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Assessment of Pathogenic Contamination and Antimicrobial Activity of Selected Herbal Medicinal Remedies in Mbarara City, South Western Uganda

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Research Article

Keywords: Herbal medicines, Contamination, Antimicrobial Activity

DOI: https://doi.org/10.21203/rs.3.rs-582967/v1

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Abstract

Background: Herbal formulations in Mbarara have been used in the treatment and management of several disease conditions extensively overtime due to low cost compared to empirical synthetic medicine, however evidenced that they can be contaminated with dangerous pathogenic organisms which are all tailored to handling practices, storage, and other environmental conditions thus, the need to further asses these herbs for safety to the consumers.

Materials and methods: Forty-five (45) liquid herbal formulations for the treatment and management of communicable infections were purchased on the open market. All Samples were cultured on plate count agar for colony counts and then subcultured on different laboratory media and then analyzed for antimicrobial activity using the agar diffusion method.

Results: Out of the 45 herbal formulations, 32(71.1 %) were contaminated while 13 (28.9 %) were not. Out of the organisms isolated from individual formulations, 19 (59.4 %) had *Bacillus subtillis and S. aureus, 4(12.5%) had C. freundi and Proteus mirabilis, 2(6.3%), C.divergens,* 1(3.1 %) *Rhodotorula*, 5(15.6%) *Aspergillus spp,* had *E. cloace*. 1 (3.1%) had *Klebsiella spp*. Of these, 29(87.9%) had contaminants within acceptable limits of less than 10^3CFU/mL, while 12(36.4%) beyond 10^3 CFU/m. Out of the 45 formulations, not even one could qualify for pharmaceutical use, all MICs all were >1000mcg/mL64.4%) were active while 16(35.6%) had no activity.

Conclusion: Herbal formulations in Mbarara are contaminated with various microbes and have very limited antimicrobial activity, herbalists therefore should be trained on good harvesting, safe handling, storage, and good manufacturing practices of these medicinal raw materials and their products, responsible authorities should enact policies and regulations to guide the herbalists and protect the public from adverse effects of consuming these unverified herbal medicinal remedies.

Background

Herbal medicines' use is the exploration of utilising homes' grown solutions for treating various forms of health disorders including but not limited to gastrointestinal, eye defects and respiratory [1]. Nearly 80% of the people in the world depend on these local medicines for primary health care needs [2-4]. In developing countries, almost hundreds of people utilise these mostly plant-exploited herbal products for microbial infections [5-7]. However, these can either be a source of great medical value or complications such as drug resistance [1, 8-10].

In East Africa and Uganda, the use of herbal medicines is a common habit with about 80% of the population accustomed to such a tradition, especially in rural settings [2, 11]. To some extent, this practice has been backed by a demonstration of antimicrobial bioactivity against microbiological agents by some of the commercially available herbal remedies [12-14].

In this regard, the Ugandan government, for instance, is encouraging the use of such formulations and an integration of their applications into the orthodox medicine framework [15, 16]. However, there are no systems in Uganda to monitor and control the herbal medicine chain of production, their hygiene conditions during production, and bioactivity spectrum, thus these products could get contaminated with pathogens leading to secondary infections [17]. Contaminants such as *Staphylococcus, Escherichia coli*, *Shigella,* and bread moulds have already been reported in some of these products. Thus, consumers of these products are at risk of exposure to such pathogens [3, 18]. This is further complicated by their unchecked administration and prescription guidelines, for instance, under or over dosing with herbal products could lead to clinical complications such as development of drug resistance and death. Therefore, this study aimed at determining the contamination levels and antimicrobial activity of randomly selected locally branded antimicrobial herbal medicinal remedies on the open market in Mbarara city, South Western Uganda.

Ethical approval

The proposal was submitted to the Department of Microbiology, Faculty of Medicine Review

Committee (FRC) and approved by the Institutional Review Committee (IRC) of Mbarara University of Science and Technology.

Results

Common microbiological contaminants of herbal formulations around Mbarara municipality.

