Preparation of Anise and Thyme Lotion for Topical Use
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Abstract :
Anise and thyme crude extract were used to prepare a lotion for topical application due to their antimicrobial, germicidal and antifungal effects. Two formulas were prepared using the mentioned natural plants, formula 2 (selected lotion) was the most acceptable one which contained veegum and xanthan gum as suspending agents in addition to other exceipients providing it good properties with high physical stability because of its flocculating, pouring, resuspending easily with sedimentation volume (F) 0.96. In addition to unchangeable odor and color with expiration date of one year. While the Preliminary clinical study was done using this lotion on 10 patients with infectious viral skin diseases, it was found that this lotion was successful as a topical preparation for allergy and mostly chicken pox.

Key words: Anise, Thyme, Crude Extract, Antimicrobial Effect.

Introduction :
Volatile oils are contained largely in the plant, they are secreted in oil cells, in secretion duct or cavities or in granular bairs.(1) These oils are used for their non-therapeutic action; flavoring e.g oil of lemon, in perfummary e.g oil of rose or as starting materials for the synthesis of other compounds e.g oil of turpentine. For therapeutic purposes they are administered externally as inhalation for catarrhs of the respiratory tract e.g thyme, eucalyptus and anise; as gargles and mouth washes e.g thymol and as transdermally for example many essential oils including those of lavender, rose mary and bergamot are employed in the practice of aromatherapy. (1, 2) It was found that the oils with high phenol content like clove and thyme have antiseptic, antibacterial and antifungal actions, while those with ether as chief constituent produce mild antispasmodic, mild insecticidal in addition to antibacterial action e.g funnel and anise. (2, 3) The constituents of many volatile oils are started to interfere with respiration and electron transport in a variety of bacteria, hence accounting for their use in food preservative and in cosmetic preparations. (4) Pharmaceutically these volatile oils can be used as extract, tincture or using the dried parts of the plant itself to be prepared for external use e.g thyme (thymi herba), creeping thyme (serpyili herba) wild oregano (origanie herba) and eucalyptus (oleum eucalypti). (4, 5) Many formulations can be used locally (external use) which include: collodions, liniments, lotions, paints, ointments and creams. (6) Lotions which are liquid preparation are easier to apply and less messy than many semisolid external preparation, they frequently contain suspended particles or emulsified liquid droplets. Depending on wether they are solid-liquid or liquid-liquid dispersions, therefore lotions can be classified as suspensions or emulsions. (6) Medicinal lotions are antiseptic and germicidal, they are used in the treatment of skin diseases and as cooling and mildly aesthetic applications for skin irritations. e.g calamine lotion and Benzyl benzoate lotions N. F. (6, 7).

Aim of the work

to prepare topical pharmaceutical liquid dosage form (lotion) from natural plants; arial part of thyme and fruit part of anise for topical applications.
Materials and Methods :-

Materials and Instruments
Anise (Pimpinella anisum) dried fruits plant.
Thyme (Thymus vulgaris) the stripped and dried leaves and flowers (aerial plant) (1) earial plant.

Preparation of stock dispersion of suspending agents:
Stock dispersion of each suspending agent used (sodium carboxy methyl cellulose, sodium lauryl sulfate, veegum and xanthan gum) was prepared by dispersing (0.8,1,2.5 and 5g m) of the suspending agents, respectively in 75 ml of distilled water (D.W) using an electric mixer at 150 r.p.m. The volume of dispersion was made up to 100ml with D.W, the resultant dispersions were allowed to hydrate for 24 hrs.

Preparation of lotion:
The preparation of lotions were shown in table (1)

<table>
<thead>
<tr>
<th>Formula 2</th>
<th>Formula 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>Quantities</td>
</tr>
<tr>
<td>Thyme and anise powder (1:1)</td>
<td>1 gm</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>0.25% w/v</td>
</tr>
<tr>
<td>Sodium carboxy methyl cellulose</td>
<td>0.5% w/v</td>
</tr>
<tr>
<td>Glycerine</td>
<td>5 ml</td>
</tr>
<tr>
<td>Alcohol 96%</td>
<td>15 ml</td>
</tr>
<tr>
<td>Rosemary spirit</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.18 gm</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.02 gm</td>
</tr>
<tr>
<td>D. W. Q. S to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

The dried fruits of anise and the dried aerial parts (leaves and flowers) of Thyme (1:1) were grinded in electrical grinder then passed through sieve 0.315inch /pore. Fine powder for these parts, methyl paraben and propyl paraben were levigated with glycerine, alcohol and a part of prepared dispersions. The remaining amount of dispersion was added in divided portions to the mixture. The mortar was rinsed several times with D. W and the rinsed volume of dispersion was added to cylinder. Finally, rosemary spirit was added, followed by sufficient quantity of D. W. to make up to the final volume (100 ml).

- Sedimentation volume (F)
Fifty ml of each formula 1 and 2 was diluted with D.W, to a volume of 100 ml in a stoppered graduated cylinder. The lotions were shaken vigorously to ensure uniformly then left undisturbed. The sedimentation volume which occurred under the influence of gravity was measured after a period of 48 hours. (11) F is the ratio of ultimate height of the sediment as lotion settles in a cylinder (Hµ) to the initial height of the total lotion (H0)(13).

