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Research Article

Diversity in the Phytochemical Profiles of *Warburgia ugandensis* Sprague from Different Populations across the Kenyan Rift Valley

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ABSTRACT

Warburgia ugandensis Sprague is one of the most utilized medicinal plants in Kenya. Due to over-exploitation for its medicinal uses, the population size of this valuable plant species has decreased to the level that warrants some conservation efforts. The aim of this study was to determine the variations in the phytochemical profiles of *W. ugandensis* extracts from different populations across the Kenyan Rift Valley. The plant materials were sequentially extracted with dichloromethane and methanol and analyzed by gas chromatography-mass spectrometry (GC-MS). Quantitative and qualitative differences were observed in the phytochemical profiles of the Kenyan populations of *W. ugandensis*. Sesquiterpenoids (32.50-58.56%) and fatty acid derivatives (9.48-22.50%) were the most dominant classes of compounds. Other classes of compounds such as phenolics, phytosterols, tocopherols, ketones and aldehydes among others were expressed in low concentrations. The major compounds included copaen-15-ol (9.83%), alloaromadendrene (9.69%), 1,4-pentadien-3-one, 1,5-diphenyl (8.40%), stigmast-5-en-3-ol, oleate (7.94%), hexadecane (6.78%), (-)-zingiberene (6.57%), n-decanoic acid (6.53%), sorbitol (6.36%), ß.-l-arabinopyranoside, methyl (6.07%), sclaral (sclareolide lactol) (5.39%) and (-)-isolongifolol (5.27%). The knowledge on phytochemical diversity is important in developing sustainable utilization and efficient conservation strategies of all genotypes of the tree species in the genus *Warburgia* from different regions in Kenya.

Keyword: *Warburgia ugandensis*; conservation; variations; phytochemical profiles

INTRODUCTION

Warburgia ugandensis Sprague (Family Canellaceae), commonly referred to as East African 'Greenheart' (pepper-bark tree) is widely distributed in the lowland rainforests and upland dry evergreen forests of Eastern and Southern Africa [1]. The tree species also occurs in secondary bushlands, grasslands and termitaria in swamp forests [1]. In Africa, *W. ugandensis* is native to Uganda, Kenya, Malawi, Democratic Republic of Congo, Ethiopia, South Africa, Tanzania and Swaziland [1, 2]. This tree species is also found in India and China [1, 3]. *Warburgia ugandensis* is a multipurpose tree which is highly beneficial for its pharmaceutical properties and is rated second highest priority medicinal plant species in Kenya according to utility and sustainable use [4].

The stem barks of this plant are generally used in traditional medicine to treat rheumatism,

toothache, diarrhea, malaria, general body pains, gastro-intestinal disorders, colds, cough, fever, sore throat, odontological, backache, constipation, gastritis, sexually transmitted diseases, abdominal pains, snake bites and respiratory problems in African countries [3, 5, 6, 7]. Moreover, the leaves are occasionally used as a spice for food [1]. Earlier pharmacological studies have confirmed antimicrobial [8-11], antioxidant and anti-inflammatory activities [12] of *W. ugandensis* leaf and stem bark extracts. Other uses and products from this tree include timber for building and furniture, mulch for soil conservation, livestock fodder, insecticide, toothbrush, antiseptic soap, veterinary medicine, ornamental, shade and resin among others [1].

Previous phytochemical investigations revealed the existence of diverse terpenoids in the bark and leaf extracts of *W. ugandensis*. Some of these terpenoids include warburganal, muzigadial, ugandenial A, ugandensidial, cinnamodial, polygodial, salutarisolide, 11α-hydroxymuzigadiolide, pereniporin B, drimenol, drimenin, mannitol, mukaadial and dendocarbins A, L and M amongst others [3, 13, 14, 15]. Analyses of bioactive components of this plant species also reported the presence of phenolics, flavonoids, alkaloids, sugar alcohols, and fatty acid derivatives [10, 16, 17]. The abundant terpenoids, particularly those drimane- and coloratane type sesquiterpenoids have been suggested to be responsible for the various medicinal activities of this plant species [3]. Some of these compounds are significant due to their potent insect anti-feedant, plant growth regulatory, cytotoxic, phytotoxic, molluscicidal, aphrodisiac, trypanocidal and piscicidal properties and activity against mitochondrial oxidative phosphorylation [18].

Chemical compositions of plants have been a major focus of many investigations worldwide and this depends on several factors such as genetic background, edaphic, climatic, elevation and topographical conditions [19-21]. Moreover, the impacts of genotype (G) and growing conditions (E) as well as their interaction (G × E) also affect the distribution and chemical composition of phytochemicals [22]. Due to the interplay among these factors, the yield and composition of plant secondary metabolites are often variable. Thus, chemical diversity or chemotypes have been reported in many medicinal plants [23-26].

