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Phytochemical, Antioxidant and Spectral Characteristics of Moringa Herbal Tea Processed Using Different Methods

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Abstract

The study examined the effect of processing methods on the phytochemical, antioxidant and the spectral characteristics of Moringa herbal tea. Fresh *Moringa oleifera* leaves were processed into moringa herbal tea using four different methods namely; Oven-dried (OD), Blanched-Oven-dried (BOD), Oxidised-Oven-dried (OOD) and solid-state Fermented Oven-dried (FOD). Phytochemical analysis consists of total phenolic, total flavonoids, saponins and alkaloids. Analysis revealed that total phenolic content was in the range of 2.278±0.041 mg/mL GAE to 1.745±0.081 mg/mL GAE. The total flavonoids were in the range of 2.93±0.001 mg/mL QAE to 2.582±0.008 mg/mL QAE. Alkaloids were estimated as follows: 22.350±0.891 % DW to 8.83±0.72 % DW and saponins, 7313±0.272 % to 2.94±0.088% DW. Antioxidant activities reveals hydrogen peroxide assay in the range of 3.315 ± 0.001% to 5.103 ± 0.004% and total and the ferric reducing/antioxidant power (FRAP) assay was in the range of 4.836± 0.067% to 7.173 ± 0.010% of AAE. Spectral findings revealed that the λ-max of herbal tea samples were in the range of 380-396 nm. Major observed FTIR frequencies were 3280.1 cm^{-1}, 2918.5 cm^{-1}, 2847.7 cm^{-1}, 1606.5 cm^{-1} and 1025.0 cm^{-1}, corresponding to; amides, alcohol, alkene, aldehydes, aromatic amines, and esters. Infusion time had no effect on the UV-Visible spectral profile of tea samples, while the processing method affected the spectral profiles for both FTIR and UV-Visible spectral profiles. These spectroscopic methods have shown to be fast, efficient and highly sensitive, they require little sample preparation, and are usually environmentally friendly.

Keywords: FTIR, UV-Visible, Characteristics, Phytochemical, Moringa herbal tea

Introduction

Tea beverages are increasingly popular health refreshments. In addition to the function of thirst satisfaction, tea beverages have many nutritional and health functions.[1]. Due to their numerous bioactive components, such as catechins, polyphenols, flavonoids, vitamins and more, tea possess many health benefits like anti-oxidants, anti-bacterial and anti-inflammation [2]. Tea is usually made from the leaves, buds, or tender stems of the plants of the genus Camellia. For some time now, the demand for herbal tea has been on the rise [3]. Herbal teas contain no *Camellia sinensis*; rather they are a combination of herbs, spices, botanicals, and natural flavours [4]. The moringa plant is well known in Nigeria and constitutes a domestic spices in several folk delicacies [5].

Herbal teas have reportedly been growing in popularity as the public shows interest in herbal therapies [6,7]. M*oringa oleifera* dried leaves are a great source of polyphenols flavonoids, the most bioactive compounds in herbal teas with some biological benefits like anti-inflammatory, anticancer, analgesics and local anesthetic [8,9].

Antioxidant have the potential to donate hydrogen ions to inhibit free radical formation and interrupt the propagation of autoxidation [10]. The high phenolic content, vitamin C and other known antioxidants in moringa leaves have contributed to its high antioxidant properties compared to other plant extracts [11]. Total ferric reducing antioxidant assay (TFRP) and hydrogen peroxide scavenging activity of moringa leaf extracts have been reported by different authors [12,13].

The Word Health Organisation (WHO) predicted that over 80% of the World's population is utilising herbal products for varieties of primary medical care [1]. Among herbal plants, *Moringa oleifera* is a multi-purpose herbal plant used as human nourishment and an alternative for medicinal purposes [14]. Due to the absence of distinctive aromas in most herbal teas like moringa, the combination of moringa with other herbs or the addition of artificial

fragrance or multiprocessing methods has been a common practice [15 ,16].

Recently, there has been a rapid improvement in the analysis of food samples by food analysts to detect adulteration, frauds, and authenticity of food samples. One of such methods has been the use of applied spectroscopy in food analysis [17,18]. UV-Vis spectroscopy have been used to discriminate between *Camellia sinensis* tea leaves [19]. To classify typical Chinese tea varieties, Fourier-transform infrared spectroscopy (FTIR) of tea polysaccharides (TPS) was used to study the diversity and heterozygosis between teas [20]. FTIR spectral effect have been reported in traditional Italian recipes prepared using different methods [21].

