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In-vitro release and *Ex- vivo* skin permeability study of Polyherbal formulation containing *Dalbergia sissoo* and *Tagetes erecta* leaves extracts

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Abstract

Polyherbal formulation has been utilized all over the world because of many medicinal and therapeutic uses, without having any side effects. Individual plant active phytochemical components are insufficient to achieve the intended therapeutic effects, so making polyherbal formulation with a number of different plants shows better synergistic effect. The present study investigated the In-vitro and Ex-vivo permeability study individually of Dalbergia sissoo, Tagetes erecta, and polyherbal formulation (having combination of both the extracts) and compared with rutin suspension. The formulation was prepared with combination of hydroalcholic extract of leaves of Dalbergia sissoo and Tagetes erecta and evaluated.

Keywords: Polyherbal, Permeability, Synergistic, Phytochemical

Introduction

The human skin is the largest organ in the body, with a surface area of around 2 square meters and a share of blood flow going through it. It acts as a permeability barrier to prevent different chemical and biological substances from being absorbed transdermally 1. Skin delivery is an efficient method of drug delivery that avoids many of the problems associated with parenteral, inhalation, and oral routes. It has piqued the interest of researchers in recent years due to these benefits for the skin 2. To maintain equilibrium, or homeostasis, within the body, the skin regulates the entry and exit of several substances, preventing moisture loss and regulating body temperature 3. Topical drug delivery entails transferring the medication from a topical substance to a locally targeted area while promoting dermal circulation throughout the body and deeper tissues 4. In the present study polyherbal formulation was formulated with the extract of *Dalbergia sissoo* and *Tagetes erecta* to evaluate the release of rutin. *Dalbergia sissoo* consists of different phytochemicals such as alkaloids, flavonoids, protease inhibitors, isoflavonoids, cyanogenic glycosides, and lectins, non-protein amino acids, amines, coumarins, phenylpropanoid, diterpenes, sesquiterpenes, and triterpenes 5,6,7. *Dalbergia sissoo* plant used to treat many diseases such as scabies, expectorant, antipyretic, disorders of leucoderma, wound healing and dysentery 8,9. *Tagetes erecta* contains quercetagetin, a quercetagetin glucoside, phenolics, syringic acid 10. The marigold plant contains terpenoids, flavonoids, quinones,

coumarins, carotenoids and volatile oils 11,12. It is effectual in case of piles, ulcers, kidney troubles, muscular pain, carminative, stomachic, scabies and liver complaints and wound healing 13. Polyherbal formulations have been utilized all over the world because of many medicinal and therapeutic uses, without having any side effects. Individual plant active phytochemical components are insufficient to achieve the intended therapeutic effects, so formulation with combination of hydroalcoholic extract of two separate plants was formulated and evaluated.

Material and Methods

Selection, Collection, Identification of plant

The plant material was collected from the local areas of Sonipat, Haryana and authentication of plants from Raw Material Herbarium and Museum, Delhi (RHMD), India.

Extraction of plant material using Soxhlet apparatus

The plants material was washed and shade dried before extraction and kept in well closed container for extraction. The dried plant material was coarsely powdered. Dried and powered leaves of *Dalbergia sissoo, Tagetes erecta* were successively defatted with petroleum ether and then placed in a thimble of soxhlet apparatus (separately). The extraction was carried out using hydroalcoholic solvent system at 40-60^{o} C temperature. After the extraction process, the extract of sample were filtered and concentrated to dryness. Extracts were collected in air tight container 14.

Preparation of polyherbal formulation 15,16

The polyherbal ointment formulation was prepared by mixing the *Dalbergia sissoo* and *Tagetes erecta* leaves extracts in 2:1 ratio incorporated in the base containing wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin.

Evaluation parameters of polyherbal formulation 17,18,19

The polyherbal formulation was evaluated by the following physicochemical parameters:

- **Colour and odour**

Visual inspection was used to evaluate colour and odour.

- **pH**

The pH of the formulation was recorded using a digital pH meter. A measured amount of the material was dissolved in purified water and left for a duration of two hours. The pH was measured three times, with the average readings being taken into account.

- **Washability**

A bit of ointment was rubbed onto the skin and let wash under running tap water. The time when the ointment was completely removed from the skin was recorded.

- **Homogeneity**

Polyherbal ointments were evaluated for homogeneity by visual appearance and touch.

