(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 June 2006 (29.06.2006)

PCT

(10) International Publication Number
WO 2006/067600 A2

(51) International Patent Classification:
A61K 9/20 (2006.01) *A61P 11/14* (2006.01)
A61K 36/19 (2006.01) *A61P 11/12* (2006.01)
A61K 36/61 (2006.01)

(21) International Application Number:
PCT/IB2005/003860

(22) International Filing Date:
22 December 2005 (22.12.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2550/Del/2004 23 December 2004 (23.12.2004) IN

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HERBAL FORMULATIONS AS COUGH LOZENGE

(57) Abstract: The present invention relates to polyherbal pharmaceutical formulations in the form of lozenges which have been found to be effective in treating and managing coughs and sore throats. The invention also relates to processes for preparation, detection and quantitative analysis of individual phytochemicals used in the polyherbal formulations.

WO 2006/067600 A2

HERBAL FORMULATIONS AS COUGH LOZENGE

Field of Invention

The present invention relates to new polyherbal pharmaceutical formulations which have been found to be effective in treating and managing cough and sore throat. The formulations comprise extracts from *Adhatoda, Syzygium* and *Eucalyptus* plants. The invention also describes processes for preparation, detection and quantitative analysis of the individual phytochemicals used in the polyherbal formulations.

Background of the Invention

Cough is the most common natural forced expiration and an important normal protective reflex activity. It is a critical physiological defense mechanism that protects the airway from noxious irritants and augments the muco-ciliary clearance of airway secretions. It is also a prime mechanism for clearing secretions from the lungs when the natural ciliary function is compromised by acute or chronic infections.

Cough is produced by inflammatory, mechanical, chemical and thermal stimulation of the cough receptors. When cough inadequately serves its protective roles, atelectasis, gas-exchange abnormalities, pneumonia, and bronchiectasis may ensue.

The warm, mucous membrane lining of the throat provides a suitable environment for invading viruses and bacteria to flourish. These organisms cause irritation and inflammation of the membrane, making the throat feel scratchy and sore. Throat inflammation due to a cold will usually diminish in 2-3 days as the body begins to conquer the infection. In the Ayurvedic system of medicine the condition is known as Kaasa. The condition may be present as a disease in itself or as a symptom of other diseases. As a symptom it is caused by the entry of dust, dirt, fumes or some other irritant in the respiratory system that is not acceptable to the body. Generally, it may be due to heavy exertion and excess intake of dry or fatless food.

The prodormal signs of cough (Kaasa) according to Ayurveda are pricking sensation, itching in the throat and difficulty in swallowing. The symptoms, type of cough and the treatment of the same may differ from patient to patient.

In the ancient classical texts, a number of herbs have been advocated for this condition viz. Vasaka (*Adhatoda zeylanica*); Tulsi (*Ocimum tenuiflorum*); Yashtimadhu (*Glycyrrhiza glabra*); Kapoorkachri (*Hedychium spicatum*); Sunthi (*Zingiber officinale*); Pippali (*Piper longum*); Kantakari (*Solanum xanthocarpum*); Malayvacha (*Alpinia galanga*); Haridra (*Curcuma longa*); Karpoor (*Cinnamomum camphora*); Guggulu (*Commiphora wightii*); Draksha (*Vitis vinifera*); Jufa (*Hyssopus officinalis*); Guduchi (*Tinospora cordifolia*); Jatiphal (*Myristica fragrans*); Gojivha (*Onosma bracteatum*); Amalaki (*Emblica officinalis*); Haritaki (*Terminalia chebula*); Vibhitak (*Terminalia bellirica*); Maricha (*Piper nigrum*); Vidanga (*Embelia ribes*); Twak (*Cinnamomum zeylanicum*); Brihati (*Solanum indicum*); Pushkarmool (*Inula racemosa*); Banafsha (*Viola odorata*); Ghrit-kumari (*Aloe vera*); Lavanga (*Syzygium aromaticum*); Talish (*Abies pindrow*); Karkatshringi (*Pistacia integerrima*); Kasamarda (*Cassia occidentalis*); Karchoor (*Curcuma zedoaria*); Bharangi (*Clerodendrum serratum*); Dugdhika (*Euphorbia thymifolia*); Soma (*Ephedra gerardiana*); Tailaparna (*Eucalyptus globulus*); Sarala (*Pinus roxburghii*) etc.

