International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

FORMULATION AND ASSESSMENT OF A HERBAL HAIR CREAM AGAINST CERTAIN DERMATOPHYTES

KAMILIA F. TAHA[1], SEHAM S. EL-HAWARY[2], HALA M. EL-HEFNAWY[3], MONA I. MABROUK[4], RANIA A. SANAD[5], MARWA Y. EL HARRIRY[6]

[1,6]Pharmacognosy Department, National Organization for Drug Control and Research (NODCAR), Giza, Egypt, [2,3]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt, [4]Microbiology Department, National Organization for Drug Control and Research (NODCAR), Giza, Egypt, [5]Pharmaceutics Department, National Organization for Drug Control and Research (NODCAR), Giza, Egypt
Email: kamilia.fouly.taha@gmail.com

Received: 21 Sep 2015 Revised and Accepted: 25 Jan 2016

ABSTRACT

Objective: Developing an herbal antifungal formulation containing eruca and garlic oils against highly resistant dermatophytes (*Malassezia fufur* AUMC No. 5173, *Microsporum canis bodin* AUMC No. 5490 and *Trichophyton mentagrophytes* AUMC No. 5501. 5501) and assessment of garlic oil thiosulfonates during the *ex vivo* percutaneous permeation through albino rat skin.

Methods: Assay of antifungal activity was performed by filter paper disc method and agar well diffusion method. The components of volatile constituents and fixed oil of eruca seeds were studied using GC/MS. Thiosulfinates in garlic oil were analyzed by HPLC/UV. Both oils were incorporated into hair cream using span 60 and brij 58 at three different concentrations (2, 4 and 6% w/w) and alliin, was *ex vivo* evaluated using albino rat skin mounted on Franz diffusion cells.

Results: The two oils have a synergistic effect on the first and additive effect on the second and the third fungi. The main constituents in eruca are 4-(methyl thio) butyl isothiocyanate (82%) for volatile constituents and erucic acid (40%) for the fixed one. The highest flux for alliin (0.337±0.0015 mg/cm^2/hr) was obtained at a 4% surfactant concentration.

Conclusion: Combination of oils has a high activity on the selected dermatophytes. Formulation of an herbal hair cream using span 60 and Brij 58 with a concentration 4% gives the highest permeation rate for alliin in garlic oil.

Keywords: Eruca, Garlic, Dermatophytes, Quantitative determination and *Ex-vivo* permeation

INTRODUCTION

Plants and herbal extracts have formed an important position in modern medicine, due to their chemical and medicinal contents found in the natural form. Their secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global health problem [1]. The increasing incidence of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards studies on the potential antimicrobial activity of plant-derived substances, an untapped source of antimicrobial chemotypes, which are used in traditional medicine in different countries [2].

There is a need to evaluate herbs scientifically for their antimicrobial activity against antibiotic-resistant microorganism in order to develop new drugs from plant origin [3]. Dermatophytes are fungi that can cause infections of the skin, hair, and nails due to their ability to utilize keratin [4]. *Malassezia furur*, *Microsporum canis bodin* and *Trichophyton mentagiophytes* are three dermatophytes known to cause severe fungal infections to hair scalp like seporretic dermatitis, ringworm, and piedra, respectively [5]. Topical antifungals are ineffective against organisms that infect the hairs. These infections are usually treated with systemic antifungals, although topical lotions or shampoos are sometimes used concurrently to decrease shedding of fungi and spores [6].

Herbal therapy for skin disorders has been used for thousands of years. In recent years, there has been a resurgence of the use of herbs due to the following reasons: the side effects of chemical drugs became apparent, there was a call to return to nature, natural remedies became a part of the green revolution, and there was a return to organic produce [7]. Herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser side effects with synthetic drugs [8].

Garlic (*Allium sativum*) is a plant known for its medicinal properties which belong to the family (Amaryllidaceae). The characteristic odor of garlic and many of its health beneficial properties: antimicrobial, antithrombotic and anti atherosclerotic, are attributed to thiosulfinates strong reactivity to free-SH groups [9, 10] and [11]. The main thiosulfinates in garlic are allicin, alliin, ajoene, dially disulfide and S-allylcysteine [12]. Garlic oil is used in hair products to reduce seborrheic dermatitis due to its antifungal activity [13].

Eruca sativa Miller is an annual species which belongs to the mustard family (Brassicaceae). Common names include rocket, arugula [14]. It contains glucosides such as allyl sulphocyanate, mineral salts and vitamin C while its seed oil contains erucic acid [15]. *E sativa* is well-known in traditional medicine for their therapeutic properties as an astringent, diuretic, digestive, emollient, tonic, and depurative, laxative, rubefacient, stimulant [16] and useful in hair growth [17]. However, the antifungal activity of *E sativa* seed oil has been poorly investigated specially against dermatophytes causing hair diseases.

Our study aimed to formulate a new herbal hair cream active on a representative range of pathogenic fungi (*Malassezia furur, Microsporum canis bodin and Trichophyton mentagiophytes*) and developing convenient analytical methods for assessment of garlic oil during the *ex vivo* percutaneous permeation through albino rat skin.

