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Preformulation Studies on Ethanolic Extract of *Pelargonium Graveolens* L'Her for Wound Management

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ABSTRACT

Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. *Pelargonium graveolens* has been used in folk medicines for the treatment of wounds. To substantiate this claim preformulation studies of ethanolic extract of *P.graveolens* have been undertaken in the present investigation. The objective of pre-formulation testing was to generate information useful to the formulator in developing stable and bio available dosage forms that can be man produced .The preformulation studies was carried out in terms of organoleptic evaluation, physiochemical evaluation, spectroscopic evaluation and biological evaluation. The tested ethanolic extract showed strong activity against clinical pathogen such as *E.coli*, *P.aeroginosa*. The result reveals that ethanolic extract of *P. graveolens* found to be suitable drug for to develop a wound dressing material for advanced wound management.

Key words: *Pelargonium graveolens*, preformulation, wound management.

1. INTRODUCTION

Preformulation is a stage of development during which the physicochemical properties of drug substance are characterized (Banker *et.al*., 2000). The goals of preformulation studies are to choose the right form of substance, evaluate its physical properties and produce a systematic

understanding of material's stability under various conditions leading to the development of optimum formulation (Brittain *et al*.,1995). The role of the preformulation team at every stage of the drug discovery and development process is to select appropriate methods to guide or alert other development teams about drug solubility and permeability issues (Sonali et al., 2013). Data from preformulation studies minimizes problems in later stages of drug development and provides necessary groundwork for successful formulation attempts thus reducing the cost of development of formulated product (Ravin *et al*., 1990).

Plant products have been used in different sectors like medical, industrial, veterinary and diagnostic applications. Although phytotherapeutic agents or phytomedicines are standardized herbal preparations that contain, as active ingredients, complex mixtures of plant materials in the crude or processed state. Phytopharmaceuticals are always mixtures of many constituents and are therefore very variable and difficult to characterize. Standardization of the preparation of herbal medicines still poses great challenges today. Therefore, critical steps have to be taken for their proper identification, screening and standardization to ensure quality (WHO, 1998)

Pelargonium graveolens L' Her is an aromatic and hairy herbaceous shrub, up to 1 m high. Leaves are prickly and carved, flowers are small, usually pink (Ana *et al*., 2014). Pelargonium species are widely used by traditional healers in the areas of Southern Africa by Sotho, Xhosa and Zulus for its curative and palliative effects in the treatment of diarrhea, dysentery, fever, respiratory tract infections, wounds, gastroenteritis, haemorrhage, kidney and bladder disorders (Anupalli *et al*., 2014). Therefore, the objective of this study was to characterize the preformulation properties of *P.graveolens* ethanolic extract in order to determine its suitability for formulation into thin films for wound management.

2. MATERIALS AND METHODS

Collection and identification of plant material

P.graveolens leaves were collected from Botanical garden of Mother Teresa Women's University, Kodaikanal, India. The fresh leaf sample was authenticated by National Institute of

Herbal Science, Plant Anatomy Research Center, Chennai with voucher specimen number PARC/2014/2050.

Preparation of extracts

The dried plant material was powered using mixer grinder and subjected to soxhlet extraction with ethanol. The residual extracts were evaporated to dryness and stored in refrigerator for further analysis.

Organoleptic evaluation

Various sensory parameters such as colour, odour, taste and nature were studied for the ethanolic extract of *P.graveolens*.

Physiochemical evaluation

Determination of pH

A 1 % w/v solution of ethanolic extract in distilled water was prepared, filtered and the pH read on a pH meter.

Determination of solubility

A 1.0 g of ethanolic extract dissolved in water and solvents such as ethanol, methanol, ethyl acetate, DMSO and observed the solubility nature of the extract.

Determination of Moisture content (loss on drying)

A 1.0 g of the extract accurately weighed and then placed in a hot air oven set at 60^{o}C and the extract dried to constant weight (approx. 3 h). It was cooled over silica gel in a desiccator and reweighed. The per cent loss in weight was taken as moisture content.

Determination of Ash content

A silica crucible was dried in an oven for 10 min and weighed on an analytical balance (AB54, Mettler Toledo, Switzerland). A 2.0 g of ethanolic extract was weighed and incinerated at a temperature not exceeding 450 °C until the sample turn into white color. After cooled down in a desiccator for 30 min, total ash content was calculated as

Ash value (%) = (W2 – W1)/M

Where, W1 = weight of the crucible, W2 = final weight of the crucible + ash, and M = initial weight of sample (Chukwuemeka etal., 2012).

