Chemistry Research Journal, 2021, 6(2):77-86

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Phytochemical Evaluation and Spectroscopic Analyses of the Extractives of the Aerial Parts of *Laggera aurita* Linn

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Abstract The plants Laggera aurita is a widely used shrub among the people of Borno state in the management of fever in children, other ethno-medicinal uses were also reported among Africans in managing diseases such as connective tissues, inflammatory conditions, sickle cell, pain, fever, anti-convulsant and jaundice, rheumatism. Almost all parts of the plants are used for medicinal purposes. The results of the preliminary phytochemical evaluation of the extractives of the aerial part of Laggera aurita Linn. revealed the presence of some of these metabolites(Terpenoids, cardiac glycosides, alkaloids, carbohydrates, flavonoids, saponins, cardiac glycosides and tannins) in the crude, n-hexane, chloroform, ethylacetate, n-butanol, aqueous portion and the column fractions. The n-butanol portion was subjected to column chromatography and Thin Layer Chromatography, where fractions with similar Rf values were pooled together, fractions 1-5 were pooled as A, 6-9 as B and 10-14 as C. Hence, fraction C having quantitative advantage to the rest was further subjected to preparative thin layer chromatography (TLC) for separation using ethylacetate: n-butanol: acetic acid: water (40:5:2.5:2.5) as solvent system to afford four distinct bands with the Rf values respectively as 0.78, 0.69, 0.53, 0.28. The separated bands were further subjected to Fourier Transform Infra Red (FTIR) and Ultra Violet (UV) spectroscopic analyses. The results of the spectroscopic analyses revealed that the sub-fraction C1, C2, C3 and C4 have maximum wavelength of absorption of ; 203 nm corresponding to saponins, sample C2 had a λ_{max} of 354 and 279 nm which were assigned as band I and II reported for flavonol class of flavonoids which are typically known to have this characteristic. The 278 nm correspond to flavan-3-ol class flavonoids, which are known to absorb only in band II due to lack of conjugation between ring B and the rest of the molecule and postulated for C3. The FTIR showed peaks at 2926 cm^{-1} C-$H_{stretch}$ (methyl), 2856 cm^{-1} C-$H_{stretch}$ (methylene), 1730 cm^{-1} (C=$O_{stretch\ carbonyl}$), 1458cm^{-1} (C=Cstretch), 1512 cm^{-1} (C=Cstretch), 3495 cm^{-1}(OH) others include 1716 cm^{-1} correspond to carbonyl stretch $(C=O)_{stretch}$, 1647 cm^{-1} corresponding to C=$C_{\ stretch}$, 1411 cm^{-1} is an C=$C_{\ aromatic\ stretch}$.

Keywords Phytochemical Evaluation, Spectroscopic Analyses, Aerial Parts, *Laggera aurita* Linn

Introduction

Laggera aurita is a member if the asteraceaea family of flowering plant, its found widely distributed in tropical and sub tropical zones of Asia and also in Africa. The plant is a ruderal shrub and is commonly found in residential area in Maiduguri, Borno state, Nigeria. Many species of genus *Laggera* are used in traditional Chinese medicine. There

are several literatures supportive of the therapeutic effects of *L. aurita* among African countries among which are Benin republic, Ghana, Cameroon Sudan and Nigeria with traditional application in the treatment and management of connective tissues, inflammatory conditions, pain, sickle cell, fever and jaundice, as well as rheumatism and showed diuretic properties among pregnant women [1-3].

The anti-bacterial and anti-oxidant effects of the ethanol extracts of *Laggera aurita* has been established. The methanol, ethyl acetate and hexane extracts of the plant *L. aurita* showed significant antimicrobial activity [4]. The methanol and hexane extract were more active while the ethyl acetate extract exhibited good antifungal activity. The hyperalgesic potential of the methanolic stem bark crude extract was reported by Olurishe and Mati [5]. The membrane stabilizing effect of the ethanol crude extract of the plant *L. aurita* has also been demonstrated [6]. Similarly, the methanol leaf extract of *Laggera aurita* possesses anti-convulsant effects and anti-epileptogenic properties which were partly attributed to sodium channel blockade and activation of serotonergic and or histaminergic pathways. Similarly, recent studies by Shehu *et al.* [7] and Fulata *et al*. [8-9] demonstrated analgesic and anti-inflammatory effects of the plant using different models. Recent publication by Fulata et al 2018 in the Nigeria journal of pharmaceutical research revealed that the n-butanol pooled column fractions of *L. aurita* Linn. possessed a great effect on inflammation and analgesic, these effects served as the basis of this paper to further examine the spectroscopic behaviours of the extractives of the plant and further propose a nucleus of the expected compound(s).

