FORMULATION AND ASSESSMENT OF A HERBAL HAIR CREAM AGAINST CERTAIN DERMATOPHYTES

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Original Article

ABSTRACT

Objective: Developing an herbal antifungal formulation containing eruca and garlic oils against highly resistant dermatophytes (Malassezia furfur 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. Thiosulfonates 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 allin, 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 eru cic acid (40%) for the fixed one. The highest flux for allin (0.337±0.0015 mg/cm²/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 allin 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 microorganisms 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 furfur, Microsporum canis bodin and Trichophyton mentagrophytes are three dermatophytes known to cause severe fungal infections to hair scalp like seborrheic 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 antiatherosclerotic, are attributed to thiosulfonates strong reactivity to free-SH groups [9, 10] and [11]. The main thiosulfonates in garlic are allin, allin, 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 sulphoaycnate, mineral salts and vitamin C while its seed oil contains eru cic acid [15]. E. sativa is well-known in traditional medicine for their therapeutic properties as 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 furfur, Microsporum canis bodin and Trichophyton mentagrophytes) 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 furfur 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 (HgCl2) and ground into powder form by using an electrical grinder and preserved in plastic bags at 4°C for further analysis.

Chemicals and standards
Acetonitrile (HPLC grade) and Brij 58, Fluka, (USA); Hexane, deionized water, petroleum ether, potassium dihydrogen-orthophosphate, methanol, benzene, concentrated H2SO4, and Stearic acid. El Nasr pharmaceutical, (Egypt); Sp 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 Jiangdi Baicao pharaceutical co. LTD. (China) and standard allin was 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 growing colonies. The plates were reinsulated for several days and checked daily to determine the CFU per milliliter. Colonies were (1:100) suspensions were vortexed again, and 0.01-ml aliquots were spread on Sabroud agar medium was poured onto each sterilized Petri dish after homogenously for investigation of antifungal activity. The concentration of oil was ranged from 625 mg to 1 g and 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
Sabroad agar medium was poured onto each sterilized Petri dish after injecting a culture of tested fungi and distributing media in Petri dish using Clevenger type apparatus. The volatile constituents were identified by comparing their retention times and mass fragmentation patterns with those of the database libraries (Wiley and NIST [Nat. Inst. St Technol., USA]). Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (GC/RI). 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 4 g 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 Agilent 1260, UV detector Agilent1200. A Phenomenex 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 sulfonic acid (A) and acetonitrile (B). The gradient conditions were: 0-5 min in 100% A, 6-20 min in 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 out on a Thermo Finnigan LQ quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with the electro spray ionization (ESI) source. [MH] 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°C and 23 V. The [MH]+ 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 (PBS) and stored in a deep freezer at -20°C 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 cm². The receptor medium was phosphate saline buffer pH 7.4 (0 g/l NaCl, 0.2 g/l KCl, 0.2 g/l KH₂PO₄ and 1.15 g/l NaHPO₄ 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 h 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, allin 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 allin in garlic oil permeated through the skin (mg/cm²) 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)

\[
PS = J/CD (1)
\]

Where, J is the drug flux at steady state and CD is the initial drug concentration in the donor cell. The lag-time (t) 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 physicochemical 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 fujar, 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. fujar 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. fujar respectively. The minimum inhibitory concentrations (MIC) of the investigated oils and combination were determined using agar well diffusion method. For M. fujar there was no significant difference between the investigated oils each separate but after combination FOE+G the fungicidal activity against M. fujar 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 ter rifine shows no significant difference in case of Mic. canis bodin and T. mentagrophytes while the sensitivity of M. fujar was higher to the combination FOE+G. These findings suggest that the combination FOE+G can be used as a substitute for synthetic antifungal ter bifine. Anise drugs as ter bifine [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. Allin isolated from garlic was tested for its potential as a treatment of dermatomycosis [26]. The presence of thiocyanatones 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 ter-bifine standard (St) on some dermatophytes

