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Research article

Anti-oxidant and Anti-bacterial activity of polyherbal formulation

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ABSTRACT

The present work aimed to evaluate anti-oxidant and anti-bacterial activity of polyherbal formulation (gel) prepared using Tulsi, Aloe vera, Neem. It has been used for skin benefits since ancient times. Formulations were tested anti-oxidant activity by DPPH radical scavenging assay and anti-bacterial activity by disk diffusion Susceptibility Testing method. The anti-oxidant activity is exhibited in percentage inhibition. F1 formulation showed maximum percentage inhibition with increasing concentration as compared to standard ascorbic acid. The anti-bacterial activity is exhibited in zone of inhibition. The F5 formulation showed highest zone of inhibition (26.5 mm) against *E. coli* and zone of inhibition (26.0 mm) against *Staphylococcus aureus* which is an indication that F5 formulation is more effective on gram positive as well as negative bacteria.

Keywords: Anti-oxidant activity, Anti-bacterial activity, DPPH assay, Disk Diffusion Susceptibility Test.

INTRODUCTION

Since the beginning of time, humans have employed natural herbals in their life to treat Increased attention has been focused on the development of polyherbal formulations because of their cost effectiveness, Currently, there are numerous treatments available that employ topical, biological, and systemic medicines. Some of the medications assist in lessening the symptoms of diseases, but they also have some adverse effects. The development of a medication with high efficacy and few adverse effects is crucial in the interim. Herbal medications are safer and more effective in reducing symptoms than allopathic ones. Any plant that includes compounds with therapeutic properties or compounds that can be utilized as building plants against microbial infections [1]. Acne, a skin disorder, develops when dead skin cells clog hair follicles. Acne vulgaris, which is defined by the development of inflammatory and non-inflammatory lesions of hair follicles and sebaceous glands, affects approximately three-fourths of individuals in the age range of 11 to 25 [2]. Acne may be brought on by hormonal imbalance, environmental conditions, Inflammatory properties, including flavonoids, phenolic acids, or a hereditary predisposition that result Gels are employed to ensure the best possible cutaneous and percutaneous medication delivery [3]. They can prevent gastrointestinal medicine-absorption issues brought on by acidic gastrointestinal conditions. Gels have the ability to prevent medication interactions with food and drink, as well as enzymatic activity. When the oral route is inappropriate, they may be used in place of oral administration of medicines. They can avoid the first to dispense some viscous oral suspensions, such as aluminum hydroxide gel [4].When compared to creams and ointments, topical administration of gels at pathological locations has significant advantages in terms of direct drug release and speedier absorption. In skin care products, single-phase gel is widely used. Organic macromolecules are uniformly Antibiotics and anti-inflammatory medications can be used orally or topically as part of current acne treatment. For mild and moderate acne, topical therapy is the first line of short-season legumes. It contains a variety of chemical components that are renowned for their antioxidant, antibacterial, and anti- and organic acids. Aloe barbadensis is a crucial ingredient [5].

Materials and Methods

Plant material

The whole parts of different plants (Tulsi, Aloe Vera, and Neem) and roots were separately dried, powdered and then extracted to use for evaluation of various physicochemical, phytochemical and pharmaceutical parameters [6].

Chemical

Ethanol, Liquid Paraffin, Carbopol 940, Propylene Glycol, Methyl Paraben, Propyl Paraben, Trietanolamin.

Apparatus

Precision balance, Hot air oven, Soxhlet apparatus, pH meter, Laboratory stirred.

Extraction of Neem leaves

Collect fresh leaves from Neem plant .Wash them with distilled water. Allow the leaves for sun drying .Place them into hot air oven at 60°C for half hour for removing excess moisture .Make coarse powder of Neem leaves with the help of mortar and pestle. Make a Pocket of tissue paper and place 10 gm of Neem powder in it. Set soxhlet apparatus and put cotton plug at bottom of the extraction chamber then put pocket on the cotton plug in extraction chamber. Pour 150 ml ethanol through condenser with the help of funnel .The ratio of solute and solvent should be 1:15.Start soxhlet apparatus and keep temperature 70°c for 6 hour .Concentrate it up to 20%. Filter the extraction through muslin cloth and sealed in pre washed ampule [7].

