



Research Article

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# FT-IR or Raman Spectroscopy Identification of the Molecular Structure of *Ficus Ovata* Plant Parts Using Different Extraction Solvents



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## Abstract

The use of plants in herbal medicine has become very popular and well documented for many hundreds of plant species, and their extracts, are used in developing countries to treat numerous diseases despite the fact that only a small number are approved for therapeutic use by the FDA. Some species such as the *Ficus* genus have been used in the treatment of infectious diseases, abdominal pain and as anti-inflammatory agents. However, the cytotoxic effects of these plants have not been studied in detail, nor have their molecular structures been identified.

**Aims:** The aim of this study was to identify the molecular structure of a range of plant parts from a number of different species of the *Ficus* genus, in addition to other plant species, using both: Fourier Transform Infrared Spectroscopy (FT-IR) and Raman spectroscopy.

**Method:** Infrared spectra of stem, leaf, bark and trunk extracts (dissolved in a range of solvents) taken from each plant species were recorded, and the wave number of the dominant peak was obtained from the absorption spectra. Based on the components present in the sample, probable assignments of the bands were then made.

**Results:** FT-IR spectra of the extracts analysed revealed the wave number of the dominant peaks obtained from the absorption spectra of the various fractions isolated from each species.

**Conclusion:** FT-IR data obtained in this study also showed that many of the extracts analysed from the various species shared similar structures.

## Introduction

Plants have a vital role to play in maintaining the environment, and sustaining life on the earth. The primary role of green plants is to take in the carbon dioxide produced on earth, and use this to produce food to feed themselves, and produce oxygen to discharge so that it can be used by all other non-green organisms [1,2]. Indeed, if this photosynthetic process was interrupted on a large-scale basis, then life on earth would not survive, so plants play a role in the existence and survival of other organisms. In addition, by taking in carbon dioxide, plants also help to reduce levels of carbon dioxide in the atmosphere, hence reducing pollution levels. An additional role of plants is to act as a main component of food webs, as they provide food for herbivores, and ultimately carnivores, while also having a role to play in the economy, as plants can be used in food, medicine, transportation, construction and fuel [3-5].

For thousands of years, plants have been recognised to play a therapeutic role in curing diseases and infections, and currently,

there is a wide range of herbal remedies available in health food shops and other outlets, which many people are using to treat numerous problems, despite the fact that most herbal and plant remedies have not been approved for use by the FDA [6]. It is now realised that the therapeutic effects of plant products are mainly due to the phytochemicals found in these plants, as many are known to have a pharmacokinetic or pharmacodynamic interaction with certain drugs [7-9]. Because of these potential benefits of using plants in medicine, many species are now being analysed to identify the phytochemicals found in them.

Despite this, however, the safety of using plants in medicine is continuously being reviewed, and much work is being done to determine not only the efficacious effects of plants, but also their potential harmful effects [10]. Although it has been purported by some herbal remedies that the use of plants as herbal medicine is safe due to the fact that it is a "natural" substance, there have been a number of publications that indicate otherwise [11,12].

Some herbal medicines are known to have resulted in severe side-effects after ingestion, which may be due to the toxic properties of the herbs or plants used, while the interactions of the plants or herbal medicine with other drugs being used by the patient can also lead to adverse effects [3,12-15]. For example, a number of severe effects, including heart attack, stroke and even death have been reported following the use of products containing Ma huang (ephedrine) and kola nut due to the interaction of the caffeine in the kola nut and the ephedrine [16]. Numerous members of the *Ficus* species have been documented to be used for both food and medicine, and their use has been most widely documented in the Middle East, where many members of this species are known to grow.

### Raman Spectroscopy

A useful technique in chemical analysis involves the use of Raman spectroscopy to obtain a fingerprint spectrum of the materials present in a substance [17]. This method of analysis can be used to obtain a chemical and morphological profile of a biological sample, and has previously been used in a number of different applications, such as the visualisation of the molecular changes in the tissue composition of the poplar plant cell wall, to characterise heterotrophic biofilm, and to identify differences in the pollen carotenoid content between species [18-21]. Raman spectroscopy can also be used to analyse the chemical composition of plant cell walls, in order to identify the molecules that contribute to their composition [8,22].

