Antimicrobial Efficacy Study Of Acmella Caulirhiza And Spermacoce Princeae Used By Postpartum Mothers In Nyamira County, Kenya

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Abstract- Puerperal sepsis is the major cause of (31%) maternal mortality. Appropriate care could prevent majority of these deaths. Exogenous and endogenous organisms are the causatives agents. Traditional medicine have been used in treating postpartum sepsis for example Acmella caulirhiza used to treat a child’s mouth sores and Spermacoce princeae used to accelerate healing of umbilical cord and to clean the system after birth.

The main objective of the present study was to determine antimicrobial activity levels of A. caulirhiza and S. princeae used by postpartum mothers in Nyamira County, Kenya.

The study area was Nyamira County where the two plant specimen and clinical specimens of 339 mothers were collected. Plant materials were identified at East Africa Herbarium. Plant specimens were transported to M.K.U. Pharmacognosy laboratory where processing were done. Clinical specimens were collected from mothers at Nyamira Level 5 Hospital. A single high vaginal swab was collected from each participant, immediately inserted in stuart’s transport medium, labeled, placed in a cooler box, triple packaged and transported to Microbiology laboratory of M.K.U. within 48 hours where processing were done. Culture, isolation, identification were done. Disc diffusion method was employed to determine antimicrobial activity in the compounds of the crude plant extracts. Data was stored in Excel spread sheet in a personal computer protected with a password. Antimicrobial activity was analyzed using descriptive statistics. Data was presented using, tables and photographs.

Results: Antibacterial activity of A. caulirhiza and S. princeae indicated that, the whole plant extracts had low antibacterial effects against S. aureus and E. coli. No antifungal effects were detected for crude extracts of the two plants.

Discussion: the two plants used by the local inhabitants of Nyamira County in treating postpartum infections indicated that, they possess low antibacterial activity against S. aureus and E. coli. However, no antifungal activity was detected from the two plants.

Recommendation: the two plants may be used in treating postpartum infections though commercially available drugs are recommended as they are highly effective. The two plants indicated low antibacterial activity against S. aureus and E. coli. However, further studies are recommended on genotoxicity of this plant extracts on S. aureus and E. coli genes. Also further research is recommended to isolate and identify pure compounds of the two plants to establish pharmacological basis of antibacterial activity shown even though it was low.

Index Terms- Traditional medicine, Disc-diffusion and antimicrobial-activity.

I. Introduction

Traditional medicine has been used in health maintenance, disease prevention and treatment (WHO, 2014). The use of medicinal plants has been known to mankind as the oldest practice of healthcare (Yogayata and Vijay, 2012). Nowadays, isolation and characterization of biologically active compounds from medicinal plants continues and drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker compounds (Marcy and Douglass, 2005). Medicinal plants used to manage postpartum complications include; Basella alba plant which belongs to Asteraceae family, is used to manage stomachache, stimulate milk production and is used to remove the placenta after birth (Jeruto et al., 2015). Toddalia asiatica and Pentas longiflora species which belongs to Rutaceae and Rubiaceae family respectively. The leaves of these herbs are used to manage Urinary tract infections (Jeruto et al., 2015).

Acmella caulirhiza is similarly known as Spilanthes acmella. It is afflowering herbal plant, which belongs to Compositae/Asteraceae family (Berhane et al., 2014). It is an annual or perennial herb. Locally it is known as Ekenyunyuntamonwana (Ekogusii) and Ajouk-olwa Salamatwe (Dholuo) (Kokwaro, 2009). It is used by different communities in Kenya and the rest of Africa to treat various medical conditions. Example in Kenya, its flowers and leaves are used to treat venereal diseases (Jeruto et al., 2015). It
is used to relieve painful sores of the mouth, gums and throat, as well as stomach ache (Kokwaro 2009). Also it is used to treat decayed teeth, gingivitis or wounds in the mouth, toothache and sore throat (Kipruto et al., 2013). The Zulu people of South Africa use A. caulirhiza as a local analgesic for toothache and to ease sensitivity of gums during dental extractions (Crouch et al., 2005).

Spermacoce princeae is flowering herbal plant which belongs to the family Rubiaceae (Augustin et al., 2015). Locally it is known as Omoutakiebo (Ekegusui), Gakungathe (kikuyu), Murkugwet (kipsigis) and Nyamwoch (Dholuo) (Kokwaro, 2009). It grows in tropical regions and it is used extensively. Normally it is used by different communities in Kenya and the rest of Africa to treat several diseases. Just to mention a few, leaves and roots are used to treat chronic asthma, cancer, mastitis in cows and venereal diseases by the Nandi people in Kenya (Jeruto et al., 2011). Another study carried out in Vihiga County, Kenya found that, cold infusion is made from leaves and drunk in the treatment of diarrhea (Antony et al., 2016). Leaves and stem are used to treat female infertility in Baham, Cameroon (Teleo et al., 2011). In Cameroon, leaves of Spermacoce princeae are warmed on fire, ground and mixed with red oil and salt, then is taken orally in treatment of kidney disease (Focho et al., 2009).