- **Viscosity**

A Brookfield viscometer was used to measure viscosity. Spindle number four was submerged in the middle of the 10 ml beaker containing the polyherbal mixture. The ointment's viscosity was evaluated at room temperature and with different speeds. To eliminate any errors that might have occurred, the reading was recorded in triplicate.

***In-vitro* release study**

Drug release is the process by which a drug leaves a drug product and is subjected to absorption, distribution, metabolism, and excretion (ADME), eventually becoming available for pharmacological action.

In-vitro drug release study of the extract of *Dalbergia Sissoo, Tagetes erecta* and Polyherbal formulation and Rutin suspension was carried out using locally developed Franz diffusion cells with diffusional area of 2.011 cm^2. An egg membrane was placed between donor and receptor compartments. An externally powered Teflon-coated magnetic bead was used to continually swirl the phosphate buffer pH 5.5 with 30% v/v ethanol that was contained in the receptor compartment. To emulate physiological conditions, the cell's temperature was kept at 37 ± 1 °C. Approximately 50 mg extract of *Dalbergia sissoo Tagetes erecta* and Polyherbal formulation and Rutin suspension (equivalent to 5 mg of rutin) was loaded on the donor different compartments of Franz diffusion cells respectively. Samples were taken out at various intervals and replaced with an identical volume of fresh buffer. The drug concentrations in aliquot were

evaluated at 280 nm against suitable blank, using UV spectrophotometer. The cumulative percent drug release was plotted as a function of time 20, 21, 22.

Method for egg membrane preparation

The material of egg shells were removed and then it was submerged in the diluted hydrochloric acid for half an hour. The egg membrane was separated manually and washed with distilled water thoroughly 23.

Drug release kinetics

The data from the in-vitro release investigation were fitted into a variety of kinetic models, including zero order, first order, Higuchi's, and Korsemeyer Peppa's model, in order to clarify the method and mechanism of drug release 21.

***Ex-vivo* permeability study**

Ex-vivo permeability study of the extract of *Dalbergia sissoo, Tagetes erecta* and Polyherbal formulations was carried out using locally developed Franz diffusion cells (Figure 1) with diffusional area of 2.011 cm^2. Rat skin was placed between donor and receptor compartments. An externally powered Teflon-coated magnetic bead was used to continually swirl the phosphate buffer pH 7.4 with 30% v/v ethanol that was contained in the receptor compartment. To mimic physiological conditions, the cell's temperature was kept at 37 ± 1 °C. The extract (50mg) of *Dalbergia sissoo, Tagetes erecta* and polyherbal formulation equivalent to 5 mg of Rutin was loaded on the different donor compartments respectively. Samples were taken out at various intervals and replaced with an identical volume of fresh buffer. The drug concentrations in aliquot were evaluated at 280 nm against suitable blank, using UV spectrophotometer 20,21,22.

The steady state flux (Jss) were calculated using following equations:

$$\text{Steady state flux (Jss)} = \frac{\text{Amount of drug permeated}}{\text{Time x Area of membrane}} = \frac{Q}{t \times A}$$

