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Research Article

Green Synthesis and Characterization of ZnO Nanoparticles for Origin Traceability of Kenyan Camellia Sinensis var. Assamica (Black Tea) using XRD, UV–Vis, and FTIR Spectroscopy

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Abstract

Kenya is one of the largest exporters of black tea, which contributes to about 26% of the country's annual export earnings. Kenya's tea market, however, is faced by a challenge of counterfeit tea products, which compromise its credibility, causing loss of revenues in the sector. The aim of this study was to develop a fingerprint model for origin traceability of Kenyan tea based on Principal Component Analysis (PCA) of the spectroscopic data of nanoparticles and aqueous extracts obtained from twenty-one tea leaves sampled from seven tea-growing regions in Kenya. The tea extracts was used to synthesize Zinc oxide, via green synthesis. The synthesized nanoparticles were characterized with an X-ray diffractometer (XRD), Fourier Transform Infrared Spectrophotometer (FTIR) and Ultra violet visible (UV-Vis). The FTIR spectral data showed strong peaks of O-H (3100-3400cm-1), C=O (1590-1619 cm-1), C=C (1510-1450cm-1), C-H (2850-2950cm-1), attributed to phenols, catechins, flavonoids, carbonyl from theaflavins, lignin in the tea extracts. The XRD spectrum revealed the wurtzite, ZnO, nanoparticles in all the samples. The crystal size of the nanoparticles was obtained via the Scherrer formula. The data obtained from FT-IR analysis was the analyzed to obtain a PCA scatter plot. The UV-Vis spectra showed that the ZnONP absorbed in the 365-400 nm region. The variance in PCA scatter plot varied from 78.59,84.27 and 88.09 % for FTIR,UV-Vis and XRD respectively. In conclusion, the PCA models from nanoparticle samples (XRD, FTIR, and UV Vis) had the highest variability indicating that the use of NPs spectral data resulted in superior origin traceability models for the Kenyan teas. The novel technique was therefore effective in geographical traceability of Kenyan tea.

Keywords: Nanotechnology, Authentication, Kenyan, Tea, Traceability, Principal Components, Clustering

1. Introduction

Tea is one of the most widely consumed agricultural beverage products, having been enjoyed for thousands of years worldwide [1]. The beverage initially originated from China and is now grown in many parts of the world. Kenya contributes 8 % of the tea, mainly black tea (*Camellia sinesis-C.assamica*), while China leads with 36 % [2]. Tea comes in various categories that include white, Oolong, Puerh, Green, Black and Yellow, with green and black tea being majorly consumed [1]. Generally, black tea is obtained from fresh leaves of Camellia sinensis strains, and the manufacturing process involves withering, rolling, fermentation, drying and sorting [3]. During the manufacturing process, some compounds are newly formed, such as theaflavins and thearubigins, which contribute to the tea colour and the taste [1]. Other metabolites such as catechins, alkaloids, polyphenols, flavonoids and polyphenols are also associated with the flavour of black tea [1]. These phytochemicals in tealeaves are used as capping and reducing agents in synthesizing nanoparticles.

Origin are important factors in identifying tea quality and

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identity. In Kenya, most black teas are identified with their origin for example, Kericho tea from Kericho region, Fahari, Gold, Eden, Melvins and Ketepa tea. Each region produced black tea with different quality depending on the climatic condition of the region, production process, sun exposure and soil type. These factors affect the concentration of phytochemicals in tea leaves. Counterfeits in the tea market has been largely experienced in both international and local level. Local counterfeits have damaged the reputation of farmers and have led to a loss of revenue in companies. At the local and international level, counterfeits in tea leaves pose a health and safety risk to consumers and an increased cost for governments. The aim of increasing their earnings has led other companies to promote their low-quality grade tea as high-grade tea from certain geographical regions by utilizing fake labelling [4]. These factors draw the attention of customers to the geographic origin of tea as key indicators of its quality.

There are various techniques used for the analysis of tea and its byproducts. Some of these are gas chromatography mass spectrometry (GC-MS), inductive coupled mass spectrometry (IC- MS), and stable isotope and metabolomics, which have been widely used to characterize tea for traceability reasons [5-7]. However, some of these methods require expensive reagents and high technical expertise that make the process economically enviable. Many studies are being conducted with an emphasis on more quick, easy, and effective methods that can offer a low-cost method for confirming the authenticity of tea by its spectroscopic characteristics and connecting this to its origin because the equipment also has high operating and maintenance costs, which make the process unmanageable for local farmers who need these services. To better highlight the difference among processed tea and their origin, IR data are subjected to PCA [8]. The PCA method has been previously used to examine sample distribution based on spectroscopic data of tea in Iran [9]. Based on the spectral position of the peaks, which is affected by elemental composition of elements the peaks position various. In tea leaves the concentration of functional group varies depending on climatic condition of the region they are obtained from [10].

In this study, spectroscopic data obtained from zinc oxide nanoparticles were considered for model development to authenticate the geographic origin of Kenyan tea because of its stability, uniformity and biocompatibility. An eco-friendly technique for synthesize of zinc oxide (ZnO), nanoparticles (NPs) using tea leaves as capping agents was applied for this study. Nanoparticles obtained were characterized with XRD, UV-Vis and FT-IR. UV-vis and FT-IR spectrophotometry to obtain the spectral data. The technique has also successfully been employed in analysis of bioactive compounds in tea. UV-Vis has been used to determine the level of caffeine in tea, and its accuracy was confirmed with high-performance liquid chromatography (HPLC) [11].

To our knowledge, this work is the first authentication effort that uses XRD, FT-IR and UV-Vis data of metal and metal oxide nanoparticles to trace the origin of tea leaves. The tea samples were all obtained from selected tea-growing regions in Kenya. The chemical precipitation method was used to synthesize nanoparticles with zinc acetate, as precursors for zinc oxide. The nanoparticles obtained were then characterized using XRD, UV-Vis and FT-IR technique.

The intensity of FT-IR peak and its functional group position rely on factors such as change in dipole moment, concentration of functional groups, and molar absorptivity [12]. In Beer-Lambart Law, absorbance is directly proportional to the concentration of species, therefore depending on the concentration of functional group present on the sample the peak intensity varies [13].

