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Research Article

Interaction Effects of Sites, Samples, Plant Parts and Solvent Types on Antimicrobial Activity of the Kenyan Populations of *Warburgia ugandensis* (Sprague)

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ABSTRACT
Warburgia ugandensis Sprague is one of the most valuable medicinal plants in Eastern, Central and Southern Africa. The roots, leaves, stem barks and heartwoods of this plant species are commonly used in traditional and modern herbal medicine to treat several diseases. Though widely used in phytomedicine, there is no published data on the interaction effects of sites, samples, plant parts and solvent types on either antibacterial or antifungal activities of the Kenyan populations of *W. ugandensis*. The extracts of this plant species exhibited antimicrobial potential against *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 90028). This was statistically confirmed by the test of normality for dependent variables with mean antibacterial and antifungal effects of 3.169±0.27 and 1.761±0.21mg/ml, respectively regardless of the interaction effects of samples, plant parts, sites and solvent types. This study demonstrated that sites (origin) of plant materials, types of solvents used for extraction, plant parts analyzed and their interactions had statistically significant effects on antimicrobial activities of *W. ugandensis* ($p \leq 0.05$). From the results of this research work, there is need for effective conservation strategies for this useful medicinal plant species in Kenya.

KEYWORDS: *Warburgia ugandensis*; phytomedicine; interactions; conservation; Kenya

INTRODUCTION

Natural products and plant secondary metabolites have been used as sources of medicinal agents for treating various human diseases such as psychiatric disorders, diabetes, cancer, coronary heart diseases and infectious diseases [1]. A report by the World Health Organization (WHO) indicated that 65-80% of the world population depends on herbal medicine for treating different illnesses [2]. To date, many plants have been shown to possess beneficial health effects such as anti-inflammatory, antimicrobial, antioxidant and anti-mutagenic properties. With emergence of multiple drug resistant strains of pathogens due to indiscriminate use of antibiotics in treatment of infectious diseases, great interests are being regenerated in traditional medicine [2].

Plants have perpetually been known to be rich sources of major bioactive compounds such as cocaine, morphine, digitalis, tubocurarine, quinine, muscarine and nicotine. Most of these compounds are useful drugs, for instance,

morphine and quinine are potent medicinal agents for treating pain and malaria, respectively. Similarly, clinically important drugs such as anticancer agent paclitaxel (Taxol) and antimalarial agent artemisinin were isolated from Pacific yew tree (*Taxus brevifolia*) and *Artemisia annua*, respectively while local anesthetics have been developed from cocaine, a psychoactive alkaloid from the leaves of Coca plants (*Erythroxylum coca* and *Erythroxylum novogranatense*) [3-5]. Some of the most important phytochemicals include terpenes, alkaloids, flavonoids, tannins, saponins and phenolics [6].

Previous studies have shown that antimicrobial activities of plants depend on the extraction procedures, type of extracts or the solvent used for extraction [7]. Several authors have reported variations on antimicrobial efficacy based on different types of extracts [8-12]. In a similar study, Sen and Batra [7] reported higher antimicrobial activity in the organic extracts of *Melia azedarach* against clinical isolates of multi-drug resistant microorganisms. Even though the modes of action of bioactive constituents of these plants are not known, it is clear that the potency of these extracts basically depends on the type of solvent used [7]. As reported by Cowan [13], most of the antimicrobials isolated from plants are normally aromatic or saturated organic molecules that can readily be solubilized in organic solvents. Therefore, it is possible to postulate that organic extracts contain non-polar residues which could be responsible for their high bactericidal and bacteriostatic effects [14].

The geographical origin or sites where plant materials are collected also influence the phytochemistry and pharmacological properties of medicinal plants. Working on the antibacterial activity of *Callistemon citrinus* and *Albizia lebbeck*, Seyydnejad *et al.* [15] reported that plants growing in different locations exhibited different medicinal activities. These differences in bioactivities were attributed to ecological or environmental factors (climatic conditions, altitude, soil types) as well as time of collection, sample preparation, age of plant and type of plant parts analyzed for therapeutic effects [15].