Determination of mineral content

Mineral contents were carried out after acid digestion of 2g of the ethanolic extract with 10ml of a mixture of nitric acid and perchloric acid (2:1 v/v) until a clear solution was obtained. The digest was allowed to cool and then transferred into a 100ml standard flask and made up to mark with de-ionized water. The mineral elements like sodium, calcium and chlorides were analyzed with atomic absorption spectrophotometer (Zafar *et al*., 2010).

Spectroscopic evaluation

FTIR spectroscopy

Fourier transform infrared spectroscopy is one of the powerful analytical techniques which offer the possibility of chemical identification. The technique is based on the simple fact that substance shows selective absorption in infrared region. After absorption of IR radiations, the molecules vibrate, giving rise to absorption spectrum. For FTIR measurement the dried pellet was grinded with potassium bromide crystals analyzed on a Perkinelmer, (Model- Frontier) FTIR spectroscopy. The FTIR spectrum was obtained in the mid IR region of 400 - 4000 cm-1 and recorded using ATR (Attenuated Reflectance Technique).

Biological evaluation

Antibacterial screening

The efficiency of extracts of *P. graveolens* leaves were evaluated against *Escherichia coli* and *Pseudomonas aeroginosa* using disc diffusion method. Muller-Hinton Agar plates were inoculated with different bacterial strains and sterile Whatman filter paper discs (3mm) were containing different extracts of *P. graveolens* (100µl). Sterile discs were placed on the plates and the plates were incubated at 37°C for 24 hours in an incubator and observe zone of inhibition.

3. RESULTS AND DISCUSSIONS

Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system. Every drug has intrinsic chemical and physical properties which has been consider before development of pharmaceutical formulation. This property provides the framework for drugs combination with pharmaceutical ingredients in the fabrication of dosage form (Kailash Vilegave et al., 2013). Drug evaluation is a verification of its identity and determination of its quality and purity and detection of nature of adulteration. Preformulation studies was carried out in terms of organoleptic evaluation, physiochemical evaluation,spectroscopic evaluation and biological evaluation.

Organoleptic evaluation

Organoleptic authentication means the study of herbal medicines using various organs of senses which includes the analysis of color, odour, taste, shape, size, texture, weight, structure, *etc*. Obviously the initial visibility, odour, color, taste, sight and smell of the plant or plant extract are specific to identify itself. Organoleptic evaluation is simplest analysis but most common practice among the practitioners and herbalists. Amber color, aromatic odour and creamy nature were observed in ethanolic extract of *P.graveolens*.

Physicochemcial evaluation

Physicochemcial evaluation includes pH, solubility, moisture content, ash content and mineral content were analysed for ethanolic extract of *P.graveolens*. The pH of the extract is slightly acidic (5.2). According to the pH-partition hypothesis, weakly acidic drugs, which exist predominantly in the unionized form at gastric pH, are well absorbed from the stomach. Although the chemistry of the extract is yet to be elucidated, it is probable that it is likely to be well absorbed from oral dosage forms in the stomach due to the weakly acidic nature. Solubility of the drug plays an important role in drug development. Ethanolic extract found to be soluble in

ethanol, methanol, DMSO and water. The percentage of the moisture content was found to be 10.1%w/w. Determination of the moisture content helps prevent degradation of drug. The ash content is generally recognized as a measure of quality for the assessment of the functional properties of foods. Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid,*etc*). Mineral content like sodium was found to be 0.002%, calcium content was 0.03% and chloride measured 0.012%. Determination of physicochemical parameters is very important in order to maintain the purity of herbal medicines (Kunle *et al*., 2012).

Spectroscopic evaluation

The FTIR spectra indicate various functional groups present at different positions. The FTIR spectrum obtained for ethanolic extract of *P.graveolens* (Fig.1) displays a number of absorption peaks, reflecting its complex nature due to biomolecules. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, carboxylic acids, amide I, alkene, non-acid carbonyl, aromatic amines, aromatics, alkynes and alkyl halides in ethanolic extract. These observations may be due to the nature of biological active components and the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for antibacterial activity.