MATERIALS AND METHODS

Materials

Microbiology

The isolates obtained from Assiut University Mycological Center (*Malassezia fufur* AUMC No. 5173, *Microsporum canis bodin* AUMC No. 5490 and *Trichophyton mentagrophytes* AUMC No. 5501). Terbinafine standard (100%) purity was kindly supplied by (NODCAR).

Plant material

Seeds of *Eruca sativa* Miller were purchased from a local herbal store and the specimen was identified in the department of botany, faculty of agriculture, Cairo University, Egypt. Seeds were surface sterilized using 1% mercuric chloride ($HgCl_2$) and ground into powder form by using an electrical grinder and preserved in plastic bags at 4ºC for further analysis.

Chemicals and standards

Acetonitrile (HPLC grade) and Brij 58, Fluka, (USA); Hexane, deionized water, petroleum ether, potassium dihydrogen-ortho-phosphate, methanol, benzene, concentrated H_2SO_4 and Stearic acid, El Nasr pharmaceutical, (Egypt); Span 60, Qualikems fine chemicals, (India); heptane sulphonic acid, lanolin and beeswax, Loba Chemie ltd., (India) and Cetostearyl alcohol, BASF Canada Inc., (Canada). All other ingredients were of analytical grade and used as received.

Deodorized garlic oil with known percent of thiosulfate (60%) was purchased from Jiangxi Baicao pharmaceutical co. LTD. (China) and standard alliin were kindly supplied by (NODCAR).

Methods

Evaluation of antifungal activity

Inoculum quantification was performed by quantitative plating on SAB to determine viable CFU per milliliter. The adjusted suspensions were vortexed and diluted 1:100 in distilled water. The diluted (1:100) suspensions were vortexed again, and 0.01-ml aliquots were spread (using a calibrated quantitative loop) onto SAB plates with a glass hockey stick. The plates were incubated at 28 to 30 °C and checked daily to determine the CFU per milliliter. Colonies were counted as soon as possible after growth became visible. For slower-growing colonies, the plates were reinsulated for several days. Inoculum quantitation for isolates needed to be performed at 24 h or less because of their rapid confluent growth. Assay of antifungal activity was performed by two methods:

Filter paper disc method

Sterile filter paper discs were soaked with the tested oils and dried 40°C for 1 hour. Discs were placed on fungi seeded plates were allowed to stand for 8 h at the refrigerator. Plates were incubated at 28°C for 3 d, after which the zone of inhibition was observed and measured. Each experiment was repeated 3 times [18].

Agar well diffusion method

Sabroud agar medium was poured onto each sterilized Petri dish after injecting a culture of tested fungi and distributed media in Petri dish homogenously for investigation of antifungal activity. The concentration of oil was ranged from 6.25 mg to 1g and incubated at 28 °C for 3 d. At the end of incubation period, minimum inhibitory concentrations were evaluated in mg/ml; Results are shown in (table 1).

Preparation of oils

***E sativa* volatile constituents**

Dried seeds (300g) were subjected to hydrodistillation for 3 h using Clevenger type apparatus. The volatile constituents were dried over anhydrous sodium sulfate and kept in a refrigerator for analysis.

***E sativa* fixed oil**

Powdered seeds were weighed accurately (210g) and subjected to extraction in a soxhlet apparatus at 60 °C using petroleum ether the extract obtained was filtered, concentrated in a rotary evaporator and dried. Saponifications of the petroleum ether extract of seeds and preparation of the fatty acid methyl esters were carried out according to [19] and [20].

Formulation of cream containing garlic and eruca oils

For the preparation of cream formulations (F1, F2 and F3) using span 60 and brij 58 as surfactant blend at three different concentrations 2, 4 and 6% w/w respectively, oil phase containing beeswax (2% w/w), cetostearyl alcohol (3% w/w), stearic acid (3% w/w), lanolin (2% w/w) and span 60 was heated up to 65 °C for melting the ingredients. The aqueous phase containing brij 58 and water was heated up to 50–55 °C. garlic and eruca oils (5% w/w for each) were added to oil phase when the temperature decreased to 50–55 °C and finally aqueous phase was mixed to the oil phase with constant stirring. The mixture was stirred for about 30 min until the homogenous cream was prepared.

Apparatus and chromatographic conditions

GC–MS analysis of volatile constituents and fixed oil

GC–MS analysis was carried out on a Gas-Chromatograph Agilent 689 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973. A fused silica capillary column (HP-5MS) 5% phenyl methyl siloxane as a nonpolar stationary phase (30 m × 0.25 mm, 0.25 μm). Temperature programming for volatile oil was from 60 °C to 180 °C at 5°C/min (5 min hold) then completed to 300 °C. Temperature programming for fatty acid methyl esters was from 120 °C to 300°C at 10°C/min (3 min hold) at 300 °C. the MSD was operated in electron impact ionization mode, scanning from *m/z* 50 to 550;the ion source temperature 250 °C and the quadrupole temperature 150 °C; solvent delay of 3 min was employed. The instrument was tuned using PFTBA (perfluoro-tributylamine). The components were identified by comparing their retention times and mass fragmentation patterns with those of the database libraries (Wiley [Wiley Int. USA] and NIST [Nat. Inst. St Technol., USA]). Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (GC/FID). Under the same conditions of GC/MS analysis and percentages obtained by FID peak area normalization method. Chromatograms are shown in (fig. 1 and fig. 2).