II. MATERIAL AND METHODS

Study Area

The study site was North Mugirango and West Mugirango constituencies of Nyamira County. The study points in West Mugirango constituency were; Sironga (0° 33'14.8536 S and 34° 58' 2.4996 E), Bonyunyu (0° 31’36.2532 S and 34° 53'20.4108 E) and miruka (0° 29'13.902 S and 34° 5320.3208 E) whereas the study point in North Mugirango constituency was; Magong’a (0° 28° 46.7724 S and 34° 57’6.4836 E) in Nyamira County. In this County, local inhabitants regularly use medicinal plants for personal and domestic animal health. Local inhabitants in this County, follow traditional beliefs and customs. Further, most inhabitants living in this area have a tendency of harvesting the medicinal plants from undisturbed vegetation. This is due to the fact that many plant species grow in the study region (Omwenga et al., 2015). Postnatal mothers use Acmella caulirhiza and Spermacoce princeae to treat child sores and to clean reproductive system respectively in women after birth. Nyamira County is one of highly populated area with approximately 912.5 Km² with a population of 598,252 and a population density of 656 persons per Km² according to (KNBS, 2009).

Plant materials collection

Acmella caulirhiza and Spermacoce princeae medicinal plant specimen were collected from West Mugirango and North mugirango constituencies in Nyamira County with acceptable bio-conservation methods (WHO, 2003a). Harvesting was done in a dry weather morning after the dew had evaporated (Prajapati et al., 2010). The two specimens were carried separately in gunny bags and transported to Pharmacognosy Laboratory of Mount Kenya University within 72 hours of collection (WHO, 2003a).

Processing of plant materials

Processing was done within 72 hours after collection. Herbarium preparations were established and the voucher specimens were processed in duplicate. They were mounted on herbarium sheets, pressed to flatten, to dry and were labeled. Voucher specimen (Number JN001 and JN002) were identified at East African Herbarium in the National Museums of Kenya on basis of morphological characteristics and compared with the voucher specimens recorded in East Africa Herbarium. Voucher specimen (Number JN001 and JN002) were deposited at Mount Kenya University Botanical Herbarium Laboratory in the school of Pharmacy. The collected materials were washed thoroughly with tap water and then air dried under a shade at room temperature for one week. When dried, the plant materials (A. caulirhiza and S. princeae) were ground into course powder using a porcelain mortar and pestle (Hena, et al., 2010). The course powder materials were labeled and stored in brown paper bags under a dry condition, away from light at room temperature till the time of extraction and phytochemical screening (Prajapati et al., 2010).

Plant extraction using organic solvents

Using a top loading Weighing Electronic Balance (Models TP-B 2000), 50 grams of the Kenyan Acmella caulirhiza and 50 grams Spermacoce princeae each powder was weighed separately and transferred into separate conical flasks, labeled with the constituency of collection, plant species and date. Then 500mls of 100% Ethyl Acetate (Loba Chemie Company Lot#L157601502) was added to cover each plant materials and covered with a stopper, then macerated in the solvent at room temperature for 48 hours with intermittent agitation. Using a funnel and Whatman filter paper No. 1 the crude extracts from each of the plant materials were strained separately into glass reagent bottles then covered with stoppers. The process was repeated with 500mls of 100% Ethanol Analar Normapur (VWR Prolabo Company Batch 12D250511) and Methanol (Loba Chemie Company Lot #B193331604). The filtrates were labeled and concentrated in a rotary evaporator at 40 degree Celsius for Ethyl acetate, 60 degree Celsius for Ethanol and Methanol respectively. Using analytical balance, empty beakers were weighed, the extracts from the distillation flask were transferred into them, labeled appropriately and the solvents were evaporated in an Oven set at appropriate temperature. Quantity of each crude plant extract paste was calculated by the formula: Plant crude residue = (weight of beaker + extract) - (weight of empty beaker). The extracted paste of each plant species examined was kept in beakers covered in a refrigerator a waiting for bioactivity assay (Afolayan et al., 2008).

