

Utilization of Essential Oil in Manufacturing of Different Types of Balm

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ABSTRACT

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Author's Contribution

All the authors contributed significantly to the research that resulted in the submitted manuscript.

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Background: Despite of great improvements and advancements in the amenities of life, alternative medicine's trends is increasing day by day. It is obvious that all plants are significantly potent as well as effective without side effects; it is therefore the designed formulation are authenticated and applied for the indication of acute respiratory illness accompanied by cough, rhinitis, muscle aches, as well as accumulation of thick sputum in chest (during bronchitis, tracheitis, and pneumonia).

Methodology: By using essential oils like menthol, eucalyptus oil, camphor, turpentine oil and clove oil three different formulation has developed i.e. classic, all natural or kids. Medicinal plants have been reported in the literature as having good and effective in cough associated with flu, runny and stiff nose, muscles aches etc. Various formulation batches were prepared and evaluated for various parameters like color, appearance, weight, pH, assay identification and microbiological activity.

Result: It is a stupendous effort to design and development of all-natural balm having natural sources including excipients which was free from *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

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INTRODUCTION

Essential oils are used as alternative medicines in respiratory illness. They have been utilized to treat cough, cold with flu and muscles ache remedies as analgesics [1]. Besides others, medicinal plants contain various essential oils which are used in alternative medicines and have been reported effective in many illnesses [2]. The essential oils have been utilized to treat cough, cold with flu and muscles ache remedies as analgesics. It has been described that plants

including *Eucalyptus globules*, *Syzygium aromaticum*, *Gaultheria procumbens* L. and *Pinus* species confers the effects of essential oils. Menthol is a naturally occurring cyclic terpene alcohol of plant origin and main ingredient of *Mentha piperita* L., menthol is endowed with analgesic properties mediated through a selective activation of κ -opioid receptors [3]. Although high dose of menthol caused sensory irritation [4], because it acts as an activator of chemical nocisensor [TRPA1/Transient receptor potential (TRP)] in humans [5]. Some over-the-counter active

ingredients can relieve the sensation of itch by interacting with the neurological pathways that propagate the signals to the brain. In particular, menthol activates a special class of cold receptors that interrupts itch sensory pathway [6]. Recent studies [7, 8] and guidelines [9] have questioned the efficacy of many oral over-the-counter (OTC) treatments for upper respiratory infection symptoms. Alternatives to oral medications are popular topical preparations that contain menthol, camphor, and eucalyptus oils and have been used in adults and children for more than a century [10]. A relaxant effect on tracheal smooth muscle was also demonstrated in rabbits and guinea pigs exposed to volatile oil of *Rosmarinus officinalis* (which contains camphor and cineol) [11]. The essential oil of *Eucalyptus globulus* oil containing cineole is the major active ingredient) has an anti-inflammatory effect on chronic bronchitis induced by lipopolysaccharide in rats and inhibits the hypersecretion of airway mucins [12]. Eugenol the main constituent of clove oil (*Syzygium aromaticum*) is used for the treatment of systemic infections of children and also decreasing body temperature and reduced fever [13]. Menthol 2.6% (Cough suppressant and topical analgesic), Camphor 4.8% (Cough suppressant and topical analgesic), Eucalyptus oil 1.2% (Cough suppressant) and turpentine oil inactively (4.68%) used in vicks vaporub [14]. Topical analgesics used in the United states patent 8304001 are made up of 3.4% Eucalyptus oil [*Eucalyptus globules*], 3.4% Peppermint oil [*Mentha piperita*], 3.4% Wintergreen oil [*Gaultheria procumbens*], 3.4% Lavender oil [*Lavandula angustifolia*], 1.7% Clove Bud oil [*Syzygium aromaticum*] 60% glycerin, and 25% water and invention is used in topical muscle and joint pain reliever, as well as a decongestant [15] (Table 1). Linkus balm has been recommended to be used in cough, associated with acute respiratory illness, rhinitis, muscles aches and accumulation of thick sputum in chest (during bronchitis, tracheitis pneumonia). Linkus balm

contains menthol, camphor, clove oil, eucalyptus oil, gaultheria oil and turpentine oil in different synthetic and plant resources with excipients to formulate the dosage form design. The present study aimed to assess the use of essential oil in manufacturing of different types of balm which could be used in alternative medicine.

