Trop J Nat Prod Res, April 2020; 4(4):172-178 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

An Investigation of the Effect of Seasonal Variation on the Phytochemical Constituents in Two *Nauclea* Species

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ARTICLE INFO

Article history:
Received 08 April 2020
Revised 18 April 2020
Accepted 26 April 2020
Published online 30 April 2020

ABSTRACT

Nauclea diderrichii and *Nauclea latifolia* are two important African medicinal plants with several known bioactive phytochemicals. However, the presence of these phytochemicals can vary depending on the season of collection. Thus, this study investigates *N. diderrichii* and *N. latifolia* leaf samples collected in October (rainy season) and February (dry season) for any disparity in their phytochemical constituents.

Pulverized samples were macerated for 24 hours in acetone. The dried extracts were subjected to phytochemical screening following standard procedures. Their Fourier transform infrared (FTIR) spectra were acquired and compared. Thin layer chromatographic analysis were carried out using the chloroform soluble fraction (CSF) and methanol soluble fraction (MSF)] of the extracts on pre-coated silica gel plates. The number of separated spots in each plate was noted after the developed plates were visualized under UV light and after spraying with vanillin-sulphuric acid reagent.

Nauclea latifolia leaf extracts showed some variations in saponin and tannin contents as they were higher in rainy season, the number of fundamental IR vibrational peaks were more in rainy season, and the number of spots on TLC for the CSF and MSF varied. For *N. diderrichii*, in both seasons, no variation was observed.

The study revealed some disparities in phytochemical constituents in *N. latifolia* leaf due to seasonal variation, while this was not so apparent with *N. diderrichii* leaf.

Keywords: *Nauclea diderrichii*, *Nauclea latifolia*, Secondary metabolites, Infrared Spectroscopy, Thin layer chromatography.

Introduction

The *Nauclea* genus belongs to the family Rubiaceae, and contains some medicinally important plants which are sources of ethnomedicinal remedies in many communities in sub-Saharan Africa.[1] While seven species of this genus are found in Africa, two prominent ones found in Nigeria and commonly used by traditional healers are *Nauclea diderrichii* (De Wild.) Merr. and *Nauclea latifolia* Smith.[1-3] A number of scientific reports are available documenting the various pharmacological properties of these plants: *N. latifolia* as antiplasmodial, antimicrobial, anthelmintic, analgesic and anti-inflammatory;[3–5] and *N. diderrichii* as antidiabetic, antileishmanial and antiplasmodial activities.[6-8]

Numerous secondary metabolites, including *β*-sitosterol, quinovic acid derivatives, indolo[2,3-a] quinolizidines-based alkaloids and phenolics, linked to the various therapeutic benefits of these two *Nauclea* species have been isolated and characterized.[1] However, it is well established that the production of the various secondary metabolites in plants is influenced to a large extent by prevailing weather condition, as this affects some environmental factors such as light, temperature and moisture in a particular season.

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Citation: Aderibigbe SA and Anowai OC. An Investigation of the Effect of Seasonal Variation on the Phytochemical Constituents in Two *Nauclea* Species. Trop J Nat Prod Res. 2020; 4(4):172-178. doi.org/10.26538/tjnpr/v4i4.9

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As a consequence of this, the amount of bioactive secondary metabolites or phytochemicals can fluctuate in different plant parts at different seasons which ultimately could alter the expected pharmacological activity.[9,10] Considering the fact that Nigeria has a tropical climate with two main seasons, this study investigated the leaves of *N. diderrichii* and *N. latifolia*, collected in the dry and rainy seasons, for any disparity in their phytochemical constituents.

Materials and Methods

Plant materials

The leaves of *N. latifolia* and *N. diderrichii* were collected from the trees growing within the University of Ibadan, Ibadan, South-West, Nigeria in October (rainy season), 2018 and February (dry season), 2019. Their identities were confirmed, and herbarium specimens deposited at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria (FHI no.: *N. diderrichii*, 111889; *N. latifolia*, 110021). The leaves were allowed to dry under shade for two weeks at ambient temperatures (26-30ºC) and ground into coarse powder with a laboratory blender (Elgento-125, China).

