% moringa, this is due to a stable protein-antioxidant activity interaction, resulting in a decrease or increasing of antioxidant activities (Gallo *et al.*, 2013). Indeed, binding to food proteins may have implications in terms of bioavailability, as the antioxidant capacity of polyphenols can be modified by the presence of proteins (Yildirim- Elikoglu & Erdem, 2018). Herbal teas of TF4, TF5 and TF6 had similar ($p > 0.05$) FRAP to the reference sample 100% moringa (Table 2). The increased FRAP in the herbal tea formulations that have a higher level of stevia is due to the high level of phytochemicals in the dried stevia leaves (Table 2).

3.4. Total antioxidant activity (TAA)

The total antioxidant activity (TAA) of all the formulated herbal tea ranged between 0.44 and 1.92 mg BHTE/g with considerable variations (Table 2). Herbal tea samples with a relatively high level of stevia (TF7 and TF8) had higher ($p < 0.05$) TAA compared to the 100% moringa (TF1). Whereas the samples with a lower level of stevia (TF2, TF3, TF4 and TF5) had lower TAA compared to the 100% moringa tea infusion is due to a stable protein-antioxidant activity interaction, resulting in a decrease or increasing of antioxidant activities (Gallo *et al.*, 2013). Indeed, binding to food proteins may have implications in terms of bioavailability, as the antioxidant capacity of polyphenols can be modified by the presence of proteins (Yildirim- Elikoglu & Erdem, 2018). Only herbal teas of TF6 had similar ($p > 0.05$) TAA to the reference sample (100% moringa). The enhanced total antioxidant activity in the herbal tea formulations that have a higher level of stevia is due to the high level of phytochemicals in the dried stevia leaves (Table 2).

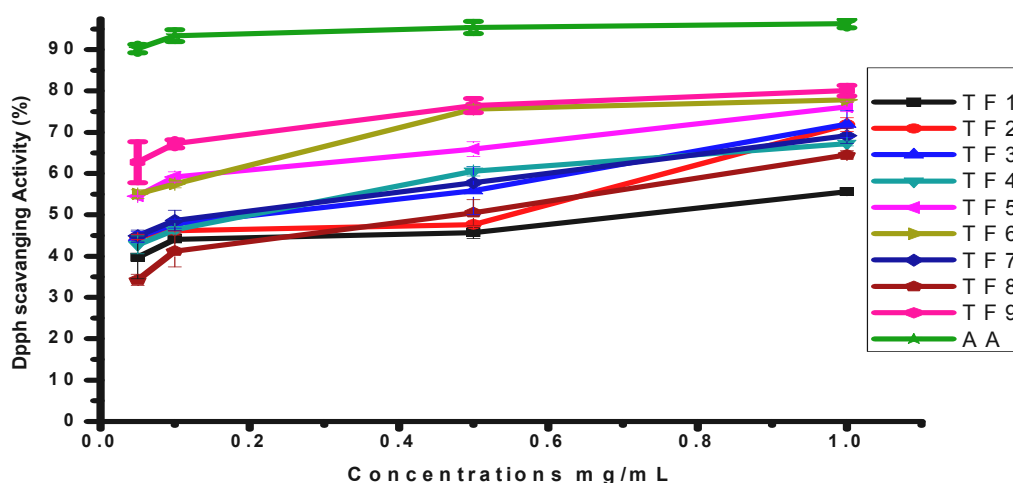


Figure 1:-2, 2- Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%) of herbal tea formulated from moringa and stevia and controls AA (L-ascorbic acid).

Table 2:-2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging IC50 values, ferric reducing power, and total antioxidant activities of herbal tea formulated from moringa and stevia

Herbal tea	DPPH scavenging (IC50, g/mL)	Ferric reducing power (mg AAE/g)	Total antioxidant (mg BHTE/g)
TF1	0.73 ± 0.02 ^a	1.01±0.07 ^f	0.44±0.03 ^f
TF2	0.55 ± 0.01 ^{ab}	1.41±0.12 ^f	0.62±0.05 ^e
TF3	0.26 ± 0.15 ^{bcd}	1.73±0.13 ^{ef}	0.69±0.03 ^e
TF4	0.21 ± 0.02 ^{dc}	2.39±0.05 ^{de}	1.04±0.06 ^d
TF5	0.05 ± 0.01 ^d	2.79±0.36 ^{cd}	1.14±0.03 ^{cd}
TF6	0.05 ± 0.03 ^d	3.42±0.39 ^{bc}	1.40±0.02 ^b
TF7	0.17 ± 0.09 ^d	4.24±0.12 ^b	1.43±0.01 ^b
TF8	0.48 ± 0.12 ^{bac}	2.27±0.02 ^{dc}	1.23±0.02 ^c
TF9	0.04± 0.01 ^d	6.67±0.25 ^a	1.92±0.02 ^a
AA	0.03±0.02 ^d		

