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Original Article

Physicochemical and antistaphylococcal evaluation of two herbal ointments from *Mikania micrantha* Kunth and *Tridax procumbens* Linn

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ABSTRACT

Objectves: This present work was carried out to formulate and evaluate herbal ointments using *Mikania micrantha* and *Tridax procumbens*. **Materials and Methods:** The extracts were prepared using maceration technique. A simple ointment base was prepared using white soft paraffin and liquid paraffin. The extracts were incorporated into the ointment base following levigation method. The amount of extract to be incorporated into the ointment base was determined according to their minimum inhibitory concentration (MIC) against *Staphylococcus aureus* MTCC 87. The formulated ointments were evaluated for several physicochemical characteristics like colour, odour, homogeneity, washability, spredability, irritation potential etc. Antistaphylococcal assay was conducted using well diffusion method. Ointment base containing white soft paraffin and liquid paraffin was used as negative control and Supragent containing Gentamicin sulphate was used as a positive control. **Results:** The formulated ointments showed satisfactory results for tested physicochemical parameters. Both ointments showed moderate antistaphylococcal activity with reference to the activity showed by Supragent. **Conclusion:** The results of this study indicate that the formulated ointments can be incorporated into India's medicine system for treating *Staphylococcus aureus* induced skin ailments in future.

Key words : *Mikania micrantha*, *Tridax procumbens*, Herbal ointment, Skin disease.

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INTRODUCTION

Skin infections considerably represent major global health issues despite being frequently ignored[1]. One of the most well-known human pathogen is *Staphylococcus aureus* because of its propensity to cause topical skin infections[2]. Health care professionals have faced difficulties to treat staphylococcal skin infections due to resistance of *Staphylococcus aureus* against widely used antibacterial medications[3]. For this reason, there is a growing urgency for discovery of new antibacterial drugs from medicinal plants. Herbs are an excellent source of both conventional and modern medicine[4]. They are able to produce secondary metabolites like alkaloids, phenols, flavonoids, terpenoids etc. These secondary metabolites has multidimensional uses in healthcare system[5]. It is an encouraging idea to administer herbal medications through the skin as it provides more surface area and is in close proximity to the circulatory as well as lymphatic system of the body. Ointments are one of the aspects of administering drugs through the skin. Typically, while formulation of an ointment, active ingredients with medicinal properties are mixed with the ointment base in a homogeneous manner. Ointment base serve as a vehicle for the medication. Ointment bases can be of different types depending on their intended use and type of active principle[6].

Mikania micrantha Kunth and *Tridax procumbens* Linn are invasive weeds that has been widely used to treat skin ailments[7-12] in traditional system of medicine. The plants belong to the family Asteraceae which is one of the largest Angiosperm family in the World. These two plants are very popular in treating complications related to wounds[11-12] and they are reported to have significant antibacterial activity against skin infection causing notorious bacterial strains[10,13]. According to our previous studies, ethanolic leaf extract of *Mikania* and methanolic whole plant extract of *Tridax* posses strong antibacterial potential against *Staphylococcus aureus*[14,15]. Since these two plants have various uses related to skin problems, the main aim of this study was to formulate herbal ointments using these two plants for topical application and evaluate its physicochemical, antistaphylococcal characteristics .

MATERIAL AND METHOD

Sample collection

Samples were collected from different parts of Paschim Medinipur. The collected samples were washed thoroughly with tap water to ensure that there was no dirt left. Samples were then dried under shade and precautions were taken to avoid direct exposure to sunlight. Shade dried samples were then ground into a very fine powder using electrical blender. The powdered samples were then stored in a zipper bag for future uses.

Authentication

The plants were identified by Botanical Survey of India, Howrah with voucher specimen number VU/AB/S-01 for *Mikania micrantha* Kunth and VU/AB/S-02 for *Tridax procumbens* Linn.

Extraction

Extraction was done following similar process used in our previous work[14,15] and it is shown is Table 1 .

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Table 1: Extraction process, used part and biological activity according to our previous study.