The plant under study, *Warburgia ugandensis* Sprague (Family: Canellaceae) is an endangered tree species which is highly utilized in herbal medicine in tropical and sub-tropical countries of Africa [16]. This tree species has a high medicinal significance both for livestock and humans, exhibiting broad spectrum antimicrobial potential. *Warburgia ugandensis* has been widely used by traditional medicinal practitioners in treating malaria, sexually transmitted diseases, hepatitis, asthma, headache, stomachache, diarrhea, constipation, tuberculosis, bronchitis and parasitic infections among other illnesses. The leaves, pods and seeds of this tree are used as fodder for livestock [17-20]. The therapeutic value of this plant species is linked to the presence of a wide variety of secondary metabolites especially sesquiterpenoids and fatty acid derivatives in its stem bark, root, leaf and heartwood extracts [12, 21, 22].

Despite the pharmacological importance of *W. ugandensis*, there is no information regarding the effects of different sites, samples, plant parts and solvent (extract) types on antimicrobial activity of its extracts from different populations in Kenya. This study will help in conservation of *W. ugandensis* in Kenya based on the statistical data on the interaction effects of sites, samples, plant parts and solvent types on antibacterial (*Staphylococcus aureus*) and antifungal (*Candida albicans*) activities. Focus will be on the bioactive populations whose plant parts would be sustainably utilized than others. Thus, the objective of this study was to determine the interaction effects of sites, samples, plant parts and solvent types on antimicrobial activity of *Warburgia ugandensis* extracts from different populations across the Kenyan Rift Valley.

MATERIALS AND METHODS

Sample Collection, Preparation and Solvent Extraction

As a continuation of the previous publication (work) on antimicrobial activity of *W. ugandensis*, the leaf and stem bark samples were randomly collected from five ecological populations of *W. ugandensis* across the Kenyan Rift Valley. The sampling sites included Karura, Londiani, Kitale, Kinale and Rumuruti forests. Four (4) trees were sampled per population and labeled T1, T2, T3 and T4. The samples were thoroughly cleaned, air dried and pulverized into fine powder. The powdered samples were

sequentially extracted with dichloromethane (DCM) and methanol (MeOH) according to the protocol of Abuto *et al.* [12]. The extracts were then concentrated at reduced pressure and temperature (35-50°C) using a rotary evaporator. The DCM and MeOH extracts were then being subjected to antimicrobial activity tests.

Antimicrobial Activity

The antimicrobial potential of *W. ugandensis* extracts was determined against *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 90028) using disk diffusion and ninety six well microtitre plate assays as previously outlined by Abuto *et al.* [12] and Mwitari *et al.* [23]. Chloramphenicol 30µg per disk (BioMerieux) and fluconazole 25µg per disk (Pfizer) served as reference drugs while 99.9% dimethyl sulfoxide (DMSO) (Sigma-Aldrich) was used as a negative control. The minimum inhibitory concentration (MIC) values (not shown in this article but available on request) were used for statistical analyses.

Statistical Analyses

Factorial ANOVA (two-, three- and four-factor ANOVAs) were used to analyze the mean interaction effects of sites, samples, plant part and solvent (extract) types on either antibacterial (*S. aureus*) or antifungal (*C. albicans*) activities of *W. ugandensis*. The MIC data were used for Factorial ANOVA computations. Tukey's HSD test was used for post-hoc analysis ($p \leq 0.05$). Data was presented as Mean±Standard Error of Mean (x±SEM).

RESULTS

Interactions of sites, samples, plant parts and extract types on antibacterial activity

This study sought to establish if sites, samples, plant parts, solvent types and their interactions had any statistically significant effect on either antibacterial or antifungal activities (dependent variables) of *W. ugandensis*. A two-way ANOVA for the interactions between site versus sample, site versus plant part, site versus solvent type, sample versus plant part, sample versus solvent type and plant part versus solvent type on either antibacterial or antifungal activity was determined. There was a statistically significant interaction between the effect of site versus sample on the antibacterial activity (*S. aureus*) (F (12, 112) = 2.657, $p < 0.05$) (Table 1). This was confirmed in the profile plot for the interaction between site and sample (Figure 1). Non-significant interactions for site versus plant part, site versus solvent type, sample versus plant part, sample versus solvent type and plant part versus solvent type ($p > 0.05$) were observed in this study as outlined in Table 1 and interaction profile plots (Figures 2, 3, 4 and 5, respectively).