Summary of the Invention

While studying some of the above mentioned medicinal plants the present inventors have come across three medicinal plants that have particularly useful medicinal properties. This combination of herbs has been selected on the basis of classical Ayurvedic literature survey and research studies conducted till date, to work synergistically against cough and sore throat. The herbs selected are as follows:

- *Adhatoda zeylanica* Medic Syn: *Adhatoda vasica*

  (Vasaka- Leaf)

- *Syzygium aromaticum* (Linn.) Merrill & Perry

  (Lavanga-Floral bud)

- *Eucalyptus globulus* Labill

  (Tailaparna-Leaf Oil)

There are innumerable cough medications of both natural and synthetic origin available in the market. Acute cough and chronic cough are symptoms of conditions that

may require medical attention. A wide variety of conditions can cause cough; about a dozen conditions are the most frequent causes. Generally, the cough syrups of synthetic origin produce good results but are always accompanied by one or another side effects like drowsiness, sedation, dryness of mouth and respiratory tract and at times can be habit forming. Considering these factors, the inventors of the present application endeavored to make a formulation that is effective and safe in all respects and can be administered to all age groups.

An ideal cough formulation should:

- Liquefy the thick mucous and at the same time help in expectorating it out
- Sooth the irritated respiratory mucosa
- Stop the irritating dry cough
- Protect the respiratory tract from allergens
- Improve the immunity of the respiratory tract.

Keeping the above points in mind, an initiative has been taken to generate a formulation to work against cough of varied etiologies, backed by conceptual and scientific studies to prove its worth.

The present inventors have a reason to believe that three indigenous herbs Adhatoda, Syzygium and Eucalyptus are time proven for their role in cough and sore throat. Some modern scientific studies also complement the properties quoted in the classical texts of Ayurveda. *Adhatoda zeylanica* has been shown to possess anti-tussive, anti-histaminic, bronchodilator and anti-bacterial properties. *Syzygium aromaticum* has been proved to be an anti-inflammatory, anti-bacterial, anti-histaminic and anesthetic agent. *Eucalyptus globulus* has proven its efficacy as a mucolytic, analgesic, anti-inflammatory and anti-histaminic agent.

The herbs have been shown to possess significant effects in etiology and symptoms of cough and keeping in mind the desirable effects and synergism of herbs, the formulation has been conceptualized to serve as an ideal cough formulation.

Ayurvedic medicines or herbal formulations are often criticized by the Western World and by regulatory agencies for the fact that there is no standardization or

quantification of active principles in the dosage form. Sometimes even the active principles are not even known, which can pose problems to health-care practitioners to prescribe the formulation without knowing the actual use or benefits of such herbal formulation. It is also a fact that sometimes more than five different herbs having different activities are combined in one formulation and the formulation is promoted as having ability to treat various ailments without logical, scientific or Ayurvedic basis.

The present inventors have also felt a need to standardize the formulation by adopting certain strict criterion for selection of raw materials, process of extraction of the herbs and manufacturing of the formulation. In this context the present inventors have embarked upon a quantification method of active phytochemicals present in the formulation, which will help provide a rational use of the formulation.

Accordingly, in one general aspect there is provided a polyherbal pharmaceutical formulation that includes an extract of *Adhatoda zeylanica Medic*, powder of *Syzygium aromaticum (Linn.)* and essential oil of *Eucalyptus globules Labill* for the treatment of cough and sore throat.

Embodiments of the formulation may include one or more of the following features. For example, the polyherbal formulation may be in the form of a lozenge that includes not more than 1 mg of Vasicine per lozenge, not more than 4 mg of cineole per lozenge, or not more than 4 mg of eugenol per lozenge. It also may contain not more than 1 mg of Vasicine, not more than 4 mg of cineole and not more than 4 mg of eugenol per lozenge.

In another general aspect there is provided a process for preparing a polyherbal pharmaceutical formulation in the form of lozenges. The process includes the steps of

a) preparing a syrup containing sugar, glucose, *Adhatoda* extract and water;

b) concentrating the syrup of step a) to get lozenge mass;

c) adding flavor, *Eucalyptus* oil and *Syzygium* clove powder to the lozenge mass of step b); and

d) preparing lozenges from the lozenge mass after performing step c).

