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Characterization of secondary metabolite families in the dichloromethane fraction of two medicinal plants by high performance thin layer chromatography and infrared spectroscopy

Kouwelton P. F. O. Koné[1,*], Yaya Soro[1], Amian B. B. Kassi[2], Carlos Vaca-Garcia[3]

[1]*Laboratoire des Procédés Industriels et de Synthèses de l'Environnement et des Energies Nouvelles, Institut National Polytechnique Felix HOUPHOUËT BOIGNY, Yamoussoukro, Côte d'Ivoire*
[2]*Laboratoire de Chimie Organique et de Substances Naturelles, Université Félix HOUPHOUËT-BOIGNY, Côte d'Ivoire*
[3]*Laboratoire de Chimie Agroindustrielle, Institut National Polytechnique de Toulouse, Toulouse, France*

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***Abstract*:**

Africa is rich in medicinal plants containing secondary metabolites, which are responsible for their biological activities. For this reason, the World Health Organization actively promotes scientific research into these bioactive substances. It is within this framework that a study of two medicinal plants from the Ivorian pharmacopoeia was conducted. These are the leaves of *Alchornea cordifolia* and the whole plant of *Phyllanthus amarus*. This study aims to identify the major families of secondary metabolites in these plants using a combined, simple, and reliable analytical approach based on High-Performance Thin-Layer Chromatography (HPTLC) and Infrared (IR) Spectroscopy, offering a rigorous complement to conventional phytochemical screening. The organs of each plant were harvested, dried in the shade, crushed, sieved, and macerated in different solvents. Liquid–liquid separation was performed on the extracts with solvents of increasing polarities. Each fraction was analysed by infrared spectrometry and then by HPTLC between 250 and 400 nm. The results show the presence of terpenoids, flavonoids, esters, and carboxylic acids in the organs of each plant. These complementary results provide valuable chemical fingerprints that allow for the identification of major functional groups and metabolite families, paving the way for future structural elucidation of the active molecules in these extracts.

***Keywords*:** HPTLC; Infrared spectroscopy; Medicinal plants; Secondary metabolites.

* Corresponding author:
Email address: *kouwelton.kone1@inphb.ci (K.P.F.O. Koné)*
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1. Introduction

Ivorian populations use traditional medicine for primary health care. The use of medicinal plants in the treatment of certain diseases raises questions about the chemical composition and the real effectiveness of these remedies. Focusing on their chemical composition, it is vital to investigate these plants for their chemical compounds.

Several methods exist in analytical chemistry to identify families of molecules or functional groups. The oldest of these is thin layer chromatography (TLC). Several authors have used it to identify compounds from natural organisms [1–4]. Devices using high-performance ultraviolet detectors have improved this identification technique [1].

In addition to TLC, another technique in analytical chemistry lies in the identification of molecules by infrared spectroscopy. It has been used several times for this objective [5–7] and remains among the methods of choice in the characterization of molecules in organic synthesis.

This work aims to combine these two techniques, chromatography and infrared spectroscopy, in the identification of families of secondary metabolites using infrared and ultraviolet absorption data tables [8].

Two plants were the focus of our attention: *Alchornea cordifolia* and *Phyllanthus amarus*. They are known in the field of traditional Ivorian medicine to cure diabetes and hypertension [9].

2. Experimental

2.1. Harvesting plant material

Harvesting of the different organs of the two plants was carried out in a forest at the National Polytechnic Institute Félix HOUPHOUËT-BOIGNY Yamoussoukro (INP-HB) coordinates 6°53'04.7" North and 5°13'54.9" West. Although a formal voucher specimen (herbarium number) was not deposited for this preliminary study, the identity was confirmed by the acknowledged botanist, Amani N'Guessan (INP-HB). The organs involved in the harvest are not the same for each plant: for *A. cordifolia* the leaves were harvested; as for *P. amarus* the whole plant was harvested. Once harvested, the various organs were dried for two weeks in a room at room temperature (between 28 and 33 °C) and relative humidity (between 50 and 80%). They were then ground into a fine powder of particle size less than 0.2 mm in diameter. The resulting powder was then packaged and kept in a cool, dry place away from light.

2.2. Extraction of secondary metabolites

Extraction of secondary metabolites from our plant material was done by maceration. The solvent used was composed of 30% distilled water and 70% absolute ethanol. For each plant, 10 g of the fine powder was macerated in the hydroethanolic solvent with a mass/solvent ratio of 1/10. The maceration time was 24 hours and the stirring speed was 600 rpm. Once the maceration was complete, the mixture was filtered on Whatman paper. The obtained solution was concentrated by evaporation on a rotary evaporator. The extract obtained after solvent removal was the raw material for fractionation.