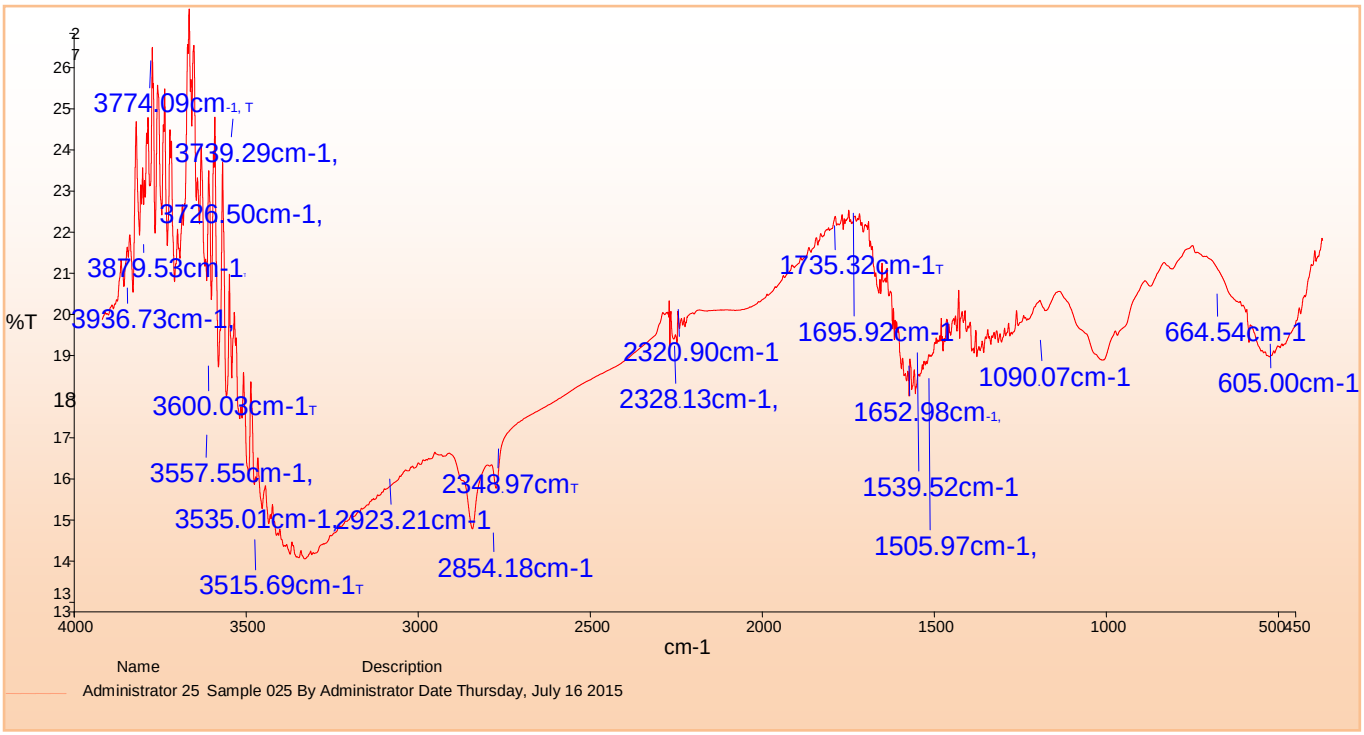

Figure 1- FTIR analysis of ethanolic extract of *P.graveolens*

Table-1 FTIR peak values and functional groups of ethanolic extract of *P.graveolens*

S.No	Peak values (cm^{-1})	Functional groups
1.	3936.73	Unknown
2.	3879.53	Unknown
3.	3774.09	Phenol
4.	3600.03	Unknown
5.	3557.55	Unknown
6.	3535.01	Unknown
7.	3515.69	Alcohol ,phenols
8.	2923.21	Alkanes
9.	2854.18	Carboxylic acids

10.	2348.97	Unknown
11.	2320.90	Unknown
12.	1735.32	Amide I
13.	1695.92	Alkene
14.	1652.98	Non-acid carbonyl
15.	1539.52	Aromatic amines
16.	1505.97	Aromatics
17.	1090.07	Alcohol
18.	664.54	Alkynes
19.	605.00	Alkyl halides

Biological Evaluation

Antibacterial screening

Chronic wounds are usually associated with the presence of bacteria which will cause delayed healing. To maintain a bacteria free wound, the drug should reduce the infection caused by resistance microorganisms such as *S.aurous,P.aeroginosa and E.coli* Therefore, *in vitro* antibacterial activity of ethanolic extract of leaves of *P.graveolens* was evaluated by disc diffusion method. The plant extract exhibited pronounced activity against all the bacterial strains tested. Results of antibacterial activity of ethanolic extract of *P.graveolens* are presented in figure-2 and table 2. *E.coli* showed maximum zone of inhibition of 1.82cm at the test concentration of 100µl in case of *P.aeroginosa* showed 1.90cm at the concentration of 50µl. Our result confirms the plant extract being active against both clinical and laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against multidrug resistant bacterial strains.

