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Fourier Transformer Infra-Red Spectrophotometer Analysis of *Warburgia ugandensis* Medicinal Herb Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya

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Abstract: Medicinal herbs are an important source of phytochemicals that offer traditional medicinal treatment of various ailments. In Kisii region, southwest Kenya, *Warburgia ugandensis* is amongst the herbs used as phytomedicine for the treatment of diabetes, malaria and pneumonia. A study was carried out on the plant in the year 2011 to 2012. The objective of this study was to identify the functional groups present in *Warburgia ugandensis* by Fourier transformer infra-red (FTIR) Spectrophotometer method of analysis. Results of the FTIR Spectra of the hexane, dichloromethane, ethyl acetate and ethanol crude extracts revealed the presence of different functional groups as follows: OH stretching for hydroxyl (3460.2-3359.8 cm^{-1}), C=O stretching for carbonyls (1751.2-1643.2 cm^{-1}), C-O stretching for alcohols (1450.4 -1049.2 cm^{-1}), carboxylic acid (1458.9-1242.1 cm^{-1}), carboxylic anhydrides (1253.6-1049.2 cm^{-1}), ethers (1242.1-1049.2 cm^{-1}), esters (1234.4-1049.2 cm^{-1}), C-N stretching for amines (1253.6-1049.2cm^{-1}), N-H stretching for amines (3460.1-3359.8 cm^{-1}), amides (3444.6-3359.8 cm^{-1}), C=C stretching for aromatic (1643.2-1531.4 cm^{-1}), C=N stretching for nitriles (2356.9-2252.7 cm^{-1}), N=C stretching for isocyanides (2137.0 -2090.7cm^{-1}), C-H stretching for alkyl (2981.7-2931.6 cm^{-1}), C-H bending for alkyl (1377.1 cm^{-1}), C-H bending for methyl (1458.1-1450.4 cm^{-1}), S=O stretching for sulphur derivatives 1384.8-1049.2 cm^{-1}), N=O stretching for nitro compounds (1377.1-1330.8 cm^{-1}), C-F stretching for organic halogens (1253.6-1049.2 cm^{-1}), C-Cl stretching (736.8-621.0 cm^{-1}), C-Br stretching (663.5 cm^{-1}) and C-I stretching (663.5 cm^{-1}). These findings indicate the presence of aldehydes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers, nitriles, isonitriles, nitro compounds, sulphur derivatives, quinones, organic halogen compounds and carbohydrates in *Warburgia ugandensis*. The medicinal value of the herb could be attributed to the presence of O-H, N-H, C-H, C=O, C-O, C-N, C=C, C=N, N=C, N=O and S=O bond stretching of the detected functional groups. The results confirm the presence of secondary plant metabolites *viz.,* alkaloids, saponins, tannins, flavonoids, steroids and terpenes, polyphenols and cardiac glycosides in the leaves of *Warburgia ugandensis*. The results confirm the presence of secondary plant metabolites *viz.,* alkaloids, saponins, tannins, flavonoids, steroids and terpenes, polyphenols and cardiac glycosides in the leaves of *Warburgia ugandensis*.

Key words: Medicinal Herbs · FTIR Spectra · Functional Groups

INTRODUCTION

Medicinal herbs are used in traditional medicine to cure various diseases. They consist of a number of biologically active ingredients therefore they are used for the treatment of a large number of infectious diseases [1, 2]. These biologically active ingredients are alkaloids, flavonoids steroids, glycosides, Terpenes, tannins and phenolic, compounds [3, 4]. They serve as source of medicine, ornamental purposes, flavouring, food additives and preservatives [5, 6]. The use of herbs in the treatment and management of diseases and disorders dates back to pre-historic days [7]. Plant extracts have been used in medical practices for the treatment of various ailments

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since ancient times [8]. The medicinal properties of various plant material and extracts have been recognized since the beginning of the 5th century [9]. Medicinal plants exist everywhere especially in Africa which offers an immense reservoir of plants that has been characterized [10]. World Health Organization (WHO) [11] described a plant as one or more with organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract [12,13]. In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds [12, 13].

The objective of this study was to identify the functional groups present in *Warburgia ugandensis* by Fourier Transformer Infra-red (FTIR) Spectrophotometer method.