MATERIALS AND METHODS

Collection of plant Materials

Extracted oil from the formulated dosage form for Linkus balm procured through the supply chain department of Herbion Pakistan Private Limited. The suppliers were Mak-Kemikal, Sabcon Chemicals, and Creatif Services from Karachi, Pakistan.

Development of Formulation

Based on literature search Linkus balm formulation was developed and manufactured for acute respiratory illness accompanied by cough, rhinitis, and muscle ache.

Preparation of Formulation

For the purpose of Linkus balm the different dosage constituents and their quantities variations were mixed with hard paraffin and soft paraffin in manufacturing vessel and heated up to 70-80 °C, till all the ingredients melted and mixed completely. After thoroughly mixing the following material was added like menthol 10.00 kg, camphor 50 kg, eucalyptus oil 3.30 kg, gaultheria oil 1.275 kg, clove oil 1.94 kg, turpentine oil 1.17 kg in the manufacturing vessel one by one with constant stirring and mixed for 5 minutes to get the homogenous solution. Filter the ingredients/solution with mesh #350 and transferred to storage container. For the development of Linkus balm all substances/material/excipients were collected. The parameters and norms for Linkus balm are given below (Table 2).

Table 1. Various types of essential oil used in the manufacture of different types of balm used in alternative medicine.

| S. No. | Herbs | Common Name | Composition A (Classic Balm) each 25 g contains | Composition B (All Naturals Balm) each 25 g contains | Composition C (For Kids Balm) each 25 g contains | Pharmacological Action |
|--------|-----------------------|-------------------|---|--|--|---|
| 1 | Eucalyptus globulus | Eucalyptus Oil | 1.5 gm (1.65ml) | 1.5 g | 0.125 g | Cough suppressant and topical analgesic, chronic bronchitis, sinusitis, fever |
| 2 | Syzygium aromaticum | Clove Oil | 1.0 gm (0.97ml) | 1.0 g | 0.0025 g | Helps in infection and reduced fever |
| 3 | Gaultheria procumbens | Oil of Gaultheria | 1.5 gm (1.275ml) | 0.75 g | --- | Topical muscles and joint pain reliever |
| 4 | Pinus Species | Turpentine Oil | 0.5 gm (0.585ml) | 0.5 g | 0.500 g | Counterirritant |
| 5 | Mentha piperita | Menthol | 2.5 gm | 5.0 g | 0.500 g | Cough suppressant and topical analgesic |
| 6 | Cinnamomum camphora | Champhor | 1.75 gm | 1.75 g | --- | Cough suppressant and topical analgesic |

Table 2. Various parameters and values for Linkus balm.

| Parameter | Norm |
|---|--|
| Appearance | Ointment of colorless or white color |
| Solubility | Soluble in hot chloroform |
| Specific gravity | 1.20 – 1.60 |
| Melting point | 30 – 60 °C |
| Uniformity of weight | 25 g ± 10% |
| Originality by GLC | On chromatograms tested solution B should be present peaks of compounds corresponding to retention periods on internal standards chromatograms I and II. |
| Quantitative Determination(GLC) | Menthol NLT 15.0% Camphor NLT 5.0% |
| Microbiological purity (SPRB, category 2.0) | Admissible contents for 1 g: Total count aerobic bacteria and fungi – not more than 10 ⁴ cfu/ g Enterobacteriaceae – not more than 10 ¹ cfu/ g <i>P. aeruginosa</i> - absent <i>S. aureus</i> – absent |

The desired concentration of oils and excipients were weighted accurately and following the above parameter like appearance, solubility, specific gravity, melting point and uniformity of weight with applying different qualitative determination (GC) and microbiological assay.

Evaluation of Formulation

Appearance

Ointment of colorless or white color. (Assessed visually).

Solubility

Accurately weighed 5 grams of sample in 100 ml volumetric flask made up the volume with hot chloroform (temperature of chloroform 50 - 55°C). The solution was cleared. (Assessed visually).

Specific gravity of 5% solution of sample in chloroform

Specific gravity was determined by dividing the weight of 5% solution of Linkus balm with the weight of same volume of water and the standard limits were set as 1.20 to 1.60.

Melting point

Melting point was determined by capillary tube method by using BioCote melting point instrument.

