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RESEARCH ARTICLE

Formulation and evaluation of antibacterial herbal ointment from aerial parts of *Sphaeranthus indicus* extract

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Abstract

Sphaeranthus indicus L., commonly known as 'Shraboni', is a member of the Asteraceae family. Traditionally, the plant has been used to cure a variety of ailments including cold and cough, fever, liver disease, mental illnesses, diabetes, wounds, and skin diseases like cellulitis, acneiform rash, green nails, boils, blisters, and more. It is also used as an anthelmintic for kids. The primary goal of this study was to formulate an herbal ointment using an ethanolic extract of *S. indicus* aerial parts and assess its antibacterial efficacy. The agar well diffusion method was used to assess the invitro antibacterial activity of prepared herbal ointments against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Other physicochemical parameters, including color, odour, PH, spreadability, consistency, solubility, washability, and acute skin irritation study, were also analyzed. Zone of inhibition was used to assess the herbal ointment's antibacterial efficacy. Different phytochemicals like alkaloids, tannins, phenols, flavonoids were present in the plant extract. Invitro antibacterial study shows that *S. indicus* has significant antibacterial activity when formulated as ointment. The herbal ointment is also better in comparison than a commercial brand of Neosporin ointment. The results are the justification for the use of the plant in tribal medicine.

Keywords: Antibacterial; Formulation; Herbal Ointment; Phytochemicals; *Sphaeranthus indicus*; Tribal medicine.

1. Introduction

The local tribal people of remote areas are mainly dependent on herbal medicine for the treatment of different human ailments. Medicinal plants also produce secondary metabolites such alkaloids, phenol, flavonoid, terpenoid etc., these secondary metabolites are responsible for curing many human ailments. With this background, the present study highlights formulation and evaluation of antibacterial herbal ointment from aerial parts of *Sphaeranthus indicus* extract. Ethanolic extract of this plant have shown antibacterial activity against gram-positive and gram-negative bacteria. Bacteria can cause many types of skin diseases i.e. rashes, eczema, acne, boils, blister etc. *Staphylococcus aureus* is one of the skin diseases producing bacteria (Lowy, 1998; Tong et al., 2015).

The plant *Sphaeranthus indicus* L. (Family Asteraceae), commonly known as shraboni is widely used in traditional medicine to treat cold, cough, fever, liver disease, mental illness, diabetes, wounds, skin disease like acneiform rash, abscesses, cellulitis, green nail etc. and also used as anthelmintic for children. From the previous study indicate that the *S. indicus* is reported to have many pharmacological activities such as anxiolytic (Ambavade et al., 2006; Galani et al., 2010), neuroleptic (Mhetre et al., 2006), sedative (Galani and Patel, 2009), immunomodulatory (Bafna and Mishra, 2004; Shekhani et al., 1990), antioxidant (Shirwaikar et al., 2006; Tiwari and Khosa, 2009; Tandon and Gupta, 2020), anti-inflammatory (Heinrich et al., 1998; Jain and Basal, 2003), analgesic (Nanda et al., 2009), antihyperglycemic (Dhar et al., 1968; Prabhu et al., 2008), hepatoprotective (Nayak et., 2007), skin disease (Sadaf et al., 2006; Jha, 2009), antimicrobial (Shaikh et al., 1986; Singh et al., 1988; Tandon and Gupta, 2020), antibacterial and antifungal (Garg and Kasera, 1982; Garg and Kasera, 1983; Vijaya and Ananthan, 1997; Naqvi et al., 1998; Dubey et al., 2000; Duraipandiyan et al., 2009), bronchodilatory (Sarpate et al., 2009), antihyperlipidemic activity (Pande and Dubey, 2009).

Different phytoconstituents such as alkaloids, phenol, flavonoids and Coumarin are present in the ethanolic extract of aerial parts of *S. indicus* which may be responsible for the antibacterial activity of the plant. The present study was carried out to formulate and evaluate the antibacterial herbal ointment from extracts of the aerial parts of *S. indicus*.

2. Material and method

2.1. Plant sample collection

Plant samples were freshly collected during flowering season from their wild habitat at Jhargram district. It was washed under running tap water and shade dried for 6-7 days. The dried plant sample was grinded to make into fine powder and stored at room temperature in an airtight container for future usage.

