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In vitro Antibacterial Efficacy of *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave* Extracts against HIV/AIDS Patients' Oral Bacteria in South-Western Uganda

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Abstract

The objective of the study was to determine the antibacterial efficacy of *Bidens pilosa* Aqueous (BPA), *Bidens pilosa* Ethanolic (BPE), *Ageratum conyzoides* Aqueous (ACA), *Ageratum conyzoides* Ethanolic (ACE), *Ocimum suave* Aqueous (OSA) and *Ocimum* suave Ethanolic (OSE) extracts on HIV/AIDS patients' oral bacteria. Healthy green leaves of the plants were collected in Ishaka Uganda, processed and portions separately extracted with hot distilled water and cold ethanol. The susceptibility, MIC and MBC of each extract were determined using standard protocols. The bacteria had significant ($p < 0.05$) respective total susceptibilities of 35 [28.7%] to BPA; 42 [34.4%] to BPE; 61

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[50.0%] to ACA; 45 [36.9%] to ACE; 38 [31.1%] to OSA; 32 [26.3%] to OSE; 105 (86.0%)] to ceftriaxone. BPE, ACA, OSA, OSE and ceftriaxone had significant MIC with [F(1, 13); $P = 0.00$ and BPA with F(1, 13); $P = 0.03$]. BPE, ACA, ACE, OSA and ceftriaxone had significant MBC with [F(1, 13); $P = 0.00$ and BPA with F(1, 13); $P = 0.01$] on the test bacteria (MANOVA). These tested medicinal plants' extracts and ceftriaxone had significant activity against oral bacteria with ACA having the best activity when compared with the control. However, the plants' extracts were resisted by some of the bacteria. These findings validate the claims of efficacy of *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave* on oral lesions of HIV/AIDS patients made by traditional healers and local people in South-Western Uganda. We recommend a detailed study of structural identities and activities of the active antibacterial principle(s) in these plants for possible new drug entities and verification of the interactive effects of the principle(s) with ARVs and cotrimoxazole used daily by HIV/AIDS patients.

Keywords

Antibacterial Efficacy, Bidens Pilosa, Ageratum Conyzoides, Ocimum Suave, Oral Bacteria, HIV/AIDS, Uganda

1. Introduction

Many drugs owe their sources more to higher plants including *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave*. than bacteria and fungi. Plants-derived broad spectrum antimicrobials are of high clinical value in the treatment of resistant microbial infections [1]. Low level of hygiene and sanitation in developing countries is responsible for increased widespread bacterial and fungal infections [2]. There were wide reports of death of about 300,000 children yearly from various under-developed regions of the globe due to diarrhoea commonly caused by *Escherichia coli*, *Shigella* spp., *Salmonella* spp., and *Yersinia*spp [3] [4]. HIV/AIDS associated infections have emerged with resistant aetiology among HIV positive UTI patients in urban Nigeria [5], and among HIV positive oral lesion patients in rural and semi-urban Uganda [6] [7] [8]. To combat the public health burden, the use of local plants with self-defensive antimicrobial phytochemicals by local communities with poor socio-economic status becomes inevitable [1]. Such plants serve as attractive alternative to expensive orthodox drugs. These plants include, but not limited to, *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave*. *Bidens pilosa* is a member of the Asteraceae family, known for its medicinal values worldwide [9]. *Bidens pilosa* is called "Enyabarashana" in Runyankole language in Uganda and "Ogwumma" in Igbo language in Nigeria. *Bidens pilosa* is a cosmopolitan, annual herb which originates from tropical Africa, America and Asia, where its roots, leaves, and seeds are reported to have antimicrobial and many other medicinal values [9]. Polyacetylenes from

Bidens pilosa have antimicrobial activity while some flavonoids have anti-inflammatory properties [10] [11] [12]. *Ageratum conyzoides* is a member of Asteraceae family, native to Central America, Carribean, Florida (USA), Southeast Asia, South China, India, West Africa, Australia and South America [13]. *A. conyzoides* is called "Butabuta" in Runyankole language in Uganda and "Eghueri-okukoatu" in Igbo language in Nigeria. The extracts have been reported to have very good antimicrobial activity. *A. conyzoides* has been used in folklore for the treatment of fever, pneumonia, cold, rheumatism, spasm, headache, wounds and burns [14] [15]. Indians use this species as a bacteriocide, anti-dysenteric, and antilithic [16]. In Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide [17] [18] [19]. The antibacterial inhibitory activities of *A. conyzoides* in the development of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* are known [15] [18] [20]. *Ocimum suave* (in the family of Lamiacaeae) and its other numerous species are native to African region (especially Tanzania and the Zanzibar archipelago, Uganda, Kenya, Ethiopia, Nigeria, Ghana, Burkina Faso, South Africa) and South Asian region [21]. It is commonly called African Bush Basil, "Omujaaja" in Runyankole language in Uganda and "Nchanwu" in Igbo language in Nigeria. It is widely used as an antiseptic, antibacterial and for many other purposes [22]-[33]. The use of *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave* as local alternative drug to treat oral bacterial lesions warrants scientific validation in line with the principles of universal primary health care services to the communities. Therefore, the objective of the study was to validate the scientific evidence for the use of *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave* in the treatment of oral lesions of HIV/AIDS patients in South-Western Uganda.