Embodiments of the process may include one or more of the following embodiments. For example, the sugar may be selected from one or more of crystal sugar of the type S-30, M-30, L-30 and/or a pharma sugar or mixtures thereof. The flavor of the components may be selected from one or more of ginger, mint, stone ginger, ice cool, lemon, honey, basil, pepper, cardamom, normal or spicy orange and/ or menthol or mixtures thereof.

In another general aspect there is provided a method for the detection and quantification of vasicine in a polyherbal pharmaceutical formulation in the form of lozenges that include extracts of *Adhatoda zeylanica Medic*, *Syzygium aromaticum (Linn.)* and *Eucalyptus globulus Labill*. The method includes:

a) crushing lozenges to fine powder, and dissolving it in water;

b) extracting the solution obtained in step a) from first organic solvents and an alkaline solution;

c) partially concentrating the extract obtained in step b) and reconstituting it in second organic solvent;

d) subjecting the solution obtained in step c) and standard phytochemical marker solution to a HPLC column; and

e) detecting and quantifying vasicine after step d).

Embodiments of the process may include one or more of the following features. For example, the first organic solvents may be one or more of alkanol, ketone, ester, ether, chlorinated hydrocarbon, aromatic, aliphatic or alicyclic hydrocarbon, and/or acetonitrile or mixtures thereof. The first organic solvents may be mixtures of chloroform, n-butanol and methanol. The alkaline solution may be ammonia gas. The second organic solvent may be one or more of methanol, ethanol, isopropanol, n-propanol, acetone, acetonitrile, diethylether, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide and tetrahydrofuran or mixtures thereof. The standard phytochemical marker solution may be prepared by dissolving a known quantity of vasicine in a known quantity of the second organic solvent.

In another general aspect there is provided a method for the detection and quantification of cineole and eugenol in a polyherbal formulation in the form of lozenges

that include extracts of *Adhatoda zeylanica Medic*, *Syzygium aromaticum (Linn.)* and *Eucalyptus globulus Labill.* The method includes:

a. crushing lozenges to a fine powder and dissolving the powder in water;

b. extracting the solution obtained in step a) from first organic solvents and an alkaline solution;

c. partially concentrating the extract obtained in step b) and reconstituting it in second organic solvent to get a test solution;

d. subjecting the solution obtained in step c) and standard phytochemical marker solution to GC column; and

e. detecting and quantifying the cineole and/or eugenol after step d).

Embodiments of the method may include one or more of the following features. For example, the first organic solvents may include one or more of alkanol, ketone, ester, ether, chlorinated hydrocarbon, aromatic, aliphatic or alicyclic hydrocarbon, and/or acetonitrile or mixtures thereof. The first organic solvents may be mixtures of chloroform, n-butanol and methanol. The alkaline solution may be ammonia gas. The second organic solvent may be one or more of methanol, ethanol, isopropanol, n-propanol, acetone, acetonitrile, diethylether, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide and tetrahydrofuran or mixtures thereof. The standard phytochemical marker solution may be prepared by dissolving a known quantity of each of cineole or eugenol in a known quantity of a second organic solvent.

The details of one or more embodiments of the inventions are set forth in the description below. Other features, objects and advantages of the inventions will be apparent from the description and claims.

Detailed Description of the Invention

Cough lozenge of the present invention comprise extracts from selected herbs along with throat soothers that provide quick relief from cough and treat all types of cough effectively so the cough does not recur again and again.

The objects of the present invention relate to new polyherbal pharmaceutical formulations which have been found to be effective in treating and managing cough and

sore throat. The formulation of the present invention ensures not only symptomatic relief from cough but also enhances the host-defense mechanism in order to strengthen the immune system so as to check the off and on paroxysms of cough and at the same time is devoid of toxic side effects. The invention also describes the process for preparation, detection and quantitative analysis of individual phytochemicals used in the polyherbal formulations.

The first aspect of the present invention provides new polyherbal pharmaceutical formulations comprising extract of *Adhatoda zeylanica Medic*, powder of *Syzygium aromaticum (Linn.)* and essential oil of *Eucalyptus globulus Labill* for the treatment of cough and sore throat.