2.3. Fractionation of the hydroethanolic extract

The residues of hydroethanolic extract were suspended in water and partitioned successively with different solvents. The solvents were chosen for their difference in polarity: hexane, dichloromethane, ethyl acetate, and butanol. The separations were performed successively as shown in Figure 1. At the end of the operation, five fractions were obtained: the hexane fraction, the dichloromethane fraction, the ethyl acetate fraction, the butanol fraction, and the aqueous residue fraction.

2.4. High-Performance Thin Layer Chromatography (HPTLC)

The thin layer chromatographic analysis was carried out on a glass plate 20 cm by 20 cm, coated with silica gel. Each plant extract had its own plate on which the different

fractions to be eluted were deposited. Spots were spaced 26.6 mm starting at 20 mm from the left edge. Spotting of fractions on the plate was done automatically by a CAMAG Automatic TLC Sampler III (ATS3). Each fraction (hexane, dichloromethane, ethyl acetate, butanol) from each plant extract was deposited onto a dedicated plate for elution. The results presented in this manuscript focus only on the dichloromethane fractions.

The chromatographic analysis was performed once as a semi-quantitative "fingerprint" screening; as the automated CAMAG system ensures high precision in deposition and elution, replicates are typically not required for this type of qualitative identification. The frontal ratio (R_f) values of the separated compounds were calculated using the standard formula:

$$R_f = \frac{\text{Distance travelled by spot}}{\text{Distance travelled by solvent front}}$$

The fractions were deposited at 30 mm from the plate bottom and the solvent front at the end of the elution was recorded at 185 mm. The detection lamp was calibrated to scan the following wavelengths: 250, 280, 310, 340, 370 and 400 nm. The eluting solvent was a hexane–ethyl acetate (1:3, v/v) binary mixture. The chromatograms were generated as PDF files on the device computer.

The wavelengths used to reveal the HPTLC spots make it possible to identify the different chromophore and auxochrome groups to correlate the infrared spectra and the HPTLC chromatograms to identify the families of secondary metabolites.

The HPTLC chromatograms were interpreted based on the UV absorption bands of the families of secondary metabolites. These results allowed us to classify the secondary metabolites of the fractions into three major families. Indeed, the absorption zones of the different spots provide information about the metabolites present. The revelation was done at six wavelengths: 250, 280, 310, 340, 370 and 400 nm. Assignment of families of secondary metabolites was based on the percentage of maximum absorption across all wavelengths for each spot based on literature wavelengths i.e. 250 and 280 nm for terpenes and sterols [10], 310 nm for alkaloids [11], and 340, 370 and 400 nm for flavonoids [12,13]. The maximum values of the frontal ratio (R_f) of the separated compounds using an eluent system (hexane/ethyl acetate: 1/3 (v/v)) and the percentages of the compounds in the mixture were obtained. The choice of the solvent is an important parameter to obtain the best separation [14] and the solvent must not absorb in the concerned UV region to avoid interference.

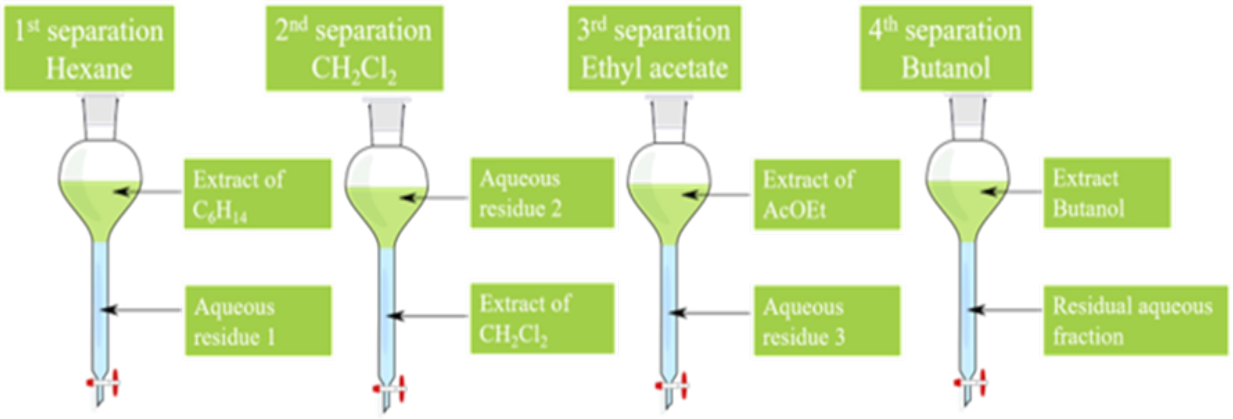

Fig. 1. Obtaining different fractions from the hydroethanolic extract.