Values are mean ± SD (n=2). Means with a different letter with column of superscripts are significantly different at p<0.05. AAE/g: Ascorbic acid equivalents per gram of dried extract; BHTE/g: Butylated hydroxytoluene equivalents per gram of dried extract;

Where TF1(100% moringa), TF2 (95% moringa and 5% stevia), TF3 (90% moringa and 10% stevia), TF4 (85% moringa and 15% stevia), TF5 (80% moringa and 20% stevia), TF6(75% moringa and 25% stevia), TF7(70% moringa and 30% stevia), TF8(65% moringa and 35% stevia), TF9 (100% stevia) and AA (Ascorbic acid).

3.5. Descriptive Sensory Analysis of the Formulated Herbal Tea

The sensory panels generated twenty-five moringa-stevia herbal tea quality descriptors and their definition, reference standards and anchors are given (Table 3). The aroma and appearance attributes were not affected (p<0.05) by the formulation (Table 4). However, taste attributes (sweetness and bitterness), after-taste attributes (sweet aftertaste) and overall sensory appeal (sweetness and sweet after-taste) properties of the herbal teas were affected (p<0.05) by the formulations. Furthermore, taste attributes (astringency, grassy taste, leafy taste and sour), after-taste attributes (leafy aftertaste, astringent aftertaste, grassy aftertaste, and lingering aftertaste) and overall sensory appeal (tea/herbal aroma) of the herbal teas were not significantly affected (p<0.05) by the formulation (Table 4).

The herbal teas formulated showed considerable variation in sweetness (Table 5). Herbal tea of TF8, TF7, and TF6 was sweeter (p<0.05) compared to reference samples TF1 (100% moringa) and GT (green tea) with no significant difference among them. Herbal teas of TF2, TF3, TF4, and TF5 were lower (p<0.05) in sweetness compared to TF8 (teas with a higher level of stevia) and were sweeter (p<0.05) than 100% moringa. Furthermore, TF2, TF3, TF4, and TF5 herbal teas were lower (p<0.05) in sweetness compared to the sample having a high level of stevia (TF8). This revealed that the addition of stevia to moringa tea infusion improved its sweetness.

The principal component analysis (PCA) of the herbal teas formulated showed that formulations with a relatively high level of stevia TF4, TF5, TF6, TF7, TF8 were aligned with sweetness (Figure 2). These herbal teas were on the same PC1 quadrant as sweetness. Furthermore, the principal component analysis (PCA) showed that teas with a low level of stevia TF2, TF3, TF1(100% moringa) and GT (green tea) were negatively associated with sweetness. Hence, the addition of stevia in herbal tea is highly associated with the sweetness of the product. The finding of this study is in agreement with Abdo, (2016) that found the sweetness equivalence ratio of stevia with sugar adopted in Ethiopia.

The herbal teas formulated showed considerable variation in bitterness (Table 5). All the formulated herbal teas were less (p<0.05) in bitterness compared to GT (green tea) and the TF1 (100% moringa) was similar (p>0.05) in bitterness with all the formulated herbal teas and green tea (GT). This shows that the addition of stevia to moringa tea infusion does significantly affect the bitterness of the herbal teas in the limit of the study. However, principal component analysis (PCA) of the herbal teas formulated revealed that formulations with relatively high level of moringa TF1 (100% moringa), TF2 and TF3 were aligned with bitterness (Figure 2). These herbal teas were on the same PC1 quadrant as bitterness. Furthermore, the PCA showed that teas with a high level of moringa TF1, TF2 and TF3 were negatively associated with bitterness.

