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Formulation and evaluation of antimicrobial activity of polyherb gel

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ABSTRACT

Ethanopharmacology and natural products provides a significant hope in drug development. Indeed today many pharmacological classes of drugs available in the market are derived from natural products as herbal drugs have less side effect and safe when compared with the synthetic drugs. The present study was undertaken to investigate the antimicrobial activity of polyherb gel. In this study we have formulated 1% polyherb gel which consists of aqueous extract of *Hibiscus sabdariffa* and aqueous extract of *Amarathus cruentus*. The base was prepared by using carbapol 940, propylene glycol-400, sodium CMC, sodium benzoate and required amount of distilled water in a quantity sufficient to prepare 10 g. The prepared formulations were evaluated for their antimicrobial activity by disk diffusion technique against *S. aureus* and *E. coli* which are indicative types of gram positive and gram negative organisms. The formulations were also assessed for appearance and homogeneity, pH, viscosity and rheological studies, spreadability. The results obtained in the present study indicate that the polyherb gel shows antimicrobial activity. As per the results obtained, it could conclude that this polyherb gel possesses antimicrobial activity.

Key words: *Polyherb gel, Hibiscus sabdariffa, Amaranthus cruentus, Antimicrobial activity, Synergistic effect*

Introduction

In India, the ayurvedic system of medicine has been in use for over three thousand years. Hippocrates, the "father of medicine" was the first to give a scientific explanation of diseases. Nowadays herbal drugs are getting more popularity due to its fewer side effects. Skin infection is very common. The appearance and reappearance of infectious diseases have become a significant worldwide concern (Wilcox and Colwell, 2005). An infectious disease is caused by various microbes or pathogens, most of them are usually microorganisms and few of them are visible by naked eyes. The most common pathogens are different types of viruses, bacteria, fungi and protozoa (Patz *et al*., 2014). *Hibiscus sabdariffa* L. (Malvaceae), extracts showed antibacterial, antioxidant, hepatoprotective, renal/diuretic effect, effects on lipid metabolism, anti-diabetic and anti-hypertensive effects among others (Ali, Badreldin and Al-Wabel, 2005). *Amaranthus cruentus* L. (Amaranthaceae), extract have reported antioxidant, antidiabetic, gastroprotective, antimicrobial, hepatoprotective and anticancer activities. Scientific evidence supports the hypothesis that several plants are composed of biologically active chemical entities and several drugs in modern day medicine are actually analogues of plant origin substances. (Ghosh *et al*., 2012; Begum *et al*., 2012; Abreu *et al*., 2012). The present study was undertaken to formulate and investigate antimicrobial activity of polyherb gel. The polyherb gel is prepared by using aqueous extract

of two plant, i.e *Hibiscus sabdariffa* and *Amaranthus cruentus* in appropriate gel base. The present study also provides novel information about synergistic antimicrobial effect of *Hibiscus sabdariffa* and *Amaranthus cruentus* against *E.coli* and *S. aurues*.

Materials and Methods

Plant materials

The plant material was collected from the local vegetable market of Panvel, Navi Mumbai in the month of September and authenticated from the Botany Department, Khalsa College Mumbai. (Specimen#: sps p 0120204061 and Specimen #: sps p 0120204009). Freshly collected leaves of *Hibiscus sabdariffa* and *Amaranthus cruentus* were dried under the shade. Dried leaves were ground to a coarse powder with an electrical blender.

Extract preparation

Two different Plants were dried, powdered and extracted with distilled water. For experimental work, two plant aqueous extracts were prepared by maceration method. 500 g of the drug were weighed and separately it was mixed with water. The whole process was kept in dark conditions for seven days with frequent shaking every 2 hours. After seven days the extract was filtered and the filtrate was concentrated by distilling of the solvent till one third. After that the extract was concentrated over water bath at a temperature not exceeding 60R C. The extract was further dried over desicator for overnight and was used further for the experiments (Pounikar *et al.*, 2012).

Gel preparation

In this study we prepared 1% w/w gel formulations, which comprised of aqueous extract of *Hibiscus sabdariffa* and *Amaranthus cruentus* in the ratio of 50:50, respectively in a base. The base was prepared by using carbapol 940, propylene glycol-400, ethanol, methyl paraben, propylparaben, EDTA, triethanolamine and required amount of water in a quantity sufficient to prepare 10g (Bhinge *et al.*, 2017).

The microorganism and growth medium

Staphylococcus aureus (MTCC 96) and *Escherichia coli* (MTCC 443) were chosen based on their clinical and pharmacological importance (McCracken and Cowsan, 1983). The bacterial and fungal stock cultures were incubated for 24 hours at 37 °C on nutrient agar. The bacteria were grown in the nutrient broth at 37 °C and maintained on nutrient agar slants at 4 °C.

Antimicrobial activity assay

The *In vitro* antimicrobial activity was performed using disk diffusion method (Tim Sandle, 2016). First the antimicrobial activity of aqueous extract of *Hibiscus sabdariffa* and *Amaranthus cruentus* were done to check synergistic effect followed by determination of antimicrobial activity of formulated polyherb gel.

Evaluation of gel

1. pH: pH of individual and polyherbal gel formulation was determined by using a pH meter (Table 3).
2. Appearance and Homogeneity: The developed individual and polyherbal gels were evaluated for physical appearance and homogeneity by visual observation (Table 3).
3. Viscosity: The viscosity of individual and polyherbal gels was measured by Brookfield viscometer (Model RVTDV II) at 100 rpm using spindle no. 6 (Table 3).
4. Spreadability: The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 125 g (Table 3).
5. Extrudability: The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 0.5 g was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).