Sample Collection

The sample was collected at No. 20 Mohammed Lawan Street adjacent State Low-Cost Housing Estate Maiduguri, Borno State, and was identified by a Taxonomist at the Department of Botany, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Sample Preparation, Extraction and Partitioning

The sample was shade dried and the root parts were removed. The aerial parts were then pulverized using wooden mortar and pestle. One thousand gram (1000 g) of the powdered materials was exhaustively extracted with a mixture of the two solvents, 80% ethanol using soxhlet apparatus at a temperature of 65 ºC. The extract was concentrated under reduced pressure and temperature. Seventy five grams (75 g) of ethanol crude extract of the aerial part of *Laggera aurita* was defatted in n-hexane. The defatted extract was then dry partitioned sequentially with chloroform and ethyl acetate and the residue later suspended in distilled water for further partitioning with n-butanol. The n-butanol and the aqueous portions were evaporated at reduced pressure using water bath. The resulting masses were then weighed and phytochemically evaluated using standard procedures as reported by Silva *et al*., [10]; Evans, [11]; Vishnoi, [12]; Sofowora, [13]; Brain and Tuner, [14]; Markham, [15] for alkaloids, anthraquinone, flovonoids, terpenoids, tannins, saponin and phlobotannins etc.

Column chromatography and Isolation and Purification

The partitioned n-butanol portion was subjected to column chromatography using the graded elution protocol through suitably mixed solvent system of graded polarities. Silica gel (60-120 mesh size) slurry was made with the solvent system established earlier. The slurry was poured time to time into the column very carefully and the silica gel was allowed to settle down to from a uniform packing. Then the stop-cock of the column was opened and the excess solvent over the column head was allowed to run. The n-butanol extract (9 g) was mixed with small amount of silica gel in a mortar to get a free flowing powder. The powdered sample was then applied carefully on the top of the prepared column and successfully eluted with solvent/ solvent system using the solvent system chloroform: methanol to separate the eluate. The eluate with same Rf value are pooled together and evaporated to dryness. When the mixture of solvent system used, the ratio of mixtures are prepared as 100:00, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 00:100. Elutes were collected in a number of conical flasks marked from fractions 1-14. Elutes were spotted successfully on TLC plate and the flasks having similar spots were combined together [16].

Spectroscopic Analyses

FT-IR Analysis

The FT-IR Spectroscopic analyses of the n-butanol column sub-fraction C preparative TLC bands were carried out using Shimadzu FTIR-8400S Fourier Transform Infrared. A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermo scientific Nicot iS5 iD1 transmission, between $4000 - 400\ cm^{-1}$ [17].

UV-Vis Analysis

The UV-Vis Spectroscopic analysis of the n-butanol column sub-fraction C preparative TLC bands were carried out using Shimadzu UV-Visible Spectrophotometer (Shimadzu, UV-1700, Pharmaspec). A little powder of plant specimen was dissolve with ethanol and a record of the amount of light absorbed by the sample as a function of the wavelength of light is displayed on the absorption spectrum, which generally, consists of absorption bands [18].

Results

Phytochemical Constituents of the Aerial Part of *Laggera aurita* Ethanol Extract and it Partitioned Portions

Table 1: Phytochemical Constituents of the Aerial Part of *Laggera aurita* Linn.