Raman spectroscopy involves gathering information on the vibrational motions of atoms in molecules, and analysis of this data can provide information on the distribution of electrons in chemical bonds in the sample analysed, the molecular environment of the sample and its molecular conformation [2,23]. The principle behind this method is that a laser beam interacts with the molecule to be analysed, and thereby interacts with the bonds of the molecule, and its electron cloud [24]. The molecule is then excited to a virtual energy state from its normal ground state by a photon, and as the molecule relaxes it returns to a different rotational state, and in doing so emits a photon [1,25]. Because there is a difference in energy between the molecule's new state and the original state, there will be a shift away from the excitation wavelength of the frequency in the emitted photon [9,26]. Because energy is transferred between molecules and photons as they interact during this process, this type of analysis is an example of inelastic scattering (or more commonly referred to in this technique as Raman scattering)

**Table 1:** The fractions analyzed by Raman spectroscopy.

Species	Fraction	Exposure Time	No. of Accumulations	Laser Power
<i>F. lutea</i>	100% methanol (stem bark)	25 seconds	2	0.1%
<i>F. ovata</i>	CH <sub>2</sub> Cl <sub>2</sub> :MeOH (1:1) (stem bark)	25 seconds	3	0.1%
<i>F. polita</i>	Methanol (stem)	30 seconds	3	0.1%
<i>F. trichopoda</i>	100% ethyl acetate (trunk)	25 seconds	3	0.1%

[3,17]. The energy in this system must remain balanced, and as a result the emitted photon is shifted to a lower frequency if the final rotational state of the molecule is more energetic than that of its original state (referred to as the Stokes shift), while on the other hand if the final rotational state is less energetic than the initial state of the molecule, the emitted photon is shifted to a higher frequency (referred to as an anti-Stokes shift) [12,27].

### Methods & Experimental Design

**Plant Material:** A number of species from the *Ficus* genus, along with species from the *Dorstenia* and *Triumfetta* genus were tested during this study. All plant species were collected from the jungle in Cameroon. The plant parts were isolated and the fractions tested.

**FT-IR Spectroscopy:** The FT-IR spectra of the various plant fractions were recorded using a Perkin-Elmer Spectrum One Universal ATR Sampling Accessory spectrometer in the region 4000-650 cm<sup>-1</sup>. [28].

**Raman Spectroscopy:** Raman measurements were obtained using a Reinshaw in Via Raman microscope. This system is based upon an upright microscope frame with a spectrometer attached. The laser provided power to the sample, through a 40 x objective. Each sample was read at a wavelength of 785nm. The fractions analysed by Raman spectroscopy, and the settings used for each, are outlined in Table 1.

**Statistical Analysis:** Absorbance levels for control samples were compared to the absorbance levels obtained for each concentration of extract used to determine if there were any significant differences between absorbance levels. In addition, results obtained for the concentrations of the extracts used were compared to each other to determine if there were any significant differences between the different concentrations.

### Results

The FT-IR spectra of the plant parts like the leaf, stem, bark and root of the plant species *F. lutea*, *F. polita*, *F. trichopoda*, *F. ovata*, *T. rhomboidea* and *D. elleptica* are shown in Figures 1-5. A number of fractions from the bark of the stem of *F. ovata* were analyzed by FT-IR and it was found that both the hexane and methanol fractions analyzed were similar in structure. The wave number of the dominant peaks obtained from the absorption spectra of each of the fractions analysed from each species are presented in Tables 1-7.

<i>T. rhomboidea</i>	Hexane (stem)	25 seconds	2	0.1%
<i>D. elliptica</i>	Methoxyumbelliform molecule	25 seconds	3	0.1%

**Table 2:** Wave number of the dominant peaks obtained from the absorption spectra of the different fractions isolated from *F. lutea*. \*Fraction incubated on HepG2 cells to determine cytotoxicity.

Functional Groups	Stem Bark	Stem Bark	*Stem Bark	Trunk	Trunk	Trunk
	Hexane Fraction	Hexane Ethyl Acetate Fraction 50%	Methanol Fraction 100%	Hexane Ethyl Acetate Fraction 50%	Ethyl Acetate Methanol Fraction 50%	Methanol Fraction 100%
O-H	3290	3291	3232			
C-H	2919	2919		2927	2930	
C=O	1707	1706		1701		
C=C	1603	1603	1605	1516		
C-NO <sub>2</sub>	1378	1378		1377		
C-NO <sub>2</sub> (asym)						1582
CC	1254	1254		1151	1228	
CH <sub>2</sub>			1441	1457		
C=S			1248	1029	1031	
C-O-C			1104			1608
N-H				3396	3331	3338
C=N					1630	

**Table 3:** Wave number of the dominant peak obtained from the absorption spectra of the different fractions isolated from *F. ovata*. \*Fraction incubated on HepG2 cells to determine cytotoxicity.

