SUBDUING THE NAIL BARRIER WITH NOVEL HERBAL PENETRATION ENHANCERS FOR TRANSUNGUAL DELIVERY SYSTEM

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Nail fungal infections are very common and also very difficult to treat because of nail morphology, deep penetrability of infectious agent inside nail plate and poor permeability of the nail plate. Transungual delivery shall be the first choice for treatment of nail infection if we get the effective penetration enhancers without causing the serious problem. In this study we tried to scanning some extracts penetration potency through the human cadaver nail plate. Five plants selected for the purpose acacia catechu, rosa hybrid, hibiscus rose-sinensis, tagetes patula, tagetes erecta. For penetration potency first defatted the nail plate with chloroform : methanol (2:1) mixture. Extracts of tagetes erecta, acacia catechu shows 100%, tagetes patula 60% and rosa hybrid 40% from dorsal side of plate. Those extracts were stable by it only. That proves that they may be used as a penetration enhancer to increase the penetration of drug.

Key words: Herbal Formulation, Penetration Enhancers, Transungual Delivery System

INTRODUCTION

Now days the cases of nail fungus infections are increasing about 26% of the population infected with nail fungus infection and very common with diabetic patients and with patients of poor circulation[1,2], although generally nail fungus infections are not causing pain but if this nail fungus infections not treated with effective manner this can lead to many systemic problems, specially person with immune problem[3]. Considering history the idea of using natural materials for human body has emerged numerously. The unifying property of natural materials is biocompatibility.

Nail fungus infections are very difficult to treat due to nail morphology and deep penetrability of infectious agent in nail plate. Again oral drug delivery for nail disorders is limited because of terrible side due to high drug concentration needed in systemic circulation for optimal *i.e.* therapeutic effect.[4,5]. Around 25 – 30% of patients relapse after treatment[1].

Nail is keratinized and compact reasonably nail plate is impermeable[6]. Nail permeability is normally poor and the drug flux by nail unit is less[7]. Nail plate act as a compact hydrogel instead of a lipophilic membrane[8]. The potency of trans ungula drug delivery system is restrained by low drug movement by the nail plate[9]. The human nail plate is highly arranged epidermal appendage made up of sulfur rich α-keratin (≈ 80%), water (10 – 30%), and lipids (0.1-1.0%). This lipid is mainly located in the dorsal and ventral layers[1]. The keratinized cell is tightly fixed with each other with desmosomes[10]. The nail has an isoelectric point between 4.9-5.4[11].

From the table number 2 it can be noticed that there is a significant difference between nail drug concentration and plasma drug concentration *i.e.* to maintain sufficient drug concentration in infective site (nail) one has to increase the drug concentration in plasma also for a long time and that may cause serious side effects. One good substitute for oral drug delivery system is transungula drug delivery system. The absorption of therapeutic agent into the nail plate in transungula delivery is highly delectable to treat the nail fungus infections. Nail permeability still quite low and restrain topical therapy. Literature shows that the aqueous or lipophilic vehicles do not change the drug penetration rate [18, 19]. Penetration enhancer may help in the case of penetration problem for transungula therapy.

MATERIAL & METHOD

Methanol was obtained from Renkem (RFCL) limited Ranbaxy, Ethanol was obtained from Changshu Yangyuan chemical (China), Chloroform was obtained from Central drug house, Centrifuge – Teknik laboratory centrifuge machine, Colorimeter – labtroices model No. 12, Hot air oven – Universal.

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Rosa Hybrid (family Rosaceae) - A rose is a woody perennial of the genus Rosa, within the family Rosaceae. Available colors are shades of red, pink, yellow, white, lavender and salmon. The bloom time is year around, temperature range for the plant 60^0 to 80^0F (16^0 to 27^0C). Flowers vary in size and shape and are usually large and showy and used as a minor source of Vitamin C[20]. Other species have been used for stomach problems, and are being investigated for controlling cancer growth[21].

Acacia Catechu - It's a 15-20 meter tall tree grown in the forests of India, Bangladesh, Nepal and Bhutan. Its bark is used for extracting color and Katha (used as traditional mouth freshening material). The constitutes like Kitechins, Tannins, glycosides and resin used as traditional mouth freshening material with beetle leaves, also used as natural dye for hair coloring and textile [22].