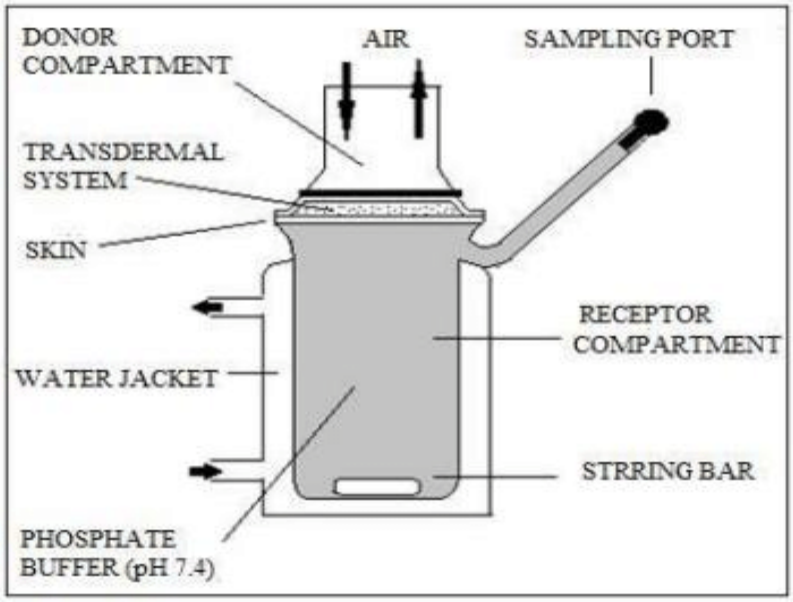

Figure 1. Franz diffusion cell 24

Results and Discussion

Evaluation parameters

Table 1. Evaluation parameters of Polyherbal ointment

S. no.	**Parameters**	**F1**	**F2**	**F3**	**F4**
1	Color	Yellowish green	Yellowish green	Yellowish green	Yellowish green
2	Odour	Characteristics	Characteristics	Characteristics	Characteristics
3	Consistancy	Smooth	Smooth	Smooth	Smooth
4	PH	6.25±0.5	6.32±0.2	6.12±0.3	6.43±0.4
6	Washability	Good	Good	Good	Good

S. no.	**Speed (rpm)**	**Viscosity cps**			
		F1	**F2**	**F3**	**F4**
1	30	20200	20700	21200	21300
2	60	1000	11200	10500	11500

***In-vitro* release study**

Polyherbal formulation showed the maximum release of 63.948% in comparison to the rutin suspension which released only 3.15% in 24hours. The higher amount of rutin released in polyherbal formulation was attributed due to its higher rutin content. Plant extract of *Tagetes erecta* and *Dalbergia sissoo* also showed significant results of 13.444% and 8.871% rutin released in comparision to the rutin suspension as shown in table 1 and graph showed in figure 2 and 3.

Table 1. *In-vitro* release

Time (hrs.)	**Suspension (Rutin)**	**Polyherbal formulation**	***Tagetes erecta* extract**	***Dalbergia sissoo* extract**
0	0	0	0	0
1	0.262	14.016	0.187	1.031
2	0.058	16.639	0.802	1.855
3	0.168	24.854	2.323	3.123
4	0.289	31.777	3.791	3.251
5	0.423	34.929	5.249	3.802
6	0.424	35.613	5.524	4.038
24	3.150	63.948	13.444	8.871

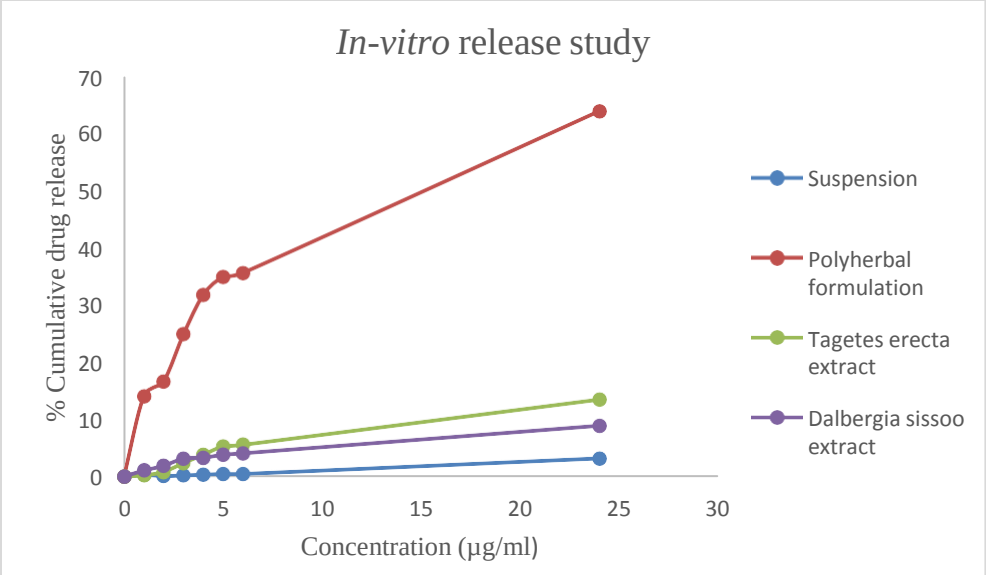

Figure 2. *In-vitro* release profile of rutin suspension, polyherbal formulation, and plant extract of *Dalbergia sissoo* and *Tagetes erecta* in 24 hours