Functional Groups	Bark of Stem	*Bark of Stem	Bark of Stem	Bark of Stem	Bark of Stem
	Hexane Fraction 50%	Methanol Fraction	CH <sub>2</sub> Cl <sub>2</sub> -MeOH Fraction (1:1)	CH <sub>2</sub> Cl <sub>2</sub> Fraction	Pyridine Fraction
N-H	3331	3328	3342	3362	
C-H	2917	2920	2927	2851	2854
C=N			1631		
C=O		1724		1710	1708
C=C	1595	1599			
CH <sub>2</sub>		1458		1458	1457
C-NO <sub>2</sub>	1377	1378	1347	1377	1377
CC	1266	1268		1268	
C-O-C (asym)	1070	1120			
C=S			1042	1029	1028
C-S (aliphatic)		741			721

**Table 4:** Wave number of the dominant peaks obtained from the absorption spectra of the various fractions isolated from *F. trichopoda*. \*Fraction incubated on HepG2 cells to determine cytotoxicity.

Functional Groups	Stem Bark	Stem Bark	*Stem Bark	Trunk
	Crude Extract Fraction	Hexane Ethyl Acetate Fraction 50%	Ethyl Acetate Fraction 100%	Crude Extract Fraction
N-H		3372	3340	
O-H	3249			3247
C-H		2924	2919	2934
C=O		1707	1707	
N=N (aromatic)	1439			
C=C			1607	1604

CH <sub>2</sub>		1453	1445	1439
C-NO <sub>2</sub>		1378	1375	
C=S	1024	1167		1245
CC	1281		1280	
C-O-C (asym)			1107	
C-O-C	825			
C-S aliphatic			767	766

**Table 5:** Wave number of the dominant peaks obtained from the absorption spectra of the various fractions isolated from *F. polita* \*Fraction incubated on HepG2 cells to determine cytotoxicity

Functional Groups	*Leaf	Stem	Stem	Stem	Root
	Crude Extract Methanol Fraction	Methanol Fraction	Hexane Ethyl Acetate Fraction 75 – 25%	Hexane Ethyl Acetate Fraction 25 – 75%	Crude Extract Methanol Fraction
N-H	3377	3340	3368	3380	3357
C-H	2850	2919	2926	2919	2851
C=O	1712	1707	1710	1708	1710
C=C		1607	1637	1515	1605
CH <sub>2</sub>	1462	1445	1451	1452	1458
C-NO <sub>2</sub>	1376	1375	1375	1376	1376
CC		1280			
C-O-C (asym)		1107			
C=S	1243		1245	1168	1243
C-S (aliphatic)	719	767	767		

**Table 6:** Wave number of the dominant peaks obtained from the absorption spectra of the various fractions isolated from *T. rhomboidea*.

Functional Groups	Stem	Stem	Stem	Stem	*Leaf
	Hexane Fraction	Acetyl Ethane Fraction	n-Butanol Fraction	Crude Extract Methanol Fraction	Crude Extract Methanol Fraction
N-H	3337	3352		3302	3348
O-H			3259		
C-H	2850	2851	2920	2920	2919
C=O	1710	1731			1709
C-NO <sub>2</sub> (asym)	1541				
C=C		1603	1603	1603	
CH <sub>2</sub>	1464	1460			1454
C-NO <sub>2</sub>	1377	1377		1377	1376
CC		1268			
C=S	1051	1120			1164
C-O-C		818			
C-S (aliphatic)	720	719			

**Table 7:** Wave number of the dominant peaks obtained from *D. elliptica* \* Fraction incubated on HepG2 cells to determine cytotoxicity, + No details specified on the parts of the plant from where each fraction was isolated.

