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A PHARMACEUTICAL STUDY ON TOPICAL FORMULATIONS OF HENNA

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ABSTRACT:

The purpose of this study was the preparation of henna extract in a suitable pharmaceutical formulation to be applied topically.

Based on microbiological studies, Hadramout -See'on (dry powder) was selected from number of henna samples collected from different places in Yemen. Hadramout-See'on (dry powder) was extracted using different solvents namely; water, methanol and chloroform separately. The inhibitory activities of various extracts of henna against two species of dermatophytic fungi (*Trichophyton violaceum* and *Microsporum canis*), one species of yeast (*Candida albicans*), one Gram-positive bacteria (*Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella*) were tested. Water extract showed the highest percentage inhibition zone particularly with the tested fungi, followed by methanol extract, while chloroform extract showed weak activity with all strains.

The best active extract of henna was formulated in traditional formulations such as ointments bases (water-soluble base, oleaginous base) and emulsion bases (o/w , w/o emulsion bases), and the in vitro release studies of active component in henna from different formulations were recorded in the following descending order :

Polyethylene glycol base > oil/water (o/w) emulsion base > water/oil (w/o) emulsion base > oleaginous base.

INTRODUCTION

The first known cultivation of Henna was in Egypt and India, around 5000 B.C.The two main cultivated varieties are *Lawsonia inermis* and *Lawsonia alba* Family: Lythraceae. Henna is widely grown in temperate zones of the world, particularly in Asia and North Africa[1,2].

In Yemen it is now well naturalized in Hadramout,Taiz,Tihama, Aden and Lahej.

Approximately 100 g of henna are needed to stain the hands and feet of an adult, and the dyeing process requires 3-6 hours. The important natural chemical dye ingredient of henna is lawsone (2-hydroxy-1,4-naphthoquinone), constituting about 1% by weight of the crushed leaves[2,4].

Qualitative phytochemical tests, and thin layer chromatography demonstrated the presence of common compounds in the plant extracts including phenols, tannins and flavonoids as major active constituents[5].

Henna is one of the most natural inexpensive, safe drugs in the world, it is safe topicaly and orally (toxic dose orally is 2.5 g/kg) it is also safe for pregnant woman . Henna might induce hemolysis in G6PD deficient male newborns[11].

Traditionally it is very effective when applied to a first or second degree burns and promotes wound healing, especially chronic wounds and ulcers, this may be due to that henna extracts showed antibacterial activity[12].Henna is not only used for cosmetics, but also for medication as well. For many years, henna has been used for the treatment of skin disorders. A potent fungicide, seborrheic dermatitis and fungal infestations are other reasons why henna is used topically on lesions in some people[6-9]. The isolated compound (lawsone) was found to possess significant anti-inflammatory, analgesic, antipyretic activity and anti-tuberculosis activity[10,13].

The aim of this study was the preparation of henna extract in a suitable pharmaceutical formulation to be applied topically.

MATERIALS AND EQUIPMENTS

Materials

Henna samples were collected from different places in yemen. Sabouraud's agar and Nutrient agar were obtained from Ministry of Health (Yemen) . Polyethylene glycol 400, polyethylene glycol 4000, span 80, were obtained from Merck Company (Germany). The following chemicals were of pharmaceutical grade:

Sodium lauryl sulphate (SLS), propylene glycol, liquid paraffin, methanol, chloroform, white soft paraffin, white bees wax, wool fat, borax, and cetyl alcohol were given as a gift from Shaphaco Pharmaceutical Ind. (Sana'a yemen):

Equipment:

UV-Spectrophotometer (Shimadzu U.V-1601 PC, Shimadzu Corporation, Japan).

Electronic Digital Balance (Metter-Toledo, Ag, CH 8606, Greifensee, Switzerland).

Dissolution apparatus, Erweka, GMBH, D-63150, Type:DT60 (Heusenstamm,Germany)

METHODS

Collection of henna samples

Fresh and dry powder samples of Henna plant were collected from different places in Yemen. Hadramout-See'on and Gaiel Bawazeer, Hajja, Taiz , Haraz, and Tihama.