<table>
<thead>
<tr>
<th>Diameter of the inhibition zone (mm)</th>
<th>Microorganism</th>
<th>VOE</th>
<th>FOE</th>
<th>GO</th>
<th>FOE+G</th>
<th>St</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malassezia fujar</td>
<td>19±1</td>
<td>22±1</td>
<td>10.67±1.52</td>
<td>38±2</td>
<td>26.3±1.15</td>
<td></td>
</tr>
<tr>
<td>Microsporum canis bodin</td>
<td>23±1</td>
<td>16.67±2.31</td>
<td>23.67±1.52</td>
<td>28.3±2.08</td>
<td>33.3±1.53</td>
<td></td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>22.8±1.89</td>
<td>24.3±2.08</td>
<td>18.6±1.59</td>
<td>39.3±2.08</td>
<td>37.3±2.08</td>
<td></td>
</tr>
<tr>
<td>Minimum inhibitory concentrations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malassezia fujar</td>
<td>0.247±0.015</td>
<td>0.233±0.015</td>
<td>0.273±0.025</td>
<td>0.121±0.006</td>
<td>0.33±0.01</td>
<td></td>
</tr>
<tr>
<td>Microsporum canis bodin</td>
<td>0.125±0.002</td>
<td>0.48±0.02</td>
<td>0.122±0.003</td>
<td>0.243±0.02</td>
<td>0.26±0.02</td>
<td></td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>0.74±0.036</td>
<td>0.25±0.003</td>
<td>0.127±0.002</td>
<td>0.247±0.035</td>
<td>0.227±0.025</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean of 3 analyses± 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 thiolental 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 (1.19) and linoleic acid methyl ester (1.07).
Table 2: GC/MS of the volatile constituents of seeds of *E sativa*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RI</th>
<th>Compounds</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>946</td>
<td>Butyl isothiocyanate</td>
<td>0.60</td>
</tr>
<tr>
<td>2</td>
<td>969</td>
<td>4-methylthio 1 butene</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>1100</td>
<td>Undecane</td>
<td>0.60</td>
</tr>
<tr>
<td>4</td>
<td>1200</td>
<td>Dodecane</td>
<td>3.50</td>
</tr>
<tr>
<td>5</td>
<td>1227</td>
<td>5-methyl thiopentanitrile</td>
<td>9.20</td>
</tr>
<tr>
<td>6</td>
<td>1447</td>
<td>4-(methylthio)butylisothiocyanate</td>
<td>82.00</td>
</tr>
<tr>
<td>7</td>
<td>1505</td>
<td>Phenyl ethyl isothiocyanate</td>
<td>0.27</td>
</tr>
<tr>
<td>8</td>
<td>1653</td>
<td>5-methyl thiopentylisothiocyanate</td>
<td>0.18</td>
</tr>
<tr>
<td>9</td>
<td>1864</td>
<td>Triacontane</td>
<td>0.19</td>
</tr>
<tr>
<td>10</td>
<td>1870</td>
<td>Hexadecanol</td>
<td>0.25</td>
</tr>
</tbody>
</table>

RI, Kovats index

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].

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

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

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

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

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

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound</th>
<th>A%</th>
<th>Rt</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S-allyl-L-cysteine sulfoxide (allin)</td>
<td>30</td>
<td>10.35</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>2</td>
<td>S-allyl-L-cysteine (deoxyallin)</td>
<td>16</td>
<td>11.77</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>3</td>
<td>γ-Glutamyl-S-allyl-L-cysteine</td>
<td>11</td>
<td>13.58</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>4</td>
<td>Diallyltiosulfinate (allicin)</td>
<td>27</td>
<td>23.27</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

A%, total area percent; Rt, retention time.
Table 5: Validation parameters of alliin in garlic oil in the three formulated creams

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>$y = 0.499x - 0.027$</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.996</td>
</tr>
<tr>
<td>Accuracy</td>
<td>101.5</td>
</tr>
<tr>
<td>LOD</td>
<td>0.06 μg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.6 μg/ml</td>
</tr>
<tr>
<td>% Recovery</td>
<td>F1=22.08 mg/g (95.8%), F2=22.73 mg/g (98.7%) and F3=22.22 mg/g (96.4%)</td>
</tr>
</tbody>
</table>

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

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

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.

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 thiosulfimates, alliin was chosen to be a 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 a 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/v%) on the skin permeation of allin 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 allin flux values were 0.310 ±0.0015, 0.337 ±0.0015 and 0.132 ±0.0065 mg/cm²/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 result 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/v 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 on the carrier solution [39]. The same results were obtained by Shoiki 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 allin 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.

CONFICT OF INTERESTS
The author reports no declaration of interest.

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