Research findings have also revealed that some of the medicinal plant features can be influenced by ecological factors such as temperature, precipitation, osmotic stress, plant competition and nitrogen content in the soil [21, 27] as well as geographical location, soil fertility, age of the plant, plant part used, time of collection and season of the year [28-30]. For instance, higher altitude and variation in soil type had significant effect in the distribution and number of volatile constituents of *Thymus serpylloides* in South Eastern Spain [21]. Similarly, a study by Matasyoh *et al.* [31] revealed that chemical composition of the essential oil of *Ocimum gratissimum* varied according to the geographical distribution and time of collection.

Currently, there is limited information on variation of secondary metabolites in different plant parts of *W. ugandensis* from different eco-geographical regions in Kenya. The knowledge gained from chemical composition of plants is useful, not only for the search of pharmaceutical products, but also because such information may be important in revealing novel sources of economic substances like tannins, oils and gums; which are precursors for the synthesis of complex substances for industrial use [27]. In addition, knowledge on the phytochemical profiles would help in identification of bioactive plants and plant parts of *W. ugandensis* from different populations in Kenya. This would ensure effective conservation and utilization of the plant species through targeted harvesting and selection of suitable genotypes. Therefore, the aim of this study was to determine the variations in the phytochemical profiles of *W. ugandensis* extracts from different populations across the Kenyan Rift Valley.

MATERIALS AND METHODS

Sample Collection and Preparation

Leaf and stem bark samples of *W. ugandensis* were randomly collected from five different populations across the Kenyan Rift Valley, namely Karura, Londiani, Kitale, Kinale and Rumuruti forests. The leaves were washed with water while the stem barks were first pre-treated by cleaning with a hard brush and then washed with water to remove dirt and soil particles before being chopped into small pieces. All samples were rinsed with sterile distilled water. The samples were then air dried away from direct sunlight for 2-3 weeks at room temperature until

they were completely dry. The dried samples were ground into fine powder using an electrical mill according to standard procedures [27]. The powder were kept in air tight polythene bags and stored at 4°C in readiness for further analyses.

Solvent Extraction

Plant powder (200g) was successively extracted using dichloromethane (DCM) and methanol (MeOH) solvents [4]. An 800ml aliquot of DCM was added into conical flasks and the flasks placed on a shaker and soaked for 48 hours. The samples were then filtered using Buchner funnel and Whatman filter paper No. 1 under vacuum. The filtrates were soaked in 800ml of DCM for 24 hours until they remained clear. The solution was then concentrated at reduced pressure using a rotary evaporator at 35°C and 45°C for DCM and MeOH extracts, respectively. This procedure was successively repeated using MeOH for the same samples.

Phytochemical Analyses

The profiles of compounds present in the extracts of *W. ugandensis* were determined using gas chromatography-mass spectrometry (GC-MS) following the previous protocol of Karau *et al.* [32]. The compounds were identified by chromatogram deconvolution and their mass spectral data matched with those of the National Institute of Standards and Technology (NIST). The percentage (%) relative abundance of each compound in the plant extract was calculated as shown by the formula below:

$$\% \text{ Relative Abundance} = \frac{\text{Peak Area of the Compound}}{\text{Total Sum of all Peak Areas}} \times 100$$

RESULTS

Comparative analyses of the profiles of phytochemicals identified in *W. ugandensis* extracts through GC-MS are presented in Figures 1-4 and Table 1. The chemical composition of plant extracts was found to be dependent on the solvent used for extraction, origin of the plant materials and plant parts analyzed. The identified compounds were classified into different classes based on their biosynthetic origins: terpenoids, fatty acid derivatives, phenolics, phytosterols, tocopherols, ketones and aldehydes among others. The chemical composition of the extracts varied greatly on the basis of classes of compounds. There were qualitative and quantitative differences as well as similarities in the chemical profiles of compounds identified in *W. ugandensis*. Some compounds were found to be common to both the leaf and stem bark extracts while others were present only in the leaf extracts and absent in the stem bark extracts and vice versa across the five populations of *W. ugandensis*. There were substantial variations in the amount of compounds present in the leaf and stem bark extracts of *W. ugandensis* (Tables 1).

All the extracts of *W. ugandensis* from the five populations were characterized by high percentage of terpenoids (sesquiterpenoids) and fatty acid derivatives as the main classes of compounds. For instance, sesquiterpenoids (38.18-58.56%) and fatty acid derivatives (11.71-19.09%) were the most dominant classes of compounds in both DCM and MeOH extracts from Karura (Figures 1-4). The major compounds in Karura DCM extracts were 1,4-pentadien-3-one, 1,5-diphenyl (8.40%; leaf extracts) and hexadecane (6.78%; leaf extracts) while the minor compound was vanillin (0.06%). In Karura MeOH extracts, n-decanoic acid (6.53%; leaf extracts), sclaral (sclareolide lactol) (5.39%; stem bark extracts) and (-)-isolongifolol (5.07%; leaf extracts) were the most abundant compounds while vitamin E (0.07%; stem bark extracts) was the least abundant compound (Table 1).