Extraction of henna samples

Each sample (500 gm) was put in a sterile polyethylene bag, sealed and put inside another sealed bag. The samples were then transferred to the laboratory. Plant materials were extracted with methanol (99.6%), chloroform and water separately. Fifty grams of plant material were dissolved in 250 ml of the used solvent (1:5 w/v) in a flask of 500 ml. The flask was shaken for one hour on shaker at 300 r.p.m. The contents of the flask were filtered through a filter paper (Whatman No.1). This procedure was repeated three times on the residue of plant. The obtained solutions of different plant extracts were evaporated to a thick mass by standing on air for a

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sufficient period of time, and in case of water extract, was left in air till dried.

Microbiological part

The microbiological activity of sterile henna extracts (filtration through Nylon membrane of 0.2 µm of por diameter) by using water, methanol, and chloroform were determined by measuring the percentage inhibition zone of each extract, on the pure cultures of the following microorganisms: Dermatophytic fungi strains such as *Trichophyton violaceum, Microsporum canis*, and *Candida albicans*. Bacterial strains such as *Escherichia coli, Staphylococcus aureus* and *Klebsiella spp*. These microorganisms were obtained from the Central Laboratory (Ministry of Health in yemen) and identified by the Department of Microbiology, Faculty of Medicine, Sana'a University .

Sterile 5 mm filter paper discs (Whatman No. 1) were saturated with each henna extract at concentration of 5000 p.p.m , and placed on the surface of 100 mm plate containing the suitable media. Sabouraud's agar medium was used for dermatophytic fungi and incubated at 27 C° for 7-10 days. Nutrient agar was used for bacterial strains and incubated at 37 C° for 24-48 hours. After incubation period, the percentage of inhibition zones were calculated.

Each plate had one control disc saturated with the solvent. Three replicates were used for each treatment.

Pharmaceutical part

Preparation of topical formulations of henna:

The following formulae were selected in which 5% of henna extract was incorporated.

a. Water soluble base

-Polyethylene glycol base: (U.S.P. XXII).

-PEG 4000	40 gm
-PEG 400	60 gm

Preparation:

On a water bath previously heated to 60°C, PEG 4000 was melted at first. To the base PEG 400 containing the drug was added. The mixture was continuously stirred until congealed and packed in a plastic Jar and stored at ambient temperature until use.

b. Oleaginous base

- White soft paraffin	95 gm
- White bees wax	5 gm.

Preparation:

An accurately weighed amount of the drug was incorporated into the melted base with continuous stirring until congealed then packed into a plastic Jar until use.

c. Emulsion bases:

i- O/W emulsion base (Beeler's base):

- White bees wax	1 gm
- Cetyl alcohol	15 gm
- Propylene glycol	10 gm
- Sodium lauryl sulphate	2 gm
- Water	72 gm

ii- W/O emulsion base [14]:

- Liquid paraffin	45 gm
- White bees wax	10 gm
- Wool fat	2 gm
- Borax	8 gm
- Water	41 gm
- Span 80	1 gm.

Preparation:

The aqueous phase and the oil phase were placed in separate containers and heated at 70°C. Henna extract was dissolved in the aqeous phase, then the aqueous phase was added to the oil phase at the same temperature with continuous stirring until cool and congealed.

Release method without a rate limiting membrane:

The drug released from a formulation through a permeable membrane (tea bag) to a receptor medium (distilled water) using dissolution apparatus was estimated, where 1 g of the of the tested formulation base containing (50 mg of henna extract) was accurately weighed and the diffusion cell was placed at the center of 1000 ml vessel containing 250 ml of distilled water. The whole diffusion unit was placed into a thermostatically controlled shaker water bath at 37°C and 100 r.p.m.

Five ml sample was pipetted from the sink solution at sutiable time intervals (5 minutes each) and assayed for its drug content at 226.4 nm using the suitable blank. At each time, equal volume of distilled water at 37°C, was replaced into the outer sink solution to keep the volume constant during the experiment.

