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FTIR FINGERPRINTING COMBINED WITH CHEMOMETRICS OF *Guiera senegalensis* LEAF POWDER

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ABSTRACT
Guiera senegalensis is a shrub that grows up to a height of 3 to 5 m and has tremendous medicinal properties. However, there is difficulty in the identification of medicinal plants leading to substitution of one plant for another. This problem can be addressed by establishing fingerprints of the medicinal plants. The aim of this research is to establish FTIR fingerprint of G. senegalensis leave. G. senegalensis leave were collected from the wild in Jigawa, Kaduna and Zamfara states, shade dried and size reduced. Samples (2 mg) were analysed in triplicate using Agilent technologies FTIR Carry 630 in the mid-IR region 4000-650 cm^{-1} at resolution 8 cm^{-1} with 16 scans. The level of similarity and differences was determined by Chemometrics using similarity and discriminant analysis. The level of differences between the samples was insignificant ($p<0.05$) as indicated by low values of eigenvalue (0.048) and canonical correlation (0.215) as well as high Wilks' Lambda value (0.954). High level of similarity between the samples was further proven by their correlation and Tuckers' congruence coefficients values, which were close to unity. However, significant ($p<0.05$) difference in the concentration of phytochemicals was observed between the samples. The Guiera senegalensis leaf samples were similar with respect to their phytochemical constituents however, differences in the phytochemical concentrations were observed.
Key words: Guiera senegalensis, Phytochemicals, FTIR fingerprint, Chemometrics and Mid-IR region

INTRODUCTION

Herbal medicines (HM) have been used time immemorial in the treatment of diseases owing to the fact that it is of natural origin, available, cheap and belief to have lesser side effects (Barnes *et al.,* 2007). Eighty percent (80 %) of the populace in developing countries depends on phytopharmaceuticals for their primary healthcare (Willcox and Bodeker 2004). One of these medicinal plants is the shrub *Guiera senegalensis* that grows up to a height of 3 to 5 m (Silva *et al.,* 2008). It is called "Sabara" (Hausa), and "Kishishi" (Kanuri) (Fiot *et al.,* 2006). This shrub has been reported to have several medicinal uses (Kerharo *et al.,* 1974; Fiot *et al.,* 2004; Diatta *et al.,* 2007). The medicinal advantages could be due to the presence of alkaloids, tannins, terpenoids, coumarins, saponins, flavonoids, cardiotonics and cynogenic hetrosides in the leaves, stem bark, fruits and root of this plant (Ficarra *et al.,* 1997; Bouchet *et al.,* 2000; Fiot *et al.,* 2006). However, environment and season of harvest among other factors are said to affect the levels of phytochemicals in plants (Verma *et al.,* 2011; Erica Biolcati *et al.,* 2013; Awwalu *et al.,* 2021). Despite the popularity and extensive use of HM, it has not been incorporated into the health policy of most countries (WHO, 2009). This is not unconnected with the reported cases of inconsistent composition and intoxication due to adulteration and or toxic component of herbal medicine (Songlin *et al.,* 2008; Junhua *et al.,* 2012). Quality control of HM, even though neglected, can address the problem of inconsistency, safety and efficacy (Junhua *et al.,* 2012). Fingerprint analysis has been accepted by the WHO as a methodology for the quality control of herbal samples (WHO, 2009; Tistaert *et al.,* 2011). It can be used for the authentication, identification and phytoequivalence of herbal products as it gives information on the chemical integrities of HM and its products (Lalit *et al.,* 2010).

The aim of this work is to carry out FTIR fingerprint analysis of *Guiera senegalensis* leaves collected from three states in northwest Nigeria.

MATERIALS AND METHODS

Collection, Identification and Processing

G. senegalensis leaf was collected from the wild in Jigawa (J), Kaduna (K) and Zamfara (Z), Northwestern Nigeria. All samples were authenticated at the Department of Botany, Ahmadu Bello University, Zaria, Nigeria and were assigned a voucher number of 1823. Samples were shade dried, size reduced using mortar and pestle, sieved through 0.5 mm mesh size and stored in a clean labeled polyethylene bag.

Spectroscopic Analysis

The FTIR spectra of each sample (2 mg) were recorded in the mid-IR region (4000-650 cm^{-1}) at resolution 8 cm^{-1} with 16 scans using FTIR Carry 630 Agilent technologies. All experiments were performed in triplicates and the mean values were used for the analysis.

Data Analysis

Chemometric techniques were then used to analyse the data. Similarity and discriminant analysis were used to determine the level of similarities and discrimination between samples using IBM SPSS statistics 20 and Microsoft excel 2007. The mean wavenumbers of ten selected major peaks from the FTIR spectra were used to determine the Correlation and Tucker's Congruence Coefficients.