2.2. Preparation of plant extract

About 10 gm of grinded fine powder was soaked in 100 ml of solvent then it was placed on a rotary shaker for 24 hours. The extract was filtered using Whatman no. 1 filter paper and the filtered extracts were evaporated to dryness using rotary flask evaporator to yield crude plant extract. Dried plant extract was stored in airtight bottles and placed in a refrigerator for phytochemical analysis and antibacterial screening.

2.3. Phytochemical screening

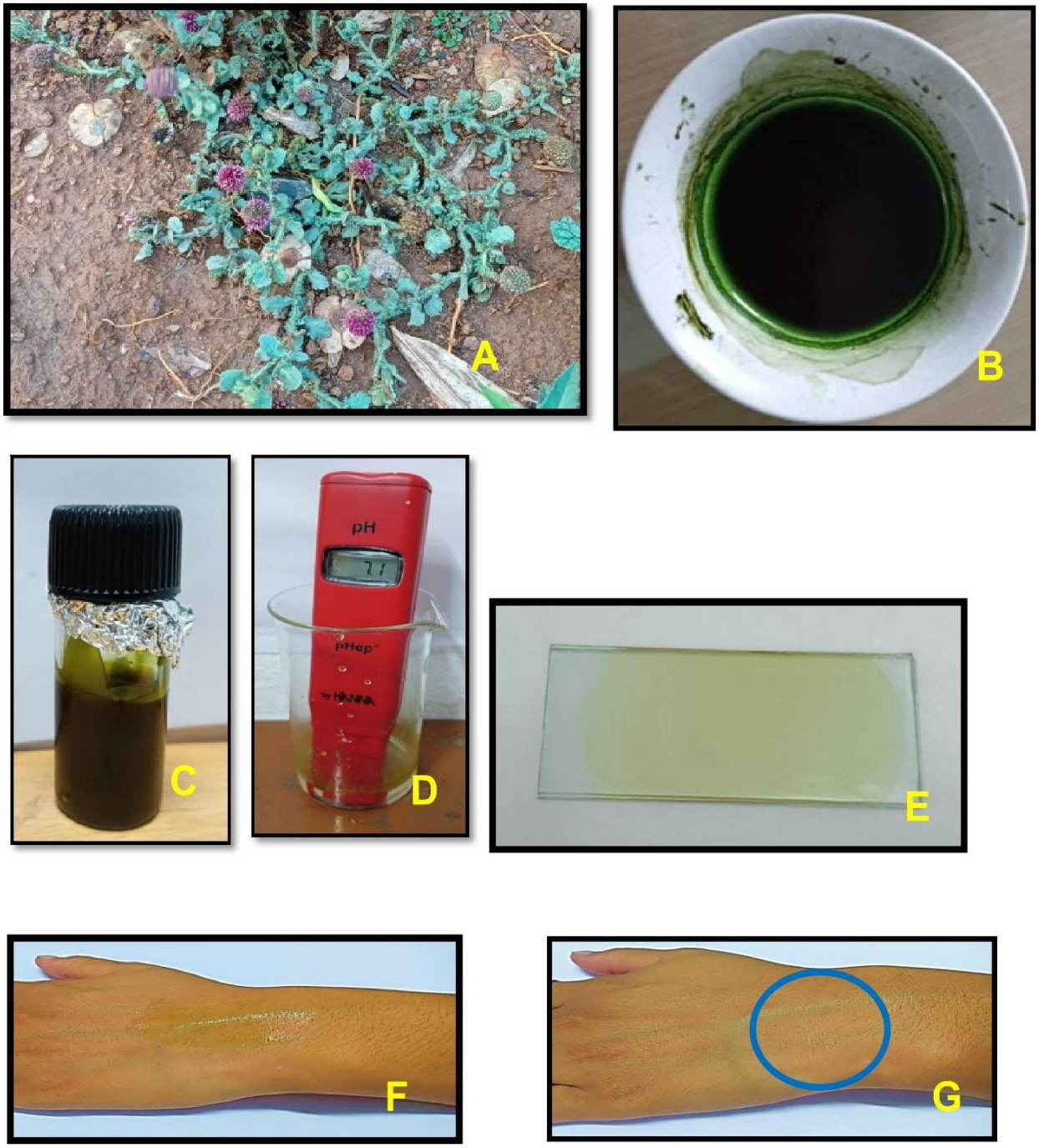

Figure 1. (A) The plant *Sphaeranthus indicus*, (B) Plant extract, (C) Formulated herbal ointment, (D) pH Determination, (E) Spreadability test, (F) Formulated ointment applied on skin to evaluate Irritation redness, edema and washability, (G) After application not generate any kind of irritation, redness and edema.