LC/MS and HPLC/UV analysis of garlic oil

Preparation of samples from creams

For garlic oil assay, about 4g of each cream sample were weighed precisely and mixed with 20 ml of saturated NaCl solution sonicated in hot water bath for 30 min for salting out. The mixture was centrifuged at 10000 rpm for 15 min, and the supernatant volumes were adjusted to 10 ml and prepared for analysis

Samples were conducted on Agilent (1260 infinity) liquid chromatography controlled by the Agilent Software Solution 1.0 and with Agilent 1260 pump, degasser Agilent1260, UV detector Agilent1200. A Phenonmex C18 column (250 mm x 4.6 mm, 5 µm) was used under 40 °C, and the peaks were monitored at 220 nm. The standards and samples were eluted using a gradient mobile phase consisting of phosphate buffer (PH 3) containing 5 mM heptane sulphonic acid (A) and acetonitrile (B). The gradient conditions were: 0-5 min 100% A, 6-20 min 70% A, 21-25 min 60% A, followed by conditioning with 100% of A for 5 min. The flow was 1.5 mL/min and an injection volume of 20.0 µl. The LC/MS of the oil was carried on a Thermo Finnigan LCQ quadripole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with the electro spray ionization (ESI) source. $[M^+H]$ ions were formed using ESI in positive mode. The sample dissolved in ammonium formate buffer at pH 3 was introduced to the ESI source by infusion at a flow-rate of 10 ml/min using a Harvard syringe pump drive (Harvard Apparatus, Holliston, MA, USA). A potential of 15.0 kV, a sheath gas flow rate at 30% and auxiliary gas flow-rate at 50% was employed. The heat and voltage of capillary were respectively maintained at 250 8C and 23 V. The $[M^+H]$ ion intensity was optimized by adjustment of the tube lens offset. Chromatograms are shown in (fig. 3).

Validation of the analytical procedures

Linearity, recovery and accuracy were carried out. All tests were performed in triplicate.

***Ex vivo* permeation studies**

Preparation of skin membrane

Male, white Albino rat was sacrificed, and the dorsal skin was excised. Hairs were removed using electric clipper; subcutaneous

tissues were surgically removed without damage to the skin. The skin samples were wrapped in aluminum foil after washing by isotonic phosphate buffer (IPB) and stored in a deep freezer at-20ºC until further experiment. All animals were treated in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care international's expectations for animal care and use/ethics committees. The study was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Skin permeation studies

For this investigation, static Franz glass diffusion cells (Microette plus, Hanson Research, USA) were used. These cells consist of donor and receptor chambers between which a dissected rat skin [21] was positioned with the epidermal side facing upward into the donor compartment; area of diffusion was 1.7 cm^2. The receptor medium was phosphate saline buffer pH 7.4 (8 g/l NaCl, 0.2 g/l KCl, 0.2 g/l KH_2PO_4 and 1.15 g/l $NaHPO_4$ in bi-distilled water), 3% (w/w) of polysorbate-20 and 0.25% (w/w) of isopropanol [22]. The receptor chamber was filled with 7.5 ml receptor media and stirred continuously at 600 rpm at 37 °C in order to ensure the skin surface temperature of 32 °C on the skin surface. Donor and receptor chambers were watertight closed with a metallic clamp, water circulation and magnetic stirring were started and a dose of 0.2 mg of each cream formulation (F1, F2 and F3) was applied to the surface of the epidermal side of the mounted skin in the donor compartment which was sealed with a screw cap to prevent evaporation of the essential oils. At time intervals of 1 hour within 12 h from the application, a 1 ml aliquot of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh receptor medium.

Permeation data analysis

For garlic oil penetration, alliin was taken as a marker. Known volumes (25, 50, 100, 250 and 500 µl) from the stock solution (1 mg/ml) were added to the receptor medium up to final volume of 7.5 ml and kept for chromatographic analysis.

The cumulative amount of alliin in garlic oil permeated through the skin (mg/cm^2) was plotted as a function of time (hours) for each formula. Flux (permeation rate) at steady-state (*J*) was calculated from the slope of the linear portion of the graph [23] and [24]. The permeability coefficient (*PS*) was calculated from Eq. (1)

$$PS = J/CD \quad (1)$$

Where, *J* is the drug flux at steady state and *CD* is the initial drug concentration in the donor cell. The lag-time *Lt* (h) is the time employed by the drug to start its diffusion through the skin in the receptor medium: it was graphically estimated by extrapolation of the steady state portion of curve *Q vs*. *t*. Data of percutaneous penetration and physiochemical parameters (flux, permeability coefficient, lag-time), averaged over three experiments are shown in (table 6). The significance of the results was checked statistically (SPSS statistics program, Release 20.0 for Windows, Chicago, IL) at ($^*P<0.05$) applying one-way ANOVA test. Post hoc multiple comparisons were carried out using the least square difference test.

