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Research Article

Phytochemical Analysis of *Warburgia ugandensis* Sprague using Fourier Transform Infra-Red (FT-IR) Spectroscopy

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ABSTRACT
The increasing resistance to convention medicine by disease agents has resulted in tremendous interest in herbal medicine. Through pharmacognosy, plants have been shown to exhibit diverse antimicrobial effects due to the presence of secondary metabolites. One such plant that has been shown to display antiplasmodial properties is *Warburgia ugandensis*, a popular plant used in herbal medicine by many Kenyan communities. *Warburgia ugandensis*, a Canaleaceae, also known as the East African greenheart, is a species of evergreen tree native to Africa and a highly valued species within the traditional health systems of the communities where it naturally grows. The plant is rich in sesquiterpenes, which have been shown to be antimicrobial. In our present study, we have established, through Fourier Transform Infra-red Spectrometry that *W. ugandensis* contains bioactive compounds including alkaloids, terpenoids, flavonoids and terpenes; justifying its use in herbal medicine and presenting it as a suitable candidate for development of a phytomedicine.

Key words: Herbal medicine, FT-IR, Pharmacognosy, Phytomedicine, Canaleaceae.

INTRODUCTION

Natural products from medicinal plants, either as isolated pure compounds or as standardized extracts, offer diverse opportunities into drug development and research. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in medicinal plants throughout the world has significantly grown[1]. Through screening, many beneficial biological effects such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity have been reported[2]. These beneficial biological activities of plant can be attributed to plant secondary metabolites which include phenolic compounds, terpenoids, alkaloids and sulphur-containing[3, 4]. Through ethnopharmacognosy, thousands of phytochemicals from medicinal plants that are safe and significantly efficacious alternatives with less adverse effects have been isolated, identified and characterized. Most plants used in screening programs are those claimed to cure certain ailments and diseases by Traditional Herbal Practitioners hence the unprecedented renewed global interest in Traditional Medicine (TM) and natural products research[5, 6]. This study was designed to investigate the bioactive antiplasmodial moieties in the stem bark of *Warburgia ugandensis. Warburgia ugandensis*, (Sprague), also known as the East African Greenheart, a Canallaceae, is a species of evergreen tree common to East African Forests[7]. Traditionally, the stem and root barks of *W. ugandensis* are used as expectorant and treatment for toothaches, constipation, diabetes and fever. It has also been used as antirepellant, anticancer, antitumour and generally as an antimicrobial agent. Its antimalarial activity has been demonstrated[8]. As a step towards development of a phytomedicine using *W. ugandensis* as one of the constituent plant material, phytochemical investigation was carried out using Fourier transform infrared spectrometry (FT-IR) to identify the active phytoconstituents responsible for observed antiplasmodial activity. A physico-chemical analytical technique, FT-IR provides a snapshot of the metabolic composition of a tissue at a given time[9], by measuring the vibrations of bonds within chemical functional groups and generating a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample[10]. FT-IR can be used to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated with molecular composition or content of the chemical group[11]. It can be

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Table 1A: FT-IR Peak Values and Functional groups of TLC Fractions from Ethyl acetate Extracts of *W.ugandensis*

Fractions										Bond Type	Comp. Type
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**		
3870.9	3822.7		3988.5	3872.8	3874.7	3861.2	3859.3	3857.4	3892.1	*	*
3801.4	3735.9		3845.8	3811.1	3824.6	3805.3	3813.0	3813.0	3805.9		
3737.8			3801.4 3743.4	3743.6	3739.7	3743.6	3747.4	3747.4	3749.7		
3458.1	3427.3	3404.1	3438.8	3423.4		3624.0				OH (s),H(b)	Alcohols, phenols
	3107.1	3101.3				3560.3				O-H (s)	Carboxylic acids
3084.0					3427.3	3456.2	3434.1	3436.9	3407.1	C-H (s)	aromatics
2923.9	2929.7	2925.8	2927.7	2925.8, 2680.9 2549.7		3388.7				O-H(s)	Carboxylic acids
2864.1	2866.0	2860.2	2862.2	2858.3		3273.0 3226.7				C-H (s) *	alkanes *
2725.2	2734.9		2736.6			3151.5				C=O:CH (s)	aldehydes
	2408.9 2283.6		2497.6	2441.7	2929.7	3111.0 2925.0	2927.7	2927.4	2929.7	*	*
			2252.7		2862.2	2858.3	2862.2	2862.2	2864.1	C≡N (s)	nitriles

(s): stretch, (b): bend, *: unknown; Frequency Peaks between 4000- 2250 cm^{-1}

used to identify specific groups (fingerprint) of compounds without separation as it is directly based on their absorption characteristics[12].