Phytochemical Evaluation	Crude	n-C_6H_{14}	CH_3Cl	EOAc	C_4H_9OH	H_2O	PCFA	PCFB	PCFC
Phlobatannins	-	-	-	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-
Carbohydrates	+	-	-	-	+	-	-	+	+
Flavonoids	+	-	-	+	+	+	-	+	+
Alkaloids	+	+	+	+	+	-	-	+	+
Terpenoids	+	+	+	-	+	-	+	-	-
Tannins	+	-	-	+	+	-	-	+	+
Saponins	+	-	+	+	+	+	-	+	+
Cardiac glycosides	+	-	+	-	+	-	+	+	+

Key: (+) Present, (–) Not detected. While ***PCFA, PCFB &PCFC*** are Pooled Column Fraction A,Pooled Column Fraction B and Pooled Column Fraction C respectively

Table 2: FT-IR Spectroscopic Data of Sub Fraction C1

S/No	Functional Group	Stretching Frequency (cm^{-1})
1	O-H stretching	3495
2	C-H stretching (methylene)	2926
3	C-H stretching (methyl)	2856
4	COOH Acidic group	2359
5	C=O stretching carbonyl	1730
6	C=C stretching	1512
7	C-O stretching carbonyl	1280
8	C-O-C stretching ether	1120

Table 3: FT-IR Spectroscopic Data of Sub Fraction C2

S/No	Functional Group	Stretching Frequency (cm^{-1})
1	O-H stretching	3504/3473
2	C-H stretching methylene	2937
3	C=O stretching carbonyl	1716
4	C=C stretching	1647
5	C=C stretching (aromatic)	1411
6	C-O-C stretching	1203/1107

Table 4: FT-IR Spectroscopic Data of Sub Fraction C3

S/No	Functional Group	Stretching Frequency (cm^{-1})
1	O-H stretching	3456
2	C-H stretching methylene	2949/2920
4	C=C stretching	1676
5	C=C stretching (aromatic)	1413
6	C-O-C stretching (ether)	1280

Table 5: FT-IR Spectroscopic Data of Sub Fraction C4

S/No	Functional Group	Stretching Frequency (cm^{-1})
1	O-H stretching	3508/3472
2	C-H stretching	2935
3	COOH acidic group	2343
4	C=O stretching carbonyl	1645
5	-C=O stretching (esters)	1265
6	>C-O stretching (ethers)	1280
7	O-CH_3 stretching (ether)	1043

Table 6: UV – Visible Spectra of the Isolated Compounds

S/no	Sample I.D	Absorption (nm)
1	C1	203
2	C2	354, 279
3	C3	278
4	C4	389

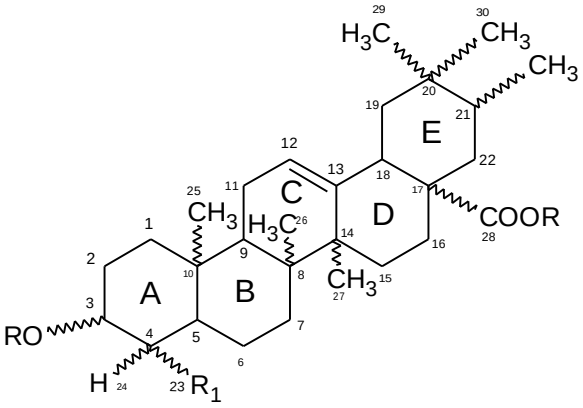
Discussion

The results of phytochemical screening of the aerial parts of *Laggera aurita* Linn revealed the presences of many bioactive metabolites. The evaluation of the crude ethanol extract showed the presence of carbohydrate, tannins, cardiac glycoside, flavonoid, terpenoid saponins and alkaloid while anthraquinone and phlabotannins were absent. The *n*-hexane tested positive for alkaloid and terpenoids, while the chloroform portion was found to contained alkaloids, terpenoids, saponins and cardiac glycosides respectively. Similarly, the ethyl acetate partitioned portion showed the presence of flavonoids, alkaloids, tannins and saponins terpenoid. The *n*-butanol partitioned portion was found to contain carbohydrate, tannins, cardiac glycoside, flavonoid, terpenoid saponins and alkaloid. The *n*-butanol column fractions *PCFA* tested positive for terpenoids and cardiac glycosides only, while *PCFB* and *PCFC* reveal the presence of carbohydrates, flavonoids, alkaloids, tannins, saponins and cardiac glycosides this results were similar with the findings of Ibrahim *et al.,* [3] and Usman *et al*., [19]. The Aqueous partitioned portion was also found to contain saponins and flavonoid.

