Spectroscopic Analyses of Alkaloids and Tannins from the Leaves of *Ficus Citrifolia*

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Abstract. *Ficus citrifolia* (Family Morracae) is known for numerous medicinal uses, including antibacterial, anti-inflammatory, analgesic, and anti-cancer. Alkaloids and tannins were isolated from crude extracts of the leaves of *Ficus citrifolia* and analysed using GCMS, FTIR and HPLC. From the ethanol, 2000 mL and 80 % methanol, 1500 mL extracts, alkaloids and tannins were isolated, respectively. Thin layer and column chromatography (TLC and CC, respectively) were employed to isolate and purify alkaloids and tannins. The ethanol (2000 mL) extract gave 22 fractions, and 20 fractions were collected from the 80 % methanol (1500 mL) extract of *F. citrifolia*. Fractions with similar retention factor (Rf) values were pooled and identified as AK1 to AK3 for alkaloids and TA1 and TA2 for tannins. The spectra of the Fourier Transform Infrared (FTIR) Spectroscopy for AK1 to AK3 showed characteristic absorption peaks between 3370 and 3470 cm^{-1}, of NH and NH_2, 1168 and 1233 cm^{-1}, C-N stretching vibrations, 726 and 895 cm^{-1}, C-H aromatic out of plane bending vibrations, all of which are expected absorption peaks for alkaloids. TA1 to TA2 showed characteristic absorption peaks between 3300.1 and 3400.1 cm^{-1}, OH stretch of phenols, 1077 and 1084 cm^{-1}, C-O stretch of alcohols, 1457.1 and 1461.1 cm^{-1}, C-C stretch (in ring) aromatic, all of which are expected absorption peaks for tannins. The Gas Chromatography-Mass Spectroscopy (GC-MS) of AK3 identified the fraction as 1,2-Benzoisothiazole-3-amine TBDMS derivative. High-Performance Liquid Chromatography (HPLC) of TA1 and TA2 treated under the same condition with a tannin standard, quercetin, showed that both fractions were quercetin. Hence, alkaloids and tannins were successfully isolated from the leaves of *F. citrifolia*. The presence of these compounds corroborates the medicinal uses of the plant.

Keywords: Alkaloids; Tannins; Ficus citrifolia; GCMS; HPLC; FT-IR.

INTRODUCTION

Medicinal plants are essential for pharmacological research and drug development. Crude extracts and isolated constituents of plants are used as therapeutic agents, as well as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. The use of foods and medicinal plants to maintain and improve health is nearly as old as humanity. Among such are members of the Ficus genus, figs, which have been cultivated for over 11,000 years, possibly predating cereal grains [1]. Many species of Ficus are used as food and for medicinal purposes in Ayurvedic and Traditional Chinese Medicine [2], owing to the presence of phytochemicals.

Natural products are a source of new phytochemicals that can be used to treat infectious diseases or as lead compounds [3] for synthesising new drugs. Plant-derived medicines have contributed immensely to human health. There are numerous examples of drugs derived from plants. Emetine, which is an isoquinoline alkaloid, was obtained from the underground part of *Cephaelis ipecacuanha* and has been used for many years as an amoebicidal drug. Quinine is another vital drug of plant origin with a long his-

tory of use. The alkaloid occurs naturally in the bark of the Cinchona tree and has helped treat malaria. Widely prescribed drugs for antimalarial drug combinations, such as chloroquine and mefloquine, are analogues of quinine [4]. Some plants have made essential contributions in areas beyond anti-infectives, such as cancer therapies. Examples include the anti-leukaemic alkaloids vincristine and vinblastine, obtained from *Catharanthus roseus* syn *Vinca roseus,* the Madagascan periwinkle [5]. A well-known benzylisoquinolone alkaloid, papaverine, has been shown to potently inhibit the replication of several viruses, including human immune virus (HIV), measles and cytomegalovirus [6].

Tannins are polymeric phenolic compounds with numerous hydroxyl groups and diverse chemical structures [7]. Hydrolysis of some tannins yields the simple, seven-carbon gallic acid; others give ellagic acid or other phenolic acids [8]. Tannins are generally divided into hydrolysable and condensed tannins. Polyphenols and related structures are responsible for the antioxidant processes in the human body system [7]. Tannic acid with low levels affords protection against polycyclic aromatic hydrocarbon-induced fore stomach and lung. Tannins of *Agrimonia japonica* have been used as antidiarrheic and hemostatic in Japan and China [7].