RESULTS AND DISCUSSION

The FTIR fingerprint analysis results are presented in the table 1 and figure 1;

Table 1: Interpretation of the FTIR Spectra of *G. senegalensis* Leaf Samples

S/No.	Wavenumber (cm^{-1})			Peak assignment
	Jigawa	Kaduna	Zamfara	
1	3280.034	3242.756	3280.291	OH stretch
2	2922.269	2922.623	2922.403	CH_2 asym. stretch
3	2851.838	2851.667	2851.667	CH_2 sym. stretch
4	2113.792	2109.701	2102.694	C≡C stretch
5	1606.617	1606.446	1606.572	C=O stretch
6	1517.324	1513.538	1513.573	C=C aromatic stretch
7	1438.915	1448.568	1438.833	C-H asym. bending
8	1230.811	230.477	1230.639	C-O Stretching
	1159.603	1159.436	1159.628	
9	1028.709	1017.49	1028.602	C-O-C Stretching

The mid-IR region (4000–600 cm^{-1}) can be divided basically into fatty acids (2800–3050 cm^{-1}), amides (1600–1800 cm^{-1}), mixed region (1250–1450 cm^{-1}) and polysaccharides (1000–1150 cm^{-1}) regions (William *et al.,* 2008). The leaf of *Guiera senegalensis* FTIR spectra collected from Jigawa, Kaduna and Zamfara revealed the presence of various functional groups (Table 1). Both O-H and N-H stretching occur around 3600-3200 cm^{-1} but can be easily differentiated based on the fact that O-H has a higher dipole moment than N-H and therefore, appears more intense and broader (this is due hydrogen bonding). The broad and moderately intense peak around 3600-3200 cm^{-1} was therefore assigned to O-H stretching (Table 1). Methylene (CH_2) symmetric and antisymmetric stretchings were identified around the region 2930-2920 and 2860-2840 cm^{-1} respectively (table 1). This sp^2 hybridized C-H stretching was further ascertained by C-H bending vibration located around the region 1455-1440 (table 1). Furthermore, the weak peak around the region 2260-2100 cm^{-1} signifies a terminal C≡C stretching (table 1). Aromatic overtones and aromatic C=C stretching at the region 2000-1650 and 1515-1505 cm^{-1} respectively reveal the presence of aromatic compounds (table 1). Carbonyl functional group was identified by the presence of C=O peak around 1260-1050 cm^{-1} (Table 1).

Table 2: Discriminant Analysis of *G. senegalensis* Leaf samples

Eigenvalues				
Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	.048[a]	100.0	100.0	.215

Wilks' Lambda				
Test of Function(s)	Wilks' Lambda	Chi-square	Df	Sig.
1	.954	1.037	2	.595

a. First 1 canonical discriminant functions were used in the analysis.

Discriminant analysis generates a discriminant function as a linear combination of measured variables, which gives maximum separability for objects from different classes (Ritz *et al.,* 2011). The closer the eigenvalue and cononical correlation values are to one, the higher the level of discrimination between samples. Samples were found not to discriminate well as indicated by the low values of both eigenvalue and cononical correlation (table 2). A small lambda is an indication that group means differ while a higher lambda (maximum value is one) occurs when the group means are equal. *Guiera senegalensis* leaf samples collected from Jigawa, Kaduna and Zamfara were found not to significantly differ ($p<0.05$) as indicated by the high Wilks' Lambda value (0.954) as shown in Table 2.

