Safety Studies of *Acmella Caulirhiza And Spermacoce Princeae* Used By Postpartum Mothers In Nyamira County, Kenya

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***Abstract*- Introduction**: 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.

Objestive: The main objective of the present study was to determine safety 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 Mount Kenya University Pharmacognosy laboratory where processing was done. Brine shrimp toxicity testing methods were employed to determine safety of the two crude plant extracts. Data was stored in Excel spread sheet in a personal computer protected with a password. Toxicity results were analyzed using the graph pad prism version 5 to estimate IC50 values. Data was presented using, tables and photographs.

Results: Brine shrimp lethality test of the two plants indicated that the plants were non-toxic.

Conclusion and recommendation: The two plants may be considered safe for use in treating puerperal sepsis though commercially available drugs are recommended as they are highly effective. The two plants may be considered safe for medicinal use and can be a potent source of complementary and modern medicine. Further studies are recommended on genotoxicity of this plant extracts on *S. aureus* and *E. coli* genes.

***Index Terms*-** Traditional medicine, brine shrimp and safety.

I. INTRODUCTION

Puerperal sepsis is a commonly pregnancy-related state which could lead to obstetric shock or death (Shagufta *et al.*, 2015). Puerperal infection has been the leading cause of maternal morbidity and mortality globally World Health Organization (2012) and it continues to be a significant public health problem worldwide, posing a major threat to women (Shagufta *et al.*, 2015). Puerperal sepsis arises in a woman, amid the onset of labour to 42 days postpartum (Seale *et al.*, 2009).

Medicinal plants play important role during birth and postpartum in several rural areas of the world (Vichith *et al.*, 2011). Traditional medicine, being the total knowledge, abilities and practices grounded on ideas, beliefs and capabilities, native to diverse cultures used to preserve health, identify, treat and prevent physical and psychological illnesses (WHO, 2008). 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 the following: *Hydrocotyle manii* and *Centella asiatica* plant species which belongs to Apiaceae family. The two plants are both used to manage abdominal pains after birth (Jeruto *et al.*, 2015).

Acmella caulirhizaI is a flowering herbal plant, which belongs to Compositae/Asteraceae family (Berhane *et al.*, 2014). Locally it is known as Ekenyunyuntamonwa (Ekegusii) and Ajuok-olwa Salamatwe (Dholuo) (Kokwaro, 2009). It is an annual or perennial herb which is used by different communities in Kenya and the rest of Africa countries to treat various medical conditions. Example in Kenya, its flowers

and leaves are used to treat venereal diseases, decayed teeth, gingivitis or wounds in the mouth, toothache and sore throat (Jeruto *et al.,* 2015; Kipruto *et al.,* 2013). It is used to relief painful sores of the mouth, gums and throat, as well as stomach ache (Kokwaro 2009). 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

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). At maturity, *Spermacoce princeae* produces white flower. Rubiaceae family grows in tropical, subtropical, temperate regions and consist more than 100 000 species divided into 600 classes. *Spermacoce princeae* is used in the Kenya to treat bacterial infections (Jeruto *et al.,* 2011). Water extracts of leaves and roots are used to treat Chronic asthma, cancer, wounds, eye problems, mastitis in cows, venereal diseases, skin diseases, pneumonia, typhoid, caterpillar bites and diarrhea (Jeruto *et al.,* 2011). The water macerate of leaves and stem is used in treating female infertility in Baham, Cameroon (Telefo *et al.,* 2011). Leaves warmed gently are crushed, diversified using red oil and salt then orally taken in treatment of kidney diseases (Focho *et al*., 2009). Leaves and roots are used to treat venereal diseases (Jeruto *et al.,* 2015). In Uganda this herb is used to quicken delivery (Jane *et al.,* 2011). For the purpose of enlightening the consumers on possible toxicological risks associated with consumption in treatment of some bacterial and fungi infections, safety of these plants were assessed using brine shrimp.

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 (0º 33’14.8536 S and 34º 58’ 2.4996 E), Bonyunyu (0º 31’36.2532 S and 34º 53’20.4108 E) and miruka (0º 29’13.902 S and 34º 5320.3208 E) whereas the study point in North Mugirango constituency was; Magong’a (0º 28’ 46.7724 S and 34º 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).