The second aspect of the present invention relates to processes for the preparation of new polyherbal pharmaceutical formulations in the form of lozenges. The process includes the steps of:

a) preparing a syrup containing sugar, glucose, *Adhatoda* extract and water,

b) concentrating the syrup of step a) to get lozenge mass,

c) adding flavor, *Eucalyptus* oil and *Syzygium* clove powder to the lozenge mass of step b), and

d) preparing lozenges from the lozenge mass after performing step c).

Syrup containing sugar and liquid glucose is prepared in a cooking chamber at a temperature range of 50° to 100°C for 10 to 60 minutes.

The sugar used is selected from the group that includes crystal sugar of the type S-30, M-30, L-30, Pharma sugar (Sucrose I.P or B.P.) and/or any other conventional sugar or mixtures thereof. Preferably, the sugar used is crystal sugar type S-30.

The flavor used is the flavor of components selected from the group that includes ginger, mint, stone ginger, ice cool, lemon, honey, basil, pepper, cardamom, normal or spicy orange, menthol and/or any conventional flavor or mixtures thereof. Preferably, the flavor used is orange flavor.

The lozenges of this invention may be of any shape. Preferably they are oval in shape and characterized by their specific weight and dimensions. The dimensions

hereinafter refer to the length, width and thickness of the lozenge. For example, the weight of the lozenges may vary in between 2.5 to 4.5 gm per lozenge. More preferably, the weight of the lozenge lies in between 3.0 to 4.0 gm. Most preferably, the average weight of the lozenges is 3.5 gm per lozenge.

The thickness of the lozenges lies in between 8 mm to 12 mm. More preferably, the thickness varies in between 9 mm to 11 mm. Most preferably, the average thickness of the lozenges is 10 mm. The width of the lozenges ranges in between 14 mm to 18 mm. More preferably, the width is 14.5 mm to 17.5 mm. Most preferably, the average width of the lozenges is 16 mm. The lozenges may be 20 to 24 mm of length. More preferably the length is between 21 to 23 mm. Most preferably, the average length of the lozenges is 22 mm.

The third aspect of the present invention provides a method for detection and quantification of vasicine in polyherbal pharmaceutical formulation in the form of lozenges, which comprises extracts of *Adhatoda zeylanica Medic*, *Syzygium aromaticum (Linn.)* and *Eucalyptus globulus Labill.* The method includes the steps of

a) crushing lozenges to fine powder, and dissolving it in water,

b) extracting the solution obtained in step a) from organic solvents and an alkaline solution,

c) partially concentrating the extract obtained in step b) and reconstituting it in second organic solvent,

d) subjecting the solution obtained in step c) and standard phytochemical marker solution to a HPLC column, and

e) detecting and quantifying Vasicine after step d).

The lozenges are crushed to a fine powder and dissolved in distilled water by sonication. It is extracted with a mixture of organic solvents and an alkaline solution.

The organic solvents are selected from the group that includes alkanols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, tert-butanol and n-octanol; ketones selected from acetone, ethyl methyl ketone, methyl isobutyl ketone and diisobutyl ketone; esters selected from methyl formate, ethyl formate, methyl acetate,

ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate and tert-butyl acetate; chlorinated hydrocarbons selected from methylene chloride, chloroform, carbon tetrachloride and ethylene chloride; aromatic hydrocarbons selected from xylene, toluene, benzene, substituted benzene, substituted toluene and substituted benzyl; aliphatic hydrocarbons such as hexane, heptane and petroleum ether; cycloalkyl hydrocarbons such as cyclopentane, cyclohexane, cycloheptane, substituted cyclohexane and substituted cycloheptane; ether selected from tetrahydrofuran, 1,4-dioxane, diethyl ether, diisopropyl ether and methyl tert-butyl ether or acetonitrile or mixtures thereof.

The alkaline solution used is any conventional solution. The alkaline solution comprising of ammonia gas is preferred.

The extract is partially concentrated and then dissolved in a second organic solvent.

The second organic solvent is selected from the group that includes methanol, ethanol, isopropanol, n-propanol, acetone, acetonitrile, diethyl ether, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide and tetrahydrofuran or mixtures thereof.