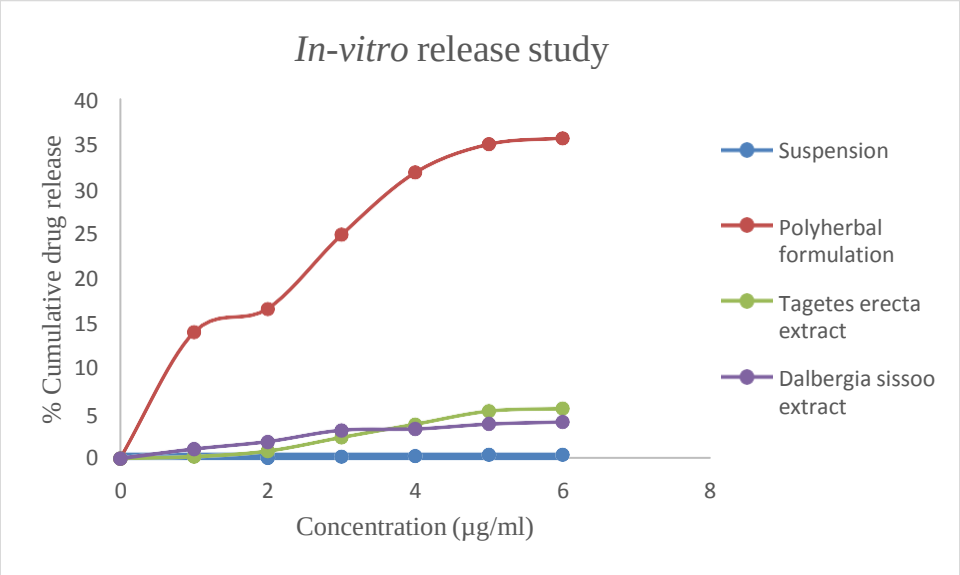

Figure 3. *In-vitro* release of rutin suspension, polyherbal formulation, and plant extract of *Dalbergia sissoo* and *Tagetes erecta* in 6 hours

Drug Release Kinetics

Several theories/kinetics models describe drug dissolution from immediate and modified release dosage forms such as Zero order, first order, Higuchi model and Korsmeyer-Peppas model. To study the drug release kinetics, data obtained from *in-vitro* drug release studies were fitted into various kinetics models. Higuchi model showed the maximum R^2 value (0.9539) as shown in table 2. This showed that the drug release profile followed by Higuchi model, i.e the rutin release from the base was diffusion controlled.

Table 2. Drug Release Kinetics

Model	**Zero order**	**First order**	**Higuchi model**	**Korsemeyer-Peppas model**
R^2 value	0.9412	0.9148	**0.9539**	0.9477

