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Research Article

**ANTIBACTERIAL ACTIVITY OF *Murraya koenigii* AGAINST
FEW *Staphylococcus* spp. AND DEVELOPMENT OF A
TOPICAL CREAM****Gouri Kumar Dash*, Mahendran Sekar, Syarifah Syamimi Putri Adiba and Afzan
Mahmad**Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of
Medicine Perak, 30450 Ipoh, Malaysia**Abstract:**

Traditional medicinal plants often offer suitable drug candidates as an alternative to antibiotics in the treatment of antibiotic-resistant bacteria. Plant extracts may be formulated to a suitable dosage form to treat the disease in an effective way. In the present study, we report the antibacterial activities of the ethanol extract of *M. koenigii* leaves against few selected *Staphylococcus* spp. such as *S. aureus* and *S. epidermidis*, methicillin resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE) together with formulation and evaluation of a topical cream containing *M. koenigii* extract. The antibacterial activity of the extract was performed by disc diffusion assay using vancomycin (30 µg) as the standard. The extract revealed good antibacterial activity against tested organisms in a concentration dependant manner. The cream was formulated by incorporating 10% w/w of the ethanol extract in the base. Physical evaluation of the cream revealed ideal characteristics of an ideal cream. Further studies may be recommended to isolate the bioactive principles from the leaves to develop newer drug moiety against MRSA and MRSE.

Keywords: *Murraya koenigii* L. Spreng, Antibacterial, MRSA, MRSE, Topical cream**Corresponding Author:****Dr. Gouri Kumar Dash,**

Professor and Head (Research and Post Graduate Studies),

Faculty of Pharmacy and Health Sciences,

Universiti Kuala Lumpur Royal College of Medicine Perak,

30450 Ipoh, Perak, Malaysia.

Tel: 0060105491614 (Mobile)**Fax:** 00605-2536634**Email:** gkdash2@gmail.com, gourikumar@unikl.edu.my

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INTRODUCTION:

Staphylococci are clustering Gram-positive non-spore forming cocci, often responsible for several diseases. One of the significant classification of *Staphylococci* depends upon their ability to produce coagulase, an enzyme responsible for blood clot formation. Two general classes that classified this species are coagulase-positive Staph (CoPS) such as *Staphylococcus aureus* and coagulase-negative Staph (CoNS) such as *Staphylococcus epidermidis* [1]. Most nosocomial infections by *S. aureus* and *S. epidermidis* have gained considerable attention due to an increase of infections caused by these strains those in recent years throughout the world [2].

According to World Health Organization (WHO), 80% of *Staphylococcus aureus* infections in some African regions are methicillin resistant (MRSA) and standard antibiotics are not effective for the treatment [3]. Prevalence of methicillin resistant *Staphylococcus epidermidis* (MRSE) has been noticed in the hospital environment and in a community [4]. Several first and second line antibiotics are rapidly becoming ineffective for treatment due to emergence of resistance. Therefore, alternative efforts are necessary to discover newer drug moiety in order to combat threat of microbial resistance to antibiotics. In light of the above growing concerns, attention is being given to the herbal medicines that may serve as autonomous antibacterial agents. Several plants used in traditional medicine have been reported to possess significant anti-MRSA activity due to presence of unique phytochemicals that they contain [5].

Murraya koenigii L. Spreng (Family- Rutaceae) commonly known as 'Curry leaves plant', the leaves of which are widely used as a condiment for cooking in Malaysia and other Asian countries. It is also one of the traditional folk remedies that contains several interesting bioactive compounds with health-promoting properties including its antibacterial activity due to the presence of high total phenolic content [6]. Previous reports on the antimicrobial activities of *M. koenigii* reveals its promising activity on several microorganisms such as *Bacillus subtilis*, *S. aureus*, *Mycobacterium bovis*, *Escherichia coli*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Streptococcus pyogenes* [7-10]. However, there are no reports in the literature on the antibacterial activity of the leaves against *S. epidermidis*, MRSA and MRSE.

In the present paper we report the antibacterial activities of the ethanol extract of the leaves against *Staphylococcus spp.* including MRSA and MRSE together with formulation and evaluation of a topical cream containing *M. koenigii* extract.

MATERIALS AND METHODS:

Plant material

Fresh leaves were collected from herbs garden around Universiti Kuala Lumpur Royal College of Medicine Perak, Tasek campus. The preserved specimens and photographs were authenticated by the botanists of Agriculture Institute of Bumbong Lima, Kepala Batas. A voucher specimen of the herbarium is preserved in our department for future reference. The collected samples were cleaned, shade dried and pulverised to coarse powder.

Bacterial strains

The *Staphylococcus spp.* used for the test were *S. aureus* (ATCC 9144), *S. epidermidis* (ATCC 12228), methicillin resistant *S. aureus* (MRSA) (ATCC 33592) and methicillin resistant *S. epidermidis* (MRSE) respectively. All the stock cultures were obtained from the Microbiology lab of Universiti Kuala Lumpur Royal College of Medicine Perak.

Extraction

About 100 g of dried leaf powder was soaked in 500 ml ethanol in round bottom flask. The mixture was boiled under reflux for 2 h to allow the components to be extracted. The final liquid extract was filtered through Whatman filter paper No.1. Following filtration, the liquid extract was concentrated to yield dried residue. The dried extract was stored in a refrigerator until further use.