MATERIALS AND METHODS

Sample Collection and Preparation: In this study the leaves of the *Warburgia ugandensis,* was collected from Kisii region, southwest Kenya. The verification of the herbal species was done by the Botanist; Egerton University. The leaves of the validated medicinal herb was then collected from their site in Kisii region and air-dried for twelve weeks to obtain constant weight. The dried sample was cut into smaller pieces and then ground into fine powder with a grinder in the Department of Food Science and Technology, Faculty of Science, Jomo Kenyatta University of Agriculture and Technology. The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

Preparation of Extracts: In order to perform FTIR analysis, thirty grams of powdered leaf samples of *Warburgia ugandensis* was extract with hexane, dichloromethane, ethyl acetate and ethanol solvents, shaken for 5 hours then kept at room temperature for 24 hours in closed 250 ml conical flask containers. The extraction process was repeated 3 times (extraction for 3 days). Then the extracts were filtered under vacuum and concentrated at reduced pressure using a rotary evaporator. The dried extracts were kept in the refrigerator at 4°C until use.

Infrared Spectroscopy Analysis: The samples of *Warburgia ugandensis* was analysed using Fourier Transformed Infrared (FTIR) Spectrophotometer model 8400 at the Chemistry Department of the JKUAT University. About 0.02 g of the air-dried samples was dissolved in hexane, dichloromethane, ethyl acetate and ethanol in different 10 ml volumetric flasks. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the FT IR and scanned through the IR region. The herbal functional groups were determined.

Data Collected: The percentage of yield extracts and the FTIR spectra of the hexane, dichloromethane, ethyl acetate and ethanol of *Warburgia ugandensis* herb was determined and recorded.

Data Analysis: The calculated t value was obtained by comparing the sum of positive (+) and negative (-) results. The critical t value is obtained from significant test table using number of samples. Differences between the critical t-value and calculated t-values of the bioactive compounds of the herbal extracts were computed. The null hypothesis was retained because the calculated t-value was more than the critical t-value at $p \leq 0.05$.

RESULTS AND DISCUSSION

In the following subsections, the results obtained for the infrared analyses are presented and discussed.

Percentage Yield: Results obtained show that the *Warburgia ugandensis* leaf extract yield was higher when ethanol was used as the extracting solvent (Table 1). However; dichloromethane extract yield was second when dichloromethane was used as the extracting solvent. On the other hand, the results also indicated that there was variation in yield with other solvents used for extraction. The differences in the extract yields from the extracted plant materials in the present analysis might be attributed to the different availability of extractable components, resulting from the varied chemical composition of plants [14].

Infrared Spectroscopy Analysis: The FTIR spectroscopic analysis showed the presence of phytoconstituants (Table 2, Figures1-4). The FTIR gives broad peaks at 3359.8, 3440.8, 3444.6 and 3460.1 cm^{-1} which indicated the presence of OH stretching [15]. The peak obtained at the 3460.1, 3444.6, 3440.8, 3363.6 and 3193.9 cm^{-1} indicated

Table 1: Shows percentage of yield extract

Extraction	Percentage of yield extract (%)			
Part of plant / solvent	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
Warburgia ugandensis	1.94	3.65	1.66	3.69

Table 2: FTIR spectra analysis of *Warburgia ugandensis*

Warburgia ugandensis		Absorption spectrum, Frequency (cm^{-1})			
Functional groups	Component (Peaks)	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
Alkanes	C-H (Stretch)	2943.2, 2873.7	2931.6	2981.7	2981.7
	-CH_2-(bend)	1458.1	1450.4	1454.2	1454.2
	-CH_3 (bend)	1377.1	1377.1	1377.1	1377.1
Alkenes	C-H (Stretch)	2943.2	2931.6	-	-
	C=C(Stretch)	1643.2, 1527.5	1643.2, 1531.4	-	-
	=C-H (bend)	-	-	-	-
Aromatics rings	=C-H(bend)	3444.6	-	-	-
	C=C(Stretch)	1643.2, 1527.5	1643.2, 1531.4,	-	-
	=C-H(Stretch)	732.9	736.8	-	-
	=C-H(bend)	-	-	-	-
Aldehydes	C=O (stretch)	1732.0	-	-	-
	C-H (Stretch)	2731.0, 2669.3	-	-	-
Ketones	C=O(Stretch)	1643.2, 1732.0	1643.2	1751.0	1654.8
Quinones	O-H(stretch	-	3440.8	-	3359.8
	C=O(stretch)	-	1643.2	-	1654.8
Esters and Lactones	C=O(stretch)	-	1728.1, 1643.2	1751.2	1654.8
	C-O(stretch)	-	1253.6	1242.1	1330.8
Carboxylic acids and their salts	O-H(stretch)	3444.6	3440.8	3460.1	3359.8
	C=O (stretch)	1643.2	1728.1, 1643.2	1751.2	1654.8
	C-O(stretch)	1458.1	1253.6, 1377.1	1242.1	1330.8
Carboxylic acid anhydrides	C=O(stretch)	-	1728.1	1751.2	-
	C-O (stretch)	-	1253.6	1242.1, 1049.2	-
Ammonium salts	N-H_4^+(stretch)	3444.6	3440.8	3460.1	3359.8
	C=O (stretch)	1458.1	1643.2	1377.1	1654.8
Esters	C=O (stretch)	1732.0	1728.1	1751.2	-
	C-O(stretch)	1149.5, 1234.4	1253.6, 1118.6, 1049.2	1242.1, 1049.2	-
Amides	C=O (stretch)	1643.2	1643.2	-	1654.8
	N-H(stretch)	3444.6	3440.8	-	3359.8
	N-H(bend)	1643.2, 1527.5	1643.3, 1531.4	-	1654.8
Anhydride	C=O (stretch)	-	-	1886.3	-
	C-O (stretch)	-	-	1242.1, 1049.2, 929.6	-
Alcohols	O-H(stretch)	3444.6	3440.8	3460.1	3359.8
Free	C-O(stretch)	-	-	-	-