MATERIALS AND METHODS

Chemicals

All solvents and chemicals used were of analytical grade, obtained from Sigma Aldrich.

Sample Collection and Preparation

The plant materials were collected from Oloolua Forests of Kajiado County and authenticated at the Herbarium, National Museums of Kenya. The stem barks at the secondary stage of growth were harvested, washed to remove physical impurities, cut into small pieces, air-dried for three weeks and pulverized. Two hundred grams (200g) of ground powder was dissolved and macerated in 1000 ml of ethyl acetate for 72h with constant shaking to enhance extraction. The solvent containing extracted components were filtered using whatmann filter paper no 1. This was repeated three times to ensure complete extraction. The filtrates were pooled and evaporated in vacuo using a rotor evaporator at reduced temperature of

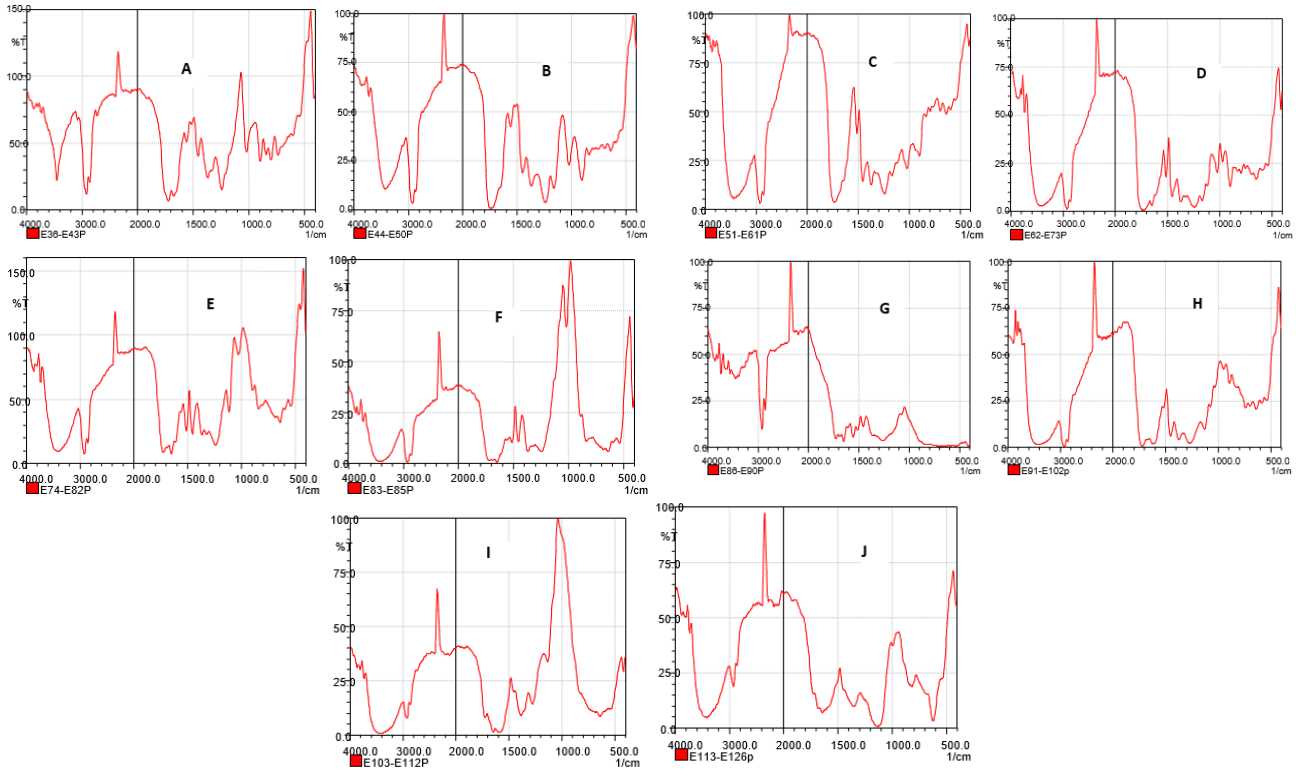

Figure 1 (A-J): FT-IR absorption spectra in the 4000-400cm^{-1} region showing percentage transmittance for TLC fractions of Ethyl acetate extracts of *W. ugandensis*

40°C. Extracts recovered were weight, put in sterile vials, and stored at 4°C until required for reconstitution.

Solvent System and Column Chromatography

Since plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation into fractions was achieved by column chromatography. Toluene and Diethylamine in the ratio of 9: 1 was determined as the most appropriate solvent system. Glass Column wet packing method was used in which 120grams of silica gel were mixed with methanol solvent to slurry. A padding of cotton was placed at the bottom of the glass column and slurry mixture allowed to settle at the bottom of column with gently tapping to allow proper packing and to get rid of air bubbles. Two (2 grams) of the extract was placed on the column and a padding of cotton placed above it. The column was first eluted by a non-polar solvent so as to allow the compounds to adsorb to the stationary phase on Thin Layer Chromatographic (TLC) plate. The polarity of the solvent was increased slowly and progressively to desorb the compounds and allow elution with the mobile phase. A total of 142 fractions were collected and pooled into 10 fractions (A to J).

Infrared spectroscopy analysis