Results and Discussion

Result

The antimicrobial activity of the aqueous extract of *Hibiscus sabdariffa* and *Amaranthus cruentus* were done to check synergistic effect against of gram

positive and gram negative organisms. Antimicrobial activity of extracts was assessed in terms of zone of inhibition of bacterial growth. The results of the antimicrobial activity are presented in Table 1, Figure 1 and Graph 1. The antimicrobial activity of formulated polyherb gel were also assessed in terms of zone of inhibition, results of the same are presented in Table 2, Figure 2 and Graph 2.

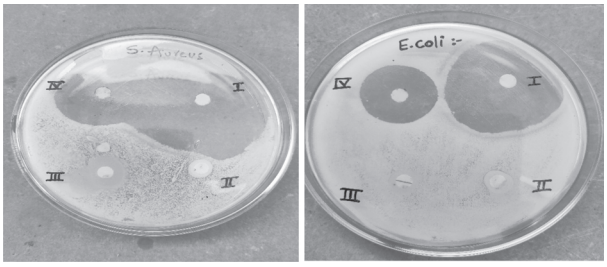
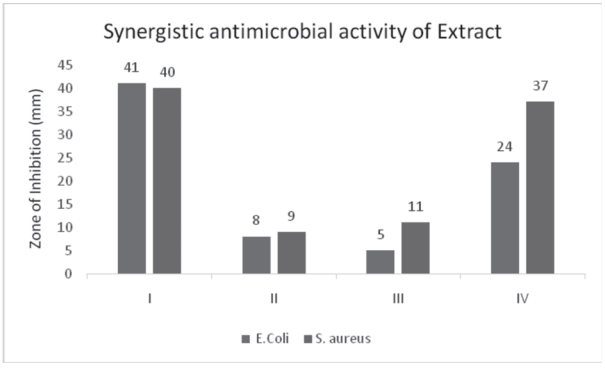


Fig. 1. Synergistic antimicrobial activity



Graph 1. Synergistic antimicrobial activity

Table 2. Antimicrobial activity of polyherb gel

Microorganism	Antimicrobial activity Zone of Inhibition (mm)	
	Standard (Clotrimazole Gel)	Candid Gel Polyherb Gel
E. Coli	9	4
S. aueus	9	8

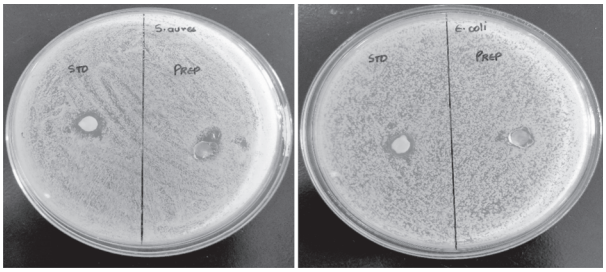
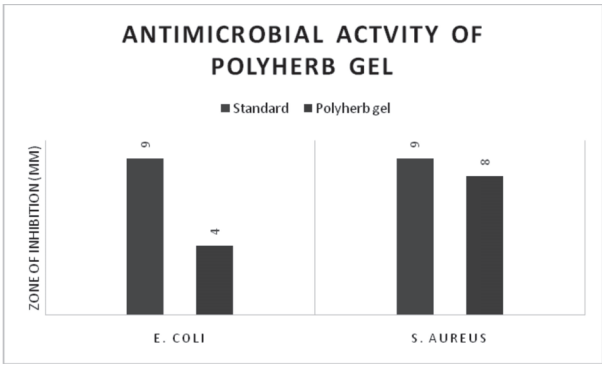


Fig. 2. Antimicrobial activity of polyherb gel



Graph 2. Antimicrobial activity of polyherb gel

Discussion

Herbal drugs are getting popularity and its pharmacological properties are reported from different part of the world. In the current study, the aqueous extract of both *Hibiscus sabdariffa* and *Amaranthus cruentus* showed good antimicrobial activity but synergistic antimicrobial activity observed when aqueous extract of *Hibiscus sabdariffa* was used in combination of aqueous extract of *Amaranthus cruentus.* This study also includes subsequent formulation of polyherb gel and the gel also showed good antimicrobial activity when compared with marketed formulation.

Table 1. Synergistic antimicrobial activity

Microorganism	Antimicrobial activity			
	Zone of Inhibition (mm)			
	Standard (Ciprofloxacin) (I)	Aqueous extract of *Amaranthus cruentus* (II)	Aqueous extract of *Hibiscus sabdariffa* (III)	*Amaranthus cruentus* + *Hibiscus sabdariffa (50:50)* (IV)
E. coli	41	8	5	24
S. aueus	40	9	11	37

Table 3. Evaluation of Polyherb Gel

Parameters	Polyherb Gel	Marketed Gel
pH	6.45	6.13
Appearance	Light brown	White
Homogeneity	Good	Good
Viscosity (cp)	42000	45000
Spreadability (spreading diameter after 1min)	39	56
Extrudability	Good	Excellent

Conclusion

As per the result obtained, it could conclude that this polyherb gel possesses antimicrobial activity. Thus can be used in the treatment of topical infectious disease. The current study also appreciates synergistic antimicrobial activity of vegetables like *Hibiscus sabdariffa* and *Amaranthus cruentus* which are widely used in Maharashtra region. Overall results of current study demonstrate that polyherb gel has good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

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