Principal Component Analysis (PCA) is a powerful statistical technique used for dimensionality reduction, feature extraction, and data visualization [14]. It simplifies large datasets by transforming them into a smaller set of linearly uncorrelated variables called principal components, while retaining as much variability as possible in the data. Scatter plot obtained from PCA has been used to distinguish rice from different countries [15,16]. The data obtained showed that the PCA could not distinguish rice samples grown in Korea and China and therefore other statistical analysis should be applied to get better results [17].

2. Materials and Methodology

For this study, a total of twenty-one samples were collected randomly from Kenya Tea Development Agency factories located in different geographical regions in Kenya as indicated in Table 1. The KTDA tea factories hence a guarantee for reliable/ unbiased data. Further representation of the sampling regions is indicated on the map of Kenya in Figure 1.

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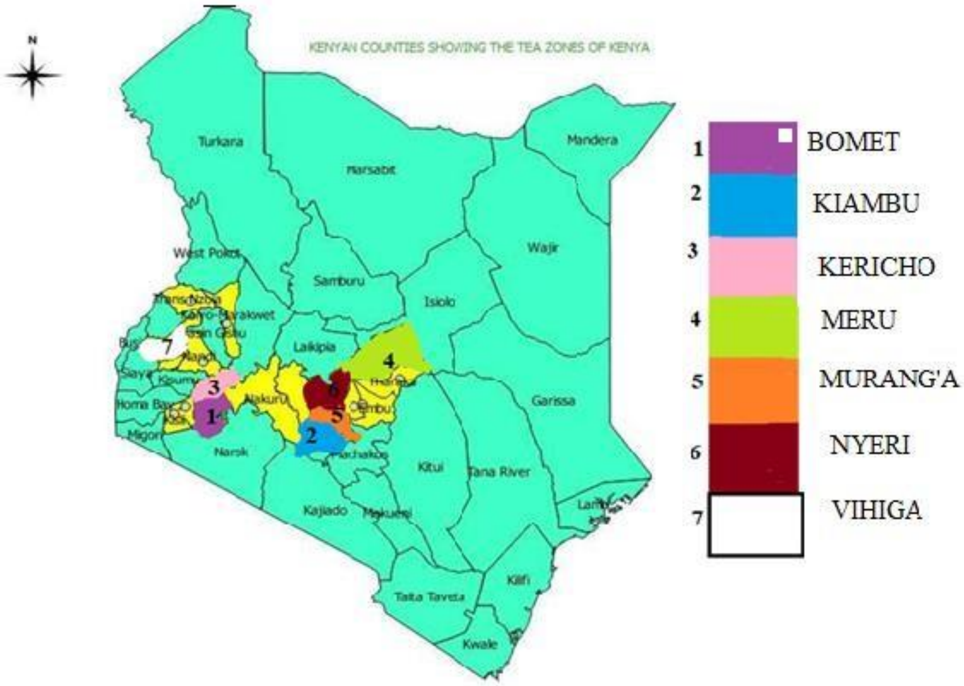

Figure 1: Tea Growing Zone Under Study.

The tea growing zones identified for tea sampling have distinct climatic conditions, coupled with different soil nutrient profiles, which greatly influence the characteristics of tea produced in the regions. Based on the varying climatic conditions and soil profile, therefore the tea leaves have metabolic differences that affect polyphenol ratios and elemental characteristics. This data is as shown in Table 1 climatic conditions of tea growing zone in Kenya.

Tea growing regions	Soil type of the regions	KTDA tea factory	Code	Altitude
Nyeri	Red volcanic soil	• Iriani • Gathuthi • Chinga • Karatina	• NYRIR • NYRGA • NYRGI • NYRKA	1800-2000
Murang'a	Red volcanic soil	• Gacharage • Kanyenyaine • Gatunguru • Makomboki	• MURGACH • MURKAN • MURGAT • MURMA	1500-2700
Meru	Red volcanic soil	• Kappa • Imenti • Weru	• MERKA • MERMA • MERWE	1500-2700
Kericho	Deep Alluvial soil	• Kerichotop • Kapkatet • Litein	• KERTOP • KERKAP • KERLIT	1800-2500
Bomet	Red volcanic soil	• Motigo • Tirgaga	• BOMO • BOTIRG	1500-2700
Kiambu	Red volcanic soil	• Mataara • Theta • Kagwe	• KIMA • KITHE • KIKA	1500-2500
Vihiga	Deep Alluvial soil	• Mundete • Nyasiogo	• VIHMU • KIRNYA	1500-2000

Table 1: Climatic Conditions of the Tea Growing Zones and Soil Type.

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3. Methods

3.1. Preparation of *Camellia Sinensis* Aqueous Extract

The extraction process was conducted by weighing 10 g of *Camellia sinensis*, which was boiled with 100 ml of deionized water for 15 minutes at a temperature of 80 ºC until the colour of water changed to light yellow. The extract obtained was filtered with Whatman grade 4 filter paper and stored for further analysis.

3.2. Preparation of 0.5 Salts

To prepare 0.5M salts, 23.625g of zinc acetate was weighed and dissolved in 100ml of deionized water in separate volumetric flasks. The salt solution obtained was then filled with deionized water to the marked neck to obtain 0.5M.

3.3. Synthesis of ZnO Nanoparticles using *Camellia Sinensis* as Capping Agent

Synthesis of ZnO nanoparticles was initiated by measuring 50 ml of Camellia sinesis extract which was mixed with 0.5M of salt solution. The solution obtained were heated for 2 hours at a temperature of 50 ºC with magnetic stirrer. The sample obtained after 2 hours were washed with analytical ethanol and dried in the Oven for 2 hours at a temperature of 90 ºC, then annealed in the furnace for 3 hours at a temperature of 500ºC ºC. The nanoparticles obtained in powder were then characterized with XRD, UV-Vis and FT-IR.