Sensory category	Attributes	Definition /reference	Scale anchors (0, 10)
Aroma	Grassy	Aromatics associated with newly cut grass.	Not grassy, intense grassy
	Leafy	Odorants typically associated with Sharp and slightly pungent aromatics	No leafy aroma, intense leafy aroma
	fruity	Containing fruity aroma	No fruity aroma, intense fruity aroma
	musty/ stale *	Having stale odor	No musty aroma, intense musty aroma
	tea	Having a normal tea smell.	No tea aroma, intense tea aroma
Appearance	clear	Transparent in color or bright	No clear look, Very clean look
	Cloudy	Not transparent or clear	Not cloudy, very cloudy
	Sediments	A collection of small particles	No sediments, much sediment
	leafy extract	Having leafy smell	No leafy extract, intense leafy extract
	Tea appearance	The act of appearing to normal tea	No tea appearance, tea appearance
Taste	Sweetness	Taste sensation typical of sucrose Reference	No sweetness, intense sweetness
	Astringency	Shriveling taste sensation on the tongue or puckering of the oral tissue	No astringency, intense astringency
	Grassy taste	Taste-related to grassy	No Grassy taste, intense Grassy taste
	Leafy taste*	Taste that has leafy	No Leafy taste, intense Leafy taste
	Sour	Taste sensation typical of acidic fruits.	No sour, intense sour
Aftertaste	Bitter	Taste sensation typical of kola nut	No bitter, intense bitter
	leafy aftertaste*	Taste of tea that persists when it is no longer present as leafy	No leafy aftertaste, intense leafy aftertaste
	sweet aftertaste*	The sweetness persists of tea as sweet after taste	No sweet aftertaste, intense sweet aftertaste
	astringent aftertaste*	A substance which draws tissue together after taste of herbal tea	No astringent aftertaste, intense astringent aftertaste
	bitter aftertaste*	Having an acrid taste usually from a basic substance after tasting herbal tea	No bitter aftertaste, intense bitter aftertaste
	grassy aftertaste*	The taste which exit after tasting herbal tea grassy	No grassy aftertaste, intense grassy aftertaste
	lingering aftertaste*	The act of waiting after tasting herbal tea	No lingering aftertaste, intense lingering aftertaste
overall evaluation	tea/herbal aroma*	Normal tea aroma	No tea/herbal aroma, intense tea/herbal aroma
	Sweetness	The taste stimulated by sucrose and other sugars, such as fructose, glucose, saccharin, Aspartame, etc.	No sweetness, intense sweetness
	sweet aftertaste*	The persistence of sweetness after taste	No sweet aftertaste, intense

Hence, the presence of high level moringa in herbal tea is highly associated with the bitterness of the product. It has been described that the presence of moringa is responsible for the increasing bitterness.

The herbal teas formulated showed considerable variation in the sweet aftertaste (Table 5). Herbal tea of TF6 TF7 and TF8 had a sweeter aftertaste ($p < 0.05$) compared to reference samples TF1 (100% moringa) and GT (green tea) with no significant difference among them. Herbal teas of TF5 had higher ($p < 0.05$) sweet after taste compared to TF2 and it was similar ($p > 0.05$) in sweet after taste to TF4, TF3 and the controls (TF1 and GT). Herbal teas of TF2 and TF3 had lower ($p < 0.05$) sweet aftertaste compared to TF6, TF7, and TF8 (teas with a higher level of stevia) and were similar ($p > 0.05$) in the sweet aftertaste to TF1 (100% moringa). This also showed that the addition of stevia to moringa results increased the sweet aftertaste of herbal tea infusions.

The principal component analysis (PCA) of the herbal teas formulated indicates that formulations with a relatively high level of stevia TF4, TF5, TF6, TF7, and TF8 were aligned with sweet aftertaste (Figure 2). These herbal teas were on the same PC1 quadrant as sweet aftertastes. Hence, it seems that the addition of stevia in herbal tea is highly associated with the sweet aftertaste of the herbal tea formulation.

The herbal teas formulated showed considerable variation in overall sensory appeal (sweetness and sweet after-taste) (Table 5). Herbal teas of TF4, TF5, TF6, TF7 and TF8, were sweeter ($p < 0.05$) in overall sensory appeal compared to control TF1 (100% moringa). TF2 and TF3 were comparable ($p > 0.05$) in sweetness to TF1 and GT (green tea). Furthermore, herbal teas of TF4, TF6, TF7, and TF7 had a sweeter aftertaste ($p < 0.05$) in overall sensory appeal compared to control TF1 (100% moringa). TF2 and TF3 were comparable ($p > 0.05$) in the sweet aftertaste to TF1 and GT (green tea). This result of overall sensory appeal showed that the addition of stevia to moringa improved the sensory quality of moringa herbal tea infusions. The PCA of the herbal teas formulated revealed that the formulations with relatively high level of stevia TF4, TF5, TF6, TF7 and TF8 were aligned with an overall sensory appeal of sweetness and sweet after-taste (Figure 2). These herbal teas were on the same PC1 quadrant as an overall sensory appeal (sweetness and sweet after-taste). This is indicative that the addition of stevia in herbal tea highly associated with the overall sensory appeal (sweetness and sweet after-taste). The finding of this study is in agreement with Abdo, (2016) that found the sweetness equivalence ratio of stevia with sugar adopted in Ethiopia.

