PHYTOCHEMICAL STUDIES OF *ACMELLA CAULIRHIZA* AND *SPERMACOCE PRINCEAE* USED BY POSTPARTUM MOTHERS IN NYAMIRA COUNTY, KENYA

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***Abstract*- Introduction:** Traditional medicine have been used in health maintenance, disease prevention and treatment 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.
Objective: The main objective of the present study was to determine phytochemical compounds of *A. caulirhiza* and *S. princeae* used by postpartum mothers in Nyamira County, Kenya.
Methodology: The study area was Nyamira County where the two plant specimens were collected. Plant materials were identified at East Africa Herbarium. Plant specimens were transported to M.K.U. Pharmacognosy laboratory where processing was done. Phytochemical analysis methods were employed to determine phytochemicals compounds in the crude plant extracts. Data was stored in Excel spread sheet in a personal computer protected with a password. Data was presented using tables and photographs.
Results: Phytochemical examinations revealed that *Acmella caulirhiza* contains flavonoids, terpenoids, coumarins and sterols compounds. On the other hand, *Spermacoce princeae* contains flavonoids, terpenoids, tannins, saponin alkaloid and glycoside compounds.
Conclusion and recommendation: The plants may be used in treating puerperal sepsis although commercially available drugs are recommended as they are highly effective. The two plants can be a potent source of complementary and modern medicine. Further research is recommended to isolate and identify pure compounds of the two plants

***Index Terms*-** Traditional medicine, phytochemical investigation, Asteraceae, Rubiaceae.

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 Douglas, 2005). Medicinal plants used to manage postpartum complications include; *Basella alba* plant which belongs to Basallaceae 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 aflowering herbal plant, which belongs to Compositae/Asteraceae family (Berhane *et al.,* 2014). It is an annual or perennial herb. Locally it is known as Ekenyunyuntamonwa (Ekegusii) andAjuok-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 relief 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 (Ekegusii), Gakungathe (kikuyu), Murkugwet (kipsigis) and Nyamwoch (Dholuo) (Kokwaro, 2009).It grows in tropical regions and it is used are 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 (Telefo *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

2.1 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^o 33'14.8536 S and 34^o 58' 2.4996 E), Bonyunyu (0^o 31'36.2532 S and 34^o 53'20.4108 E) and miruka (0^o 29'13.902 S and 34^o 5320.3208 E) whereas the study point in North Mugirango constituency was; Magong'a (0^o 28' 46.7724 S and 34^o 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 Km^2 with a population of 598,252 and a population density of 656 persons per Km^2according to (KNBS, 2009).

2.2 Plant materials collection

Acmella caulirhiza andSpermacoce 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).

Fig. 1: The two medicinal plant materials collected

(A) *Acmella caulirhiza* **(B) *Spermacoce princeae***

2.3 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).

2.4 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.,* 20008).

2.5 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 distilled water in beakers of 400ml capacity on Hot Plate set at 100^0 C for 5 minutes. The extracts were cooled, using a funnel and Whatman filter paper (No. 1) they were filtered and freeze dried according to Pikal *et al.,* (2010), to extract dry powders from the aqueous solutions of the two plants. Freeze-drying was done in the following steps; freezing, primary drying and secondary drying. Primary drying involves; evacuating the system, increasing shelf temperature resulting to product temperature $2–3^0$ C below collapse temperature. Secondary drying involves; removing unfrozen water from the solute phase by desorption through raising temperatures. The dry and lyophilized extracts were weighed and stored in a freezer for bioactivity testing (Bibi *et al.,* 2012).

2.6 Phytochemical investigation

Phytochemical analysis for various secondary metabolites of the plant extract was done using methods described below. The groups of phytochemical compounds investigated were alkaloids, flavonoids, tannins, coumarins, steroids, sterols, saponins, terpenoids and glycosides.

Test for tannins

Ferric Chloride Test: *Acmella caulirhiza* 0.5 grams and 0.5gms *Spermacoceprinceae* dry powdered samples were boiled in 10ml distilled water on a hot plate separately and the extracts were filtered. 2ml portion of each filtrate was measured and 3drops of 0.1% ferric chloride solution was added. Formation of a green colored solution shows tannins presence (Kiran and Prasad 2015).

Test for coumarins

Onto separate filter papers, a few drops of ammonia were added and then a drop of *Acmella caulirhiza* and *Spermacoceprinceae* extracts were added separately. Fluorescence on the paper shows coumarins presence (Sangeetha *et al.,* 2014).