Extraction of plant materials

The ground plant materials were extracted by macerating 50 g of each leaf material with 100 mL of acetone for 24 hours. The supernatants were filtered through Whatman No. 1 filter papers, concentrated using a rotary evaporator into smaller volumes and dried in vacuum oven at 40°C for 48 hours. The extracts were categorized for experimental purpose as shown in Table 1.

Table 1: Categorization of the *Nauclea* species leaf extracts

Nauclea species	Plant codes	Leaf extracts*	
		Dry season	Rainy season
N. diderrichii	ND	ND I	ND II
N. latifolia	NL	NL I	NL II

* I – Dry season; II – Rainy season

Phytochemical screening
Samples of the four extracts, in duplicates, were subjected to phytochemical evaluations using standard procedures adopted from Sofowora (1993) and Evans (2009).[11,12]

Fourier transform infrared (FTIR) spectroscopic analysis of extracts
The leaf extracts of the plants were subjected to FTIR analysis. The IR spectra were obtained in the scanning wavenumber ranging from 650–4000 cm^{-1}. Aliquots (1 mg) of each extract were grinded into powdery form before triturating with 100 mg of potassium bromide (KBr) and then hydraulically compressed into translucent KBr discs. The resulting discs were placed, in turn, in a cell holder and mounted on the FTIR spectrophotometer (FTIR Spectrum BX II, Perkin-Elmer) to acquire the spectrum. The observed fundamental vibration peaks in the spectra were noted and overall IR spectra profiles compared between the seasons for any variation.

Fractionation of extracts and thin layer chromatographic (TLC) analysis of fractions
Ten milligrams (10 mg) each of the plants extracts was dissolved in 3 mL of chloroform and filtered to generate chloroform soluble fraction (CSF). The residue, after being washed with chloroform (1 mL) twice, was dissolved in methanol (1 mL) and tagged methanol soluble fraction (MSF). Aliquots (5 µL) of the CSF and MSF were spotted twice (band spotting) on pre-coated silica gel plates. The plates were developed in TLC chamber previously saturated with different solvent systems [for CSF – n-hexane/EtOAc (35:15); for MSF – EtOAc/MeOH/H_2O (40:5:4.4). The number of separated components in each plate was noted after visualizing under UV light (254 and 365 nm), and after spraying with vanillin-sulphuric acid reagent (0.1 g vanillin, 28 mL methanol, 1 mL sulphuric acid) followed by heating at 105ºC for 5 minutes. The overall TLC chromatograms were compared between the seasons for any variation.

Results and Discussion

Choice of extraction solvent
The choice of acetone as the extraction solvent was informed by some of its unique properties as well as from literature. Its high solvent strength (5.1, the same as that of methanol), low viscosity, intermediate polar characteristic, ease of removal and good safety profile were attractive advantages.[13-15] In addition, the solvent has been demonstrated to be better at extracting more bioactive constituents of medicinal plants relative to other common solvents.[15,16] A previous work on *N. diderrichii* leaf had used three different extracting solvents (*n*-hexane, ethyl acetate and methanol), and even the result showed that over 90% of compounds extracted were in methanol.[2] However, methanol, a polar hydrophilic solvent, has been shown to extract more polar primary metabolites like carbohydrates and amino acids in addition, thus increasing extraction mass relative to the bioactive secondary metabolites present in the plant materials.[16,17]

Phytochemical screenings
The results of phytochemical screenings of the extracts are shown in Table 2. The secondary metabolites present include alkaloids, tannins, steroids, terpenes, saponins, and flavonoids. The absence of glycosides is understandable in the light of the use of acetone for extraction. Glycosides being polar molecules would easily be extracted out by polar, hydrophilic solvents such as ethanol, methanol and water. This fact is corroborated by Isa *et al*.[2] where the methanol leaf extract of *N. diderrichii* indicated the presence of glycosides while being absent in ethyl acetate and *n*-hexane extracts. In addition, glycosides such as strictosamide, vincosamide, quinovic acids which have been isolated from various *Nauclea* species, were isolated by hydrophilic solvents or mixtures containing at least an hydrophilic solvent.[18-21] However, it should be noted that an exhaustive extraction with acetone, which was not employed in this study, could make possible the extraction of these glycosides. *N. latifolia* leaf extracts showed variations in their saponins and tannins contents as increased thickness of foam formation (for saponins) and higher intensity in blue-black colour formation (for tannins) were observed with NL II compared to that of NL I. Ncube *et al*.[9] found a related result as phenolics and saponins concentrations were higher in winter than in other seasons. Also, the results indicated the presence of steroids, terpenoids, flavonoids and alkaloids with relatively the same degree of colour changes or precipitate formation of these metabolites in both seasons. With *N. diderrichii*, no variation was observed as there seems to be consistent levels of these metabolites present in ND I and ND II in both seasons.

