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Medicinal potential of *Commiphora sponthulata, Hugonia casteneifolia* and *Raphidiocystis chrysocoma* indigenous to Tanzania

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Abstract: Herbal medicines constitute a potentially important resource for new and safe drugs for the management of microbial infections and other diseases. This study aimed at examining whether or not crude plant extracts of *Hugonia casteneifolia*, *Commiphora sponthulata* and *Raphidiocystis chrysocoma* have antimicrobial activities on some pathogenic bacterial isolated from sick domesticated animals. Agar diffusion technique was used to determine the inhibition of microbial growth and broth dilution technique was used to determine MIC and MBC. The study has shown that *R. chrysocoma* extract is very potent against *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli*, *H. casteneifolia* is potent against *S. agalactiae* and *S. aureus*, and *C. sponthulata* extract is potent against *Salmonella gallinarum* only. All the three plant extracts are non-cytotoxic. This is the first scientific evaluation of the veterinary medicinal potential of the *H casteneifolia, C. sponthulata* and *R. chrysocoma* from Tanzania, providing the baseline for further investigations towards discovering new drugs.

Keywords: Antimicrobial activity, *Commiphora sponthulata*, *Hugonia casteneifolia*, *Raphidiocystis chrysocoma*, Tanzania, veterinary.

INTRODUCTION

Unlike countries such as China, India and Korea, Tanzania has minimal or non-existent authentic records of its pre-colonial utilization of medicinal plants. Despite this, Tanzania has a reputation in the use of

medicinal plants in traditional medicine, which dates back to time immemorial[1]. The minimal information available, however, keeps on disappearing with the deaths of traditional healers who are invariably tradition bound to secrecy. Parallel to this, most of the plant species of medicinal and aromatic value are constantly threatened as a result of industrialization, villagelization and other exploitations. There is a need to pinpoint such potential plants and hopefully, in so encourage the governmental authorities and human community as a whole to conserve the plants.

Commiphora spanthulata Mattick belongs to the family BURSERACEAE from which several plant species exhibits medicinal potential[2, 3]. The genus *Commiphora* has for many years been medically known in Tanzania as dressing for wounds, for chest diseases, for toothache by the Chaga[3]. *Commiphora spanthulata* Mattick is a spiny tree 4 - 8 m tall with a well-defined cylindrical trunk. This species is commonly distribution in area with altitude 550 - 1100 m and rainfall 450 - 600 mm. However, there is no literature available on any member of the genus to have been tested against microbial isolates of veterinary importance.

Raphidiocystis chrysocoma (Schumach.) C. Jeffrey belongs to the family CUCURBITACEAE. It is a perennial climber up to 6 m with herbaceaus stem. The plant species commonly inhabits in lowland rain forests with altitude between 300 - 1370 m. Literatures available do not report on any medicinal value of this plant species.

Hugonia casteneifolia Engl belongs to the family LINACEAE. There have been reports on few species in this family that exhibit medicinal potential[2]. They include *Ochothocosmus candidus* Hallier f. used in the treatment of colds, chronic cough, whooping cough and inflammable eyes. *H. casteneifolia* Engl is a small tree, scandent shrub or liane 2 - 7 m high. The species is commonly found in dry evergreen or riverine forests persisting in secondary bushland, and altitude between 7 and 150 m. In Tanzanian *H. casteneifolia* is found in Usambara mountains (Tanga region), eastern slop of Udzungwa mountains in Morogoro region, Pugu forest in Coastal region and Pangajuu in Zanziba[4]. In East Africa the plant is used as remedy for intestinal worms, malaria and fevers[3, 4]. Some hugononediterpenoids have been isolated from the root back of this plant. The hugonones are related to mytenone, which is a bis-nor-deterpene that was isolated from Mytenus dispermum[5]. The rootbark extract of *H. casteneifolia* exhibits antifungal and anti mosquito larvae[5] and strong brine shrimp toxicity and hugorosenone has been reported to be the active principle[6]. However, there is no documented information about antibacterial activity tests on *H. casteneifolia* against microorganisms of veterinary importance.

As an effort to contribute to the world-wide interest in searching for new antimicrobial agents from higher plants, this study aimed at examining if crude extracts of *Hugonia casteneifolia* (LINACEAE), *Commiphora sponthulata* (BURSERACEAE), *Raphidiocystis chrysocoma* (CUCURBITACEAE) have antimicrobial activities on some of the pathogenic microorganisms isolated from sick domesticated animals. The study has shown that plant extracts are potent against some bacterial isolates of veterinary importance. This is the first scientific report on the potential of these indigenous plants as medicines against bacterial infections in veterinary. The plants should be protected against overexploitation, and further studies towards developing new medicines should be carried out.