4. Results and Discussion

4.1. Characterization Techniques

X-ray diffractometer (XRD) model ARL EQUINOX 100 was used at 40Kv, 0.9mA, at a scanning range of 20°-100° and an interval time of 240 seconds to obtain the crystal structure and phase component of the synthesized nanoparticles. The data obtained was plotted in origin, analyzed. Compared to those of the standard in the Joint Committee on powder diffraction standards (JCPDS) [18]. Derby Scherer equation was used to calculate the crystal size of nanoparticles formed. Fourier transform infrared spectroscopy (FT-IR) Shimadzu model was used to determine the functional groups present in the nanoparticles. Ultraviolet visible spectroscopy evolution one plus model was used to determine the optical properties of nanoparticles.

4.2. X-ray Diffraction Analysis (XRD)

4.2.1. Zinc Oxide Nanoparticles (ZnONPs)

The XRD pattern of ZnO prepared from *Camellia sinesis* from selected regions in Kenya is shown in Figure 2. The pattern depicts a hexagonal wurtzite structure, and it matched with the Joint Committee (JCPDS card no 00-036-1451). No other phase was observed, thus confirming the formation of single-phase ZnO nanoparticles. The intensity of the peak at plane 101 was the highest; the broadening of this peak was used to estimate the crystal size using Debye Scherrer formula in equation 1. When *Camellia sinesis* from Nyeri was used as a capping agent the intensity of the peak increased and its broadness was small, confirming that the ZnONPs obtained had a relatively good crystalline structure. ZnONPs prepared using tea extracts from Vihiga region had a peak shift towards higher 2θ degrees, whereas ZNONPs from tea extracts from Bomet shift 2 theta to a lower angle. Pure ZnO or uncapped ZnONPs had the lowest peak intensity.

The broadness of the peaks varied depending on the region where the leaf extract was obtained. As observed in Figure 2b, the nanoparticles prepared with tea extract from Meru had broader peak than those from Bomet, and they both shifted to a lower angle. The broadness of the peak was smaller for samples prepared with tea extract from Nyeri and Meru. Each peak had different broadness depending on the region where the capping agents and this can be used to trace the origin of tea since they all have unique broadness. This observation was consistent with results reported by Jepngetich, et al. when *Citrus reticulata* peel extract was used to synthesize nanoparticles, the intensity of the peak increased because of good crystal structures. Figure 2b shows the intensity of the ZnO at plane 101 prepared using *Camellia sinesis* extract from selected regions in Kenya. ZnO capped with tea samples from Kiambu had major baseline shift or humps as shown in Figure 2b, which can be associated with the presence and involvement of phytochemicals such as polyphenols and caffeine.

$$D = \frac{K\lambda}{\beta_{hkl} Cos\theta} \text{........................ (Equation 1)}$$

Where K is a constant 0.9, λ is the wavelength commonly 0.15406 nm for Cu ka radiation, β is the full width at half maximum (FWHM) and is the Bragg's angle that is the half of the 2 theta value from the XRD peak.

The crystal size of all nanoparticles prepared with *Camellia sinesis* decreased when compared with uncapped ZnO nanoparticles, similar results has been reported for ZnO NPSs prepared using tea leave extract [19]. Pure ZnO had an average crystal size of 19.70 nm and the lowest average crystal size of capped ZnO was 12.93 nm for samples from Kiambu (Table 2). Tea samples from each region produced ZnO nanoparticle with unique properties indicating variables percentage composition of polyphenols and caffeine in samples.

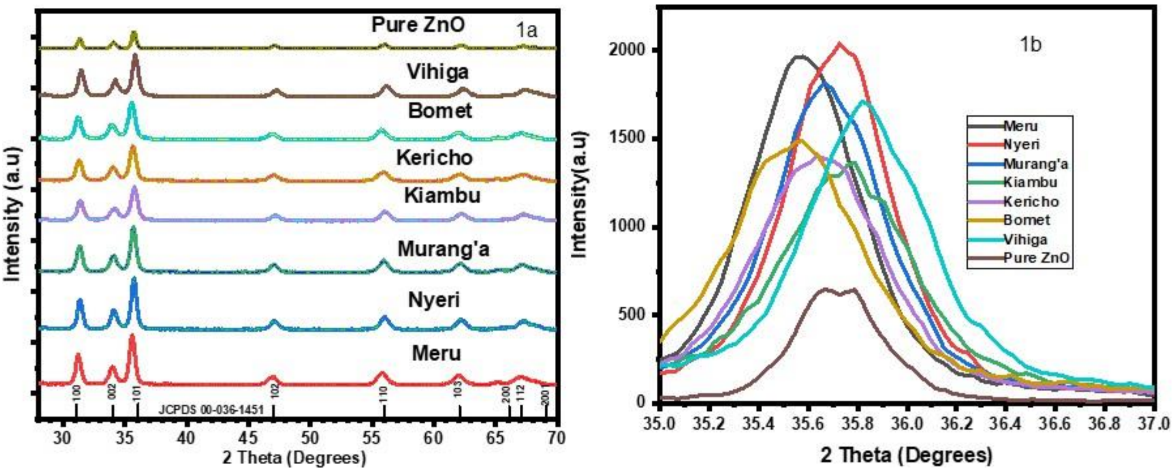

Figure 2(a): XRD Spectra of Uncapped and Capped ZnONPs, (b) Enlarged XRD Plane 101.

Table 2 shows the spectral data of zinc oxide nanoparticles synthesized from tea extracts. As was observed, there was variation in particles sizes and full half at maximum from different samples obtained from different tea growing zones.