Screening for Antibacterial Activity

The extract was screened for antibacterial activity by disc diffusion method using *S. aureus*, *S. epidermidis*, MRSE and MRSA as the test organisms. Actively growing log phase cultures were streaked on to sterile agar plates containing sterile culture medium. Ready-made sterilized discs of size 6 mm were used, each having maximum capacity of 30 μ l. The extract was loaded on the sterile discs at concentrations of 100 μ g/ml and 200 μ g/ml respectively. A standard disc of the antibiotic vancomycin (30 μ g) was used as positive control and the solvent as negative control [11]. The plates were incubated for 24 h at 35°C and the diameters of inhibition of zones were measured. The zones of inhibition referred to the clear zones surrounding the discs. The experiment was done in triplicate and the mean value of the zones of inhibition was taken as the final reading.

Formulation of Cream

An oil-in-water (O/W) emulsion based cream was formulated (Fig.1). The formulation components are listed in Table 1. For the preparation of the cream, the components such as stearic acid, cetyl

alcohol and liquid paraffin were taken in a porcelain dish and melted at 70 °C. The other components such as glycerine, triethanolamine, and distilled water were taken in another container and heated the mixture to 70 °C. The aqueous phase was added to the oil phase in a mortar and triturated continuously until a smooth cream was produced. The extract of *M. koenigii* was incorporated in the cream base at a concentration of 10% w/w.



Fig. 1: Formulation of cream containing *M. koenigii* leaf extract

Table 1: Formula for oil-in-water (O/W) emulsion base

Ingredients	Quantity
Stearic acid	11 g
Cetyl alcohol	4 g
Liquid paraffin	4 g
Glycerine	4 g
Triethanolamine	2 g
Distilled water	75ml

Evaluation of the Cream

The formulated cream was evaluated for various physical parameters such as determination of type of cream, organoleptic properties, pH, viscosity, homogeneity, after feel, removal, irritancy test etc.

Organoleptic properties

The appearance of the cream was judged by its color, odour, opacity and roughness [12].

Type of cream

The formulated cream was applied on skin and then washed under tap water with minimal force to remove the cream.

pH

About 0.5 g of the cream was weighed accurately in a 100 ml beaker and dispersed in 45 ml of water. The pH of the dispersion was measured at 27 °C using the calibrated pH meter [13].

Homogeneity

The test was done by physical touch with hands and rubbing between two fingers to check presence of gritty particles [12].

After feel

After applying the herbal cream on skin the properties like emollient nature, slipperiness and the amount of cream left after applying to the skin was checked [12, 14].

Removal

The removal of the cream applied on skin was done by washing under tap water with minimal force to remove the cream [12].

Irritancy test

The cream was applied on left hand dorsal side surface of 1 cm² and observed in equal intervals up to 24 h for irritancy, redness and edema if any [12].

Stability studies

The formulated cream was stored at different temperatures such as 8 °C ± 0.1°C in a refrigerator and at 25°C ± 1°C and 40 °C ± 1 °C in incubators for 4 weeks. The above parameters were again studied for the formulation after the incubation period [13].

RESULTS AND DISCUSSION:

Antibacterial activity

The results of antibacterial activity of the ethanol extract of the leaves of *M. koenigii* against *S. aureus*, *S. epidermidis*, MRSE and MRSA are presented in Table 2. The results of the study showed that the extract is effective in all tested strains of *Staphylococcus* in a concentration dependant manner. The study further reveals that the active components responsible for such activity may serve as better candidates for treating *Staphylococcus* infections because the leaves of *M. koenigii* are edible and the antimicrobial components may be free from toxicity.

Table 2: Antibacterial activity of the ethanol extract of *M. koenigii* leaves

Treatment	Concentration	Zone of inhibition (mm)			
		SA	SE	MRSA	MRSE
Solvent	-	0.00	0.00	0.00	0.00
Vancomycin	30 µg/disc	17.80	16.40	13.60	15.51
<i>M. koenigii</i> extract	100 µg/ml	7.59	7.36	4.51	5.70
	200 µg/ml	8.68	8.80	6.60	7.60

SA= *Staphylococcus aureus*, SE= *Staphylococcus epidermidis*, MRSA= Methicillin-resistant *Staphylococcus aureus*, MRSE= Methicillin-resistant *Staphylococcus epidermidis*

Table 3: Evaluation of formulated cream

Parameter	Observations
Appearance	Semisolid
Colour	Pale green colour
Odour	Pleasant
Type of cream	Oil-in-water (O/W)
pH	7.6
Homogeneity	Homogenous, smooth and free from gritty particles
After feel	Emollient and no residue left
Removal	Easily removed with water
Irritancy test	No irritancy after 24 h of application
Spreadability	Good
Stability	Stable for 4 weeks

Evaluation of the Cream

The result of evaluation of the formulated cream is presented in Table 3. The overall evaluation report reveals an ideal topical formulation containing the extracts of *M. koenigii*. The formulation was non greasy, homogenous, non irritant, easily washable with a pleasant odour. All these physical properties remained unaltered when tested after 4 weeks of the stability study.

CONCLUSION:

The therapeutic effectiveness of medicinal plants rests on their unique phytochemicals. *M. koenigii* is a very popular plant due to its application as a condiment in cooking foods in the kitchen and restaurants. As reported earlier, the leaves are rich with phenolic components which are reported to possess promising antibacterial activity. In this study we evaluated the antibacterial activity of the ethanol extract of the leaves against selected *Staphylococcus* species using disc diffusion method. The results of the study revealed that the extract possess antibacterial activity against tested organisms. Further, we formulated and evaluated a topical cream containing *M. koenigii* extract. The physical properties of the formulated cream

revealed ideal characteristics of a cream. Further studies may be recommended to isolate the bioactive principles from the leaves to develop new drug candidates against MRSA and MRSE.

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