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).

Plate 3.2 Two medicinal plant materials collected

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

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.,* 20008).

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).

Brine Shrimp Lethality Assay using *Artemia salina* larvae

Artificial sea water was prepared by dissolving 33gms of sera sea salt (USA GB) in 1 liter of distilled water. Brine shrimps were hatched in a round-shaped plastic container which contained a divider in between the chambers (dark small chamber and light larger chamber) with several holes. Prepared sea water (200ml) was poured into both chambers of the container and 5grams of *Artemia salina* eggs (Original Great Salt Lake USA) were introduced into the dark small chamber. It was covered and a light source provided, aerated well and incubated for 48 hours at room temperature (22-29 degrees Celsius). Active larvae (nauplii) were hatched. The free hatched larvae were attracted by light source and migrated from small hatching compartment into the illuminated larger hatching chamber through a divider in between and were collected and used for Brine Shrimp Lethality assay.

Evaluation of toxicity of *Acmella caulirhiza* and *Spermacoce princeae* in *Artemia salina* larvae was performed according to Sharma *et al.,* (2013). Samples were prepared by dissolving 0.1 g of each (methanol, ethyl acetate, ethanol and aqueous) crude extract in 9ml of distilled water in different test tubes and serial diluted to make the concentration range of 10 µg/ml, 100 µg/ml, 1000 µg/ml then tested by adding 10 brine shrimp larvae. Positive control containing 10 brine shrimp larvae and 10 ml artificial sea water was set. Also a negative control containing 10 brine shrimp larvae and 10 ml dimethyl Sulphoxide was set (Naidu *et al.,* 2014). Experiments were done in triplicate and toxicity was evaluated by inspecting and counting survived larvae after 24 hours using a magnifying glass. Abbott's formula Somayeh *et al.,* (2015), was used to calculate percentage lethality as shown; Percentage lethality = [(Test − Control)/Control] × 100. IC50 values were estimated using a Graph pad Prism version 5 (Misonge *et al.,* 2015).

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. The toxicity results were analyzed using the Graph pad Prism version 5 to estimate IC50 values. Data was presented using photograph and tables.

Ethical Considerations

Ethical clearance was obtained from Mount Kenya University E.R.C and NACOSTI before commencement of the study. Toxicity testing was in M.K.U Pharmacognosy laboratory.

III. FINDINGS

Evaluation of Toxicity Levels of the Crude Plant Extracts Using Brine Shrimp

Brine shrimp toxicity is a method of testing bioactive chemicals in natural plant extracts and it is based on the killing ability of test compounds on a zoological organism- brine shrimp (*Artemia salina*). Table 4.3a, LC50 values of ethyl acetate and ethanol crude plant extract of *Acmella caulirhiza* had low toxic effects on brine shrimp larvae while methanol and aqueous crude plant extract of the same plant indicated it had no toxic effects on brine shrimp larvae. The classification of toxicity levels was done according to Misonge *et al.,* (2015) methods.

Plate 2a: a Container used to hatch *Artemia salina* nauplii

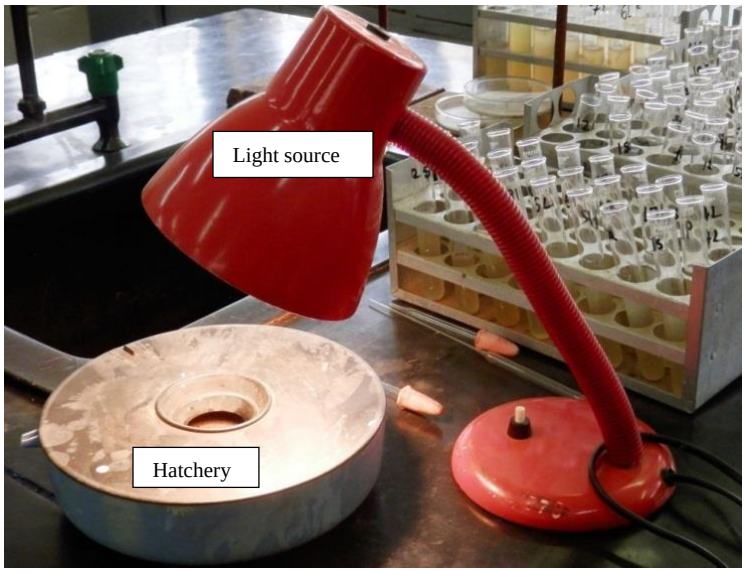

Plate 2b: a hatched *Artemia salina* nauplius

Table 4.3a: Percentage Mortality of brine shrimp *Artemia salina* nauplius Treated with *Acmella caulirhiza* Crude Plant Extracts and their IC50