Sample	Plane	2θ(°)	FWHM (°)	Crystal size (nm)
Pure ZnO	100	31.34	0.3937	20.96
	002	34.09	0.46290	17.95
	101	35.71	0.41216	20.2
Meru	100	31.23	0.50966	16.1
	002	33.99	0.61688	13.4
	101	35.58	0.51998	16.04
Nyeri	100	31.36	0.48752	16.92
	002	34.12	0.53684	15.48
	101	35.71	0.50691	16.4
Murang’a	100	31.33	0.54109	15.20
	002	34.08	0.61927	13.41
	101	35.67	0.54949	15.12
Kiambu	100	31.38	0.60206	13.7
	002	34.14	0.72011	11.5
	101	35.78	0.61024	13.6
Kericho	100	31.29	0.58216	14.17
	002	34.04	0.71611	11.6
	101	35.63	0.60382	13.81
Bomet	100	31.22	0.60063	13.7
	002	33.96	0.66836	12.4
	101	35.54	0.62515	13.34
Vihiga	100	31.46	0.55782	14.79
	002	34.22	0.59722	13.92
	101	35.83	0.58378	14.30

Table 2: XRD Data ZnO NPs (Crystal Size, FWHM and 2θ) Capped with Tealeaves Extracts from Selected Regions in Kenya.

4.3. UV-Vis Analysis

4.3.1. Zinc Oxide Nanoparticles

The synthesized NPs were further investigated for their UV-Vis absorption properties and pure samples of each metal oxide was used as controls. The size, morphology, phytochemical composition and synthesis method of NPs are known to affect their UV-Vis absorption spectra. [20]. In addition, the amount and type of capping agent is reported to affect the crystal size of nanoparticles [21]. For this study, the amount of capping agent and synthesis method used was the same for all NPs, but the capping agent (tea leaves extracts) used was obtained from different regions thus the difference in phytochemical components which has influence on the absorption region in the UV-Vis spectrum. Figure 5 shows the UV-vis spectra of ZnONPs synthesized using tea leaves from selected tea-growing regions in Kenya. Pure ZnO nanoparticles displayed a sharp absorption edge at wavelength around 365-400nm similar to a previous report [22]. Thus, the data observed in Figure 3 is in agreement with the standard values. The absorption intensity and absorption edge of capped ZnO nanoparticles varied depending on the region where the tea leaves were obtained. The absorption intensity of ZnONPs capped with tealeaves extracts from Murang'a region had the highest intensity of 2.7 at a wavelength of 368 nm while ZnONPs capped with sample extracts from Vihiga had the lowest absorption intensity of 0.9 at a wavelength of 374 nm. Pure ZnONPs used as the control had the lowest absorption intensity and its absorption edge shifted to a higher wavelength. The effects of capping agents can be clearly outlined from this data. The UV-Vis absorption spectra of ZnO capped with tealeaves from Kiambu, Meru and Murang'a had multiple small peaks, which suggest that these samples had multiple or wide distribution of particle sizes and aggregates [23]. The absorption edge of ZnONPs capped with tealeaves extracts from Vihiga, Nyeri and Kericho were found to shift to a higher wavelength of 374, 372 and 370 nm respectively, which were close to the value for pure ZnONPs. The ZnONPs capped with tea leaves from Bomet, and Kiambu, Meru and Murang'a absorbed at a wavelength of 367and 368, respectively, but the intensity of absorption peaks varied.

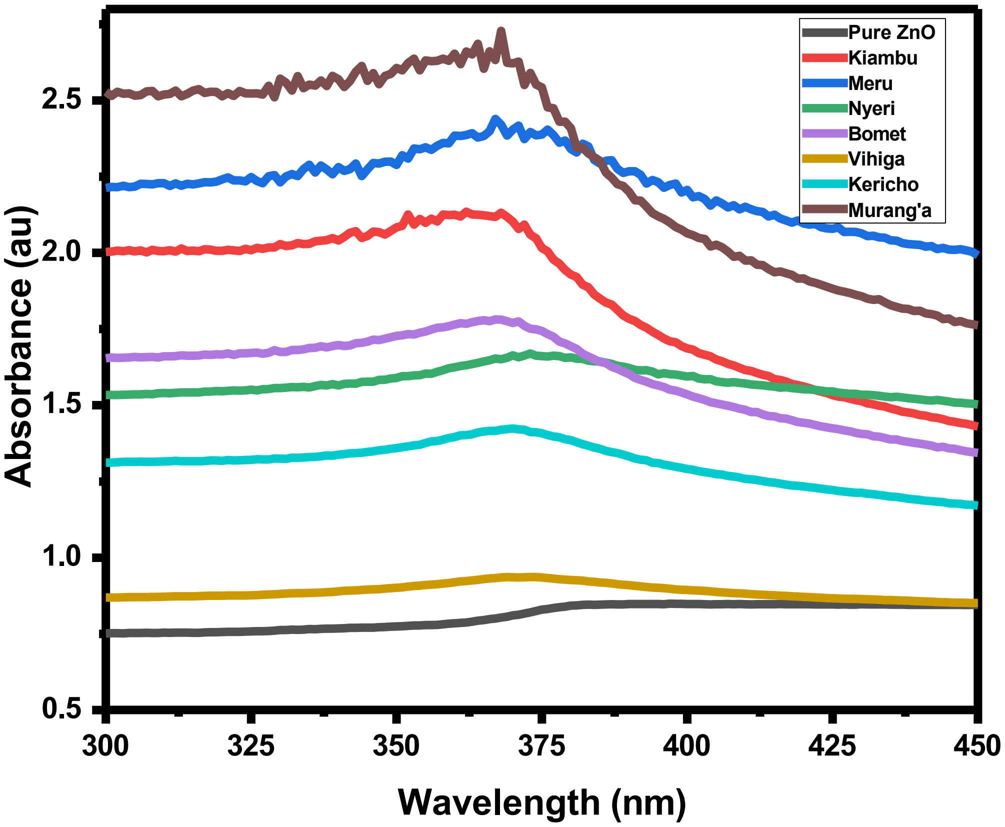

Figure 3: UV-vis Spectra of Pure and Capped ZnONPs Capped with Tealeaves Extracts from Selected Regions in Kenya.

4.4. Characterization of ZnO, using FTIR Spectroscopy

4.4.1. Characterization of ZnONPs

The NPs were further characterized using the FTIR (Figure 4) shows the FT-IR spectra of pure and capped zinc oxide nanoparticles. The samples absorbed in both the fingerprint and functional group regions. Pure or uncapped ZnO nanoparticles were used as a reference sample.