Furthermore, the result of the column chromatographic analysis of the n-butanol partitioned portion yielded fourteen different fractions which were later pooled on the basis of their *Rf* values as follows; fraction 1-5 pooled as *PCFA*, 6-9 as *PCFB* and 10-14 as *PCFC* respectively. The pharmacological, qualitative and quantitative advantage of sub-fraction *PCFC* favoured its selection for isolation using preparative thin layer chromatographic (TLC) [8-9]. Thus, *PCFC* which was eluted in the solvent regions of 20:80 to 00:100 $CHCl_3$: MeOH mixtures was subsequently separated using EtOAc: *n*.BuOH: Acetic Acid: Water (40:5:2.5:2.5) solvent system to afford four distinct bands with the *Rf* values respectively as 0.78, 0.69, 0.53, 0.28 respectively.

The FTIR spectra of sub-fraction C1 in fig. 1 showed a peak at 2926 cm^{-1} C-$H_{stretch}$ (methylene), 2856 cm^{-1} C-$H_{stretch}$ (methyl), 1730 cm^{-1} (C=$O_{stretch}$ carbonyl), 1458cm^{-1} (C=$C_{stretch}$), 1512 cm^{-1} (C=$C_{stretch}$) 1280 cm^{-1} (C-$O_{Stretch}$ carbonyl), 1120 cm^{-1}(C-O-$C_{Stretch}$), 1031 cm^{-1} (CH_2 Out of plane bend), 3495 cm $^{-1}$(OH) which are all in agreement with earlier findings of Asha *et al.,* [20] which suggested saponin. The exceptional band observed in the IR spectra at 2359 cm^{-1} could be due to the presence of acidic groups (COOH) as earlier reported by Wagh Dipali *et al.,* [21]

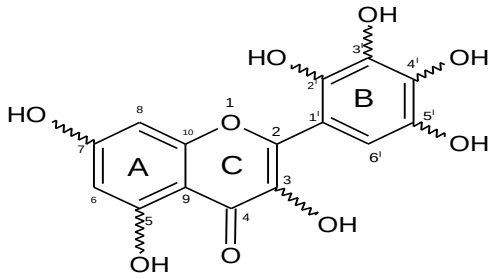
who also suggested saponins. According to Deore *et al.,* (2009) majority of saponins has to be traced at lower UV wavelength ranging from 200 to 210 nm hence, 203 nm absorption wavelength reported for this sample is suggestive of saponins.



Proposed Triterpenoidal saponin nucleus

The interpretation of FTIR spectra of sub-fraction C2 in fig. 2 revealed the presence of five significant bands which are essential in the interpretation of FTIR spectra of flavonoids. 1716 cm^{-1} correspond to carbonyl stretch $(C=O)_{stretch}$, 1647 cm^{-1} correspond to $C=C_{\ stretch}$, 1411 cm^{-1} is an $C=C_{\ aromatic\ stretch}$, and 1203 cm^{-1} are due to $C\text{-}O\text{-}C_{\ deformation}$ and 1107 cm^{-1} is believed to be due to C-O-C stretching vibration which all corroborates with the findings of Maciej *et al.*, [22] and Hao *et al.,* [23]. The broad band observed between 3504 cm^{-1} and 3473 cm^{-1} are as a result of $O\text{-}H_{\ Stretch}$ which is indicative of occupation of position 3 or 5 on the flavones ring nucleus as opined by Maciej *et al.,* [22].

Conclusively the band at 2937 cm^{-1} is $C\text{-}H_{aliph.\ stretch}$ as reported earlier by Coates, [24] and Mistry, [25]. The typical UV-Vis spectra of flavonoids include two absorbance bands. Band I lies in the 310-350 nm range for flavones, while for flavonols it is between 350 and 385 nm. Band II, found in the 250-290 nm range. The UV visible observed for the sample showed an absorption maxima at 354 and 279 nm which correspond to the two regions of flavonoids [26]. Flavonoids are often hydroxylated in positions 3, 5, 7, 2', 3', 4', and 5'. Methyl ethers and acetyl esters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose [27].