Requirements or Standard limits: 30 - 60°C

Quantitative determination (menthol and camphor)

Limit: menthol contents should not be less than 15%, and camphor – not less than 5%.

Tested solution preparation

Linkus balm (500 mg) was taken in a 250 mL volumetric flask, in which 22 mL of ethyl acetate was added. This mixture was mixed on magnetic stirrer for 10 minutes at 25°C. The mixture was cooled at 8°C and filtered through a filter paper. Dilute the filtrate up to 25 mL volumetrically with ethyl acetate. Filter the resulting solution

(solution A). The 5 mL of solution A was diluted up to 10 mL volumetrically with ethyl acetate (solution B).

Preparation RSO solutions

Menthol and camphor standards were dissolved in ethyl acetate yielding concentrations of 1mg / 10 mL for each standard separately.

External standard

Mix 2 ml of RSO menthol solution and 1 ml of RSO solutions of camphor in a 10 ml volumetric flask, made up to the volume with ethyl acetate (solution B).

Conditions

Injection volume was 1 µl for all analysis, detector was flame-ionization (FID), and column was Teknokroma (TRB-5, 30m x 0.25 x 0.25um, capillary column)

For the determination of menthol and camphor solution B was used. Column thermostat temperature was programmed: 50°C for 15 minutes, then increase up to 180°C at rate of 20°/min. Detector's and injector's temperatures were 200°C. Carrier gas was nitrogen with flow rate of 30 cm³/min, hydrogen and air consumption were 30 cm³/min, and 600 cm³/min. Contents X of menthol and camphor (in%) calculated by formula:

$$X = \frac{S \cdot m_0 \cdot 25 \cdot 10}{S_0 \cdot m \cdot 10 \cdot 5} \cdot 100$$

Where

S– area of sample, S₀ –area of standard, m – amount of sample (mg), m₀ – amount of standard (mg).

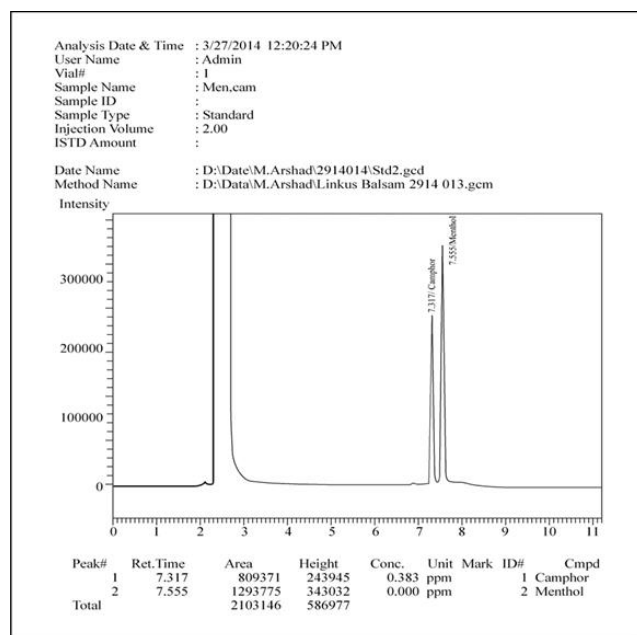


Figure 1. Analysis of camphor and menthol with flame-ionization.

Microbiological purity

The antimicrobial activities of different formulations were determined by the admissible content which was about 1 g; while the total count was aerobic bacteria and fungi not more than 10^4 cfu/g. Enterobacteriaceae spp. was – not more than 10^1 cfu/g. Further evaluated that *Pseudomonas aeruginosa* was absent as well as *Staphylococcus aureus* was also absent. Preparation in conditions of test did not has antimicrobial action.

Evaluation of pre-clinical dermatologically toxicity studies

Evaluation of pre-clinical dermatological toxicity studies had been initialed in the Animal House of Herbion. Whereas the test drug sample (Linkus balm) was collected from the Research and Development Department, Herbion Pakistan (Pvt.) Limited.

Experimental Animals

Albino Guinea pigs (300 – 400 g) were obtained from animal house facility of Herbion Pak. Pvt. Ltd. They were housed under standard environmental conditions i.e. $25 \pm 1^\circ\text{C}$ and 12 h

dark / light cycle. Food and water were available *ad libitum*. Food and water were available *ad libitum*. The experiments were performed in accordance with the guidelines provided by the National Institute of Health (NIH publication no. 85-23, revised 1985) for the care and use of laboratory animals.