Functional Groups	*+DE1	+DE6	+DE13	+DE14	+Methoxyumbelliform
	O-H	3117	3116	3115	3123

C-H	2972	2921	2983	2891	2974
C=O	1725	1715	1703	1719	1703
C=C	1587			1546	
C=N					1611
CH <sub>2</sub>	1455			1456	
C-NO <sub>2</sub>	1359	1342	1342	1341	1351
C-O-C (asym)	1099	1082	1082		1122
C-O-C		838	939	825	838
C-S (aliphatic)	760	746	747	750	750
N-H		3436	3436	3413	
N=N (aliphatic)		1576	1576		1557

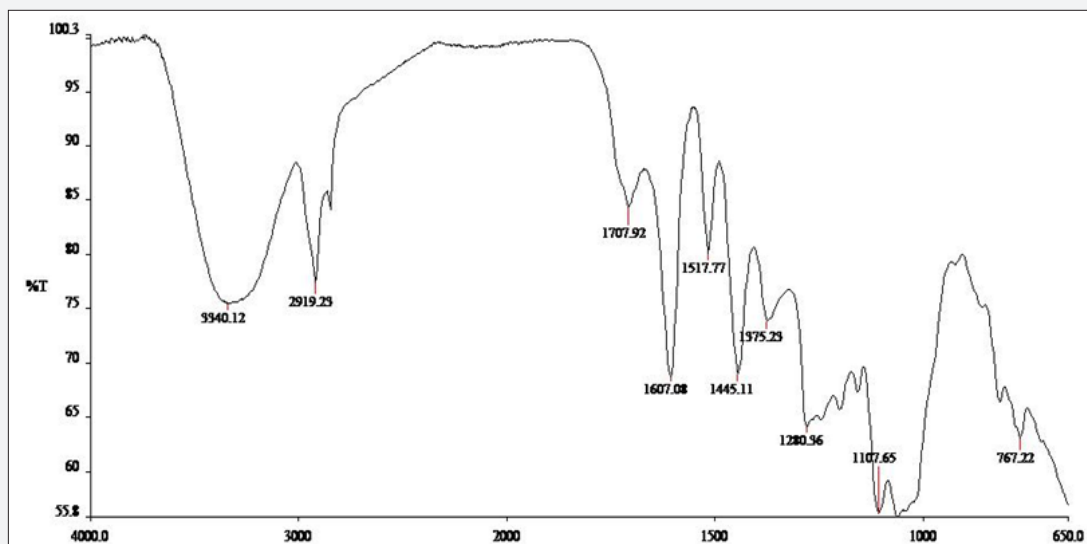


Figure 1: FT-IR spectrum of the methanol crude extract isolated from the leaf of *F. polita*.

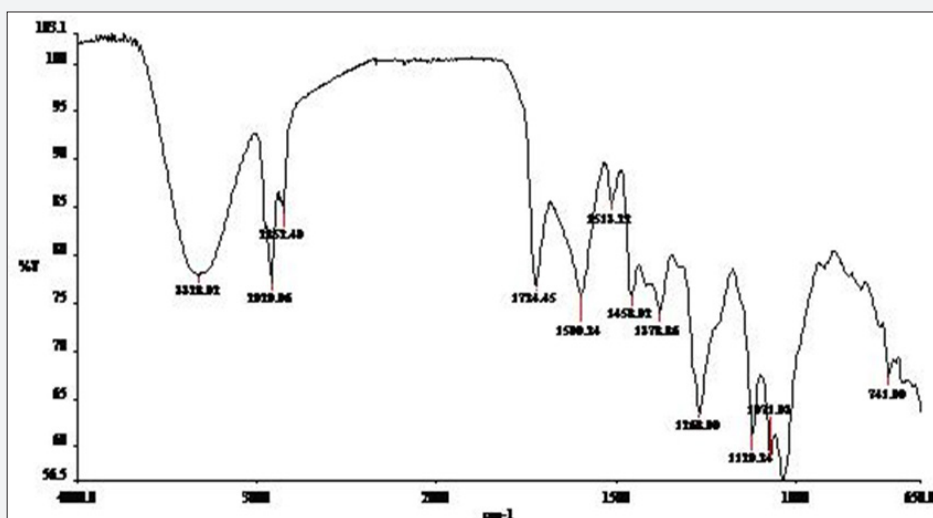


Figure 2 : FT-IR 2. spectrum of the 100% ethyl acetate fraction of the stem bark of *F. trichopoda*.