The Chinese milk scandal and the horsemeat scandal have prompted the vulnerability of the food supply system to adulteration and authenticity checks and frauds [22]. Nowadays, the most common food categories susceptible to any type of food fraud are; olive oil, fish, organic foods, milk, grains, honey and maple syrup, coffee and, spices, wine and fruit juices, and teas [22]. The scandals concerning fraudulent foods have increased the pressure on food laboratories to develop fast and reliable screening methods for the detection of food fraud. One of the most commonly used screening techniques utilised by both industry and governmental laboratories for food fraud currently is Fourier-Transform IR (FTIR). This study seeks to determine the Phytochemicals, antioxidant activities and the UV-Visible and FTIR spectral characteristics of moringa herbal tea processed using different methods.

Materials and Methods

Materials and instrument used in this study include; PASCO automated spectrophotometer (PS-2600), FTIR spectrophotometer (FTIR-8400S Shimazu Co, Ltd. Japan), distilled water, empty tea bags (60x60 mm), filter paper material, 0.25 mm pore size sieve, pH meter (OAKLON, SN:1564140), aluminium foil, Whatman Filter papers and electronic balance (Max 180 g, 0.0001 g),electric oven (GEN-LAB E8A76788), centrifuge machine (K3, 13036-14).

The reagents used include; Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one), Galic acid (3,4,5-trihydroxybenzoic acid), 2,6-dichloroindophenol Acid, Folin- Ciocalteu reagent, potassium hydroxide, sodium hydroxide, aluminium chloride, Hydrogen peroxide, phosphate buffer (pH 7.4), 1% $K_4Fe(CN)_6$, 0.1% $FeCl_3$, 10% TCA and ascorbic acid. All chemicals and solvents used in the study were of analytical grade

Sample collection and preparation

Fresh sample of *M. oleifera* leaves was collected from a moringa farm, located at Amla village, Otukpo. Plant sample was authenticated at Botany laboratory Benue State University, Makurdi.

Fresh Samples of moringa were washed with tap water and allowed to drain properly. Subsequently, it was divided into four equal portions and processed to herbal tea powders using different methods. The first portion of the leaf was blended to reduced particle size and facilitates the drying process. It was later dried in a GEN LAB oven at 50^0C for 8 hours to a constant weight [23], (OD). The second portion was subjected to wet blanching for 3 minutes to halt polyphenol oxidase reaction before drying as in the previous sample (BODM). The third portion was first blended, in this case not just to increase the surface area for proper and faster drying, but to facilitate natural enzymatic oxidation. This sample was exposed to air (oxygen) for 18 hours for a dark colouration to be observed. This method mimics the processing of Oolong tea [24] (OOD). The fourth portion of the leaf was first processed as in OD above, and later subjected to solid-state fermentation using *saccharomyces cerevisiae (*yeast) bought from Jumia online shopping mall, as a starter for 72 hours thereafter, oven-dried at 50^0C for 8 hours (FOD). Powdered samples were packaged in air tight plastic-containers, stored at room temperature, away from direct sunlight.

Determination of phytochemicals

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC) of the various organic crude extracts spectrophotometrically in terms of Gallic acid equivalent (GAE)[25]. The total flavonoid content was determined by spectrophotometric method, using quercetin acid as standard, aluminium chloride as reagent [26] . Alkaloids and saponins were estimated gravimetrically [27,28].

Determination antioxidant Properties

The hydrogen peroxide scavenging activity of moringa leaf ethanol extract was determined spectrophotometrically at 230 nm [8]. The scavenging activity is measured in percentage with respect to change of absorbance as the antioxidant compounds in extracts oxidises hydrogen peroxide to water.

The ferric reducing power assay method is based on the principle that constituents with reduction potentials in a sample, react with potassium ferricyanide and reduces it to potassium ferrocyanide, which then reacts with ferric chloride to form ferric–ferrous complex that has absorption at 700 nm. Ascorbic acid was often used as reference standard and results expressed as percentage of ascorbic acid equivalent [29].