In this study, we isolated and carried out a spectroscopic analysis of some natural product compounds (tannins and alkaloids) in the leaf extract of *Ficus citrifolia*. Although alkaloids and tannins are widely studied, to our knowledge, there is no commercial report on the GCMS, FTIR and HPLC analysis of alkaloids and tannins isolated from *Ficus citrifolia*.

METHOD

This study used fresh leaves of *Ficus citrifolia*. All chemicals used were analytical grade. Chromatographic fractionations were monitored with TLC plates (Layer: 0.22 mm, silica gel 60-200 mesh size with fluorescent indicator UV254). Spectroscopic data were collected using UV, GCMS, HPLC, and FTIR spectrophotometers.

Sample Collection, Preparation and Extraction. The plant materials were collected from a garden within the Bauchi Ring Road Senior Staff Quarters, University of Jos, Nigeria. The plant was taxonomically identified as *Ficus citrifolia* at the Herbarium unit of the Department of Botany, Federal College of Forestry, Jos, Plateau State, Nigeria, where a specimen with voucher No FHJ253 was deposited. The leaves were manually collected, washed in clean cold water and air-dried for 20 days. The dried sample was pulverised using mortar and pestle and sieved with a mesh size of 2.0 mm. The powder was kept in an incubator in a clean, dry bottle for further use.

The leaf powder was divided into two parts, 500 g each, for extraction. The two parts were treated separately with different solvents for crude extraction and further isolation of tannin and alkaloids by column chromatography. In the first part, cold maceration was employed using 80 % methanol. The extract was filtered through a white silk cloth, and the filtrate collected was further filtered through Whatman paper no 1. The methanol was evaporated under reduced pressure at 40 °C using a rotary evaporator [9].

In the second part, pulverised leaves (500 g) of *Ficus citrifolia* were soaked in ethanol for 48 hours at room temperature. The ethanol extract was filtered using Whatman filter paper No 1 and concentrated using a rotary evaporator at 40 °C.

Both crude extracts were finally dried in an air oven at 50 °C and stored in a desiccator for further use.

Phytochemical screening. The extracts were screened for secondary metabolites using standard procedure [10]. One gram of the ethanol extract was heated with 5 ml of 0.1N HCL for alkaloids. The filtrate was divided into three portions. Five drops of Dragendorf's reagent (Solution of Potassium Bismuth Iodide) were added to the first portion. The formation of a red precipitate, if observed, indicated the presence of alkaloids. Wagne's reagent was added to the second portion drop-wise. The formation of a brown/reddish precipitate indicates the presence of alkaloids. Mayer's reagent (Potassium Mercuric Iodide) was added drop-wise to the third portion. If observed, the formation of a yellow-coloured precipitate indicates the presence of alkaloids.

For tannins, to 1 g of the 80 % methanol extract, 20 ml of water was added, boiled and filtered. The filtrate was again adjusted to 20 ml with water. A small quantity of the adjusted solution was made up to 5 ml with water, and a few drops of 0.1 % w/v ferric chloride solution were added. If observed, the formation of a bluish-black colour

indicates the presence of phenols. Two drops of bromine water were added to another 1mL of the adjusted solution. Colour changes or formation of white precipitate, if observed, indicates the presence of tannins.

Isolation by Chromatography. The crude ethanol extract was subjected to column chromatography and eluted with 100% n-hexane, hexane-ethyl acetate (80:20, 70:30, 60:40, 50:50), ethyl acetate (100 %) and methanol (100 %) gradients.

A silica gel 70-230 mesh slurry was made with the eluting solvent (n-hexane) and packed into the glass column. The tap was opened to allow excess solvent to run off. The leaf extract was dissolved in the methanol and loaded on the silica gel slurry with a pipette. As soon as the cake began to form on the column, glass wool fibre was placed on top of the extract, and the eluting solvent was added. Fractions were collected in volumes of 25 ml. Further elution was done with increasing concentration gradients [11].