The obtained test solution is subjected to a HPLC column for the detection and quantification of vasicine.

For the detection and quantification of vasicine, the standard phytochemical marker solution is prepared by dissolving an accurately known quantity of vasicine in an accurately known quantity of a second organic solvent as mentioned above. The solution can be sonicated and then made up to a desired fixed volume using the same solvent. This solution is then used as the reference standard solution.

The presence of vasicine is detected and quantified through HPLC.

The fourth aspect of the present invention provides a method for the detection and quantification of Cineole and Eugenol in the polyherbal formulation in the form of lozenges, which comprises extracts of *Adhatoda zeylanica Medic*, *Syzygium aromaticum (Linn.)* and *Eucalyptus globulus Labill.* The method further includes the steps of

a) crushing lozenges to a fine powder and dissolving it in water,

b) extracting the solution obtained in step a) from organic solvents and an alkaline solution,

c) partially concentrating the extract obtained in step b) and reconstituting it in second organic solvent to get a test solution,

d) subjecting the solution obtained in step c) and standard phytochemical marker solution to GC column, and

e) detecting and quantifying Cineole and Eugenol after step d).

The steps a) to e) are performed in similar manner as described for the detection and quantification of vasicine.

The test solution obtained in step c) is subjected to a GC column for the detection and quantification of Cineole and Eugenol.

For the detection of Cineole and Eugenol, the standard phytochemical marker solutions are prepared by dissolving an accurately known quantity of each of cineole or eugenol in an accurately known quantity of a second organic solvent as mentioned in the third aspect of present invention. These standard solutions are then used as reference standard solutions.

The presence of cineole and eugenol are detected and quantified by using gas chromatography (GC).

The present invention also provides a method of use of the pharmaceutical formulation in the form of lozenges which comprises *Adhatoda zeylanica* extract, *Syzygium aromaticum* powder and *Eucalyptus globules* oil to a mammal in need thereof for oral use for

1. Liquefying the thick mucous and at the same time expectorating it out,
2. Soothing the irritated respiratory mucosa,
3. Stopping the irritating dry cough, and
4. Protecting the respiratory tract from allergens.

The following examples are intended to exemplify various aspects of the inventions but not to limit the scope of the inventions described herein.

Example 1: Manufacturing Procedure of Cough Lozenge

1. Grinding of *Syzygium aromaticum* Clove

The cloves (10.284 Kg) were ground in a suitable grinder and sifted through 16# sieve fitted to a mechanical sifter into double polythene-lined drums. The retained material was re-ground and sifted again to obtain 16# powder. The material retained over 16# was discarded.

2. Preparation of Syrup

a) Purified water (225 L) was taken into a stainless steel steam (jacketed) dissolving tank. The tank was heated to 60-70°C and the agitator of the tank started. Sugar (550 Kg; S-30) was added to this hot water. The sugar was heated and mixed continuously until was completely dissolved. To the resultant solution, liquid glucose (549 Kg) was added and mixed for half an hour. During mixing of liquid glucose, the temperature was maintained in a range between 75 – 85°C. The obtained syrup was passed through 100# sieve into the holding tank.

b) To a stainless steel tank fitted with a stirrer, purified water (40 L) was taken and Vasaka (Adhatoda, 28.57 Kg) extract was added under slow stirring and the stirring was continued for 15-20 minutes. The extract solution was passed through a 100# sieve into the holding tank containing the syrup of stage 2(a) and the contents were mixed for 15-20 minutes.

3. Flavour–Medicament Preparation

To a clean and dried stainless steel container (20 L), Eucalyptus oil (2.25 Kg) and orange cool flavour–RANORC (Symrise) (3.5 Kg) were added and mixed together using a stainless steel spatula for 15-20 minutes. The liquid was filtered through double-layered 100# nylon cloth and then transferred to the liquid dosing equipment.

