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ASSESSEMENT OF LARVICIDAL ACTIVITY AND SKIN PENETRABILITY OF TULSI

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ABSTRACT

Prevalence of mosquito borne diseases is one of the world's most health hazardous problems like malaria, filariasis, etc. Also there are certain penetration enhancers available in market e.g. dimethyl sulfoxide, but they have disadvantages that they should be used below 1 % concentration due to its adverse effects. Natural penetration enhancers are rarely available in market but yet not studied for their full strength. Present study deals with larvicidal (Larvae: *Aedes aegypti*) activity and skin penetration activity of two different species of Tulsi i.e. *Ocimum sanctum* Linn. and *Ocimum tenuiflorum* Linn. (Labiatae). Tulsi contains mainly volatile oil which is composed of different types of terpenes in the form of apiol, anethole, eugenol, etc. Terpenes proved to have antibacterial activity, fungicidal activity, mosquito repellent activity, etc. Terpenes may also possess to have skin penetration activity of active drug due to their effect as reported i.e. counter – irritant. Both the species of Tulsi showed significant results in larvicidal as well as skin penetration activity as compared to standard drugs.

Key words: Tulsi, *Ocimum sanctum, Ocimum tenuiflorum*, Apiol, Anethole.

INTRODUCTION

There are many synthetic skin penetration enhancer available in the world market e.g. DMSO (Dimethyl Sulfoxide). But they are having many side effects on the skin like allergic reaction, irritation and so on. So we have alternative use of herbal plants. If this occurs, no side effect on the skin and we can use herbal plants in disease condition in the forms of paste, cream, gel and ointment (Elias PM, 1983).

There are mainly three layers of the human skin; Epidermis, Dermis and Subcutaneous (Casey G, 2002).

Subcutaneous layer is the inner most layer of the skin consisting of adipose tissues and blood capillaries for systemic circulation of drug. Its main function is store fat and give thermal insulation to internal structure.

Dermis is the middle layer of skin, composed of mainly connective tissue. Blood capillaries, nerves, glands and hair follicles are embedded in dermal tissue. The function of this layer is synthesis of major constituents of skin layer like proteins, collagen and elastin. This also supports epidermis and also connected to sub cutaneous through blood capillaries.

Epidermis is the upper most layer of the skin. It is entirely cellular and has four strata: (A) the basal layer (*Stratum germinativum*): This is lower most layer, it constantly divides and the daughter cells are pushed up towards the surface. (B) The prickle cell layer (*Stratum spinosum*). *(C) The Granular Layer (Stratum granulosum*). (D) The Horny Layer (*stratum corneum*) (Potter B, 1966).

This is the outermost layer of skin having the keratinized and dead cells. This is a barrier of the skin, gives mechanical protection to the underlying living tissues, provides defense against microorganisms and harmful chemicals and controls in and out of water and electrolytes.

Possible pathways of penetration through the skin barrier are shown in the figure no.1 (Bary BW, 1987): (1) Across the intact horny layer, (2) Through the hair follicles with the associated sebaceous glands, or (3) The sweat gland (Fartasch M *et al.*, 1993).

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Accordingly, a molecule may use two diffusional routes to penetrate normal intact human skin: (1) The appendageal route and (2) the Trans epidermal route.

The appendageal route comprises transport via the sweat glands and hair follicles with their associated sebaceous glands. These routes circumvent penetration through the stratum corneum and are therefore known as shunt routes. Although these routes offer high permeability, they are considered to be minor importance because of their relatively very small surface area, approximately 0.1% of total skin area. Penetration enhancers mainly deal with Trans epidermal transport. The efficacy of topically applied active ingredient is often suboptimal because the transport into the skin is slow due to the resistance of the outermost layer of the skin, stratum corneum. Thus, a variety of means have been studied in attempts to overcome this barrier (Johannes P Venter *et al*., 2001).

Approaches to penetration enhancement (Patel MN, 2010): (A) Physical approach; (A-1) Stripping of stratum corneum, (A-2) Hydration of stratum corneum, (A-3) Iontophoresis, (A-4) Phonophoresis, (A-5) Thermal energy. (B) Chemical approach; (B-1) Synthesis of lipophilic analogs, (B-2) Delipidization of stratum corneum, (B-3) Coadministration of skin penetration enhancer. (C) Biochemical approach; (C-1) Synthesis of bio convertible prodrug, (C-1) Coadministration of skin metabolism inhibitor (Lampe MA *et al*., 1983).

This investigation deals with the chemical approach with co-administration or skin penetration enhancer. Penetration enhancer increases penetration of drug into skin through mainly transepidermal route which is shown in fig no 2. This route consists of two potential micro routes of penetration: (1) Trans cellular pathway (or intracellular), (2) Intercellular pathway (Cevc G *et al*., 1996).