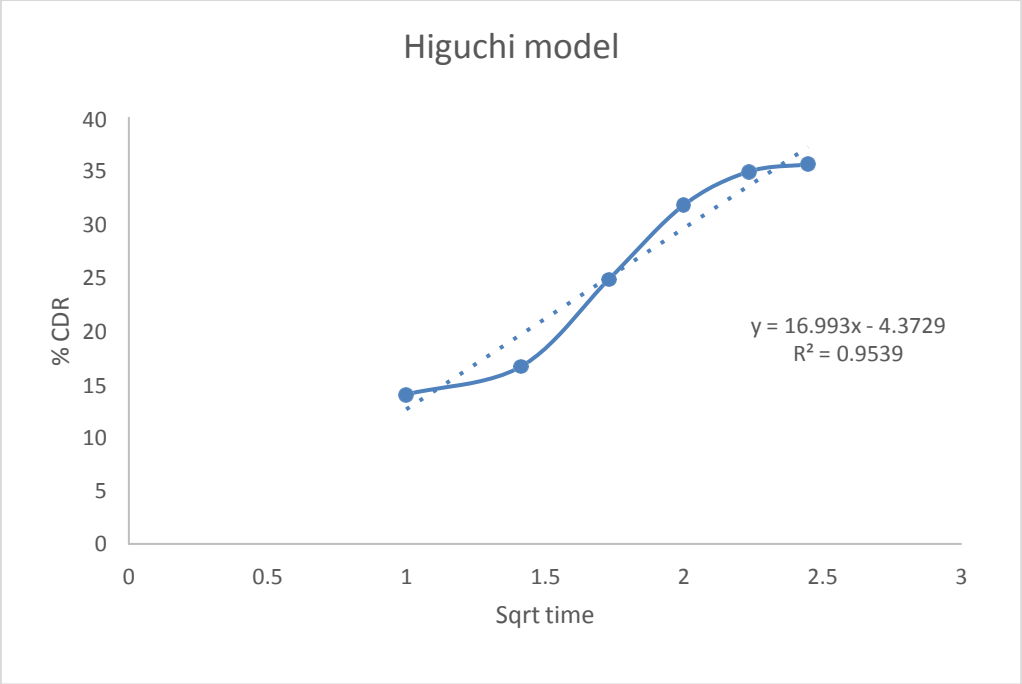

Figure 4. Graph shows the Higuchi model

***Ex- vivo* permeability study**

Ex- vivo permeability study showed that the Polyherbal formulation has the maximum flux i.e 0.21µg cm^{-2}/ h. This showed that Polyherbal formulation has the maximum release in comparison to the rutin suspension. Plant extract of *Tagetes erecta* and *Dalbergia sissoo* also showed significant results in comparision to the suspension of rutin as shown in table 3 and graph showed in figure 5.

Table 3. Steady state flux (Jss)

	Rutin Suspension	**Extract of *Tagetes erecta***	**Extract of *Dalbergia sissoo***	**Polyherbal formulation**
Flux (µg cm^{-2}/ h)	0.0015	0.035	0.028	0.21

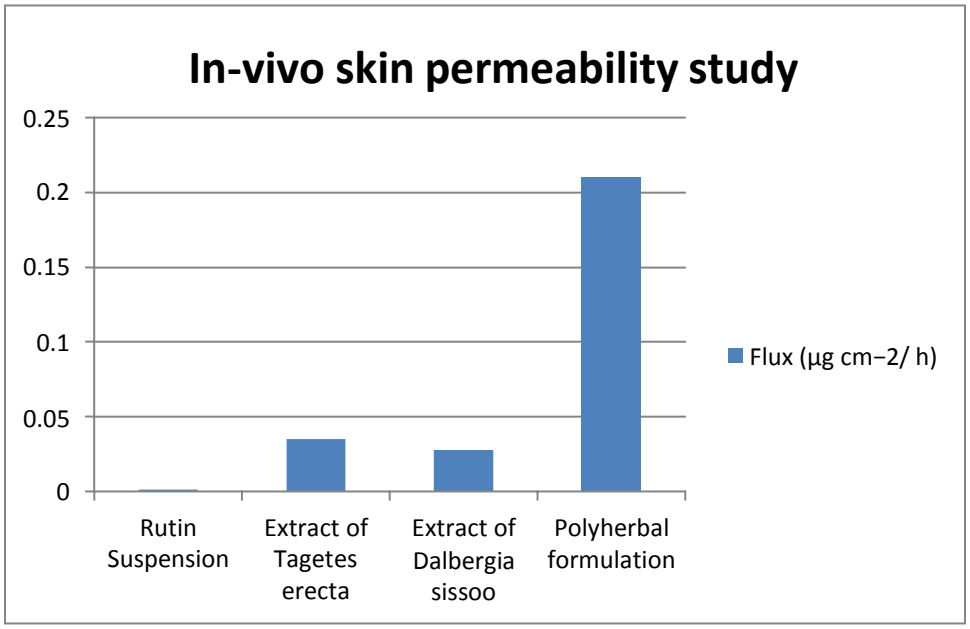

Figure 5. Graph shows the flux (Jss)

Conclusion

The present study evaluated the *In-vitro* and *Ex-vivo* permeability study individually of *Dalbergia sissoo, Tagetes erecta,* and polyherbal formulation (having combination of both the

extracts) and compared with rutin suspension. Polyherbal formulation showed the maximum release of 63.948% in comparison to the rutin suspension which released only 3.15% in 24 hours. The higher amount of rutin released in polyherbal formulation was attributed due to its higher rutin content. Drug release kinetics studies were also performed and the data obtained from *in-vitro* drug release studies were fitted into various kinetic models. Higuchi model showed the maximum R^2 value (0.9539). This showed that the drug release profile followed by Higuchi model, i.e the rutin release from the base was diffusion controlled. *Ex- vivo* permeability study showed that the Polyherbal formulation has the maximum flux i.e 0.21µg cm^{-2}/ h. This showed that Polyherbal formulation has the maximum release in comparison to the rutin suspension. Plant extract of *Tegetes erecta* and *Delbergia sisso* also showed significant results in comparision to the suspension of rutin.

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