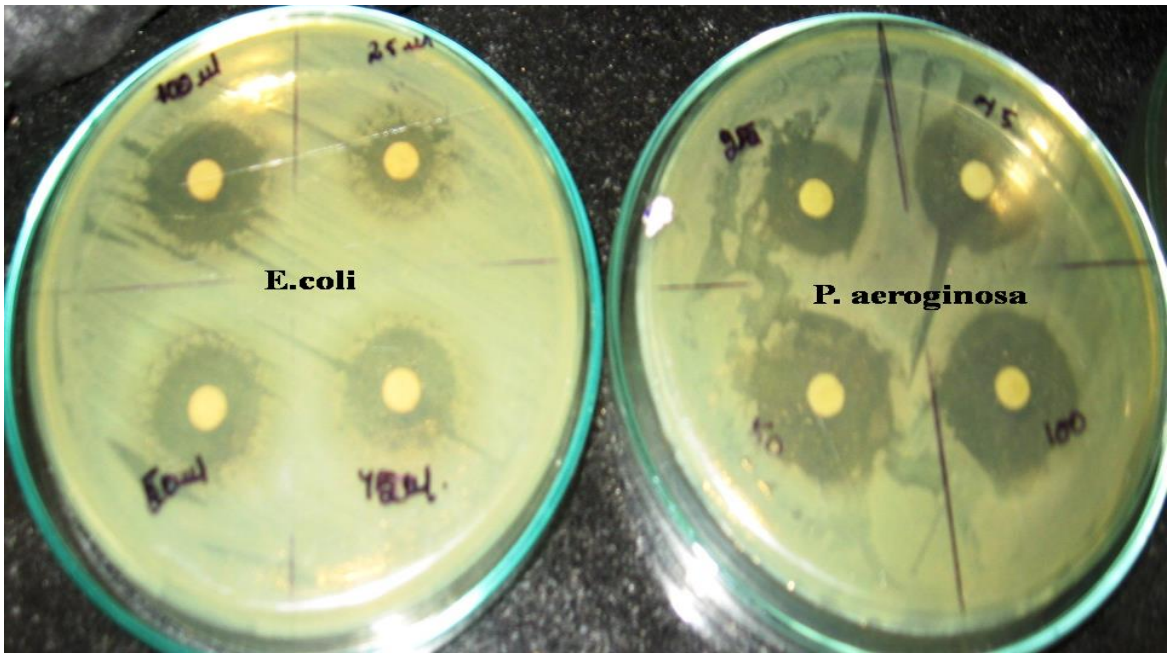

Figure-2 Antibacterial screening of ethanolic extract of *P.graveolens*

Table-2 Preformulation evaluation of ethanolic extract of *P.graveolens*

S.No	Tests	Ethanolic extract	Limits
1.	**Organoleptic evaluation**		
	colour	Amber	
	odor	Aromatic	-
	nature	creamy	
2.	**Physiochemical evaluation**		
	pH of 1% solution	5.2	4.50 - 5.60
	solubility	Soluble in ethanol,methanol,DMSO & water	
	moisture content	10.1	<14%
	ash content	2.2	2%
	mineral content		
	sodium	0.002	<0.2%
			<0.65

	calcium chlorides	0.03 0.012	<0.40
3.	**Spectroscopic evaluation** FTIR	Alcohol ,phenols , alkanes, carboxylic acids alkynes,non-acid carbonyl, aromatics, alkyl halides	-
4.	**Biological evaluation** Antibacterial screening *E.coli* *P.aeroginosa*	 1.82cm MIC for 100µl 1.90cm MIC for 50 µl	-

4. CONCLUSION

This study demonstrates the feasibility of developing drug into the nanofilm for advanced wound management. The physiochemical and biological properties manifested by ethanolic extract of *P.graveolens* in this study substantiate its use in wound healing and validates its commercial exploitation in preparation of wound dressing materials. To facilitate clinical development and to reduce attrition rate, a thorough study of physicochemical properties of drug candidates is desired. This also serves as the foundation for developing robust formulations.

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