Forty five (45) herbal formulations (samples) were selected for this study, 20 (52.6 %) of the samples had *Bacillus subtillis,* 6 (15.8%) *Citrobacter freundii,* 5(13.2 %) *Enterobacter cloacae,* 2(5.3 %) with *Citrobacter divergens,* and 1(2.6 %) *Aspergillus species, 1(2.6 %) Rhodotorula,* 1(2.6 %) *Staphylococcus aureus,* 1(2.6 %) *Klebsiella spp* and 1(2.6%) *Proteus mirabilis all* being represented in equal measure as shown in **Tables** 1 and 2.

Table 1: List of bacterial and fungal microbial contaminants as per recruited coded herbal sample formulations.

Sample	Total bacterial count (CFU/mL).	Fungal count (CFU/mL).	Isolate.	REFFERENCE RANGES.	
				Acceptable Limit	Contaminated
W01	0	0	-	$<10^3$	$> 10^3$
W02	0	0	-	$<10^3$	$> 10^3$
W03	0	0	-	$<10^3$	$> 10^3$
W04	0	0	-	$<10^3$	$> 10^3$
W05	33	0	*B.subtillis*	$<10^3$	$> 10^3$
W06	$1x10^4$	0	*B.subtillis*	$<10^3$	$> 10^3$
W07	$1x10^5$	0	*C.freundi*	$<10^3$	$> 10^3$
W08	158	0	*E.cloace*	$<10^3$	$> 10^3$
W09	0	156	*Aspergillus spp,Rhodotorula*	$<10^3$	$> 10^3$
W10	$1x10^4$	0	*C.freundi*	$<10^3$	$> 10^3$
W11	3	0	*B.subtillis*	$<10^3$	$> 10^3$
W12	18	0	*B.subtillis*	$<10^3$	$> 10^3$
W13	197	0	*B.subtillis*	$<10^3$	$> 10^3$
W14	0	0	-	$<10^3$	$> 10^3$
W15	60	0	-	$<10^3$	$> 10^3$
W16	$1x10^5$	0	*E.cloace*	$<10^3$	$> 10^3$
W17	60	0	*B.subtillis , S.aureus*	$<10^3$	$> 10^3$
W18	67	0	*B.subtillis*	$<10^3$	$> 10^3$
W19	0	0	-	$<10^3$	$> 10^3$
W20	$1x10^6$	0	*C.divergens*	$<10^3$	$> 10^3$
W21	$1x10^5$	0	*E.cloace*	$<10^3$	$> 10^3$

W22	22	0	*B.subtillis*	$<10^3$	$> 10^3$
W23	$1x10^5$	0	*B.subtillis*	$<10^3$	$> 10^3$
W24	31	0	*B.subtiliis*	$<10^3$	$> 10^3$
W25	0	0	-	$<10^3$	$> 10^3$
W26	$1x10^4$	0	*P.mirabilis.,C.freundi*	$<10^3$	$> 10^3$
W27	0	0	-	$<10^3$	$> 10^3$
W28	0	0	-	$<10^3$	$> 10^3$
W29	36	0	*B.subtillis*	$<10^3$	$> 10^3$
W30	156	0	*B.subtillis*	$<10^3$	$> 10^3$
W31	170	0	*B.subtillis*	$<10^3$	$> 10^3$
W32	$1x10^4$	0	*C.freundi*	$<10^3$	$> 10^3$
W33	95	0	*B.subtillis*	$<10^3$	$> 10^3$
W34	64	0	*B.subtillis*	$<10^3$	$> 10^3$
W35	$1x10^5$	0	*B.subtillis*	$<10^3$	$> 10^3$
W36	206	0	*B.subtillis*	$<10^3$	$> 10^3$
W37	$1x10^4$	0	*B.subtillis*	$<10^3$	$> 10^3$
W38	162	0	*E.cloace*	$<10^3$	$> 10^3$
W39	0	0	-	$<10^3$	$> 10^3$
W38	0	0	-	$<10^3$	$> 10^3$
W40	0	0	-	$<10^3$	$> 10^3$
W41	0	0	-	$<10^3$	$> 10^3$
W42	147	0	*E.cloace*	$<10^3$	$> 10^3$
W43	205	0	*Klebsiella spp*	$<10^3$	$> 10^3$
W44	$1x10^5$	0	*C.divergens*	$<10^3$	$> 10^3$
W45	107		*B.subtillis.*	$<10^3$	$> 10^3$

