Research on the Herbal Formulation "Hectasor Ointment" Topical Dosage form Used against Psoriasis

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Abstract

Psoriasis is recurring skin disorder due to the accelerated cell growth characterized by thick, silvery, red and painful patches. It may be genetic due to malfunction of immune system. It is an uncomfortable disease and painful affecting patients life bearing social stigma .

There is no appropriate treatment for psoriasis, however corticosteroids, immuno suppressant and phototherapy coal tar treatment are used to cure the disease but having side effects research still required to formulate new drugs not only from synthetic origin but also from the natural origin. In this regard, some herbs according to ancient literature are considered beneficial as reliable treatment in psoriasis, but clinical findings are still lacking. Moreover some herbal remedies available in the literature are used in the treatment of psoriasis *Capsicum anum L, Scutellaria baicalensis, Calendula officinalis, Hypericum perforatum, Psoralea corylifolia, Phellodendron chinensis and Sophora flavescense* as both monoherbal and in polyherbal remedies.

A polyherbal Hectasor ointment is composed of seven culinary herbs (i.e. *Azadirachta indica L, Psoralea corylifolia L, Punica granatum L, Foeniculum vulgare, Uncaria gambir, Curcuma amada, Mentha piperita*). The topical dosage form was prepared with tar obtained from these culinary herbs in the emollient base.

1. INTRODUCTION

Modernized medical phenomenon have provided many technologies like Magnetic Resonance Imaging (MRI), chemotherapeutic agents, organ transplantation, laser surgery, dialysis and other several life saving devices. This incredible advancement has often made people lose sight of the history of healing. Humans have not always considered technology to heal or cure many disorders. However people relied on a link between nature and its healing power. Nature was part of routine life and people were closely familiar with it. For example, before recorded

history, plants have been used for food, medicine, clothing, house making and, tools as well as in religious ceremonies. The World Health Organization (WHO) also refers to herb as "people's medicines" (Fried, B. and Sherma *et al* ; 1994). These can be used alone or in combinations with other herbs to achieve desired pharmacological effects including bitter principles ,minerals, mucilages, tannins, volatile oils, vitamins, phenols,amines, alkaloids, saponins, anthocynins, anthoquenons, glycosides, coumarins and others. These are the chemicals present in the herbs and produce specific medicinal effect on the human body. These active principles have prime role in herbalism and in formulating new herbal dosage form as well as in prescribing them for remedial purpose.

Medicinal herbalism includes five basic systems Arabic, Chinese, Indian or Ayurvedic, Egyptian-Greco-Roman, and Native American. Because of these systems, herbs can be classified in different ways. Some systems still classify herbs containing plant parts, botanical family,morphology or colors but Chinese system have complex classification system based on "chi" or "body energy concepts". This classification strategy is successful in association with the human body to proper herb usage. For the substitution of herbs a simple method is used to classify herbs. In this method, herbs are classified by their active principles, which is easier to id entify herbs using 'taste' and 'smell' and become useful when needed to replace them. Despite of many available options, herbal remedies still remain one of the most widely prescribed remedies throughout the world. In the condition like psoriasis Hectasor ointment is ensuring its effectiveness and it is accessible to the patients.

2. MATERIALS AND METHOD:

To prepare the Hectasor ointment a step-wise approach was taken, the procedure includes following steps: Selection of herbs, Design of formulation, Chemical Evaluation of formulation and stability studies.

In the first step, a literature search was performed to assess the Traditional use of particular herbs in Psoriasis. Based on the data available following seven herbs (Table-1) were selected and as it's a formulation prepared from the extract of seven herb named hectasor ointment, used against Psoriasis.

Below is the different parts of the herbs which were used in the preparation of ointment. *Azadirachta Indica* L. (seed), *Curcuma amada* (Rhizome), *Foeniculum vulgare mill* (fruit), *Psoralea corylifolia* L. (seeds), *Uncaria gambir* (extract), *Mentha piperita* (leave), *Punica granatum* L. (seeds with pulp).

2.1. Collection and Identification

The tree of *Azadirachta indica L. (*neem) grows in the University of Karachi, Karachi, Neem's seed were collected from Karachi University campus, seeds of the plant *Psoralea corylifolia* L., *Punica granatum* L., *Foeneculam vulgare Mill;* rhizomes of the plant *Curcuma amada* extract *Uncaria gambir* from local market essential oil of the plant *Mentha piperita* were purchased from Herbal market , Saddar, Karachi and all plants and their parts were used identified by Prof. Dr. Ghazala H.Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi with specimen nos. 00B34, 00B35, 00B36, 00B37, 00B38, 00B39 and 00B40. Specimen of all plants deposited in the herbal meusem department of Pharmacognosy, Faculty of Pharmacy University of Karachi.

