Research Article

FORMULATION AND EVALUATION OF POLY HERBAL IMMUNOSTIMULANT PROTECTANT CREAM

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ABSTRACT

The present study was focussed on the latest development regarding immunostimulant activity of poly herbal based formulation, it has less side effects when compared to the allopathic medicines. Immunostimulants are used to stimulate or potentiate the weapons of your immune system. The component of immune system including both innate and adaptive immune responses. There are large number of medicinal plants and its individual phytoconstituents have a major role to play in curing certain immune diseases causing body against infections without producing any adverse effects. The aqueous plant extract of polyhedral drugs have been possible for immunostimulant properties. Some of the traditionally Indian medicinal plants are *Acorus calamus, Alpinia officinarum, Azadirachta indica, Glycyrriza glabra, Hedyotis corymbosa, Justica adhatoda, Piper longum, Rosa foetida and Zingiber officinalis*. This polyhedral drugs being used as immunostimulant, antibacterial, antifungal, anti-inflammatory and antioxidant activity. This polyhedral drugs was subjected to preliminary phytochemical screening of the extracts presence of alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids and phenolic content. Antibacterial activity against E.Coli, S.Aureous, antifungal activity against A.Fumigatus, C.Albicans.

Keywords: Immunostimulant activity, Polyherbal, Antibacterial, Antifungal, Anti-inflammatory, Antioxidant.

INTRODUCTION

Our immune system is increasingly exposed to detrimental effects that are immune suppressive, environmental consequences, unhealthy living, and chronic illnesses. The arrival of COVID-19 has finally led us to realize the importance of having immunity checks, as it has been demonstrate that if a person's immune system is strong, this fatal virus will not be able to harm the individual easily.

The present scenario of chronic infections has made us realize the importance of immune defence and the need for prophylactic treatments that serves as a multi target therapy to combat various infections. The earliest attempts to develop suitable medication for immuno stimulation were based on traditional remedies which embodied the accumulated experience of several centuries. Medicinal plants are already being used prophylactically as standardized and efficacy-optimized preparations for the treatment of various recurrent infections. Therefore it is necessary to develop new treatment options with immune modulatory activity and also possessing anti-bacterial and anti-viral properties to combat harmful microorganisms and also to improve the overall well-being of the patient. They act at different levels and multiple links of the immune system and can act simultaneously on multiple targets to improve immunity. At the same time, herbs can also enhance the patient's resistance, improve the body's immune function, and improve the quality of life. Moreover, natural medicines have fewer side effects as immunopotentiators. Our Current project aims at developing a glycerine based polyherbal protectant cream that contains potential herbs mentioned in Ayurvedic texts and are scientifically proven for its immunostimulant effects and also for the treatment of various Respiratory ailments.

Why Polyherbal Protectant Cream?

The pandemic has an accelerating demand for hand sanitizers. Most liquid hand sanitizers contain a large amount of ethyl or isopropyl alcohol. When used too frequently, alcohol-based hand sanitizers can wash away skin's natural oils, which can cause drying and cracking. Dehydrated skin can be unattractive and irritating, and it is likely to cause one or more of the following symptoms:

- Itching
- Slight to severe flaking, scaling, or peeling
- Fine lines or cracks
- Gray, ashy skin color
- Redness
- Deep cracks that may bleed

Most hand sanitizers on the market contain a high volume of alcohol, enough to be considered a fire hazard. In fact, “alcohol-based hand sanitizers are classified as Class I Flammable Liquid substances. Opportunistic pathogens such as bacteria, viruses and fungi can survive on inanimate surfaces for long periods of time and items such as watches, pens, and mobile phones are permanent surfaces for transmission of these types of bacterial infections. Possibility of bacterial transmission is much more in tight shake hand than in loose shake hand. Responding to the increasing concerns of dry, aging hands and those suffering from atopic eczema and contact dermatitis, we formulated a polyherbal protectant cream using potent herbs clinically proven to be anti-viral and antibacterial to inhibit and neutralize harmful pathogens. Also, it is formulated to be intensely nourishing and hydrating to eliminate skin dryness, redness and irritation.

MATERIALS AND METHODS

The following dried powders of the selected herbs are commercially available and are purchased from a local shop in Ooty.