Fourier transform infrared spectroscopic analysis of extracts
Infrared spectroscopy as a physico-chemical technique detects the stretching and bending of bonds within functional groups. An IR spectrum of a pure organic compound is a complexity of vibrational nodes and serves as its unique fingerprint. However, for plant extracts, which is a mixture of phytochemicals, the spectrum is fundamentally a sum of the spectra of the individual constituent compounds. Here, it provides a snapshot of the chemical composition of the extract at a given time.[22,23] The FTIR spectra of the Nauclea species leaf extracts reveals many prominent fundamental vibrational peaks which provide information about possible characteristic functional groups/chemical bonds present in their phytochemical constituents.
The IR absorption frequency peaks of the extracts are presented in Table 3 and their IR spectra presented in Figure 1 (*N. diderrichii*) and Figure 2 (*N. latifolia*). The chemical bonds include N-H, O-H, C-H, C=N, C=O, C=C, C-O, C-N and are present in the various classes of phytochemicals already shown to be present in the extracts (Table 2). For ND I and ND II, the results revealed equal number of fundamental vibration peaks in both leaf samples. In addition, the overall profile of their IR spectra appears to match (Figure 1). Thus, seasonal variation appears not to alter the phytochemical composition of *N. diderrichii* leaf. But for *N. latifolia* leaf, there was disparity in the number of fundamental vibration peaks (NL I, 12; NL II, 16). Also, their overall IR spectra do not match (Figure 2). The vibrational peaks that were present in NL II but absent in NL I include 2638.00, 2030.00, 899.00, and 813 cm^{-1}. However, the exact chemical bonds they represent could not be ascertained as they are weak vibrations that may be coming from sum tones/overtones or fingerprint vibrations from hundreds of different bending motions.[23] Beyond the variation, however, peaks 3388 -3419 cm^{-1} represent the merged O-H and N-H vibrations that might be coming from phenolic and indole fragments in some of the phytochemicals present. The C=O (1700.47-1721.43 cm^{-1}) chemical bond results from vibration of ketone and amide fragments; C=C (1611.33-1627.00 cm^{-1}) chemical bond may be coming from aromatic fragments including benzene, pyrrole, and (1443.5-1448.17 cm^{-1}) from

Table 2: Phytochemical constituents present in the acetone leaf extracts of the two *Nauclea* species in both dry and rainy seasons

Phytochemical	Inference			
	ND I	ND II	NL I	NL II
Saponins	+	+	+	++
Tannins	++	++	+	++
Flavonoids	+	+	+	+
Alkaloids	+	+	++	++
Steroids	++	++	+	+
Terpenoids	+	+	+	+
Glycosides	-	-	-	-

* ND – *N. diderrichii*; NL – *N. latifolia*; I – Dry season; II – Rainy season; − = not detected; + = low; ++ = high; +/++: degree of colour intensity or precipitate observed.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Assignments of absorption peak frequency data from FTIR spectra of the leaf extracts of the two *Nauclea* species collected in the dry and rainy seasons