Name of the plant	Parts used	Solvent	Extraction method	Reported activity
Mikania micrantha Kunth	Leaves	Ethanol	Maceration	Antistaphylococcal[14]
Tridax procumbens Linn	Whole plant	Methanol	Maceration	Antistaphylococcal[15]

Formulation of herbal ointment

The ointments were formulated following slightly modified standard protocol[16]. Ointment base was prepared by heating white soft paraffin and liquid paraffin at 6:4 ratios. They were allowed to melt together in a melting pan at 70˚C. Semisolid extracts were mixed with the ointment base and stirred gently at 40˚C. Stirring of the mixture was continued until it became homogenous. Amount of extract to be incorporated into ointment base was decided according to minimum inhibitory concentration of the respective extracts which was determined in our previous works[14,15]. After that the mixture was cooled and allowed to solidify. It was stored in a container for further evaluation. Ointments produced from *Mikania* and *Tridax* were named as MM and TP respectively.

Physicochemical evaluation of herbal ointments

Following parameters were used to evaluate the formulated herbal ointments :

Colour and odour

Colour and odour of the formulated ointments were examined visually.

pH determination

1 gm of formulated ointment was dissolved in 50 ml of distilled water with continuous stirring and the pH was measured using digital pH meter.

Homogeneity

Homogeneity of the formulations was determined following standard protocols[17].

Spreadability test

The spreadability of ointments was determined using glass slide method[18]. About 0.5 gm of formulated ointment was placed between two slides and homogenous weight was given to the upper slide. Spreadability was measured as the time required separating two slides. The shorter the time required to separate, the greater the spreadability.

Stability

Stability of the ointments was determined at 2˚C, 25˚C and 37 ˚C for 28 days.

Solubility

Solubility of the ointments was checked with different solvents like hot water, water, ethanol, ethyl acetate and chloroform.

Washability

A small amount of ointment was applied to the skin and washed off with water to check the washability of the ointments.

Irritation test

Small amount of ointment was placed and rubbed into the skin to examine irritation potential.

Antistaphylococcal evaluation

The antistaphylococcal activity of the formulated ointments was assessed following agar well diffusion method[19]. Autoclaved Muller-Hilton agar was placed in a volume of 15-20 ml onto identical sized glass petriplates where it was left to solidify. Using a sterilised cork borer, four wells were carved into the Muller Hilton agar that had hardened. Using a sterile spreader, standardized inoculums of *Staphylococcus aureus* MTCC 87 was evenly distributed on the surface of agar plates. 0.2 gm of formulated ointment was melted in small beaker under aseptic conditions. Melted ointments were placed into their respective wells and allowed to solidify. Ointment base was used as a negative control and Supragent containing 0.1% Gentamicin sulphate was used as a positive control. The amount of positive and negative control was same as the formulated ointments. The petri-plates were incubated in a incubator for 24 h at 37ºC. After incubation the plates were observed and the zone of inhibition around the well was measured in mm.

RESULTS AND DISCUSSIONS

A significant step towards valuing medicinal plants to treat various disorders in the healthcare system is herbal formulation. Additionally, the most effective way to treat diseased skin topically is by using herbal ointments. In order to develop herbal ointments, the active components are added to the ointment base. In this current work, the ingredients used to prepare the herbal ointments are shown in the table 2. Formulated ointments also showed satisfactory results on the evaluated physicochemical parameters. Stability of the formulated ointments do not changed in various conditions like 2˚, 25˚and 37˚C which supports the prolonged use of an ointment. The result is displayed in the table 3.

The effectiveness of these herbal formulations against *S. aureus*, a significant representative of skin illness[2], was examined using well diffusion method. In terms of zone of inhibition (mm), the antistaphylococcal activities of prepared ointments were assessed. The results showed that *Staphylococcus aureus* was moderately susceptible to formulated ointments as compared to supragent (Table 4). Even

Table 2: Ingredients of formulated herbal ointments.