Determination of UV-visible spectral profiles

The UV-Visible spectral of the moringa teas were determined using a PASCO (PS-2600) automated spectrophotometer. Each tea bag containing 2 g of moringa herbal tea was immersed in five beakers of 100 mL boiling distilled water each for different time intervals, ranging from 30 to 180 seconds respectively. This procedure was repeated for all the moringa herbal tea samples and Lipton tea samples. After infusion the brews were allowed to cool to room temperature; thereafter the spectrum of each sample was determined using an

automated SPACO UV-Visible spectrometer. The system software (PASCO scientificSpectrometrySpectrometry.exe) records the solution number and the generated each spectrum accordingly.

FTIR spectral profiles

The FTIR analysis was carried out using the spectral scanning in the range: of 4000 – 650 cm^{-1}at a resolution of 8 cm^{-1}. Each sample (2 mg) was incorporated into 200 mg of KBr (spectroscopic grade) powder to be pressed into 1 mm pellets for FT-IR measurement [30]. The absorbances spectral were recorded from OMNICESPV6 software. As a first step, the FTIR spectra were analysed for the spectral band positions to identify the signatures of the major functional groups. An assignment of the main bands was carried out by analysing the acquired spectra and by comparing them with those in the literature [21]. The tea solutions of varying infusion time analysed for maximum wavelength (λ-max) of absorbance. The FTIR spectral and the different functional groups were determined from the spectral peaks [31].

Statistical analysis

Results were reported as mean values ± SD. Levels of significance was tested using one way ANOVA using the Statistical Package of Social Science (SPSS)-23 software. The Posthoc Tukey Test was used to compare the mean values among groups. P<0.05 were considered statistically significant.

Results and Discussion

Table1: Phytochemical Composition of Moringa Herbal Tea Samples

Sample	phenolics (mg/ mL GAE)	Flavonoids (mg/mL QAA)	Alkaloids (% DW)	Saponins (%DW)
OD	2.278^{a} ±0.041	2.932^{a} ± 0.001	22.350^{a}±0.891	7.313^{a} ± 0.272
BODM	2.009^{a}±0.005	2.582^{a}±0.008	10.861^{a}±1.043	7.223^{b} ± 0.735
OOD	1.234^{a}±0.144	2.870^{a}±0.043	8.688^{b}±0.704	6.158^{c}±0.336
FOD	1.745^{c}±0.081	2.939^{a}±0.02	8.82^{c}± 0.720	2.948^{d}±0.088

OD=Oven-dried moringa herbal tea powder, BODM = Blanched-oven dried Moringa herbal tea powder, OOD= Oxidised-oven dried moringa herbal tea powder, FOD= Fermented-oven dried Moringa herbal tea powder. Values represent the mean with ±SD. Means in the same column with different superscripts are significantly different at $p < 0.05$; GAE = Galic acid equivalent, QAE = Quercetin acid equivalent, DW = dry weight

Table 2: Antioxidant Assays of Moringa Herbal Teas Samples

Samples codes	H_2O_2 (%)	FRAP (% AAE)
OD	3.268^{c}± 0.556	6.250^{b} ± 0.00
BODM	3.315^{b} ± 0.001	7.173^{b} ± 0.010
OOD	5.103^{a} ± 0.004	6.255^{b} ± 0.064
FOD	3.194^{a} ± 0.024	4.836^{a} ± 0.067

OD = Oven-dried moringa herbal tea powder, BODM = Blanched-oven dried Moringa herbal tea powder, OOD= Oxidised-oven dried moringa herbal tea powder, FOD= Fermented-oven dried Moringa herbal tea powder. Values represent the mean ±SD. Means in the same rows with different superscripts are significantly different at $p < 0.05$. FRAP = ferric reducing antioxidant assay, AAE= ascorbic acid equivalent

Table 3: UV-Visible Spectral Data for Moringa Herbal tea Samples and Commercial Tea

Samples	λ-max (nm)
OD	398
BODM	396
OOD	380
FOD	396
LPT	396

Oven-dried moringa (OD), Blanched-Oven-dried moringa (BODM), Oxidised-Oven-dried (OOD), Fermented-oven-dried (FOD), commercial tea (LPT), Maximum wavelength of Absorbance (λ-max)