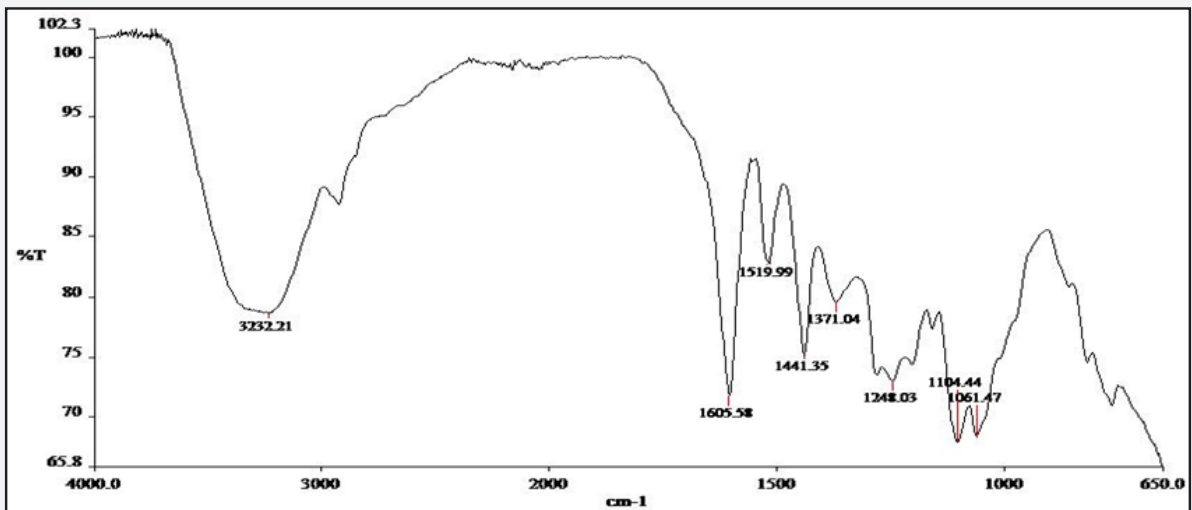


Figure 3 : FT-IR 3. spectrum of the methanol fraction of isolated from the bark of the stem of *F. ovata*.

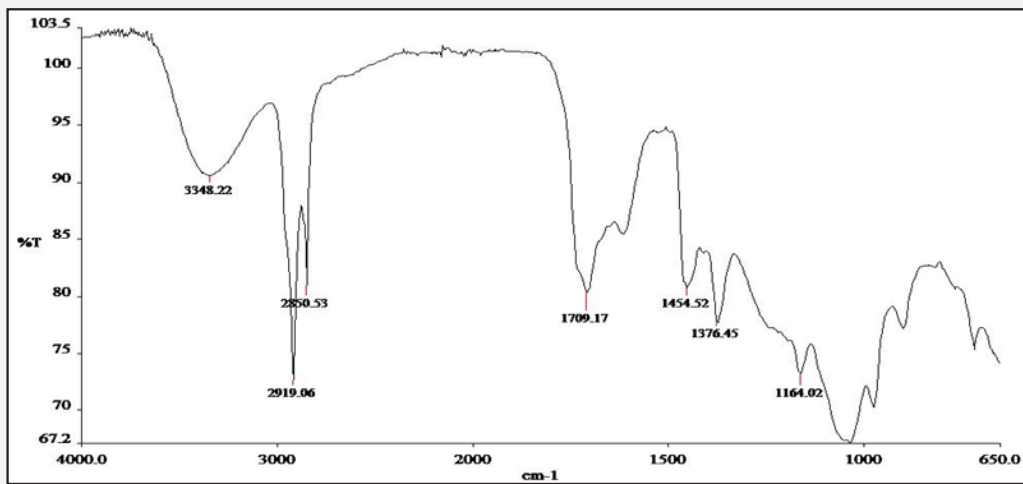


Figure 4 : FT-IR spectrum of the 100% methanol fraction isolated from the stem bark of *F. lutea*.