Name of the ingredient	Amount (gm)	
	MM (Ethanolic leaf extract)	TP (Methanolic whole plant extract)
White soft paraffin	12	12
Liquid paraffin	8	8
Crude extract	0.4	0.8

Table 3: physicochemical characteristics of formulated ointments.

physicochemical parameters	Observation	
	MM	TP
Colour	Dark brownish green	Light greenish
Odour	Characteristics	Characteristics
pH	7.2	7.1
Spreadability (Seconds)	6	5
Solubility	Soluble in hot water with stirring, Partially soluble in normal water, highly soluble in ethanol, chloroform, ethyl acetate with stirring	Soluble in hot water with stirring, Partially soluble in normal water, highly soluble in ethanol, chloroform, ethyl acetate with stirring
Washability	Good	Good
Stability	Stable at 2˚, 25˚and 37˚C.	Stable at 2˚, 25˚and 37˚C.
Irritation	Non irritant.	Non irritant.

Table 4: Antistaphylococcaal activities of formulated ointments.

Test Organism	Zone of Inhibition (MM ointment containing 2% ethanolic leaf extract of Mikania micrantha)	Zone of Inhibition (TP ointment containing 4% methanolic whole plant extract of Tridax procumbens)	Zone of Inhibition (Supragent containing 0.1% Gentamicin sulphate)	Zone of Inhibition (Ointment base)
***Staphylococcus aureus* MTCC 87**	14 mm	16 mm	18 mm	-

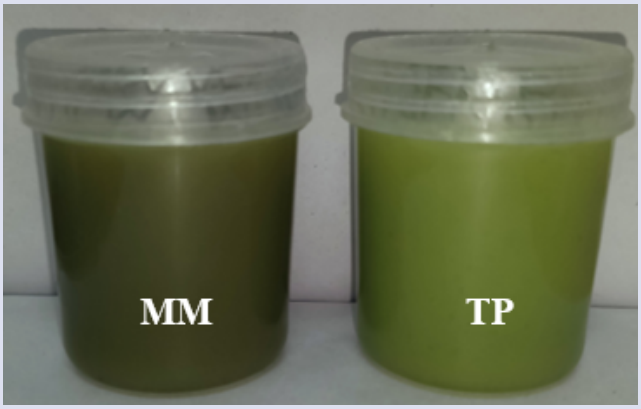

Figure 1. Formulated herbal ointments.

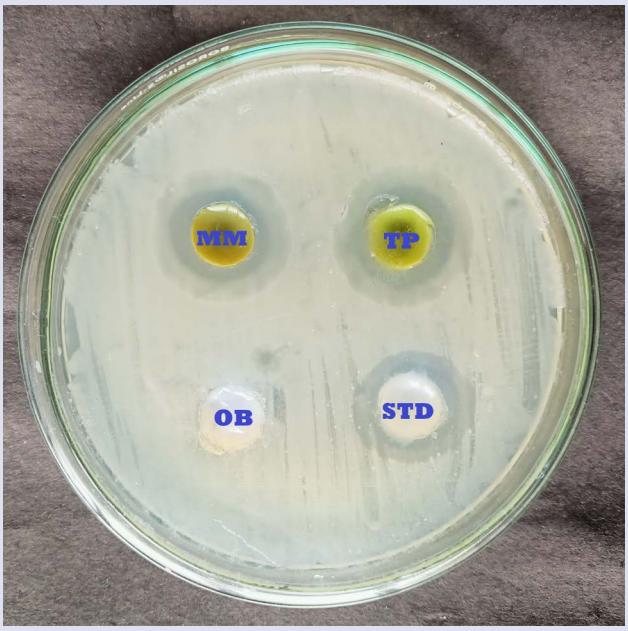

Figure 2. Antistaphylococcus activity of herbal formulations; MM - *Mikania micrantha* ointment, TP- *Tridax procumbens* ointment, STD - Standard drug Supragent, OB - Ointment base.