Dermatological toxicity

Guinea pigs of either sex ($n = 3 / \text{sex}$) were shaved to remove hairs. The test substances 2 or 3 g/kg and reference Woodward baby chest rub 2 or 3g/kg were applied topically for 7 days. The un-treated animals were used as control. Mortality and dermatological changes were observed for 1 week. The animals were weighed before the commencement of test and at the end of the test.

RESULTS

Effects of test product Kids Chest Balm on toxicity on mortality in Guinea pigs

The test product Linkus balm did not cause mortality in guinea pigs at the given doses. The skin did not inflame, scaly and sensitized as compared to reference decongestants and analgesic balm (Woodward chest rub) and untreated animals (Figure 2-4). It was found that test product is appeared to be dermatologically safe.

The pre-clinical study was conducted on both the test Linkus balm and the control (Woodward) drugs on healthy guinea pigs in the Animal Laboratory of Herbion Pakistan (Pvt.) Limited. It was found that test product is appeared to be dermatologically safe.

DISCUSSION

Linkus Balm was evaluated in term of appearance and found color less or white in color with solubility 5% solution in chloroform at temperature 50-55 C. The specific gravity of 5% solution in chloroform was 1.20-1.60 at the same time the melting point was 30-60°C. The

uniformity of weight was $25 \text{ g} \pm 10 \%$ although the novelty was checked through GLC, on chromatograms tested solution B should displayed peaks of compounds corresponding to retention periods on internal standards chromatograms I and II. Quantitatively determination evaluated values were menthol not less than 15.0% and camphor not less than 5.0%. By analyzing antimicrobial activity it was found that new developed Linkus balms had an inhibitory effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It also shows satisfactory zone of inhibition compared with control sample.

The present study conclusively proved that newly developed Linkus balm was successfully designed, developed and assessed quantitatively and anti-microbiologically. In addition, the quantity of active ingredients like menthol, and camphor were determined by using Gas Chromatography (GC). Hence Linkus balm could be used as better and safe substitution of natural's world because it contained all naturals' ingredients including excipients. In previous studies it was described that menthol helps for decrease in congestion by stimulating trigeminal cold receptors which binds to the TRPA8 receptor (a temperature receptor), (TRPM8; CMR1) [16,17] and traditional method of cold and pain relief with combination of eucalyptus [18-20] and act of K-receptor. Although the recent antitussive activity of menthol has been established in guinea pigs [21]. Despite the fact that camphor used here in our product as antiseptic, analgesic, antipruritic, counterirritant and rubefacient [22,23]. Even as inhalants effects of camphor when applied topically helps to recover the symptoms of cold [24,25]. It helps to improve antitussive activities, nasal decongestant and expectorant [26]. It is reported that eugenol 3 is the main component of (*Syzygium aromaticum*) which helps in different infection in children, and reduced fever [27]. The combination therapy of (camphor, menthol, and eucalyptus oils) helps in relief of nighttime cough, congestion, and helps to improve sleep

when compared with petrolatum or no treatment [28]. The American patent with different combination helps for reliving joint pain as well as decongestant. The essential oil containing turpentine oil, eucalyptus and peppermint oil in which turpentine used as counterirritant, whilst eucalyptus oil as topical counterirritant and peppermint oil as inhalation. Dosage forms of Linkus balm is prepared for various evaluation parameters such as general appearance (white to color less), solubility (in hot chloroform), specific gravity (1.20-1.60), melting point (30-60°C) and uniformity of weight ($25 \text{g} \pm 10\%$) with different quantitative analysis like GC through which quantity of menthol is NLT 15.0% and 2.0%, camphor. Pre-clinical test was developed to check the sensitivity by topical application in guinea pigs while the comparative market brand was baby chest rub by Woodward and found to be clinically safe.

CONCLUSION

The biomarker such as menthol and camphor of Linkus balm were evaluated quantitatively through GC and determined as, Menthol not less than 15.0% and Camphor not less than 5.0 %. Linkus balm showed effective and improved efficacy. It is a stupendous effort to design and development of all-natural balm having natural sources including excipients which was free from *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Despite the fact that pre-clinical study Linkus Balm showed that it is effective dermatologically and safe in children.

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