Table 4: FTIR Spectral Data of Moringa Herbal Tea Samples and Commercial Tea

Group frequency (cm^{-1})	Functional Group	Observed Frequencies (cm^{-1})					Assignment
		OD	OOD	FOD	BODM	LPT	
3700-3500	Amide	3693.8	-	3675.2	3679.1	-	N-H
3500-3100	Alcohols and phenols	3280.1	3272.6	3280.1	3280.1	3265.1	Hydrogen bonded OH group
3000-2850	Alkanes	2918.5 2847.7	2918.5 2851.4	2918.5 2847.7	2914.8 2847.7	2922.2 2851.4	H-C-H Asymmetric stretch
2260-2100	Alkyne	2161.9	-	2109.7	-	-	C≡C
1710-1665	aldehydes	1606.5	-	1733.2	1733.2	-	C=O
1680-1640	alkene	1606.5	-	1621.4			C=C
1550-1475	nitro	1408.9	1595.3	-	1375.4	1513.3	N-O Asymmetric Stretch
1470-1350	Alkanes	-	1408.9	1420.1 1375.4	1420.1	1446.2	C-H bent
1335-1250	Aromatic amines	1315.8 1233.7	1319.5	-		1364.2 1319.5	C-N
1250-1020	Aliphatic amines	1025.0	-	1233.7	101.1	1144.3 1025.7	C-N Stretch
1300-1000	Esters	1025.0 1233.7	1237.5 1010.1	1013.8 -	101.1	1606.5	C-O
900-675	Aromatics 719.4		-	-	719.4	820.0	C-H out of plane deformation

Oven-dried moringa (OD), Blanched-Oven-dried moringa (BODM), Oxidised-Oven-dried (OOD), Fermented-oven-dried (FOD), and commercial tea (LPT)