The GC-MS analyses of DCM leaf and stem bark extracts of *W. ugandensis* from Kinale population also revealed diversity in the phytochemical profiles. Kinale extracts were also largely composed of sesquiterpenoids (36.22-48.41%) and fatty acid derivatives (11.43-17.70%) as the most dominant classes of compounds (Figures 1-4). The most abundant compound in Kinale DCM extracts was hexadecane (5.16%; leaf extracts while the least dominant compound was α-curcumene (0.06%). For Kinale MeOH extracts, the most abundant compound was 3-cyclopentylpropionic acid, 3,5-dimethylphenyl ester (5.03%; stem bark extracts) while the minor compound was (+)-α-tocopherol, O-methyl- (0.05%; leaf extracts) (Table 1).

Table 1: Percentage (%) Relative Abundance (R.A) of Compounds Identified in *W. ugandensis* Extract from the Five Populations across the Kenyan Rift Valley

Name of compound	Karura (%) R.A				Kinale (%) R.A				Kitale (%) R.A				Londiani (%) R.A				Rumuruti (%) R.A			
	DCM		MeOH		DCM		MeOH		DCM		MeOH		DCM		MeOH		DCM		MeOH	
	LV	SB	LV	SB	LV	SB	LV	SB	LV	SB	LV	SB	LV	SB	LV	SB	LV	SB	LV	SB
Copaene-15-ol	0.90	0.38	0.45	1.01	2.42	1.05	0.37	0.19	2.23	1.37	0.93	0.08	0.47	9.83	0.38	0.34	1.12	0.37	0.39	0.42
Epizonarene	0.32	1.16	0.25	1.90	0.23	1.37	1.23	2.01	1.42	1.89	1.18	0.43	0.21	0.29	1.56	0.25	1.91	2.40	1.81	9.42
α-bergamotene	0.11	0.16	0.75	0.93	1.19	0.67	1.91	0.82	0.32	1.23	0.33	0.67	0.44	0.28	0.14	0.12	0.97	2.39	3.42	1.57
Himachala-2,4-diene	0.44	0.89	0.14	0.19	0.19	0.76	0.39	0.80	1.71	2.02	0.42	1.42	2.49	0.56	1.30	0.91	0.99	1.75	0.77	1.96
Calamenene	0.12	0.16	0.87	1.56	0.09	2.28	0.22	1.50	1.34	0.10	1.01	0.42	0.48	1.06	1.39	1.96	0.29	1.45	0.92	1.02
(-)-zingiberene	0.38	2.53	1.44	1.76	1.30	0.22	1.06	1.80	0.33	1.22	0.49	0.86	1.67	0.14	1.37	1.23	1.94	1.76	0.36	0.16
Hexadecane	6.78	0.21			5.16	0.44			5.27	0.28			3.05				5.67	0.30		
7-epi-α-selinene	0.97	1.48	1.73	1.24	0.86	0.08	1.12	0.47	0.17	0.98	0.51	2.21	0.26	1.80	0.59	1.01	1.21	0.95	1.89	0.90
Drimenin	0.32	0.26	0.31	0.19					1.14	1.61		0.16	0.22							
D-mannitol			1.69	1.17			1.14	1.53			4.26		0.83	0.10	0.79	0.16	6.36		4.08	
δ-cadinene	1.06	0.58	1.20	0.32	0.43	1.53	0.12	1.11	0.28	1.14	0.92	2.06	1.49	1.66	1.09	2.91	0.04	1.78	019	2.82
Ar-himachalen-2-ol				0.18					0.24	0.30										
α-terpineol	0.35	0.08	1.03	0.11	0.34	0.27	0.24	0.04	1.09	0.05	0.95	1.06	0.26	0.03	0.36	0.98	0.12	1.25	0.84	0.09
Berkheyaradulene	0.25						1.10				0.68									
Nerolidol 2	0.69	1.26	0.53	0.69	1.17	1.36	0.84	0.83	2.40	1.42	0.98	0.92	0.01	0.82	0.32	1.03	0.46	1.07	0.79	1.38
γ-costol	0.54	0.84	0.29	0.41	0.74	1.21	1.82	0.25	0.91	0.75	0.57	0.96	0.34	0.09	1.14	1.10	0.07	0.37	3.82	0.57
Germacrene B		0.11			0.32					0.70	0.82			0.88	1.14			1.66	0.95	1.93
(-)-isolongifolol	3.51	2.14	5.07	0.38	1.31	0.35	0.70	0.65	1.34	1.14	1.19	0.79	1.31	0.18	1.30	1.09	3.08	0.81	1.01	1.65
δ-bisabolene	0.22	0.37	1.53	1.3	0.76	0.37	0.56	1.33	0.84	0.43	0.36	0.18	1.49	0.20	0.99	1.82	0.22	1.03	0.39	1.00
Caryophyllene	0.49	0.35	0.43	0.19	1.44	1.84	1.09	1.79	1.95	2.20	0.42	0.49	0.54	4.29	1.36	1.25	0.99	0.48	1.11	0.42