Air-dried sample of ethyl acetate extract from *W. ugandensis* was analyzed for identification of characteristic functional groups using Fourier Transform Infrared (FT-IR) spectrophotometer (Shimadzu 8400) at the Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology. A small quantity (0.1g) of extract sample and 0.025g of dry potassium bromide (KBr) were homogenized using mortar and pestle. A portion of the homogenized mixture was placed on the disc and pressed using a mini hand press to form a KBr thin film and the disc was placed in the FT-IR spectrophotometer in which spectra was measured by accumulating 64 scans at 4 cm^{-1} resolution in the spectral range of 4000 to 400 cm^{-1}. Percentage transmittance was plotted against wavelengths (Figure 1A-J). The FT-IR spectra were used to identify the functional groups of active metabolites based on the peak values in the infra red region (Table 1A-C).

RESULTS AND DISCUSSION

Strong and broad absorption band of hydroxyl occurred at 3431.1, 3458.1, 3427.3, 3404.1, 3438.8, 3423.4, 3624.0, 3427.3, 3456.2, 3436.9 and 3407.1cm^{-1}, indicating the presence of O-H stretching in all fractions, depicting the presence of alcohols and phenols. The FT-IR Peaks obtained at 3107.1, 3101.3, 2931.6, 2923.9, 2929.7, 2925.8, 2925.8, 2680.9, 2549.7, 3273.0, 3226.7, 3151.5, 3111.0, 2543.9, 2675.1, 2362.6, 2376.1 and 2592.2cm^{-1} showed C=O stretching for Carboxylic acids. While Peaks obtained at 3388.7, 1645.2, 1614.9, 1610.5, 1596.9, 1645.2 cm^{-1} are N-H bending for primary amines with 898.8, 896.8, 906.5, 900.2, 900.7, 894.9cm^{-1} N-H wagging that indicated the presence of secondary amines. Moreover, the medium peaks generated at 2738.7, 2721.4, 2725.2, 2725.2, 2734.9 and 2736.61cm^{-1} represent H-C=O: C-H stretching for aldehydes, with strong absorption peaks at 1689.5, 1724.2, 1731.6, 1735.8, 1735.8 and 1728.1cm^{-1} are assigned to C=O stretching vibration in carbonyl compounds; which may be characterized by the presence of high content for unsaturated aldehydes, esters and ethers[13]. The medium peaks at 1244.0, 1244.4, 1164.9, 1164.1, 1170.7, 1168.8, 1128.3, 1124.4, 1120.6, 1116.7, 1026.1, 1022.2, 1024.1 and 1024.1cm^{-1}, represent C-N stretching for aliphatic amines. The observed sharp peaks at 1515.9, 1514.0, 1521.0cm^{-1} for N-O asymmetric stretching and N-O symmetric medium stretching at 1355.9cm^{-1}, revealed the presence of nitro compounds. Other strong peaks at 1334.6, 1325.0 cm^{-1} C-N stretching indicates the presence of Aromatic amines. Also present were the medium band for Alkanes; C-H stretching at 2929.7, 2925.0, 2858.3, 2927.7, 2927.4, 2929.7, 2864.1, 2866.0, 2860.2, 2862.2 and 2858.3 cm^{-1}; medium C-H bending for alkanes at peaks 1458.1, 1454.2, 1462.5, 1452.3 cm^{-1} and C-H