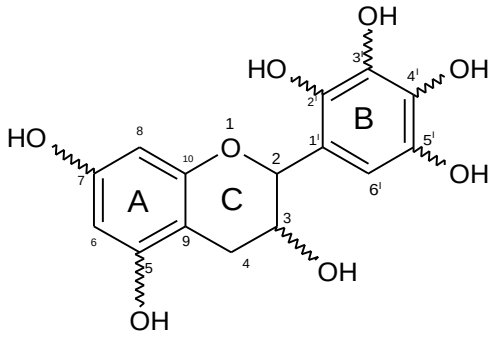
Proposed Flavone nucleus

More so, the FTIR spectra of sub-fraction C3 in fig 3 showed a strong intensity broad band that in the region of 3456 cm^{-1} which represent $O\text{-}H_{stretch}$ and band between 2949-2920 cm^{-1} represent $C\text{-}H_{stretch}$ (methylene), likewise the vibrational band at 1676 cm^{-1} correspond to $C=C_{\ stretch}$, 1413 cm^{-1} is due to aromatic stretch while 1232 cm^{-1} and 1074 cm^{-1} represent C-O-C groups commonly noticed in flavan nucleus (catechin) corroborative with the earlier studies by Maola *et al.*, [28]; Hao *et al.,* [23]; Randhan *et al.,* [29]; Okoye, [30].

Moreover, another spectroscopic tool vital in the elucidation of compounds is the UV spectroscopy which measure the maximum wavelength of absorption of a compound. The Flavan-3-ols, proanthocyanidins and dihydrochalcones classes of flavonoid show mainly one absorption maximum around 270–290 nm (Band II) [31-32]. The absence or

low absorption maximum of Band I is probably caused by the lack of conjugation between ring B and the rest of the molecule (no double bond in ring C) [33].

The UV-Vis observed for the sample showed absorption maxima at 278 nm which corroborates with the flavan-3-ol class flavonoids which absorbed in this region. The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). Flavanols differ from flavanones by hydroxyl group at the 3- position and a C2–C3 double bond as well as ketonic representation at position 4 [34]. Sequel to the earlier presentations, it has been evaluated by several workers that Flavonoids are often hydroxylated in positions 3, 5, 7, 2', 3', 4', and 5'. Methyl ethers and acetyl esters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose [35].



Proposed Flavan-3-ol nucleus

The IR spectral data of sub-fraction C4 in fig 4 exhibited absorption in the range of 3817 cm^{-1} -1043 cm^{-1}. The spectrum showed a peak at 3817 cm^{-1} for free hydroxyl group (-OH) and a broad band in the range of 3508 cm^{-1}-3473 cm^{-1} suggestive of OH group with hydrogen bonding, 2363.4 cm^{-1} for carboxylic acids (COOH), while the sharp peak at 1645 cm^{-1} indicated the presence of (C=C) group in the sample which are in lined with earlier findings [24, 36-37]. The peaks between 1300 cm^{-1} -1000 cm^{-1} at 1265 cm^{-1} and 1107 cm^{-1} indicate the presence of –C=O (esters) and -C-O (ethers) groups in the compound. Further presence of a peak at 1043 cm^{-1} is clear evidence for the presence of another ester group C=O(O-CH_3) in the isolated compound. In FTIR spectrum, aliphatic C-$H_{stretching}$ (CH_3) was observed at 2935 cm^{-1}. The peak at 1107 cm^{-1}corroborates to the earlier presentation by Sharma *et al.*, [38]; Wagh Dipali *et al.,* [21] who suggest that peaks around 1265-1043 are characteristic of the presence of OCH_3. According to Deore *et al*., [39], majority of saponins has to be traced at lower UV wavelength ranging from 200 to 210 nm, but, Negri and Tabasch, [40] reported that carbonyl group in saponins absorb in the range of 280-300nm and ethylenic double bond appears at the 195-198 nm. However, saponins isolated by Asha *et al.,* [20] were reported to have higher values of 417 nm and 425 nm hence, 389 nm absorption reported for this sample may likely be due to saponins following the earlier arguments by preceding authors.

Appendix 1. FTIR Spectra of Sub-Fraction C1

Figure 1 shows the FTIR spectra of sub-fraction C1obtained from the most active n-butanol column fraction C TLC with the RF value of 0.78.