2. Materials and Methods

2.1. Drugs and Reagents

These included ceftriaxone susceptibility discs (Biomerieux® France) and ethanol (Lobachemie® South Africa).

2.2. Study Design

The study was experimental in design. Clinical oral bacterial isolates [6] from the Microbial Bank, Department of Microbiology and Immunology, Kampala International University-Western Campus (KIU-WC) were supplied by the Unit for analysis.

2.3. Sample Size and Sampling Technique

Sample size of 100 out of the available 610 clinical bacterial isolates [6] were selected using systematic-random sampling technique in a 1:6 ratio. Subsequently, a duplicate of each of the selected isolates was made to serve as a backup. Then

22 isolates of the standard bacteria (a pair) were selected to serve as control for the 11 species of bacterial isolates under study based on whether the selected isolate was a gram negative or gram positive bacterium. This made a grand total of 122 bacterial isolates [7].

2.4. Confirmation of Bacterial Viability and Identity

MacConkey agar, Chocolate agar and Blood agar were used as the primary media to grow the 122 samples to get fresh viable isolates. The viability of the previously identified isolates and standard bacteria were confirmed by growing the isolates on MacConkey agar, Chocolate agar and Blood agar [34] [35] [36] [37] [38]. To further confirm microbial identities, Chrom-agar orientation and carbohydrate assimilation tests were done using the analytical profile index (API) testing kits (Biomerieux® SA France, INS005517) and results were read using apiweb TM identification software.

2.5. Selection and Identification of Plants for Antibacterial Activity Testing

A survey was conducted in Ishaka and its environs which showed that 97.0 (80.8%), 118.0 (98.3%) and 95.0 (79.2%) out of 120 traditional healers and local people respectively used *Bidens pilosa*, *Ageratum conyzoides*and *Ocimum suave* to treat oral lesions associated with HIV/AIDS disease [unpublished data]. A sample of each of the plants was locally identified by the traditional healers and local users of the herbs. The plants were botanically identified by a botanist at the Department of Biology of Mbarara University of Science and Technology, Mbarara, Uganda and their voucher specimens numbered MUST-DB-0001, MUST-DB-0002 and MUST-DB-0003 respectively for *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave* were deposited in the Departmental Herbarium for reference purposes.

2.6. Collection, Processing and Preparation of Plant Extract

Fresh healthy, green leaves of *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave* were collected from Ishaka area of Western Uganda during rainy season in February 2015. The method of extraction used by traditional healers and local users of the herbs were replicated in this study. The aqueous extract was made with 200 g of the powdered plant material in 1 Litre of distilled water for an hour hot extraction. The ethanolic extract was made with 200 g of the plant material in 1Litre of absolute ethanol for a 72-hour cold extraction. The filtrate of the aqueous extract was dried in a water bath at 100˚C whereas the ethanolic extract filtrate was dried in a hot air oven at 40˚C. The dried extracts were then stored at −20˚C without exposing them to freeze-thaw cycles and humidity [39]. The aqueous extract was always freshly reconstituted in distilled water while ethanolic extract suspension was always reconstituted with minute quantity of Tween 80 in distilled water for use in the studies.

2.7. Susceptibility Testing

An overnight culture was used to make a bacterial suspension corresponding to a 1.5 McFarland standard whose turbidity was estimated with a densitometer for precision. The suspension was then homogenized and used immediately to determine the sensitivity, MIC and MBC of all the purified isolates according to the methods defined by Cheesbrough [34]. The diluted extracts and ceftriaxone discs were used to determine their antibacterial activities (bacterial sensitivity and resistance patterns on isolates) in accordance with the CLSI modified Kirby-Buaer tube dilution and agar well diffusion methods [40]. The standard reference organisms of the American Type Culture Collection such as ATCC 25923 *Staphylococcus aureus*, ATCC 25922 *Escherichia coli* and ATCC 27853 *Pseudomonas aeruginosa* were selected as the controls for pyogenic bacteria, enterobacteriaceae and *Pseudomonas aeruginosa* respectively.

2.8. Broth Preparation for MIC

Mueller Hinton agar (MHA) was prepared and tested for sterility overnight. Freshly prepared suspension made from 18 - 24 hour sub-cultured bacterial strains was inoculated on the MHA. Sterile cork borer was used to make holes on the MHA. Then, the sensitivity and resistance patterns, MIC and MBC were determined following the standard conventional method [34]. The specific concentrations of a stock solution of the extracts "BPA, BPE, ACA, ACE, OSA and OSE" (0.5 g/mL); and ceftriaxone injection (200 mg/mL) were prepared by diluting in 1mL of water for injection in sterile plain tubes. Then, a 200 µL of the stock solution of the extracts and ceftriaxone was each pipetted separately into each of the holes made on the media, incubated for 18 - 24 hours and then zones of inhibition were determined using a transparent ruler and recorded for determination of sensitivity and resistance patterns. Afterwards, serial dilutions ranging from 0.5 to 0.004 strength of each of the stock solutions were prepared with 1mL of distilled water in each case [41] [42]. Then, micropipettes were used to add 200 µL of each dilution into respective holes on the agar plate for agar well diffusion beginning with the lowest dilution. The plates and the tubes were incubated at 37˚C for 18 - 24 hours, checked for growth and zone of inhibition in the petri dishes and then recorded.

