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IN VITRO ANTIBACTERIAL ACTIVITES, SAFETY STUDIES AND PHYTOCHEMICAL SCREENING OF DREGEA SCHIMPERI CLARK (ASCLEPIADACEAE) EXTRACTS

Onyancha Jared M.[1,2*], Wakori E.W.T.[1], Moriasi Gervason A.[3], Waiganjo Bibianne W.[4], Kisengi John M.[2], Arara Lameck N.[1] and Ng'etich Japhet K.[5]

[1]Mount Kenya University, School of Pharmacy, Department of Pharmaceutics and Pharmacognosy, P.O Box 342-01000, Thika, Kenya.

[2]Kenya Methodist University, School of Medicine and Health Sciences, Department of Pharmacy, P.O Box 267-60200, Meru, Kenya.

[3]Mount Kenya University, School of Medicine, Department of Medical biochemistry, P.O Box 342-01000, Thika, Kenya.

[4]Mount Kenya University, Department of Research Grants and Endowments, P.O Box 342-01000, Thika, Kenya.

[5]Kenya Medical Research Institute, Centre for Traditional Medicine and Drug Research P.O Box 54840 00200, Nairobi, Kenya.

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***Corresponding Author**
Onyancha Jared M.
Mount Kenya University, School of Pharmacy, Department of Pharmaceutics and Pharmacognosy, P.O Box 342-01000, Thika, Kenya.

ABSTRACT

Plants synthesize phytochemical compounds for protection against environmental stress and diseases. Some of the phytochemicals are used for curative and preventive medicine. Over 90% plant materials are used in traditional medicine to treat diseases in human and veterinary diseases. Since a great percentage of plant materials used lack sufficient scientific data to back their healing claims, the current study focused on the evaluation of antimicrobial, cytotoxic and phytochemical properties of methanolic and water extracts of *Dregea schimpheri* leaves and husks. *D. schimperi* has many ethnomedical claims including the management of infectious diseases. Disc diffusion method was used to assay for antimicrobial activities of the methanolic and water extracts of leaves and husks against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus pumilus*, *Pseudomonas aerugnosa* and

Escherichia coli. Cytotoxicity of the extracts was determined by use of brine shrimp lethality test. Qualitative phytochemical screening of the husks and leaves was performed with standard phytochemical tests. Antibacterial results were tabulated as mean zone of inhibition ± SEM. LC_{50} values for brine shrimp lethality test were estimated using Graphed Prism Version 5 statistical software. Phytochemical screening observations were also tabulated. Most extracts exhibited antibacterial activity against *S. aureus*, *M. luteus*, *B. pumilus*, *E. coli* and *P. aurignosa*. Water and methanol husk extracts of *D. schimperi* were found to be cytotoxic (LC_{50} <100µg/ml) while all leaf extracts had moderate to low toxicity. Phytochemical screening revealed presence of alkaloids, phenols, tannins in both leaf and husk powders. Saponins and anthraquinones were present in leaf extracts but absent in the husks. The antibacterial and cytotoxic activities are attributed to the presence of these secondary metabolites. Further studies aimed at isolating the bioactive compounds with antibacterial and cytotoxic properties are recommended. In *vitro* and in *vivo* toxicity studies are also necessary.

KEYWORD: Disc diffusion, Zone of inhibition, Brine shrimp lethality test, LC_{50}.

INTRODUCTION

In spite of the fact that natural products research has been intensively conducted, it is still far from exhaustion. Scientists have been in the fore front of efforts to obtain bioactive compounds from medicinal plants, of which only approximately 15 % of medicinal plants have been investigated (WHO, 2013). Health of individuals and most communities in the world has been fostered by the crucial role of medicinal plants. The ultimate value of medicinal plants is attributed to the fact that they have secondary metabolites (phytochemicals) which either singly or in synergy confer their medicinal properties. These metabolites prompt physiological activities within the human body bringing about healing (WHO, 2013). A number of classes of phytochemicals elicit pharmacological actions in humans and impact preventive and/or curative effects. The major classes of phytochemicals that have antimicrobial effects are phenolics, alkaloids, lectins, polypeptides, polyacetylenes, terpenoids and essential oils (WHO, 2013; Ekhaise and Okoruwa, 2001). The Asclepiadaceae family has been reported for various biological activities including antibacterial, anti-inflammatory, antitumor, antioxidant among others (Zakaria *et al*., 2001; Venkatesh *et al*., 2003; Tatiya *et al*., 2010). Plants in this family are herbs, shrubs and rarely trees and are characterized by a milky or clear latex substance which they secrete. The family contains

about 250 genera whose members are spread in over 2000 species. These plants are distributed in tropical and subtropical regions, especially in Africa and Southern America. The genus *Dregea* synonymous to *Marsidenia* belongs to this family and consists of 200 to 300 species (Schmelzer and Gurib-Fakim, 2013).