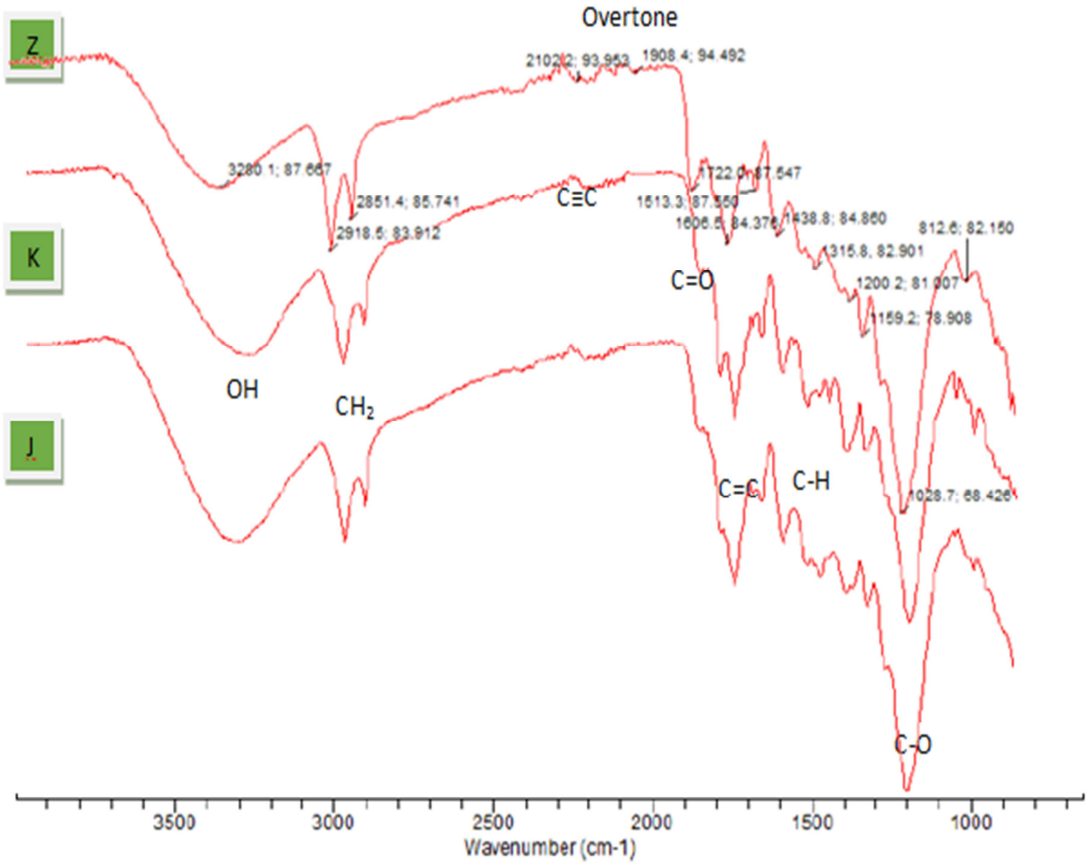

Figure 1: Overlay FTIR Spectra of *G. senegalensis* Leaf samples

Table 3: Similarity Analysis of *G. senegalensis* Leaf Samples

Sample	Correlation coefficient	Tucker's Congruence Coefficient
Jigawa	1	1
Kaduna	0.9999	0.9999
Zamfara	0.975	0.9961

The three *Guiera Senegalensis* Leaf samples were found to be similar as observed in the overlay FTIR spectra at both the functional and fingerprint region (figure 1). This similarity can be supported by Chemometric techniques, which involves the use of mathematical and statistical methods for the extraction of useful information from physical and chemical phenomena involved in a process (Singh *et al.,* 2013). A strong correlation was observed between the three samples as indicated by both Correlation and Tucker's Congruence Coefficients values (table 3). Degree of similarity is considered excellent, good, borderline, poor or terrible when the Congruence Coefficients is within the range of 1.00-0.98, 0.98-0.92, 0.92-0.83, 0.82-0.68 and < 0.68, respectively (MacCallum *et al.,* 1999). Therefore, the similarity between the three *Guiera senegalensis* leaf samples collected from Jigawa, Kaduna and Zamfara was excellent as their Tucker's congruence coefficients values were within range of 1.00-0.98 (table 3).

Table 4: Comparison between phytochemical levels using Transmittance (%) of Ten Selected FTIR Peaks in the Spectra of *G. senegalensis* Leaf Samples

S/No.	Wavenumber (cm^{-1})	Transmittance (%)		
		Jigawa	Zamfara	Kaduna
1	3280-3242	77	74	87.5
2	2922.6 -2922.2	76	73	84
3	2851.8-2851.7	80	77	86
4	2113-2102	94	93.5	96
5	1606.5-1606.4	72	72	84
6	1517-1513	79	80	87.25
7	1448-1438	73	75	85
8	1230.8-1230.4	68	70	81
9	1159.6 -1159.4	68.5	67	79
10	1028-1017	51	50	69

Transmittance (%) values at the various regions were significantly ($p<0.05$) different between the various samples. The concentration of the secondary metabolites as revealed by transmittance (%) of the various functional groups were found to significantly ($p<0.05$) differ among the various samples (table 4). Sample collected from Kaduna had the highest secondary metabolites concentration while that from Zamfara had the lowest (table 4).

CONCLUSION

The FTIR fingerprint analysis revealed that the *Guiera senegalensis* leaf samples were similar with respect to their phytochemical constituents however, differences in the phytochemical concentrations were observed.

Conflict of Interest: No conflict of interest associated with this work.

Contribution of Authors: We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Awwalu, S.; conceived the study, designed the study, collected the data, analysed the data, and wrote the manuscript Kassoum, D.B.; collected the data Nasir, I.; collected the data and analysed the data.

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