Table 3:-The lexicon used to describe the sensory properties of herbal tea formulated from moringa and stevia leaf

(Lee and Chambers, 2007), Attributes without symbol indicators were developed by the panel.

Table 4:-One-way ANOVA table and summary of significance for herbal tea formulated from moringa and stevia

Sensory category	Attributes	Formulated Herbal Tea
Aroma	Grassy	NS
	Leafy	NS
	fruity	NS
	musty/ stale	NS
	tea	NS
Appearance	clear	NS
	Cloudy	NS
	Sediments	NS
	leafy extract	NS
	Tea appearance	NS
Taste	Sweetness	***
	Astringency	NS
	Grassy taste	NS
	Leafy taste	NS
	Sour	NS
	Bitter	***
Aftertaste	leafy aftertaste	NS
	sweet aftertaste	***
	astringent aftertaste	NS
	bitter aftertaste	NS
	grassy aftertaste	NS
	lingering aftertaste	NS
overall evaluation	tea/herbal aroma	NS
	Sweetness	***
	sweet aftertaste	***

*** P-value < 0.001; ** p-value < 0.01, *p-value < 0.05, NS (not significant)

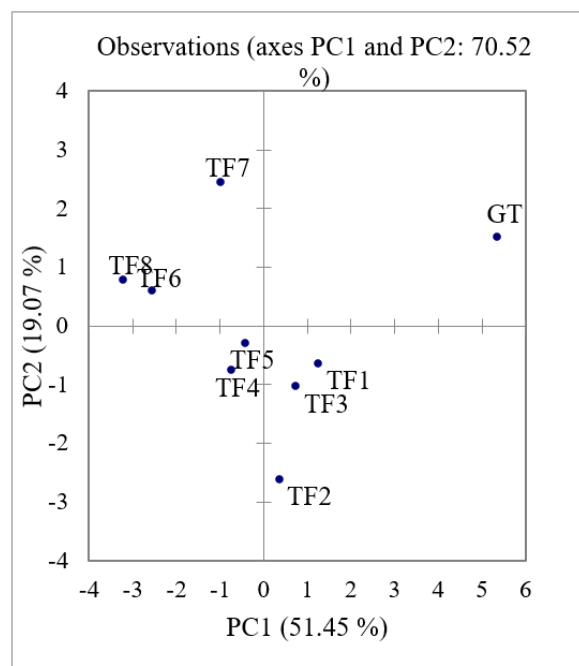
Table 5:-Descriptive sensory profile of herbal tea formulated from moringa and stevia leaf