Table 1B: FT-IR Peak Values and Functional groups of TLC Fractions from Ethyl acetate Extracts of *W.ugandensis*

Fractions										Bond Type	Compounpd Type
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**		
	2135.1		2206.4							-C=C- (s)	alkynes
			2050.2		2738.7	2675.1	2362.6	2376.1	2721.4	*	*
			2002.0		2543.9	2592.2					
			1922.9								
1724.2	1731.6	1735.8	1735.8	1728.1	2497.6	2399.3		2327.9	2329.3	C-O (s)	sat.aliphatic, Aldehydes
1676.0		1647.1	1651.0	1652.9	2447.5					-C=C- (s)	alkenes
				1614.9	2260.4	2206.4				N-H (b)	1 amines
1560.3	1560.3			1564.2						*	*
1515.9	1515.9	1514.0	1515.9	1514.0		2125.4			2183.3	N-O asy (s)	Nitro cpds
1458.1	1452.3	1458.1	1458.1	1458.1						C-H (b)	alkanes
1371.3	1371.3	1375.2	1373.2		2088.8	1730.0	1965.7	1924.8	2098.4	*	*
							1724.2	1728.1			
				1369.4		1689.5				C-H (r)	alkanes
1334.6				1325.0	1651.0	1651.0	1651.0	1651.0		C-N (s)	Aromatic amines
						1610.5		1596.9	1645.2	*	*
1244.0	1242.1	1244.4	1242.1	1242.1						C-O	Esters, ethers

(s): stretch, (b): bend, (w): *: unknown; Frequency Peaks between 2800- 1150 (cm^{-1}).

Table 1C : FT-IR Peak Values and Functional groups of TLC Fractions from Ethyl acetate Extracts of *W.ugandensis*

Fractions										Bond type	Compound type
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**		
1022.2	1164.1	1170.7	1168.8	1116.7	1560.3	1558.4	1521.0	1566.1		C-N (s)	aliphatic
	1024.1	1124.4	1120.6		1515.9	1514.0	1454.2	1462.5			amines
		1022.2	1024.1		1458.1	1458.1					
					1371.3			1380.9		*	*
			974.0						1355.9	=CH (b)	alkenes
896.8	906.5	900.2	900.7	894.9	1247.9	1265.2	1272.9	1278.7		N-H (w)	1,2 amines
					1022.2	1078.1	1124.4	1126.4	1137.9	*	*
							1076.2	1078.1			
844.8	839.0	837.0,	796.5,		840.9		898.8	894.9	997.1	C-Cl	Alkyl halides
802.3	754.1	796.5	756.0								
738.7	694.3	767.6									
							746.4	748.3	817.8	*	*
		698.2			642.3		617.2	648.0	624.9	C=C:C (b)	alkynes
603.7	638.4	638.4	651.9	644.2	594.0	542.0			543.9	C-Br	Alkyl halides
					538.1						
545.8	592.1	596.0	594.0	596.0	416.6	416.6	422.2		410.8	*	*
		547.7	542.0	542.0							
			416.6								