Decoctions and infusions from root are used to treat problems of urine retention, constipation, infectious diseases, abdominal pain during pregnancy, relieving breast pain in women, and as aphrodisiac in men. Aerial part infusions have been used to treat snakebites (Ichikawa, 1987; Kokwaro, 2009; Schmelzer and Gurib-Fakim, 2013).

Dregea schimperi syn *Marsdenia schimperi,* it is a robust climber, 1-5 m tall. Leaves are broad- ovate to circular and merely-tomentose beneath. Inflorescences cymose and loose, stalked. Flowers can be white or yellow, corolla 8-12 mm. Fruits follicles (narrowly) ovoid, 6-8 by 2-4 cm, pods with numerous wrinkles but not winged. It occurs in dry forest margins, riverine woodland and bushland near forest edges (Agnew, 1994 and Beentje, 1994). Despite the numerous folkloric claims and traditional medicinal uses of *D. schimperi,* there is missing scientific data to validate these claims. This study was done to evaluate antibacterial potential of *D. schimperi* for use in the management of infectious bacterial strains. Evaluation of phytochemical classes and establishment of safety levels, using the brine shrimp lethality test were also done in this study.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh plant materials (aerial parts) of *Dregea schimperi* were collected at the entrance of The Ark gate of Aberdares National park, Mweiga-Nyeri. The voucher specimen (number Ms-JO-1-2013) was prepared in duplicate, labeled and deposited at Mount Kenya University Herbarium and also at the East Africa Herbarium at the National Museums of Kenya.

Preparation of extracts

The plant materials were air dried and ground into a moderately coarse-textured powder and extracted by organic solvents sequentially by maceration, starting with petroleum ether and then successively with dichloromethane, a mixture of dichloromethane: methanol (1:1) and methanol. The aqueous extract was prepared by hot maceration. Organic extracts were filtered and reduced *in vacuo,* and thereafter they were concentrated to dryness in the oven at 30 ^{O}C. The aqueous extracts were freeze dried, weighed and yield calculated. Extracts were

kept under refrigeration at 4 ^{O}C until use. Test extracts were prepared at a concentration of 100 mg/ml using DMSO as a solvent for antimicrobial assay and 1000 µg/ml for brine shrimp lethality tests.

Antimicrobial assay of *D. schimpheri* extracts

Test microorganisms

Master cultures of the microorganisms: *Staphylococcus aureus* (NTCC 07447), *Bacillus pumilus* (NTCCO8241), *Micrococcus luteus* (NTCC010716), *Escherichia coli* (ATCC10536) and *Pseudomonas aeruginosa* (NTCC.PF2275) were obtained from the National Quality Control Laboratory (NQCL) microbiology.

Preparation of culture media and culturing of microorganisms

Nutrient media for growth of the test microorganisms were prepared as per the manufacturer's instructions, sterilized and left to cool at 50 ^{O}C. Each of the subcultured microorganism was suspended in 5 ml of sterilized distilled water and 1.5 ml of the suspension inoculated into 150 ml growth media so as to produce inoculated agar with approximately 1 x 10^{6} colony forming units per ml. The inoculated nutrient media were then rapidly but carefully poured into six petri dishes using a 20 ml measuring cylinder in such a manner as to deliver 20 ml of inoculated agar with uniform thickness of 3 mm in each petri dish. The layered agar was allowed to cool so as to set into a firm gel suitable for plating out (Mwitari *et al*., 2013).

Disc diffusion technique

A disc diffusion method was used to evaluate antimicrobial activity levels. A symmetric paper template with six circles drawn in a hexagonal array was used to aid in punching six cylindrical wells into the layered media using an improvised cork borer of 6 mm diameter. The 12 test extracts together with the positive and negative controls were applied into the wells using a fixed volume micropipette set to deliver 20 µl per well. Dimethyl sulfoxide (DMSO) was used as a negative control for the extracts while gentamicin (0.32 mg/ml) was used as a positive control for antibacterial activity. Each application was done in triplicate for quality control. A pre-diffusion period of one hour was allowed to facilitate diffusion of the applied solutions into the inoculated media before the petri dishes were incubated for 18 h at 37 ^{O}C. The diameters of the zones of inhibition were then measured using an electronic digital caliper and captured by photography (Mwitari *et al*., 2013).

The brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence of cytotoxic effects in the crude extracts of *Dregea schimpheri*. Solutions of the extracts were made in DMSO at concentration of less than 1µl/ml, a volume of 5 ml brine salt solution at concentrations of 1000µg/ml, 100µg/ml, 10µg/ml, 1µg/ml and 0µg/ml in triplicates was used to incubate the nauplii for 24 h. Ten brine shrimp larvae were placed in each of the triplicate vials. After 24 h, dead nauplii were examined against a lighted background and the average number of the surviving nauplii in each test tube was determined and recorded.

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites. The investigated phytochemicals were alkaloids (Mayer's and Drangedorff's reagents), cardiac glycosides (Kedde and Keller-Killian tests), saponin glycosides (frothing and haemolytic tests), anthracene glycosides (Borntrager's test for combined and free anthraquinones), phenols (ferric chloride test) and tannins (Soni and Sheetal, 2013; Evans, 2009).

Data analysis

Antibacterial activity results were presented as mean ± SEM of zones of inhibition of three replicates and then compared with that of the standard by calculation of activity index. LC_{50} values of brine shrimp lethality test were estimated using Graphed Prism Version 5 statistical software.

RESULTS

Extraction using different solvents resulted to different percentage yields, the mixture of dichloromethane/methanol (50%) realized the highest yield for leaves (5.23%), petroleum ether, dichloromethane, methanol sequential (extract) and methanol (direct extract) had percentage yields of 1.1, 1.2, 1.3 and 3.5 respectively. Similary, the husk extracts had varied extraction values, dichloromethane, petroleum ether, methanol (sequential extract), methanol (direct extract) and mixture of dichloromethane/methanol (50%) percentage yields recorded as 6.8, 6.6, 6, 4 and 3.7 respectively.

Antimicrobial activity

Most extracts in this study demonstrated varied antibacterial activity ranges against *S. aureus*, *M. luteus*, *B. pumilus*, *E. coli* and *P. aurignosa*. Dichloromethane and a mixture of

dichloromethane/methanol (50:50) extracts of *D. schimperi* leaf extracts were active against *P. aurignosa* (Table1). Methanol extract of *D. schimperi* husk was active against *P. aurignosa. S. aureus* and *Bacillus pumilus*. *E. coli* exhibited resistance to seven of the twelve extracts and showed sensitivity to five extracts (Table 1). Water extracts of *D. schimperi* leaf were inactive against all the tested bacteria strains while water extract of the husk was observed to be active (Table 1).

Table 1: Antibacterial activity of *Dregea schimpheri* extracts

Test solution Extracts (100 mg/ml)	**Plant part**	**Zones of inhibition (*mm) ±SEM**				
		S. aureus	***M. luteus***	***B. pumilus***	***E. coli***	***P. aurignosa***
Petroleum ether	Husk	9.3±0.3	8.5±0.5	8.3±0.3	8.2±0.1	8.2±0.4
	Leaf	10.4±0.0	10.4±0.1	10.2±0.3	9.4±0.4	10.2±0.1
Dichloromethane	Husk	9.3±0.1	9.3±0.3	9.1±0.1	8.3±0.1	10.7±0.1
	Leaf	10.4±0.1	10.6±0.1	10.4±0.0	10.3±0.5	14.0±0.1
Dichloromethane/methanol	Husk	9.8±0.2	10.7±0.1	9.4±0.4	9.4±0.2	11.1±0.1
	Leaf	10.3±0.3	10.8±0.1	11.3±0.3	10.7±0.01	13.5±0.3
Methanol (sequential extract)	Husk	9.5±0.1	8.3±0.3	10.1±0.1	8.4±0.3	12.7±0.3
	Leaf	9.9±0.0	10.1±0.1	10.3±0.5	8.3±0.0	10.6±0.5
Methanol (direct extract)	Husk	9.7±0.0	8.4±0.1	10.0±0.1	8.5±0.4	13.3±0.2
	Leaf	11.0±0.1	10.9±0.0	10.5±0.3	8.0±0.1	10.8±0.3
Water	Husk	11.1±0.1	9.7±0.3	11.1±0.2	10.0±0.1	12.2±0.6
	Leaf	8.8±0.3	9.2±0.0	9.2±0.0	8.4±0.1	8.5±0.4
Gentamycin (0.32 mg/ml)		22.5±0.1	22.8±0.7	25.9±0.2	23.4±0.6	30.9±0.1
DMSO		8.1±0.1	8.0±0.2	8.3±0.5	8.0±0.1	8.3±0.2