For the 80 % methanol leaf crude extract (polyphenols/tannins), elution was carried out using chloroform-ethyl acetate (80:20, 70:30), ethyl acetate (100 %), ethyl acetate-methanol (50:50) and methanol (100 %) gradients.

The fractions collected were monitored by spotting on precoated Thin-Layer Chromatographic (TLC) plates and viewing them under visible UV light (254 nm). The fractions were combined according to similarity in Rf values, weighed, kept in dried vials, and stored in a desiccator for further analysis [12].

FTIR spectrophotometry of alkaloids and tannins. Fractions from the ethanol and 80 % methanol extracts of *Ficus citrifolia* that were confirmed to contain alkaloids or tannins based on TLC chromatograms were subjected to IR spectrophotometry to get information on the functional groups present.

Gas Chromatography-Mass Spectrometry of alkaloids. Gas Chromatography – Mass Spectrometry, which characterised alkaloids. GC-MS was recorded in a GCMS-2010 Shimadzu instrument operating in EI mode at 70 ev. A Restek-5MS column (30 m x 0.25 mm x 0.25 µm) was used. The oven temperature program was 100 ° to 250 °C at 5 °C min-1 and held for 5 min at 250 °C and from 250 °C to 280 °C at 10 °C min-1 and held for 10 min at 280 °C. The injector temperature was 250 °C with normal injection mode. The flow rate of carrier gas helium was 1.21 ml min^{-1}. The identification of alkaloids was confirmed by comparing the mass spectral data with those of authentic compounds and data obtained from the literature [13].

High-Performance Liquid Chromatography (HPLC) Analysis of Tannins. All solvents used in HPLC experiments were "HPLC grade". High-performance liquid chromatography (HPLC) analysis was performed using an HPLC system from Shimadzu (Prominence) equipped with a UV–VIS detector (SPD-20-AV), Aproma (Promasil) C18 250 mm × 4.6 mm, five µm pores column and CTO-20AC column oven. Accurately weighed 0.0025 g of each tannin fraction (TA1 & TA2) was transferred to a 25 ml volumetric flask and dissolved in methanol (10 ml). Volume was made up to the mark with methanol to obtain a concentration of 1000 µg/ml vortexed for 5 minutes and then filtered using a 0.42 µm membrane filter and injected into the column. Isocratic elution was used with a mobile phase of methanol and 0.20% phosphoric acid 60:40 (%, v/v) at 25°C. The flow rate was 1 ml/min, and injections were 20 µL in volume at a wavelength of 360 nm. Quercetin was used as standard.

RESULTS AND DISCUSSION

Extraction. The ethanol crude extract for the isolation of alkaloids yielded 35 g, 7%, while that of methanol/water (80%) for the isolation of tannin yielded 12.5g, 2.5%.

Phytochemical Screening. Phytochemical screening on both crude extracts indicated the presence of alkaloids, tannins and phenols.

Isolation by Chromatography. The ethanol and 80% methanol extracts were subjected to column chromatography, and fractions were collected. From the column chromatography separation of alkaloids from ethanol crude extract, 22 fractions of 25 ml each were collected. Based on analytic TLC, fractions with identical Rf values were combined to yield three fractions (AK1, AK2 and AK3). When compared with standard Rf values for alkaloids, the Rf values fell within the range [12]. For methanol extract, 20 fractions were collected and based on analytic TLC, fractions with similar Rf values were combined to yield two fractions (TA1 and TA2). When tested with 1% Ferric chloride solution, these fractions gave a greenish colour, which confirmed the presence of tannins.

Fourier Transform – Infrared Spectroscopy (FT-IR. The FT-IR of AK1, AK2 and AK3 displayed characteristic alkaloid peaks between 3370 and 3470 cm^{-1}, of NH and NH_2, 1168 and 1233 cm^{-1}, C-N stretching vibrations, 726 and 895 cm^{-1}, C-H is aromatic out of plane bending vibrations, which are expected absorption peaks for alkaloids, as shown in Tables 1-3.