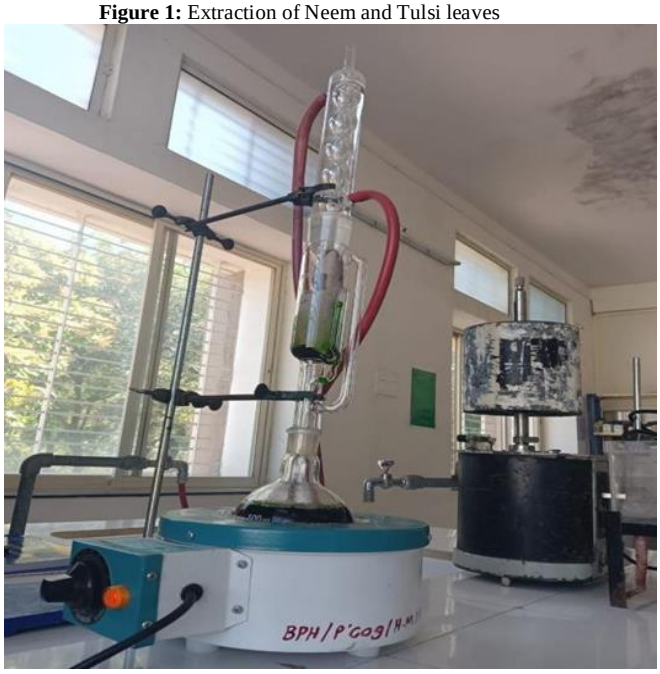
Extraction of Tulsi leaves

Collect fresh leaves from Tulsi plant. Wash them with distilled water .Allow the leaves for sun drying .Place them into hot air oven at 60°C for half hour for removing excess moisture. Make coarse powder of Tulsi leaves with the help of mortar and pestle. Make a Pocket of tissue paper and place 10 gm of Tulsi powder in it. Set soxhlet apparatus and put cotton plug at bottom of the extraction chamber then put pocket on the cotton plug in extraction chamber. Pour 150 ml ethanol through condenser with the help of funnel. The ratio of solute and solvent should be 1:15.Start soxhlet apparatus and keep temperature 70°c for 6 hour. Concentrate it upto 50 %. Filter the extraction through muslin cloth and sealed in pre washed ampule [8].

Extraction of Aloe-Vera

Collect fresh and thick Aloe Vera leaves wash them with distilled water. Dry leaves in hot air oven for 30 minute at 60°c.Remove upper part with sterile knife remove gel with the help of sterile knife .filter the gel through muslin cloth and F3, F4, and F5) in the test tube and final volume of 3 ml was sealed in air tight container [14].

Figure 1: Extraction of Neem and Tulsi leaves



Formulation of polyherbal gel

Take 50 ml of water in beaker. Add 0.2 gm of methyl paraben and 0.2 ml of propyl paraben. Dissolve it by stirring. Dispersed 1 gm of Carbopol uniformly with continuous siring. Allow the mixture for soaking overnight. Add 0.2 ml of propylene glycol. And stir with the help of laboratory stirrer. Add 0.1, 0.3, 0.5, and 0.7, 0.9 ml of Neem and Tulsi extract in F1, F2, F3, F4, and F5 beaker respectively. And then dispersed 0.5, 1, 1.5, 2, and 2.5, 3 ml of Aloe Vera gel into F1, F2, F3, F4, and F5 beaker respectively.

Evaluation of antioxidant activity

The antioxidant activity of Polyherbal Gel was determinedby in-vitro methods such as free radical scavenging activity (FRSA) using 1,1-diphenyl-2- picryl – hydrazyl (DPPH) scavenging activity. The assay was carried out in triplicate, and average values were considered.

In-vitro free radical scavenging activity (FRSA) using DPPH method

DPPH, commonly known as 1,1-diphenyl-2-picrylhydrazyl, is a cell permeable, stable free radical used to assess the ability of compounds to act as free radical scavengers to measure the antioxidant activity. The reaction of DPPH withan antioxidant or reducing compound produces the corresponding hydrazine DPPH2, which can be followed bya color change from purple to pale yellow [11]. The free radical scavenging activity of both the extracts was measured by 1.1 Diphenyl-2-picryl-hydrazil (DPPH) [12]. 0.1 mM solution of DPPH was prepared in methanol, and 1 ml of it was added to different Polyherbal Gel formulations (F1, F2) made with methanol. The mixture was shaken vigorously and

allowed to stand at room temperature for 30 min. Absorbance of the resulting mixture was measured at 517 nm against methanol as blank, by using a UV-visible spectrophotometer (Systronics, 2203, Japan). Each sample was then measured in Triplicate and results were represented as mean. The ascorbic acid was used as a standard antioxidant in this method. Percentage of DPPH free radical scavenging activity (FRSA) was determined as follows:

$$\% \text{ (FRSA)} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Antibacterial activity

Anti-bacterial assay was performed using disk diffusion Susceptibility Testing (Kirby-Bauer Method) [15, 16].