RESULTS AND DISCUSSION

Evaluation of antifungal activity

The viability of (*Malassezia fufur, Microsporum canis bodin* and *Trichophyton mentagrophytes*) at various concentrations ranging from 0 (control) to 100% dilutions of garlic oil (GO), eruca volatile constituents and fixed oil (VOE and FOE) alone and in combination (FOE+G) were estimated using filter paper disc method. (table 1) showed that GO was the most effective in the inhibition of *M. canis bodin* but in the case of *T. mentagrophytes* and *M. fufur* VOE and FOE were the most active. The combination FOE+G increased the zone of inhibition 16.67% in case of *M. canis bodin*, 37.5% and 45% in case of *T. mentagrophytes* and *M. fufur* respectively. The minimum inhibitory concentrations (MIC) of the investigated oils and combination were determined using agar well diffusion method. For *M. fufur* there was no significant difference between the investigated oils each separate but after combination FOE+G the fungicidal activity against *M. fufur* was increased twice showing a synergistic effect while in the case of *M. canis bodin* and *T. mentagrophytes* the effect was additive. Comparing the MIC values of the combination FOE+G with the St terbinafine shows no significant difference in case of *Mic. canis bodin* and *T. mentagrophytes* while the sensitivity of *M. fufur* was higher to the combination FOE+G. These findings suggest that the combination FOE+G can be used as a substituent for synthetic antifungal terbinafine. Azole drugs as terbinafine (standard) are widely used in the treatment of dermatomycosis [25], but can cause various side effects and drug resistance to the patients. Hence, plant extracts can be used as an alternative for chemical drugs. Allicin isolated from garlic was tested for its potential as a treatment of dermatomycosis [26]. The presence of thiosulfonates of garlic and isothiocyanates of eruca may be the cause for the significant combination activity compared to the synthetic antifungal drug.

Table 1: Effect of eruca fixed (FOE) and volatile (VOE) oils, garlic oil (GO) eruca and garlic combination (FOE + G) and terbinafine standard (St) on some dermatophytes

Diameter of the inhibition zone (mm)					
Microorganism	**VOE**	**FOE**	**GO**	**FOE+G**	**St**
Malassezia fufur	19 ± 1^{b}	22 ± 2^{c}	10.67 ± 1.52^{a}	38 ± 2^{e}	26.33 ± 1.15^{d}
Microsporum canis bodin	23 ± 1^{b}	16.67 ± 2.3^{a}	23.67 ± 1.52^{b}	28.33 ± 2.08^{c}	33.33 ± 1.53^{d}
Trichophyton mentagrophytes	22.8 ± 1.89^{b}	24.3 ± 2.08^{b}	18.6 ± 1.53^{a}	39.3 ± 2.08^{c}	37.3 ± 2.08^{d}
Minimum inhibitory concentrations (mg/ml)					
Malassezia fufur	0.247 ± 0.015^{bc}	0.233 ± 0.015^{b}	0.273 ± 0.025^{c}	0.1215 ± 0.006^{a}	0.33 ± 0.01^{d}
Microsporum canis bodin	0.125 ± 0.002^{a}	0.48 ± 0.02^{c}	0.122 ± 0.003^{a}	0.243 ± 0.02^{b}	0.263 ± 0.02^{b}
Trichophyton mentagrophytes	0.74 ± 0.036^{c}	0.25 ± 0.03^{b}	0.127 ± 0.002^{a}	0.247 ± 0.035^{b}	0.227 ± 0.025^{b}

Each value represents the mean of 3 analysis±S. D. In the same row, the presence of different letter indicating a significant difference between oils by using one way ANOVA using SPSS software (version 20) followed by DMRT at $P<0.05$.

GC/MS analysis

Volatile constituents distilled from seeds of *E sativa* yielded a clear colorless liquid (0.4 %). GC/MS (fig. 1) revealed the presence of ten degradation products from glucosinolates [27], which represent 96.52% of the total constituents. The major compounds were 4-(methylthio) butyl isothiocyanate (erucin) which represent 82% and 5-methyl thiopental nitrile (9.2%) of the total oil, respectively (table 2). These results were in accordance with the previous report of the essential oil composition of *E. sativa* leaves [28].

Analysis of *E sativa* fatty acid methyl esters (table 3) representing 75.3% of the total lipids and their GC/MS analysis (fig. 2) resulted in the identification of ten compounds constituting 97.97% of the total fraction in which saturated and unsaturated fatty acids represent 13.04% and 86.96% respectively(monounsaturated fatty acids 55.7%and polyunsaturated fatty acids 31.26%).

The major fatty acid identified were erucic acid methyl ester represent (40%), oleic acid methyl ester (15.7), cis-11-eicosenoic acid methyl ester (11.9) and linolenic acid methyl ester (10.07).

Table 2: GC/MS of the volatile constituents of seeds of *E sativa*

S. No.	RI	Compounds	Area %
1	946	Butyl isothiocyanate	0.60
2	969	4-methyl thio1 butene	0.10
3	1100	Undecane	0.60
4	1200	Dodecane	3.50
5	1227	5-methyl thiopentanonitrile	9.20
6	1447	4-(methylthio)butyl isothiocyanate (erucin)	82.00
7	1505	Phenyl ethyl isothiocyanate	0.27
8	1653	5-methyl thiopentylisothiocyanate	0.18
9	1864	Triacontane	0.19
10	1870	Hexadecanol	0.25