Table 2: Continue

Warburgia ugandensis Functional groups	Absorption spectrum, Frequency (cm^{-1}) Component (Peaks)	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
H-Bonded	C-O(stretch)	1149.8, 1377.1	1253.6, 1377.1, 1450.4	1049.2, 1377.1, 1454.2	1049.2, 1384.8,
Carbohydrates	O-H(stretch)	-	-	3460.1	3359.8
	C-O(stretch)	-	-	1049.2	1049.2
Phenols	O-H(stretch)	3444.6	3440.8	3460.1	3359.8
Free	C-O(stretch)	-	-	-	-
H-Bonded	C-O(stretch)	1377.1	1377.1	1242.1, 1377.1	1384.8
Ethers	C-O (stretch)	-	-	1242.1, 1049.2	1049.2
1° and 2° amines	N-H(stretch)	3444.6, 3193.9	3440.8	3460.1	3359.8
	C-N(stretch)	1234.4, 1149.5	1253.6	1049.2, 1242.1	1049.2
	N-H(bend)	1643.2, 1531.4	1643.2, 1531.4	1569.9, 848.6	1654.8
Nitriles	C=N (stretch)	-	2302.8	2356.9	2252.7
Isocyanides	N=C (stretch)	-	2090.7	2090.7	2137.0
Nitro compounds	N=O	1377.1	-	1377.1	1384.8, 1330.8
Mercaptans, Thiophines, Thiolacids	S-H(stretch)	2611.4	-	-	2538.1
Sulphides,	C-S(stretch)	-	-	-	663.5,
Disulphides,	-S-S-(stretch)	-	-	-	432.0
Thioacids	C=S(stretch)	-	-	-	1049.2
Sulphonic acids	O-H(stretch) of SO_3H	-	-	3460.1	-
	S=O(stretch)	-	-	1242.1	-
Sulphonate esters	S=O(stretch)	-	-	1242.1	1384.8
Sulphate esters and salts	S=O(stretch)	-	-	1242.1	1384.8
Thioamides and Thioureas	N-H (stretch)	-	-	-	3359.8
	C=S (stretch)	-	-	-	1384.8
Sulphonamides	N-H(stretch)	-	-	-	3359.8
	S=O(stretch)	-	-	-	1384.8
Sulphoxides	S=O(stretch)	-	-	1049.2	1049.2
Halides	C-F	-	1253.6	1049.2	1049.2
	C-Cl	-	736.8	1242.1	621.0
	C-Br	-	-	-	663.5
	C-I	-	-	-	663.5

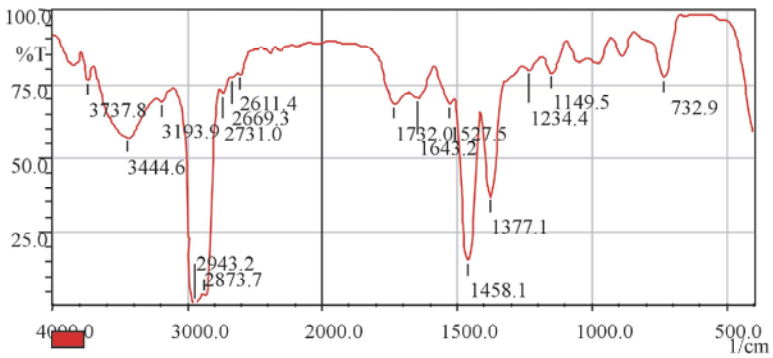

Fig. 1: FTIR spectrum hexane crude extract of *Warburgia ugandensis*.