2.5. Infrared spectroscopy

The various fractions (hexane, dichloromethane, ethyl acetate, butanol and aqueous residue) were analysed by infrared spectroscopy. This analysis takes place in two stages: preparation of the tablet to be analysed and the analysis itself. A mass of 5 to 10 mg of the sample to be analysed was weighed, then the mass was completed to 300 mg with potassium bromide (KBr). The mixture was mixed and then manually ground into a fine powder for 3–5 minutes until a uniform mixture was obtained, which was then pressed with a hand press up to 200 bars for 30 seconds. A pellet was obtained by crystallization of the powder. This pellet was introduced into the optical chamber of the spectroscope for analysis. A neutral pellet (containing only KBr) was used for levelling the device. A computer coupled to the device collected the spectrum of the sample. In the case of a liquid sample, ZnSe pellets were used. They were rinsed beforehand with chloroform, then a drop of the sample was deposited between two ZnSe pellets. They were then introduced into the optical chamber for analysis.

Infrared (IR) spectra were used to identify functional groups of secondary metabolites based on peak values in the 4000–500 cm^{-1} region. An IR spectrum provides information on the characteristic group in the molecule studied. Each absorption band is associated with a type of bond which allows identification of the characteristic group. The following symbols and their meanings were used: ν (stretching), ω (out-of-plane bending), τ (twisting), δ (bending), a (asymmetrical), s (symmetric). The chemical bond affected by the vibration frequency is in bold. The wavenumbers are expressed in cm^{-1}. Assignments were made according to Belboukhari *et al.* [15], Bulama *et al.* [16], Rolere *et al.* [17], Silverstein *et al.* [18], and Vijayalakshmi and Ravindhran [19].

3. Results and discussion

3.1. Analysis of the dichloromethane fraction from hydroalcoholic extracts of *Alchornea cordifolia* by HPTLC

The HPTLC chromatogram was obtained for the dichloromethane fraction (F_{DA}) from the hydroalcoholic extract of *Alchornea cordifolia*. The spectra were measured at six wavelengths (250, 280, 310, 340, 370 and 400 nm) depending on the desired secondary metabolite families [20]. The results of the analyses are summarized in Figure 2 and Table 1.

3.2. Discussion

The assignment of secondary metabolite families based solely on UV-Vis absorption profiles (e.g., maximum absorbance at 250 nm, 340 nm, or 310 nm) is considered indicative rather than conclusive, due to the potential for overlapping chromophores and interference from multiple co-eluting compounds. For this reason, the IR spectroscopic analysis was utilized to provide crucial complementary evidence regarding the functional groups present within these identified families. Figure 2 shows differences in the chromatographic imprints of six spots in the dichloromethane fraction of *Alchornea cordifolia*. The spots do not all absorb at the six wavelengths of the study and this is related to the nature of their chromophore groups. Indeed, the absorption characteristics of organic molecules in the ultraviolet (UV) region depend on the electronic transitions that can occur and the effect of the atomic environment on the transition [18]. The results obtained show that the number of metabolites detected is related to the wavelength used and the nature of the fraction. The chromatogram of the dichloromethane fraction of *Alchornea cordifolia* has six spots with frontal ratios between 0.01 and 0.91.