RESULTS AND DISCUSSION

Microbiological part

A preliminary microbiological study was done in order to confirm difference in the inhibition zone of henna extract obtained from either dry powder or green leaves using water as extraction solvent (data are not shown). Results showed no significant differences with some priority in inhibition zone of dry powder extract than green leaves. Traditionally henna was used as dry powder, accordingly the experimental study done on the extract of dry powder of henna.

Also in a preliminary experimental study against *C.albicans, T.violaceum , M. canis, , S. aureus, E. coli* and *Klebsiella* to select the more active extract of henna collected from different places in Yemen as a dry powder using water as extraction solvent, (data not shown) the results showed that:

Hadramout-See'on > Hadramout (Ghiel Bawazeer) > Hajja > Taiz > Haraz > Tihama.

To select the best solvent for dry powder collected from Hadramout -See'on the inhibition zone on three species of dermatophytic fungi namely: *Microsporum Canis, Trichophyton violaceum, Candida albicans*, and three species of bacteria one Gram-positive *Staphylococcus aureus*, two Gram-negative *Escherichia coli, Klebsiella spp* were also tested.

Results in table (1) revealed that henna extracts have antimicrobial (antifungal, antibacterial) activity on the dermatophytic fungi, Gram-positive and Gram-negative bacteria, and it is species dependent. The percentage of inhibition zones of different strains was water extract > methanol extract > chloroform extract.

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Table (1): Effect of different extraction solvents on (Hadramout -See'on dry powder henna extract (5000 ppm) on the growth of different strains.

Extraction Solvent	Percentage of inhibition zone(mm) of different strains					
	Control	*Microsporum Canis*	*Trichophyton violaceum*	*Candida albicans*	*Staph. aureus*	*E.coli*	*Klebsiella*
Water	0	100	100	21	39	35	33
Methanol	0	89	85	18	35	33	32
Chloroform	0	45	42	9	15	12	11

Pharmaceutical part

The henna water extract was chosen to be formulated in topical bases, to exert its expected action from different topical preparations. It is important that the vehicle is able to release the active ingredients.

Selection of different topical bases as vehicles for henna extract depends on several factors such as polarity, viscosity, and homogenicity. For this purpose traditional classes of topical bases were investigated which included water-soluble bases, emulsion bases (w/o and o/w emulsions) and oleaginous bases .

As a general rule in ointment formulations if the drug is held firmly by the vehicle, the rate of release of the drug is slow. The release of the drug from ointments can be altered by modifying the composition of the vehicle. A greater release of drug is expected when there is less affinity of the drug for the base[15].

Results illustrated by Fig.1 clearly show that the rate and percentage amount of drug released from polyethylene glycol base is greater than that released from the other bases, in which the percentage of henna released reached 100% within 10 min. The high diffusion rate of henna from water soluble ointment bases that contain mainly polyethylene glycol may be due to diffusion of distilled water through the tea bag and formation of water-PEG solution which increases the solubility and accordingly the rate and extent of henna release. The high release of henna from water-soluble base may be attributed to the high solubility of henna extract in PEG which is water soluble, and the easily permeation of henna from the PEG base through the permeable tea bag to the receptor media. similar observations involving the use of PEG base ointment have been made with respect to the release of sorbic acid[16], salicylic acid[16], and benzocaine[17].

Fig. 2. shows that the percentage of the water henna extract released from o/w emulsion base, is higher to some extent than the w/o emulsion base (Fig.3) which could be due to the formation of a continuous contact between the external phase of the o/w emulsion and the distilled water, in which the percentage of henna released reached 80% within 30 min[14].

Alternatively the presence of an oily vehicle as an external phase in w/o emulsion will result in formation of an occlusive film, which will result in a retardation of the permeation of the drug molecules into the sink solution[14].

Fig. 4. shows the release of water henna extract from the oleaginous base. Oleaginous ointment bases contain primarily white soft paraffin with several additional lipoidal constituents which favor the retention of the drug in the base.