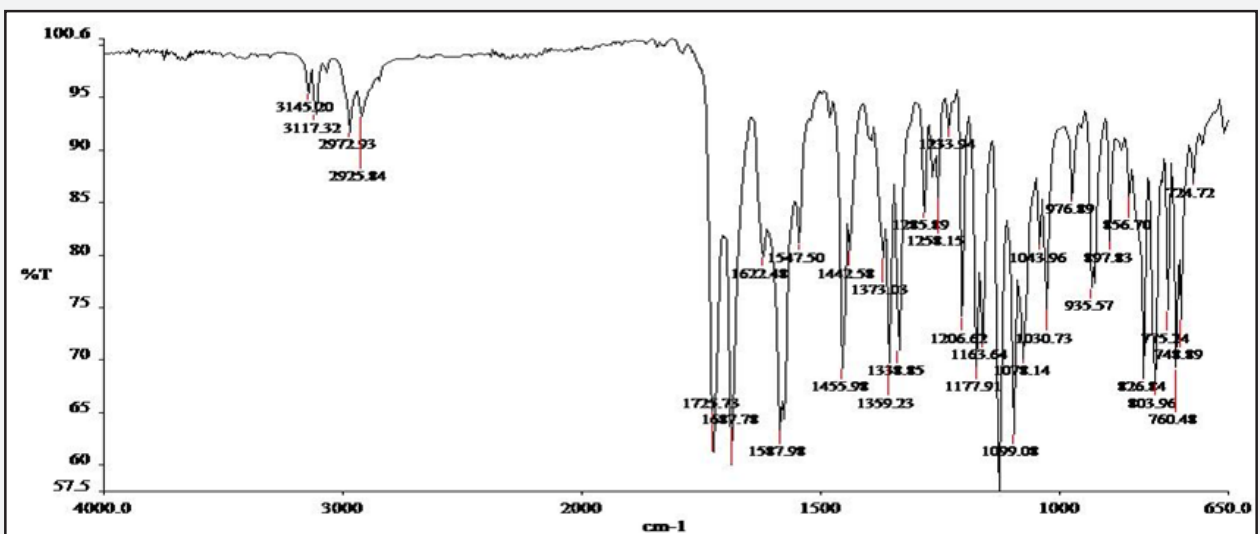


Figure 5 : FT-IR 5. Spectrum of the methanol crude extract isolated from the leaf of *T. rhomboidea*.

### Analysis of Wave Number of Dominant Peak from Various Plant Fractions

For *F. lutea*, a number of fractions of both the stem bark and trunk were analysed by FT-IR and the wave number of the dominant peak obtained from the various fractions were outlined in Table 2. For each fraction of the stem bark analysed, a strong absorption band around 3232-3291 was observed, and may be due to the presence of an O-H group. In addition, each fraction of the stem bark analysed for *F. lutea* also showed a C=C group present. For both the hexane fraction, and hexane ethyl acetate fraction of the stem bark of *F. lutea*, very similar functional groups were observed, with results indicating that a C-H, C=O, and a C-NO<sub>2</sub> group are present in both fractions. The 100% methanol fraction of the stem bark of *F. lutea* was shown to contain a CH<sub>2</sub>, C=S and C-O-C group. A number of fractions of the trunk of *F. lutea* were also analysed by FT-IR. Absorption spectra obtained from various fractions analysed are outlined in Table 7. The functional groups common to both the hexane ethyl acetate fraction and ethyl acetate methanol fraction include a C-H group and N-H group. Results also indicate that for the hexane ethyl acetate fraction of the trunk of a C=C, C=O, C-NO<sub>2</sub>, CH<sub>2</sub>, and C=S group was also present, while FT-IR results for the ethyl acetate methanol fraction of the trunk showed a C=N and C=S group present. A methanol fraction of the trunk of *F. lutea* was also analysed, and results indicate that the functional groups present include a C-O-C, N-H and C-NO<sub>2</sub>.

The bark of the stem of *F. ovata* was isolated and analysed by FT-IR. A number of fractions were analysed and the wave numbers of the dominant peaks are outlined in Table 3. The functional groups common to each fraction include the C-H and C-NO<sub>2</sub> group. The functional groups present in the hexane fraction of the bark of the stem of the *F. ovata* include the N-H, C=C, C-NO<sub>2</sub> and C-O-C asym group, while in the methanol fraction of the bark of the stem of *F. ovata* FT-IR results indicate that N-H, C=O, C=C, CH<sub>2</sub>, C-NO<sub>2</sub> and C-O-C asym groups are present. A CH<sub>2</sub>Cl<sub>2</sub>-MeOH fraction of the bark of the stem of *F. ovata* was also analysed, and the functional groups detected include the N-H, C=N, C-NO<sub>2</sub> and C=S groups, while in the CH<sub>2</sub>Cl<sub>2</sub> fraction of the bark of the stem analysed showed N-H, C=O, CH<sub>2</sub>, C-NO<sub>2</sub> and C=S present. In addition, a pyridine fraction of the bark of the stem of *F. ovata* was also analysed and results indicate that C=O, CH<sub>2</sub>, C-NO<sub>2</sub> and C=S are present.