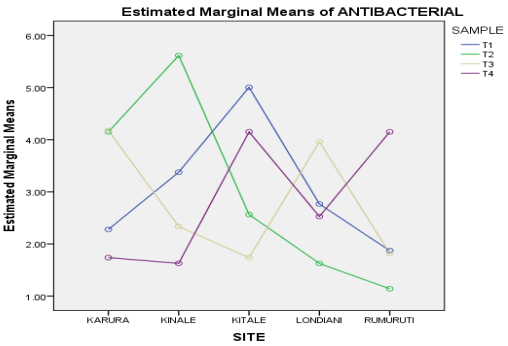

Fig. 1: Profile plot for the interaction between sites and samples

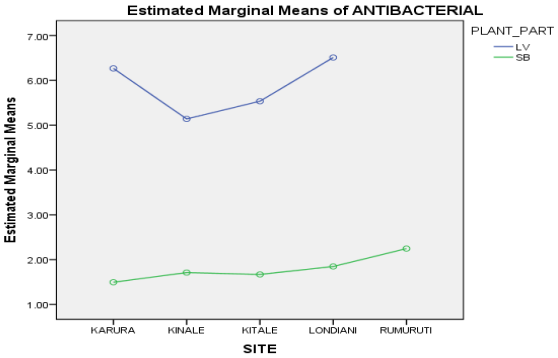

Fig. 2: Profile plot for the interaction between sites and plant parts

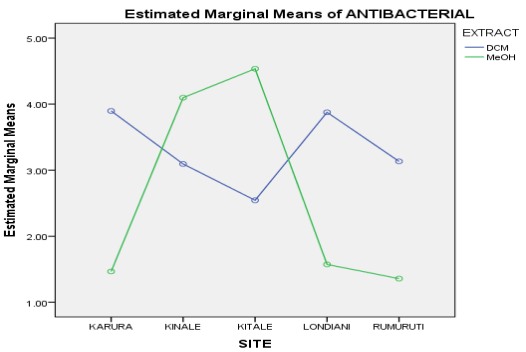

Fig. 3: Profile plot for the interaction between sites and extract types

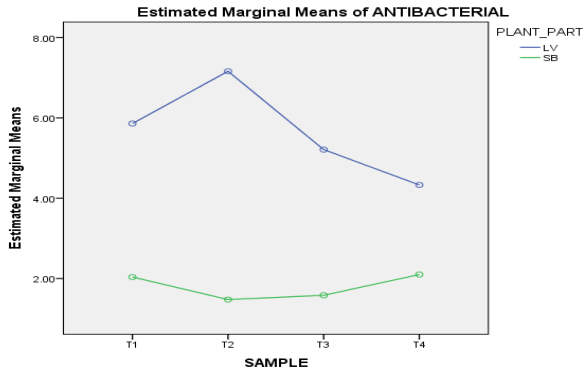

Fig. 4: Profile plot for the interaction between samples and plant parts

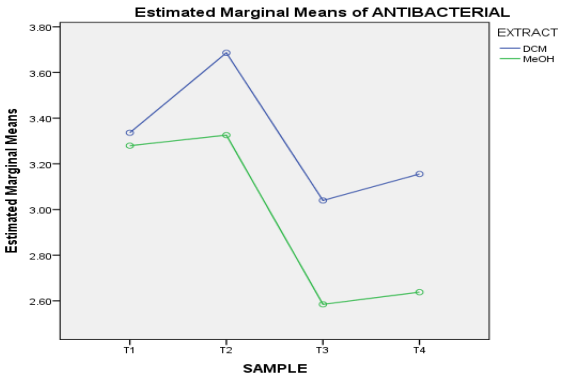

Fig. 5: Profile plot for the interaction between samples and extract types

Other significant interactions were also observed following three-way ANOVA for site versus sample versus plant part on antibacterial activity (F (6, 112) = 3.542, $p < 0.05$). Other interactions for site versus sample versus solvent type, site versus plant part versus solvent type, and sample versus plant part versus solvent type on antibacterial activity were statistically insignificant ($p > 0.05$) (Table 1). There was also significant difference in the mean antibacterial (*S. aureus*) effects between the different plant parts (leaves and stem barks) of *W. ugandensis* (F (1, 112) = 63.012, $p < 0.001$) (Table 1). On the contrary, multiple comparisons revealed non-significant difference in the mean antibacterial effects for the different sites (Karura, Kinale, Kitale, Londiani and Rumuruti) (Table 1 and 2, respectively), samples (T1, T2, T3 and T4) (Table 1 and 3, respectively) and solvent types (DCM and MeOH) (Table 1) ($p > 0.05$). All the subsets were homogeneous for both the sites and samples, respectively.