S/no.	Absorption peaks (cm^{-1})*		Probable chemical bond (s)**	Absorption peaks (cm^{-1})*	
	ND I	ND II		NL I	NL II
1.	3784.28	3783.61		3781.80	3781.33
2.	3397.00	3419.00	N-H; O-H, broad; SV	3388.00	3395.00
3.	2925.33	2926.35	C-H, aliphatic; SV	2926.52	2927.62
4.	2863.35	2860.61	C-H, aliphatic; SV	2861.33	2863.88
5.	-	-		-	2638.00
6.	2360.00	2356.47	C≡N	2360.12	2360.53
7.	-	-		-	2030.00
8.	1700.47	1727.34	C=O; SV	1721.16	1721.43
9.	1612.12	1611.76	C=C; SV	1611.33	1627.00
10.	1519.10	1517.67	N-H; BV	-	-
11.	1447.48	1448.17	C=C (Pyridine); SV	1446.20	1443.50
12.	1369.63	1371.45	CH_3; BV	1369.57	1363.73
13.	1271.96	1271.45		-	-
14.	1171.33	1169.87	C-N; SV	1181.00	1200.00
15.	1109.00	1107.79	C-O; SV	1074.21	1068.26
16.	-	-		-	899.00
17.	-	-		-	813.00
18.	775.27	774.11		-	-
19.	723.23	725.91		726.00	720.00
20.	662.54	668.46		-	-
21.	602.00	603.66		-	-

* ND – *N. diderrichii*; NL – *N. latifolia*; I – Dry season; II – Rainy season. SV- Stretching Vibration; BV- Bending Vibration
** Reference: [23]

Table 4: The number of components observed on TLC chromatograms of CSF and MSF obtained from acetone leaf extracts of the *Nauclea* species in both dry and rainy seasons

Nauclea species*	No. of components in fractions**	
	CSF	MSF
ND I	12	5
ND II	12	5
NL I	10	12
NL II	11	11

* ND – *N. diderrichii*; NL – *N. latifolia*; I – Rainy season; II – Dry season. ** CSF – Chloroform soluble fraction; MSF – Methanol Soluble fraction.

pyridine fragments.[23] Many of these fragments are present in nauclefine-based indolo[2,3-a]quinolizidine derivatives and strictosamide-based indolo[2,3-a]quinolizidine derivatives in these Nauclea species.[1]

Thin layer chromatographic analysis of fractions

The TLC fingerprints, as visualised under UV light (at 365 nm) and after spraying with vanillin-sulphuric acid reagent, of the CSFs and MSFs obtained from the leaf extracts are shown in Figure 3, while the number of phytochemical compounds (based on the observed spots) in each plate is shown in Table 4. The fractionation of the acetone extracts afforded the gross separation of the phytochemicals into two broad categories, namely; non-polar fraction (CSF) and polar fraction (MSF). Overview of the TLC chromatograms revealed some variations in which a few compounds were present in one season but absent in another season with respect to the *N. latifolia* (Figure 3). This observed disparity in the number of compounds in the CSFs and MSFs with respect to NL I and NL II (Table 4) is probably due to seasonal variation. A study by Ramírez-Briones *et al*.[24] using HP-TLC find similar kind of variation in metabolic fingerprints in two contrasting Diospyros species. However, this was not observed with *N. diderrichii* samples as their TLC fingerprints seem to be homogenous, and a consistent number of components found in the samples collected in the both seasons.

From the phytochemical screenings, FTIR and TLC analyses of the two plant species, seasonal variation was found to alter the phytochemical constituents of *N. latifolia*, but not so in *N. diderrichii*. One implication of this change in phytochemical constituents may be that the pharmacological or biological properties of the plant might vary depending on the season of collection. A number of studies have reported changes in bioactivity as plant samples collected in different seasons showed some disparities in their phytochemical compositions.[9,25]

Conclusion

The study showed possible seasonal variation in the phytochemicals in the *N. latifolia* leaf extracts, while there was none for *N. diderrichii* leaf extracts.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