2.2. Extraction:

The seeds of plant source *Azadirachta indica L.* (100g), *Psoralea corylifolia L.* (100g),

Punica granatum L. (100g), *Foeniculum vulgare Mill* (100g) *Curcuma amada* (100g), *Uncaria gambir* (50g) were weighed, granule separately followed by percolation method in menthol for 15-20 days watered occasionally shaking practiced on every second day. The solutions were filtered through Whatsmann filter paper. This process was repeated thrice and all three filtrates were collected, evaporated at specific pressure and controlled temperature 40°C through rotary evaporator. A dark brown thick semi solid residue (100g) was obtained. This residue was lyophilized for the complete removal of water from the extract at low pressure to almost solid crystalline consistency.

2.3. Identification of Chemical Constituents by Color Reaction

The following physiochemical analysis of crude drug (tar) was carried out for identification of chemical constituents present in the crude drug:

Table 1. Existence of naturally occurring compounds in crude drug material

S. no	Chemicals	Observation	Result	Inference	Duration of response
1	Alkaloid's Dragendroff Test	Orange ppt	Alkaloid present	+++	Quick
2	Froth test for saponins	No reaction	Saponins absent	---	Quick
3	Lead acetate test for tannins	Yellowish white ppt	Tannins present	+++	Quick
4	Libermann-Burchard test for triterpenes	Dark pink solution	Interpene present	+++	Quick
5	Salwaski test for Sterols	Prominent purple ring is formed	Sterols present	+++	Quick
6	Test for Flavanoids	Ribbon pink tomato red color	Flavanoids present	+++	Quick
7	Test for Protein	Powder insoluble in ethanol/water	Protein present	+++	Quick

2.4. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is an analytical method that is widely used for the separation, isolation, identification, and quantification of components present in a mixture. Components of the mixture are carried through the stationary phase by the smooth flow of a mobile phase. Separations are based on differences in elution rates among the sample components (Fried, B. and Sharma *et al* ; 1994).

TLC was chosen over other chromatographic methods because of various

simple, quick and cheap procedure that can be used for the analysis of mixtures. TLC is a mode of liquid chromatography in which the sample is applied as a small spot or streak to the origin of a thin adsorbent layer such as silica gel, alumina, cellulose powder, polyamides, ion exchangers or chemically bonded silica gel supported on a glass, plastic, or metal plate. This layer consists of finely divided particles and constitutes the stationary phase. The mobile phase is a solvent or a mixture of organic and/or aqueous solvents in which the spotted plate is placed. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or pressure (Fried, B. and Sharma *et al* ; 1994, Skoog *et al*; 1998).

TLC separations take place in the "open" layer, with each component having the same total migration time but different migration distances. Plates can be visualized, depending on the chemical structure of the compounds at visible light, UV-254 nm and 365 nm or by using spray reagents (Wagner *et al* ; 1996).

The effectiveness of the separation depends on the mixture to be separated, the choice of the mobile phase and the adsorption layer (Fritz *et al* ; 1987).

The term retention factor Rf, is used to describe the chromatographic behavior of sample solutes. The Rf value for each substance is the distance it has moved divided by the distance the solvent front has moved. Usually, the centre of each spot is the point taken for measurement. Comparison of Rf values makes it possible to research complex mixtures qualitatively. The extent of the surface of the spot is a measure for the quantity of the material present (Fritz *et al* ; 1987).

The selection of a solvent for application of the sample can be a critical factor in achieving a reproducible chromatography with distortion free zones. In general, the application solvent should be a good solvent for the sample and should be as volatile as possible and more non-polar (Fried, B. and Sherma *et al* ; 1994). The solvents chosen during this research for extraction were therefore methanol chloroform.

Silica gel was chosen as stationary phase, since it is an efficient adsorbent for the TLC separation of most of the plant extracts and plant drug extracts (Barbetti *et al* 1990, 6-Houghton et al., 1996).

Thin layer chromatography (TLC) was employed in this study to analyze the compounds present in the crude plant extract of methanol.