Table 1: Factorial ANOVA for tests of between-subjects effects on antibacterial activity
Dependent Variable: Antibacterial effects (*S. aureus*)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	1267.373[a]	55	23.043	3.154	0.000
Intercept	1817.369	1	1817.369	248.717	0.000
Site	31.421	4	7.855	1.075	0.372
Sample	42.169	3	14.056	1.924	0.130
Plant Part	**460.426**	**1**	**460.426**	**63.012**	**0.000**
Solvent Type	2.672	1	2.672	0.366	0.547
Site * Sample	**232.964**	**12**	**19.414**	**2.657**	**0.004**
Site * Plant Part	26.962	3	8.987	1.230	0.302
Site * Solvent Type	48.175	4	12.044	1.648	0.167
Sample * Plant Part	26.868	3	8.956	1.226	0.304
Sample * Solvent Type	30.380	3	10.127	1.386	0.251
Plant Part * Solvent Type	0.000	1	0.000	0.000	0.995
Site * Sample * Plant Part	**155.280**	**6**	**25.880**	**3.542**	**0.003**
Site * Sample * Solvent Type	28.802	8	3.600	0.493	0.859
Site * Plant Part * Solvent Type	6.803	1	6.803	0.931	0.337
Sample * Plant Part *	5.960	2	2.980	0.408	0.666

Solvent Type					
Site * Sample * Plant Part * Solvent Type	0.123	1	0.123	0.017	0.897
Error	818.381	112	7.307		
Total	3772.512	168			
Corrected Total	2085.755	167			

a. R Squared = 0.608 (Adjusted R Squared = 0.415).

Interactions **(in bold)** are significant at $p \leq 0.05$

Table 2: Multiple comparisons on effects of sites on antibacterial activity

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Karura	Kinale	-0.4703	0.62476	0.943	-2.2026	1.2620
	Kitale	-0.3864	0.60444	0.968	-2.0624	1.2895
	Londiani	0.0742	0.71234	1.000	-1.9010	2.0493
	Rumuruti	0.8408	0.71234	0.763	-1.1343	2.8160
Kinale	Karura	0.4703	0.62476	0.943	-1.2620	2.2026
	Kitale	0.0838	0.59138	1.000	-1.5559	1.7236
	Londiani	0.5444	0.70130	0.937	-1.4001	2.4889
	Rumuruti	1.3111	0.70130	0.340	-.6334	3.2556
Kitale	Karura	0.3864	0.60444	0.968	-1.2895	2.0624
	Kinale	-0.0838	0.59138	1.000	-1.7236	1.5559
	Londiani	0.4606	0.68325	0.962	-1.4339	2.3551
	Rumuruti	1.2273	0.68325	0.381	-.6672	3.1217
Londiani	Karura	-0.0742	0.71234	1.000	-2.0493	1.9010
	Kinale	-0.5444	0.70130	0.937	-2.4889	1.4001
	Kitale	-0.4606	0.68325	0.962	-2.3551	1.4339
	Rumuruti	0.7667	0.78033	0.863	-1.3970	2.9303
Rumuruti	Karura	-0.8408	0.71234	0.763	-2.8160	1.1343
	Kinale	-1.3111	0.70130	0.340	-3.2556	0.6334
	Kitale	-1.2273	0.68325	0.381	-3.1217	0.6672
	Londiani	-0.7667	0.78033	0.863	-2.9303	1.39706

Based on observed means; the error term is Mean Square (Error) = 7.307.

Table 3: Multiple comparisons on effects of samples on antibacterial activity

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
T1	T2	-0.1962	0.57996	0.987	-1.7087	1.3164
	T3	0.4326	0.57996	0.878	-1.0799	1.9452
	T4	0.3534	0.59138	0.933	-1.1890	1.8957
T2	T1	0.1962	0.57996	0.987	-1.3164	1.7087
	T3	0.6288	0.58987	0.711	-0.9096	2.1672
	T4	0.5495	0.60111	0.797	-1.0182	2.1173
T3	T1	-0.4326	0.57996	0.878	-1.9452	1.0799
	T2	-0.6288	0.58987	0.711	-2.1672	0.9096
	T4	-0.0793	0.60111	0.999	-1.6470	1.4884
T4	T1	-0.3534	0.59138	0.933	-1.8957	1.1890
	T2	-0.5495	0.60111	0.797	-2.1173	1.0182
	T3	0.0793	0.60111	0.999	-1.4884	1.6470

Based on observed means; the error term is Mean Square (Error) = 7.307.