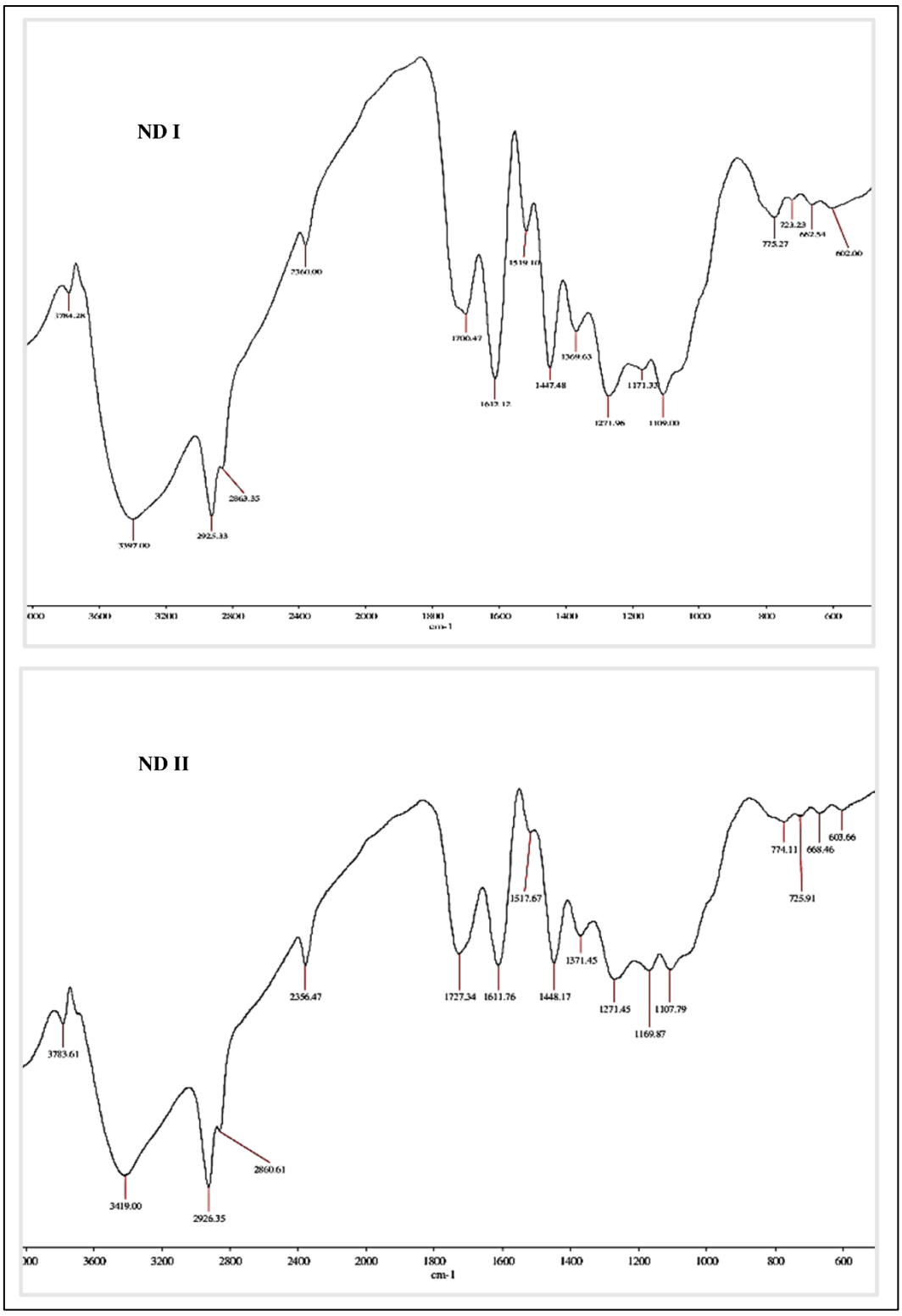

Figure 1: FTIR spectra of *N, diderrichii* (ND) collected in dry and rainy seasons.
I – Dry season; II – Rainy season