Table 2: Summary of common microbial contaminants in the samples herbal formulations

Contaminants	*Number of herbal formulations with contaminants*	*Percentage (%)*
Bacillus subtillis	20	52.6
Citrobacter freundi	6	15.8
Enterobacter cloaceae	5	13.2
Aspergillus species	1	2.6
Rhodotorula	1	2.6
Staphylococcus aureus	1	2.6
Citrobacter divergens	2	5.3
Klebsiella spp	1	2.6
Proteus mirabilis	1	2.6
Total	**38**	

Levels of contamination in herbal formulations on the open market around Mbarara city.

In this study, we graded in accordance with the individual contaminants as shown in Table 3 below. However, only formulations with *B. subtillis* 4(8.8%), *C. freundi* 4(8.8%), *C. divergens* 2 (4.4%), *E. cloacae* 1 (2.2%), and P. *mirabilis* 1 (2.2%) showed highly an unacceptable level of contamination. While contamination with the organism within acceptable limits were *B. subtillis* 16(35.6%), *C. freundi* 2(4.4%), *Enterobacter cloace* 4(4.5%), *Rhodotorula and Aspergillus spp* 1(2.2%), *Staphylococcus aureus* 1(2.2%), *Klebsiella spp* 1(2.2%), *Proteous mirabilis* 1(2.2%) as shown in Table 3.

Table 3: levels of contamination in herbal formulations

No	*Contaminant*	*Number of herbal formulations and their Level of contamination(n=45)*		
		No contamination 0 (cfu/ml)	Acceptable limit(1-1000)cfu/ml	Highly contaminated Greater than 1000cfu/ml
1	*Bacillus subtillis.*	25 (55.6%)	16(35.6%)	4 (8.8%)
2	*Citrobacter fruendi*	39(86.7%)	2(4.4%)	4(8.9%)
3	*Enterobacter cloace*	40(93.3%)	4(4.5 %)	1(2.2%)
4	*Rodotorula and Aspergillus spp*	44(97.8%)	2(2.2%)	0(0.0%)
5	*Staphylococcus aureus*	44(97.8%)	1(2.2%)	0(0.0%)
6	*Citrobacter divergens*	43(95.6%)	0(0.0%)	2(4.4%)
7	*Klebsiella spp*	44(97.8%)	1(2.2%)	0(0.0%)
8	*Proteous mirabilis*	44(97.8%)	0(0.0%)	1(2.2%)

Antimicrobial activity profiles against target organisms.

Our study found that Out of the 45 herbal formulations, 29 (64.4%) were active while 16(35.6%) had no activity against all standard organisms, of the 29 active herbal formulations, 26(89.6%) of the herbal formulations were active against *staphylococcus aureus*, 11(37.9%) *against Candida albicans*. 9(31.03%) against *E.coli* .The least activity was noted against *Pseudomonas aeruginosa* where 6(20.7%) of the herbal formulations acted against these microorganisms as shown in **Tables 4** and **5** below.

Table 4: In vitro antibacterial activity of the herbal formulations (n=45)

Sample	*Zone of inhibition in mm(antimicrobial activity)*			
	S.aereus ATTC 25923	*E. coli* ATTC 25922	*P.aeruginosa* ATTC 27583	*C. albicans* ATTC 10231
W01	13	0	5	0
W02	12	3	3	0
W03	13	0	0	0
W04	9	0	0	4
W05	0	0	0	0
W06	7	0	3	0
W07	0	0	3	0
W08	0	0	0	0
W09	0	0	0	0
W10	8	0	0	0
W11	0	0	0	4
W12	0	0	0	0
W13	0	0	0	0
W14	8	0	0	0
W15	12	0	0	0
W16	6	0	0	6
W17	0	0	0	0
W18	0	0	0	0
W19	7	0	0	0
W20	4	0	0	8
W21	0	0	0	0
W22	7	7	0	0
W23	17	0	0	0
W24	17	0	0	0
W25	0	3	0	3