- *Acorus calamus*
- *Azadirachta indica*
- *Rosa foetida*
- *Justicia adhatoda*
- *Glycyrrhiza glabra*
- *Piper longum*
- *Zingiber officinal*
- *Alpinia officinarum*
- *Hedyotis corymbosa*


Extraction (Triple Kinetic maceration process)

In this process the collected herbs are macerated thrice by using menstrum which is divided into three parts in such a manner that same volume is used for each maceration.

The powdered plant materials were macerated by using water (aqueous) as the solvent. The extracts were then concentrated to dryness under reduced pressure and controlled temperature to yield a dark brown semisolids.

The percentage yield was found to be 14% w/w. Finally all the extracts were stored in desiccator till further use.

$$\textbf{Percentage yield} = \frac{\textit{Weight of extract blend}}{\textit{Weight of the powder}} \times 100$$

$$= \frac{187.000}{1350.000} \times 100$$

$$= 13.85\ \%\ w/w$$

RESULTS

Physical Properties of Poly Herbal Extract Blent

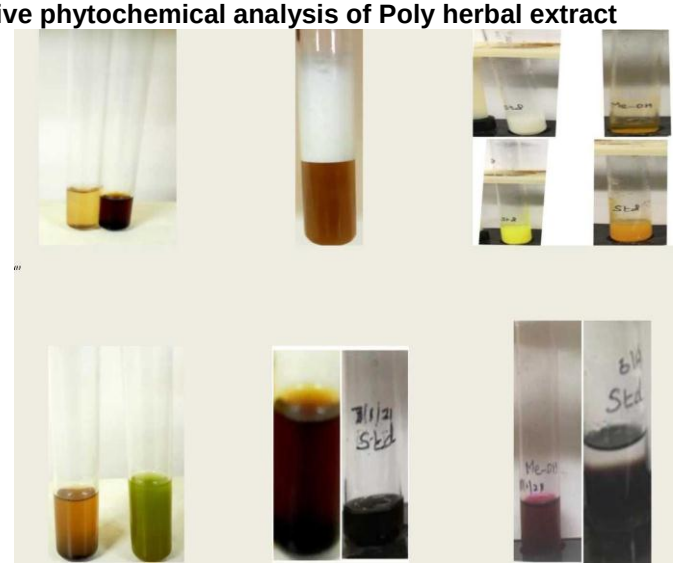
Colour - Dark brown
Odour - Sharp, pungent and aromatic
Texture -Semi solid
pH - 5.1

Method of preparation of Poly Herbal Protectant Cream

S. No.	Ingredients	Official Formula (100 gm)	Working Formula (10 gm)
1	Plant Extract	20 gm	2 gm
2	Bees wax	47 gm	4.7 gm
3	Liquid Paraffin (Heavy)	30 gm	3 gm
4	Borax	2 gm	0.200 gm
5	Sodium Benzoate	1 gm	0.100 gm

Plant extract was dissolved in boiling water along with borax, then bees wax and liquid paraffin were melted together. The aqueous phase was added to the hydrophobic phase with constant stirring and when the temperature dropped to 45^0C perfume and preservatives were added.

Preliminary qualitative phytochemical analysis of Poly herbal extract



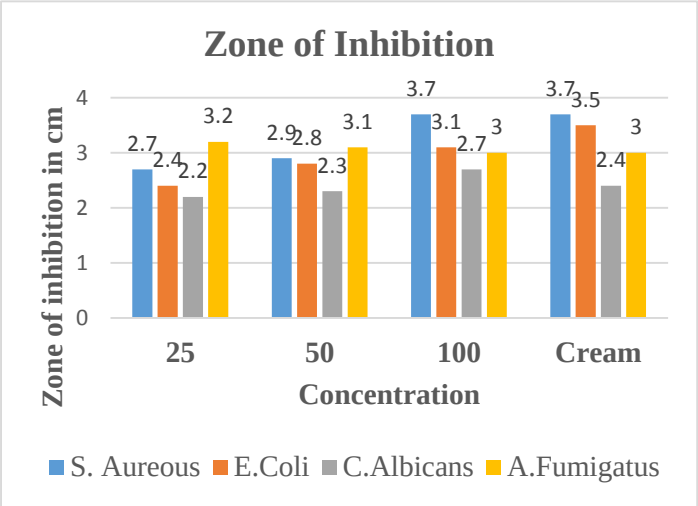
The concentrated poly herbal extracts were subjected to chemical tests as per the standard methods for the identification of the various constituents like Alkaloids, Glycosides, Tannins, Flavonoids, Saponins and Carbohydrates.