2.9. MIC and MBC Determination

MIC was determined by recording the smallest concentration of the drug that inhibited growth of microorganisms. The prevalent high turbidity in the tubes did not permit direct determination of MBC. Samples were therefore taken with sterile swab sticks from cleared zones of inhibition considering each specific dilution on the petri dishes and then smeared onto a fresh MHA medium and incubated for 18 - 24 hours and then results were checked for MBC as the smallest concentration of the drug that killed the organisms which corresponds with the plate in which the smallest concentration of the drug did not permit the regrowth of the organisms [41].

2.10. Data Analysis

The MS Excel 2010 version was used to enter the duplicates of the data and transferred to SPSS Version 20.0 for analysis. Results were expressed as mean ± SD. Chi square test was used to test the significance ($P < 0.05$) of susceptibility patterns of the oral bacteria to the medicinal plants' extracts and ceftriaxone. MANOVA, test of between subject effects and pairwise comparisons were done to determine variations among groups in MIC and MBC tests with $P < 0.05$ considered as significant.

3. Results

3.1. Susceptibility of Oral Bacteria

In susceptibility testing of 44 isolates of *Staphylococcus aureus* (Table 1), *Bidens* pilosa, *Ageratum* conyzoides and *Ocimum* suave extracts were found to be active on *Staphylococcus aureus*. More importantly, 24 [54.5%] of isolates of *Staphylococcus aureus* were susceptible to aqueous extract of *Ageratum conyzoides*. Thirty three [75%] of the *Staphylococcus aureus* isolates were fully vulnerable to the effect of ceftriaxone which was a reference antibiotic. *Staphylococcus saprophyticus* was 100% susceptible to aqueous extract of *Bidens pilosa*, aqueous extract of *Ageratum conyzoides*, ethanolic extract of *Ageratum conyzoides* and ceftriaxone. *Streptococcus mutans* isolates were found to be reasonably sensitive to aqueous extract of *Ageratum conyzoides*at 5 [55.6%]; to ethanolic extract of *Ocimum suave*at 6 [66.7%] and to ceftriaxone at [8 (88.9%)]. *Streptococcus pneumoniae* had full susceptibility of 21 [100%] to ceftriaxone and majorly of 5 [23.8%] susceptibility to ethanolic extract of *Ageratum conyzoides*. The isolate of non-haemolytic streptococcus showed full susceptibility [100%] to aqueous and ethanolic extracts of *Bidens pilosa* and ceftriaxone. *Bacillus cereus* was totally sensitive [100%] to aqueous extract of *Ageratum conyzoides*, ethanolic *Ocimum* suave and ceftriaxone. All the isolates of *Staphylococcus aureus* ATCC 25293 showed 100% susceptibility to aqueous and ethanolic *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave*. Five [55.6%] isolates of *E. coli* were susceptible to ethanolic extract of *Bidens pilosa.* Three [33.3%] isolates of *E. coli* were sensitive to ethanolic extract of *Ageratum conyzoides*. *E. coli* was found to be 6 [66.6%] susceptible to ceftriaxone. *Salmonella pullorum* was only sensitive to ethanolic extract of *Bidens pilosa*. Aqueous extract of *Ageratum conyzoides* and ceftriaxone respectively exhibited major activities on *Klebsiella pneumoniae* at 7 [77.8%] and 8 [88.9%]. *Proteus mirabilis* were 100% susceptible to ceftriaxone and 50% susceptible to ethanolic extract of *Ageratum conyzoides*. The reference *E. coli* ATCC 25922 had 8 [100%] susceptibility to all the extracts and ceftriaxone. *Pseudomonas aeruginosa* were 100% susceptible to ceftriaxone; and 50% sensitive to ethanolic extracts of *Ageratum conyzoides* and *Ocimum suave*. Conversely, *Pseudomonas aeruginosa* ATCC 27853 demonstrated 100% susceptibility to all the medicinal plants in addition to ceftriaxone.

Table 1. Susceptibility patterns of oral bacterial isolates to antibacterial medicinal plants' extract/agent.