Interactions of sites, samples, plant parts and solvent types on antifungal activity:

Analysis by two-way ANOVA revealed significant interaction between the effects of site versus solvent type on the antifungal (*C. albicans*) activity (F (3, 84) = 5.445, $p < 0.05$) (Table 4). This was confirmed in the profile plot for the interaction between site and solvent (extract) type (Figure 6). There was also a statistically significant interaction between the

effects of sample versus solvent type on the antifungal activity (F (3, 84) = 3.278, $p < 0.05$) (Table 4 and Figure 7, respectively). Other significant interactions were also observed following three-way ANOVA for site versus sample versus solvent type on the antifungal activity of *C. albicans* (F (5, 84) = 2.808, $p < 0.05$). Non-significant interactions were also observed following two- and three-way ANOVAs for plant part versus solvent type, site versus sample versus plant part, sample versus plant part, site versus plant part and site versus sample on the antifungal activity ($p > 0.05$). Other non-significant interactions were also observed following three- and four-way ANOVAs for site versus plant part versus solvent type, sample versus plant part versus solvent type, site versus sample versus plant part versus solvent type on antifungal activity (Table 4).

Multiple comparisons on the effects of sites revealed significant difference in the mean antifungal (*C. albicans*) activity among the different sites (Karura, Kinale, Kiate, Londiani and Rumuruti) (F (4, 84) = 3.250, $p < 0.05$) (Table 4 and 6, respectively). Similarly, there was a significant difference in the mean antifungal effect between the different solvent types (DCM and MeOH) (F (1, 84) = 6.162, $p < 0.05$) (Table 4). Conversely, there was no significant difference in the mean antifungal activity between the different samples (T1, T2, T3 and T4) (Table 4 and 5, respectively) and plant parts (leaves and stem bark extracts) with all the subsets being homogeneous for the sites and samples, respectively. The mean antibacterial (*S. aureus*) and antifungal (*C. albicans*) effects of extracts of *W. ugandensis* from the five populations were 3.169±0.27 and 1.761±0.21mg/ml, respectively regardless of the interaction effects of sites, samples, solvent types and plant parts on antibacterial or antifungal activities as shown by the test of normality for the dependent variables (Table 7).

Table 4: Factorial ANOVA for tests of between-subjects effects on antifungal activity
Dependent Variable: Antifungal effects (*C. albicans*)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	367.603[a]	41	8.966	2.294	0.001
Intercept	265.304	1	265.304	67.880	0.000
Site	**50.816**	**4**	**12.704**	**3.250**	**0.016**
Sample	12.854	3	4.285	1.096	0.355
Plant Part	6.068	1	6.068	1.553	0.216
Solvent type	**24.085**	**1**	**24.085**	**6.162**	**0.015**
Site * Sample	51.391	12	4.283	1.096	0.375
Site * Plant Part	2.824	2	1.412	0.361	0.698
Site * Solvent Type	**63.850**	**3**	**21.283**	**5.445**	**0.002**
Sample * Plant Part	7.056	3	2.352	0.602	0.616
Sample * Solvent Type	**38.434**	**3**	**12.811**	**3.278**	**0.025**
Plant Part * Solvent Type	0.008	1	0.008	0.002	0.965
Site * Sample * Plant Part	5.856	1	5.856	1.498	0.224
Site * Sample * Solvent Type	**54.869**	**5**	**10.974**	**2.808**	**0.021**
Site * Plant Part * Solvent Type	0.000	0	.	.	.
Sample * Plant Part * Solvent Type	0.000	0	.	.	.
Site * Sample * Plant Part * Solvent Type	0.000	0	.	.	.
Error	328.309	84	3.908		
Total	1086.738	126			
Corrected Total	695.912	125			

a. R Squared = 0.528 (Adjusted R Squared = 0.298)