All samples absorbed at 3296 cm^{-1} except for ZnO capped with tea leaves extract from Vihiga. The absorption peaks in this wavenumber were very broad and it correspond to the O-H group of alcohol and polyphenols present in the capping agent [24]. Given that the intensity of the peaks depends on the concentration of phytochemicals present in the capping agent, this observation therefore, could imply that the Vihiga tea has distinctly unique phytochemical profile when compared to the other teas, and probably it has the lowest levels of polyphenols.

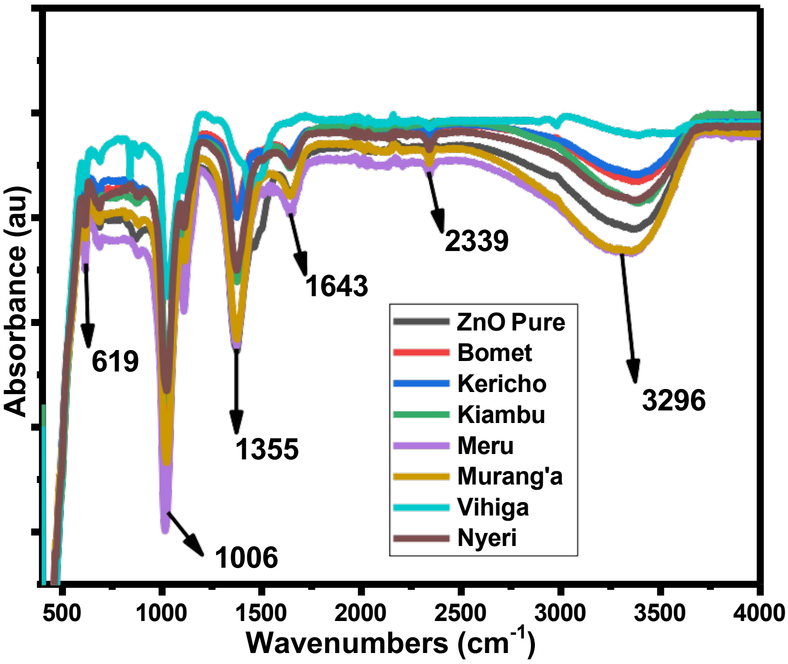

Figure 4: FTIR spectra of *Camellia Sinensis* Capped ZnONPs.

ZnONPs capped with tea leaves from Murang'a and Meru had the highest peak intensity 3296 cm-1. The peaks observed at 2339 cm-1 correspond to symmetric and asymmetric C-H stretching of methyl groups. At 1643 cm-1 the peak observed corresponds to the bending vibration of amide group of the proteins present in plant extract. This peak is less intense in all the samples but more pronounced than the peak at 1643 cm-1. A sharp peak was observed in all samples at 1355 cm-1, with the capping agent from Bomet and Kericho having similar intensity. This peak corresponds to the C-N of the amino acids or the amine group present in the phytochemicals of the capping agent. The intensity of this specific peak for the capping agent was highest for NPs capped by tea extracts from Meru and Murang'a. The peak with the highest intensity in all samples was at 1006 cm-1 that corresponds to stretching vibration of C-O of ethers and polysaccharides. At casual consideration, it can be assumed that FT-IR data of the ZnONPs cannot be an effective technique for origin tracing of the tea samples since the samples absorb in the same regions and had relatively similar peak intensities. However, the FTIR data can be made more useful by subjecting it to PCA, as it shall be discussed in subsequent sections. It was observed that when the extracts of Tea leaves from Kiambu were used as capping agents the ZnO nanoparticles absorption wavelengths tend to shift more significantly to higher wavenumbers making it easy to distinguish these teas from other samples. At the lower wavenumbers the most important peak is observed at 619 cm-1 is assigned to the stretching vibration of metal-oxide bond (Zn-O) [25].

4.5. Fingerprint Model for Origin Traceability of Kenyan Tea from Principal Component Analysis (PCA) of the Spectroscopic Data of NPs and Aqueous Extracts

4.5.1. XRD PCA Clustering

4.5.1.1. PCA Score Scatter Plot of Capped Zinc Oxide Nanoparticles (ZnONPs)

Tealeaves from twenty-one regions were used as capping agents for the synthesis of zinc oxide nanoparticles. The XRD data obtained was subjected to PCA, unsupervised technique to evaluate the difference in the properties of capped ZnONPs based on the effects of phytochemicals. Each dot in the plot represent a sample and the rectangle groups NPs with similar XRD patterns. Phytochemicals present in capping agents affects structural properties of nanoparticles, the peak intensity, peak position and crystal size thus subjecting the data to PCA is expected to result in output that helps distinguish the samples. This technique is sensible to crystalline phases, if important origin markers were present in trace amount of amorphous the data will not reflected thus the need for more than one technique to confirm. Figure 5 shows two dimensional scatter plot of the scores obtained for PC1 and PC2. A total of 88.09% variance was obtained as summarized in (Table 4.4). The colored rectangles group capped ZnONPs based on XRD pattern, the clusters vary depending on the region where the capping agent (tealeaf) used was obtained. The red rectangle belong to ZnONPs capped with tea leaves from Kericho, the cluster is located at the negative PC1 and positive PC2 and is highly distinct from the other samples making it to be easily traced. When tea samples from Kiambu and Meru were used as capping agents the PCA data cluster at the bottom right of quadrant having negative PC1 and PC2. ZnONPs capped with tea leave extracts from Kiambu cluster close to origin whereas the

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ZnONPs capped from tea leaves from Meru cluster in the far bottom indicating altered properties such as crystal size and peak intensities.

Tea leaves from Bomet, when used as capping agents the capped ZnONPs had a strong PC1 effect, which might have resulted in unique peak shifts or changes in structural properties depending on the phytochemical profiles. The cluster point of ZnONPs capped with tealeaves from Murang'a, Nyeri and Vihiga were located in the right quadrant of the score plot. The closer the samples means that the nanoparticles have almost similar properties such as peak position and peak intensity, which contribute to crystallographic properties of nanoparticles depending on the components of capping agent used. Samples from Murang'a were located in the central right, which suggest a moderate variance in PC1 and PC2. Samples from Nyeri were slightly influence by PC2. The extracted eigenvector B had greater effect on PC2 variance, feature C points directly to right and was a major contributor to PC1 and explains the horizontal separation in samples (Table 3 and Table 4) Feature D point towards the upper left where samples prepared with tea leaves from Kericho is. (Table 4) summarizes the extracted eigenvectors for capped ZnONPs. The total variance account to 100% for PC1, PC2 and PC3 which confirms that all differences were factored in.