Table 1 – Ftir Peaks For AK1

Peaks	Frequency (cm^{-1})	Bond Type/Functional Group
1	3470.2	N-H amine
2	2985.6	C-H Aliphatic symmetry stretching vibrations
3	1736.9	C=O stretch saturated aliphatic
4	1371.7	C-H rock alkanes
5	1233.7	C-N stretching vibrations

Table 2 – Ftir Peaks For Peaks For AK2

Peaks	Frequency (cm^{-1})	Bond Type / Functional Group
1	3370.5	NH Amine
2	2858.1	C-H Aliphatic symmetry stretching vibrations
3	1714.3	C=O stretch saturated aliphatic
4	1457.1	C-H bend alkanes
5	1374.7	C-H rock alkanes
6	1168.2	C-N stretching vibrations
7	1039.2	C-O ether stretching vibrations
8	977.6	=C-H bend alkene
9	727.7	C-H aromatic out-of-plane bending vibration

Table 3 – Ftir Peaks For Peaks For AK3

Peaks	Frequency (cm^{-1})	Bond Type / Functional Group
1	3395.6	NH_2 Amine
2	2922.2	C-H stretch Alkanes
3	1736.9	Esters, Saturated aliphatic
3	1481.1	C-C stretch (in ring) aromatic
4	1379.7	C-H rock alkanes
4	1215.1	C-N stretching vibrations
5	726.8	C-H aromatic out-of-plane bending vibration

The FT-IR of TA1 and TA2 also showed characteristic absorption peaks between 3300.1 and 3400.1 cm^{-1}, OH stretch of phenols, 1077 and 1084 cm^{-1}, C-O stretch of alcohols, 1457.1 and 1461.1 cm^{-1}, C-C stretch (in ring) aromatic, all of which are expected absorption peaks for tannins, as shown in Tables 4-5. [14]

Table 4 – Ftir Peaks For Peaks For TA1

Peaks	Frequency (cm^{-1})	Bond Type/Functional Group
1	3300.1	OH stretch H-bonded (Phenols)
2	2924.1	C-H stretching vibrations of the aromatic ring
4	1640.0	C=C stretch of alkene
5	1461.1	C-C stretch (in ring) aromatic
6	1352.1	C-H rock alkanes
7	1294.2	C-O stretch of carbonyl
8	1251.2	C-O stretch of alcohol
9	945.6	=C-H bend alkene aromatic out of plane bending vibration

Table 5 – Ftir Peaks For Peaks For TA2

Peaks	Frequency (cm^{-1})	Bond Type/Functional Group
1	3400.1	OH stretch H-bonded (Phenols)
2	2923.3	C-H stretching vibrations of the aromatic ring
4	1711.5	C=O stretch of carboxylic acid
5	1457.1	C-C stretch (in ring) aromatic
6	1376.7	C-H rock alkanes
7	1276.2	C-O stretch of carbonyl
8	1124.2	C-O stretch of alcohol
9	732.5	aromatic out-of-plane bending vibration

Ultra Violet-Visible Spectroscopy. UV-visible spectroscopy is a beneficial technique for the analysis of fractions. The UV-visible spectrum of AK1, AK2, AK3, TA1 and TA2 are shown in Figure 1.

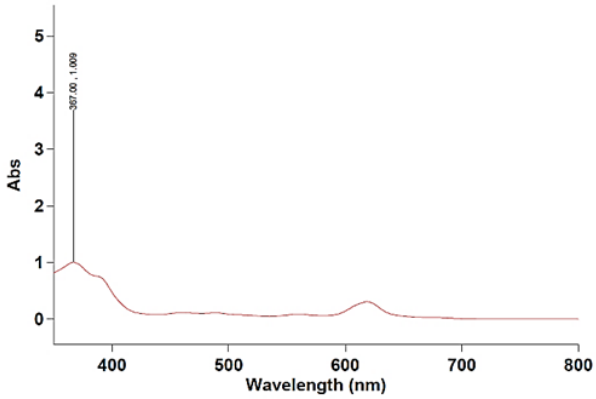

Figure 1 – UV-Visible Spectrum of AK1

UV-VS Analysis. The UV-Vis spectrum of AK1 peaks at the wavelength of 367nm with an absorbance of 1.009. The spectrum shown in Figure 2 for AK2 identifies the maximum wavelength at 369nm with an absorbance of 3.412. The UV-visible spectrum for AK3 in Figure 3 shows a maximum wavelength at 369 nm with an absorbance of 3.422. The UV-visible spectrum of TA1 (Figure 4) identifies a maximum wavelength at 367 nm with an absorbance of 2.421. The UV-visible spectrum of TA2 (Figure 5) shows a maximum wavelength at 366nm with an absorbance of 1.378.