RI, Kovates index

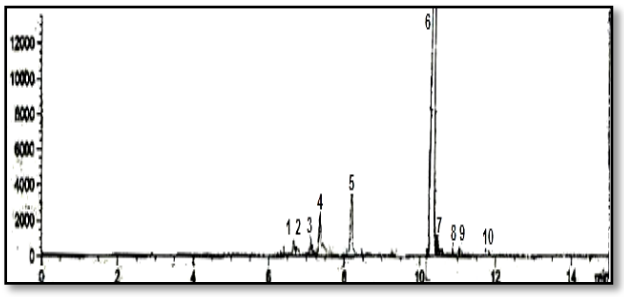


Fig. 1:GC/FID of the volatile constituents of seeds of *E sativa*

Table 3: GC/MS of the fatty acids of seeds of *E sativa*

Peak No.	Rt	Compounds	Rel%
1	6.3	Stearic acid methyl ester	5.01
2	6.8	Palmitic acid methyl ester	0.60
3	7.2	Oleic acid methyl ester	15.70
4	7.3	Linoleic acid methyl ester	9.29
5	7.4	Linolenic acid methyl ester	10.07
6	8.05	cis-11-Eicosenoic acid methyl ester	11.90
7	8.15	Heptadecanoic acid methyl ester	1.22
8	8.8	Erucic acid methyl ester	40.00
9	8.9	Nervonic acid methyl ester	1.68
10	9.55	Lignoceric acid methyl ester	2.84

Rt, retention time(min); Rel %, relative area percent

Although some chromatographic methods have been developed for the determination of thiosulfinates in garlic [29]; this study attempted to establish a chromatographic method for determination of garlic oil thiosulfinates in the formulated creams. HPLC chromatograms of garlic oil, alliin and creams are shown in (fig. 3)

Analysis of deodorized garlic oil was run on HPLC-UV and the peak identities were further confirmed by their total ion chromatograms of LC-MS according to method of [30].

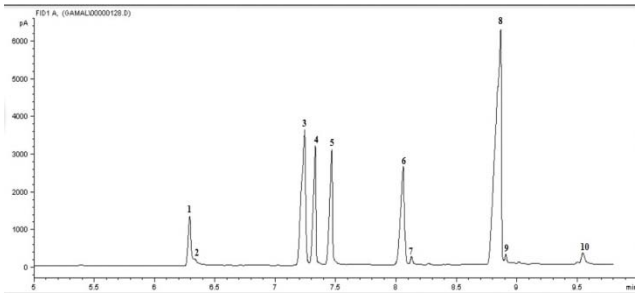
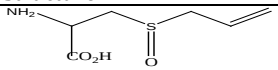
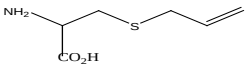
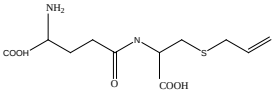
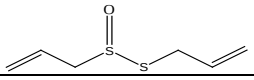


Fig. 2: GC/FID chromatogram of the fatty acid methyl esters of E sativa seeds oil

The HPLC chromatogram revealed the identification of four compounds. The major was alliin with a percent (30%) followed by allicin (27%). The identified compounds, their retention times and area percents are listed in (table 4). For the quantitative determination of alliin, a gradient elution analysis system was used. Good peak resolution was achieved within 25 min. Calibration curve of alliin was in a good linear correlation with a correlation coefficient of 0.997-0.999 within a range of 5-50.0 µg/ml for alliin in cream. The LOQ was 1.6 µg/ml and LOD, which represented the lowest detectable concentration of the analytes (S/N>3) was 0.06. For the three formulations, recoveries of the selected marker (alliin) were calculated it was high in the low and moderate concentrations of surfactant, but it decrease at the high concentration. The validation parameters of alliin in the three formulated creams are listed in (table 5).

Table 4: Identified thiosulfinates in garlic oil using Lc/Ms

Peak No.	Compound	A%	Rt	Structure
1	S-Allyl-L-cysteine sulfoxide (alliin)	30	10.35	
2	S-Allyl-L-cysteine (deoxyalliin)	16	11.77	
3	γ-Glutamyl-S-allyl-L-cysteine	11	13.58	
4	Diallylthiosulfinate (allicin)	27	23.27	

A%, total area percent; Rt, retention time.

Table 5: Validation parameters of alliin in garlic oil in the three formulated creams

Validation parameters	Results
Regression equation	*y =0.499x-0.027*
r^2	0.996
Accuracy	101.5
LOD	0.06 µg/ml
LOQ	1.6 µg/ml
% Recovery	F1=22.08 mg/g (95.8%), F2= 22.73 mg/g (98.7%) and F3= 22.22 mg/g (96.4%)

LOD, limit of detection (µg/ml); LOQ, limit of quantification (µg/ml). F1, F2 and F3 are cream formulations with surfactant concentrations 2, 4 and 6 w/w% respectively