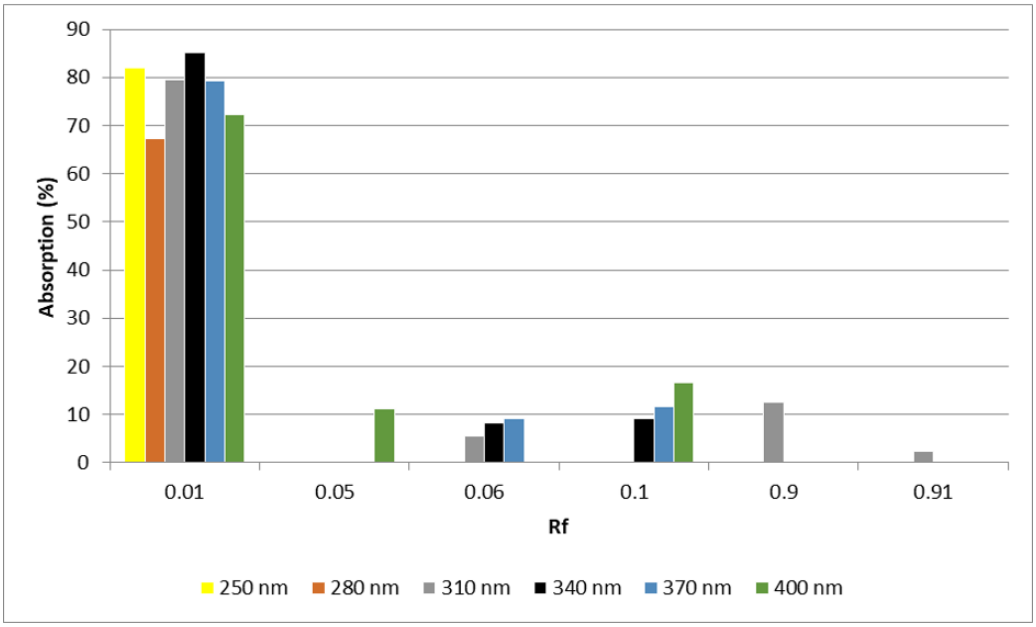

Fig. 2. HPTLC chromatogram of the dichloromethane fraction of *Alchornea cordifolia*.

Table 1
Chemical composition of the dichloromethane fraction of *A. cordifolia* based on HPTLC analysis.

Spot	% Absorption at different wavelengths (nm)						$R_{f\max}$	Proportion (%)	Major metabolite family
	250	280	310	340	370	400			
1DA	82.02	67.30	79.58	85.12	79.24	72.31	0.01	76.99	Flavonoids
2DA	0.00	0.00	0.00	0.00	0.00	11.10	0.05	1.30	Flavonoids
3DA	0.00	0.00	5.57	8.26	9.18	0.00	0.06	3.50	Flavonoids
4DA	0.00	0.00	0.00	9.18	11.58	16.59	0.10	4.39	Flavonoids
5DA	0.00	0.00	12.53	0.00	0.00	0.00	0.90	13.36	Alkaloids
6DA	0.00	0.00	2.32	0.00	0.00	0.00	0.91	0.46	Alkaloids

The most abundant (76.99%) spot 1DA has a maximum absorption percentage (85.12) at 340 nm which indicates that it is composed of flavonoids, followed by spot 5DA ($R_f = 0.90$) (13.36%) which shows a maximum absorption percentage (12.53) at 310 nm indicating that it contains alkaloids (Figure 2, Table 1). The analysis of all the spots shows that spots 1DA, 2DA, 3DA and 4DA consist of flavonoids and represent a total proportion of 86.18%. The 5DA and 6DA spots consist of alkaloids and represent a total proportion of 13.82%. In total, the dichloromethane fraction contains two families of desired metabolites with a more pronounced tendency for flavonoids followed by alkaloids. This fraction does not contain terpenes and sterols detectable by this method.

HPTLC was used as a useful tool for analytical validation of new natural product forms and allowed the determination of an amount of adulterant compound slightly higher than that contained in a Viagra tablet [21]. This analytical technique was successfully applied for identification and quantification of illegally added sibutramine in certain slimming products on the market [22]. It has been applied for simultaneous estimation of Telmisartan and Ramipril as combined doses [23] or β-sitosterol-D-glucoside and withaferin A [24]. This technique was used in preparation for the separation and isolation of flavonoids from *Launaea procumbens* [25].

3.3. Fraction analysis from the hydroalcoholic extracts of *Phyllanthus amarus* by HPTLC

The HPTLC chromatogram was obtained for the dichloromethane fraction (F_{DP}) from the hydroalcoholic extract of *Phyllanthus amarus*. The spectra were measured at six wavelengths (250, 280, 310, 340, 370, and 400 nm) depending on the desired secondary metabolite families. The results of the analyses are summarized in Figure 3 and Table 2. The HPTLC chromatogram has been interpreted as before. The overall results are recorded in Table 2.

3.4. Discussion

Figure 3 shows the chromatographic imprints of the spots (ten in total) in the dichloromethane fraction from the hydroalcoholic extracts of *Phyllanthus amarus*. The spots do not all absorb at the six wavelengths of the study and this is related to the nature of their chromophore groups.