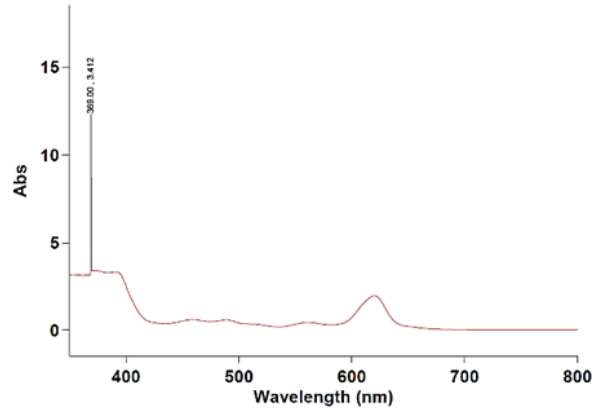

Figure 2 – UV-Visible Spectrum of AK2

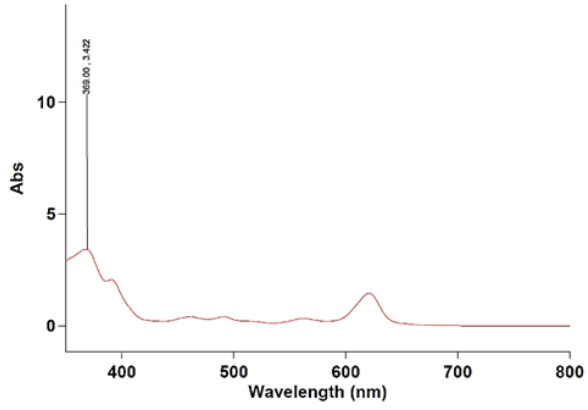

Figure 3 – UV-Visible Spectrum of AK3

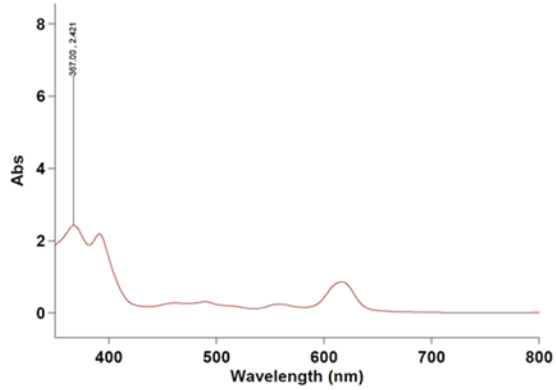

Figure 4 – UV-Visible Spectrum of TA1

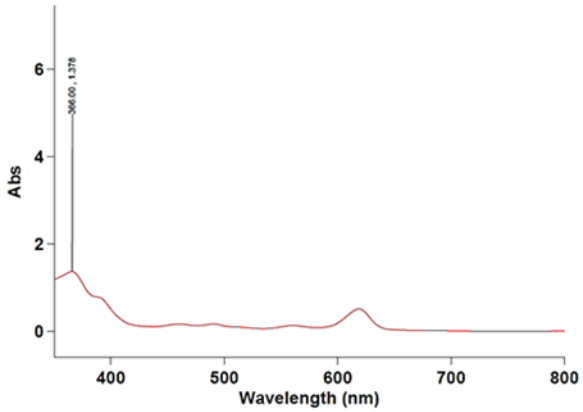

Figure 5 – UV-Visible Spectrum of TA2

According to authors [15], there is an indication of the presence of unsaturated groups and heteroatoms such as S, N, and O in the UV-VIS spectrum that shows the appearance of one or more peaks in the region from 200 to 400 nm. It can be inferred that the fractions AK1 to AK3 suggest alkaloids, while TA1 to TA2 suggest phenolic tannins.