Tagetes Erecta (family Asteraceae) - *Tagetes erecta*, the Mexican marigold, also called Aztec marigold, is a species of the genus *Tagetes*. This plant reaches heights of between 50–100 cm (20–39 in). The Aztecs gathered the wild plant as well as cultivating it for medicinal [23]. *T. Erecta* is grown to extract lutein; a common yellow/orange food colour (E161b).The essential oil of the flower contains antioxidants[24].

Tagetes Patula (family Asteraceae) - The flower is an annual, occasionally reaching 0.5 m by 0.3 m. In some climates it flowers from July to October. The leaves of all species of marigold include oil glands. The oils are pungent[25]. The essential oil is being investigated for antifungal activity; including treatment of candidacies [26] and treating fungal infections in plants[27,28].

Rosa-Sinensis (family Malvaceae) - It is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide. Hibiscus flower preparations are used for hair care. The flowers themselves are edible and are used in salads in the Pacific Islands. Hibiscus rosa-Sinensis has been shown to function as an anti-solar agent by absorbing ultraviolet radiation[29].

Extraction procedure from plant sources

Collect the selected plants and part of selected plants, extraction of desired extracts were done by pawing the different parts (petals of *Rosa hybrid*, *Hibiscus Rosa-Sinensis*, *Tagetes Patula*, *Tagetes Erecta* and powder of *Acacia Catechu*) in methanol, ethanol or chloroform for time according to the plant or plant's part type to draw out the colors from the plant's part. Decant the methanol with the dissolved constituents of dipped plants or part of plants. Centrifuged the extracted methanol, ethanol or chloroform with 5000 rpm the un-dissolved part or other solid part separated out after the sample centrifuged.

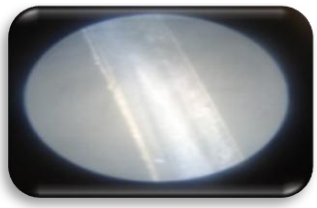
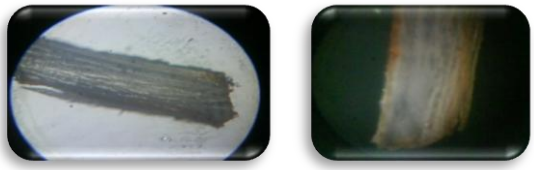


Fig 1: T.S. of cadaver human nail plate



At zero time **After 1 hr**

Fig 2: Transverse section of human cadaver nail treated with *Acacia Catechu* extract

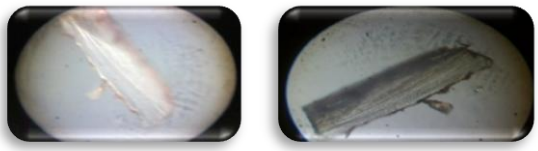


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Fig 3: Transverse section of human cadaver nail treated with *Rosa hybrid* extract

Collection of nail plate

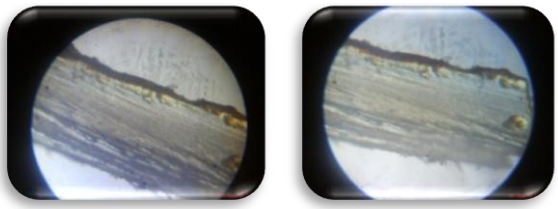
Healthy nail tip pieces were collected from the fingers of hefty volunteers (20 females volunteers of age about 20 years) using nail clipper.

Preparation of the nail plates[30]

For each nail plate, clinical information (age and sex) was recorded. Before using the nail plates were kept and allowed to equilibrate with the room temperature

and other conditions, cleaned with a mild liquid detergent. Thoroughly rinsed with distilled water and dried at 45^0C to a constant weight. Only female fingernails (index, middle and

ring finger) were use because they are already reported to be more comparable in size, weight, and thickness and more reproducible within the same donor (Lehman, personal communication). For each nail plate sample, the dry weight and thickness were measured. Thickness was measure at three points with Vernier caliper and averaged for each nail. Defatting of nail plates[31] Cleaned nail pieces were defatted by placing them in a beaker containing chloroform: methanol (2:1) mixture (10ml) and stirred for a period of 12 hr.