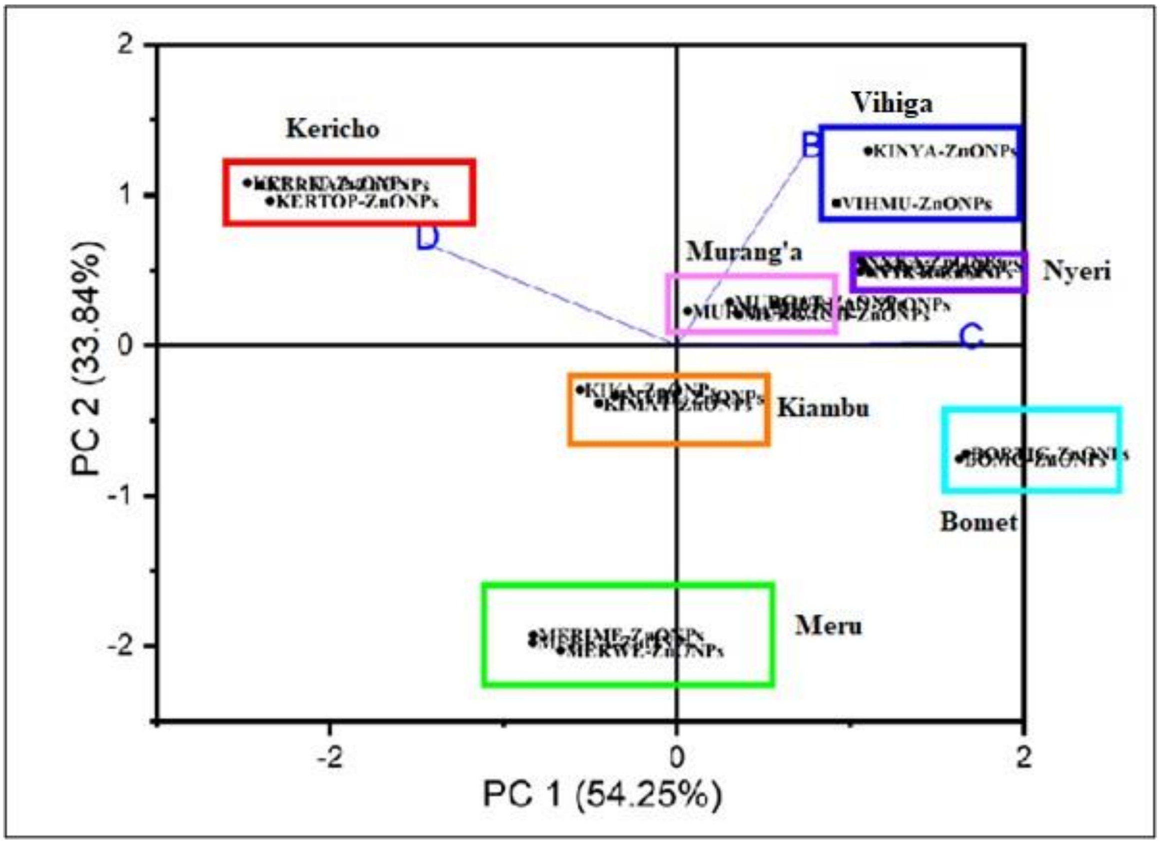

Figure 5: PCA Scatter Plot of *Camellia Sinesis* Capped ZnONPs.

PC	Eigenvalue	Percentage of Variance	Cumulative
1	1.62739	54.25%	54.25%
2	1.01519	33.84%	88.09%
3	0.35742	11.91%	100.00%

Table 3: Eigenvalues of the Correlation Matrix for Camellia Sinesis capped ZnONPs XRD Data.

Eigen Vectors	Coefficients of PC1	Coefficients of PC2
B	0.31948	0.88309
C	0.71123	0.01617
D	-0.62617	0.46893

Table 4: Extracted Eigenvectors for Capped ZnONPs.

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4.6. UV-Vis PCA Clustering

4.6.1. PCA Scatter Plot of *Camellia Sinesis* Capped ZnONPs UV-Vis Data

Zinc oxide nanoparticles have high catalytic and photochemical activities and has received considerable attention due UV blocking and antimicrobial activities. UV-Vis spectra data of tea extracts has been used to classify tea in narrow geographical regions [25]. The UV-vis absorbance data of ZnONPs capped with tea leaf extracts from different tea growing region in Kenya were subjected to PCA analysis. The energy of UV light is strong, so the UV spectrum obtained had more stable and much useful information. This method has been used to previous to determine the aging of four different insulating oils [26]. A two dimensional cluster plot shown in (figure 6) was obtained. A total of 84.27 % variance was obtained as summarized in (Table 5). In the PCA output, the nanoparticles clustered depending on the region where the capping agent was obtained. When tea leaf extracts from Bomet and Kericho were used as a capping agent, the UV-Vis data clustered in the negative PC1 and positive PC2, indicating that the capping agents had similar phytochemical profile properties. The two regions, Kericho and Bomet, are both from Rift Valley province, and they are neighbouring, thus there is a possibility of similar climatic conditions. Samples from Meru and Nyeri had positive PC1 and PC2 values and were significantly influenced by eigenvector C.