Solvent extracts of *Acmella caulirhiza*	Dimethyl Sulphoxide	1000 µg/ml	100 µg/ml	10 µg/ml	Sea salt death=0 µg/ml	IC50 values µg/ml
Ethyl acetate	100%	50%	30%	20%	0%	1000
Ethanol extract	100%	50%	30%	30%	0%	1000
Methanol extract	100%	30%	0%	0%	0%	>1000
Aqueous extract	100%	20%	0%	0%	0%	>1000

Brine shrimp (*Artemia salina*) were used to assess toxicity of *Spermacoce princeae* crude plant extracts. Table 4.3b, LC50 values of ethyl acetate crude plant extract of *Spermacoce princeae* had low toxic effects on brine shrimp larvae while ethanol, methanol and aqueous crude plant extract of the same plant indicated it had no toxic effects on brine shrimp larvae.

Table 4.3b: Percentage Mortality of Brine Shrimp *Artemia salina* nauplius Treated with *Spermacoce princeae* Crude Plant Extracts and their IC50

Solvent Extracts of *Spermacoce princeae*	Dimethyl Sulphoxide	1000 µg/ml	100 µg/ml	10 µg/ml	Sea salt death=0 µg/ml	IC50 values µg/ml
Ethyl acetate	100%	50%	30%	20%	0%	1000
Ethanol extract	100%	40%	40%	30%	0%	>1000
Methanol extract	100%	30%	30%	10%	0%	>1000
Aqueous extract	100%	30%	20%	10%	0%	>1000

Table 4.3 c: Classification of toxicity

Classification	Extremely toxic	Very highly toxic	Highly toxic	Moderately toxic	Lowly toxic	Particularly non-toxic
LC Values 50	≤1 µg/ml	1-100 µg/ml	100- 200 µg/ml	200-500 µg/ml	500-1000 µg/ml	≥1000 µg/ml
Extract	None	None	None	None	Ethanol in *A. caulirhiza.* Ethyl acetate in the plants.	Ethanol in *S. princeae.* Aqueous and methanol twoin the two plants.

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. Antimicrobial drug discovery from natural medicinal plants are expected to be effective against multi drug resistant microorganisms (Gemechu *et al.*, 2015).

Evaluation of Toxicity Levels of the Crude Plant Extracts Using Brine Shrimp

Brine Shrimp Lethality Assay is the most suitable method for monitoring biological activities of several plant species. This technique is suitable for preliminary assessment of toxicity levels of crude plant extracts. It is based on the killing ability of the test compounds on a zoological organism-brine shrimp (*Artemia salina*). Lethal dose 50 values of methanol and aqueous extracts of *A. caulirhiza* indicated that

the plant is non-toxic while those of ethyl acetate and ethanol extracts indicated low toxic effects on brine shrimp. On the other hand IC50 values of aqueous, methanol and ethanol extracts of *S. princeae* indicated the plant was non-toxic while ethyl acetate extract showed low toxic effects on brine shrimp. These results concur with those of Augustin *et al.*, (2015), in which he reported that aqueous extract was non-toxic in mice and rats. Therefore the two plants are considered safe.

Conclusion

Brine shrimp toxicity testing confirmed that, the two plants can be considered safe for use as medicine. Brine shrimp toxicity study lays a foundation for ethnobotanical and pharmacological investigations for new drug discovery.

Recommendation

A. caulirhiza and *S. princeae* plants may be used in treating puerperal sepsis as they can be considered safe. The two plants may be considered safe for medicinal use and can be a potent source of complementary and modern medicine. Further studies are recommended on genotoxicity of this plant extracts on *S. aureus* and *E. coli* genes.

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