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

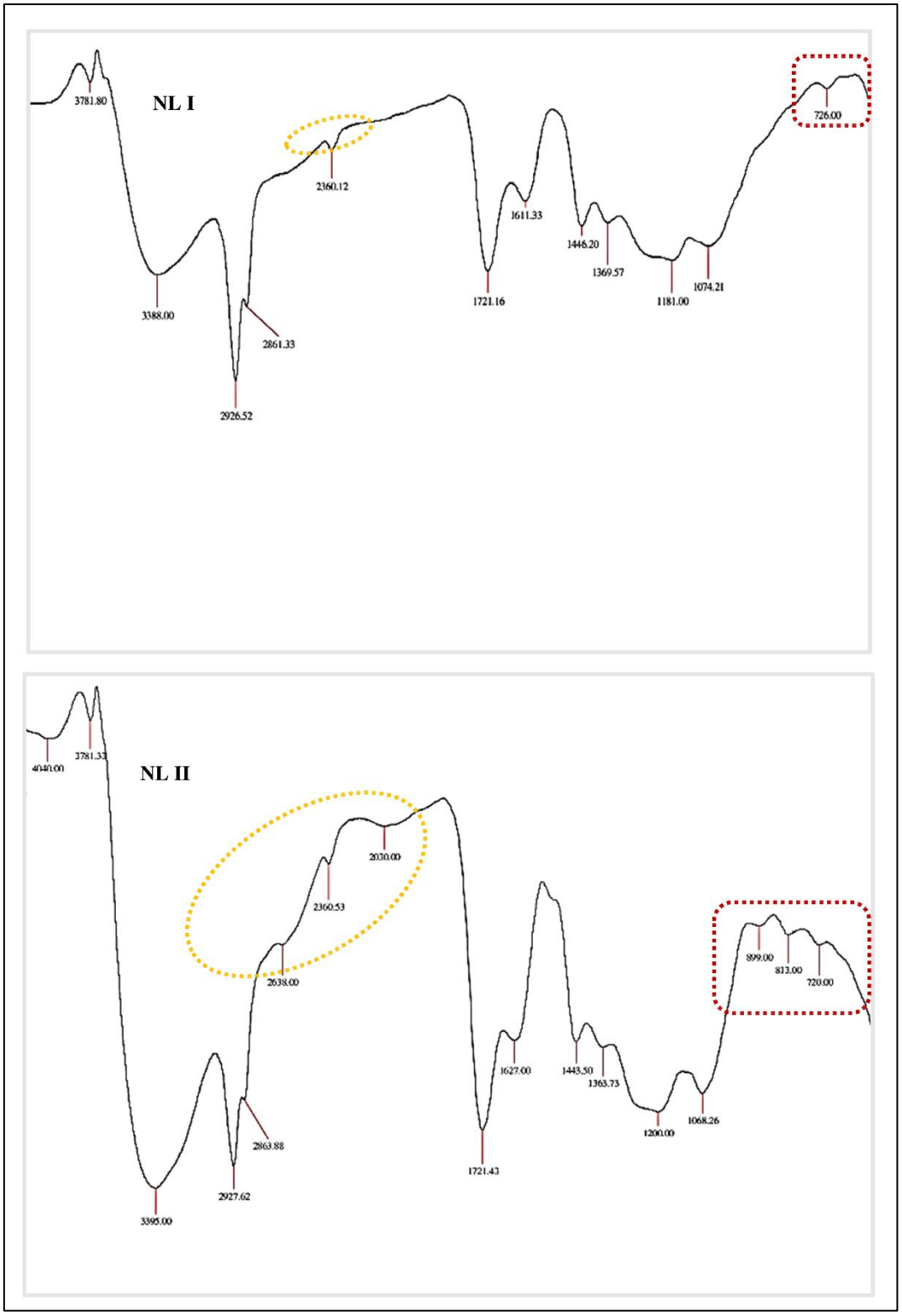

Figure 2: FTIR spectra of *N. latifolia* (NL) collected in dry and rainy seasons. Dash lines represent points of variation between the samples. I – Dry season; II – Rainy season