Phytochemicals	Status
Alkaloids	+
Glycosides	+
Saponins	+
Flavonoids	+
Tannins	+
Carbohydrates	+

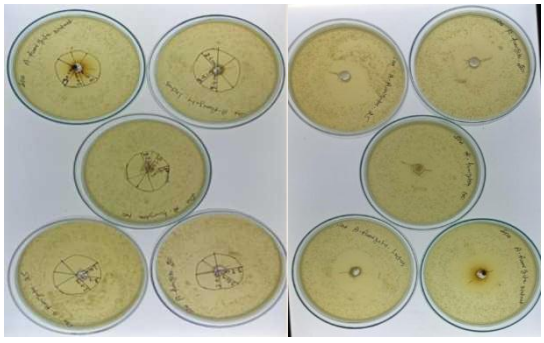
Antimicrobial studies for extracts Blent and formulations by using cup plate method. (Antibacterial, Antifungal)

The aqueous poly herbal extracts blunt and the formulation (protectant cream) showed reasonably good anti-bacterial activity at different concentration (100, 50, 25 µg/ml), as well as solution of ampicillin at concentration (100 µg/ml) against both gram positive and gram negative organism by cup plate method. The zone of inhibition ranging from 2.4 to 3.7 cm diameter.

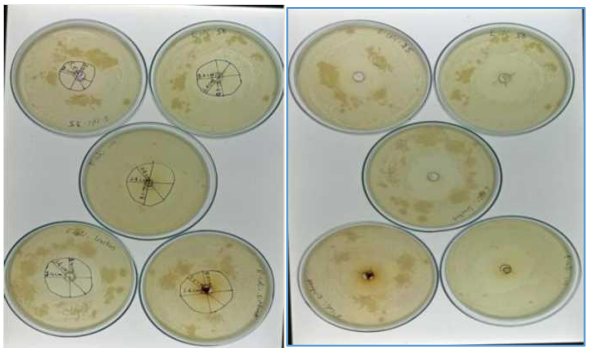
Microorganism	Extract blent			Cream	Std
	25	50	100		
Gram positive S.Aureous	2.7	2.9	3.7	3.7	3.2
Gram negative E.Coli	2.4	2.8	3.1	3.5	3.4
C.Albicans	2.2	2.3	2.7	2.4	2.6
A.Fumigatus	3.2	3.1	3.0	3.0	3.2



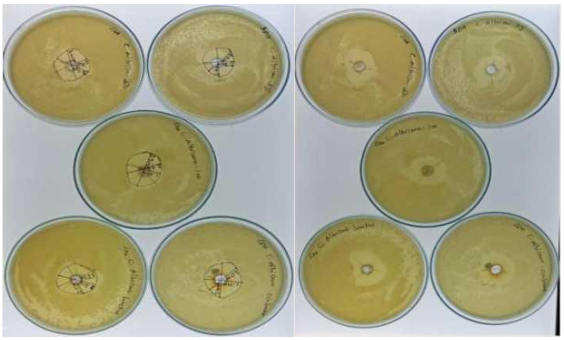
Among the aqueous poly herbal extract blunt and the formulation (protectant cream) are tested for antifungal activity. It showed good anti-fungal activity at different concentration (100,50,25 µg/ml), as well as solution of fluconazole at concentration (100 µg/ml) against fungi organism by cup plate method. The zone of inhibition ranging from 2.2 to 3.7 cm diameter.



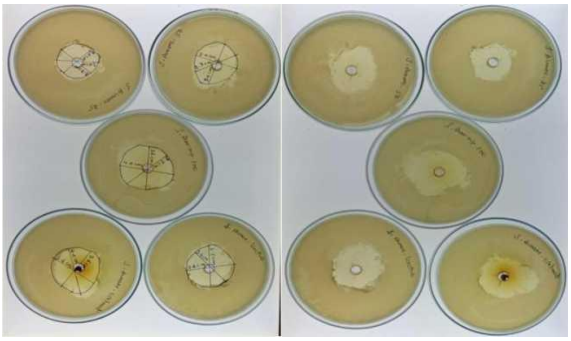
Zone of inhibition of E.Coli (Gram –Ve Bacteria)