Sensory category	Attributes	Formulations								GT
		TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8	
Aroma	Grassy	3.83 ^a	3.44 ^a	3.39 ^a	3.61 ^a	4.06 ^a	3.44 ^a	3.61 ^a	2.44 ^a	3.39 ^a
	Leafy	6.44 ^a	6.33 ^a	6.22 ^a	6.56 ^a	6.11 ^a	5.61 ^a	6.44 ^a	5.94 ^a	5.22 ^a
Appearance	fruity	2.28 ^a	1.61 ^a	1.44 ^a	1.28 ^a	1.39 ^a	1.56 ^a	1.28 ^a	1.39 ^a	2.17 ^a
	musty/ stale	0.78 ^a	0.61 ^a	0.61 ^a	0.50 ^a	0.39 ^a	0.50 ^a	0.50 ^a	0.50 ^a	1.39 ^a
	tea	2.89 ^a	2.33 ^a	2.89 ^a	3.33 ^a	3.28 ^a	3.44 ^a	2.67 ^a	4.11 ^a	2.67 ^a
	clear	5.22 ^a	5.01 ^a	5.44 ^a	5.56 ^a	5.50 ^a	4.94 ^a	4.89 ^a	5.89 ^a	6.72 ^a
	Cloudy	3.89 ^a	3.94 ^a	4.28 ^a	3.78 ^a	4.11 ^a	4.39 ^a	3.89 ^a	3.83 ^a	3.06 ^a
	Sediments	3.72 ^a	2.17 ^a	2.56 ^a	2.50 ^a	2.83 ^a	3.39 ^a	4.67 ^a	3.22 ^a	2.83 ^a
	leafy	3.83 ^a	3.39 ^a	3.78 ^a	3.33 ^a	4.11 ^a	3.72 ^a	3.67 ^a	2.94 ^a	4.44 ^a
	extract	3.67 ^a	4.22 ^a	3.94 ^a	4.83 ^a	4.44 ^a	4.89 ^a	4.11 ^a	5.89 ^a	5.11 ^a
Taste	Tea appearance	3.67 ^a	4.22 ^a	3.94 ^a	4.83 ^a	4.44 ^a	4.89 ^a	4.11 ^a	5.89 ^a	5.11 ^a
	Sweetness	1.39 ^c	2.50 ^{de}	3.33 ^{ce}	5.44 ^{bc}	4.61 ^{bc}	7.00 ^{ab}	7.39 ^{ab}	7.83 ^a	3.44 ^{cde}
	Astringency	4.61 ^a	5.22 ^a	4.44 ^a	5.00 ^a	5.17 ^a	4.28 ^a	4.89 ^a	4.61 ^a	5.89 ^a
	Grassy taste	6.28 ^a	5.67 ^a	5.50 ^a	6.11 ^a	6.33 ^a	6.39 ^a	5.28 ^a	6.28 ^a	5.11 ^a
	Leafy taste	3.06 ^a	3.39 ^a	3.28 ^a	3.06 ^a	3.44 ^a	3.39 ^a	2.56 ^a	3.06 ^a	3.22 ^a
	Sour	1.72 ^a	1.50 ^a	1.78 ^a	1.50 ^a	1.89 ^a	1.61 ^a	1.83 ^a	1.72 ^a	2.78 ^a
Aftertaste	Bitter	4.06 ^{ab}	2.50 ^b	2.89 ^b	2.78 ^b	2.67 ^b	1.94 ^b	2.44 ^b	2.06 ^b	7.06 ^a
	leafy	5.67 ^a	5.22 ^a	5.00 ^a	5.39 ^a	5.94 ^a	5.72 ^a	5.50 ^a	5.39 ^a	3.94 ^a
	aftertaste	5.67 ^a	5.22 ^a	5.00 ^a	5.39 ^a	5.94 ^a	5.72 ^a	5.50 ^a	5.39 ^a	3.94 ^a
	sweet	2.83 ^{bc}	2.28 ^c	3.50 ^{bc}	4.39 ^{abc}	4.94 ^{ab}	6.61 ^a	6.61 ^a	6.50 ^a	3.44 ^{bc}
	aftertaste	2.83 ^{bc}	2.28 ^c	3.50 ^{bc}	4.39 ^{abc}	4.94 ^{ab}	6.61 ^a	6.61 ^a	6.50 ^a	3.44 ^{bc}
	astringent	4.94 ^a	4.17 ^a	4.56 ^a	4.33 ^a	4.72 ^a	4.06 ^a	4.39 ^a	3.83 ^a	4.78 ^a
	aftertaste	4.94 ^a	4.17 ^a	4.56 ^a	4.33 ^a	4.72 ^a	4.06 ^a	4.39 ^a	3.83 ^a	4.78 ^a
	bitter	3.44 ^a	2.83 ^a	3.61 ^a	3.11 ^a	2.83 ^a	3.00 ^a	3.00 ^a	2.22 ^a	5.39 ^a
	aftertaste	3.44 ^a	2.83 ^a	3.61 ^a	3.11 ^a	2.83 ^a	3.00 ^a	3.00 ^a	2.22 ^a	5.39 ^a
	grassy	2.72 ^a	2.78 ^a	3.06 ^a	2.72 ^a	3.33 ^a	3.11 ^a	3.06 ^a	1.94 ^a	3.56 ^a
overall evaluation	aftertaste	2.72 ^a	2.78 ^a	3.06 ^a	2.72 ^a	3.33 ^a	3.11 ^a	3.06 ^a	1.94 ^a	3.56 ^a
	lingering	6.39 ^a	4.94 ^a	5.44 ^a	5.50 ^a	6.44 ^a	6.78 ^a	6.89 ^a	6.61 ^a	5.94 ^a
	aftertaste	6.39 ^a	4.94 ^a	5.44 ^a	5.50 ^a	6.44 ^a	6.78 ^a	6.89 ^a	6.61 ^a	5.94 ^a
tea/herbal	3.94 ^a	3.67 ^a	3.78 ^a	4.78 ^a	4.50 ^a	5.50 ^a	4.94 ^a	5.78 ^a	4.11 ^a	
aroma	3.94 ^a	3.67 ^a	3.78 ^a	4.78 ^a	4.50 ^a	5.50 ^a	4.94 ^a	5.78 ^a	4.11 ^a	
Sweetness	1.50 ^d	2.89 ^{cd}	3.67 ^{bcd}	4.78 ^{ab}	4.33 ^{bc}	6.94 ^{ab}	5.83 ^a	6.67 ^a	3.44 ^{cd}	
sweet	2.11 ^c	2.89 ^{de}	3.83 ^{cde}	4.67 ^{abcd}	4.17 ^{cde}	6.28 ^{ab}	5.61 ^{abc}	6.61 ^a	3.33 ^{cde}	
aftertaste	2.11 ^c	2.89 ^{de}	3.83 ^{cde}	4.67 ^{abcd}	4.17 ^{cde}	6.28 ^{ab}	5.61 ^{abc}	6.61 ^a	3.33 ^{cde}	