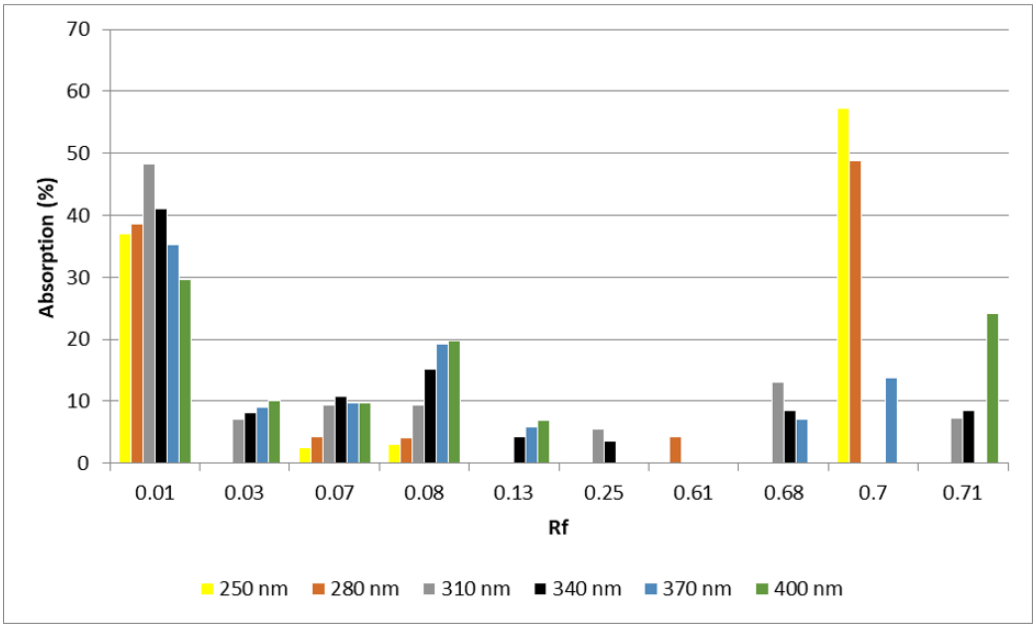

Fig. 3. HPTLC chromatogram of the dichloromethane fraction of *Phyllanthus amarus*.

Table 2
Chemical composition of the dichloromethane fraction of *Phyllanthus amarus* based on HPTLC analysis.

Spot	% Absorption at different wavelengths (nm)						$R_{f\max}$	Proportion (%)	Major metabolites family
	250	280	310	340	370	400			
1DP	37.10	38.65	48.25	41.06	35.19	29.54	0.01	38.09	Alkaloids
2DP	0.00	0.00	7.14	8.12	9.01	10.00	0.03	5.20	Flavonoids
3DP	2.55	4.31	9.31	10.78	9.78	9.65	0.07	7.35	Flavonoids
4DP	3.10	4.04	9.37	15.12	19.24	19.83	0.08	11.11	Flavonoids
5DP	0.00	0.00	0.00	4.32	5.85	6.83	0.13	2.63	Flavonoids
6DP	0.00	0.00	5.57	3.62	0.00	0.00	0.25	1.34	Alkaloids
7DP	0.00	4.19	0.00	0.00	0.00	0.00	0.61	0.89	Terpenes and sterols
8DP	0.00	0.00	13.15	8.58	7.11	0.00	0.68	4.21	Alkaloids
9DP	57.25	48.80	0.00	0.00	13.83	0.00	0.70	22.90	Terpenes and sterols
10DP	0.00	0.00	7.21	8.41	0.00	24.14	0.71	6.27	Flavonoids

Indeed, the absorption characteristics of organic molecules in the UV region depend on the electronic transitions that can occur and the effect of the atomic environment on the transition [18]. The chromatogram of the dichloromethane fraction of *Phyllanthus amarus* has ten spots with maximum frontal ratios between 0.01 and 0.71. Spot 1DP (R_f = 0.01), the most abundant (38.09%), has a maximum absorption percentage (48.25) at 310 nm which indicates that it is composed of alkaloids, followed by spot 9DP (R_f = 0.70) (22.90%) which shows a maximum absorption percentage (57.25) at 250 nm indicating that it is composed of terpenes and sterols (probably terpenes because of the polarity of this fraction) (Figure 3, Table 2). The analysis of all the spots shows that spots 1DP, 6DP and 8DP consist of alkaloids and represent a total proportion of 43.64%. Spots 2DP, 3DP, 4DP, 5DP and 10DP consist of flavonoids and represent a total proportion of 32.56%. Spots 7DP and 9DP consist of terpenes and sterols (probably terpenes because of the polarity of this fraction) and represent a total proportion of 23.79% (Figure 3, Table 2). In total, the dichloromethane fraction contains the three families of desired metabolites with a more pronounced tendency for alkaloids followed by flavonoids and terpenes and sterols.

We did not find similar data in the literature to compare with our results. However, the study of the ethanol extract of *Evolvulus alsinoides* [26] by HPTLC revealed the presence of steroids, terpenoids and glycosides in this extract.

HPTLC was used as a useful tool for analytical validation of new natural product forms [21]. This analytical technique was applied with successful identification and quantification of illegally added sibutramine in certain slimming products on the market [22]. It has been applied for simultaneous estimation of Telmisartan and Ramipril as combined doses [23] or β-sitosterol-D-glucoside and withaferin A [24]. This technique was used in preparation for the separation and isolation of flavonoids from *Launaea procumbens* [25].