Test for flavonoids

Ferric chloride test; 2ml *Acmella caulirhiza* and 2ml *Spermacoceprinceae* crude extracts were separately treated with 5 drops of Ferric chloride solution each. Formation of a blackish red colour shows flavonoids presence (Kiran and Prasad 2015).

Tests for steroids

Liebermann test: To 1ml *Acmella caulirhiza* and 1ml *Spermacoceprinceae* extract solution in 10ml chloroform solution separatery, 3drops of acetic anhydride and Sulphuric acid solutions were added slowly from the side of test tube separately. Observation of a brown ring at the junction of the two layers and the upper layer turns green separating the liquids shows steroids presence (Kiran and Prasad, 2015).

Test for sterols

Liebermann test: Few grams of *Acmella caulirhiza* and *Spermacoceprinceae* in separate test tubes were dissolved in 0.5 ml hot acetic anhydride and 0.5 ml of chloroform was added. Observations of blue-green ring at interphase a positive reaction (Sabri *et al.,* 2012).

Testing for saponins

Froth test: Using a top loading weigh balance, 0.5 grams *Acmella caulirhiza* and 0.5gms *Spermacoceprinceae* powder were weight, placed in separate test tubes and 10ml distilled water added to each. Then themixture was shacked and left to stand. Persistent froth shows saponins presence (Kiran and Prasad 2015).

Test for terpenoids

Five ml *Acmella caulirhiza* and 5ml *Spermacoceprinceae* extracts were mixed separately in 2ml chloroform and then 3ml concentrated sulphuric acid was added. At the interface a reddish brown color shows terpenoids presence (Ablude, 2001).

Test for glycosides

Keller-kiliani test; *Acmella caulirhiza* (50mg), and 50mg *Spermacoceprinceae*extracts in separate test tubes, 2ml and1ml glacial acetic acid and ferric chloride solution were added respectfully. The contents were heated and cooled then transferred to a test tube containing 2ml concentrated sulphuric acid. Formation of a brown ring at the interface (presence of deoxy sugar characteristic of cardenolides) and observation of pale green colour in the upper layer (steroidal nucleus) shows glycoside presence (Kiran and Prasad 2015).

Test for alkaloids

Using a top loading weigh balance, 0.5gms of *Acmella caulirhiza* and 0.5gms *Spermacoceprinceae* dried powdered sample free extract were measured, agitated with 3mls diluted hydrochloric acid and filtered separately. Then the filtrates were tested for alkaloids using dragendoff's reagent: To 2mls of *Acmella caulirhiza* and *Spermacoceprinceae* filtrate in separate test tubes, 2 mL Dragendorff's reagent was added to each. A prominent yellow precipitate shows presence of alkaloids (Harborne, 2005).

2.7 Data Analysis and Presentation

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. Data was presented using tables and photographs.

2.8 Ethical Considerations

Ethical clearance was obtained from Mount Kenya University Ethical Review Committee and NACOSTI before commencement of the study. Phytochemical analysis was conducted in Mount Kenya University Pharmacognosy laboratory.

III. FINDINGS

Quantity of crude plant extracts

Table 1: % yields of extract obtained by different extraction solvent

Percentage yield					
Plant species	**Part used**	**Ethyl Acetate**	**Ethanol**	**Methanol**	**Aqueous**
Acmella caulirhiza	Whole plant	1.68 (2.1%)	3g (3.75%)	4g (5%)	2.45g (12.25%)
Spermacoce princeae	Whole plant	2g (2.5%)	4g (5%)	5(6.25%)	3g (15%)