EXPERIMENTAL

Plant material: The *H. casteneifolia*, *C. sponthulata* and *R. chrysocoma* crude extracts were supplied by Prof M. Nkunya's research group in the Department of Chemistry of the University of Dar es salaam (UDSM), and the extracts were kept in AMU/MBB UDSM laboratories refrigerators at -4 ^{o}C

for 13 years. The known concentration of the crude extract was tested for *in vitro* activity against microbial pathogens isolated from sick domesticated animals.

Isolation and culturing of the test microorganisms: The diagnosis of microbial infections in sick domestic animals, specimen taking, isolation and identification of the microorganisms were done in the laboratory of the Department of Veterinary Microbiology and Parasitology of Sokoine University of Agriculture. Bacteria *Streptococcus agalactiae*, *Staphylococcus aureus*, *Salmonella gallinarum* and *Escherichia coli* isolates were used. The media used were as follows: *S. gallinarum* and *E. coli* each onto MacConkey agar (MA) Petri-dishes, *S. aureus* and *S. agalactiae* each streaked onto sterile nutrient agar (NA) and blood agar (BA) petri-dishes. The culture media were prepared according to manufacture's specifications in slunts or culture plates. The plates were incubated, and observed for growth and purity after the 24 h at 37°C. The observation was recorded, and the positive result plates were stored at 4°C. Sub-culturing of pure strains was done at UDSM microbiology laboratory monthly, with the aim to maintain the strains.

Preparations of the pathogen inocula: Pure cultures of *S. aureus*, *S. gallinarum* and *E. coli* were grown on sterile NA slant, *S. agalactiae* was maintained on BA slant at 37°C for 24 h. A loopful from the aforementioned pure culture slant was inoculated into 100 ml of sterile broth medium in 200-volumetric flask, which was then incubated at 37°C, for 24 h for the bacteria or for 120 h for the dermatophytic fungi in a shaking incubator. The broth culture was diluted to make an inoculum of approximately 10^5 C.F.U/ml. Determination of the number of cells in the broth cultures was done by the viable cell count method according to Grigorova and Norris[7].

Antimicrobial activity assays: Both the agar diffusion and broth (or tube) dilution techniques were used to test for the antimicrobial activity of the plant extracts as described by Lyantagaye and Magingo[8]. The agar diffusion technique was used to determine zones of inhibition of the microbial growth; both the Agar Well Method (AWM) and Disc Method (DM) were used. The broth dilution was used to determine minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC).

C**ytotoxicity test: T**he presence and levels of toxicity of the active plant crude extracts were determined by the Brine Shrimp Test (BST) method described by Lyantagaye and Magingo[8] and Meyer *et al.*[9] , by which a crude extract is considered to be cytotoxic if it kills at concentrations less or equal to 240 µg/ml.

RESULTS AND DISCUSSION

The results (**Tables-1to 3)** provide evidence of the efficacy of extracts from these plants to treat bacterial infections. *R. chrysocoma* extract has been shown to be very potent against Gram-positive bacteria *S. agalactiae* (MIC 57 µg/ml), *S. aureus* (MIC 60.5 µg/ml) and Gram-negative bacteria *E. coli* (MIC 31.2 µg/ml). It is also worthy noting that another Gram-negative bacteria *S. gallinarum* is resistant to *R. chrysocoma* extract. The results also show that *H. casteneifolia* is potent against the Gram-positive *S. agalactiae* (MIC 74.5 µg/ml) and *S. aureus* (MIC 62.5 µg/ml). The results also show that *C. sponthulata* extract activity is very selective towards *S. gallinarum* only (MIC 250 µg/ml). The extracts did not show any toxicity against the brine shrimps *Artemia* naupli **(Table 4).**

This study aimed at examining if crude plant extracts of *H. casteneifolia*, *C. sponthulata* and *R. chrysocoma* have antimicrobial activities on some pathogenic bacterial isolated from sick domesticated animals. The results (**Tables 1to 3**) provide evidence of the efficacy of extracts from these plants to treat bacterial infections. The observed resistance of *S. gallinarum* to *R. chrysocoma* extract treatment suggests that whatever type of active compound in the extract has no receptors in *S. gallinarum* cell. The

observation that *H. casteneifolia* is potent only against the Gram-positive *S. agalactiae* (MIC 74.5 µg/ml) and *S. aureus* (MIC 62.5 µg/ml) may suggest that one among the factors contributing to the activity of the plant extract has to do with the structure and interaction in the bacterial cell membrane. The results also show that *C. sponthulata* extract activity is very selective towards *S. gallinarum* only (MIC 250 µg/ml). The plants have never before been reported to have antimicrobial activity against the tested bacterial strains of veterinary importance, hence no literature available to compare.