Bacterium	No. of isolates	S/R/I	Antibacterial medicinal plants'extract/agent (No (%))						
			BPA (0.5 g)	BPE (0.5 g)	ACA (0.5 g)	ACE (0.5 g)	OSA (0.5 g)	OSE (0.5 g)	Ceftri (30 µg)
Staphylococcus aureus	44	S	7 (15.9)	9 (20.5)	24 (54.5)	10 (22.7)	9 (20.5)	1 (2.3)	33 (75.0)
		R	36 (81.8)	35 (79.5)	0 (0.0)	22 (50.0)	34 (77.2)	33 (75.0)	11 (25.0)
		I	1 (2.3)	0 (0.0)	20 (45.5)	12 (27.3)	1 (2.3)	10 (22.7)	0 (0.0)
Staphylococcus aureus ATCC 25293	12	S	12 (100.0)	12 (100.0)	12 (100.0)	12 (100. 0)	12 (100.0)	12 (100.0)	11 (91.7)
		R	–	–	–	–	–	–	1 (8.3)
Staphylococcus saprophyticus	1	S	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
		R	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
Escherichia coli	9	S	1 (11.1)	5 (55.6)	0 (0.0)	3 (33.3)	1 (11.1)	1 (11.1)	6 (66.7)
		R	8 (88.9)	3 (33.3)	0 (0.0)	0 (0.0)	4 (44.4)	5 (55.6)	0 (0.0)
		I	0 (0.0)	1 (11.1)	9 (100.0)	6 (66.7)	4 (44.4)	3 (33.3)	3 (33.3)
Escherichia coli ATCC 25922	8	S	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)
Salmonella pullorum	1	S	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		R	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)
		I	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella pneumoniae	9	S	0 (0.0)	2 (22.2)	7 (77.8)	1 (11.1)	0 (0.0)	0 (0.0)	8 (88.9)
		R	9 (100.0)	7 (77.8)	0 (0.0)	7 (77.8)	8 (88.9)	7 (77.8)	0 (0.0)
		I	0 (0.0)	0 (0.0)	2 (22.2)	1 (11.1)	1 (11.1)	2 (22.2)	1 (11.1)
Streptococcus mutans	9	S	1 (11.1)	2 (22.2)	5 (55.6)	1 (11.1)	4 (44.4)	6 (66.7)	8 (88.9)
		R	8 (88.9)	7 (77.8)	1 (11.1)	8 (88.9)	5 (55.6)	3 (33.3)	1 (11.1)
		I	0 (0.0)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Streptococcus pneumoniae	21	S	2 (9.6)	0 (0.0	1 (4.8)	5 (23.8)	2 (9.6)	1 (4.8)	21 (100.0)
		R	19 (90.4)	18 (85.6)	7 (33.3)	16 (76.2)	18 (85.6)	19 (90.4)	0 (0.0)
		I	0 (0.0)	3 (14.4)	13 (61.9)	0 (0.0)	1 (4.8)	1 (4.8)	0 (0.0)
Non-haemolytic streptococcus	1	S	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
		R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
		I	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Proteus mirabilis	2	S	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)
		R	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	0 (0.0)
		I	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pseudomonas aeruginosa	2	S	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	2 (100.0)
		R	2 (100.0)	1 (50.0)	2 (100.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)
		I	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pseudomonas aeruginosa ATCC 27853	2	S	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)
Bacillus cereus	1	S	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
		R	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
		I	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	122	S	35 (28.7)	42 (34.4)	61 (50.0)	45 (36.9)	38 (31.1)	32 (26.3)	105 (86.0)
		R	85 (69.7)	75 (61.5)	8 (6.6)	53 (43.4)	76 (62.3)	73 (59.8)	13 (10.7)
		I	2 (1.6)	5 (4.1)	51 (41.8)	24 (19.7)	8 (6.6)	17 (13.9)	4 (3.3)

KEY: BPA = *Bidens pilosa* Aqueous, BPE = *Bidens pilosa* Ethanol, ACA = *Ageratum conyzoides* Aqueous, ACE = *Ageratum conyzoides* Ethanol, OSA = *Ocimum suave* Aqueous and OSE = *Ocimum suave* Ethanol.

In summary, all the bacteria used for the test were susceptible; 61 [50%] to aqueous extract of *Ageratum conyzoides*, 45 [36.9%] to ethanolic extract of *Ageratum conyzoides*, 42 [34.4%] to ethanolic extract of *Bidens pilosa*, 38 [31.1%] to aqueous extract of *Ocimum suave*. 35 [28.7%] to aqueous extract of *Bidens pilosa*, 32 [26.3%] to *Ocimum suave* and 105 [86.0%] to ceftriaxone (Table 1). The Pearson Chi-square test with [$P = 0.00$]; Likelihood ratio test with [$P = 0.00$] for medicinal plants; and [$P = 0.03$] for antibacterial agent respectively revealed significant antibacterial effects for all the medicinal plants and ceftriaxone.

3.2. Minimum Inhibitory Concentration (MIC)

From Table 2, the results of the tests revealed that *Streptococcus mutans*, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Bacillus cereus* and non-haemolytic streptococcus were sensitive to aqueous extract of *Bidens pilosa* with a mean range of MICs from 0.01 ± 0.04 µg/ml to 1.00 ± 0.00 µg/ml. *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella pullorum* and *Klebsiella pneumoniae* were sensitive to the ethanolic extract of *Bidens pilosa* with a mean range of MICs from 0.04 ± 0.0 µg/ml and 1.00 ± 0.00 µg/ml. The antibacterial activities of the ethanolic extract of *Bidens pilosa* were generally better than those of the aqueous extract of *Bidens pilosa*. Aqueous extract of *Ageratum conyzoides* minimally and sensitively inhibited the growth of *Salmonella pullorum*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus cereus*at 0.06 ± 0.00 µg/ml. The ethanolic extract of *Ageratum conyzoides* minimally and sensitively inhibited the growth of *Streptococcus mutans* at 0.02 ± 0.03 µg/ml), *Klebsiella pneumoniae* at 0.13 ± 0.00 µg/ml, *Salmonella pullorum* at 0.13 ± 0.00 µg/ml and *Proteus* mirabilis at 0.13 ± 0.00 µg/ml. The aqueous extract of *Ocimum suave*hada minimal sensitive inhibition of growth of *Staphylococcus aureus* at 0.07 ± 0.1 6 µg/ml, *Streptococcus mutans* at 0.17 ± 0.18 µg/ml and *Streptococcus pneumoniae* at 0.06 ± 0.13 µg/ml. The ethanolic extract of *Ocimum suave* had MICs against *Staphylococcus saprophyticus* at 0.06 ± 0.16 µg/ml, *Streptococcus pneumoniae* at 0.06 ± 0.15 µg/ml, *E. coli* at 0.50 ± 0.35 µg/ml and *Klebsiella pneumoniae* at 0.06 ± 0.17 µg/ml. Ceftriaxone was observed to have better MICs against most of the tested bacteria than the extracts. This implies that all the aqueous and ethanolic extracts of *Bidens pilosa*, *Ageratum conyzoides*, and *Ocimum suave* minimally inhibited the growth of most oral bacteria (Table 2). Multiple analysis of variance of the MICs using any of the Pillai's Trace, Wilk's Lambda, Hotelling's Trace or Roy's Largest Root tests showed a high significant activity against the bacteria tested [$P = 0.000$], with an observed power of 100%, thus we reject the null which says there is no observed antibacterial activity of antibacterial agents against the bacteria tested; and accept the alternative hypothesis which says that antibacterial agents have antibacterial activity against the tested bacteria. Multivariate effects of plant extracts against