4. Lozenge Mass Preparation

The syrup was transferred from the holding tank (step 2 product) into the cooking chamber of Bosch Manufacturing Line Cooker. The syrup was cooked into the cooking chamber at up to 140 $\pm$ 2°C and it was transferred into the vacuum chamber of a Bosch make manufacturing line where vacuum was applied at –0.6 to –0.7 bar. The lozenge mass

prepared was then passed into the mixing extruder, where Flavour–Medicament (5.75 kg) solution and 8.572 kg Lavanga (Syzygium *aromaticum)* powder were dosed. The Flavour–Medicament liquid doser speed was adjusted at desired point to feed appropriate volume suitable for lozenge mass out-put per minute. The Lavanga powder doser was adjusted to feed appropriate quantity suitable for lozenge mass out-put per minute. Both the dosers (liquid and powder) were switched on. The liquid and powder were allowed to mix with the vacuum cooked lozenge mass in the mixing extruder.

5. Lozenge Formation and Cooling

The mixed lozenge mass of stage 4 was allowed to pass onto the moving stainless steel cooling conveyer and then it was passed through the batch roller to form a rope that followed the rope sizer by adjusting the desired thickness of the rope. The lozenge rope was passed into lozenge forming machine of a Bosch make manufacturing line fitted with oval shaped dies to form lozenges of appropriate size, shape and weight. The average weight of formed lozenges and adjustment of the machine were checked, as needed, as per the required standard average weight. The average weight was checked every 30 minutes. The formed lozenges were passed onto a three deck-cooling conveyer and then through the metal detector. The lozenges were collected in polybags and allowed to cool to room temperature. The lozenges were stored in an area maintained at 25°C and 50% relative humidity till pillow packing was done.

Shape of lozenge: Oval

Weight: 3.5 gm

Example 2: Detection and Quantification of Vasicine from the Lozenge

Preparation of Vasicine Reference Standard Solution

A reference standard sample of Vasicine (around 5.0 mg) was taken in a 10 ml volumetric flask. Methanol (5.0 ml) was added to it and sonicated in an ultrasonic water bath to dissolve, and if required it was heated on a boiling water bath for a few seconds. The solution was cooled at room temperature and methanol was added up to the mark of volumetric flask. The resulting solution (2 ml) was taken into a 25 ml volumetric flask and the volume was made up with methanol. The resulting solution was used as reference standard solution for Vasicine.

Preparation of Extraction Media

Chloroform (250 ml; AR Grade), n-butanol (30 ml; AR Grade), methanol (10 ml; AR Grade) and ammonia solution (2.5 ml; AR grade) were measured individually using graduated measuring cylinders and were transferred separately to a 500 ml volumetric flask. This mixture was used as extraction media for test solution preparation.

Preparation of Test Solution

Two Lozenges were crushed to a fine powder with the help of mortar and pastel and transferred to a 250 ml beaker. Distilled water (20 ml) was added to the beaker and sonicated in an ultrasonic water bath to dissolve the lozenges. The contents of the beaker were transferred to a 250 ml separating funnel through a cotton plug. The beaker was rinsed with 10 ml of water and the washing was transferred through the same cotton plug to the same separating funnel. The solution obtained was extracted thrice with each 70 ml of extraction media. The organic layer was separated and passed over anhydrous sodium sulphate. The organic layer was evaporated up to 15 ml on a rotary evaporator/water bath and the contents of vessel were transferred to a 25 ml volumetric flask. The vessel was rinsed with 4-5 ml of methanol and the washings were transferred to the same volumetric flask. Methanol was added to it up to the mark of volumetric flask. The resultant solution was used as a test solution.

Procedure:

System Suitability Criteria:

The instrument was set as per the condition prescribed and the following system suitability test was performed:

1. The relative standard deviation of the reference standard solution in triplicate injections (Vasicine) should not be more than 2 %.

The instrument used was a Gradient High Performance Liquid Chromatographic System with an attached UV detector (Shimadu with class LC 10A software). If the instrument passed the system suitability test, then 10µL of test solution was injected twice and the chromatograms were recorded. The mobile phase used in the HPLC column [C8, 250 mm X 4.6 mm, 5µ (Intersil) or equivalent] was a mixture of buffer solution and acetonitrile in a ratio of 82:18 respectively. The buffer solution used was a mixture of 0.1 gm hexane

sulphonic acid, 75 ml of water and 2 ml of acetic acid. The flow rate was 1 ml/min and the run time was 18 minutes. The retention time observed for Vasicine was approximately 8 to 10 minutes. The chromatograms for the test solution were recorded at a wavelength of 281 nm. These chromatograms were compared from the chromatograms obtained for reference standard solution and the percentage content of Vasicine in the cough lozenge was calculated.