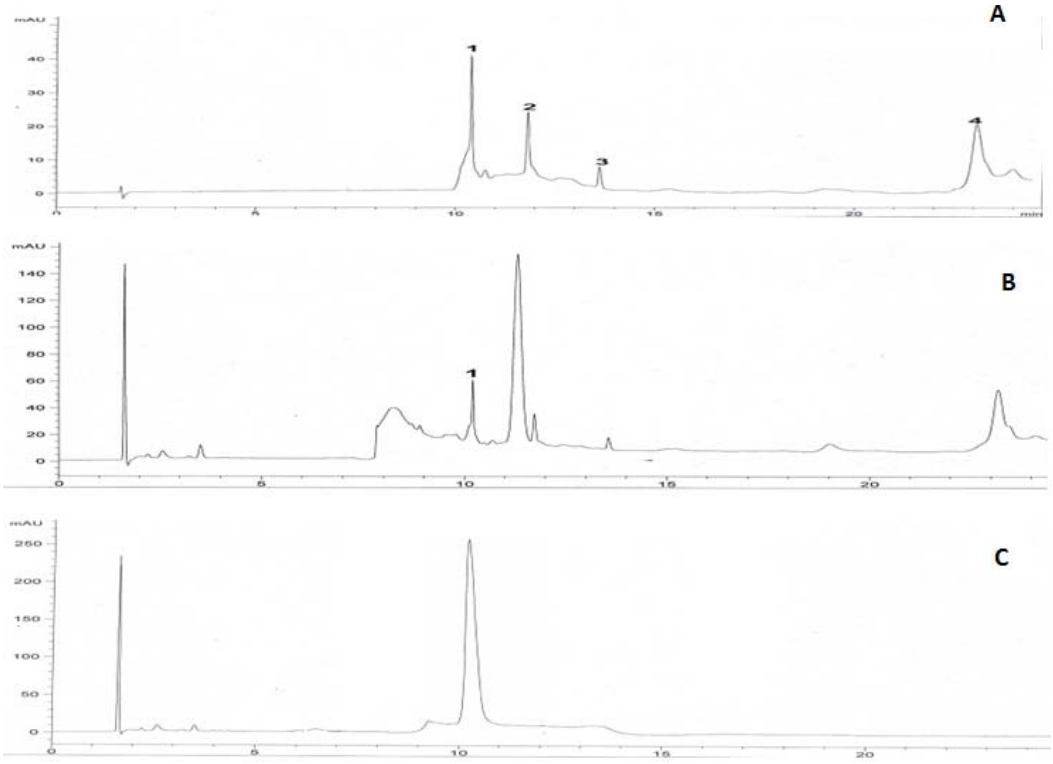


Fig. 1: HPLC chromatograms of A: garlic oil, B: formulation 2 and C: alliin (10.3 min)

***Ex vivo* percutaneous permeation**

Table 6: Donor concentration (CD), lag-time (Lt), flux (J) and permeability coefficient (PS) through rat skin of alliin released from the three cream formulations F1, F2 and F3

Donor formulation*	CD [mg/m]	Lt [h]	J [mg/cm²/h]	PS [cm/h]
F1	5.17	1.87	0.310±0.0015	0.060±0.0003
F2	5.32	0.05	0.337±0.0015	0.063±0.0003
F3	5.20	1.90	0.132±0.0065	0.025±0.0013

F1, F2 and F3 are cream formulations formulated with surfactant mixture (span 60 and Brij 58) concentrations of 2, 4 and 6 w/w% respectively

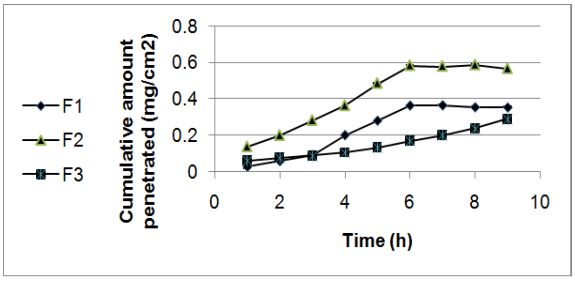


Fig. 2: Time course of the mean cumulative amount of alliin permeated through rat skin. Key: (♦) formulation 1(F1), (▲) formulation 2 (F2) and (*) formulation 3(F3). Each point represents mean±S. D. of 3 diffusion experiments

Despite the benefits of thiols in the skin, their use in skin applications has been somewhat limited due to their distinctive odor, limited cutaneous delivery and poor solubility [31]. Studying the permeation of garlic oil thiosulfinates, alliin was chosen to be permeation marker rather than allicin although they are nearly similar in percent (table 4). This may be attributed to its chemical structure as allyl cysteine sulfoxide in which cysteine acts as penetration enhancer [32]. The presence of sulfoxide group make it slightly similar to synthetic sulfoxides (dimethyl sulfoxide and decyl methyl sulfoxide) which have been reported extensively in the literature as penetration enhancers [33], a range of mechanisms have been suggested for their skin penetration enhancement properties including displacement of bound water from keratin, extraction of skin lipids, changes in keratin conformation and/or interaction with lipid alkyl chains in the stratum corneum [34]. The presence of eruca oil in

formulated creams increased thiosulfonates diffusivity through the partially delipidised SC due to the high content of unsaturated fatty acids (86.96%) in which oleic and linoleic acids represent (25%) [35]. It was thus postulated that since the oil contains different types of fatty acids, a synergistic effect cannot be ruled out [36].

The effect of increasing the non-ionic surfactant mixture span 60 and Brij 58 concentration (2, 4 and 6 w/w%) on the skin permeation of alliin in garlic oil from the cream formulations (F1, F2 and F3) in the presence of eruca oil was evaluated. The percutaneous permeation profiles of the tested cream formulations are shown in (fig. 4) and their related parameters consisting fluxes, lag times and permeability coefficients are tabulated in (table 6).