Table 2. MIC of local antibacterial medicinal plants and commercial agent against oral bacterial isolates.

Bacterium	Minimum inhibitory concentration of locally used medicinal plants (Mean ± SD (μg/ml))					
		BPA	BPE	ACA ACE	OSA OSE	CEFTRI WATER/ETH
Staphylococcus aureus	0.16 ± 0.37	0.08 ± 0.18	0.24 ± 0.17	0.15 ± 0.20	0.07 ± 0.16	0.00 ± 0.00 0.16 ± 0.10 0.00 ± 0.00
Staphylococcus aureus ATCC 25293	0.31 ± 0.20	0.13 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.25 ± 0.00	0.13 ± 0.00 0.06 ± 0.00 0.00 ± 0.00
Staphylococcus saprophyticus	0.50 ± 0.00	0.50 ± 0.00	0.13 ± 0.00	0.25 ± 0.00	0.50 ± 0.00	0.06 ± 0.16 0.06 ± 0.00 0.00 ± 0.00
Escherichia coli	0.12 ± 0.33	0.08 ± 0.08	0.10 ± 0.15	0.26 ± 0.20	0.18 ± 0.24	0.50 ± 0.35 0.11 ± 0.08 0.00 ± 0.00
Escherichia coli ATCC 25922	0.10 ± 0.00	0.10 ± 0.00	0.06 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.50 ± 0.00 0.06 ± 0.00 0.00 ± 0.00
Salmonella pullorum	0.00 ± 0.00	0.10 ± 0.00	0.06 ± 0.00	0.13 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 0.13 ± 0.00 0.00 ± 0.00
Klebsiella pneumoniae	0.00 ± 0.00	0.08 ± 0.16	0.06 ± 0.00	0.06 ± 0.09	0.00 ± 0.00	0.06 ± 0.17 0.11 ± 0.08 0.00 ± 0.00
Streptococcus mutans	0.01 ± 0.04	0.04 ± 0.06	0.11 ± 0.15	0.02 ± 0.03	0.17 ± 0.18	0.18 ± 0.19 0.04 ± 0.03 0.00 ± 0.00
Streptococcus *pneumoniae*	0.06 ± 0.22	0.11 ± 0.20	0.11 ± 0.17	0.09 ± 0.16	0.06 ± 0.13	0.06 ± 0.15 0.07 ± 0.04 0.00 ± 0.00
Non-haemolytic streptococcus	1.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.00	0.13 ± 0.00	0.50 ± 0.00	0.25 ± 0.00 0.13 ± 0.00 0.00 ± 0.00
Proteus mirabilis	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.00	0.13 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 0.06 ± 0.00 0.00 ± 0.00
Pseudomonas aeruginosa	0.00 ± 0.00	0.25 ± 0.35	0.06 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.35 0.03 ± 0.00 0.00 ± 0.00
Pseudomonas aeruginosa ATCC 27853	0.13 ± 0.00	0.13 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00 0.03 ± 0.00 0.00 ± 0.00
Bacillus cereus	0.50 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.13 ± 0.00	0.50 ± 0.00	0.13 ± 0.00 0.06 ± 0.00 0.00 ± 0.00

KEY: BPA=*Bidens pilosa* Aqueous, BPE = *Bidens pilosa* Ethanol, ACA = *Ageratum conyzoides* Aqueous, ACE = *Ageratum conyzoides* Ethanol, OSA = *Ocimum suave* Aqueous and OSE = *Ocimum suave* Ethanol, CEFTRI = Ceftriaxone; WATER = Distilled Water and ETH = Ethanol.

bacteria dependent on linearly independent pairwise comparisons among the estimated marginal means were done. From the tests results of in-between subject effects and pairwise comparison tests when $P < 0.05$ was rated as statistically significant, it showed that BPE. ACA, OSA, OSE and ceftriaxone with F statistic [1, 13]; $P = 0.00$ and BPA with F statistic [1, 13]; $P = 0.03$ had significant bacterial inhibitory effects compared to ACE with F statistic [1, 13]; $P = 0.24$ without significant bacterial inhibitory effects.