Interactions **(in bold)** are significant at $p \leq 0.05$

Table 5: Multiple comparisons on effects of samples on antifungal activity

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T2	0.4129	0.48872	0.833	-0.8681	1.6940
	T3	-0.0553	0.54142	1.000	-1.4744	1.3639
	T4	-0.3709	0.48872	0.873	-1.6520	0.9101
T2	T1	-0.4129	0.48872	0.833	-1.6940	0.8681
	T3	-0.4682	0.52098	0.806	-1.8338	0.8974
	T4	-0.7839	0.46598	0.339	-2.0053	0.4375
T3	T1	0.0553	0.54142	1.000	-1.3639	1.4744
	T2	0.4682	0.52098	0.806	-0.8974	1.8338
	T4	-0.3157	0.52098	0.930	-1.6813	1.0499
T4	T1	0.3709	0.48872	0.873	-0.9101	1.6520
	T2	0.7839	0.46598	0.339	-0.4375	2.0053
	T3	0.3157	0.52098	0.930	-1.0499	1.6813

Based on observed means; the error term is Mean Square (Error) = 3.908.

Table 6: Multiple comparisons on effects of sites on antifungal activity

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Karura	Kinale	-0.0546	0.54142	1.000	-1.5640	1.4548
	Kitale	1.1540	0.59074	0.298	-0.4929	2.8009
	Londiani	0.9171	0.61643	0.573	-0.8015	2.6356
	Rumuruti	**1.5803**	**0.53037**	**0.030**	**0.1016**	**3.0589**
Kinale	Karura	0.0546	0.54142	1.000	-1.4548	1.5640
	Kitale	1.2086	0.56249	0.210	-0.3596	2.7768
	Londiani	0.9717	0.58942	0.471	-0.6716	2.6149
	Rumuruti	**1.6348**	**0.49872**	**0.013**	**0.2445**	**3.0252**
Kitale	Karura	-1.1540	0.59074	0.298	-2.8009	0.4929
	Kinale	-1.2086	0.56249	0.210	-2.7768	0.3596
	Londiani	-0.2369	0.63502	0.996	-2.0073	1.5335
	Rumuruti	0.4263	0.55186	0.938	-1.1123	1.9648
Londiani	Karura	-0.9171	0.61643	0.573	-2.6356	0.8015
	Kinale	-0.9717	0.58942	0.471	-2.6149	0.6716
	Kitale	0.2369	0.63502	0.996	-1.5335	2.0073
	Rumuruti	0.6632	0.57929	0.782	-0.9518	2.2782
Rumuruti	**Karura**	**-1.5803**	**0.53037**	**0.030**	**-3.0589**	**-0.1016**
	Kinale	**-1.6348**	**0.49872**	**0.013**	**-3.0252**	**-0.2445**
	Kitale	-0.4263	0.55186	0.938	-1.9648	1.1123
	Londiani	-0.6632	0.57929	0.782	-2.2782	0.9518

Based on observed means; the error term is Mean Square (Error) = 3.908.
The mean differences (**in bold**) are significant at $p \leq 0.05$.

Table 7: Test of normality for dependent variables (antibacterial and antifungal effects)

		Antibacterial effects	Antifungal effects
N	Valid	168	126
	Missing	72	114
Mean		3.1686	1.7612
Std. Error of Mean		0.27266	0.21020
Std. Deviation		3.53406	2.35951
Skewness		2.238	2.724

Std. Error of Skewness	0.187	0.216
Kurtosis	5.049	10.016
Std. Error of Kurtosis	0.373	0.428

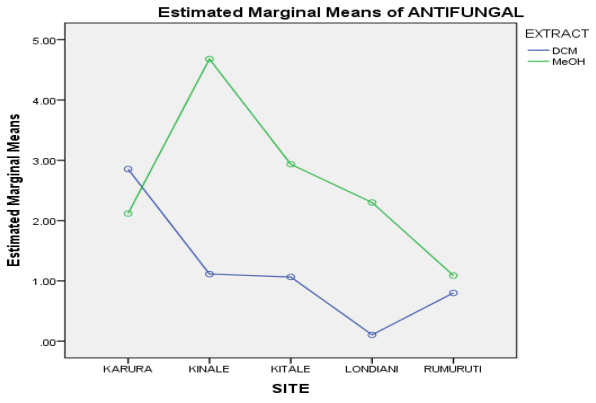

Fig. 6: Profile plot for the interaction between sites and extract types.