Values are Mean ± standard deviation (n=2). Values in a row with different letters in superscript are significantly different (p<0.05).

Where TF1(100% moringa), TF2 (95% moringa and 5% stevia), TF3 (90% moringa and 10% stevia), TF4 (85% moringa and 15% stevia), TF5 (80% moringa and 20% stevia), TF6(75% moringa and 25% stevia), TF7(70% moringa and 30% stevia), TF7(65% moringa and 35% stevia) and GT (green tea).

A



B

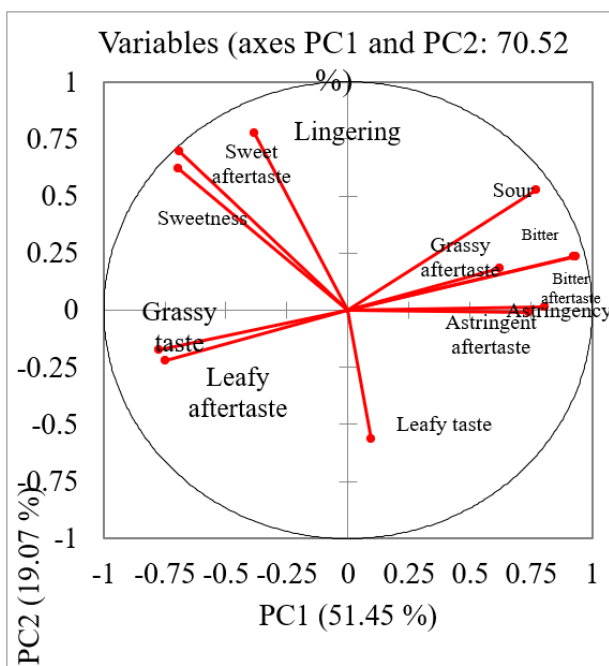


Figure 2:-Principal component analysis of herbal tea with different formulations and their taste, Aftertaste and overall evaluation sensory quality

A: Tea formulations: TF1(100% moringa), TF2 (95% moringa and 5% stevia), TF3 (90% moringa and 10% stevia), TF4 (85% moringa and 15% stevia), TF5 (80% moringa and 20% stevia), TF6(75% moringa and 25% stevia), TF7(70% moringa and 30% stevia), TF8(65% moringa and 35% stevia) and GT (green tea).

B: PCA Loadings: Sweetness, sweet aftertaste, lingering, sour, grass after taste, bitter, bitter after taste, astringency, astringency after taste, grassy taste, leafy taste, and leafy after taste.

4. Conclusions

This study to investigate the sensory and chemical composition of moringa (*moringa stenopetala*) leave tea with partial replacement of stevia (*stevia rebaudiana bertonii*) leaves. The result of sensory properties all the formulated herbal teas have the similar in aroma and appearance, especially herbal teas brewed from TF5, TF6, TF7 and TF8 were the most preferred in sweetness and sweetness after taste compared to that of TF2, TF3, TF4, TF1 and GT (Green tea). The blending of moringa and stevia produced herbal teas with the most appealing sensory characteristics as compared with herbal tea brewed from only moringa and green teas which are regularly consumed. The results of the antioxidants show that the herbal tea brewed from 20-35% of stevia had the highest DPPH scavenging, ferric reducing power and total antioxidant activities than the herbal tea brewed from 100% moringa herbal tea infusion. This implies that the formulated teas could have better sensory and nutraceutical benefits. It concludes that using these formulated herbal teas due to their sensory, nutraceutical, and economical advantage to the consumer rather than using moringa alone or with table sugar is evident from this work.