Linoleic acid	1.39	0.98	0.14				0.44	0.51					3.37				0.35	0.98	0.52	0.58
Oleic acid				0.10	0.06				0.33	0.10			0.98	0.43	0.77				1.05	1.03
Selina-3,7(11)-diene				0.10					0.18	0.28	0.20	0.40	0.37							
δ-cadinene	0.16	1.33	0.71	0.57	1.21	1.87	0.39	1.19	2.13	0.56	0.59	0.94	0.33	0.96	0.05	0.16	0.52	1.33	1.71	1.97
α-muurolene	0.17	0.21	0.68	0.19		0.28				0.12				0.86		0.67				
α-cubebene	1.86	1.35	1.14	0.31	0.07	0.20	0.51	0.08	0.81	0.14	0.15	0.41	1.79	0.18	1.89	0.04	0.76	1.07	2.01	1.03
δ-humulene	0.36	1.68	1.03	0.36	0.39	1.44	0.41	0.92	0.37	1.10	1.29	0.52	1.20	1.46	1.05	0.73	1.04	1.19	1.01	0.87
Longifolenaldehyde	0.69	0.31	0.49	0.40	1.31	0.94	3.15	1.11	2.18	0.30	0.77	0.42	1.37	1.03	1.94	0.6	0.37	0.86	0.23	0.51
Geranyl linalool	0.27	0.11	0.28	0.46	0.12	2.03	0.38	0.60	1.05	0.08	0.47	2.52	0.39	1.35	0.70	0.95	1.34	0.41	1.69	2.65
Geranylgeraniol	0.33	0.08	0.24	0.59	0.50	1.04	0.43	0.15	0.60	0.10	0.03	0.34	0.91	0.92	1.32	1.33	0.43	0.07	0.59	0.38
Manool	0.45	0.28	0.55	0.58	0.37	1.98	1.44	0.55	2.20	0.59	0.29	0.52	0.47	1.04	2.04	2.24	1.7	0.92	0.47	0.59
Phytol	0.51	0.03	1.90	1.04	0.98	0.39	2.78	2.34	0.54	1.65	2.01	2.11	1.25	1.04	0.06	0.96	1.12	1.05	1.85	1.03
Drimenol	1.05	4.04	2.02	1.15	0.59	1.03	2.35	1.65	1.65	0.98	2.47	2.50	2.08	0.34	0.06	1.85	1.40	0.22	0.04	0.01
palmitic acid	4.37	1.79	2.37	1.55	3.30	1.58	2.92	2.86	2.00	1.59	1.43	3.20	0.97	1.44	3.19	4.21	0.28	2.87	3.09	3.44
n-decanoic acid			6.53			0.15	0.11		0.11			6.12			1.94	0.47	1.69	1.64	5.70	1.51
1-decanol, 2-hexyl-	0.56	0.46	0.33	0.56	0.51	0.20	0.46	0.75	0.20	0.09	1.22	1.98	0.77	0.34	0.89	0.94	1.29	0.70	0.20	0.94
Octadecanoic acid	0.45	0.31	0.36	0.33	0.45	0.58	0.52	0.29	1.18	0.31	0.61	0.40	0.14	0.02	1.52	1.59	0.64	0.34	0.15	1.01
Schottenol	2.31	1.40	2.36	1.38	1.77	0.40	1.99	1.61	2.12	0.06	2.08	1.24	0.05	0.39	1.79	0.59	0.38	1.12	1.16	0.09
Pollinastanol	1.28			0.15	0.13				0.16	0.12			0.33	0.52	1.73	1.27				
δ-tocopherol	0.33	0.40	0.24	0.21	0.26	1.89	0.18	0.14	0.17	1.22	0.16	0.11	0.08	0.48	0.97	1.40	0.59	0.36	0.91	1.98
Guaiacol	1.63	1.52	0.93	1.57	0.24	1.28	1.26	1.33	3.92	1.54	1.49	1.85	0.23	0.58	1.48	2.05	0.78	3.35	3.02	4.78
Stigmast-5-en-3-ol, oleate	0.13	0.28	0.20	0.17	0.08	1.04	0.06	1.76	0.47	0.13	0.59	0.95	1.04	2.03	1.08	0.01	0.07	0.48	0.02	0.07
Stigmasterol acetate	0.21	0.58	0.68	0.48	0.22	0.38	0.38	1.04	1.58	0.04	0.18	0.37	0.39	0.67	0.48	0.28	0.35	0.85	0.92	1.59
Squalene	1.18	0.26	3.35	4.33	0.87	0.39	0.10	0.34	0.64	1.68	0.16	1.32	1.04	0.14	0.09	0.76	2.01	1.90	0.85	0.27
(-)-δ-elemene	0.28						0.28				0.81	0.13			0.68	0.28				
δ-patchoulene				0.45	0.76	0.76	0.47					0.46		1.32	0.49	0.17	3.50		0.51	
Isolongifolene oxide		0.23		0.12	0.25	1.28		0.15	1.05	0.29	0.46		0.50	1.09		0.19	0.60	3.91	0.19	0.58
Alloaromadendrene	0.18		1.86	2.92		3.74								1.87	0.30			9.69		
α-santalene	0.13	0.07	0.31	0.33		0.30				0.30			0.06		0.25		0.18		1.13	
Bakkenolide A			2.27	4.28		0.86	1.54	1.34			0.66		0.39	0.26	0.52		1.06	0.49		
γ-himachalene	0.28	0.52	0.18	1.19	0.16	1.87	1.06	1.09	2.20	0.11	2.76	1.98	4.88	1.09	0.12	0.68	2.03	1.08	1.50	0.93
cis-muurola-3,5-diene	0.08				0.21		2.22	0.20			0.44		0.30	0.10	0.18	0.10		1.58		0.56