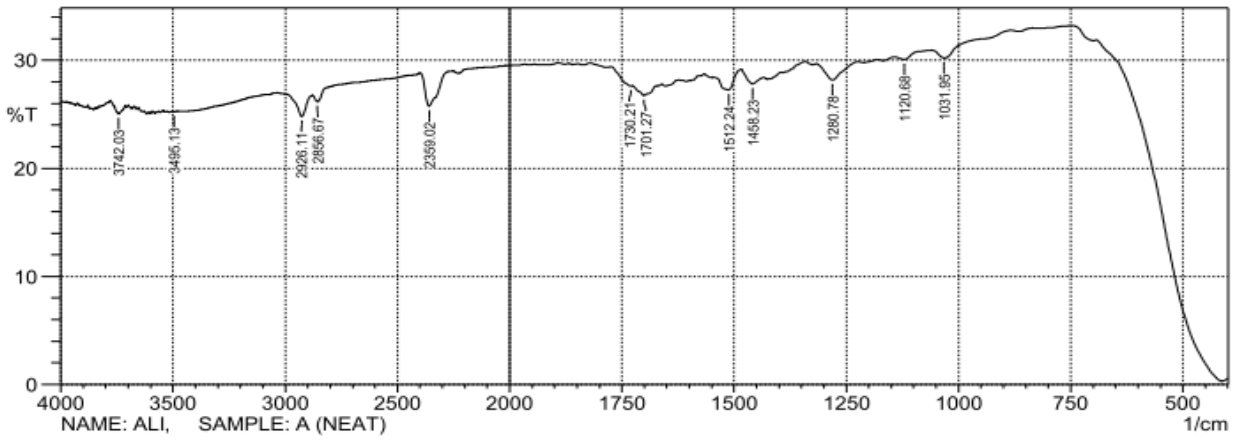


Figure 1: FTIR spectrum of sub-fraction C1

Figure 2 shows the FTIR spectra of sub-fraction C2 obtained from the most active n-butanol column fraction C TLC with the RF value of 0.69.

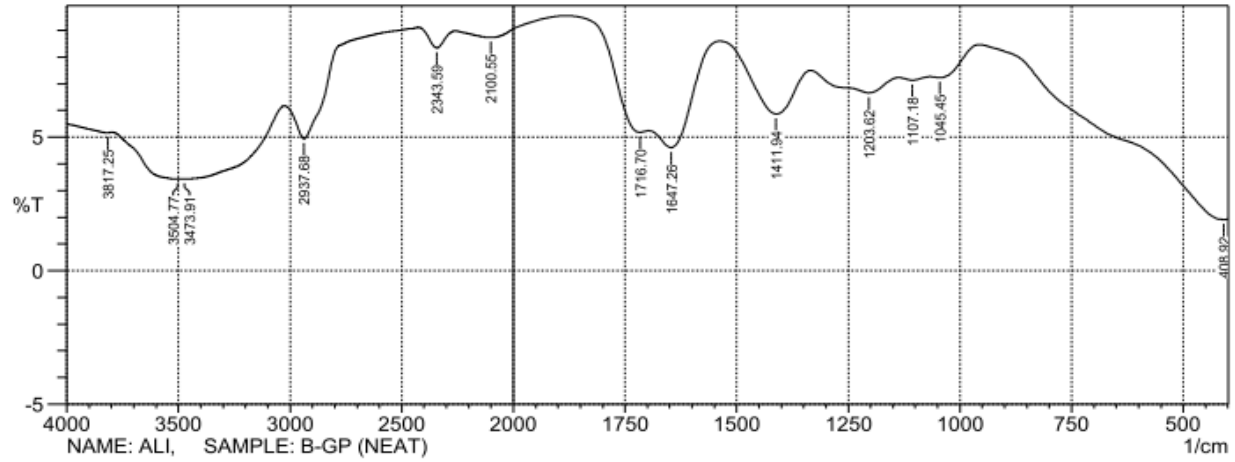


Figure 2: FTIR Spectrum of sub-fraction C2

Figure 3 shows the FTIR spectra of sub-fraction C3 obtained from the most active n-butanol column fraction C TLC with the RF value of 0.53.