Based on this finding of this study the optimization of aroma and taste of moringa (*moringa stenopetala*) leave tea with partial replacement of stevia (*stevia rebaudiana bertonii*) leaves and the characterization chemical composition of dried moringa and stevia leaves, formulation of herbal teas and analyzing of their sensory properties and antioxidant (DPPH scavenging activity, ferric reducing assay power and total antioxidant) activity. However, there is a need to carry out further herbal tea composition profile using GS-MS, HPLC and UPLC to explore the potential chemicals present in the formulated herbal tea. The determination of steviol glycoside, antimicrobial activities, the commercialization and promotion of herbal tea including this product.

5. Acknowledgments: The authors would like to thanks, the Ethiopian Institute of Agricultural Research, for allowing as to pursue the work and financial support and Hawassa University for allowing us the laboratories and other facilities for doing this work.

6. Conflicts of Interests: -The authors declare that there are no conflicts of interests regarding the publication of this paper.

7. REFERENCES

Abdo, B. 2016. Sweetness equivalence ratio of Stevia (*Stevia Rebaudiana Bertonii*) with sugar adopted in Ethiopia. *IJABBR*, 4(1), 58-61.

- Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., Hayat, I., Ahmed, I. 2013. Chemical composition and sensory evaluation of tea (*Camellia sinensis*) commercialized in Pakistan. *Pak. J. Bot*, 45(3), 901- 907.
- Ahmad, U., Ahmad, R. 2018. Nutritional, Physicochemical and Organoleptic Evaluation of Low-Calorie Muffins Using Natural Sweetener Stevia (*Stevia rebaudiana* Bertoni). *Journal of Nutrition & Food Sciences*, 8(673), 2.
- Alakali, J., Ismaila, A., Alaka, I., Faasema, J., Yaji, T. 2016. Quality evaluation of herbal tea blends from ginger and *Pavetta crassipes*. *European Journal of Medicinal Plants*, 1-8.
- Azadi Gonbad, R., Afzan, A., Karimi, E., Sinniah, U.R., Kumara Swamy, M. 2015. Phytoconstituents and antioxidant properties among commercial tea (*Camellia sinensis* L.) clones of Iran. *Electronic Journal of Biotechnology*, 18(6), 433-438.
- Brand-Williams, W., Cuvelier, M.-E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30.
- Chew, K., Khoo, M., Ng, S., Thoo, Y., Aida, W.W., Ho, C. 2011. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. *International Food Research Journal*, 18(4), 1427.
- De-Heer, N.E.A. 2011. Formulation and sensory evaluation of herb tea from *Moringa oleifera*, *Hibiscus sabdariffa*, and *Cymbopogon citratus*. *Journal of Ghana Science Association*, Vol. 15 No. 1, 2011.
- Einstein, M.A., 1991. Descriptive techniques and their hybridization. In: Lawless HT, Klein BP, Editors. Sensory science theory and applications in foods. New York: Marcel Dekker; pp. 317-338.
- Gallo, M., Vinci, G., Graziani, G., De Simone, C., Ferranti, P. 2013. The interaction of cocoa polyphenols with milk proteins studied by proteomic techniques. *Food research international*, 54(1), 406-415.
- Gasmalla, M.A.A., Yang, R., Amadou, I., Hua, X. 2014. Nutritional composition of *Stevia rebaudiana* Bertoni leaf: effect of drying method. *Tropical Journal of Pharmaceutical Research*, 13(1), 61-65.
- Glærum, N. 2012. Usage and acceptance of *Moringa stenopetala* in the diet in Ethiopia, Universitetet i Agder; University of Agder
- Goyal, S., Samsher, G.R., Goyal, R. 2010. Stevia (*Stevia rebaudiana*) a bio-sweetener: a review. *International Journal of Food Science Nutrition*, 61(1), 1-10.
- Gupta, E., Purwar, S., Sundaram, S., Rai, G. 2013. Nutritional and therapeutic values of *Stevia rebaudiana*: A review. *Journal of Medicinal Plants Research*, 7(46), 3343-3353.
- Habtemariam, S., Varghese, G. 2015. The extractability of rutin in herbal tea preparations of *Moringa stenopetala* leaves. *Beverages*, 1(3), 169-182.
- Hamza, T.A., Azmach, N.N. 2017. The miraculous moringa trees: From nutritional and medicinal point of views in tropical regions. *Journal of Medicinal Plants Studies*, 5(4), 151-162.
- Hicks, A. 2009. Current status and future development of global tea production and tea products. *Austral Journal*, 2009, 12.
- Horžić, D., Komes, D., Belščak, A., Ganić, K.K., Iveković, D., Karlović, D. 2009. The composition of polyphenols and methylxanthines in teas and herbal infusions. *Food chemistry*, 115(2), 441-448.
- Huang, Y.L., C.C. Chen, Y.J. Chen, R.L. Huang, and B.J. Shieh. 2001. Three xanthenes and a benzophenone from *Garcinia man gostana*. *J. Nat. Prod.* 64: 903–906.
- Ihekoronye, A.I. and P.O. Ngoddy, *Integrated food science and technology for the tropics*. 1985: Macmillan.
- Ilyas, M., Arshad, M., Saeed, F., Iqbal, M. 2015. Antioxidant potential and nutritional comparison of *Moringa* leaf and seed powders and their tea infusions. *Journal of Animal and Plant Sciences*, 25(1), 226–233.
- Kassahun, B.M., Kebede, W., Gebremeskel, H., Zigene, Z.D. 2012. Performance of *Stevia (Stevia Rebaudiana Bertoni)* for morphological and economic traits under different ecologies of Ethiopia.
- Kazimierczak, R., Hallmann, E., Rusaczonok, A., Rembialkowska, E. 2015. Polyphenols, tannins and caffeine content and antioxidant activity of green teas coming from organic and non-organic production. *Renewable Agriculture and Food Systems*, 30(3), 263-269.
- Killedar, S.G., Pawar, A.V., Suresh Killedar, C. 2017. Preparation of Herbal Tea from Mulberry Leaves. *Journal of Medicinal Plants*, 5(2), 325-328.
- Koh, G.Y., Chou, G., Liu, Z. 2009. Purification of a water extract of Chinese sweet tea plant (*Rubus suavissimus* S. Lee) by alcohol precipitation. *Journal of agricultural and food chemistry*, 57(11), 5000-5006.
- Kumar, S., Pandey, A.K. 2013. Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, 2013.
- Le, K., Chiu, F., Ng, K. 2007. Identification and quantification of antioxidants in *Fructus lycii*. *Food Chemistry*, 105(1), 353-363.
- Lee, J., Chambers, D.H. 2007. A lexicon for flavor descriptive analysis of green tea. *Journal of Sensory Studies*, 22(3), 256-272.
- Lim, Y., Lim, T., Tee, J. 2007. Antioxidant properties of several tropical fruits: A comparative study. *Food chemistry*, 103(3), 1003-1008.