Aqueous extraction of crude plant material

Aqueous extracts of Acmella caulirhiza and Spermacoce princeae was made from crude plant material according to Bibi et al. (2012) by weighing 20 grams of Acmella caulirhiza and 20 grams of Spermacoce princeae. They were boiled separately in 400mls...
3.8.1 Bacteria and Fungi Isolation and Characterization

3.8.1.1 Staphylococcus species

Inoculum was streaked on Nutrient agar (Oxoid, UK) in a laminar air flow hood. The plates were labeled with the name of the culture medium, participant identification number. The plates were incubated aerobically at 37°C for 18-24 hours. After an overnight, gram stain was done on the growth. Then the isolates were sub cultured into mannitol salt agar (Oxoid, UK) and the plates were incubated aerobically at 37°C for 24 hours. Then sub cultured into nutrient agar (Oxoid, UK) and re-incubated aerobically at 37°C for 24 hours after which biochemical tests such as catalase and coagulase test were conducted to identify Staphylococcus aureus species (Gayle and Reginald, 2016).

3.8.1.2 Escherichia species

Inoculum was streaked on Nutrient agar (Oxoid, UK) plates in a laminar air flow hood. The plates were labeled with the name of the culture medium, participant identification number and the date. The plates were incubated aerobically at 37°C for 18-24 hours. Identification of the bacteria was done based on their colonial morphology and Gram staining characteristics. Then the isolates were sub cultured into macConkey agar (Oxoid, UK) and the plates were incubated aerobically at 37 degree Celsius for 24 hours. Then biochemical tests such as motility, indole lysine decarboxylation, lysine deaminase in motility indole lysine medium, glucose fermentation and gas production in triple sugar iron agar slant and citrate utilization in citrate agar slant, were conducted

3.8.1.3 Candida species

Inoculum was streaked on sabouraud dextrose agar (Oxoid, UK) in a laminar air flow hood. The plates were labeled with the name of the culture medium, participant identification number and the date. The plates were incubated aerobically at 25°C for 3 days after which, grams stain and germ tube test were done (Cheesbrough, 2006). Then the yeast isolates were sub cultured onto chromogenic candida agar (Oxoid, CM 1002) plates and then sub cultured into corn meal agar (Oxoid, UK). Lastly, biochemical test was done using API Candida System to identify Candida albicans species. Budding yeast cells (Gram stain) indicates Candida species (Haw et al., 2012).

3.8.1.3.1 Germ tube test

Small test tubes were labeled with participant identification number and 0.5ml human serum was inoculated with suspected Candida cultures colony and then incubated aerobically at 37°C for 2 hours. After incubation, a drop of serum yeast culture was placed on a glass slide using a Pasteur pipette and covered with a cover slip then examined using x10 and x 40 objectives with condenser iris diaphragm closed to give good contrast for germinating blastospores (Kumar, 2010).

3.8.1.3.2 Candida albicans colonies on CHROMagar Candida

CHROMagar Candida is a chromogenic medium for speedy identification of Candida species within 24 to 48 hours on basis of colony colors (Odds and Davidson, 2000). On this medium Candida albicans forms green colonies. This medium differentiates C. albicans from other Candida species. This medium provides presumptive identifications of C. albicans (Odds and Davidson, 2000; Dilek et al., 2012). Chromogenic candida agar (Oxoid, CM 1002) plates were prepared according to the manufacturers' instructions. The plates were labeled with the name of the culture medium, participant identification number. Using inoculating loop, a single colony from a pure culture was inoculated onto chromogenic candida agar (Oxoid, CM 1002) media and incubated at 35°C for 2-3 day (Dilek et al., 2012).
3.8.1.3.3 Test for Chlamydospore formation on Corn Meal Agar
Corn meal agar stimulates sporulation, suppressing certain other fungal growth and promotes morphological features in *C. albicans* and differentiates it from other *Candida* species. A significant characteristic of *Candida albicans* is the capability to form chlamydospores on corn meal agar which is significant in identification of *Candida albicans* (Michael and Burton, 2011).

Corn meal agar (Oxoid, UK) plates were prepared according to the manufacturer’s instructions in a laminar flow hood. The plates were labeled with the name of the culture medium, participant identification number and the date. The yeast cultures were inoculated on corn meal agar (Oxoid, UK). The plates were incubated aerobically at 25°C for 3 days. After incubation a colony from the culture was picked and prepared on a glass slide then, was covered with a cover slip and examined using x10 and x 40 objectives with condenser iris diaphragm closed suitably to give good contrast. Chlamydospores which appear thick-walled at the ends of pseudo hyphae, large and round indicates *Candida albicans*. Also pseudo hyphae (budding yeast cells in chains) that produce clusters of asexual blastoconidia which are round at the cell junctions shows *Candida albicans* (Michael and Burton, 2011).