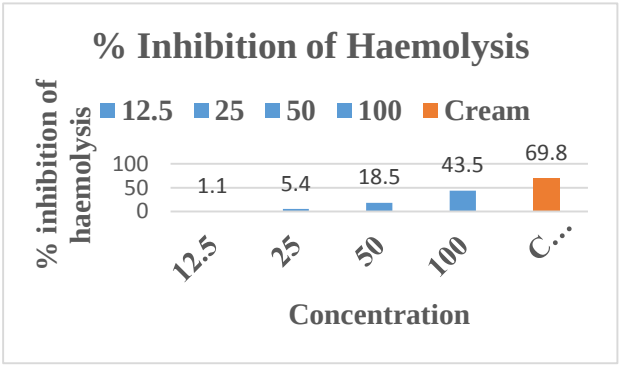
Zone of inhibition of A. Fumigatus (Fungi)



Zone of inhibition of S.aureous (Gram +Ve Bacteria)



Zone of inhibition of C. Albicans (Fungi)



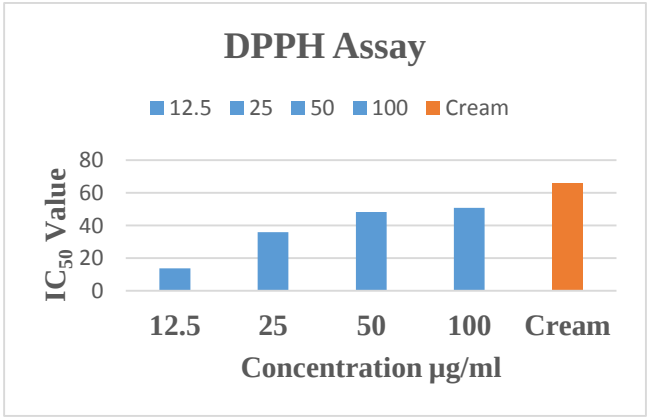
Anti-inflammatory activity of extracts using different concentrations by HRBC method

The anti inflammatory activity of poly herbal formulation was assessed by in vitro HRBC membrane stabilization method. Blood was collected from healthy goats. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100ml) and centrifuged with isosaline. To 1ml of HRBC suspension, equal volume of test drug in four different concentrations, 100, 50, 25 and 12.5 µg/ml was added. All the assay mixtures were incubated at 37^{0}C FOR 30 minutes and centrifuged. The Haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 540 nm. The percentage inhibition of Haemolysis was calculated by the formula has given below:

$$\textbf{\% inhibition of haemolysis} = \frac{OD1-OD2}{OD1} \times 100$$

OD1- Optical density of test soution
OD2- Optical density of control

Concentration (µg/ml)	Percent of haemolysis (%)
12.5	1.1
25	5.4
50	18.5
100	43.5
Cream (200 µg/ml)	69.8



Anti-oxidant activity of extracts using different concentrations by DPPH method

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solution used are 1000 to 1.95 µg/ml. The plates were incubated at 37^0C for 20 minutes and the absorbance of the well was measured at 540 nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated. IC_{50} (Inhibtory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals.

$$\textbf{Percentage of inhibition} = \frac{Control - Sample}{Control} \times 100$$

Concentration (µg/ml)	IC_{50} value (%)
12.5	13.8
25	35.9
50	48.2
100	50.9
Cream	66.01

DISCUSSION

The pandemic has been accelerating demand for immunity boosting products and ingredients. The human immune system, despite having its own sophisticated defense mechanisms, is inferior to bacteria and viruses with respect to adaptability. The extract blend were subjected to phytochemical analysis and was found to contain glycosides, alkaloids, tannins and flavonoids. The aqueous poly herbal extracts and the formulation (protectant cream) are showed reasonably good anti-bacterial activity at different concentration (100, 50, 25 µg/ml) against both gram positive and gram negative organism by cup plate method. The zone of inhibition ranging from 2.4 to 3.7 cm diameter. Aqueous poly herbal extract and the formulation (protectant cream) are tested for antifungal activity. It showed good anti-fungal activity at different concentration (100,50,25 µg/ml) against fungi organism by cup plate method. The zone of inhibition ranging from 2.2 to 3.7 cm diameter.

CONCLUSION

The prepared extract blunt and formulation were subjected to various evaluation parameters and it was found to have satisfying results. The extract blunt showed good antibacterial and fungal properties on five strains of micro-organisms and also potent anti-oxidant property with IC50 value of 39.94. The extract blunt showed significant results on in-vitro anti-inflammatory study. Thus studies reveal that the prepared formulation can be used as an immune booster as it showed good antimicrobial and anti-inflammatory potential, Further studies are to be performed to validate the stability parameters of the formulation.

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