Penetration enhancers have been found to solvate the horny layer and also to bring about configurational changes in skin protein structure, with resultant swelling. Swelling may open channels within the stratum corneum, thereby lowering its effectiveness as a barrier to certain drugs. Due to swelling, extraction of lipid from stratum corneum occurs and it causes disruption of lipid bilayer structureand loosening of horny cells. Thus making the stratum corneum more permeable for drug by Trans epidermal route (Chaudhary Heena *et al*., 2012; Gupta SP *et al*., 2004).

Tulsi which is a very useful herbal plant because of their various pharmacological actions and also its volatile nature may be used as skin penetration activity and also for larvicidal activity (Kokate CK, 2006).

MATERIALS AND METHODS

Collection: both *Ocimum sanctum* and *Ocimum tenuiflorum*, in the flowering stage were collected from the campus of Sigma Institute of Pharmacy in the month of September in the morning.

Extraction: the essential oils were extracted by hydro distillation using Clevenger Apparatus.

Storage: The isolated oils were subsequently dried over anhydrous sodium sulfate and stored in ampoule with cotton plug under refrigeration until analyzed & tested (Williams AC, Bary BW, 2004).

Larvicidal Activity: Portion of essential oils (5 to 500 µL/ml) from both species were placed in a beaker (50 ml) and dissolved in H_2O: DMSO (98.5:1.5).25 Instar III larvae of A. aegypti were collected to each beaker. After 24 hours, at room temperature, the numbers of dead larvae were counted and the lethal percentage calculated. A control experiment using DMSO and water was carried out separately for each sample. Three independent experiments were conducted (Mohomed Anees A, 2008).
For the preparation of salicylic acid ointment, salicylic acid, benzoic acid and ointment base were taken in 6%, 3% and 90% concentration respectively as shown in table no. 1. The ointment was prepared by levigation technique and salicylic acid was used as active ingredient and Benzoic acid was used as preservative.

Diffusion study: This study was carried out by Franz diffusion cell. This study is carried out to evaluate penetration enhancement activity of *Ocimum sanctum* oil and comparison with Std. solution of synthetic penetration enhancer (DMSO).The release and skin permeation kinetics of drug can be evaluated using a two compartment diffusion cell assembly under identical condition. Franz diffusion cell consists of a receptor and a donor compartment, between these two compartments there is a membrane onto which human or animal skin is placed. There is a provision for maintaining temperature.

Procedure

Different concentrations 1%, 2% and 4% of *Ocimum sanctum* oil in methanol solution were prepared. Diffusion cell was set up vertically with continuous flow of water. Fresh rat skin was made free from epithelial layer and hair. Receptor compartment of diffusion cell was filled with phosphate buffer (pH 6.8). After that donor compartment, which was filled with ointment, was put on skin. The sample was collected after interval of 30, 60, 120, 180, 240, 300 and 360 min by using oral feeding needle and measured the absorbance in UV Spectrophotometry. Result was incorporated in the form of % cumulative release of active drug i.e. Salicylic Acid. Diffusion study was also done by using 1% DMSO solution and compare with results obtained by *Ocimum sanctum* oil of different solution.

RESULTS

Total no. of larva taken= 25
% Larvicidal Activity of *Ocimum sanctum* oil and

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Ocimum tenuiflorum oil in concentrations 75, 125, 250, 500 and LC_{50} µL/ml are 4, 16, 36, 60 and 322.61 for *Ocimum sanctum* oil and 8, 20, 36, 64 and 291.29 for *Ocimum tenuiflorum* respectively is shown in table no. 2.
Skin penetration activity:
Salicylic Acid Ointment:

Phosphate buffer pH = 6.4
λmax = 297 nm
Calibration Curve for solution without apiol in concentrations of 2, 4, 6, 8, 10, and 12 with absorbance 0.121, 0.168, 0.238, 0.284 and 0.356 is shown in table no. 3.

Table 1. Preparation of salicylic acid ointment

Sr. No.	Ingredients	Quantity
1	Salicylic acid	6%
2	Benzoic acid	3%
3	Ointment base	90%

Table 2. % larvicidal activity of *O. sanctum* and *O. tenuiflorum*

Concentration (µL/ml)	*Ocimum sanctum* Oil	*Ocimum tenuiflorum* Oil
75	4	8
125	16	20
250	36	36
500	60	64
LC_{50}	322.61	291.29

Table 3. calibration curve for solution without apiol

Concentration (µg/ml)	Absorbance
2	0.121
4	0.168
6	0.238
8	0.284
10	0.356

% cumulative release of salicylic acid for the above solution for time periods of 30, 60, 120, 180, 240, 300 and 360 min was calculated to be 0.054, 0.226, 0.765, 1.675, 2.899, 4.386 and 6.016% respectively as shown in table no. 4.