Fig.5. shows the amount of water henna extract released from different topical pharmaceutical preparations which can be arranged in the following descending order:

PEG base > o/w emulsion base > w/o emulsion base > oleaginous base.

CONCLUSION

From this study it can be concluded that: Water and methanolic extracts of henna have antimicrobial activity on the dermatophytic fungi, gram-positive and gram-negative bacteria, with some priority for water henna extract and it is species dependent.

The amount of henna released from different formulations was arranged in the following descending order:

PEG base> o/w emulsion> w/o emulsion > oleaginous base

Henna water extract is better to be formulated in PEG (water-soluble)bases and o/w emulsion bases respectively.

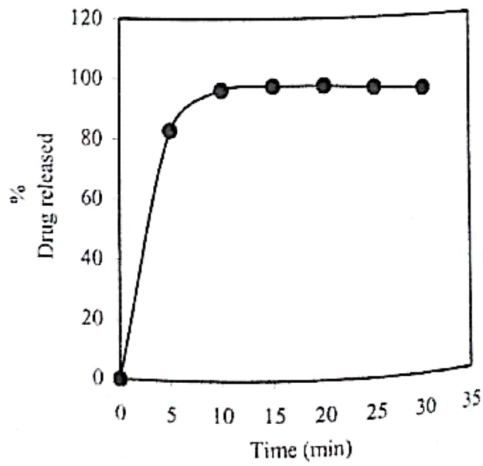

Fig. 1: *In vitro* drug release from polyethylene glycol base into distilled water at 37°C.

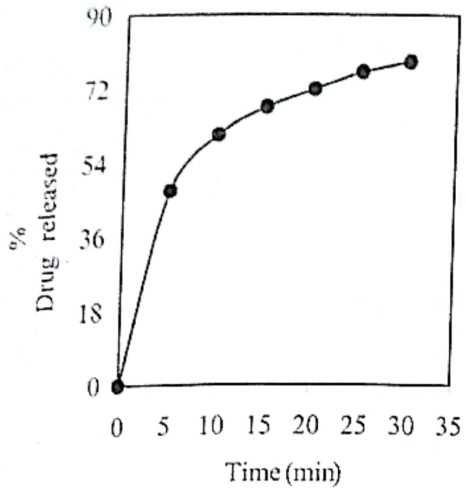

Fig. 2: *In vitro* drug release from o/w emulsion base into distilled water at 37°C.

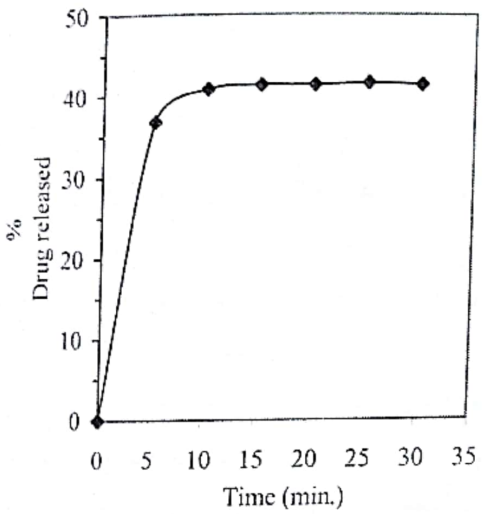

Fig. 3: *In vitro* drug release from w/o base into distilled water at 37°C.

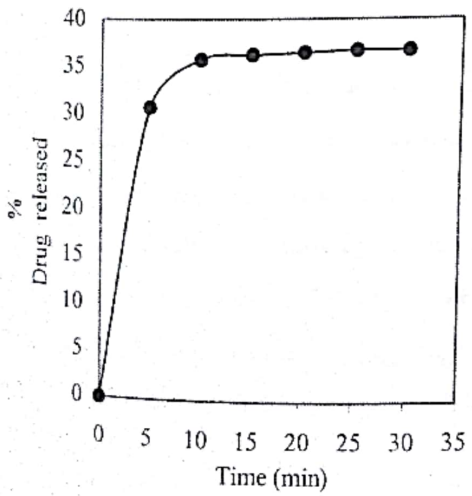

Fig. 4: *In vitro* drug release from oleaginous base into distilled water at 37°C.