3.3. Minimum Bactericidal Concentration (MBC)

From Table 3, the best mean bactericidal activity of aqueous extract of *Bidens pilosa* against *Streptococcus pneumoniae* was 0.05 ± 0.22 μg/ml, against *Staphylococcus aureus* was 0.16 ± 0.37 μg/ml and against *E. coli* was 1.00 ± 0.00 μg/ml. At higher doses, it also had antibactericidal activity on *Staphylococcus saprophyticus*, *Salmonella pullorum*, non-haemolytic streptococcus and *Pseudomonas aeruginosa.* The ethanolic extract of *Bidens pilosa* exerted bactericidal effect on all the bacterial isolates ranging between 0.11 ± 0.32 μg/ml and 0.50 ± 0.71 μg/ml except on those of *Streptococcus mutans*, *Proteus mirabilis* and *Bacillus cereus*. The aqueous extract of *Bidens pilosa* generally had better bactericidal activity on quite a number of the bacteria used in the study than the ethanolic extract of *Bidens pilosa*. The aqueous extract of *Ageratum conyzoides* recorded bactericidal activity on all the bacterial isolates tested with better activity on gram positive

Table 3. MBC of local antibacterial medicinal plants and conventional agent against oral bacterial isolates.

Bacterium	Minimum bactericidal concentration of locally used medicinal plants Mean ± SD (µg/ml)							
	BPA	BPE	ACA	ACE	OSA	OSE	CEFTRI	WATER/ETH
Staphylococcus aureus	0.16 ± 0.37	0.11 ± 0.32	0.69 ± 0.38	0.50 ± 0.51	0.07 ± 0.16	0.36 ± 0.49	0.18 ± 0.13	0.00 ± 0.00
Staphylococcus aureus ATCC 25293	0.06 ± 0.00	0.13 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.06 ± 0.0	0.06 ± 0.00	0.00 ± 0.00
Staphylococcus saprophyticus	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00
Escherichia coli	0.11 ± 0.33	0.44 ± 0.53	0.78 ± 0.44	0.89 ± 0.33	0.33 ± 0.50	0.33 ± 0.50	0.44 ± 0.24	0.00 ± 0.00
Escherichia coli ATCC 25922	0.10 ± 0.00	0.50 ± 0.00	0.13 ± 0.00	0.50 ± 0.00	0.13 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.00 ± 0.00
Salmonella pullorum	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00
Klebsiella pneumoniae	0.00 ± 0.00	0.22 ± 0.44	0.69 ± 0.37	0.22 ± 0.44	0.00 ± 0.00	0.11 ± 0.33	0.44 ± 0.24	0.00 ± 0.00
Streptococcus mutans	0.00 ± 0.00	0.00 ± 0.00	0.92 ± 0.25	0.11 ± 0.33	0.17 ± 0.18	0.44 ± 0.53	0.46 ± 0.25	0.00 ± 0.00
Streptococcus pneumoniae	0.05 ± 0.22	0.14 ± 0.36	0.36 ± 0.42	0.19 ± 0.33	0.06 ± 0.13	0.14 ± 0.36	0.26 ± 0.23	0.00 ± 0.00
Non haemolytic streptococcus	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.00	1.00 ± 0.00	0.25 ± 0.00	0.00 ± 0.00
Proteus mirabilis	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.09	0.00 ± 0.00
Pseudomonas aeruginosa	0.50 ± 0.71	0.50 ± 0.71	0.63 ± 0.53	0.50 ± 0.71	0.00 ± 0.00	0.50 ± 0.71	0.31 ± 0.27	0.00 ± 0.00
Pseudomonas aeruginosa ATCC 27853	0.13 ± 0.00	0.50 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.06 ± 0.00	0.13 ± 0.00	0.06 ± 0.00	0.00 ± 0.00
Bacillus cereus	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.00	0.00 ± 0.00	0.13 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00

KEY: BPA=*Bidens pilosa* Aqueous, BPE = *Bidens pilosa* Ethanol, ACA = *Ageratum conyzoides* Aqueous, ACE = *Ageratum conyzoides* Ethanol, OSA = *Ocimum suave* Aqueous and OSE = *Ocimum suave* Ethanol, CEFTRI = Ceftriaxone; WATER = Distilled Water and ETH = Ethanol.

bacteria. The optimal bactericidal activity achieved by ethanolic extract of *Ageratum conyzoides* against *Streptococcus mutans*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa* ranged from 0.11 ± 0.33 µg/ml to 0.50 ± 0.71 µg/ml. The aqueous extract of *Ocimum suave* killed the isolates of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus mutans*, *E. coli*, non-haemolytic streptococcus and *Staphylococcus saprophyticus* at minimum concentrations ranging from 0.06 ± 0.13 µg/ml to 0.50 ± 0.00 µg/ml. Generally, the aqueous extract of *Ocimum suave* exhibited better bactericidal activity on the gram positive bacteria than on the gram negative bacteria. The ethanolic extract of *Ocimum suave* was able to kill *Klebsiella pneumoniae* and *Streptococcus pneumoniae* at minimum concentrations of 0.11 ± 0.33 µg/ml and 0.14 ± 0.36 µg/ml. The bactericidal activity of aqueous extract of *Ocimum suave* was generally better than that of ethanolic extract of *Ocimum suave*. The aqueous extract of *Ocimum suave* was seen to have a relatively overall superior bactericidal activity to aqueous extract of *Bidens pilosa*. ethanolic extract of *Ageratum conyzoides*, aqueous extract of *Ageratum conyzoides*, ethanolic extract of *Bidens pilosa* and *Ocimum suave*. Ceftriaxone had better bactericidal activity on *Staphylococcus saprophyticus*, *Salmonella pullorum*, *Bacillus cereus*, *Proteus mirabilis* and non-haemolytic streptococcus than the aqueous and ethanolic extracts of *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum*