GC-MS Analysis of AK3. The gas chromatogram of AK3 revealed a peak that strongly indicates an alkaloid. The peak with a retention time of 27.2517 mins matches fragment by fragment with suggestions from the NIST library. The molecular ion peak can be observed at m/z 264, and the base peak at m/z 207. Compared with the spectrum of 1,2-Benzisothiazol-3-amine (TBDMS derivative) suggested by the NIST library, both peaks are observed. It has a molecular formular of $C_{13}H_{11}N_2SSi$. The suggested fragmentation pattern for the compound is shown by the scheme presented in Figure 1; the molecular formulae of the fragments are shown in Table VI. The reason for the unwanted peaks could be noise from the equipment.

Analysis of 1,2-Benzisothiazol-3-amine, TBDMS derivative, alongside the information provided by the FTIR spectrum, showed that all major functional groups were present (Table 6). The MS fragmentation scheme of 1,2-Benzisothiazol-3-amine terms isolated from AK3 are shown in Figure 6.

Table 6 – Suggested GCMS fragmentation pattern of 1,2-Benzothiazole-3-amine TBDMS derivative.

m/z for 1,2-Benzoisothiazole-3-amine isolated from AK3	Suggested Fragmentation ($C_aH_bN_cS_dSi_e$)
264	$C_9H_{11}N_2SSi^+$
207	$C_7H_5N_2SSi^+$
177	$C_6H_2N_2S^+$
134	$C_6H_2^+$

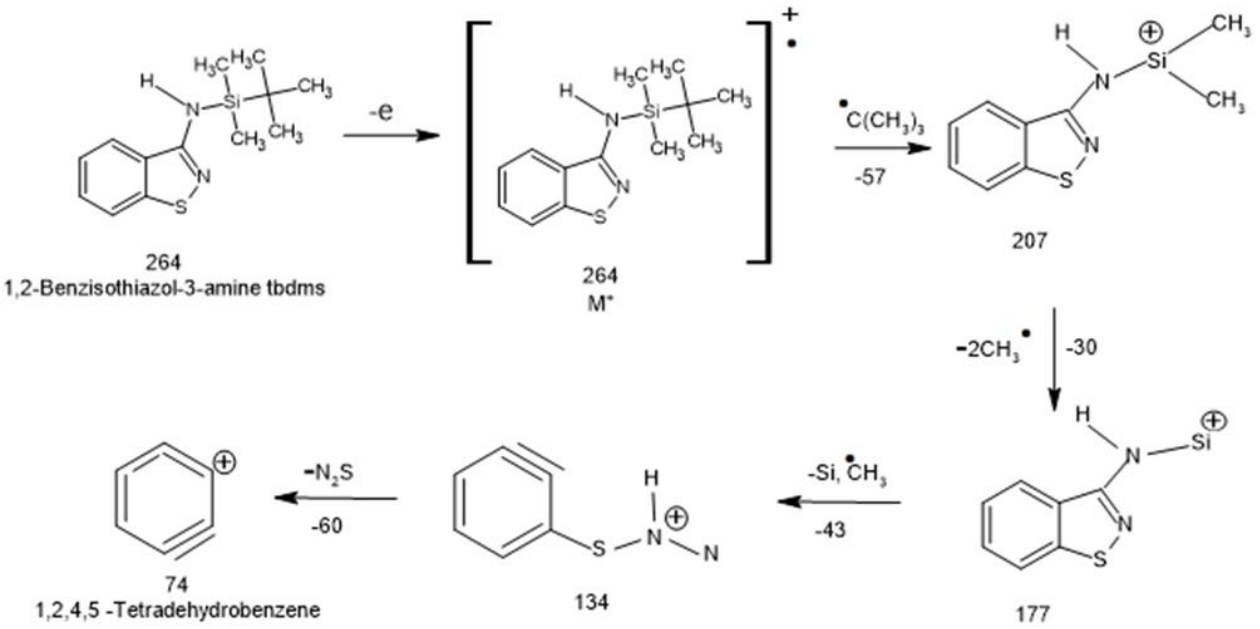

Figure 6 – MS fragmentation scheme of 1,2-Benzisothiazol-3-amine TBDMS isolated from AK3

High-Performance Liquid Chromatography (HPLC) of TA1 and TA2. The HPLC chromatogram was used to identify tannins. This was done by matching the retention time and absorption wavelength (UV-visible) of fractions (Figures 7 and 8) with that of standard (Figure 9) [16].