3.5. Identification by infrared spectroscopy of the dichloromethane fraction of *Alchornea cordifolia*

This study was conducted on the dichloromethane fraction of *Alchornea cordifolia*. The dichloromethane fraction of *Alchornea cordifolia* is noted as F_{DA}. The spectrum is given in Figure 4.

The F_{DA} spectrum (Figure 4) shows the presence of bands at 3421.5 cm^{-1} (ν **NH** and **OH**), 2921.5 (ν_a-**CH_2**-), 2850.5 (ν_a-**CH_2**-), 1715 (ν -(**C=O**)-**OR**), 1617 (ν -(**C=C**)-), 1513.5 (ν **Ar**), 1463.5 (δ-**CH**), 1318 (δ_s-**CH_3**), 1200 (ν_a -**O-C=C**-), 1038.5 (ν **CN**). All these results show the presence of amine and hydroxyl groups, aliphatic chains, carbon structures with multiple bonds and carbonyl groups (ester).

3.6. Discussion

All the results obtained for the dichloromethane fraction show the presence of the amine function indicated by two bands at 3421.5 (ν **NH**) and 1038.5 (ν **CN**) indicating the presence of alkaloids [27]. The bands at 1377.5 (δ -**CH_3**) and 1620 (ν -(**C=C**)-) indicate the presence of terpenes [28, 29]. The presence of the broad band at 3421.5 (υ **O–H**) as well as bands at 1200 (ν_a -**O–C=C**-) and at 1513.5 (ν **Ar**) indicates the presence of a phenolic group in this fraction.

The simultaneous presence of the hydroxyl and amine groups at 3421.5 cm^{-1} gives a superposition of their usual absorption bands. It can be noted that the spectrum does not have 815, 824, 872 or 882 cm^{-1} bands attributed to the terminal methyl group of sterols [16, 30], hence the absence of this family in this fraction.

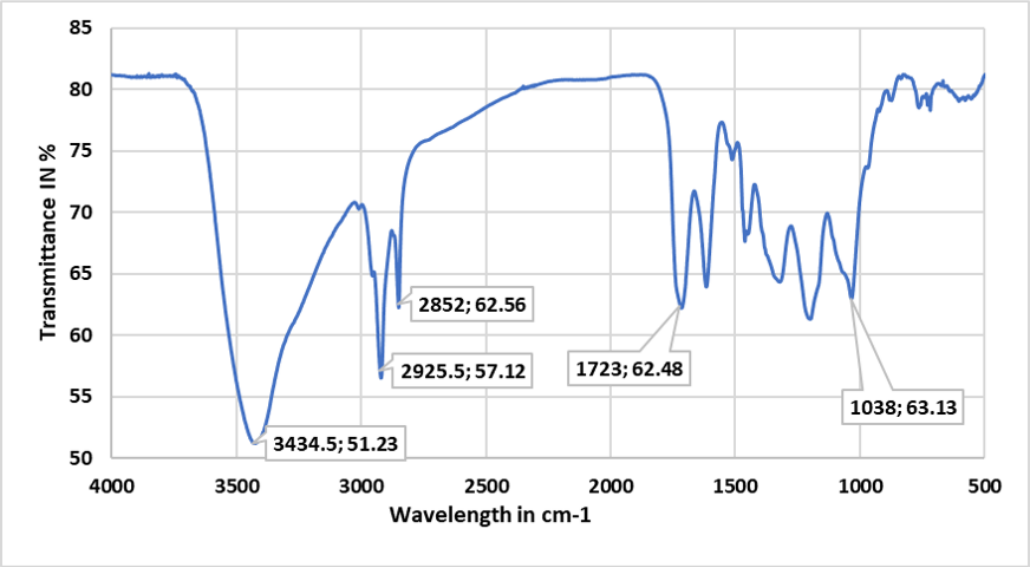

Fig. 4. Infrared spectrum of the dichloromethane fraction of *Alchornea cordifolia*.