if the TP ointment gave slightly larger zone of inhibition than the MM ointment, the antistaphylococcal activity of the MM ointment will be considered higher because less amount of crude extract was incorporated into the ointment base during formation of the MM ointment than TP ointment. The effectiveness of the formulated herbal ointments is comparable to earlier studies where alcoholic extracts were used in the formulation[20-22]. This suggests that phytocompounds for herbal ointments may be extracted effectively using polar solvents. The effectiveness of herbal ointments may be attributable to the bioactive antibacterial compounds that are present in their respective extracts. Several antibacterial compounds are already reported to be present in the extracts of *Mikania micrantha* and *Tridax procumbens*[23,24,25]. Thus, we anticipate that both these formulations, with special emphasis to MM ointment, may be successful in addressing the pathophysiology of topical skin diseases. However, more research is required to evaluate the effectiveness of these formulations employing in vivo tests which might open the door to human clinical trials for the administration of topical medicine.

CONCLUSION

One of the critical organs that need special care to avoid infection from microorganisms is the human skin. The formulated herbal ointments inhibited *Staphylococcus aureus* which is one of the major concerns for skin related issues. physicochemical characteristics of the formulated ointments were satisfactory as it can spread quickly into the skin, easily washable, non irritant and stable at various conditions. So, it can be concluded that both herbal ointments are promising candidates for drug development in future. Furthermore, research in medicinal plants is required to combat diseases in our society.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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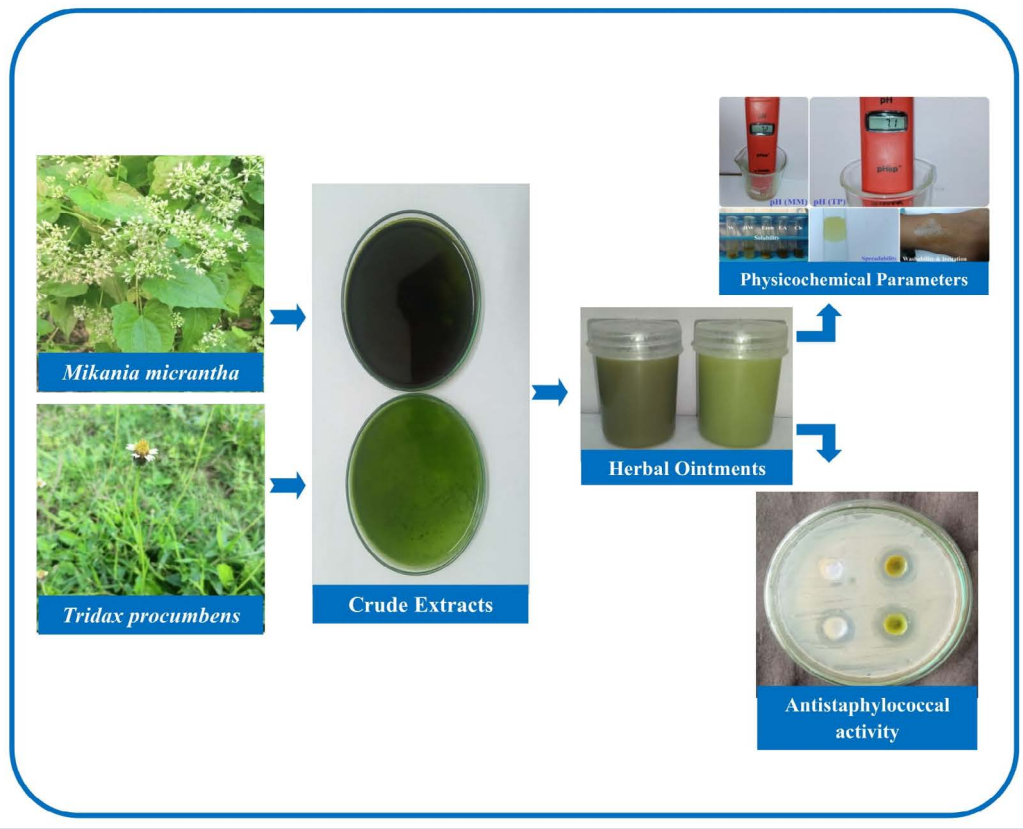
GRAPHICAL ABSTRACT



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