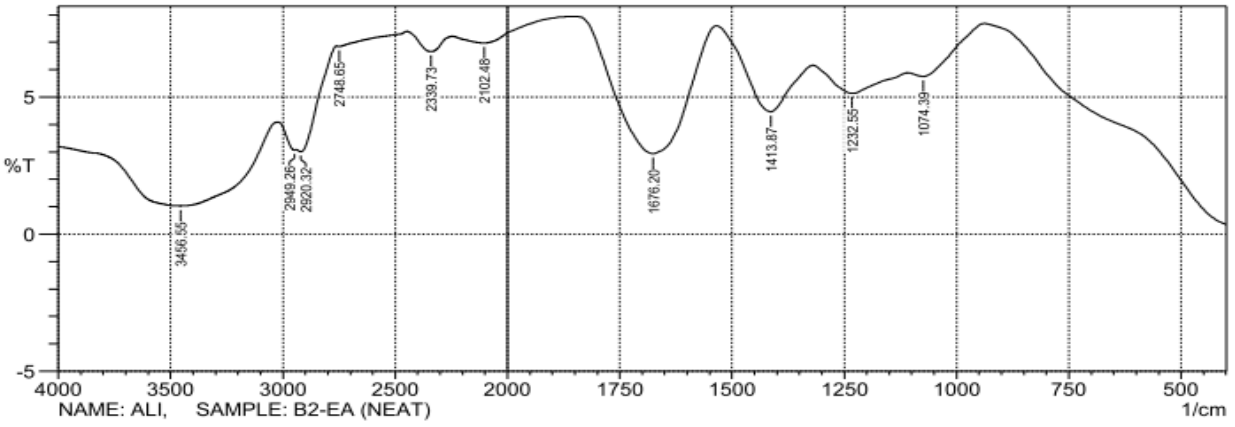


Figure 3: FTIR spectrum of sub-fraction C3

Figure 4 shows the FTIR spectra of sub-fraction C4 obtained from the most active n-butanol column fraction C TLC with the RF value of 0.28

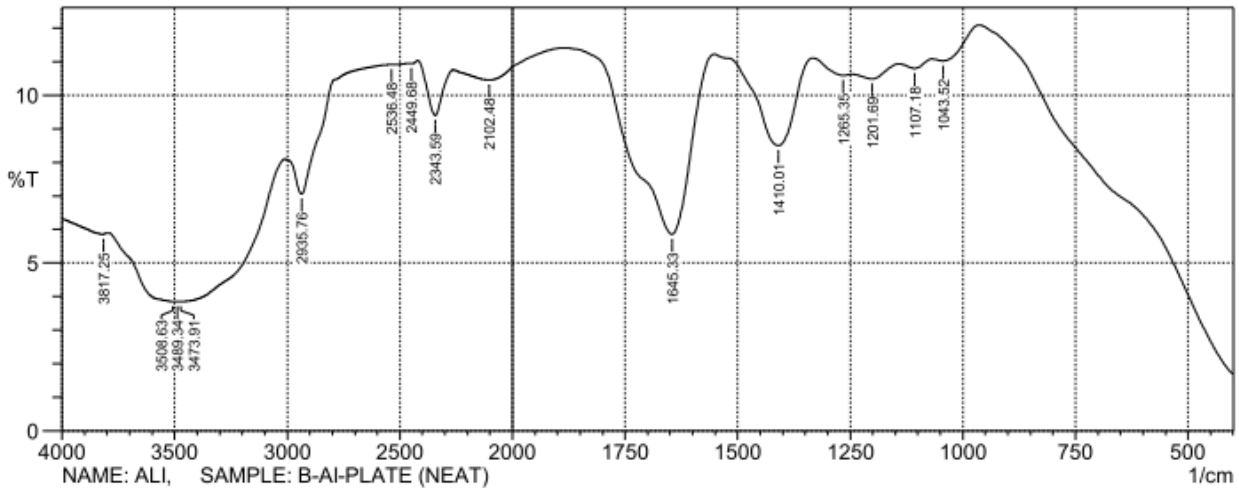


Figure 4: FTIR Spectrum of sub-fraction C4

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