W26	0	0	0	0
W27	20	18	0	19
W28	22	16	5	0
W29	33	0	0	0
W30	0	0	0	0
W31	0	0	0	0
W32	4	0	0	8
W33	6	0	0	0
W34	0	0	0	0
W35	0	0	0	0
W36	4	3	0	8
W37	0	0	0	0
W38	4	0	0	0
W39	0	0	0	0
W38	4	0	0	0
W40	17	20	24	32
W41	4	7	0	6
W42	0	0	0	0
W43	0	0	0	0
W44	6	3	0	8
W45	3	0	0	0
Ciprofloxacin 0.002gm/ml	**22**	**37**	**32**	
Fluconazole 2mg/ml				**42**

NOTE: zone of inhibition means the herbal formulation was active.

***Table 5*: Antimicrobial activity profiles (MICs) of different herbal formulations**

Susceptible microorganism to the herbal formulation	*Number of herbal of formulations(n=45)*	*Percentage (%)*	*MIC≤100MCG*
Staphylococcus aureus	26	89.7%	0
Eschericia.coli	8	27.6%	0
Pseudomonas aeruginosa	6	20.7%	0
Candida albicans	11	37.9%	0

Table 6: Concentrations of different herbal extracts and their respective minimum inhibitory concentrations. (n=45)

Herbal formulation	INDICATION	Conc(mcg/ml)	ACTIVITY(MIC in mcg/ml)			
			STANDARD ORGANISM ATCC STRAIN			
			S. aureus 25923	*E. coli* 25922	*P. Aeruginosa* 27583	*C. albicans* 10231
W01	Candida, syphilis and UTIs	$9.2x10^5$	$2.3x10^5$	-	$2.3x10^5$	-
W02	Candida and UTI	$13.1x10^5$	327500		$65.5x10^4$	-
W03	Candida, UTI and itching	$9.6x10^5$	$4.8x10^5$		-	-
W04	sore throat, asthma and bronchitis	$11.2x10^5$	-	-	-	$5.6x10^5$
W05	Diarrhea	$8.6x10^5$	-	--	-	-
W06	Cough	$10.3x10^5$	$51.5x10^4$		-	-
W07	Syphilis	$7.7x10^5$	-		-	-
W08	Cough and diarrhea	$13.3x10^5$	-	-	-	-
W09	Cough	$9.0x10^5$	-	-	-	-
W10	Ulcers, stomach gas and worms	$11.8x10^5$	$29.5x10^4$		-	-
W11	Mental disorder, typhoid, UTI	$10.8x10^5$	-	-	-	-
W12	worms and UTI	$12.1x10^5$	-	-	-	-
W13	liver intoxication, food poisoning	$2.9x10^4$	$7.3x10^3$	-	-	-
W14	cough, sore throat, bronchitis	$11.8x10^5$	$29.5x10^4$	-	-	-
W15	bacterial infection	$2.9x10^4$	$1.813x10^3$	-	-	-

W16	malaria and bacterial infection	$2.9x10^4$	$7.250x10^3$	-	-	-
W17	worms and diarrhea	$2.9x10^4$	-	-	-	-
W18	diarrhea, dental carries,	$9.9x10^5$	-			
W19	allergy, infections, insomnia,	$11.5x10^5$	$5.75x10^5$	-	-	-
W20	Wounds	$2.9x10^4$	-	-	-	-
W21	stomach pain and diarrhea	$2.9x10^4$	-	-	-	-
W22	stomach pain and diarrhea	$8.0x10^5$	$4.0x10^5$	-	-	-
W23	gonorrhea, UTI and candida	$2.9x10^4$	$1,8x10^3$	-	-	-
W24	Cough and sore throat	$2.9x10^4$	$1.8x10^3$	-	-	-
W25	stomach ache	$1.21x10^6$	-	-	-	-
W26	Diarrhea	$1.07x10^6$	$6.69x10^4$		-	-
W27	Candida	$1.02x10^6$	$6.38x10^4$		-	$6.375x10^3$
W28	UTI, syphilis, chest pain	$11.2x10^6$	$1.4x10^5$	-	-	-
W29	Cough	$2.9x10^4$	$1.813x10^3$			
W30	Candida, cough, diarrhea and UTI	$2.9x10^4$	-	-	-	-
W31	Cough	$2.9x10^4$	-	-	-	-
W32	Candida, gonorrhea, syphilis	$2.9x10^4$	-	-	-	$3.625x10^3$
W33	Candida, gonorrhea,	$2.9x10^4$	-	-	-	-