Similar trend of chemical polymorphism was observed in Kitale extracts where sesquiterpenoids (36.61-48.60%) and fatty acid derivatives (10.28-17.07%) were the most dominant classes of compounds (Figures 1-4). The principal component in Kitale DCM extracts was hexadecane (5.27%; leaf extracts) while the least prominent compound was α-terpineol (0.05%; stem bark extracts). Subsequently, n-decanoic acid (6.12%; stem bark extracts) was the most abundant compound in Kinale MeOH extracts while artemisia triene (0.05%; leaf extracts) was the least abundant compound (Table 1).

Chemical diversity was also observed in Londiani extracts with sesquiterpenoids (36.75-48.57%) and fatty acid derivatives (9.48-17.09%) being the most dominant classes of compounds (Figures 1-4). Based on percentage relative abundance, the most dominant compound in Londiani DCM extracts was copaen-15-ol (9.83%; stem bark extracts) while the least dominant compound was (+)-α-tocopherol, O-methyl- (0.04%; leaf extracts). On the other hand, the most prominent compound in Londiani MeOH extracts was 3-cyclopentylpropionic acid, 3,5-dimethylphenyl ester (5.27%; leaf extracts) while the least prominent component was (+)-mecambroline (0.05%; stem bark extracts) (Table 1).

Similarly, sesquiterpenoids (32.50-54.55%) and fatty acid derivatives (10.23-22.50%) were the most dominant classes of compounds in Rumuruti (Figures 1-4). Based on peak area percentage, the most abundant compounds in Rumuruti DCM extracts were alloaromadendrene (9.69%; stem bark extracts), stigmast-5-en-3-ol, oleate (7.94%; stem bark extracts) and (-)-zingiberene (6.57%;

stem bark extracts) while the minor compound was α-muurolene (0.06%; leaf extracts). For MeOH extracts, the most dominant compounds were 1,2,3-benzenetriol (7.95%; leaf extracts), sorbitol (6.36%; leaf extracts), ß.-l-arabinopyranoside, methyl (6.07%; leaf extracts) and n-decanoic acid (5.70%; leaf extracts) while the least dominant constituent was (+)-mecambroline (0.03%; stem bark extracts) (Table 1).

This study reports for the first time compounds that were found to be common in all the DCM and MeOH leaf and stem bark extracts of *W. ugandensis* from the five populations across the Kenyan Rift Valley. These compounds included α-terpineol; copaene-15-ol; himachala-2,4-diene; calamenene; (-)-zingiberene; 7-epi-α-selinene; ß-cadinene; α-bergamotene; eudesma-5,11(13)-dien-8,12-olide; nerolidol; γ-costol; bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-; (-)-isolongifolol; ß-bisabolene; cycloisolongifolene; caryophyllene; δ-cadinene; (-)-δ-panasinsine; epizonarene; α-cubebene; γ-himachalene; ß-humulene; longifolenaldehyde; procerin; hexadecanoic acid, methyl ester; geranyl linalool; geranyl-geraniol; phytol; drimenol; n-hexadecanoic acid; 1-decanol, 2-hexyl-; octadecanoic acid; 3-keto-ß-ionone; stigmast-5-en-3-ol, oleate; ergosta-4,6,22-trien-3.beta.-ol; stigmasterol acetate; schottenol; pollinastanol; squalene; ß-tocopherol; 2-acetyl-5-methylfuran; phellopterin; isolongifolene 9-hydroxy-; 1,Z-5,E-7-dodecatriene; vitamin E and 1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z.