*mm-Mean diameter zones of inhibition in mm, SEM- Standard error of the mean, DMSO-Dimethyl sulfoxide

Brine shrimp lethality

Cytotoxicity studies of *D. schimperi* extracts against brine shrimp exhibited varied LC_{50} values, dichloromethane and methanol husk extracts were the most cytotoxic with LC_{50} value of 52.1 µg/ml. It was however noted that the other husk extracts were not cytotoxic, as shown in Table 2. All extracts of *D. schimperi* leaves were cytotoxic.

Table 2: LC_{50} values (µg/ml) of *Dregea schimperi* extracts

Extract	**LC_{50} values of plant parts**	
	Husks	**Aerial**
Petroleum ether	>1000 µg/ml	886.2 µg /ml
Dichloromethane	52.1 µg/ml	610.6 µg/ml
Dichloromethane/methanol	>1000 µg/ml	537.0 µg /ml
Methanol (sequential extract)	>1000 µg/ml	321.8 µg /ml
Methanol (direct extract)	52.1 µg/ml	602.1 µg /ml

Qualitative phytochemical analysis

Phytochemical screening revealed varied classes of secondary metabolites in both the husks and leaves. Alkaloids, compounds with deoxy sugars and lactone rings, tannins and phenols were present in both the leaves and husk, however anthraquinones and saponins were present in leaves and absent in the husks as shown in Table 3.

Table 3: Qualitative results for phytochemical tests of various parts of *Dregea schimperi*

Phytochemicals	Part of the plant	
	husks	leaves
Alkaloids		
Mayer's test	+	+
Drangendorff test	+	+
Glycosides		
Borntragers test	-	+
Modified Borntrager's test	-	+
Keller-killian test	+	+
Kedde test	+	+
Saponins	-	+
Tannins	+	+
Phenols	+	+

Key: absent (-); present (+)

DISCUSSION

The current finding (*in vitro* antibacterial and cytotoxic activities) and detection of classes of secondary metabolites are reported for the first time. However, some plants from the genus *Dregea* have been found to have good antibacterial activity, these plants include organic extracts of *Dregea volubilis* leaves (Venkatesan and Anton, 2013; Purushoth *et al*., 2013). The antibacterial activity of the water and organic extracts were evaluated and their potency assigned according to inhibition zone diameter as follows; no activity (<7 mm), active (8–11 mm) and very active (>12mm) respectively, according to Mwitari *et al.*, (2013). Antibacterial activity of the twelve extracts from *D. schimperi* husks and leaves were equally active against both Gram positive and Gram negative bacteria, it is indicative that antibacterial activity is not due to the bacterial cell wall properties.

Cytotoxicity studies indicated that dichloromethane and methanol (direct) extracts of the husk were toxic with LC_{50} < 100 µg/ml. All other husk extracts were non-toxic (LC_{50} > 1000 µg/ml). All leave extracts were of moderate to low toxicity. Phytochemical screening reported the chemical class of phytochemicals that are known to have antimicrobial and toxic properties. The antimicrobial and cytotoxic activities of this plant is attributable to the

presence of the alkaloids, saponins and phenolics class of phytochemicals that were detected in the current study (Harvey *et al.*, 2000; Newman *et al.*, 2007).

CONCLUSION

The findings of this study provide scientific data for validation of the ethnomedical uses of *D. schimperi* extracts. It is used in management of infectious diseases, wounds including eczema, snakebite among other ailments. The fact that most of the extracts were active against the tested microorganisms, provides baseline for recommending this plant for its use in various ethnic groups for management of infectious diseases and related conditions. A number of extracts demonstrated safety toward brine shrimp nauplii, this is also preliminarily indicative that the extracts are safe when used for short periods. Activities of the extracts are due to the present groups of phytochemicals that were detected. The researchers from this study advocate for further work on bioassay-guided isolation, purification and characterization of the active compounds of *Dregea schimperi*. Additionally, elucidation of the possible mechanism(s) of action as well as other *in vitro* and *in vivo* toxicity studies of the plant extracts should be determined.

Conflict of interest

The authors declare that there are no conflicts of interest.

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