	syphilis					
W34	Diarrhea, appetite, itching anus, worms, Stomach ache	$2.9x10^4$	$1.813x10^3$	-	-	-
W35	Boils and wounds	$2.9x10^4$	$1.8x10^3$	-	-	-
W36	Wounds and sore throat	$2.9x10^4$	-	-	-	-
W37	Stomach gas	$2.9x10^4$	-	$1.813x10^3$	-	-
W38	Cough, stomach ache, diarrhea	$2.9x10^4$	-	-	-	-
W39	clean fallopian tube, man power	$1.22x10^6$	-	-	-	-
W40	Mouth wash and antibacterial	$1.02x10^6$	$12.75x10^4$	$12.75x10^4$	$1.275x10^5$	$1.275x10^5$
W41	Tonsillitis, allergy, flu, cough	$8.1x10^5$	-	-	-	-
W42	Pressure ,cough asthma	$1.21x10^6$	-	-	–	-
W43	Diarrhea	$2.9x10^4$	-	-	-	-
W44	Cough, UTI and stomach pain	$2.9x10^4$	-	-	-	$3.625x10^3$
W45	Cough, UTI	$2.9x10^4$	-	-	-	-

Discussion

In this study, herbal preparations contained varying degrees of both bacteria and fungi as common contaminants .This has also been reported in similar studies by Archibong and colleagues [20-22]. Of

concern is the high levels of contaminants such as *B. subtillis, C. freundi, C. divergens, E. cloacae* and *P. mirabilis* although some had acceptable values for *Enterobacter cloacae, Rhodotorula and Aspergillus spp, Staphylococcus Aureus, Klebsiella spp* according to WHO standards. This is similar to studies done in Mwanza with the exception of *Shigella* and *Salmonella* that we didn't obtain [23, 24].

Contamination of these herbal preparations could be resulting from the methods involved in the production processes as previously reported by [20, 25, 26]. Storage conditions, methods of drying herbal raw materials such as roots, leaves and stems, bark, improper handling, and the soil from which raw materials are obtained may contribute to the presence of these contaminants in the herbal products.

Presence of microorganisms in therapeutic herbal preparations incapacitates the pharmacological activity of the herbal products and contributes to secondary infections to the consumers. Previous studies have reported that the majority of these isolates are implicated in causing various disease conditions including gastritis. The cultivation of high numbers of *Bacillus subtilis* could be because of inadequate heat processing methods, improper handling of the products, and contaminated processing equipment. Our results are in agreement with that obtained by Oluwatoyin and colleagues [24, 27] which found that most herbal remedies were contaminated because of improper handling of the products, and contaminated processing equipment used in the production process.

Bacillus subtilis is not considered a human pathogen nor is it toxigenic like some other members of the genus, however in immune-suppressed individuals, it's implicated in various systemic infections such as endocarditis, meningitis [26-28].

On the other hand, the presence of members of the *Enterobacteriaceae* family such as *C. freundi , C. divergens , E. cloacae , P. mirabilis, Enterobacter cloacae* and *Klebsiella spp* is an indication of fecal contamination. This revealed poor hygienic practices of the herbalists in the process of production and handling of these products [24, 25, 29]. These results are similar to those done in Croatia and Tanzania, .They are known food borne pathogens and thus implicated in food poisoning and diarrhea diseases [25, 30, 31].