In contrast, samples from Murang'a exhibited variable properties; for instance, sample MURGACH had positive PC1 and negative PC2, while samples MURGAT and MURMA had negative PC1 and PC2 values. The variation in cluster position depends on UV Vis regions where the phytochemicals absorb. This means that the concentration and level of phytochemicals in a tea sample extract from the Murang'a region varied. The nanoparticles cluster in the central region of the cluster plot, which suggest the variation was moderate as compared to other clusters. When tealeaf extract from Vihiga region was used as capping agent clustering occurred in the lower quadrant far from other samples implicating unique absorption properties. ZnOnPs capped with tealeaf extracts from Kiambu had Negative PC1 and PC2; the nanoparticles did not cluster in the same position because of different absorption properties. Nanoparticles that cluster in positive PC1 were influenced heavily by absorbance intensity and other eigenvectors. Capping agents from Meru were greatly influenced by eigenvector C. Eigenvector B and E had negative coefficient in PC2 as summarized in (Table 4.10) a clear separation was observed for nanoparticles prepared with tea extract from Vihiga and Kiambu region.

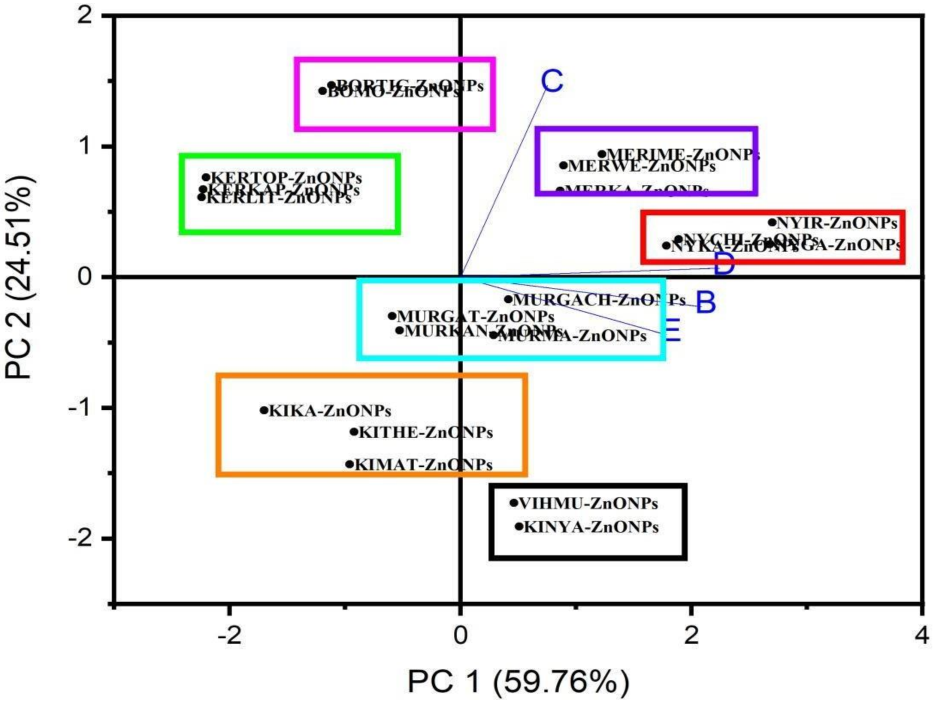

Figure 6: UV-Vis PCA Scatter Plot of *Camellia Sinesis* Capped ZnONPs.

PC	Eigenvalue	Percentage of Variance	Cumulative
1	2.39041	59.76 %	59.76%
2	0.98059	24.51%	84.27%
3	0.52488	13.12%	97.40%
4	1.10415	2.60%	100.00%

Table 5: Eigenvalues of the Correlation Matrix for Camelia Sinesis Capped ZnONPs UV- Vis Data.

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Eigenvector	Coefficients of PC1	Coefficients of PC2
B	0.57598	-0.1453
C	0.20773	0.9464
D	0.61939	0.04389
E	0.49138	-0.28511

Table 6: Extracted Eigenvectors for Capped ZnONPs UV-Vis Data.

4.7. FT-IR Plot Clustering

4.7.1. FTIR PCA Scatter Plot of *Camellia Sinesis* Capped with ZnONPs

FT-IR PCA scatter plot of ZnO nanoparticles capped with tealeaves from the 7 growing areas is represented in (Figure7) The scatter plot had a total of 78.59 % variance as summarized in (Table 6). In the scatter plot, samples from Kericho and Bomet cluster in close proximity with negative PC1 and moderate PC2. The FT-IR spectra of the two samples showed nearly identical peaks suggesting similar concentration of their constuent phytochemicals. Samples from Vihiga region had negative PC1 and PC2 and was easily distinguished from the rest. The negative PC variance suggest that the teas from Vihiga region had lower concentration of capping phytochemicals as compared to other teas. When tea leave extract from Kiambu was used as capping agent the nanoparticles obtained cluster in positive PC2 and neutral PC1the nanoparticle cluster in a very close range with each other suggesting uniformity in phytochemicals present in the extract. Eigenvector G influenced the nanoparticle synthesized with tea extract from Murang'a and the samples had positive PC2 and PC1. The strong positive PC1 suggest large concentration of functional groups in the sample. These nanoparticles could be easily distinguished from other nanoparticles. Samples from Meru and Nyeri had negative PC2, the PC1 variance was strongly positive and moderate in Nyeri and Meru samples respectively. Eigenvector D and F influenced the cluster position for samples from Meru and Nyeri. (Table 4.17) summarizes the strength of each eigenvector coefficient in the PC1 and PC2 variance.