Fig. 2: Preliminary phytochemical screening of *Acmella caulirhiza* whole plant

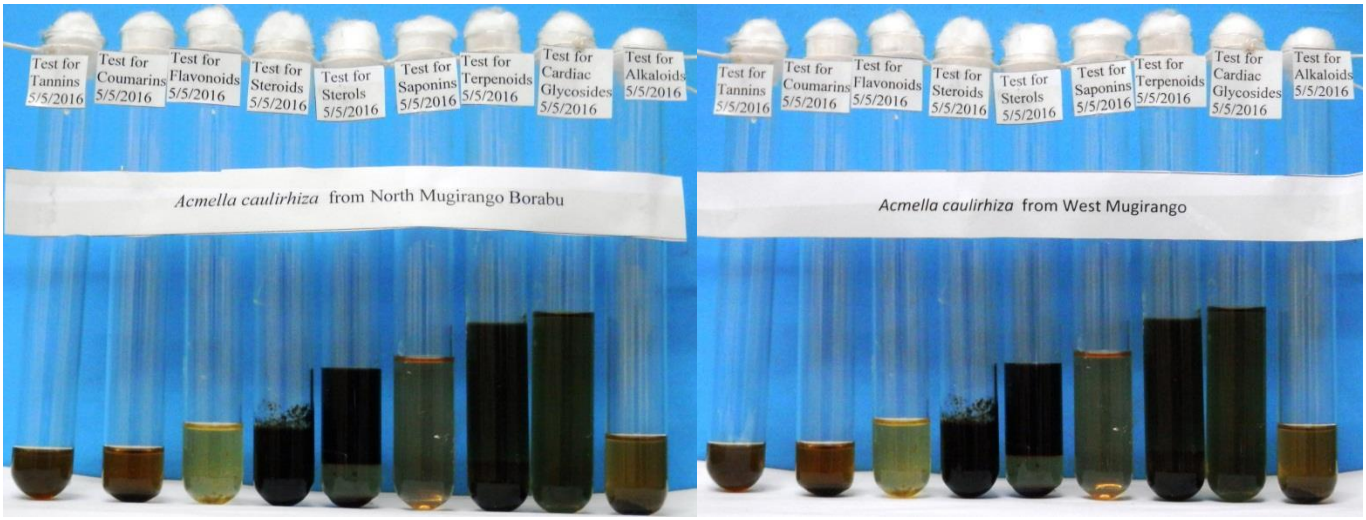
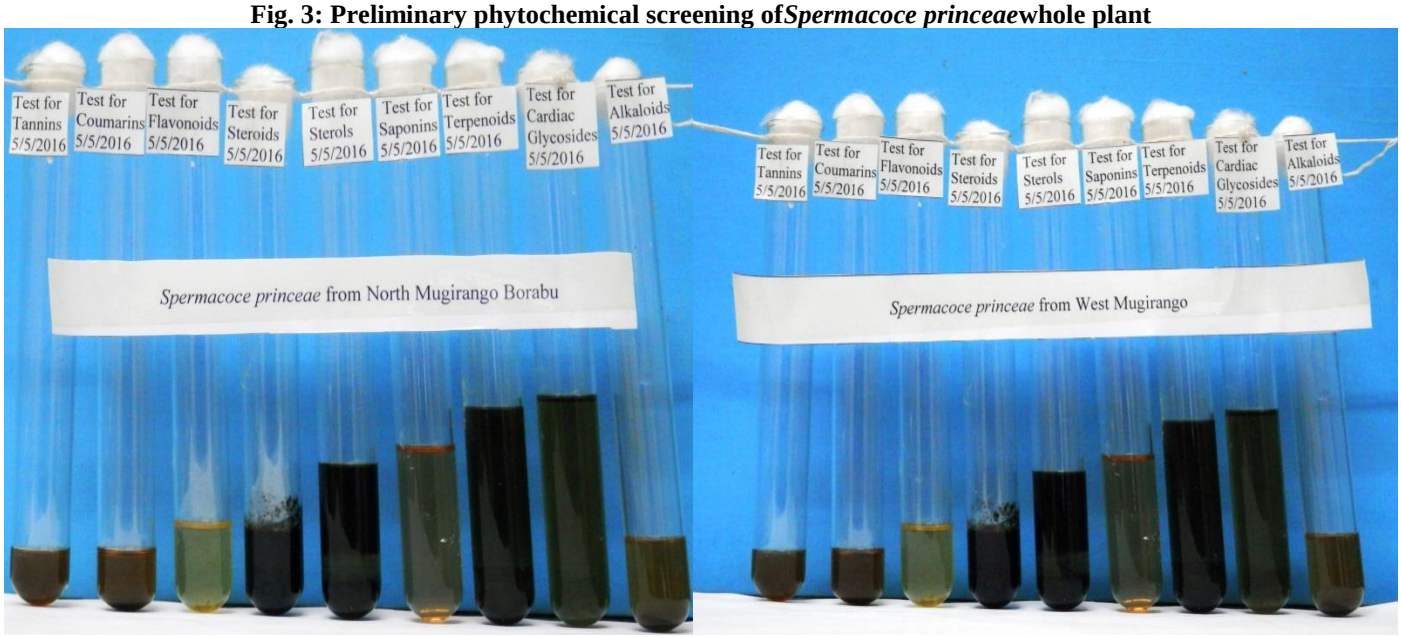