Disc diffusion method

Disc diffusion method was followed to test the antibacterial activity of different poly herbal formulations. The paper disc having the same diameter absorbed the concentration of extract. After impregnated the disc on agar plate, it diffuses their drug and in case of drug sensitive bacteria make a clear area that is known as Inhibitory area or zone of inhibition. The medium used for the activation of the microorganisms was nutrient broth. The nutrient agar media was used for the antimicrobial test. All the culture media were prepared and treated according to the manufacturer guidelines (Hi Media Laboratories Ltd., Mumbai, India). *Staphylococcus aureus and E. Coli*. Were used as test organism for the activity. Cefotaxime (10µg/ml) was used as positive control for gram positive bacteria while Gentamicin (10µg/ml) for gram negative bacteria. The plates were incubated at 37°C for 24 hours [13].

RESEULT AND DISCUSSION

As we know that the free radical and reactive species become an important etiological factor in the pathogenesis of several diseases. Although the numbers of antioxidants are available to reduce the risk associated with free radicals, efficacy and safety of synthetic antioxidants have become a concern among scientist and important current issue in discovery of natural antioxidants [14]. Studies suggested that plant derived bioactive constituents having antioxidant activity such as vitamins, alkaloids, tannins, terpenoids, phenolic compounds, flavonoids and coumarins play a key role in the management of several diseases like neurodegenerative disorders, diabetes and cardiovascular disorders [15, 16]. Plant derived herbal drugs becomes a promising alternative to the available synthetic anti-oxidants. Formulations showed gradual increasing percentage inhibition with increasing concentration at 517 nm in spectrophotometer as antioxidant by DPPH assay. Ascorbic acid showed gradual increase in percentage inhibition with increasing concentration (Table-1). Determination of anti-bacterial activity is based on recording zone of inhibition. The result shows that the different poly herbal formulations were effective against gram positive & gram-negative bacteria (Table-2).

Table-1: Antioxidant activity of different Polyherbal formulations (Gel) by DPPH method.

Sample	Concentration (µg/ml)	Absorbance (Mean ± S.E.M.)	% Inhibition
Ascorbic acid (Standard)	50	0.272	48.09
	100	0.225	57.06
	200	0.182	65.26
F1	50	0.372	29.0
	100	0.301	42.55
	200	0.259	50.07
F2	50	0.357	31.87
	100	0.291	44.46
	200	0.265	49.42
F3	50	0.362	30.91
	100	0.312	40.45
	200	0.285	45.61
F4	50	0.368	29.77
	100	0.317	39.5
	200	0.295	43.7
F5	50	0.372	29.0
	100	0.322	38.54
	200	0.305	41.79

Results are represented as Mean ± S.E.M., and Control OD at 517 nm 0.524

Table 2: *In vitro* antibacterial activity of different Polyherbal formulations (Gel).

Sample	Zone of inhibition in mm Mean±S.D.	
	Staphylococcus aureus	E. Coli
Cefotaxime (10µg/ml)	28.0±1.3	-
Gentamicin (10µg/ml)	-	27.5±1.2
F1 (100µg/ml)	20.0±1.1	22.0±1.4
F2 (100µg/ml)	22.3±1.2	23.0±1.5
F3 (100µg/ml)	24.2±0.8	23.5±0.5
F4 (100µg/ml)	25.0±0.8	24.5±0.6
F5 (100µg/ml)	26.0±1.1	26.5±1.3

Results are represented as Mean ± S.D.

CONCLUSION

The development of polyherbal formulations has drawn increasing attention due to its historical roots, economic viability, and patient compliance. Gels are becoming more and more popular as compared to other semisolid preparations, including ointments, creams, pastes, *etc.* they can give controlled release and are more stable. Making gels can result in improved absorption, which increases medicinal drugs' bioavailability. Gels' long-term stability features open up possibilities for their beneficial application to patients. Gels are simple to make, but extensive drug and excipient modification is required to produce a stable, effective, and secure product. On the basis of findings

of the present study, it can be concluded that the polyherbal gel formulations have a good anti-oxidant and anti-bacterial potential. Additionally, the exact mechanism of action of the gel on the skin can be explored through extensive pharmacological tools at the molecular level so that it would be an effective way to use in a rational way.

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