At zero time **After 1 hr**

Fig 4: Transverse section of human cadaver nail treated with Hibiscus *Rosa-Sinensis* extract

Composition	**Dorsal**	**Intermediate**	**Ventral**
Calcium	High	Low	High
Phospholipids	High	Low	High
Disulphide bounds	Low	High	High
Sulfhydryl groups	High	Low	High
Acid Phosphatase activity	Low	High	High
Keratin fibers orientation to the growth axis	60% perpendicular 40% parallel	All perpendicular	60% perpendicular 40% parallel

Table 1 [12, 13] Comparison of the nail layers composition and orientation of the keratin fibers.

Molecule	**Plasma Drug Concentration (mg/L)**	**Nail Drug Concentration**	**Dose**
Itraconazole	0.272	600 – 900 ng/g	200 mg/d One week repeated after 21 day interval
Terbinafine	0.03 – 1.39	250 – 1000 ng/ g	250 mg/d
Fluconazole	0.3 – 2.7	8.5 – 9.5 µg/g	150 mg/d

Table-2 [15, 16, 17]-Antifungal drug's concentration in different locations used to treat onychomycosis.

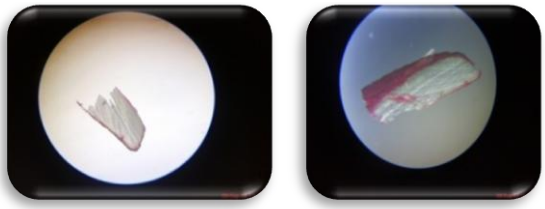
S.N.	Extracts used	Human cadaver Nail plate Thickness	Dry Weight of Human cadaver nail plate	Optical Density of applied extract	Temperature
1.	Acacia catechu	220 µm	45.8 mg	0.01	Room Temperature
2.	Rosa Hybrid	220 µm	46 mg	0.03	
3.	Hibiscus Rosa-Sinensis	220 µm	46 mg	0.4	
.	Tagetes Patula	218 µm	45 mg	0.02	
5.	Tagetes Erecta	218.5 µm	45.12mg	0.8	

Table: 3 - Conditions for permeation study

Permeation study

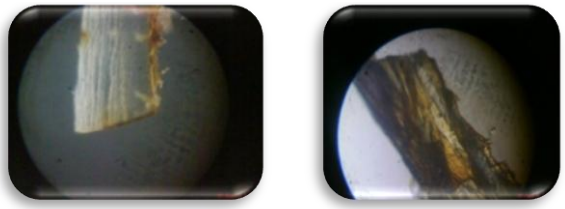
Took the piece of nail plate and apply the slurry of extracted extracts from the different sources separately in the dorsal side of nail plate, and left it for 24 hr for penetrate the extracted extracts according to its potential to penetrate deep inside the nail plate structure. Cut the transverse section of nail treated with extract and observed under compound microscope with magnification of 10X for examine the level of penetration of extracts. Extracts from different sources were subjected to the stability study for seven weeks with change in pH profile (digital pH meter), color density (digital colorimeter) and maximum absorbance wavelength value (UV- visible spectrophotometer). As if pH value of the extract get change that mean there may be some deviation in the ionic concentration of the medium, which may alter the penetration power of the extract as ionic concentration highly, influence the penetration through the nail plate. If the color concentration get change from the initial value that mean some type of

breakdown of the extracting material or change in the solubility of the extracted material in the vehicle or any indication of microbial growth in the medium, which may highly affect the penetration power of the extract and also the stability of formulation in which that extract likely to be use. The third parameter studied was λmax which clearly structure specific that is if the structure of extracted material gets change the λmax also get change from the initial value.



At zero time **After 1 hr**

Fig 5: Transverse section of human cadaver nail treated with *Tagetes Patula* extract



At zero time **After 1 hr**

Fig 6: Transverse section of human cadaver nail treated with *Tagetes Erecta* extract

RESULTS

The selected plant sources successfully extracted with the used method and selected solvents and they shows a considerable good stability to the experimented data's specially Tagetes Erecta and Acacia catechu as they do not change their λmax and pH for a long time, and in respect of others (table 4) they were also good but they need some preservative if planed to be formulated as they presented a powerful penetration in the human cadaver nail plate which is the only limitation for trans ungula delivery.