Fig. 3: Preliminary phytochemical screening of*Spermacoce princeae*whole plant



Determination of Phytochemical Compounds in Crude Plant Extracts of *A. caulirhiza* and *S. princeae*

Phytochemical examinations revealed that *Acmella caulirhiza* contains flavonoids, terpenoids, coumarins and sterols compounds. However, tannins, saponin and glycoside were absent. On the other hand, *Spermacoce princeae* contains flavonoids, terpenoids, tannins, saponin alkaloid and glycoside compounds. However, sterols and coumarins were not found.

Table 2: Phytochemical Analysis of *Acmella caulirhiza* and *Spermacoce princeae* from Nyamira County

Phytochemical compounds	Flavonoids	Terpenoids	Coumarins	Sterols	Tannins	Saponins	Alkaloids	Glycoside	Steroids
Acmella caulirhiza	+	+	+	+	-	-	-	-	-
Spermacoce princeae	+	+	-	-	+	+	+	+	-

IV. 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.

Determination of Phytochemical Compounds in Crude Plant Extracts of *Acmella caulirhiza* and *Spermacoce princeae*

Phytochemical examinations revealed that, *Acmella caulirhiza* contains flavonoids, terpenoids, coumarins and sterols phytochemical compounds. However, tannins, saponin and glycoside were absent. The presence of coumarin compound in this plant is consistent with other study Chhabra *et al*., (1989). Other findings indicates that, *A. caulirhiza*leaves and flowers contain spilanthol compounds believed to prevent bacterial pathogens in addition to numbing the pain when used in toothache therapy (Neil and Jerald, 2005). Besides, *A. caulirhiza* plant is believed to treat sore throat and stomachache traditionally (Kipruto *et al.*, 2013; Giday *et al.*, 2010; Njoroge and Bussmann, 2006; Chhabra *et al.*, 1989).

On the other side, *Spermacoce princeae* contained flavonoids, terpenoids, tannins, saponin alkaloid and glycoside phytochemical compounds. However, sterols and coumarins were not found. This result concurs with the phytochemical results reported by Jeruto *et al.*, (2011) on phytochemical compounds present in this plant.*S. princeae* is used traditionally to clean reproductive system after birth.Other findings have shown that *S. princeae* treats venereal diseases, pneumonia, typhoid, Chronic asthma, cancer, wounds, eye

problems, venereal diseases, diarrhoea skin and kidney diseases (Jeruto *et al.,* 2011). This validates therapeutic value of this plant, in traditional management of typhoid, pneumonia and eye infections(Jeruto *et al.,* 2011).

Therapeutic value of medicinal plants is determined by the presence of phytochemical compounds having certain functional and pharmacological activity (Geeta *et al.,* 2012). Flavonoids compounds have been reported to have antimicrobial activity (Pandey *et al*., 2010; Cowan, 1999). Example flavonoids such as flavone and flavonol glycosides, apigenin, isoflavones, galangin flavanones, and chalcones have been revealed to possess effective antibacterial activity (Cushnie and Lamb, 2005). A new flavanone, 7-hydroxy-6,8-dimethoxyflavanone, showed anti-mycobacterial activity against *M. tuberculosis* H37Ra at 50 µg/mL MIC value (Prawat *et al.,* 2013). Mode of action results from their ability to inactivate microbial cell envelope transport proteins, enzymes and adhesins (Kumar and Pandey 2013; Mishra *et al*., 2009; Cowan, 1999). Lipophilic flavonoids may disrupt microbial membranes (Mishra *et al*., 2009; Cowan, 1999). Flavonoids inhibit cytoplasmic membrane function and they inhibit DNA gyrase and hydroxyacyl-acyl carrier protein dehydratase activities (Zhang *et al.,* 2008).

Terpenoids have antimicrobial activity against bacterial and fungi (Omojate *et al.,* 2014; Ghoshal *et al.,* 1996). Example petalostemumol terpenoid exhibited excellent effects against *Bacillus subtilis* and *Staphylococcus aureus* (Omojate *et al.,* 2014). They also control *Listeriamonocytogenes* Cowan, (1999). Terpenoids derived from essential oil inhibitory 60% fungi while they inhibit 30% bacteria (Omojate *et al.,* 2014; Mohd *et al.,* 2014). Terpenoids act by disrupting cellular membrane by lipophilic compounds (Cowan, 1999; Hatice and Ayse 2014). Coumarin, has been reported to contain antimicrobial activity against *Candida albicans* and chronic infections (Thornes *et al*., 1982). Example coumarin compound isolated from *Angelica lucida* L. is effective against oral pathogens such as *Streptococcus viridians* and *Streptococcus mutans* (Widelski *et al.,* 2009). They act by activating other cells of immune system stimulating macrophages, indirectly affecting the disease causing agents (Casley *et al.,* 1997).