The wave numbers of the dominant peaks obtained from the various fractions obtained for *F. trichopoda* are outlined in Table 4. For both the hexane ethyl acetate fraction and ethyl acetate fraction of the stem bark of *F. trichopoda*, similar functional groups including the N-H, C-H, C=O, CH<sub>2</sub>, and C-NO<sub>2</sub> being detected in both. The crude extract fraction of *F. trichopoda* that was analysed showed that the O-H, N=N (aromatic), C=S and C-O-C groups were present. In addition, the crude extract of the trunk of *F. trichopoda* was also analysed and the functional groups detected include the O-H, C-H, C=C, CH<sub>2</sub> and C=S groups.

Fractions of the leaf, stem and root of *F. polita* was analysed by FT-IR and the wave numbers of the dominant peaks obtained are outlined in Table 5. A number of functional groups were found to be common in each of the fractions analysed, including the N-H, C-H, C=O, CH<sub>2</sub>, and C-NO<sub>2</sub> groups. In addition to each of these groups, C=S and C-S aliphatic groups were also detected in the crude extract of the leaf of *F. polita* which was analysed, and a C=S group was also detected in the crude extract of the root of *F. polita*. In addition to each of the common functional groups detected, a C=S group was detected in each of the hexane ethyl acetate fractions (75 - 25% and 25 - 75%) of the stem of *F. polita*, with a C-S aliphatic group being detected in the methanol and hexane ethyl acetate (75 - 25%) fractions of the stem of *F. polita*.

A number of extracts of *T. rhomboidea* were analysed by FT-IR and the wave number of the dominant peaks obtained are outlined in Table 6. A number of functional groups were found to be common between both the hexane and acetyl ethane fractions of the stem of *T. rhomboidea* that were analysed, including N-H, C-H, C=O, CH<sub>2</sub>, C-NO<sub>2</sub> and C-S aliphatic groups. A number of functional groups were also found to be common between the n-butanol and crude extract methanol fraction of the stem including the C-H and C=C groups. An O-H group was also detected in the n-butanol fraction of the stem and a C-NO<sub>2</sub> detected in the crude extract methanol fraction of the stem of *T. rhomboidea*. A crude extract methanol fraction of the leaf of *T. rhomboidea* was also analysed and it was found that N-H, C-H, C=O, CH<sub>2</sub>, C-NO<sub>2</sub> and C=S were present.

The wave numbers of the dominant peaks of the various fractions of *D. elliptica* that were analysed are outlined in Table 7. The functional groups that were found to be common between each fraction analysed include the C-H, C=O, C-NO<sub>2</sub>, C-O-C and C-S aliphatic groups. In addition, an O-H group was detected in all fractions tested apart from the methoxyumbelli form fraction. A C-O-C asym group was also detected in all fractions apart from the DE14 fraction, and a C-O-C group detected in all fractions apart from the DE1 fraction. An N-H group was detected in the DE6, DE13 and DE14 fractions, while an N=N aliphatic functional group was detected in the DE6, DE13 and methoxyumbelli form fractions.

### Discussion

The FT-IR spectrum of each plant fraction was obtained, and the functional groups present in each fraction were then compared. The trunk and the stem bark were isolated from *F. lutea*, and various fractions of each were analysed by FT-IR. It was found that for both the hexane and hexane ethyl acetate fraction for the stem bark of *F. lutea*, very similar functional groups were present, indicating that these fractions are very similar in structure. Both of these fractions contain O-H, C=O, C=C and C-NO<sub>2</sub> but the methanol fraction of the stem bark of *F. lutea* appears different in structure as it contains the functional groups CH<sub>2</sub>, C=S and C-O-C. Three fractions of the trunk of *F. lutea* were also analysed, but little similarity was observed between

each of these fractions, with the hexane ethyl acetate fraction containing C-H, C=O, C=C, C-NO<sub>2</sub>, CH<sub>2</sub> and C=S groups, the ethyl acetate methanol fraction containing C-H, C=S and C=N groups and the methanol fraction containing C-O-C and N-H groups.