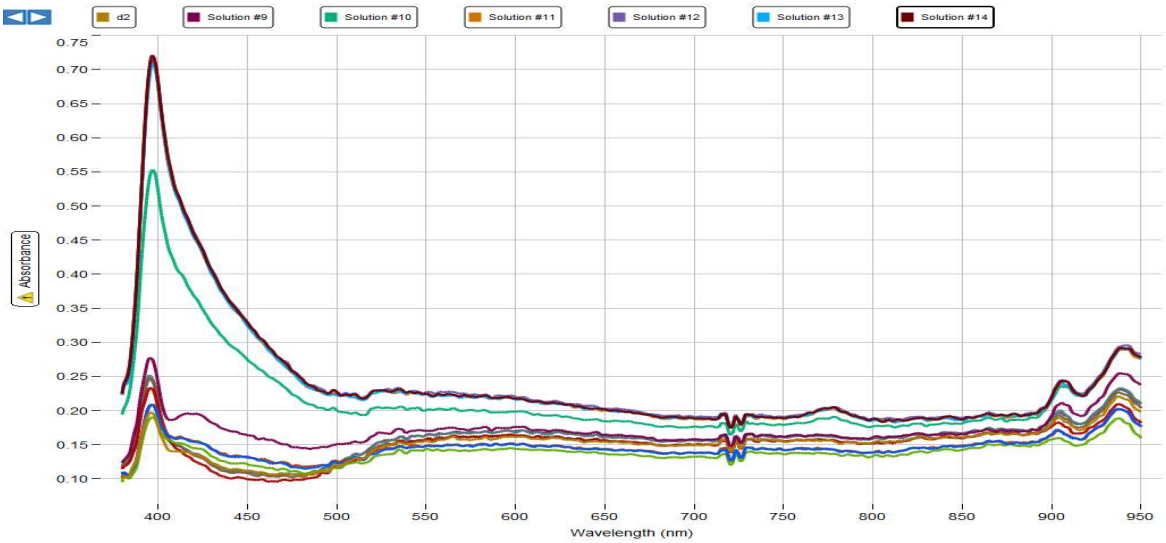

Figure 1: UV-Visible Spectral Profile of Oven-dried Moringa Herbal Tea

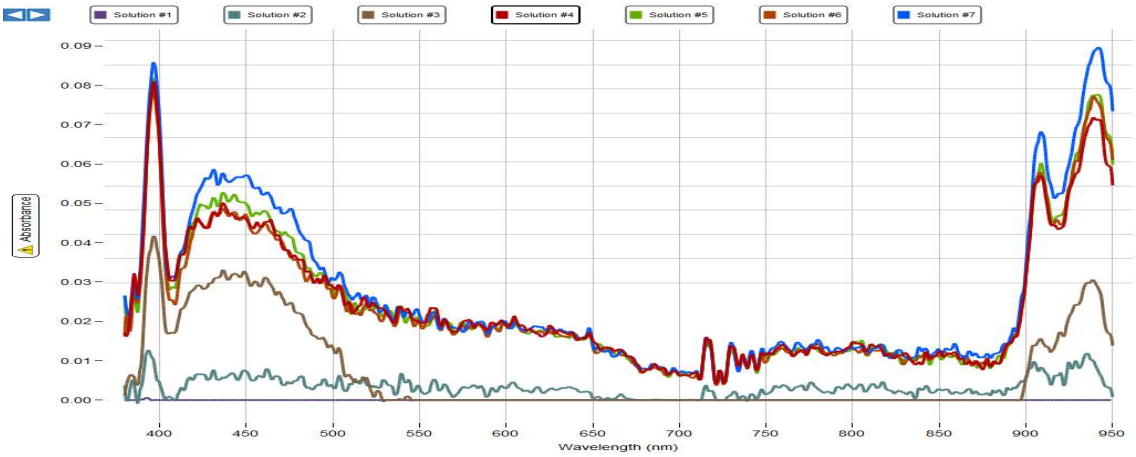

Figure 2: UV-Visible Spectral Profile for Fermented-Oven-dried Moringa Herbal Tea

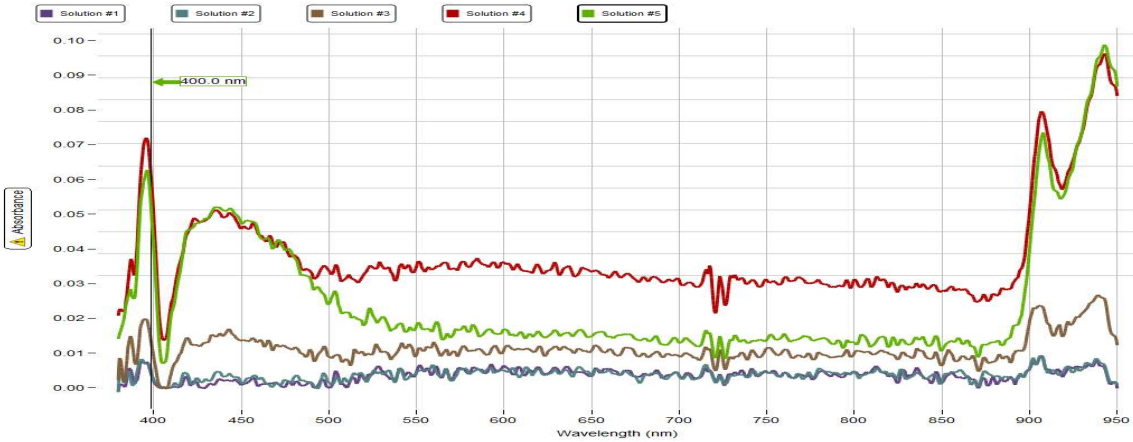

Figure 3: UV-Visible Spectral Profile of Commercial Tea

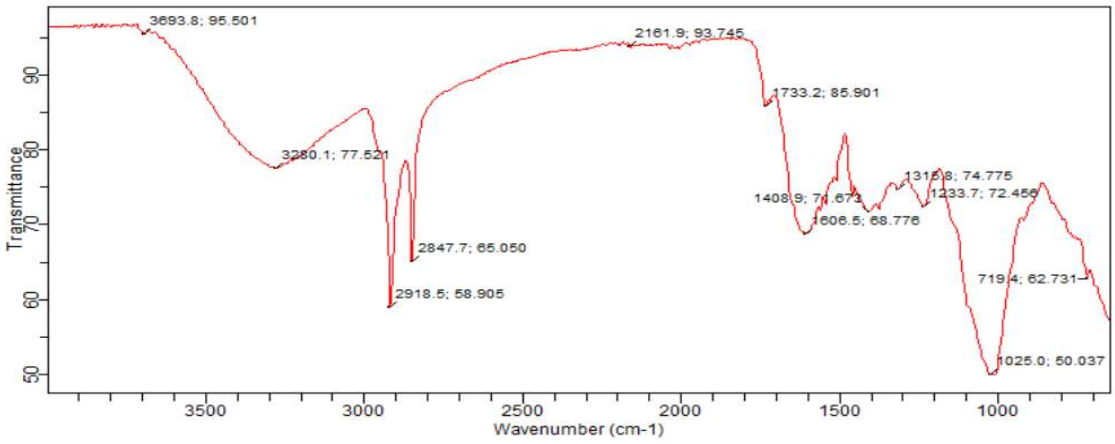

Figure 4: FTIR Spectrum of Oven-dried Moringa Herbal Tea (OD)