The results revealed that garlic oil penetration marker alliin flux values were 0.310±0.0015, 0.337±0.0015 and 0.132±0.0065 mg/cm^2/hr for F1, F2 and F3 respectively. These results showed that F2 formulated with 4% surfactant mixture exhibited an increase in garlic oil permeation compared with F1 containing 2% surfactant mixture. This could be due to the important role played by nonionic surfactants include the polyoxyethylene alkyl ether (Brij58 and Span60) [37] to enhance the flux of materials permeating through biological membranes by two possible mechanisms [38]. Initially, the surfactants may penetrate into the intercellular regions of stratum corneum, increase fluidity and eventually solubilize and extract lipid components. Secondly, penetration of the surfactant into the intercellular matrix followed by interaction and binding with keratin filaments may results in a disruption within the corneocyte that enhancing the thermodynamic coefficient of the drug allowing it to penetrate into the cells more effectively.

Also, it was observed that as the content of surfactant mixture increased from 4% w/w to 6%w/w, the skin permeation rate of garlic oil decreased. This may be due to the oil droplets emulsions which were abundantly covered with the surfactant molecules at the high surfactant concentration. Moreover, it is possible that in the presence of high surfactant concentrations, there are also excess surfactants that may inhibit diffusion of the oil to the carrier solution [39]. The same results were obtained by Shokri *et al.* [40] who established that the enhancement of the skin transport occurs at low concentrations of the surfactant (1% w/w), but this is seen to decrease at higher concentrations. In addition, this is in accordance with [38] who observed the decrease in drug permeation upon increasing the concentration of the nonionic surfactant Tween 80 in the formulation. This was attributed to the reduction of the thermodynamic activity of the drug (which is the driving force for penetration) due to its higher affinity to the vehicle upon increasing surfactant concentration (41).

CONCLUSION

Antimicrobial studies showed that combination of garlic and eruca fixed oils has a high activity on the selected dermatophytes. Formulation of an herbal hair cream using the non-ionic surfactant mixture span 60 and Brij 58 with a concentration 4% gives the highest permeation rate for alliin in garlic oil which is necessary for treatment of the hair fungal infections. The various constituents of the oils such as thiosulfates, unsaturated fatty acids and thiocyanates may be the cause for the significant combination activity compared to synthetic antifungal creams.

CONFLICT OF INTERESTS

The author reports no declaration of interest.

REFERENCES

1. Khoobchandani M, Ojeswi BK, Ganesh N, Srivastava MM, Gabbanini S, Matera R, *et al.* Antimicrobial properties and analytical profile of traditional *Eruca sativa* seed oil: comparison with various aerial and root plant extracts. Food Chem 2010;120:217-24.
2. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol 2012;7:979-90.
3. Simoes M, Bennett RN, Rosa EAS. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. National Public Radio 2009;26:746-57.
4. Tra FHB, Koné MW, Kouamé NF. Antifungal activity of erigeron floribundus (Asteraceae) from côte d'Ivoire, West Africa. Trop J Pharm Res 2008;7:975-9.
5. Dias MFRG, Quaresma-Santos MVP, Bernardes-Filho F, Amorim AGF, Schechtman RC, Azulay DR, *et al.* Update on therapy for superficial mycoses: review article part I. An Bras Dermatol 2013;88:764–74.
6. The Center for Food Security and Public Health. Dermatophytosis. Iowa state university, College of Veterinary Medicine, (Ames, Iowa); 2013. Available from: www.cfsph.iastate.edu. [Last accessed on 20 Aug 2015].
7. Shenefelt PD. Herbal treatment for dermatological disorders. In: Benzie IFF, Wachtel-Galor S. Editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton (FL): CRC Press; 2011. p. 383.
8. Sumit K, Vivek S, Sujata S, Ashish B, Rapid I. Herbal cosmetics: used for skin and hair. Inventi Rapid: Cosmeceuticals; 2012.
9. Qi R, Wang Z. Pharmacological effects of garlic extract. Trends Pharmacol Sci 2003;24:62-3.
10. Juszkiewicz A, Zaborska A, Łaptaś A, Olech Z. A study of the inhibition of jack bean urease by garlic extract. Food Chem 2004;85:553–8.
11. Hiyasat B, Sabha D, Grotzinger K, Kempfert J, Rauwald JW, Mohr FW, *et al.* Antiplatelet activity of *Allium ursinum* and *Allium sativum*. Pharmacology 2009;83:197-4.
12. Stool A, Seebeck E. *Allium* compounds. I. Alliine, the true mother compound of garlic oil. Helv Chim Acta 1949;31:197-9.
13. Ledezma E, De Sousa L, Jorquera A. Efficacy of ajoene, an organosulphur derived from garlic, in the short-term therapy of tinea pedis. Mycoses 1996;39:393-5.
14. Bianco VV. Rocket, an ancient underutilized vegetable crop and its potential. In: Padulosi S. Editors. Rocket Genetic Resources Network. Report of the First Meeting, Lisbon, Portugal; 1994.
15. Nuez F, Hernández Bermejo JE. Neglected horticultural crops. In: Hernándo Bermejo JE, León J. Editors. Neglected Crops: 1492 from a different perspective. Plant Production and Protection Series 26. FAO, Rome, Italy; 1994. p. 303-32.
16. Filocamo A, Paterniti Mastrazzo G, Maimone P, Buongiorno L, Catania S, De Pasquale R, *et al. Eruca sativa* Mill: phytochemical profile and antimicrobial properties of rocket leafy salads. Planta Med 2010;76:WSI_1.
17. Al Khalil S. A survey of plants used in jordanian traditional medicine. Pharm Biol 1995;33:317-23.
18. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of clinical microbiology. 6th ed. ASM, Washington, DC; 1995.
19. Vogel AI. Practical organic chemistry. 3rd ed. London: Longmans, Green; 1961.
20. Johnson AR, Davenport JP. Biochemistry and methodology of lipids. New York: Wiley; 1971. p. 35.
21. Dhawan B, Aggarwal G, Harikumar SL. Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. Int J Pharm Investig 2014;4:65-76.
22. Gabbanini S, Lucchia E, Carli M, Berlini E, Minghetti A, Valgimiglib L, *et al. In vitro* evaluation of the permeation through the reconstructed human epidermis of essentials oils from cosmetic formulations. J Pharm Biomed Anal 2009;50:370-6.
23. Patel RP, Gaiakwad DR, Patel NA. Formulation, optimization, and evaluation of a transdermal patch of heparin sodium. Drug Discoveries Ther 2014;8:185-93.
24. El Meshad AN, Tadros MI. Transdermal delivery of an anti-cancer drug via W/O emulsions based on alkyl poly glycosides and lecithin: design, characterization, and *in vivo* evaluation of the possible irritation potential in rats. AAPS PharmSciTech 2011;12:1-9.
25. Araújo CR, Miranda KC, Fernandes OFL, Soares AJ, Silva MRR. *In vitro* susceptibility testing of dermatophytes isolated in goiania, brazil, against five antifungal agents by broth microdilution method. Rev Inst Med Trop 2009;51:9-12.
26. Aala F, Yusuf UK, Jamal F, Rezaie R. Antimicrobial effects of allicin and ketoconazole on trichophytonrubrum under *in vitro* condition. Braz J Microbiol 2012;43:2.
27. Kim SJ, Ishii G. Glucosinolate profiles in the seeds, leaves and roots of rocket salad (*Eruca sativa* Mill.) and anti-oxidative