2.3.1. *Preliminary qualitative phytochemical analysis*

Preliminary qualitative phytochemical analysis was carried out following the standard method described by Harborne (1998).

2.4 Herbal ointment formulation

2.4.1. Preparation of herbal ointment

The herbal ointment was prepared following the standard method described by Mhatre et al (2014), and Sekar and Rashid (2016). At first 10 gm of the ointment base was prepared by weighing the finely grated white soft paraffin and liquid paraffin at 6:4 ratio which were placed on water bath to melt the paraffin. Then the liquid form of homogeneously mixed paraffin was slightly cooled down to make the ointment base. After that 0.5 gm of ethanolic crude plant extract was mixed to the ointment base and then the

Table 1. Composition of the herbal ointment from ethanolic extract of aerial parts of *Sphaeranthus indicus*.

Sl. No.	Components	Amount (gm)
1.	Ethanolic extract of aerial parts of *S. indicus*	0.5
2.	White soft paraffin	6
3.	Liquid paraffin	4

Table 2. Phytochemical analysis of ethanolic extract of aerial parts of *Sphaeranthus indicus*

Plant species	Usable parts	Solvent used	Phytochemicals								
			Alk	Car	Fla	Phe	Phlo	Sap	Tan	Ter	Cou
Sphaeranthus indicus	aerial part	Ethanol	+++	+++	++	+	-	-	-	-	+

Table 3. Physicochemical parameters of the herbal ointment from ethanolic extract of aerial parts of *Sphaeranthus indicus*

Physicochemical parameters	SI ointment (5%)
Colour	Dark green
Odour	Characteristic
pH	7.1
Consistency	Smooth
Spreadability (Seconds)	3.7
Solubility	Slightly soluble in normal water, miscible in hot water, ethanol and chloroform with stirring.
Stability	Stable at 2°C, 25°C and 37°C with pH 7.1
Washability	Good
Diffusion study	3 cm in 5 min
Irritation, redness and edema test	Not generate any kind of irritation, redness and edema.

Table 4. Antibacterial activity of herbal ointment from *Sphaeranthus indicus* against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Plant species	Plant Parts used	Microorganism	Solvent used	Zone of inhibition (mm)		Standard ointment-Neosporin
				Concentration (5%)		
				0.2gm	0.4gm	
Sphaeranthus indicus	Aerial parts	*Pseudomonas aeruginosa*	Ethanol	8.33±0.57	16.33±1.154	6.66±2.081
		Staphylococcus aureus		-	13.83±0.763	10.33±

homogenous mixture was allowed to cool and solidify to get a smooth ointment, then finally stored in airtight container (Table 1).

2.5. Evaluation of herbal ointment

2.5.1. Physicochemical parameters

The evaluation of herbal ointment was carried out by using the following physicochemical parameters.

Colour and odour: Colour and odour of herbal ointment was examined by visual examination.

pH: The pH of prepared herbal ointment was determined by using digital pH meter. 1 gm of ointment was dissolved in distilled water and pH was measured.

Spreadability: The spreadability of prepared herbal ointment was determined by using two glass slide and weight block. Formulated ointment was placed between two glass slide and weight block was placed on the glass slide for 5 min to compress the ointment. The time in seconds required to separate the two slides was taken as a measure spreadability[34].

Consistency: Smooth consistency was observed.

Solubility: Soluble in water alcohol, chloroform and ethyl acetate.

Stability: The stability test was carried out in different temperature condition (Stable at 2°C, 25°C and 37°C) for four months.

Washability: The washability of prepared herbal ointment was determined by using water.

Diffusion study: The diffusion study was carried out by using Agar plate. The whole Bored was created at the centre of the petriplate and ointment was placed in it. The time required for the preparation of ointment to get diffuse was recorded.