3.8.1.3.4 Biochemical identification of fungi
A commercial Analytical Profile Index (API) Candida System for the identification of yeasts (BioMérieux, France) was used to confirm the species of isolated yeast. A total of 12 biochemical tests were performed using this system. API Candida is a standard used to identify 18-24 hours yeasts. It consist 10 tubes containing dehydrated substrates, which allow 12 identifications which entail sugar acidification or enzymatic reactions to be performed. The end point of these reactions is the color change which is read visually following the Reading Table and referring to the list of profiles in the package insert.

Preparation of the inoculum: Yeast suspensions were prepared from 18-24 hours culture of *S. aureus* on corn meal agar (Oxoid, UK) plates. Using a wire loop, three well isolated and identical colonies were picked and emulsified in an ampule of API Sodium chloride 0.85 % Medium (2 ml). Yeast suspension whose turbidity equivalent to 3 McFarland were prepared and they were compare with a turbidity control. These suspensions were used immediately after preparation. Strip [incubation box (tray and lid)] preparation was done according to the manufacturer’s instructions. To create a moist atmosphere, distilled water (5ml), was spread into the tray (honey-combed wells). The strip was inserted in the incubation box and tilting incubation box slightly forward (Monget et al., 1995).

Inoculation of the strip: Inoculum was distributed into each tube of the test strip using a pipette avoiding formation of bubbles by position the pipette on the edge of the cupule. The first 5 tests (GLU to RAF) and the last test (URE) were covered with mineral oil. Immediately after inoculation, incubation box was covered and aerobically incubated at 38°C for 18-24 hours. Negative control was set containing a strip inoculated with sterile distilled water (Monget et al., 1995).

3.8.1.3.5 Reading and Interpretation of API Candida System test Results
After 18-24 hours of incubation, API Candida system results was read based on color change in each test strip, confirming from the reading table in the package insert and recording them on result sheet (as + or –) provided by the manufacturer (Monget et al., 1995).

3.9 Determination of Antimicrobial Activity of the two plants extracts
3.9.1 Determination of Antibacterial Activity
*In vitro* antibacterial activities of plant extracts was evaluated using Kirby-Bauer technique Bauer, (1998) based on traditional uses of the two plants by disc diffusion method using *Escherichia coli* and *Staphylococcus aureus* clinically isolated from Nyamira County Referral Hospital. ATCC 25923 (*S. aureus*) and ATCC 25922 (*E. coli*) from Kenya Medical Research Institute were used for quality control.

Inoculum was prepared using direct colony suspension method (EUCAST, 2015). Using a sterile loop, four morphologically similar colonies from overnight cultures (18-24 hours) were picked and suspended in 5ml sterile physiological saline (Sodium chloride 0.85%) in separate test tubes for *Escherichia coli* and *Staphylococcus aureus*. A smooth suspension of the content was made using a vortex mixer (EUCAST, 2015). They were diluted with sterile physiological saline and their turbidity adjusted using UV-Visible Spectrophotometer (model; T60U PG Instrument) [OD625nm 0.08-0.12 (1cm light path)] to match 0.5 MacFarland standard (EUCAST, 2015). This obtained a microbial suspension of (1-2) x 10⁸ CFU/mL for both bacterial. Mueller Hinton agar plates were labeled and inoculated within 15 minutes of adjusting the turbidity. The lid was left ajar for 3 - 10 minutes at room temperature in laminar hood to allow any excess surface moisture to be absorbed.

Discs of 6 mm diameter were obtained by cutting Whatman No. 1 filter-paper (Maidstone, UK) using a paper punch. They were put in a bijou bottle, wrapped in aluminum foil paper and autoclaved at 121°C for 15 minutes. Each disc was impregnated with 20µl of each plant extract at varying concentration of 100mg/ml-500mg/ml (Dora et al., 2012). The coated discs on petri dishes separately were covered and left in aseptic condition under the laminar air flow to dry. The discs were aseptically transferred to the inoculated plates using sterile forceps. A volume (20µL) dimethyl Sulphoxide solvent was used as a negative control for *Staphylococcus aureus* and *Escherichia coli*. Ceftriaxone (30 µg) was used as positive controls for *Escherichia coli* while Co-trimoxazole (25µg) was used as positive controls for *Staphylococcus aureus*. Finally, agar plates were labeled, inverted and incubated at 37°C for 24 hours (EUCAST, 2015). Experiments were done in triplicate. Inhibition zones were determined and the mean values of the three replicates recorded to the nearest whole millimeter.