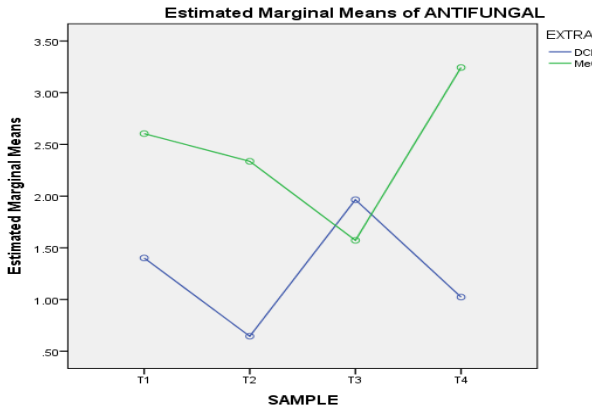

Fig. 7: Profile plot for the interaction between samples and extract types

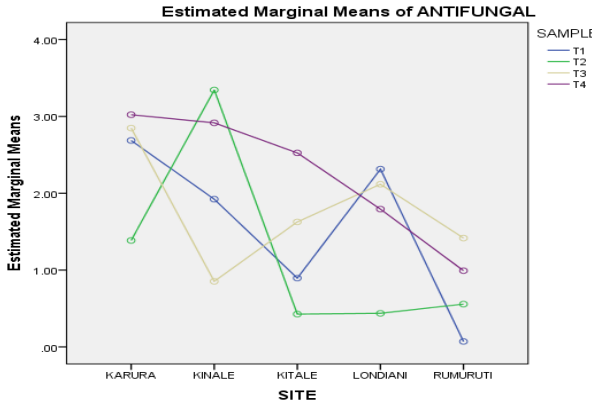

Fig. 8: Profile plot for the interaction between sites and samples

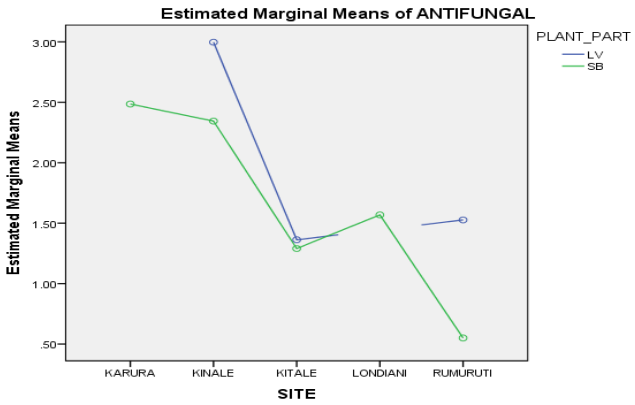

Fig. 9: Profile plot for the interaction between sites and plant parts.

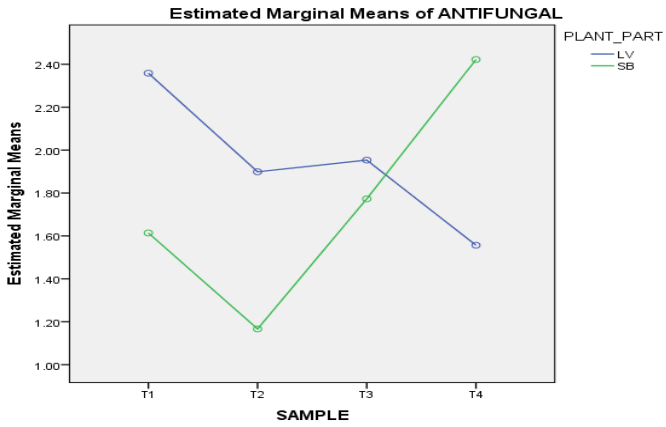

Fig. 10: Profile plot for the interaction between samples and plant parts

DISCUSSION

So far, there is no comparative study available on the interaction effects of sites, samples, plant parts and solvent types on either antibacterial or antifungal activities of the Kenyan populations of *W. ugandensis*. The extracts of *W. ugandensis* exhibited antimicrobial potential against *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 90028). This was evident in the test of normality for dependent variables (antibacterial and antifungal effects) where the extracts of *W. ugandensis* displayed mean antimicrobial activities of 3.169±0.27 and 1.761±0.21mg/ml against *S. aureus* and *C. albicans*, respectively regardless of the interaction effects of samples, plant parts, sites and solvent types on antibacterial and antifungal properties. The observed differences in the mean antibacterial and antifungal effects

suggested that *C. albicans* (1.761±0.21mg/ml) was more susceptible to the plant extracts as compared to *S. aureus* (3.169±0.27mg/ml).