Pre-coated TLC plates were used. The extracts were applied as a band on TLC plate about 1.3 cm from the edge (spotting line), using 20 capillary tubes. The mobile phase saturated with chloroform/methanol = 8:2 (v/v). TLC separation was performed at room temperature, i.e. 18-23°C. After sample application of 3 of Hectasor extracts the plates were placed vertically into a solvent vapor saturated TLC chamber. The spotting line was about 0.5 cm from the developing solution. After the mobile phase had moved about 80% from the spotting line, the plate was removed from the developing chamber.

2.5. Detection Methods:

The eluted spots, representing various fractions/compounds, were visualized by different detection methods .i.e

iodine vaporization, visualization seen by naked eye.

Visualization by U V light on different wave lengths such as 254nm and 366nm.

TLC plate was sprayed with Dragendorff reagent. This reagent was prepared by dissolving 8 g of

potassium iodide (KI) in 20 ml water and by adding it to 0.85 gram of bismuth nitrate ($BiNO_3$) dissolved in 10 ml 17.4 M glacial acetic-acid to which 40 ml D.D. water was poured to detect the alkaloid substances in the material. The plate was sprayed with cerium ammonium sulphate (CAS) reagent. This reagent was prepared by mixing 100mg of CAS with 5% concentrated H_2SO_4 solution in water. This solution is used to determine the existence of sterols, terpenes, hydrocarbons, esters and other low melting compounds. After drying the plate for 5-10 minutes under a fume hood it was heated at 100°C for 3 minutes to visualize all such compounds.

2.6. Formulation of "Hectasor Ointment"

Topical dosage form "Hectasor Ointment" was prepared in two steps that are:

i. Solubility of Tar

ii. Mixing

2.6.1. Solubility of Tar

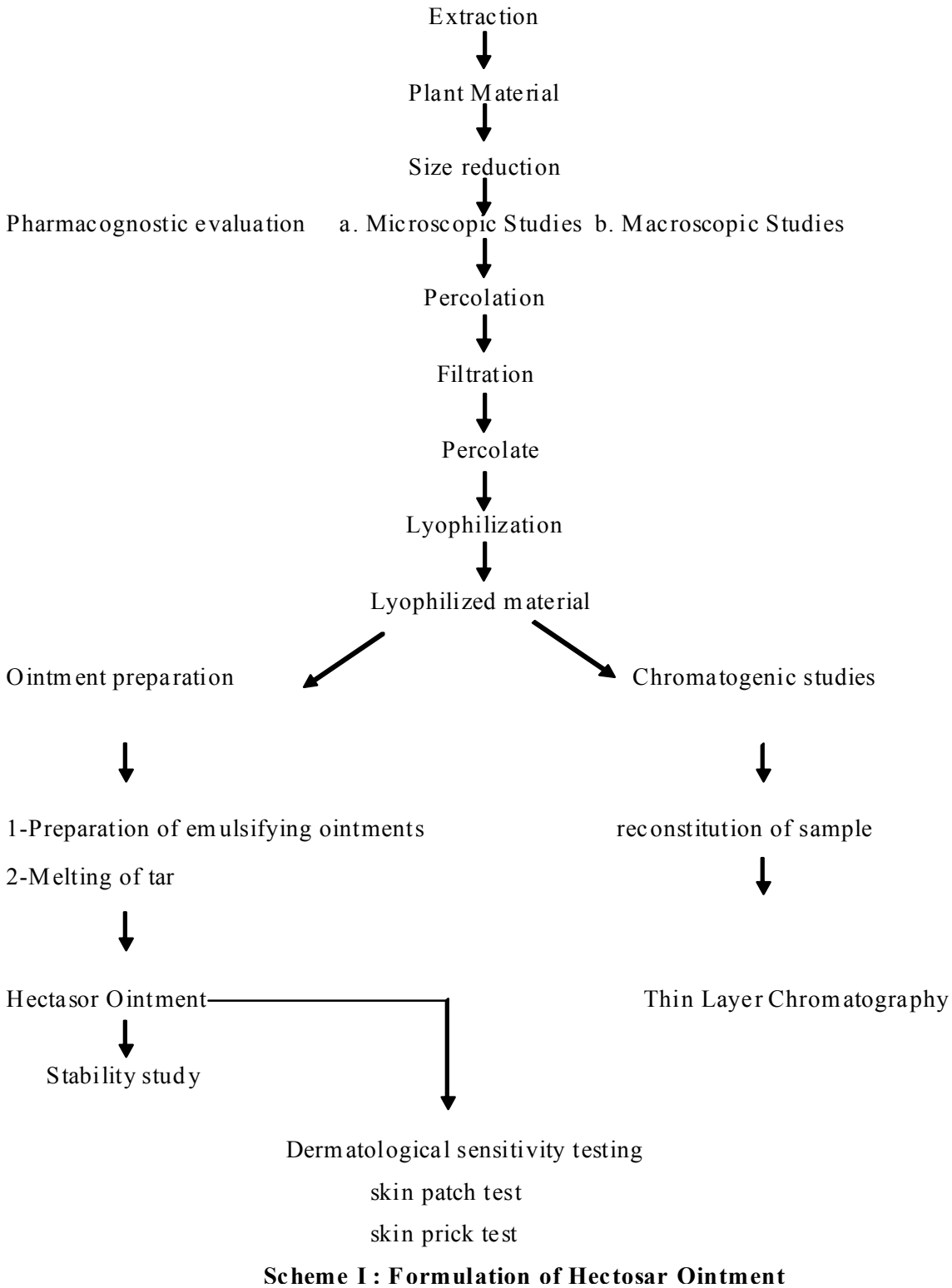
Solubility of tar is major step in the formulation of Hectasor Ointment