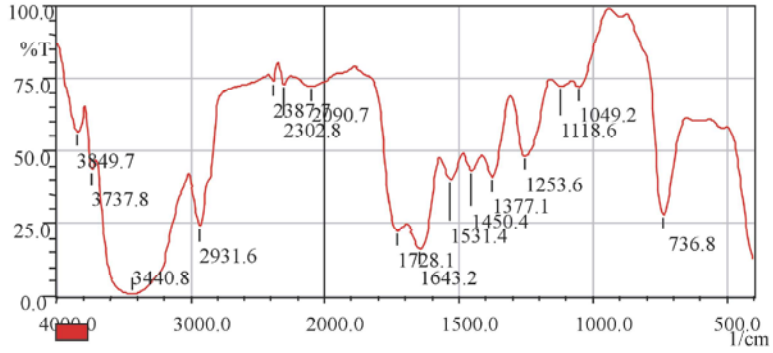

Fig. 2: FTIR spectrum dichloromethane crude extract of *Warburgia ugandensis*.

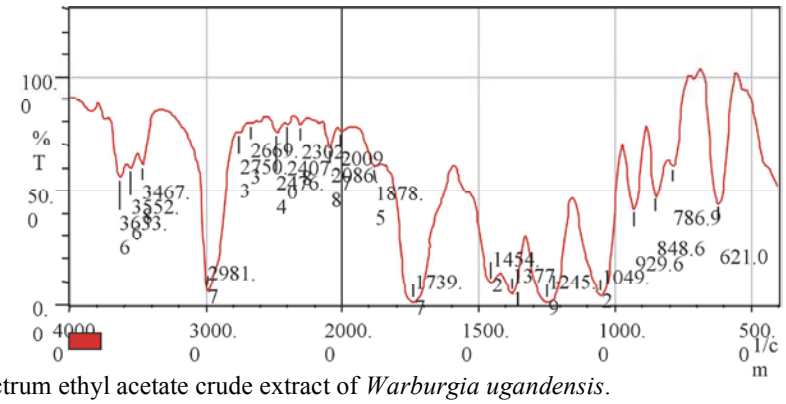

Fig. 4 35: FTIR spectrum ethyl acetate crude extract of *Warburgia ugandensis*.

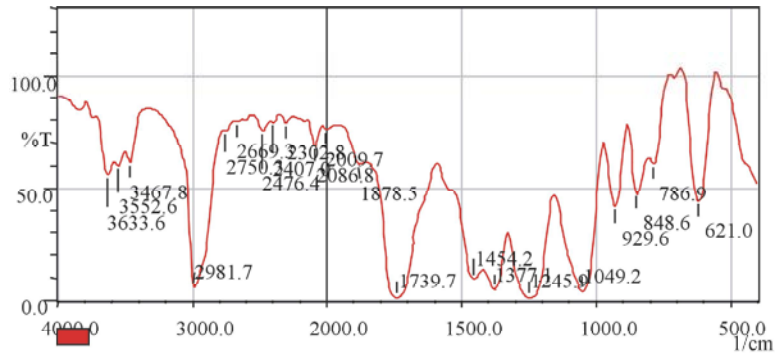

Fig 4 36: FTIR spectrum ethanol crude extract of *Warburgia ugandensis*.