3.9.2 Determination of Antifungal Activity of the two plants extracts
*In vitro* antifungal activity of the two plant extracts were evaluated using Kirby-Bauer technique Bauer, (1998) based on traditional uses of the two plants by disc diffusion method using *Candida albicans* clinically isolated from Nyamira County Referral Hospital. American Type Cultures Collections (ATCC 90028) of *Candida albicans* from Kenya Medical Research Institute were used for quality control. Antifungal activity was done on Mueller-Hinton agar (Oxoid UK).
Inoculum was prepared using direct colony suspension method (EUCAST, 2015). Using a sterile loop, four morphologically similar colonies from overnight cultures (24 hours) on Sabouraud dextrose agar (Oxoid UK) were picked and suspended in 5ml sterile physiological saline (Sodium chloride 0.85%) in test tubes. A smooth suspension of the content was made using a vortex mixer (EUCAST, 2015). It was diluted with sterile physiological saline and the turbidity was adjusted using UV-Visible Spectrophotometer (model; T60U PG Instrument) {OD$_{625nm}$ 0.08-0.12 (1cm light path)} to match 0.5 MacFarland standard (EUCAST, 2015). This obtained a yeast suspension concentration of (1-5) x 10$^6$ CFU/mL for Candida albicans (EUCAST, 2015).

Mueller Hinton agar plates were labeled and inoculated within 15 minutes of adjusting the turbidity. The lid was left ajar for 3 -10 minutes at room temperature in laminar hood to allow any excess surface moisture to be absorbed.

Discs of 6 mm diameter were obtained by cutting Whatman No. 1 filter-paper (Maidstone, UK) using a paper punch. They were put in a bijou bottle, wrapped in aluminum foil paper and autoclaved at 121 degree Celsius for 15 minutes. Each disc was impregnated with 20µl plant extract at a concentration of 100mg/ml-500mg/ml (Dora et al., 2012). The coated discs on Petri dishes separately were covered and left in aseptic condition under the laminar air flow to dry. The discs were aseptically transferred to the inoculated plates using sterile forceps. Dimethyl Sulphoxide solvent (20µL) was used as negative control while Nystatin (Himedia india, SD025-ICT) (100µg) was used as positive control for Candida albicans. The plates were labeled, inverted and incubated at 37° C for 20-24 hours. Experiments were done in triplicate. The zone diameters were measured manually then the mean values of the three replicates recorded to the nearest whole millimeter (EUCAST, 2015).

3.9.3 Measurement of inhibition zones and interpretation of results

After incubation, antimicrobial potentials of crude plant extracts against test organisms were assessed by determining zones of inhibition (indicated by clear zones of growth inhibition) around the discs diameter (including the diameter of the disk) and recorded in millimeters using a ruler on the undersurface of the plate without opening the lid. The zones of inhibition diameter were measured to the margin of heavy growth. Then the zones of inhibition were compared with the zone-size interpretative of standard growth inhibition zones and recorded as susceptible, intermediate, or resistant to each crude drug according to inhibition size (CLSI, 2015).

Data Analysis and Presentation

The questionnaires were checked for completeness at the end of each data collection session. Data was stored in Excel spread sheet in a personal computer protected with a password. Also a flash disk secured with a password was used as a backup. After incubation, the zones of inhibition diameter were measured in mm and the averages results of the triplicate experiments were calculated. Activity of each extract was interpreted as; < 6mm no activity, 6.1mm- 8 mm little activity), 8.1mm-10mm moderate activity, 11mm-12mm active, >12 mm very active. The Data was presented using tables.

3.12 Ethical Considerations

Ethical clearance was obtained from Mount Kenya University E.R.C, NACOSTI and Nyamira Level 5 Hospital before commencement of the study. Participants who met inclusion criteria were informed on the study aspects. Only those who signed informed consent form were included in the study and confidentiality was maintained. Antimicrobial activity was done in Microbiology laboratory in M.K.U Research Centre while plant processing was conducted in M.K.U Pharmacognosy laboratory.

Findings

IV. Characterization of Clinical Isolates Causing Postpartum Infections

Out of 339 samples examined, 18.29% postpartum mothers were infected whereas 81.71% remaining postpartum mothers had no potential pathogens isolated. Microbial isolates of gram positive S. aureus (8.26%), gram negative E. coli (4.13%) and fungi C. albicans (5.9%) were isolated from the postpartum mothers.

4.1.1 Staphylococcus species

The characterization and identification of Staphylococcus spp. was done according to Gayle and Reginald, (2016) and Chesbrough (2006) methods. It was determined that Staphylococcus aureus was one of the causative agent of postpartum.

4.1.2 Escherichia species

The characterization and identification of Escherichia spp. was done according to Feng et al., (2011) and Chesbrough (2006). It was determined that Escherichia coli was one of the causative agent of postpartum.