Table-1: Agar Well Method (AWM) results showing the growth inhibition zones ± SD of plant crude extracts against four different bacterial strains of veterinary importance obtained by the Agar Well Method. n = 10.

Plant species	Plant part	Sample code	Zone of microbial growth inhibition			
			S.agalactiae	*S.aureus*	*S.gallinarum*	*E.coli*
C.spanthulata	Stem	23	0	0	7.90 ± 1.45	0
R.chrysocoma	fruit	24	16.20 ± 1.03	20.20 ± 0.79	0	22.60 ± 1.43
H.casteneifolia	leef	32	15.40 ± 1.35	12.80 ± 1.94	0	0

Table-2: Disc (on agar) Method (DM) results showing the growth inhibition zones ± SD of plant crude extracts against four different bacterial strains of veterinary importance obtained by the Disc Method. n = 10.

Plant species	Plant part	Sample code	Zone of microbial growth inhibition			
			S.agalactiae	*S.aureus*	*S.gallinarum*	*E.coli*
C.spanthulata	Stem	23	0	0	4.30 ± 0.86	0
R.chrysocoma	fruit	24	14.10 ± 1.27	18.10 ± 0.96	0	20.20 ± 0.91
H.casteneifolia	leef	32	12.60 ± 0.85	10.30 ± 1.02	0	0

Table-3: Broth Dilution Method (BM) results showing the MIC and MBC values of some plant crude extracts, in µg/ml, against bacterial isolates of veterinary importance. Each assay was done ten times.

Plant species	Plant part	Sample code	*S.agalactiae*		*S.aureus*		*S.gallinarum*		*E.coli*	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
C.spanthulata	Stem	23					250	500		
R.chrysocoma	fruit	24	57	86.5	60.5	62.5			31.2	125
H.casteneifolia	stem	32	74.5	125	62.5	250				

Table-4: The Brine Shrimp Test (BST) results showing the cytotoxicity of the plant crude extracts to *Artemiasalina* larvae, and their IC_{50} at 24 h treatment.

Plant species	Plant part	Sample code	% Deaths at 24 h for different concentrations (µg/ml)						
			8	**24**	**40**	**80**	**120**	**240**	**IC_{50}**
C.spanthulata	Stem	23	0	0	0	50	70	90	75
R.chrysocoma	fruit	24	0	0	0	0	10	20	NT
H.casteneifolia	stem	32	0	0	0	5	20	30	NT

NT = non toxic.

The observed antimicrobial activity of these crude extracts could be attributed to multiple compounds interacting synergistically, antagonistically, and additively, etc.[10]. The study findings provide promising leads for the isolation of antimicrobial compounds; and are therefore worth investigating further using bioassay guided fractionation.

The observed lower activity in DM **(Table 1)** as compared to AWM (**Table 2)** implies that sensitivity of a test method is also proportional to the amount of active agent(s) applied to the agar[11]. Holding capacity of a 7 mm diameter disc of Whatman filter paper could not equal the amount of extract 50 µl loaded in the agar well. The lower disc capacity the less the amount of the antimicrobials available to diffuse through inoculated agar media, hence smaller zones of inhibition[12].

The absence of toxicity against the brine shrimps *A. salina* (**Table 4)** suggests that the extracts of the three plants are fairly selective for infective organisms. *A. salina* has been suggested for use as a model organism for several preliminary evaluations of pharmacological and ecotoxicological activities of compounds before[13, 14]. Intuitively, it is therefore tempting to suggest that their use may not cause serious side effects on the host body cells[14- 16]. However, earlier investigations[6, 9] reported the presence strong anti mosquito larvae and strong brine shrimp toxicity in *H. casteneifolia* rootbark extracts, attributed to the presence of hugorosenone. This shows that different anatomical parts of a plant may contain different bioactive compounds.

CONCLUSION

In conclusion the study reveals a promising medicinal potential for possible therapeutic use of the three plants against pathogenic bacteria. However, further studies should be done to ascertain their potential for their clinical application. Such potential plants should be protected in their natural environments, and if possible be propagated in botanical gardens to avoid their disappearances.

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