Calculations: Calculated percentage content of Vasicine in cough drop (lozenge) was as follows:

$$\frac{A_{SPL}}{A_{STD}} \times \frac{D_{STD}}{D_{SPL}} \times \frac{P}{100} \times 100$$

Where,

A_{SPL} –Peak area corresponding to the Vasicine from test chromatogram

A_{STD} – Peak area corresponding to the Vasicine from reference standard chromatogram

D_{SPL} – Dilution of sample (mg/ml)

D_{STD} – Dilution of reference standard solution (Vasicine)

P – Potency of the Reference standard (in % w/w)

Results:

Experiment No.	Vasicine (mg/lozenge)
1	0.49
2	0.51
3	0.54

Example 3: Detection and Quantification of Cineole and Eugenol from the Lozenge

Preparation of Cineole Standard Solution

Cineole reference standard (50.0 mg) sample was weighted accurately in a 100 ml volumetric flask. Methanol (70 ml) was added to it and it was sonicated in an ultrasonic water bath for 2-3 minutes to dissolve and then the volume of volumetric flask was made up with methanol. The resulting solution was used as standard solution for Cineole.

Preparation of Eugenol Reference Standard Solution

Eugenol reference standard (50.0 mg) sample was weighted accurately in a 100 ml volumetric flask. Methanol (70 ml) was added to it and it was sonicated in an ultrasonic water bath for 2-3 minutes to dissolve and then the volume of volumetric flask was made up with methanol. The resulting solution was used as standard solution for Eugenol.

Preparation of Test Solution

The preparation of test solution was the same as described in Example-2.

Procedure

System Suitability Criteria:

The instrument was set as per the condition prescribed and the following system suitability tests were performed:

1. The relative standard deviation of the reference standard solutions (i.e., Cineole and Eugenol) in triplicate injections should not be more than 2 %.

2. Resolution between Cineole and Eugenol peaks should not be less than 2 (for this test reference standard solutions of Cineole and Eugenol were mixed in 1:1 ratio).

The instrument used was GC Chromatograph with FID (Perkin- Elmer Clarus-500). If the instrument passed the system suitability test, then 1µL of test solution was injected three times and the chromatograms were recorded. It was preferred to keep the temperature of the Injector at 220°C. The column used was a DB-WAX (60M x 0.25 mm x 0.25 micron). Nitrogen gas was used as the carrier gas and the flow rate was maintained at 1 ml/min. The oven temperature was kept at 120°C to 220°C. The detector temperature was kept at 280°C. Methanol was used as a diluent. The chromatographs of test solution and

reference standard solution were recorded and the percentage content of Cineole/Eugenol in the cough drop (lozenge) was quantified.

Calculations

The percentage content of Cineole and/or Eugenol in the cough drop (lozenge) was calculated as follows:

$$\frac{A_{SPL}}{A_{STD}} \times \frac{D_{STD}}{D_{SPL}} \times \frac{P}{100} \times 100$$

Where,

A_{SPL} –Peak area corresponding to the Cineole/ Eugenol from test chromatogram

A_{STD} – Peak area corresponding to the Cineole/Eugenol from reference standard chromatogram

D_{SPL} – Dilution of sample (mg/mL)

D_{STD} – Dilution of reference standard solution (Cineole/Eugenol)

P – Potency of the Reference standard (in % w/w)

Results:

Experiment No.	**Cineole (mg/lozenge)**	**Eugenol (mg/lozenge)**
1.	2.20	3.52
2.	3.43	3.24
3.	2.84	3.66

While the present invention has been described in terms of its specific aspects, certain modifications and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the present invention.

We claim:

1. A polyherbal pharmaceutical formulation comprising extract of *Adhatoda zeylanica Medic*, powder of *Syzygium aromaticum (Linn.)* and essential oil of *Eucalyptus globules Labill* for the treatment of cough and sore throat.