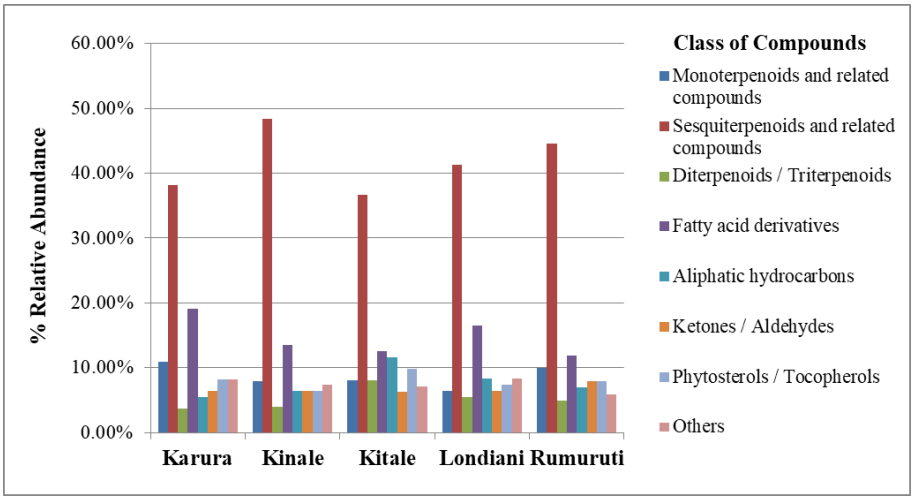

Fig. 1: Comparison of chemical classes identified in *W. ugandensis* DCM leaf extracts

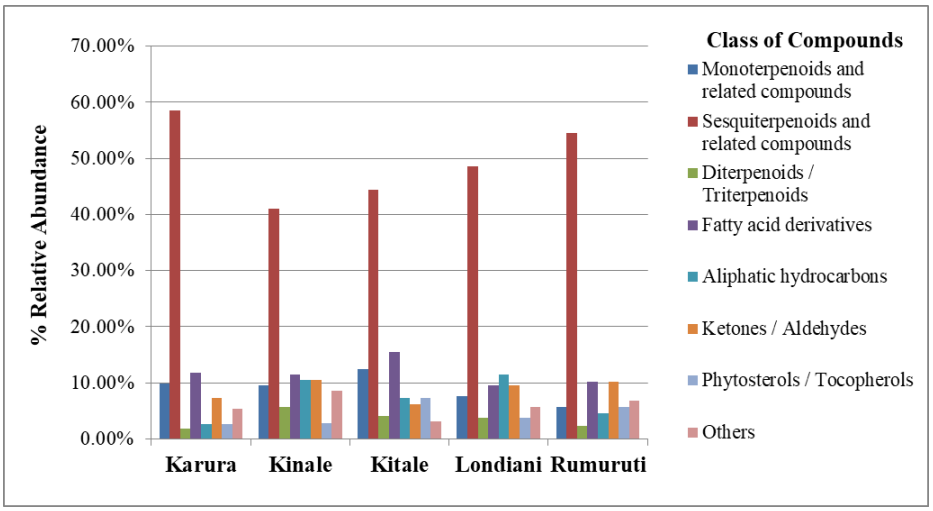

Fig. 2: Comparison of chemical classes identified in *W. ugandensis* DCM stem bark extracts

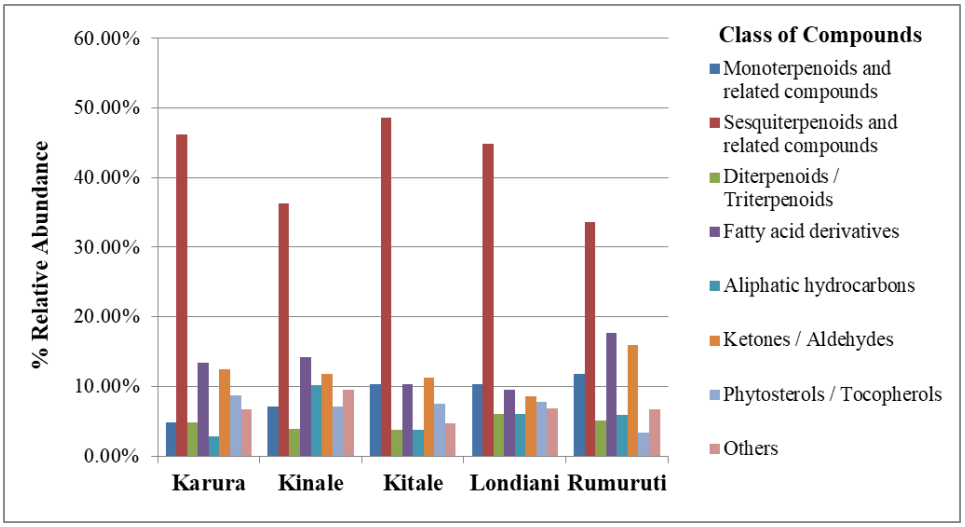

Fig. 3: Comparison of chemical classes identified in *W. ugandensis* MeOH leaf extracts