Tar obtain from freeze-drying was very hard. We used various solvents in this regard to soluble and make formulation in ointment consistency.

Solvent	**Solubility**	**Inference**
Water	Not soluble	Clear Solution
Water (heat)	Not soluble	Clear Solution
Liquid Paraffin	Not soluble	Clear Solution
Liquid paraffin(heat)	Partially soluble	Dense appearance
Propylene glycol	Not soluble	Clear Solution
Ethanol	Partially soluble	Dense appearance
Methanol	Soluble	Dark brown
Dimethyl sulfoxide	Freely soluble	Thick Dark brown

2.6.2. Method of Formulation (Mixing)

Accurately weigh w/w both the active ingredient and excipient i.e. hydrophobic ointment base and tar. Formulation was formed in two steps. In first step, tar was taken in beaker and melted in water bath after melting of tar pour it on mixing the tile and add specific amount of hydrophobic base and mix it by means of spatula. When tar and base are homogenized, transfer it into the ointment jar and label it as "Hectasor Ointment" (Scheme - I).



Scheme I : Formulation of Hectosar Ointment

2.7. Stability Studies of Topical Dosage Form "Hectasor Ointment"

Stability studies of herbal ointments are very important for developing stable topical dosage form. Word "stability" describes the ability of a specific formulation in a specific closure system (container) remaining within its chemical, physical, microbiological, therapeutic and toxicological levels in a define period of time.

First criteria in stability studies is no change in physical qualities of formulation, physical changes may include any change in appearance, consistency and change in weight.

There are several factors that affect stability of dosage form including temperature, light, and humidity. Degradation varies with temperature and humidity. Ointment consistency changes due to adsorption of moisture in the base.

Any chemical, physical and microbial change in formulation alters the therapeutic activity of compound and may lead to severe adverse effects, so the assessment of stability studies of any dosage form is a prime concern in designing of a new product.

2.8. Effect of Temperature and Humidity

For the determination of shelf life of topical dosage form Hectasor Ointment stability study was performed. In this study we check the effect of temperature and humidity on extreme conditions and carefully observe the gradual changes appearing in the dosage form.

3. RESULTS AND DISCUSSION

Sample: Randomly selected three samples of weight 25gm, 50gm and 100gm.

Control: 25gm sample refrigerated at controlled temperature for 12months.

In this study, first determine the effect of temperature on ointment and observe the changes that occur and then determine the effect of humidity in a controlled room temperature and observe the effect of humidity along with temperature, Results from these observations give us an idea about the stability of dosage form.

Table 2. Effect of temperature on topical dosage form for stability study.

Weight (g)	Hectasor Ointment				
	Months				
	1	3	6	9	12
25	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+

+ no change in formulation

Table 3. Effect of humidity in a controlled room temperature

Weight in g	Hectasor Topical Ointment														
	30%					50%					60%				
	Hours					Hours					Hours				
	1	6	12	18	24	1	6	12	18	24	1	6	12	18	24
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\+ no change in appearance and consistency.

4. CONCLUSION

At different temperatures stability of product was checked and confirmed by physico chemical examinations such as consistency of ointment, colour appearance and odour observed by organoleptic evaluation, and the product found was almost stable except a slight melting of ointment observed at 40 °C and 75% humidity.

5. REFERENCES

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