- Mingarro, D., Acero, N., Llinares, F., Pozuelo, J., Mera, A.G., Vicenten, J., Morales, L., Alguacil, L., Perez, C. 2003. Biological activity of extracts from *Catalpa bignonioides* Walt. (Bignoniaceae). *Journal of Ethnopharmacology*, 87, 163-167.
- Munoz, A.M. and Civille, G.V. 1998. Universal, product, and attribute specific scaling and the development of common lexicons in descriptive analysis. *Journal of Sensory Studies*, 13(1), 57-75.
- Omogbai, B.A., Ikenebomeh, M. 2013. Microbiological characteristics and phytochemical screening of some herbal teas in Nigeria. *European Scientific Journal*, ESJ, 9(18).
- Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, 269(2), 337-341.
- Ravikumar, C. 2014. Review of herbal teas. *Journal of Pharmaceutical Sciences and Research*, 6(5), 236.
- Safdar, N., Sarfaraz, A., Kazmi, Z., Yasmin, A. 2016. Ten different brewing methods of green tea: comparative antioxidant study. *Journal of Applied Biology & Biotechnology* Vol, 4(03), 033-040.
- Sánchez - Moreno, C., Larrauri, J.A., Saura - Calixto, F. 1998. A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, 76(2), 270-276.
- Teye, E., Owusu, P.F., Darko, R.O., Ackah, F.K. 2017. Evaluation of composite tea made from roselle (*Hibiscus sabdariffa*), ginger (*Zingiber officinale*) and turkey berry (*Solanum torvum*). *African Journal of Food and Integrated Agriculture*, 1(1), 39-44.
- Wharf, C., & Kingdom, U. 2010. Glossary on herbal teas, 44(July), 1-5.
- Yildirim-Elikoglu, S., Erdem, Y.K. 2018. Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. *Food reviews international*, 34(7), 665-697.