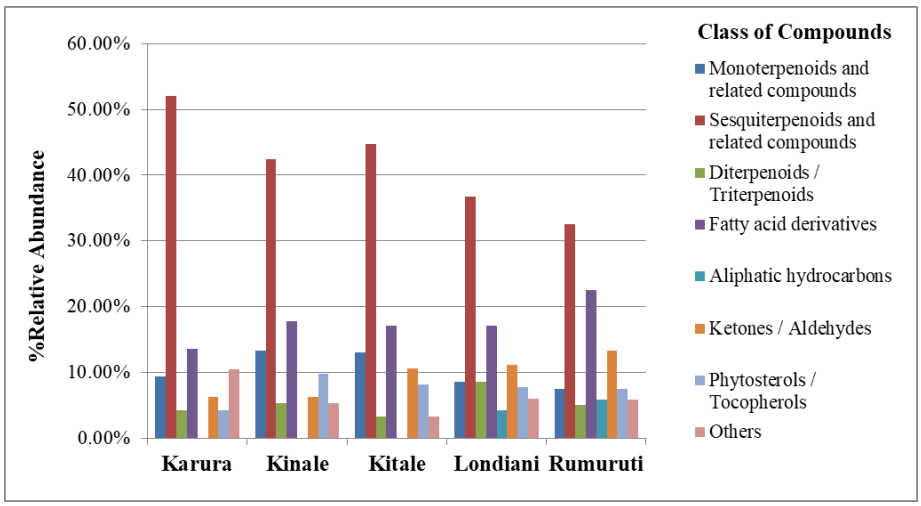

Fig. 4: Comparison of chemical classes identified in *W. ugandensis* MeOH stem bark extracts

DISCUSSION

Previous phytochemical and pharmacological studies have reported the presence of diverse secondary metabolites in the extracts of *W. ugandensis*. These metabolites are linked to the medicinal properties of this plant species [3, 15, 17]. The present study shows that *W. ugandensis* is a rich source of phytochemicals as depicted by the presence of several classes of compounds in its leaf and stem bark extracts. This study revealed variations in the profiles of compounds identified in *W. ugandensis* extracts. Sesquiterpenoids and related compounds were the main class of compounds identified in this plant species. This was followed by fatty acid derivatives. Other classes of compounds identified in the extracts of *W. ugandensis* including phytosterols, tocopherols, phenolics, ketones and aldehydes among others were expressed in low concentrations. This pattern of compound class distribution was consistent in all the five populations of *W. ugandensis* across the Kenyan Rift Valley. These observations support the findings of previous studies which reported the abundance of terpenoids and fatty acids in the leaves and stem barks of *W. ugandensis* [3, 15]. Similar consistencies in compositional patterns of compound classes were observed in *Warburgia salutaris* and *Warburgia stuhlmannii* in South Africa [33].

Qualitative and quantitative differences as well as similarities were observed in the chemical composition of *W. ugandensis* extracts. Some compounds were common to both the leaf and stem bark extracts while others were only present in the leaf and absent in the stem bark

extracts and vice versa. The occurrence of compounds common to all the plant parts of *W. ugandensis* from different populations could be as a result of sharing of similar constitutive genes that influence phenotypic characteristics of the plant [34]. The differences in composition of bioactive compounds in the leaf and stem bark extracts of *W. ugandensis* from different populations in Kenya could be attributed to different expression levels of genes that confer the biosynthesis of various plant secondary metabolites (PSMs) [35]. Both the type and quantity of many PSMs vary greatly across physical and biotic environments due to local adaptation, genotypic sorting and selection across habitats. However, much of this diversity is attributed to phenotypic plasticity of genotypes in response to variations in resources for growth including soil nutrients, soil moisture, light, atmospheric carbon dioxide and the presence or absence of enemies, competition or mutualists. Such changes to concentration of PSMs in plant tissues may be as a result of specific up- or down-regulation of their biosynthesis [36].

There were variations in the numbers of compounds present in the leaf and stem bark extracts of *W. ugandensis* from the five populations across the Kenyan Rift Valley. The amounts of compounds were distinctly different between the leaf and stem bark extracts. For instance, the numbers of compounds in the stem bark extracts were slightly higher compared to the leaf extracts in almost all the five populations. This could be due to dramatic differences in the expression levels of unigenes between the leaves and stem barks of *W. ugandensis*. Many unigenes could have been specifically expressed in the stem barks compared to the leaves [3]. In this context, the term unigene refers to a cluster of genes that perform a particular function like biosynthesis of plant compounds. Saronjic [16] also reported higher amounts of compounds in the stem barks of *W. ugandensis* compared to those in the leaves. These results are also in agreement with the findings of Kuglerova *et al.* [8] who noted that the stem barks of *W. ugandensis* contained other compounds (or different combinations of compounds) not present in the leaves. These could be due to the ecological conditions of the regions where *W. ugandensis* samples were collected [37].