4.1.3 Candida species

The characterization and identification of Candida spp. was done according to Haw et al., (2012) and Chesbrough (2006). Germ tube test was done according to Kumar, (2010). The yeast were sub-cultured on chromogenic candida agar according to Odds and Davidson, (2000) and Dilek et al., (2012). Chlamydosporation formation was done on corn meal agar according to Michael and Burton, (2011). Analytical Profile Index (API) Candida System (BioMérieux, France) was used to identify Candida species according to Monet et al., (1995). It was determined that Candida albicans was one of the causative agent of postpartum and the isolate had a profile number; 7002, Table 4.1 obtained with the API Candida System test strip, was identified as Candida albicans.
Plate 4.1: API Candida System for C. albicans Identification
   a) Shows API suspension medium (BioMérieux, France)
   b) Shows API Candida System test strip (BioMérieux, France)
   c) Candida albicans Reaction on API Candida System kit (BioMérieux, France)

Table 4.1: Candida albicans result codes from API Candida System kit test

<table>
<thead>
<tr>
<th>API Candida System Result sheet coded</th>
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<tr>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
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<tr>
<td>+ + - - - - - - + -</td>
</tr>
<tr>
<td>1 2 4 1 2 4 1 2 4 1 2 4</td>
</tr>
<tr>
<td>GLU GAL SAC TRE RAF βMAL αAMY βXYL βGUR URE βNAG βGAL</td>
</tr>
<tr>
<td>7 0 0 2</td>
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Table 1a: Antibacterial Activity of A. caulirhiza against S. aureus and E. coli

Acmella caulirhiza

<table>
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<th>Extracts</th>
<th>Negative control 20 µl</th>
<th>100 mg/ml</th>
<th>200 mg/ml</th>
<th>300 mg/ml</th>
<th>400 mg/ml</th>
<th>500 mg/ml</th>
<th>Co-trimoxazole (CT) 25 µg</th>
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<td>6</td>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>25</td>
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</table>
Antibacterial activity of *A. caulirhiza* extracts against *S. aureus*, *E. coli* (isolates), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) indicated a similar results.

Table 1b: Antibacterial Activity of *S. princeae* against *S. aureus* and *E. coli*

<table>
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<th>200 mg/ml</th>
<th>300 mg/ml</th>
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Antibacterial activity of *S. princeae* extracts against *S. aureus*, *E. coli* (isolates), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) indicated a similar results.
Table 1c: Antifungal Activity of *A. caulirhiza* and *S. princeae* against *C. albicans*

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<th>300 mg/ml</th>
<th>400 mg/ml</th>
<th>500 mg/ml</th>
<th>Nystatin (NS) 100 µg</th>
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Antifungal activity of *A. caulirhiza* and *S. princeae* extracts against *C. albicans* isolates and *C. albicans* (ATCC 90028) indicated a similar result.

V. DISCUSSION

Medicinal plants usage is increasingly popular among the Gusii community of Nyamira County. Many medicinal plants grow around the homestead and have been used naturally for many years by traditional healers to control common health problems. Antimicrobial drug discovery from natural medicinal plants are expected to be effective against multi drug resistant microorganisms (Gemechu et al., 2015).

Characterization of Clinical Isolates Causing Postpartum Infections

*Staphylococcus aureus* was characterized and identified by culture, gram stain, biochemical techniques and serology. Thus, it was identified as one of the causative agent of postpartum infections. Other findings corroborate this finding that *S. aureus* is the common microorganism isolated from postpartum mothers (Bako et al., 2012; Nahid et al., 2009).

*Escherichia coli* was characterized and identified by isolating it in pure form using nutrient agar, and using selective/differential growth condition such as macConkey agar. Staining reaction on gram stain was used to differentiate gram negative from gram positive and to identify the shape of bacteria. Colonial morphology of *E. coli* was identified and cultural characteristics were also observed. Metabolism involving the requirement of oxygen and the capacity to form pigments and biochemical techniques were performed. Thus *E. coli* was identified as one of the causative agent of postpartum infections. Other findings have reported that *E. coli* is the common microorganism isolated from postpartum mothers (Bako et al., 2012; Nahidet al., 2009).