(S): Stretch, (B): Bend, (R): (W): Wag, *: Unknown; Frequency Peaks between 1600- 400 (cm^{-1})

medium rocking at 1369.4cm^{-1}. The double bond -C=C- medium stretching at 1678.0, 1676.0, 1647.1, 1651.0 and 1652.9 cm^{-1} as well as the double bond -C=C sharp bending at 974.0, 997.1, 894.9 and 898.8 cm^{-1} depicted the availability of alkenes. Alkynes were represented by -*C*≡*C*- weak stretching at 2206.4, 2183.3, 2135.1, and 2125.4 cm^{-1}; and -C≡C: C-H strong and broad bending at 698.2cm^{-1}. A strong haoalkene, C-Cl appeared at 840.9 cm^{-1}. Based on the functional groups identified, The FT-IR spectroscopic analysis of ethyl acetate fractions from *W. ugandensis* (Table1A-C) revealed the presence of alkaloids due to N-H stretch at 3388.7 (G), 1645.2 cm^{-1} (J), 1614.9 (H, E), 1610.5 (G), 1596.9 (I); N-H bending 898.8 (H), 896.8 (A), 906.5 (B), 900.7 cm^{-1} (D, C), 894.9 cm^{-1} (I, E); C-N stretch at 1334.6 cm^{-1} (A) and 1325.0 cm^{-1} (E) cm^{-1} which are fingerprint peaks found in primary, secondary as well as tertiary amines. Indeed, these alkaloids were contained in all fractions apart from F (Table2). Also predominantly present were the terpenoids and flavanoids as indicated by peaks for C=O at 2738.7, 2721.4, 2725.2, 2725.2, 2734.9 and 2736.61cm^{-1}, represented as H-C=O: C-H stretching for aldehydes, as well as C=O stretching at 1724.2, 1731.6, 1735.8 and 1735.8cm^{-1} for saturated aliphatic compounds. The C=O stretching at 1689.5 cm^{-1} pointed at α and β-unsaturated aldehydes or ketones. The unsaturated aromatic lactones with C=O at the 1735.8, 1730.0, 1728.1 and 1689.5 cm^{-1} indicated the presence of coumarin glycosides, with the nitrile peak at 2252.7 cm^{-1} at C≡N stretch, showing the presence of cyanogenic glycosides. The presence of phenolic compounds were depicted by O-H stretch at peak values 3431.1, 3458.1, 3427.3, 3404.1, 3438.8, 3423.4, 3624.0, 3427.3, 3456.2, 3436.9 and 3407.1cm^{-1}. Anthraquinones were present as aromatic ethers with C-O stretch at 1244.4, 1244.0 and 1242.1cm^{-1}. The medium band for C-H stretch at 2929.7, 2925.0, 2858.3, 2927.7, 2927.4, 2929.7, 2864.1, 2866.0, 2860.2, 2862.2 and 2858.3 cm^{-1}; the -C=C- medium stretch at 1678.0, 1676.0, 1647.1, 1651.0, 1652.9; and -C=C- sharp bending at 974.0, 997.1, 894.9, 898.81 cm^{-1} revealed large quantities of terpenes. Interestingly, a number of peak values (Table 1A-C) remains un identified and would therefore require further spectroscopic analysis to determine the bond types they represent. They would then be isolated identified characterized and their molecular structures elucidated as they may be novel and responsible for the observed antimicrobial activities of *W. ugandensis*.

CONCLUSION

The FT-IR spectrometry revealed the presence of O-H, N-H, C-H, C=C, C≡C, C=O, C≡N, C-Cl, C-Br representing the functional groups of carboxylic acids, alcohols and phenols, amines, alkanes, alkenes, alkynes, esters, nitriles and organic halogens. These functional groups represents various secondary metabolites such as alkaloids, terpenes, flavonoids, cardiac glycosides, polyphenols and terpenoids which are responsible for the observed antiplasmodial activity, thereby presenting them as alternative antimalarial treatment and justifying their use in herbal medicine. Further analysis involving, isolation, quantification, characterization and structural elucidation of these biologically active compounds that will be useful in the development and formulation of a phytomedicine for treatment of malaria as well as in pharmaceutical research and development.

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CONFLICTS OF INTERESTS

No competing interests exist.

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