2. The polyherbal formulation of claim 1, comprising a lozenge comprising not more than 1 mg of Vasicine per lozenge.

3. The polyherbal formulation of claim 1, comprising a lozenge comprising not more than 4 mg of cineole per lozenge.

4. The polyherbal formulation of claim 1, comprising a lozenge comprising not more than 4 mg of eugenol per lozenge.

5. The polyherbal formulation of claim 1, comprising a lozenge comprising not more than 1 mg of Vasicine, not more than 4 mg of cineole and not more than 4 mg of eugenol per lozenge.

6. A process for preparation of polyherbal pharmaceutical formulation in the form of lozenges, wherein the process comprises of

a) preparing a syrup containing sugar, glucose, *Adhatoda* extract and water;

b) concentrating the syrup of step a) to get lozenge mass;

c) adding flavor, *Eucalyptus* oil and *Syzygium* clove powder to the lozenge mass of step b); and

d) preparing lozenges from the lozenge mass after performing step c).

7. The process of claim 6 wherein the sugar comprises one or more of crystal sugar of the type S-30, M-30, L-30 and/or a pharma sugar or mixtures thereof.

8. The process of claim 6 wherein the flavor used is the flavor of components selected from one or more of ginger, mint, stone ginger, ice cool, lemon, honey, basil, pepper, cardamom, normal or spicy orange and/ or menthol or mixtures thereof.

9. A method for the detection and quantification of vasicine in a polyherbal pharmaceutical formulation in the form of lozenges that include extracts of *Adhatoda*

zeylanica Medic, Syzygium aromaticum (Linn.) and *Eucalyptus globulus Labill*, wherein the method comprises:

a) crushing lozenges to fine powder, and dissolving it in water;

b) extracting the solution obtained in step a) from first organic solvents and an alkaline solution;

c) partially concentrating the extract obtained in step b) and reconstituting it in second organic solvent;

d) subjecting the solution obtained in step c) and standard phytochemical marker solution to a HPLC column; and

e) detecting and quantifying vasicine after step d).

10. The method of claim 9 wherein the first organic solvents comprise one or more of alkanol, ketone, ester, ether, chlorinated hydrocarbon, aromatic, aliphatic or alicyclic hydrocarbon, and/or acetonitrile or mixtures thereof.

11. The method of claim 9 wherein the first organic solvents comprise mixtures of chloroform, n-butanol and methanol.

12. The method of claim 9 wherein the alkaline solution comprises ammonia gas.

13. The method of claim 9 wherein the second organic solvent comprises one or more of methanol, ethanol, isopropanol, n-propanol, acetone, acetonitrile, diethylether, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide and tetrahydrofuran or mixtures thereof.

14. The method of claim 9 wherein the standard phytochemical marker solution is prepared by dissolving a known quantity of vasicine in a known quantity of the second organic solvent.

15. A method for the detection and quantification of cineole and eugenol in a polyherbal formulation in the form of lozenges that include extracts of *Adhatoda zeylanica Medic, Syzygium aromaticum (Linn.)* and *Eucalyptus globules labill*, wherein the method comprises:

a. crushing lozenges to a fine powder and dissolving the powder in water;

b. extracting the solution obtained in step a) from first organic solvents and an alkaline solution;

c. partially concentrating the extract obtained in step b) and reconstituting it in second organic solvent to get a test solution;

d. subjecting the solution obtained in step c) and standard phytochemical marker solution to GC column; and

e. detecting and quantifying the cineole and/or eugenol after step d).

16. The method of claim 15 wherein the first organic solvents comprises one or more of alkanol, ketone, ester, ether, chlorinated hydrocarbon, aromatic, aliphatic or alicyclic hydrocarbon, and/or acetonitrile or mixtures thereof.

17. The method of claim 15 wherein the first organic solvents comprise mixtures of chloroform, n-butanol and methanol.

18. The method of claim 15 wherein the alkaline solution comprises ammonia gas.

19. The method of claim 15 wherein the second organic solvent comprises one or more of methanol, ethanol, isopropanol, n-propanol, acetone, acetonitrile, diethylether, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulphoxide and tetrahydrofuran or mixtures thereof.

20. The method of claim 15 wherein the standard phytochemical marker solution is prepared by dissolving a known quantity of each of cineole or eugenol in a known quantity of a second organic solvent.