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

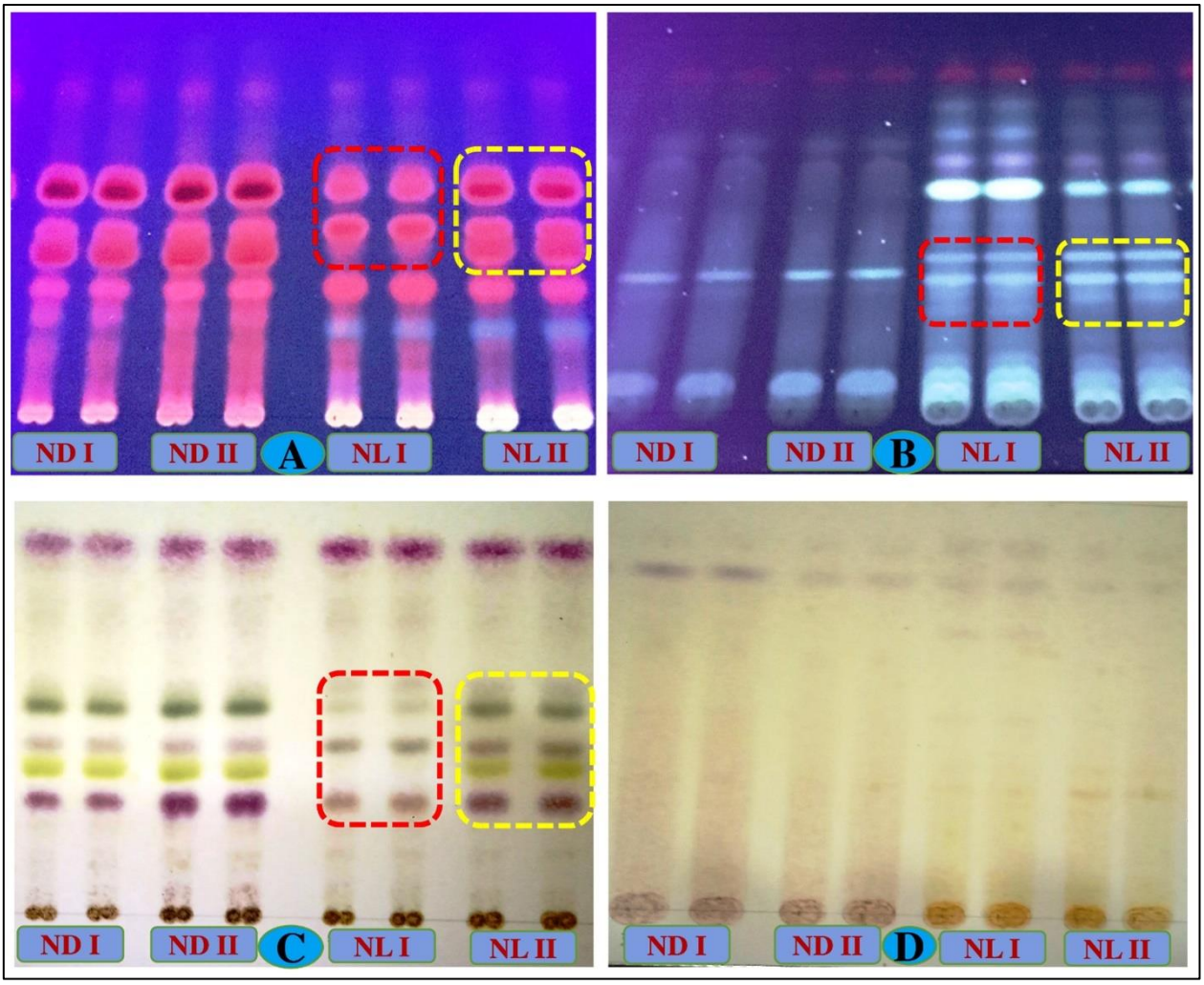

Figure 3: TLC chromatograms of the *Nauclea* species visualized under UV light at 365 nm (A & B), and after spraying with vanillin-sulphuric acid reagent (C & D) followed by heating at 105ºC for 5 minutes.

A and B are chloroform soluble fractions; B and C are methanol soluble fractions. Dash lines represent points of variation between the same species collected at different seasons. A and B are chloroform soluble fractions; B and C are methanol soluble fractions. * ND – *N. diderrichii*; NL – *N. latifolia*; I – Dry season; II – Rainy season

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to thank the reviewers for their comments and suggestions. Thanks to Prof O.A. Adegoke for guidance received towards the manuscript preparation, and assistance received from Mr. Owolabi, the plant curator, Botanical Garden, University of Ibadan, towards the collection of the plants.

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