Thus  \( F = \frac{H_\mu}{H_0} \)

When the ultimate volume of the sediment is smaller than the original volume of lotion, the sedimentation volume is less than 1. If this value can be made to approach unity, the product becomes in flocculation equilibrium and show no supernatant on standing so it is pharmaceutically acceptable. Therefore 50 ml of each the selected formulas should be diluted with distilled water to a volume of 100 ml in a stoppered cylinder. The lotion was shaken vigorously to ensure uniformity, then left undisturbed. The sedimentation volume which occurred under the influence of gravity was measured every 4 hours for a period of 48 hours. (10)

Resuspendability of lotions (12, 13).

The resuspendability of formula 1 and 2 was evaluated quantitatively. The efforts of redispersion required to convert the sedimented system to homogenous lotion by shaking the cylinder manually rated as resuspendable, resuspendable with difficulty or not resuspendable.

Rheogram (11,13):
Rheogram was obtained at 40 °C with Brookfield DV-H+ pro viscometer which read viscosity versus shear rate.

Assay of the volatile oils (14, 15):
TLC technique was used to determine the active ingredients present in the prepared lotion in comparison with standard powder of 2 plants (1:1) which was done by diluting the lotion to 200 ml using D.W, filtering, then the powder on filter paper was macerated in ether for 3 days. On the other hand 0.5 gm of standard powder (1:1 dried from of anise and dried aerial parts of thyme was also macerated in ether over night, then the products of both were concentrated by evaporating of ether, then TLC technique was done using benzene and chloroform (1:1) as mobile phase, while anisaldehyde was used as spraying reagent to calculate the retarded factor (RF) value of spots produced of both lotion and standard powder. RF value is directly propotional to the remaining quantities of active ingredients.

Stability and Shelf life (13):

9
The same method mentioned above was used in which the total amount of stored lotion (50 ml) was diluted, filtered and macerated in ether then TLC technique was done to determine the stability of prepared lotion stored at 40 °C, 50 °C and 60 °C for four months by measuring the RF values of all samples by calculating their quantities in comparison with standard lotion stored at room temperature.

**Preliminary clinical study:**
The prepared lotion was applied topically to 10 patients twice daily those with skin disease (allergy, infection, chicken pox …) (1).

**Statistical Analysis:**
Analysis of variance ANOVA table and X^2 test (chi-square) was used to examine the effect of some parameters.

**Results and Discussions**

**Formulation of the lotion:**
Lotions are usually suspensions of solids in an aqueous medium. A few lotions are, in fact, emulsions or solutions (6). A wide variety of ingredients may be added to the preparation to produce better dispersions (6). Methyl cellulose or sodium carboxy methyl cellulose will localize and hold the active ingredient in contact with the affected site and at the same time be rinsed off easily with water. However they are usually added to lotion, cataplasms and burn medications to produce tough and flexible films. Surfactants as sodium Lauryl sulfate can also be used in preparation of lotions to induce the flocculation of particles in dispersion (i.e formation of a loose aggregation of particles). (7, 13).

Therefore formula 1 was prepared using sodium lauryl sulfate and sodium carboxy methyl cellulose as a suspending agents at concentrations of 0.25% w/v and 0.5% w/v respectively (16).

While in formula 2, the veegum and xanthan gum combination was used to control flocculation (17). The following excipients additives were also added to the prepared lotions: alcohols for its drying and cooling effect, glycerine to keep the skin moist for a considerable period of time, rose mary spirit as an odouring agent, methyl paraben and propyl paraben as preservatives (7, 18).

**Sedimentation volume (F) and Resuspendability:**
Formula 1 had F=0.2 and was resuspended while formula 2 had F=0.96 and was easily resuspended (19). Depending on physical properties, formula 2 was selected to study its rheogram, shelf life and clinical effect.

**Rheogram:**
Rheogram of formula 2 is represented in figure (1). The profile showed that the viscosity of formula 2 lotion decreases with increasing rate of shear, therefore, the results illustrated that the selected formula exhibited pseudoplastic flow due to the blend of veegum and xanthan gum. This is resulted to get a formula with good viscosity, physical stability and smooth flow characteristics. Such blend was well suited to stabilizing all types of fluid suspensions and lotions (17, 19, 20).

**Study of shelf life (T 10%)** (9, 8, 13):
The accelerated studies at higher temperatures (40 °C, 50 °C, and 60 °C) were employed to predict the breakdown of active ingredients that may occur over prolonged periods of storage at normal shelf condition as shown in figures 2 and 3. The loss of anise and thyme active ingredients is directly proportional to the concentrations remaining with respect to time, it is called a first order reaction.

**Figure (1): Rheogram of formula 2**

**Figure (2): Accelerated stability study of anise crude extract in prepared lotion at elevated temperatures**
Figure (3): Accelerated stability study of thyme crude extract in prepared lotion at elevated temperatures.