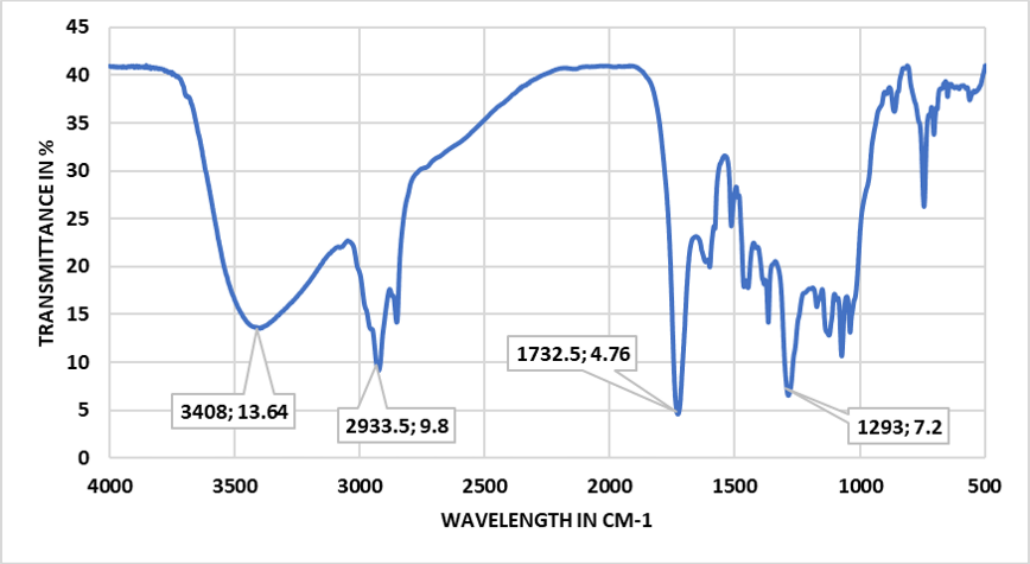

Fig. 5. Infrared spectrum of the dichloromethane fraction of *Phyllanthus amarus*.

3.7. Identification by infrared spectroscopy of the dichloromethane fraction of *Phyllanthus amarus*

The dichloromethane fraction of *Phyllanthus amarus* is noted as F_{DP}. The spectrum is given in Figure 5.

The F_{DP} spectrum (Figure 5) shows the presence of bands at 3401.5 cm^{-1} (υ **NH** and υ **OH**), 2923.5 (ν_a-**CH_2**-), 2853.5 (ν_a-**CH_2**-), 1731 (ν -(**C=O**)-**OR**), 1602 (ν -(**C=C**)-), 1515 (ν **Ar**), 1446.5 (δ-**CH**), 1366.5 (δ_s-**CH_3**), 1285 (ν **N–CO**), 1174.5 (ν_a-**O–C=C**-), 1123.5 (ν **C–C** + ω-**CH_2**-), 1074 (τ-**CH_2**-), 1039 (ν **CN**), 862.5 (ω -**CH_3**), 745.5 (ω-**CH**, **Ar**). All these results show the presence of amine and ether groups, aliphatic chains, carbon structures with multiple bonds and carbonyl groups (esters).

3.8. Discussion

All the results obtained for the dichloromethane fraction show the presence of the amine function indicated by two bands at 3401.5 (ν **NH**) and 1039 (ν **CN**) indicating the presence of alkaloids [27].

The bands at 1366.5 (δ -**CH_3**) and 1602 (ν -(**C=C**)) also indicate the presence of terpenes [28, 29]. The presence of a broad band at 3401.5 (υ **OH**) as well as bands at 1174.5 (ν_a -**O–C=C**-), at 1515 (ν **Ar**) and

at 745.5 (ω -**CH**, **Ar**) indicates the presence of a phenolic group in this fraction. The simultaneous presence of the hydroxyl and amine groups gives a superposition of their usual absorption bands. The spectrum shows a band at 862.5 (ω-CH_3) that is attributed to the terminal methyl group of sterols and reflects the presence of this family in the dichloromethane fraction [16, 30].

3.9. Combined interpretation for *A. cordifolia*

3.9.1. HPTLC Findings

The HPTLC chromatogram of the DCM fraction of *A. cordifolia* (Figure 2) showed a predominant chemical profile dominated by **Flavonoids** (86.18%) and a lesser contribution from **Alkaloids** (13.82%), based on their characteristic λ_{max} (Table 3). Notably, **no spots were detected in the UV range specific to terpenes or sterols** (250 nm to 280 nm), suggesting their absence or concentration below the HPTLC detection limit.

3.9.2. IR Spectroscopy Corroboration and Discrepancy

The IR spectrum (Figure 4) strongly supported the HPTLC findings regarding the major families. The DCM fraction showed strong bands at 3421.5 cm^{-1} (ν-NH/OH) and 1038.5 cm^{-1} (ν-CN), confirming the presence of **Alkaloids** (amine derivatives). The presence of Phenolic groups (characteristic of **Flavonoids**) was confirmed by the ν-OH (3421.5 cm^{-1}) and bands in the 1513.5 cm^{-1} region (aromatic C=C stretching).