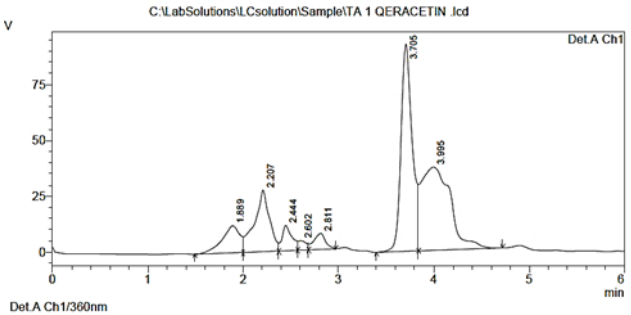

Figure 7 – HPLC Chromatogram of TA1

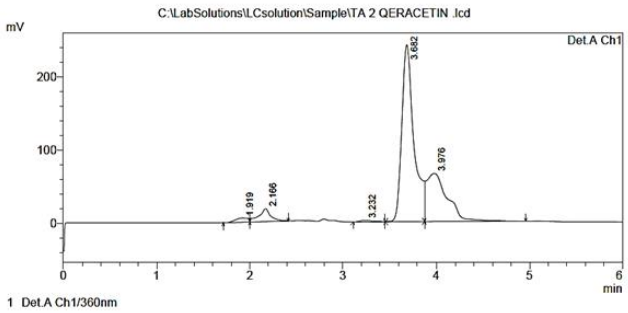

Figure 8 – HPLC Chromatogram of TA2

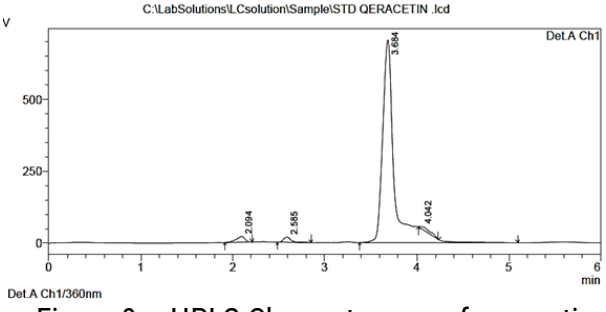

Figure 9 – HPLC Chromatogram of quercetin standard

HPLC Analysis of TA1 and Quercetin Standard. The peaks (with the given retention times) for quercetin are 2.094, 2.585, 3.684 (solid), and 4.042 minutes. The peaks for TA1 appeared at 2.207, 2.444, 3.705 (very strong) and 3.995 mins, respectively. The difference between the peaks in the chromatogram of quercetin standard and that of TA1 is 0.021, 0.141, 0.021 and 0.047 mins, respectively, as well as an additional peak with a retention time of 1.889 mins for TA1 might be attributed to the presence of impurity or lower phenolics in TA1 [16].

HPLC Analysis of TA2 and Quercetin Standard. The HPLC chromatogram of TA2 revealed five peaks, while the quercetin standard revealed four. At 3.682 mins, the chromatogram of TA2 revealed a peak that strongly matches that of the peak of quercetin standard at 3.884 mins.

Quercetin is an example of flavonoids, and flavonoids are units of condensed (polyflavonoids) tannins [17]. Quercetin standards and TA2 are both absorbed at the same wavelength of 360 nm on the UV-visible. Therefore, TA2 is identified as quercetin, a unit of condensed tannin.

CONCLUSIONS

This study confirms that the leaves of *Ficus citrifolia* contain alkaloids, one of which is 1,2-Benzisothiazol-3-amine and quercetin, a unit of condensed tannins. A literature review within our reach showed that an attempt has yet to be made to isolate and characterise alkaloids and tannins from the leaves of *Ficus citrifolia*. In this study, we isolated bioactive compounds and conducted a spectroscopic analysis of the leaves of *Ficus citrifolia.*

The outcome of this research is novel. Both isolated compounds could provide a new lead for synthesising and producing novel and health-friendly therapeutic agents.

More secondary metabolites could be isolated from the leaves *of Ficus citrifolia,* and complete chemical studies, including NMR and physicochemical analysis, could be conducted. Researchers could also perform pharmacological studies on the isolated compounds to fully understand their medicinal properties.

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