suave when compared with control organisms. Multivariate analysis using any of Pillai's Trace, Wilk's Lambda, Hotelling's Trace or Roy's Largest Root tests showed a high statistically significant bactericidal activity against the bacteria tested [$P = 0.000$], with an observed power of 100%, thus we reject the null hypothesis which says there is no observed bactericidal activity by phyto-antibacterial agents against the bacteria tested; and hence we accept the alternative hypothesis which says that antibacterial agents have bactericidal activity against the tested bacteria as represented in **Table 3**. Multivariate effects of bacteria dependent on linearly independent pairwise comparisons among the estimated marginal means were done. From the results of in-between tests, it showed that BPE, ACA, ACE, OSA and ceftriaxone with F [1, 13]; $P = 0.00$ and BPA with F [1, 13]; $P = 0.01$ had significant bactericidal effects compared to OSE with F [1, 13]; $P = 1.25$ which did not exert any significant bactericidal effects on the test bacteria. From pairwise comparisons tests, it showed that BPA, BPE, ACA, ACE and OSA did not have statistically significant difference in bactericidal activity against *Salmonella pullorum*, *Staphylococcus saprophyticus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, non-haemolytic streptococcus, *Proteus mirabilis*, *Streptococcus mutans*. *Streptococcus pneumoniae* and *Staphylococcus aureus.*

4. Discussion

Higher plants have contributed immensely to sources of many pharmaceuticals dispensed today in pharmacies, with very limited number envisioned for antimicrobial uses because majority of antibiotics in the global market today emanated from bacterial and fungal sources [1]. But today, we argue that clinical microbiologists have two reasons to be interested in the antimicrobial plant extracts because they inhibit microorganisms via multiple targets antithetical to antibiotics with effective short life span due to resistance from frequent and indiscriminate use of antibiotics resulting from higher rate of infections due to low level of hygiene and sanitation in Africa and so higher plant antimicrobials can be of great value in treating multi-resistant microorganisms [2]. This neglect of research on higher plant-derived antimicrobials has resulted in widespread multi-resistant infections; that about 300,000 children die annually in some places owing to common intestinal diarrhoeal diseases caused by *E. coli*, *Shigella* spp., *Salmonella* spp., and *Yersiania* spp [3] [4]. In most of these affected areas, people resort to the use of local and indigenous plants as the possible option to treat the infections due to high cost and non-availability of effective conventional drugs and microbial resistance. The plants possess unlimited potentials in synthesizing aromatic secondary metabolites such as phenolics, terpenoids and essential oils, alkaloids, lectines and polypeptides, and polycetyles to defend the plants against microorganisms; amongst which phenols are the most reportedly documented ones with potential antimicrobial activity [1] [43]. Instances of these phenolic compounds include catechol or caffeic acid, quinones, flavonoids, flavonols, fla-

vones and coumarins. Tannins are examples of polymeric phenols [44]. Polymerization via air oxidation perhaps, is the reason for the phenolics' observed antimicrobial activity of the plants through iron deprivation of hydrogen bonding with vital proteins especially microbial enzymes [43]. Therefore, an important feature central to the plant phytochemicals' toxification and detoxification is their polymerization size [45]. Many assays including agar disc diffusion or agar well diffusion as modified [46] have frequently been employed in microbiology laboratories in screening for the antimicrobial activities of plants' extracts and serial broth microdilution assay [40] has been in use in quantifying the antimicrobial activity of plants' extracts and determining MIC and MBC. Many plants which have been screened in laboratories for antimicrobial activities are often selected sequel to their traditional uses which was also the case in our study. The plants were ascertained to be used in the treatment of oral lesions of HIV/AIDS patients in South-Western Uganda by traditional healers and local people. The most frequently used plants as ascertained were eventually selected for the study and they included *Bidens pilosa*, *Ageratum conyzoides* and *Ocimum suave*. The plant extracts were also prepared in the same way the traditional healers and locals prepare them for use. Hence, the antimicrobial susceptibility and smallest concentration of each of the antibacterial medicinal plants that inhibited (MIC) or killed (MBC) the oral bacterial isolates of HIV/AIDS patients were determined in the study using the agar well diffusion and serial dilution test methods as described in the study methods. The results of the study proved that higher plants can produce effective antimicrobial agents because the tested BPA, BPE, ACA, ACE, OSA and OSE extracts have antibacterial properties as claimed by the traditional healers and local people who treat oral lesions of HIV/AIDS patients in South-Western Uganda with the extracts of the plants. These findings corroborate the reports that polyacetylenes from *Bidens pilosa* possess antimicrobial activity while some flavonoids from the plant possess anti-inflammatory properties [9] [10] [11] [12]. The findings in this study also attest to the previously reported studies on antibacterial activities of *Ageratum conyzoides* [20]. Another study verified its inhibitory activities against *in vitro* development of *Staphylococcus aureus* using ether and chloroform extracts [15] and this was corroborated by another study which confirmed its inhibitory activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* using methanolic extract of the whole plant [18]. The findings equally confirm the reports that *Ocimum* suave has been used in the treatment of dysentery, dyspepsia, skin diseases, bacterial, and fungal infections [22] [33]. The findings of the study therefore lay credence and validation to the claims made by the traditional healers and local people of South-Western Uganda that *Bidens pilosa* Aqueous, *Bidens pilosa* Ethanolic, *Ageratum* conyzoides Aqueous, *Ageratum* conyzoides Ethanolic, *Ocimum suave* Aqueous and *Ocimum suave* Ethanolic can be used to treat some bacteria infected oral lesions of HIV/AIDS patients. The gram positive bacteria were reported often to be more susceptible to