Not only did we obtain the above but also *Staphylococcus aureus* was also identified in this study, *S. aureus* has also been reported in similar study done in Brazil and Kenya [24, 25] except that its prevalence was higher compared to our study and its implicated in scalded skin syndrome, wound abscess, and gastroenteritis[25, 32].

Microbial analysis of these samples also indicated the presence of molds, especially *Aspergillus* and *Rhodotorula species*. These have also been reported in similar studies done in Brazil [25, 33]. Since fungi are ubiquitous in nature, their presence reveals that it could be because of improper storage and handling practices of raw materials during pre-and post-harvesting and processing periods. *Rhodotorula* spp is known to have high affinity for plastics, therefore its presence indicates poor packaging of the products since the majority of the herbal preparations were sold in unauthentic plastic bottles which appeared to have been used for other products such as commercial mineral water [34, 35].

With all negative impacts of the contaminants mentioned above, *Rhodotorula* was't left out. Previously it was considered nonpathogenic but has emerged as pathogenic opportunists that may colonize susceptible patients, and it causes bloodstream infections, meningeal and skin infections [36-38]. Aspergillus spp causes a range of diseases referred to as áspergillosis with a variety of clinical syndromes, the spectrum of these illness depending on host pathogen interactions. These include invasive pulmonary aspergillosis, chronic pulmonary aspergillosis, and allergic Broncho pulmonary aspergillosis.[39-41].

Invitro- antibacterial activity in this study was carried out on targeted standard organisms, namely, *S.aureus* **ATTC 25923, E. coli ATTC 25922**, *P.aeruginosa* ATTC **27583** and *C. albicans* **ATTC 10231**.

Herbal extracts have proven antibacterial activity in studies reported previously [26, 42-44]. This is similar to what we obtained in this study. We established that majority of the extracts were active against *S .aureus*, followed by *E.coli, C .albicans,* and least against *P.aeruginosa*. This is in agreement with other studies by Shu and collegues [26, 43].

However, effectiveness was established by determining Minimum Inhibitory Concentrations, in this context, the most effective crude extract regarded as sensitive should have an MIC of <100mcg/mL, and that >1000mcg/mL is regarded as resistant[45, 46] .In our study, none of the samples had an MIC <100mcg/mL, all were >1000mcg/mL. This could be because of the inadequate and improper purification methods involved in extraction.

Our results are similar to those obtained in Northern Peru[47, 48] but contrally from those obtained by Wong and colleagues[49, 50].

Conclusion

Herbal formulations in Mbarara are contaminated with various microbes and have very limited antimicrobial activity, herbalists therefore should be trained on good harvesting, safe handling, storage, and good manufacturing practices of these medicinal raw materials and their products, responsible authorities should enact policies and regulations to guide the herbalists and protect the public from adverse effects of consuming these unverified herbal medicinal remedies.

Materials And Methods

Study site.

Study was undertaken in Mbarara city in major divisions of Nyakayojo, Nyamitanga, Kakoba, Kakiika, Kamukuzi, and Biharwe.

Study design.

Cross-sectional laboratory-based study.

Sampling.

Herbal products were randomly sampled at market price and the choice of herbal formulations was obtained by asking the vendors the herb treating a particular infection in local language and in this case skin infections such as boils, diarrhoea, stomach pain, respiratory tract, genital, urogenital and gastrointestinal tract infections were considered. Five out of the six divisions of Mbarara Municipality were randomly selected for sampling of herbal products.

Sample size determination.

A pilot study was done in two divisions of Mbarara Municipality to identify the common herbal remedies on the market and it was found out that Kakoba division had 11 different types of herbs treating different infections and Nyamitanga division had 7 different herbal remedies for different ailments giving a total of 18 herbal remedies for each division in the municipality with average of 9 therefore a total of 45 samples was used for the study.

Inclusion criteria.

Liquid formulations branded to treat cultivatable etiological agents and not expired at the time of conducting the study.

Exclusion criteria.

Herbal remedies used to treat non-communicable diseases.