the presence of N-H stretching. It gives a strong peak at 2873.7, 2931.6, 2943.2, 2981.7 cm^{-1} which indicated the presence of C-H stretching. The peak obtained at the 1643.2, 1654.8, 1728.1, 1732.0, 1751.2 and 1886.3 cm^{-1} indicated the presence of C=O stretching. The peak obtained at the 1458.1, 1454.2, 1450.4, 1384.8, 1377.1, 1330.8, 1253.6, 1242.1, 1234.4, 1149.5, 1149.8, 1118.6 and 1049.2 cm^{-1} indicated the presence of C-O stretching. The peak obtained at the 1234.4, 1253.6, 1242.1, 1149.5 and 1049.2 cm^{-1} indicated the presence of C-N stretching [16]. The peak obtained at the 1643.2, 1527.5 and 1531.4 cm^{-1} indicated the presence of C=C stretching in aromatic system found in the herb. While the peak obtained at the 2356.9, 2302.8 and 2252.7 cm^{-1} indicated the presence of C=N stretching. The peak obtained at 2090.7 and 2137.0 cm^{-1} indicated the presence of N=C stretching. However, the peak obtained at 1384.8, 1377.1 and 1330.8 cm^{-1} indicated the presence of N=O stretching. The peak obtained at the 1384.8, 1242.1 and 1049.2 cm^{-1} indicated the presence of S=O stretching. The peak obtained at 1253.6 and 1049.2 cm^{-1} indicated the presence of C-F stretching. The peak obtained at 1242.1, 736.8 and 621.0 cm^{-1} indicated the presence of C-l stretching. The peak obtained at 663.5 cm^{-1} indicated the presence of both

C-Br and C-I stretching. The FT-IR Spectroscopic analysis of *Warburgia ugandensis* (Table 2), revealed the presence of alkaloids due to N-H stretch at the 3444.6, 3440.8, 3460.1, 3359.8 cm^{-1}, C-N stretch at the 1253.6, 1242.1. 1377.1, 1330.8 cm^{-1} and N-H bend at the 1527.5, 1643.2, 1531.4, 1569.9, 1654.8 cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the hexane, dichloromethane, ethyl acetate and ethanol crude extract. The saponins were found to be present due to the presence of C=O stretch at the 1728.1, 1751.2 cm^{-1} and C-O stretch at the 1242.1, 1253.6 cm^{-1} in the dichloromethane and ethyl acetate crude extracts as carboxylic acid anhydrides. The unsaturated aromatic lactones with C=O stretch at the 1728.1, 1751.2 cm^{-1} and C-O stretch at the 1253.6, 1242.1 cm^{-1}, which occurred either in the free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the dichloromethane and ethyl acetate crude extracts. The tannins were absent as free phenols with O-H stretch and C-O stretch in the hexane, dichloromethane, ethyl acetate and ethanol extract. The polyphenols were found to be present with O-H (stretch) at the 3444.6, 3440.8, 3460.1, 3359.8 cm^{-1} and C=O (stretch) at the 1377.1, 1253.6, 1242.1. 1384.8 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract. Anthraquinones were present as aromatic ethers with C-O stretch at 1253.6, 1242.1 and 1049.2, 1053.1 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at the 1728.1, 1751.2, 1654.8 cm^{-1} and C-O stretch at the 1253.6, 1242.1, 1049.2 cm^{-1} were due to the presence of terpenoids and steroids for dichloromethane, ethyl acetate and ethanol extract. The presence of quinones revealed that Flavonoids were present with O-H (stretch) at the 3440.8, 3359.8 cm^{-1} and C=O (stretch) at the 1643.2, 1654.8 cm^{-1} for dichloromethane and ethanol extract. The terpenes were present with C-H (Stretch) at the 2943.2, 2931.6 cm^{-1}, C=C (Stretch) at the 1643.2, 1527.5, 1531.4 cm^{-1} and =C-H (bend) at the 1458.1, 1450.4 cm^{-1} for the analysed extract. The Cyanogenic glycosides were present due to the presence of C=N (stretch) at the 2302.8, 2356.9, 2252.7 cm^{-1} for dichloromethane, ethyl acetate and ethanol extract while N=C (stretch) at the 2090.7, 2137.0 cm^{-1} was for Isothiocyanate glycosides for dichloromethane, ethyl acetate and ethanol extract. The presence of components C=O stretch at the 1728.1, 1751.2 cm^{-1} and C-O stretch at the 1253.6, 1242.1cm^{-1} of dichloromethane and ethyl acetate extract, revealed the presence of cardiac glycosides.

CONCLUSIONS

The functional groups present in *Warburgia ugandensis* herb are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers, nitriles, isonitriles, quinones, organic halogen compounds and carbohydrates. All these compounds (except carbohydrates) belong to secondary plant metabolites as per researcher explanations [23 - 25]. These were confirmed by FTIR spectrophotometer study that predicted the presence of the groups: O-H, N-H, C-H, C=O, C-O, C-N, C=C, S=O, C=N and N=C stretching. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, isonitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Warburgia ugandensis*. Further studies are needed with this herb to identify the unknown functional groups, isolate, characterize and elucidate the structure of the bioactive compounds which are responsible for the antimicrobial activity and other medicinal values.

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