3.9.3. Addressing the inconsistency (Terpenes)

In contrast to the HPTLC profile, the IR analysis also revealed bands characteristic of **Terpenes**, specifically bands at 1377.5 cm^{-1} (δ-CH_3, gem-dimethyl group) and 1620 cm^{-1} (ν-C=C, unsaturation in the terpene skeleton). **This apparent contradiction is discussed as follows:** The HPTLC system, highly optimized for polar and moderately polar compounds, may have failed to resolve or detect very non-polar terpenes/sterols present in low concentrations in the DCM fraction. However, the IR technique detects the functional groups of **all compounds present** in the bulk sample. The IR bands confirm the molecular **skeleton** of terpenes (methyl, C=C groups) even if their concentration is below the HPTLC UV-Vis detection limit. This underscores the **complementary value** of IR spectroscopy in providing a comprehensive chemical **fingerprint** of the functional groups that might be missed by a single chromatographic method.

Table 3
HPTLC characterization of secondary metabolite families in the dichloromethane fraction of *A. cordifolia*.

Metabolite Family	Area (%)	λ_{max} (nm)	HPTLC Justification
Flavonoids	86.18	340	λ_{max} within the 320–390 nm range (Band II), typical of flavonoid compounds.
Alkaloids	13.82	310	λ_{max} within the 300–330 nm range, generally associated with chromophoric systems of certain alkaloids.
Terpenes/Sterols	Absence	N/A	No significant absorption detected within the 250–280 nm range.

3.10. Combined interpretation: *Phyllanthus amarus* DCM fraction

For *P. amarus*, the HPTLC profile suggested a more balanced mixture of families: Alkaloids (43.64%), Flavonoids (32.56%) and Terpenes/Sterols (23.79%) (Table 4). The IR spectrum **provides strong confirmation for all three families**:

- **Alkaloids & Flavonoids (Concordance):** Bands at 3400.92 cm^{-1} (ν OH/NH), 1037.10 cm^{-1} (ν C–N or ν C–O), 1515.08 cm^{-1} (aromatic C=C).
- **Terpenes/Sterols (Concordance):** The bands at 2925.14 and 2852.57 cm^{-1} (ν C–H aliphatic) and 1368.16 cm^{-1} (δ-CH_3) confirm the presence of long aliphatic chains. Furthermore, the band at 863.49 cm^{-1} (ω-CH_3), often associated with the fingerprint region of sterols (such as phytosterols), is consistent with the HPTLC result of 23.79% for this family.

It is important to note that the δ-CH_3 bands (1368.16 cm^{-1}) are non-specific and found in all aliphatic molecules. However, their co-occurrence with the HPTLC detection at 250 nm and other specific bands allows for a high-confidence preliminary attribution of the terpene/sterol family within the context of this combined approach.

The secondary metabolite families characterized in the present study, namely flavonoids, alkaloids and terpenes/sterols, are consistent with phytochemical classes widely reported in the literature for medicinal plants investigated in the context of metabolic disorders, including type II diabetes. Numerous studies have highlighted the recurrent involvement of these compound families in plant-based research related to glycemic regulation and associated mechanisms [31–33]. Nevertheless, the present work remains strictly limited to chemical characterization at the metabolite family level, and no direct pharmacological inference can be drawn from the analytical data presented.

4. Conclusion

This study enabled the characterization of major secondary metabolite families present in the dichloromethane fractions of *Alchornea cordifolia* and *Phyllanthus amarus* using a complementary High-Performance Thin Layer Chromatography (HPTLC) and Infrared (IR) spectroscopy approach. The results revealed a predominance of flavonoids and alkaloids in *A. cordifolia*, while *P. amarus* exhibited a more diverse chemical profile including alkaloids, flavonoids and terpenes/sterols.

Table 4
HPTLC characterization of secondary metabolite families in the dichloromethane fraction of *Phyllanthus amarus*.

Metabolite Family	Area (%)	λ_{max} (nm)	HPTLC Justification
Alkaloids	43.64	310	λ_{max} within the 300–330 nm range, commonly attributed to chromophoric systems of alkaloids.
Flavonoids	32.56	340–400	λ_{max} within the 340–400 nm range (Band II), characteristic of flavonoid structures.
Terpenes/Sterols	23.79	250–280	λ_{max} within the 250–280 nm range, typical of terpenoid and sterol compounds.

The combined interpretation of UV–Vis absorption behavior and IR functional group analysis provides a consistent and coherent basis for metabolite family-level characterization. This analytical strategy constitutes a rapid, instrument-based complementary approach to conventional phytochemical screening, without claiming structural elucidation or biological activity assessment.

The metabolite families identified in this work agree with phytochemical classes frequently reported in the literature for medicinal plants studied in relation to metabolic disorders, including diabetes. However, the present study does not establish any pharmacological activity, and further investigations involving targeted bioassays and advanced analytical techniques such as LC–MS or NMR would be required to support such claims.

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