*Candida albicans* was characterized and identified by culture, gram stain and germ tube test. The yeasts were sub-cultured on chromogenic candida agar then on corn meal agar to differentiate *C. albicans* based on colony colors and chlamydospore formation respectively. *C. albicans* colonies appeared green indicating a positive result. On corn meal agar, chlamydospores appeared thick-walled at the ends of pseudohyphae, large and round. Budding resulted in chains of cells called pseudohyphae that produced clusters of round, asexual blastoconidia at the cell junctions were observed. Analytical Profile Index (API) Candida System was used to identify *Candida* species. The reactions obtained were coded into numerical profile groups of three where by 1, 2 or 4 was assigned to each one. A 4-digit numerical profile was obtained by adding together the numbers corresponding to the positive reactions within each group. The isolate with profile number: 7002 obtained with the API Candida System test strip was identifies as *Candida albicans*. It was determined that *Candida albicans* was one of the causative agent of postpartum infection. Other findings have reported that *Candida albicans* cause 6.8% microbial urinary tract infections related to postpartum infections (Payam et al., 2010).
Other studies have indicated that micro-organisms causing postpartum infections are indigenous to the female genital tract (Nahid et al., 2009). This is justified from this study because mixed bacterial and fungal agents were isolated from high vaginal specimen and this implicates endometritis diagnosis in women clients. This has a significant public health consequence since physicians, frequently face difficulties of knowing exact cause of postpartum infections in the process of giving the right treatment. In this study, postpartum infections were prevalent because out of 339 participants examined, 18.29% (62 persons) postpartum mothers were infected whereas 81.71% had no potential pathogens isolated. Gram positive *S. aureus* (8.26%), gram negative *E. coli* (4.13%) and fungi *C. albicans* (5.9%) were isolated. This finding concurs with those of Ahnfeldt-Mollerup, (2012), who reported that puerperal sepsis prevalence was between 5–24% depending on the different socio-cultural conditions.

**Antibacterial Activity of Acmella caulirhiza and Spermacoce princeae against *S. aureus* and *E. coli***

Neigher aqueous nor ethyl acetate crude plant extracts of *A. caulirhiza* indicated antibacterial activity against *S. aureus* and *E. coli* in contrast to positive controls. Although aqueous plant extract is mostly used, alcoholic solvents have been reported to provide consistent activity (Das et al., 2010). Ethanol and methanol crude plant extracts of *A. caulirhiza* indicated that they had low activity against *S. aureus* and *E. coli*.

*A. caulirhiza* ethanol crude plant extract indicated its highest antibacterial activity at concentration 500mg/ml (8mm) and its lowest activity at concentration 200mg/ml (7mm) against *S. aureus*. While it indicated its highest antibacterial activity at concentration 300mg/ml (7.3mm) and its lowest activity at concentration 100mg/ml (6.1mm) against *E. coli*. This means ethanol crude plant extract of *A. caulirhiza* has low activity against *S. aureus* and *E. coli*. Antibacterial activity with the increase of the concentration observed is in agreement with the earlier studies (Banso and Adeyemo, 2007).

Similarly, methanol crude plant extract of *A. caulirhiza* indicated its highest antibacterial activity at concentration 500mg/ml (8mm) and its lowest activity at concentration 300mg/ml (7mm) against *S. aureus*. While it indicated its highest antibacterial activity at concentration 400mg/ml (6.6mm), its lowest activity was at concentration 500mg/ml (6.3mm) against *E. coli*. This means *A. caulirhiza* methanol crude plant extract has low activity against *S. aureus* and *E. coli*. The results of this study corroborate and/or oppose data reported in literature. Kipruto et al., 2013, conducted a study which opposes the finding of the present study in which *A. caulirhiza* chloroform extract indicated inhibitory effects against *S. aureus* and *E. coli*, whereas Bethwell (2012), found no antibacterial activity of methanol extracts of *A. caulirhiza* against *S. aureus* and *E. coli*. In reference to positive controls co-trimoxazole at concentration 25 µg had the highest antibacterial activity (25mm) for *S. aureus* while ceftriaxone had highest antibacterial activity at concentration 30 µg (21mm) for *E. coli* hence commercially available antibiotics are still effective as compared to crude plant extracts.

Antibacterial activity of ethyl acetate crude plant extract of *S. princeae* indicated no antibacterial activity against *S. aureus* and *E. coli* in contrast to positive controls. However, aqueous crude plant extract of the same plant at higher concentration (500mg/ml) indicated low antibacterial activity (6.3mm and 6.5mm zone of inhibition) against *S. aureus* and *E. coli* respectively. This point out that, aqueous crude plant extract of this plant has little activity against *S. aureus* and *E. coli* at higher concentration. On the other hand, ethanol crude plant extracts of *S. princeae* indicated a moderate antibacterial activity against *S. aureus* and *E. coli* from concentration 200mg/ml onwards. An antibacterial effect with the increase of the concentration observed is in agreement with the earlier studies (Mahmoud et al., 2014). Similarly, methanol crude plant extracts of *S. princeae* indicated a moderate antibacterial activity against *E. coli* and *S. aureus* from concentration 200mg/ml and 300mg/ml onwards respectively. This indicates that even if this plant is used during postpartum, the plant has little activity against *S. aureus* and *E. coli*.