Table 4. % cumulative release of salicylic acid

Time (min)	Absorbance	Conc.	Cumulative release	% cumulative release
30	0.0731	0.656	0.054	0.054
60	0.115	2.067	0.172	0.226
120	0.246	6.478	0.539	0.765
180	0.378	10.92	0.910	1.675
240	0.490	14.69	1.224	2.899
300	0.584	17.85	1.487	4.386
360	0.635	19.57	1.630	6.016

% cumulative release of salicylic acid for 1% concentrated solution for time periods of 30, 60, 120, 180, 240, 300 and 360 was calculated to be 0.4608, 1.1458, 2.1258, 3.4108, 4.9348, 6.6718 and 8.5468% respectively as shown in table no. 5.

Table 5. %cumulative release of salicylic acid for 1% concentrated solution

Time (min)	absorbance	Conc.	Cumulative release	% cumulative release
30	0.218	5.53	0.4608	0.4608
60	0.298	8.22	0.685	1.1458
120	0.403	11.76	0.980	2.1258
180	0.512	15.43	1.285	3.4108
240	0.597	18.29	1.524	4.9348
300	0.673	20.85	1.737	6.6718
360	0.722	22.50	1.875	8.5468

% cumulative release of salicylic acid for 2% concentrated solution for time periods of 30, 60, 120, 180, 240, 300 and 360 min was calculated to be 0.578, 1.364, 2.684, 3.963, 5.762, 7.676 and 9.708% respectively as shown in table no. 6.

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Table 6. %cumulative release of salicylic acid for 2% concentrated solution

Time (min)	absorbance	Conc.	Cumulative release	% cumulative release
30	0.260	6.94	0.578	0.578
60	0.334	9.44	0.786	1.364
120	0.453	13.44	1.120	2.484
180	0.581	17.75	1.479	3.963
240	0.695	21.59	1.799	5.762
300	0.736	22.97	1.914	7.676
360	0.778	24.39	2.032	9.708

% cumulative release of salicylic acid for 4% concentrated solution for time periods of 30, 60, 12, 180, 240, 300 and 360 min was calculated to be 0.730, 0.960, 1.302, 1.636, 2.110 and 9.747 respectively as shown in table no. 7.

Table 7. %cumulative release of salicylic acid for 4% concentrated solution

Time (min)	absorbance	Conc.	Cumulative release	% cumulative release
30	0.314	8.76	0.730	0.730
60	0.396	11.52	0.960	0.960
120	0.518	15.63	1.302	1.302
180	0.637	19.64	1.636	1.636
240	0.781	24.49	2.040	2.040
300	0.806	25.33	2.110	2.110
360	0.898	28.43	2.369	2.369

% cumulative release of salicylic acid for 1% std. DMSO solution for time periods 30, 60, 120, 180, 240, 300 and 360 min was calculated to be 0.553, 1.373, 2.521, 3.972, 7.702, 8.731 and 9.747 respectively as shown in table no. 8.

Table 8. %cumulative release of salicylic acid for 1% std. DMSO solution

Time (min)	absorbance	Conc.	Cumulative release	% cumulative release
30	0.251	6.64	0.553	0.553
60	0.346	9.84	0.820	1.373
120	0.463	13.78	1.148	2.521
180	0.571	17.42	1.451	3.972
240	0.689	21.39	1.782	7.702
300	0.748	23.38	1.948	8.731
360	0.783	24.55	2.045	9.747

Table 9. % Cumulative release of salicylic acid

solution	% Cumulative release of salicylic acid
Blank	6.016
1%	8.5468
2%	9.708
4%	11.147
Std. DMSO	9.747

Results revealed that volatile oil obtained from plant increases penetration ability of the active drug. The volatile oil increases penetration in dose dependent manner as compared to the reference standard DMSO.

DISCUSSION AND CONCLUSION

% Cumulative release of salicylic acid in solution without apiol, 1% concentrated solution, 2% concentrated solution, 4% concentrated solution and 1% std. DMSO solution was found out to be 6.016, 8.5468, 9.708, 11.147 and 9.747 respectively as shown in table no. 9.

From the study, it can be concluded that the active terpenes present in volatile oil are responsible for larvicidal activity as well as skin penetration ability. We can use these natural resources for preventing the vector borne diseases such as malaria, dengue. Also this volatile oil may be used for development of better analgesic or anti-inflammatory formulation for increasing the penetration of active drug through skin which tend to increase pharmacological action of the same.

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