Acute skin irritation, Redness and edema test: The skin irritation, Redness and edema effect on skin was determined through direct application of the ointment on the skin.

2.5.2. In-vitro Antibacterial assay

2.5.2.1 Bacterial strain and culture conditions

The bacterial strains like *Staphylococcus aureus* (*S. aureus* MTCC 87) and *Pseudomonas aeruginosa* (MTCC 741) were taken for antibacterial screening. They were cultured in nutrient broth media in aerobic condition at 37.9°C.

2.5.2.2. In vitro antibacterial screening of herbal ointment

The antibacterial activity of the formulated ointment was assessed following agar well diffusion method [35] against the bacterial strains viz, *Staphylococcus aureus* (*S. aureus* MTCC 87) and *Pseudomonas aeruginosa* (MTCC 741) were taken for antibacterial screening. Ointment base was used as a negative control and Neosporin containing Neomycin and polymyxin B sulfates was used as a positive control. The petri-plates were incubated in an incubator for 24 h at 37°C. After incubation the plates were

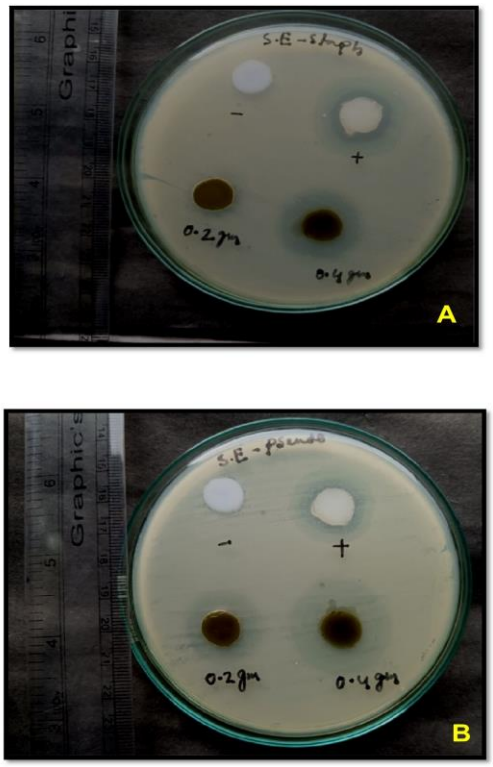

Figure 2. A. Antibacterial activity of herbal ointment from *Sphaeranthus indicus* against *P. aureginosa,* B. Antibacterial activity against *S. aureus*

observed and the zone of inhibition around the well was measured in mm.

3. Result and discussion

3.1. Preliminary qualitative phytochemical analysis

The results showed that the ethanolic extract of aerial parts of *Sphaeranthus indicus* contained phytochemically important compounds like alkaloids, carbohydrate, flavonoid and phenol (Table 2). The presence of these phytoconstituents may be used as a novel source of modern medicine.

The herbal ointment was prepared from ethanolic extract of *Sphaeranthus indicus*. The ointment was deep green in colour and PH was constant about 7.1. This ointment did not generate any kind of irritation, redness and edema when applied to the skin (Table 3 and Figure 1). The result of in vitro antibacterial activity of herbal ointments from ethanolic extract of *Sphaeranthus indicus* was given in Table 4. The in-vitro antibacterial activity of herbal ointments of *Sphaeranthus indicus* showed excellent activity against both tested bacteria i.e. *Pseudomonas aeruginosa* and *Staphylococcus aureus* and it was found that the formulation of ethanolic extract of *Sphaeranthus indicus* showed more zone of inhibition compared to the standard drug, Neosporin (Figure 2) which is in agreement with earlier findings of Jha et al (2009).

4. Conclusion

This study concluded that antibacterial herbal ointment from aerial parts of *Sphaeranthus indicus* can be used as a broad-spectrum antibiotic ointment due to the excellent zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This ointment was stable after 4 months. In a word *Sphaeranthus indicus* has a possibility to become a novel source of antibacterial medicine in near future.

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Authors' contributions

All the authors have equally contributed in research design, data generation and manuscript draft.

Conflicts of Interest

The authors declare no conflicts of interest

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