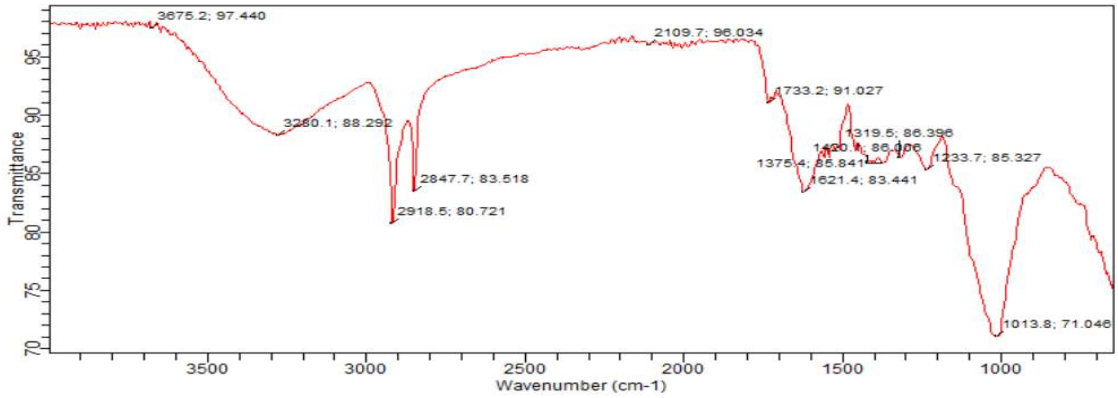

Figure 5: FTIR Spectrum of Fermented-Oven-Dried Moringa Herbal Tea (FOD)

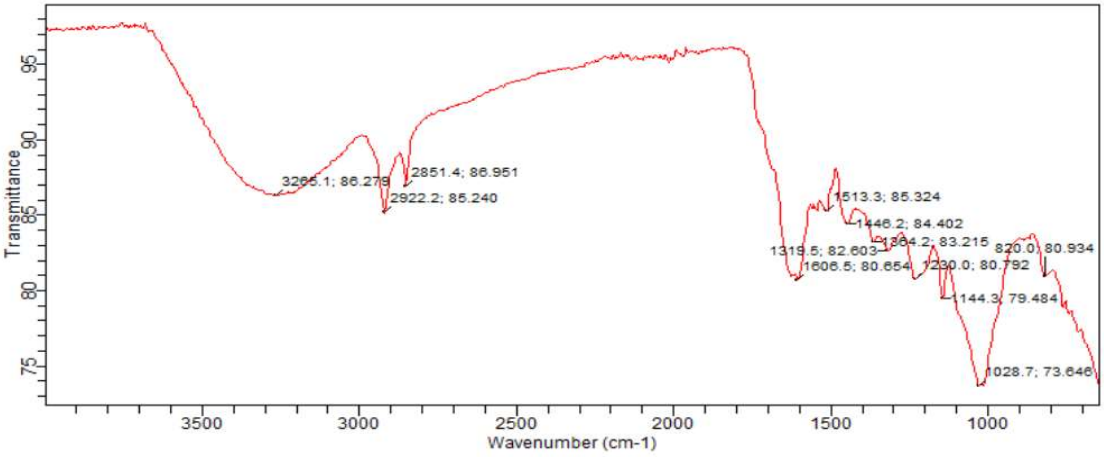

Figure 6: FTIR Spectrum of a Commercial Tea (LPT)

Phytochemical composition of moringa herbal tea samples

The results of the phytochemical estimation of the various moringa tea powders are presented in Table 1. Processing methods have a significant role in the phenolic contents. No significant effect was observed for the flavonoids contents. The amount of total flavonoids and phenolic content of the herbal tea was higher than that of other herbal teas like hibiscus tea, but lower than that of commercial green tea [32]. The alkaloids and saponins in sample OOD and FOD could be attributed to oxidation and solid state fermentation. It has earlier been reported in literature that processing methods like wet blanching and drying methods have an effect on the chemical composition of moringa leaves [11].