activities of intact plant powder and purified 4-methoxyglucobrassicin. J Soil Sci Plant Nutr 2006;52:394-400.
28. Miyazawa M, Maehara T, Kurose K. Composition of the essential oil from the leaves of *Eruca sativa*. Flavour Fragrance J 2001;17:187–90.
29. Pârvu M, Pârvu AE, Vlase L, Casian OR, Pârvu O, Puşcaş M, *et al.* Allicin and alliin content and antifungal activity of *Allium senescens* L. ssp. *Montanum* (F. W. Schmidt) Holub ethanol extract. J Med Plants Res 2011;5:544-9.
30. Arnault JP, Christide`s, Mandon N, Haffner T, Kahane R, Auger J, *et al.* High-performance ion-pair chromatography method for simultaneous analysis of alliin, deoxyalliin, allicin and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. J Chromatogr A 2003;991:69–75.
31. Barel AO, Paye M, Maibach HI. Handbook of cosmetic science and technology. 4th ed. Taylor and Francis group (NY); CRC Press; 2014. p. 273.
32. Babu RJ, Kikwai L, Jaiani LT, Kanikkannan N, Armstrong CA, Ansel JC, *et al.* Percutaneous absorption and anti-inflammatory effect of a substance P receptor antagonist: spantide II. Pharm Res 2004;21:108-13.
33. Williams AC, Barry BW. Penetration enhancers. Adv Drug Delivery Rev 2004;56:603-18.
34. Lane ME. Skin penetration enhancers. Int J Pharm 2013; 447:12-21.
35. Ibrahim SA, Kevin Li S. Efficiency of fatty acids as chemical penetration enhancers: mechanisms and structure enhancement relationship. Pharm Res 2010;27:115-25.
36. Amina S, Mirb SR, Kohlia K, Alib B, Ali M. A study of the chemical composition of black cumin oil and its effect on penetration enhancement from transdermal formulations. Nat Prod Res 2010;24:1151-7.
37. Kamboj S, Saini V, Bala S. Formulation and characterization of drug loaded nonionic surfactant vesicles (Niosomes) for oral bioavailability enhancement. Sci World J 2014. Doi.Org/10.1155/2014/959741. [Article in Press]
38. Butani D, Yewale C, Misra K. Amphotericin B topical microemulsion: formulation, characterization, and evaluation. Colloids Surf B 2014;116:351-8.
39. Sakulku U, Nuchuchua O, Uawongyart N, Puttipipatkhachorn S, Soottitantawat A, Ruktanonchai U, *et al.* Characterization and mosquito repellent activity of citronella oil nanoemulsion. Int J Pharm 2009;372:105-11.
40. Shokri J, Nokhodchi A, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Jalali MB, *et al.* The effect of surfactants on the skin penetration of diazepam. Int J Pharm 2001;228:99–107.
41. Javadzadeh Y, Hamishehkar H. Enhancing percutaneous delivery of methotrexate using different types of surfactants. Colloids Surf B 2011;82:422-6.