The mathematical expression is:

\[-\frac{dc}{dt} = KC\]

where \(C\) is the concentration of intact active ingredients remaining.

\(t\) is time

\((dc/dt)\) is the rate at which the intact active ingredients degrades, and \(K\) is the specific reaction rate constant. The integrated and more useful form of equation:

\[\log C = -\frac{Kt}{2.303} + \log C_0\]

The degradation rate constants \((k)\) were calculated from slopes of straight lines. These degradation rate constants are summarized in Table (2).

Table (2): Degradation Rate Constants \((K)\) and RF Values of Both Anise and Thyme Crude Extracts in Formula 2 at Different Temperatures.

<table>
<thead>
<tr>
<th>Temperatures ((^\circ C))</th>
<th>(K) ((\text{month}^{-1}))</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anise</td>
<td>Thyme</td>
</tr>
<tr>
<td>40</td>
<td>17 x 10^{-3}</td>
<td>10.6 x 10^{-3}</td>
</tr>
<tr>
<td>50</td>
<td>25.5 x 10^{-3}</td>
<td>15.6 x 10^{-3}</td>
</tr>
<tr>
<td>60</td>
<td>38 x 10^{-3}</td>
<td>24 x 10^{-3}</td>
</tr>
</tbody>
</table>

To determine \(t\) 10%, Arrhenius plots were constructed to predict the degradation rate constants at 25 \(^\circ C\) \((K_{25})\) for both anise and thyme crude extracts. Figures 4 and 5 predicted the rate constants of formula 2 which were found to be equal to 8.6 x 10^{-3} and 6.2 x 10^{-3} month^{-1} respectively.

Thus \[T_{10\%} = \frac{0.104}{K_{25\circ C}}\]

The shelf lives of both anise and thyme crude extracts in formula 2 were 1.0 and 1.3 years respectively. Therefore the expiration date of formula 2 was about 1 year.

Figure (4): Arrhenious plot for shelf life estimation of anise crude extract in the lotion.
Figure (5): Arrhenious plot for shelf life estimation of thyme crude extract in the lotion.

As shown in scheme 1\(^{(21)}\), thyme extract contains chemical materials thymol and carvacrol which known as phenol types that are held responsible for antiseptic properties of thyme.\(^{(3)}\) Thyme extracts according to the results shown in table (2) were significantly (P<0.05) more stable than anise extracts which also have anti-infection and anti-inflammation effects.\(^{(1,21)}\) Anise extracts which contain fenchone (a bicyclic monoterpen) and anisatin ketons are affected by accelerated studies at higher temperatures.\(^{(1,21)}\)

**Scheme (1)**

_Thymol_ and _Carvacrol_ are the active components in thyme extract. Fenchone and Anisatin are the active components in anise extract.

**Preliminary clinical study:**
The preliminary results of clinical study shown in table 3 suggest that formula 2 can be used for treatment of infectious viral skin disease such as chickenpox which was caused by varicella zoster virus (VZV); this viral disease was highly contagious and common disease throughout the world, most patients ages with chickenpox were ranged between 6-10 years.\(^{(22)}\) Significant period healing differences (P<0.05) depending on age with rapid clinical improvement was achieved during period of treatment (7-14 days) due to the antimicrobial, antifungal and germicidal properties of anise-thyme crude extracts due to essential oil compositions (thymol, carvacrol, anisatin and fenchone) with disappearance of all signs of this disease when this lotion was given topically two times/day with antipyril syrup.\(^{(23, 24, 25)}\)

<table>
<thead>
<tr>
<th>PATIENT NO.</th>
<th>AGE (YEAR)</th>
<th>SEX</th>
<th>DISEASE</th>
<th>DOSE TOPICALLY</th>
<th>DURATION OF TREATMENT (DAY)</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>8</td>
<td>Male</td>
<td>Chicken pox</td>
<td>Apply two times /day</td>
<td>14</td>
<td>rapid healing</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>7</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>9</td>
<td>Female</td>
<td>Chicken pox</td>
<td>=</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>7</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>4</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>7</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>6</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>10-10</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>7</td>
<td>Female</td>
<td>Chicken pox</td>
<td>=</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>10</td>
<td>Male</td>
<td>Chicken pox</td>
<td>=</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

**Table (3): The results of Preliminary Clinical Study Using Formula 2 on 10 Patients with Infectious Skin Diseases.**

-The Degree of healing depend the relief of the patients skin from itching, redness and pustules.

**Conclusion:**
Crude extracts from the dried fruits of anise and the dried aerial parts of thyme were used as natural plants for preparing of successful anise-thyme lotion for topical infectious skin diseases. Formula 2 with veegum and xanthan gum suspending agents was the selected formula for preparing lotion since it was flocculated, poured, resuspended easily with F value 0.96, and it had good odor, color with expiration date of 1 year. Addition it was very effective in many infectious and virus skin diseases mainly itching and chicken pox.

**Acknowledgement:**
The authors gratefully acknowledge D. Fakher Fakhri for his aids in preliminary clinical study.
Synthesis of anti-inflammatory aromatic Schiff bases

References:
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