This study also reports the occurrence of varying concentrations of fatty acid derivatives in the leaves and stem barks of *W. ugandensis*. These include tetracosanoic acid, methyl ester; octadecanoic acid (stearic acid); hexadecanoic acid, methyl ester; tridecanoic acid; hexadecanoic acid (palmitic acid); 9-octadecenoic acid, (E) (oleic acid); 9,12-octadecadienoic acid (Z,Z) (linoleic acid) and hexadecanoic acid, 2-methylpropyl ester. This observation correlate with the findings of Wang *et al.* [3] who reported the presence of oleic acid, linolenic acid and linoleic acid in *W. ugandensis* even though their proportions varied between the leaf and stem bark extracts. Saronjic [16] also reported varying concentrations of arachidonic acid, decanoic acid and palmitic acid in the extracts of *W. ugandensis*. The occurrence of diverse metabolites in *W. ugandensis*, specifically fatty acids and terpenoids could be linked to the difference in the expression levels of fatty acid desaturases and terpernoid synthases; which are key structural enzymes involved in biosynthesis of unique backbones of fatty acids and terpenoids, respectively [3].

Significant solvent-dependent differences in compound profiles in different parts of *W. ugandensis* were observed. The solvent effects identified in this study revealed that MeOH extracted higher numbers (amounts) of compounds from *W. ugandensis* compared to DCM regardless of the plant parts analyzed. These findings support the work of Iloki *et al.* [38] who reported higher numbers of compounds in MeOH extracts of *Phoradendron californicum* (oak and mesquite) compared to extraction yields of other solvents such as DCM, ethanol, hexane and ethyl acetate. Sun and Ho [39] also showed that MeOH was the most effective solvent in extracting phytoconstituents of oat bran. The content of phytochemicals were higher in MeOH, a more polar solvent than DCM, hence MeOH seems to be a good solvent for the recovery of optimum yield of phytochemicals in different parts of *W. ugandensis*. Remarkable differences were also observed in the percentage content of some of the major and minor compounds in the leaf and stem bark extracts of *W. ugandensis*. These could be attributed to the type of solvent used, its polarity index and the solubility of the compounds in the extraction solvent as well as the plant part analyzed and origin of the plant samples [40].

The general chemical profile of *W. ugandensis* extracts from the five populations was found to be quite different from what was previously reported. Compounds that were earlier reported to occur in this tree species such as warburganal, muzigadial and polygodial [41, 42] were not identified in the present study. The major compounds identified in the present study were notably different from the main compounds observed by Oladipupo *et al.* [33] in South Africa. In their paper, Oladipupo *et al.* [33] reported myrcene (27.5%), limonene (16.9%), (E)-ß-ocimene (11.1%) and (Z)-ß-ocimene (9.5%) as the major compounds while in this study, copaen-15-ol (9.83%), alloaromadendrene (9.69%) and 1,4-pentadien-3-one, 1,5-diphenyl (8.40%) were the main compounds. Contrary to the documented literature, most of the major compounds in this study were dominant in the leaf extracts compared to the stem bark extracts. Therefore, the leaves should be used as a replacement for the stem barks in phytotherapy. These variations in literature data may not only reveal the difference in plant parts analyzed and extraction method used, but also the influence of geographic, climatic and genetic factors on the chemical composition of plants. In fact, there have been many reports that the chemical compositions of plants are variable between seasons, sites, altitudes, species, and ages, even in individual plants [37, 43].

Genetic diversity has been recognized as a source of chemical variation in plants [22]. Since the plant materials in this study were collected from different eco-geographic locations across the Kenyan Rift Valley, it is likely that the plants could have undergone some genetic differentiation as a consequence of local adaptation to different ecological conditions emanating from random genetic drift [44]. It has been documented that plant populations that are distantly or geographically isolated usually evolve separately as they adapt to new ecological habitats. This leads to alterations in allele frequencies hence genetic variation and diversity in their chemical profiles as well as in the presumed medicinal properties [45]. A study by Gachie *et al.* [46] also revealed variations in the yield and composition of crude bark extracts of *Prunus africana* trees from different provenances in Kenya. These differences were attributed to the geographic and genetic isolation among the tree populations.

CONCLUSION

The present study confirms that *W. ugandensis* is a rich source of phytochemicals as depicted by the presence of several classes of compounds in its leaf and stem bark extracts. The abundance of these phytochemicals, especially terpenoids and fatty acid derivatives could be linked the medicinal activities associated with this plant species. There were variations in the profiles of compounds identified in *W. ugandensis* extracts. The varied chemical composition was dependent on the solvents used for extraction, sampling sites and plant parts analyzed. All the extracts of *W. ugandensis* from the five populations across the Kenyan Rift Valley were characterized by high percentage of sesquiterpenoids and fatty acid derivatives as the dominant classes of compounds. There were substantial variations in the amount of phytochemicals present in the leaf and stem bark extracts of *W. ugandensis*. Due to the presence of high amount of bioactive compounds in the leaf and stem bark extracts of this plant species, there is need for sustainable utilization and efficient conservation strategies of the Kenyan populations of *W. ugandensis*.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest from the authors regarding publication of this article.

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