The extracted material applied to human cadaver nail plate of 218 – 220 µm thickness and of 45- 46 mg dry weight i.e. almost equal density at the room temperature (table 3). The optical density of applied extracts was form 0.01 to 0.8. The extracts shows up to 100 percent penetration in the human cadaver nail plate in the case of Tagetes Erecta and Acacia catechu (figure 2,6) and 40 percent for Rosa Hybrid (figure 3) and 60 percent for Tagetes Patula (figure 5) but only 2 percent in the case of Hibiscus Rosa-Sinensis (figure 4).

CONCLUSION

As from the literature and general observation based on the experiment it can be concluded that the natural penetration enhancers (methanolic extract of *Acacia Catechu, Rosa hybrid,Tagetes Patula, Tagetes Erecta*_) sweep over the biggest barrier of the formulation for transungual drug delivery systems. They shows satisfactory penetration capacity which may be suitable for transungula formulation. The extracted natural penetration enhancer shows a noticeable good stability for the examined parameter though they are not effective for prolong period of time but safer then the keratolytic chemicals. These potential penetration enhancers shall be used in development of suitable transungula delivery system.

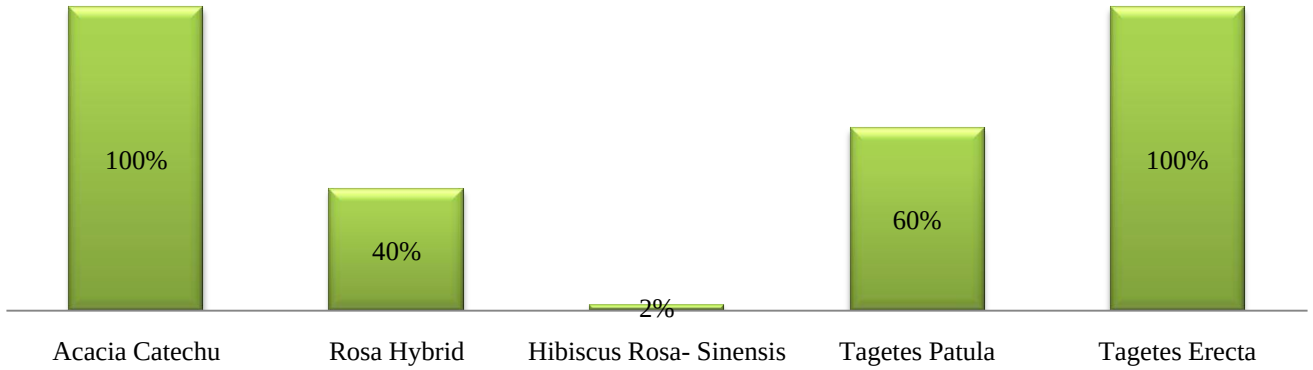


Fig 7: percent penetration of plant extracts.

Sr. No.	Penetration Enhancers	Factor Observed	Observance								
			1st day	7th day	2nd week	3rd week	4th week	4th week	5th week	6th week	7th week
1.	*Acacia catechu*	pH	5.6	5.6	5.6	5.6	5.6	5.6	4.5	4.5	4.5
		Color density	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02
		λ_{max} (nm)	292	292	292	292	292	292	292	293.4	293.3
2.	*Rosa Hybrid*	pH	5.6	5.6	5.6	5.6	5.6	5.6	3.4	3.4	3.4
		Color density	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
		λ_{max} (nm)	211	211	211	211	211	211	211	211	211
3.	*Hibiscus Rosa-Sinensis*	pH	5.6	5.6	5.6	5.6	3	3	2.8	2.8	2
		Color density	0.4	0.4	0.4	0.27	0.27	0.25	0.22	-	-
		λ_{max} (nm)	260	260	260	255	255	245	230	-	-
4.	*Tagetes Patula*	pH	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		Color density	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
		λ_{max} (nm)	280	280	280	280	280	280	280	280	280
5.	*Tagetes Erecta*	pH	5.6	5.6	5.2	5.2	5.2	5.2	5.2	5.2	5.2
		Color density	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
		λ_{max} (nm)	275	275	275	275	275	275	277	277	277

Table 4: Stability profile of extracts

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Received	4th February 2015
Revised	16th February 2015
Accepted	21st February 2015

J. App. Pharm. Res., 3 (2); 2015: 16 – 21