*S. princeae* ethanol crude plant extract indicated its highest antibacterial activity at concentration 300mg/ml (7mm) and its lowest activity at concentration 200mg/ml and 500mg/ml (6.3mm) against *S. aureus*. While it indicated its highest antibacterial activity at concentration 200mg/ml (8.3mm) and its lowest activity was at concentration 500mg/ml (6.6mm) against *E. coli*. This indicates that *S. princeae* ethanol crude plant extract has little activity against *S. aureus* and *E. coli* even if this plant is used during postpartum. Similarly, methanol crude plant extract of *S. princeae* indicated its highest antibacterial activity at concentration 400mg/ml (7.6mm) and its lowest activity was at concentration 300mg/ml (6.3mm) against *S. aureus*. While it indicated its highest antibacterial activity at concentration 300mg/ml and 400mg/ml (7mm) its lowest activity was at concentration 500mg/ml (6.3mm) against *E. coli*. This indicates that, methanol crude plant extract of *S. princeae* has little activity against *S. aureus* and *E. coli*. In reference to positive controls, co-trimoxazole 25 µg had the highest antibacterial activity (25mm) for *S. aureus* while ceftriaxone had its highest antibacterial activity at concentration 30 µg (21mm) for *E. coli* hence commercially available antibiotics are still effective as compared to crude plant extracts.

In general, ethanol was indicated as the best solvent in extracting active ingredients from these plants for antibacterial screening. These results are consistent with earlier reports that, *A. caurhiza* was active against *S. aureus* and it is used to treat toothache, sore throat, earache and stomachache (Kipruto et al., 2013). Other findings have shown that *S. princeae* water extracts of leaves and roots are used to treat wounds, eye problems, venereal diseases, skin diseases, pneumonia, typhoid and diarrhea (Jeruto et al., 2011). There was no significant difference between the clinical isolates (*S. aureus* and *E. coli*) and the standard *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922).

**5.2.2 Antifungal Activity of Acmella caulirhiza and Spermacoce princeae against Candida albicans.**

*A. caulirhiza* and *S. princeae* at concentration 100mg/ml 200mg/ml, 300mg/ml, 400mg/ml and 500 mg/ml indicated no antifungal activity against *C. albicans*. These findings concur with other studies that *A. caulirhiza* does not have antifungal activity against *C. albicans* even at high concentration (Kipruto et al., 2013). In reference to positive control Nystatin at concentration 100µg had the
highest antifungal activity (25mm) for *C. albicans* hence commercially available antifungals are still effective as compared to crude plant extracts. There was no significant difference between the clinical isolates (*C. albicans*) and standard *C. albicans* (ATCC90028).

VI. CONCLUSION

The two plants (*A. caulirhiza* and *S. princeae*) used by the local inhabitants of Nyamira County in treatment of child sores and to clean reproductive system after birth indicated that, they were not efficacious but possess some antibacterial activity even though they are used in treatment of postpartum infections. Antibacterial activity of the two plants varied depending on the extraction solvent used. Aqueous and ethyl acetate extracts were the weakest extraction solvents though ethanol and methanol extraction solvents indicated they were the best solvents in obtaining activity from the two medicinal plants. Alcoholic extracts indicated some activity against *S. aureus* and *E. coli*. Ethanol extracts had the highest zone of inhibition while ethyl acetate extracts had the least zone of inhibition. Manifestation of antibacterial activity from the two medicinal plants investigated indicates that the two plants can be a potent source of alternative medicines. Plants based antimicrobials have enormous therapeutic potential. *Acmella caulirhiza* and *Spermacoce princeae* plants represent unexploited source of compounds with antimicrobial activity which could be a resource for development of natural therapeutic drugs. No antifungal activity was detected on the two medicinal plants. Antimicrobial activity evaluation lays a foundation for ethnobotanical and pharmacological investigations for new drug discovery.

VII. RECOMMENDATION

Pregnant women are encouraged to deliver at the health facility to reduce morbidity and mortality caused by postpartum infections. The two plants may be used as medicine and can be a potent source of complementary and modern medicine although their antimicrobial activity is low. *A. caulirhiza* and *S. princeae* plants may be used in treating puerperal sepsis though commercially available drugs are recommended as they are highly effective. The two plants indicated some antibacterial activity against *S. aureus* and *E. coli*. However, further studies are recommended on genotoxicity of this plant extracts on *S. aureus* and *E. coli* genes. Also further research is recommended to isolate and identify pure compounds of the two plants to establish pharmacological basis of antibacterial activity shown even though it was low.

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