Raman spectroscopy has been used to characterize pollen carotenoids with in situ and high-performance thin-layer chromatography supported resonant [28]. A number of fractions from the bark of the stem of *F. ovata* were analysed by FT-IR and it was found that both the hexane and methanol fractions analysed were similar in structure, as both were found to contain N-H, C-H, C=C, C-NO<sub>2</sub> and C-O-C asym groups. The CH<sub>2</sub>Cl<sub>2</sub> and pyridine fractions were also found to be similar, both containing C-H, C=O, CH<sub>2</sub>, C-NO<sub>2</sub> and C=S groups. The CH<sub>2</sub>Cl<sub>2</sub>-MeOH fraction of the bark of the stem of *F. ovata* was found to contain the functional groups C-H, C=N, C-NO<sub>2</sub> and C=S groups so its structure appears different to the other fractions of this species. Extracts of the leaf, stem and root of *F. polita* were analysed by FT-IR. Each of the fractions analysed for the stem showed similar structures, containing the functional groups N-H, C-H, C=O, C=C, CH<sub>2</sub>, and C-NO<sub>2</sub> groups. The crude extract methanol fraction of the leaf that was analysed was found to contain the functional groups N-H, C-H, C=O, CH<sub>2</sub>, C-NO<sub>2</sub> and C=S while the crude extract methanol fraction of the root contains the groups N-H, C-H, C=O, C=C, CH<sub>2</sub>, and C=S groups.

For *F. trichopoda* the FT-IR spectrum of different fractions of the trunk and bark of the stem were examined. The crude extract fraction of the trunk was analysed, and the O-H, C-H, C=C, CH<sub>2</sub> and C=S functional groups were found to be present. A number of functional groups were found to be shared between the hexane ethyl acetate and ethyl acetate fractions of the stem bark, including the N-H, C-H, C=O, CH<sub>2</sub>, and C-NO<sub>2</sub> groups, indicating both these fractions have similar structures. The FT-IR spectrum of the crude extract fraction of the stem bark which was obtained showed that the functional groups O-H, N=N (aromatic), C=S and C-O-C are present, indicating that this fraction is somewhat different in structure to the other fractions of the stem bark. Determination of glucose and ethanol after enzymatic hydrolysis and fermentation of biomass has been elucidated using Raman spectroscopy [29,30].

*T. rhomboidea* was also analysed by FT-IR, and the FT-IR spectrum obtained for both the hexane and acetyl ethane fractions of the stem indicates that each of these fractions are very similar in structure as both share the functional groups N-H, C-H, C=O, CH<sub>2</sub> and C=S groups. Analysis of the n-butanol fraction of the stem was shown to contain the groups O-H, C-H and C=C, while the crude extract methanol fraction contains the N-H, C-H, C=C and C-NO<sub>2</sub> groups. The crude extract methanol fraction of the leaf of *T. rhomboidea* was shown to contain the groups N-H, C-H, C=O, CH<sub>2</sub> and C=S. A number of extracts of *D. elliptica* were also analysed by FT-IR. Analysis of the spectra of the DE6, DE13 and DE14 fractions showed that each of these fractions share very similar structures due to the functional groups that were

common between each fraction. The DE1 fraction was also analysed and showed that O-H, C-H, C=O, C=C, CH<sub>2</sub> and C-NO<sub>2</sub> groups are present, while in the methoxyumbelli form fraction it was found that C-H, C=O, C=N, C-NO<sub>2</sub> and C-O-C groups were present. One sample from each plant species was also analysed by Raman spectroscopy, and a Raman spectra was obtained for each of the fractions analysed.

## Conclusion

FT-IR data obtained in this study showed that many of the extracts analysed from the various species shared similar structures. It was found that for both the hexane and hexane ethyl acetate fraction for the stem bark of *F. lutea*, very similar functional groups were present, indicating that these fractions were very similar in structure. This study also showed that Raman spectroscopy is a difficult method to establish, and it requires much time to optimize the correct procedures for its use in the analysis of the molecular structures of plant fractions. However, FT-IR is a suitable method for the analysis of the functional groups present in different fractions of plant species, and it allows comparisons of the molecular structures of different plant fractions to be made.

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