Previous researchers working on antimicrobial activities of crude extracts of *W. ugandensis* also reported higher bioactivities against C. *albicans* compared to *S. aureus* [24, 25, 26]. The varied antimicrobial activities could be linked to the enhanced defense mechanisms acquired by these microorganisms besides the differences in the strains of pathogens under investigation [27]. The difference in sensitivities of *C. albicans* and *S. aureus* to the plant extracts could be associated with the diversity in diffusibility of phytochemicals through the culture media as well as the morphological constitution of these microorganisms; which could further influence their reaction to the plant extracts [28, 29].

This study revealed statistically significant differences in the interaction effects among samples, solvent types, sites and plant parts on antimicrobial activities of *W. ugandensis*. Studies have shown that biological activities of plant extracts can be significantly influenced by the effects of interactions among different factors [6, 30]. Significant interactions for the effects of samples, sites, solvent types and plant parts on antifungal activities were observed in this study ($p < 0.05$) while non-significant interactions for sites and samples on antibacterial activities were also noted in the present study ($p > 0.05$). These diverse findings could be explained by the fact that the type and level of antimicrobial activity displayed by any plant material is influenced by the interactions of factors such as temperature, harvesting time, soil type, test strains, drying methods and storage conditions among other aspects [31]. For instance, a relatively high temperature of 50^{o}C and above which is normally generated during plant tissue grinding or pulverization can denature chemical constituents of the plant and affect the level of biological activity and chemical composition of secondary metabolites extracted from the plant tissues [31].

According to Figueiredo *et al.* [32], plant origin (sites), plant parts analyzed, type of solvent used and their relationships are crucial parameters to consider when analyzing plants for their medicinal properties. Solvent types (for example chloroform, ethanol, acetone, hexane and water) among others are known to affect plant extract antimicrobial activities due to polarity differences between solvents [33]. Such differences in antimicrobial potential with regard to the solvent types were observed in DCM and MeOH extracts of *W. ugandensis* in this study.

Multiple comparisons revealed varied outcomes in the mean antibacterial and antifungal effects for different sites (Karura, Kinale, Kitale, Londiani and Rumuruti), samples (T1, T2, T3 and T4), solvent types (DCM and MeOH) and plant parts (leaves and stem barks) of *W. ugandensis*. The differences in antimicrobial activities with regard to plant parts could be linked to the synergistic or antagonistic actions of various secondary metabolites such as fatty acids, flavonoids and sesquiterpenoids present in this plant species [22, 34, 35]. According to Chanda and Kaneira [36], the bioactive components are normally accumulated as secondary metabolites in all plant cells or tissues but their concentrations could greatly vary in different plant parts. Moreover, the dissimilarity in the mean antimicrobial effects of the different plant parts and samples of *W. ugandensis* against *S. aureus* and *C. albicans* could be due to variation in the exposure level of the plant extracts to the test microorganisms [24].

The similarity or diversity in the interaction effects of different sites on the mean antimicrobial activities of *W. ugandensis* could be due to the interplay among geographical, environmental or ecological factors [37]. Some of these factors include changes in altitude and climatic conditions. Genetic differentiation, segregation or disjunction among different populations of *W. ugandensis* could also contribute to the varied interaction effects of different variables on the mean antibacterial or antifungal activities of *W. ugandensis* extracts [16].

CONCLUSION

The outcome of this research work reaffirms the antimicrobial potential of the Kenyan populations of *W. ugandensis*. Based on the statistical evidence of the test of normality for dependent variables (antibacterial and antifungal effects), the extracts of *W. ugandensis* displayed higher antimicrobial activity against *C. albicans* as compared to *S. aureus*. Therefore, this suggests that *C. albicans* was the most

susceptible pathogen to the plant extracts. Moreover, this study established that origin (sites) of plant materials, types of solvents used for extraction, plant parts analyzed and their interactions had statistically significant effects on antibacterial and antifungal activities of *W. ugandensis* extracts. From the findings of this study, there is need for conservation of this valuable medicinal plant species in Kenya.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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