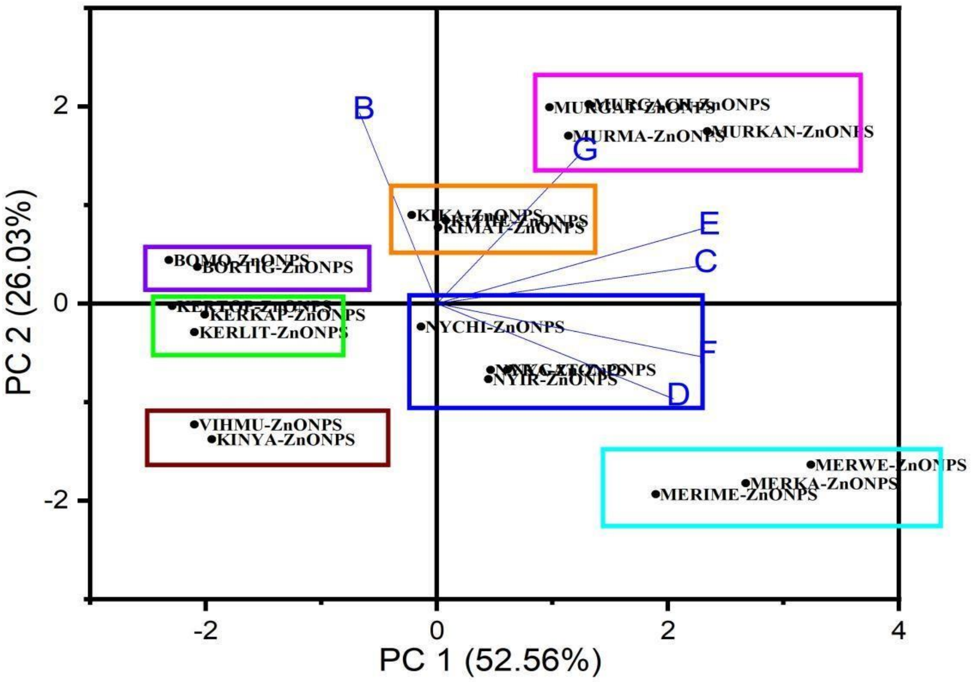

Figure 7: FTIR PCA Scatter Plot of *Camellia Sinesis* Capped ZnONPs.

PC	Eigen value	Percentage of variance	Cumulative
1	3.15334	52.56%	52.56%
2	1.56198	26.03%	78.59%
3	0.54972	9.16%	87.75%
4	0.39444	6.57%	94.32%
5	0.2589	4.31%	98.64%
6	0.08163	1.36%	100.00%

Table 7: Eigenvalues of the Correlation Matrix for *Camellia Sinesis* Capped ZnONPs FTIR Data.

Eigenvector	Coefficients of PC1	Coefficients of PC2
B	-0.1428	0.68331
C	0.48644	0.13481
D	0.43599	-0.34186
E	0.49315	0.27085
F	0.48984	-0.19229
G	0.26413	0.53638

Table 8: Extracted Eigenvectors for Capped ZnONPs.

5. Conclusion

Green synthesis of ZnONPs were successfully achieved using tea leaves from selected regions in Kenya. The nanoparticles obtained were characterized with XRD, FT-IR and UV-Vis to identify the structural and functional groups present the sample. When tea leaves from Nyeri were easily distinguished from the rest with XRD analysis due to their unique peak intensity, crystal size and different peak broadness. This was also confirmed with UV-vis spectoscopic data as the intensity of absorption differed from the rest. ZnO nanoparticle synthesized with tealeaf extract from Murang'a differed from the rest based on absorption intensity and the absorption edge. In addition, the ZnO nanoparticles prepared with tealeaf from Vihiga had the lowest absorption peak and were distinguishable from the rest of the samples without subjecting the data to PCA.

The approach used in this work was eco-friendly and cost effective for producing zinc oxide nanoparticles utilizing Camellia sinesis (Black tea) extracts as both a reducing agent and capping agent. A successful synthesis process for the nanoparticles was confirmed using X-ray diffraction (XRD), Fourier Transform infrared (FTIR) spectroscopy, and Ultraviolet-Visible (UV-Vis) spectroscopy that showed their formation, crystalline nature and optical properties.

XRD analysis unambiguously established the crystalline nature of the synthesized nanoparticles. The diffraction peaks observed for ZnO nanoparticles perfectly indexed to their respective growing zones. Zinc oxide corresponds to card no 00-036-1451. The sharpness of the diffraction peaks suggested good crystallinity, which was due to capping agent applied from different region in Kenya and the quality of the peaks differed confirming concentration of the phytochemicals present in tea extracts vary per region.

FTIR spectroscopy provided showed various functional groups (O-H, C=O, C-O) from the phytochemicals present in the black tea extracts confirming their reducing effect of metal ions and the binding of phytochemicals to the nanoparticles surface, confirming their role in stabilizing the nanoparticles and preventing agglomeration.

UV-VIS spectroscopy further corroborated the formation of the respective nanoparticles by exhibiting characteristic surface Plasmon Resonance (SPR) peaks. For Ag nanoparticles, ZnONPs displayed absorption edge at 365-400nm after capping with tea leaves extracts. The UV-Vis spectra showed strong absorption in the UV-Vis spectral region, with distinct absorption edge corresponding to their respective tea extracts.

For origin traceability, this research revealed that PCA effectively reduced the dimensionality of spectroscopic data while retaining the most significant variance. This allowed for easier visualization and comparison of data from the various tea samples. The clustering of spectral data in the PCA plot indicated distinct groups corresponding to different geographical origins. This suggested that each tea extract from different regions imparted unique spectral features to the tea, which can be applied for origin verification. Samples from the same region clustered in the same position in the PCA scatter plot, showing a fingerprint model reliable for tracing the origin of Kenyan tea, ensuring authenticity.

Furthermore, this research presents a reliable biogenic synthesis technique for ZnO, nanoparticles utilizing the plentiful and sustainable resource of Camellia sinensis (black tea). The comprehensive characterization by XRD, FTIR and UV-Vis spectroscopy confirmed the distinct optical properties, thus laying a strong foundation for exploring the potential application of these nanoparticles in biological materials of economic importance. The approach further provides a strong framework for the development of models for tracing the origins of Kenya tea. The findings underscore the potential of chemical fingerprints in ensuring the integrity of the tea products, which is critical on the global market where there is an increasing concern for product authenticity and quality.

Since tea extract was used to synthesize the nanoparticles, the concentration of phytochemicals in each samples might have varied and might not be detected by spectroscopic techniques. The XRD technique also analyze the crystal structure and not the amorphous which might have been containing the key elements for origin traceability. To improve the outcomes other multivariate techniques such as orthogonal partial least squares discriminant analysis (OPLS-DA) and linear discriminant analysis (LDA) should be used to further confirms the outcomes.

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for giving me access to various equipment to carry out my analysis.

Conflict of Interest

The authors declare no conflict of interest.

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