plant extracts than the gram-negative ones [2] [47] [48]. This is in agreement with the results of our study because most of the plant extracts exhibited much better antibacterial activity on gram positive bacteria than on gram negative bacteria and *Pseudomonas aeruginosa*. Indeed, this greater activity on gram positive bacteria might be because gram positive bacteria have only an outer peptidoglycan layer which is not an effective barrier [49]. The gram negative bacteria have an outer phospholipid membrane that makes the cell wall impermeable to lipophilic solutes, while the porines constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da [50]. The screening of extracts for antimicrobial activity *in vitro* often involves reference and clinical strains of microorganisms isolated from pathological products or those that demonstrate resistance to several antibacterials. Previous works in South-Western Uganda involving reference and pathological bacteria have demonstrated huge antibacterial resistance burden amongst HIV/AIDS patients in relation to high level resistance of oral bacteria associated with HIV/AIDS patients to standardised antibacterial discs and commercially available antibacterial agents [6] [7] [8]. However, some of the oral bacteria resisted the activity of some of the plants implying that antibacterial resistance is still a great public health burden in rural areas of developing nations including South-Western Uganda. This therefore calls for incisive and in-depth search into the plant world to obtain the effective and efficient antimicrobial drug entities endowed by nature to solve the present challenges of huge cost and non-availability of effective drugs, antimicrobial resistance, high morbidity, discomfort, extended hospitalizations, poor quality of life and possible death facing HIV/AIDS patients, and burden of poor patient management facing healthcare professionals and managers, ministries of health and governments in developing world as handlers of the challenges.

5. Conclusion and Recommendations

It can be concluded that *Bidens pilosa* Aqueous, *Bidens pilosa* Ethanolic, *Ageratum* conyzoides Aqueous, *Ageratum* conyzoides Ethanolic, *Ocimum suave* Aqueous and *Ocimum suave* Ethanolic have antibacterial activities including bacteriostatic and bactericidal potentials. These findings therefore validate the claims made by the traditional healers and local people of South-Western Uganda that *Bidens pilosa* Aqueous, *Bidens pilosa* Ethanolic, *Ageratum* conyzoides Aqueous, *Ageratum* conyzoides Ethanolic, *Ocimum suave* Aqueous and *Ocimum suave* Ethanolic can be used to treat bacteria infected oral lesions of HIV/AIDS patients. We hence recommend an isolation, identification and structural elucidation of the bioactive antibacterial principle(s) in these plants for possible new drug entities, verification of the interactive effects of the principle(s) with ARVs and cotrimoxazole used daily by HIV/AIDS patients.

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Ethical Approval

Ethical approval was pursued and acquired from The AIDS Support Organisation (TASO) Kampala, Uganda National Council for Science and Technology (UNCST) and Mbarara University of Science and Technology Institution's Research and Ethics Committees. These clearances allowed us to use the clinical isolates supplied by the Microbiology Department, KIU_WC.

Competing Interests

Authors have declared no conflicts of interest exist.

Authors' Contributions

The authors collaborated in carrying out the work as follows: JOCE initiated the study. Authors JOCE, EA, AAA, TS, JCE, OED and FB collected the data. Authors JOCE, MN, SOO, EA, COO and FB designed the study, wrote andcorrected the protocol.Authors KIK, JKT and JOCE wrote the protocol and thefirst draft of manuscript, searched for literature, analyzed resistance, MIC andMBC data, read through the data and made corrections.Authors MN, SOO,EA and FB managed the experimental processes, read through and madecorrections to the manuscript draft. Authors COO, TS, JCE, OED and AAA read through and made corrections to the manuscript draft. All authors read and approved the manuscript for publication.

Consent

Written consent was sought and secured from the Kampala International University Microbiology Research Laboratory, Ishaka, Bushenyi where the isolates were being kept with the permission of the scientist who previously researched on these organisms.

Consent to Publish

The authors have given their consents to publish this article.

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Abbreviations

HIV = Human Immunodeficiency Virus
AIDS = Acquired Immunodeficiency Syndrome
ARVs = Antiretrovirals
MHA = Mueller Hinton Agar
API = Analytical Profile Index
MIC = Minimum Inhibitory Concentration
MBC = Minimum Bactericidal Concentration
MBCs = Minimum Bactericidal Concentrations
MANOVA = Multiple Analysis of Variance
p (p value) = Probability value
KIU-WC = Kampala International University Western Campus
NCCLS = National Council for Clinical and Laboratory Standards
CLSI = Clinical and Laboratory Standards Institute
TASO = The AIDS Support Organisation
UNCST = Uganda National Council for Science and Technology.
SPSS = Statistical Package for Social Sciences
UTI = Urinary Tract Infections
BPA = Bidens pilosa Aqueous
BPE = Bidens pilosa Ethanol
ACA = Ageratum conyzoides Aqueous
ACE = Ageratum conyzoides Ethanol
OSA = Ocimum suave Aqueous
OSE = Ocimum suave Ethanol