Antioxidant properties of moringa herbal tea samples

The ferric reducing or the antioxidant power (FRAP) assay and the hydrogen peroxide antioxidant activities of the moringa teas samples vary significantly with the processing methods Table 2. Lowest FRAP was recorded in the BODM sample; this may be due to the effect of the sample treatments. In the reducing power assay, the presence of reductants or antioxidants in the samples resulted in the reduction of Fe^{3+} to Fe^{2+} by donating an electron [12]. H_2O_2 is a reactive oxygen species produced in the body, toxic to the body tissues event at low concentration of 10 µM. Phenolic compounds or other antioxidants present in samples were able to donate electrons to hydrogen peroxide, neutralizing it to water.

Spectral characterisation of moringa herbal tea samples

The study established that processing methods affected the UV-Visible spectral profile of Moringa herbal teas. The infusion time have no effect on the spectral profiles of herbal teas. The λ-max of moringa herbal teas differs slightly from one sample to another. The λ-max was in the range of 380 to 396 nm. The λ-max of commercial tea was observed to be 396 nm similar to that of the FOD sample. Despite the colour variation of the samples, the UV-Visible spectral has some similar features. A maximum wavelength (λ-max) was closed to each other. LPT also exhibited a similar spectrum. A lower peak was observed between 450 to 500 nm for LPT. For FOD sample, second amplitude was observed at 430 to 470 nm. OOD sample has its peculiar second amplitudes at a wave

length range of 550 to 650 nm. The variation in spectral observation can be attributed to the different processing methods of the herbal tea. This implies that UV-Visible spectral can be used to distinguish between Moringa samples prepared with different methods, discriminate between different tea types, and also screen for adulterations or fraud. Similar reports have stated that this suggested analytical method could serve as an alternative to expensive methods that require monotonous sample preparation [33,34]. Underlined advantages of the method were simplicity, little time, and low cost [35]. Report has earlier stated that spectroscopic methods were useful in analysis of simultaneous processes that require in-time response [36].

The FTIR spectral varied slightly from each other based on the signal strength of various functional groups present (Table 4). All the moringa samples have a similar fingerprint region on the FTIR spectrum. Broad peaks were observed at 3280.1 cm^{-1}, corresponding to OH functional group for all the moringa herbal teas. In LPT, the OH peak was observed at 3265.1 cm^{-1} at 86.279 transmittances. The double peaks observed in between 3000 cm -1 to 2500 cm^{-1} are a characteristic of the alkane functional group. Most the observer frequencies were 3280.1 cm^{-1}, 2918.5 cm^{-1}, 2847.7 cm^{-1}, 1606.5 cm^{-1} and 1025.0 cm^{-1}. Functional groups include alcohols, amides, alkanes, aldehydes, aromatic amines and, esters. Processing methods affected the FTIR spectral profile of the Moringa herbal teas, but the fingerprint region of the herbal teas was different from that of commercial tea. Observed frequencies of 3675.2 cm^{-1}, 369.8 cm^{-1} and 3679.1 cm^{-1} assigned to amide functional group were observed in FOD, OD, and BODM. This could be attributed to processing methods and tea variety. This suggests that FTIR can be used to rapidly discriminate between sample tea types, detect adulterations and deceptions in tea samples. Discrimination of tea varieties by FTIR has been reported in literature with similar advantages [20]. It is a fast, easy, and generally, cost-effective method to detect food adulteration. Researchers have also reported that FTIR can provide information about the geographical origin of certain foods which can be useful for investigating authenticity [22]. FTIR methods have also been applicable in the quality evaluation of Java tea from different regions, discriminatory capacity of FTIR was noticed relating tea quality to cultivated regions [37]. The effect of preparation, cooking methods and time of cooking was observed in FIRT analysis of some Italian traditional recipes. According to the authors, with FTIR a clear-cut classification of the food groups was possible [21].

Conclusion

Processing methods have a significant role in the phytochemical composition and the antioxidant activity of moringa herbal tea. Processing methods also have an effect on the UV-Visible and FTIR spectral profile of moringa herbal teas. Spectral data shows that irrespective of the processing methods, the λ-max of samples falls within rang (380 to 390 nm). The fingerprint regions of the moringa herbal teas were alike but different from that of commercial tea. Fourier Transform Infrared (FTIR) spectroscopic methods can be applied to food products and have shown to be fast, non-destructive, and highly sensitive, to require little or no sample preparation, and usually not to require the use of harmful chemical agents. In addition, FTIR methods also allow for the fingerprinting of food products based on their overall composition and present excellent repeatability.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest

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