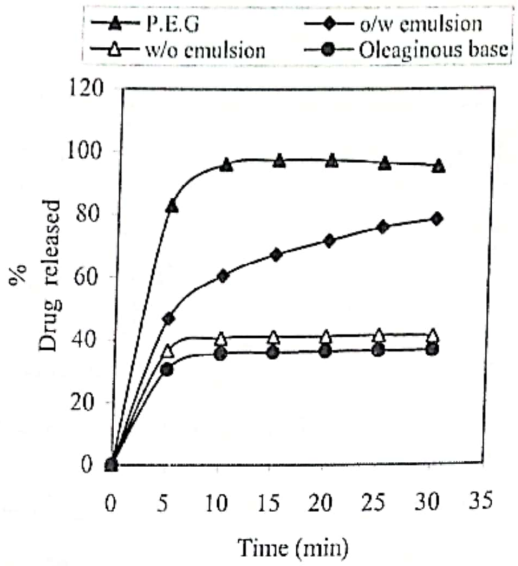

Fig. 5: *In vitro* release of henna from different ointment bases into distilled water at 37°C.

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دراسة صيدلانية لصياغات موضعية من الحناء.

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الغـرض من هذه الدراسة هو الوصول لأفضل مستحضر صيدلاني موضعي لمستخلص الحناء, والذي جمع من عدة مناطق مختلفة في الجمهورية اليمنية , وذلك بعد دراسة فعالية المستخلصات المختلفة على ثلاثة أنواع من الفطريات المسببة للأمـراض الجلدية ونوع واحد من الخمائر , ونوع واحد من البكتريا الموجبة الجرام واثنتين من البكتريا السالبة الجرام.

وينقسم هذا البحث إلى جزئين :

الجزء الأول:

ويشمل استخلاص المادة الفعالة في الحناء ودراسة التأثير الفطري والبكتيري لأحسن مستخلص:

تـم الاسـتخلاص بعدة مذيبات حيث أظهر كل من المستخلص المائي والميثانولي للحناء تأثيراً مثبطاً بدرجة عالية للفطريات والبكتريا المختبرة,وذلك مع إظهار بعض التفوق للمستخلص المائي, في حين أظهرت خلاصة الكلوروفورم تأثيرا ضـعيفا علـى كل من الفطريات والبكتريا المختبرة. كذلك وجد أن مستخلص الحناء من المسحوق الجاف أكثر فعاليةً من مسـتخلص الأوراق الخضراء .كما وجد أن ترتيب الفعالية للمستخلص المائي من المسحوق الجاف للحناء والذي جمع من عدة مناطق مختلفة في اليمن كالآتي:

حضرموت (سيؤن) > حضرموت(غيل باوزير) > حجة > تعز > حراز > تهامة.

الجزء الثاني:

ويشمل تحضيرافضل مستخلص من الحناء في عدة قواعد موضعية صيدلانية مختلفة ودراسة انطلاق المادة الفعالة من الحناء:

تـم اختيار المستخلص الأكثر فعالية (للمستخلص المائي من المسحوق الجاف – حضرموت سيئون) ليكون موضع الدراسـة حـيث تـم صياغته في عدة صيغ صيدلانية موضعية مختلفة من المراهم (مرهم يذوب في الماء ومرهم دهني) والمستحلبات (زيت في الماء ، ماء في الزيت).

تمـت دراسة انطلاق الحناء من القواعد الموضعية المختلفة وفي أوقات متدرجة في جهاز معد لذلك وصولا إلى حساب النسبة المئوية المنطلقة من الحناء في كل وقت و لكل قاعدة على حدة حيث أظهرت النتائج لانطلاق مادة الحناء من الصيغ الموضعية المحضرة الترتيب التنازلي الآتي:

قاعدة عديد اثيلين جليكول > مستحلب الزيت في الماء > مستحلب الماء في الزيت > القاعدة الدهنية