Data collection instruments/methods:

Data collection commenced following a thorough explanation of the study objectives.

Microbial analysis.

Preparation of culture media.

All dehydrated media were prepared according to the manufacturer's instructions. They were mixed with distilled water and dissolved by gentle heat to boil. The media were sterilized in an autoclave at 121˚C for 15 min at 15 pounds of pressure. The sterile media were then dispensed into sterilized petri dishes and allowed to cool. The sterility of the prepared media was checked by incubation of blindly selected plates at 37˚C for 24 hrs.

Determination of the presence of bacterial contaminants.

Microbial parameters of significance such as colony forming units were determined by dispensing 1mL of the herbal formulation into the middle of a sterile culture plate using a microliter pipette followed by addition of 15-20 mL of plate count agar. The mixture was gently shaken back and forth then round to allow uniform mixing of the agar and herbal extracts. It was left to stand for 30 minutes to solidify, and

each of the samples were incubated at 37o, C for 48 hrs. CFUs were then determined by manual counting of distinct colonies and expressed in colony forming units per/mL.

Identification of bacterial and fungal contaminants.

Colonies that grew on plate count agar media were sub-cultured on blood agar, MacConkey or Sabouraud Dextrose Agar and incubated for 24 hrs at 37^{o} C for bacterial growth while 25-30^{o} C for 3 days for fungal growth. Pure cultures were identified based on morphological features, cultural characteristics, staining properties, and biochemical reactions. Lactophenol blue stain was used to identify moulds with aid of microscope [19].

Determination of antimicrobial activity of selected herbal product samples using agar diffusion assay.

The herbal samples were further examined for antimicrobial activity against selected bacterial and fungal isolates, respectively.

All samples were tested against reference bacterial strains, namely, *E. coli* **ATCC 25922,** *S. aureus* **ATCC 25923,** *P. aeruginosa* **ATCC 27583,** and *Candida albicans* **ATCC 10231.**

Individual colonies of standard organisms were inoculated on Mueller Hinton agar for bacteria using sterile swabs, two holes of 8 mm diameter and equidistant from each other were bored into the plates using a sterile glass cork borer. A volume of 1 mL of herbal formulation and 1 mL of control drug *ciprofloxacin* for bacterial activity was dispensed into the holes and left to stand for 5 minutes to allow adequate diffusion of the samples. The plates were then taken for incubation at 37°C for 24hrs.

The diameter of the zones of inhibition around each hole in the plate were measured in millimetres. The same procedure was repeated for the test with *C. albicans* **ATCC 10231** but using Sabouraud Dextrose Agar and Fluconazole as control drugs and incubation at 25-30°C for 48hrs.

Determination of minimum inhibitory concentrations (MICs) of the herbal formulations using broth dilution assay.

To determine the herbal MICs, 1 mL of the original herbal formulation was serially diluted in the ratio of 1/2, 1/4, 1/8, 1/16, 1/32 using sterile water in bijou bottles, these were then seeded on to MHA for bacteria and SDA for fungal standards. The dilutions which inhibited growth of the test organisms were read as the MICs for each of the herbal formulations.

Declarations

Ethical consideration.

Ethical approval was sought from Research Ethics Committee (REC) of Mbarara University of Science and Technology (reference number MUREC 1/7).

We blinded the products during practical procedures, analysis of samples, and dissemination of results of the study to ensure anonymity of the manufacturer and source of the product using letter codes followed by figures representing the sample number according to the sample size and this was W01-W45.

Availability of data and materials

Data and materials are readily available from the corresponding author upon request.

Authors' contributions.

GW, BM contributed in study conception and design, CO and KM Collected data, MC, HZ, IM, and GM participated in the laboratory analysis, KK and CO carried out data cleaning and analysis. MB and FT wrote the first draft of the manuscript while HI reviewed the manuscript and supervised the whole research process.

Conflict of Interest

The authors declare that they have no Conflict of interest.

Acknowledgments

We are grateful to our research assistants and staff of Mbarara University Microbiology department for all support given to us during data collection.

Funding

We did not receive funding for this study.

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