Sterols have been reported to contain antimicrobial activity. Examples stem sterols of *Withania somnifera* is reported to have antimicrobial effects against *P. aeruginosa.* As wellstem sterol of *Euphorbia hirta* and *Terminalia chebula* is active against *S. aureus* (Geeta *et al.,* 2012). There is no information on antimicrobial mode of action of plant steroids and sterols. Tannins have been reported to contain bactericidal activity against gram positive organisms such as *S. aureus* and used for the treatment of diarrhea an example of enteric diseases (Omojate *et al.,* 2014; Chung *et al.,* 1993). Tannins have been used as antiseptic whose activity is due to manifestation of the phenolic group. Tannin-rich plants have been shown to be toxic to filamentous fungi, yeasts, and bacteria (Omojate *et al.,* 2014). Example tannins extracted from *Solanum trilobatum* plant have been reported to possess high antibacterial activity against *Staphylococcus aureus* and *Proteus vulgaris* at 2.5mg/ml concentration (Doss *et al.,* 2009). Tannin compounds act by interfering with bacterial cell wall causing disintegration of bacterial colonies thus inhibiting microbial growth (Erasto *et al.,* 2004). They also act by forming complexes with proteins and polysaccharide through hydrogen bonding, hydrophobic bond and covalent bond resulting to inactivation of microbial adhesion, enzymes and cell envelope transport proteins (Haslam, 1996; Haslam *et al.,* 1988). Also they have the ability to inactivate microbial adhesins, enzymes, cell envelope and transport proteins (Omojate *et al.,* 2014). Condensed tannins have been confirmed to bind to cell walls of ruminal bacteria, thus inducing bacterial stasis and protease activity (Jones *et al*., 1994).

Saponins have been reported to have antimicrobial activity (Moyo *et al*., 2012). Example Yucca saponin have antibacterial activity against *Staphylococcus aureus* and *Lactobacillus* (Tanaka *et al.,* 1996). Mode of action is by altering with the permeability of cell walls hence they exert toxicity on all tissues. Also they form complexes with cell membranes hence elicit changes in cell morphology leading to cell lysis (Moyo *et al*., 2012).

Alkaloids have antibacterial properties against gram-positive and gram-negative bacteria (Donald *et al*., 2016). Example alkaloids Chloroform and ethanol extracts from *Callistemon citrinus* leaves have been reported to have antibacterial activity against *Staphylococcus aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* via disc diffusion methods (Krishna *et al.,* 2012; Donald *et al.,* 2016). They act by disrupting cell wall membrane (Cowan, 1999). Glycosides: have been reported to contain antibacterial activity. Example glycosides compounds extracted from *Caesalpinia coriaria* (Jacq) Willd exhibited antibacterial activity against *E.coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* (Anandhi *et al.,* 2014). Glycosidic compounds (G1) extracted from *Citrus laurantifoia* L. fruits exhibited broad spectrum antibiotic effects against *Staphylococcus aureus*, *Streptococcus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Sameerah *et al*., 2013). Mode of action glycoside act by causing leakage of cellular materials by breaking the outer membrane. Also glycosides inhibit the respiration and growth of pathogenic micro-organisms by entering the inner membrane and inactivating the enzyme system dehydrogenase (Anandhi *et al.,* 2014).

V. Conclusion and Recommendation

Demonstration of phytochemical compounds in the two plants such as flavonoids, terpenoids, coumarin, sterols, tannins, saponin, alkaloids and glycosides validates their use in management of puerperal sepsis. Mode of action of compounds present in the two plants indicates that the plants have therapeutic potential. Phytochemical compounds analysis lays a foundation for ethnobotanical and pharmacological investigations for new drug discovery.

A. caulirhiza and *S. princeae* plants may be used in treating puerperal sepsis. The two plants may be used as medicine and can be a potent source of complementary and modern medicine. Further research is recommended to isolate and identify pure compounds of the two plants

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