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Original Article

Polyherbal Extract Based Linkus Lozenges for Symptomatic Relief: Design, Development and Evaluation

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ABSTRACT

Objective: The development of modern dosage form such as lozenges associated with an extended period of local remedy with beneficial therapeutic effect was the objective of this study. The polyherbal extract based lozenges include *Viola odorata L, Cordialatifolia L, Menthapiperita L., Adhatodavasica, Glycyrrhizaglabra, Piper longum, Hyssopusofficinalis, Alpiniagalangal* which is envisioned for cough and sore throat.

Method: The preparation of extract, manufacturing of lozenges batch, processing technique with extract based herbal formulation and expansion for analytical standardization including qualitative and quantitative determinations were carried out.

Result: Vasicine was analysed qualitative and quantified through HPTLC and quantitative analysis of flavonoids by UV visible spectrophotometry, which was not less than 0.02 mg per lozenges and 0.080 mg respectively.

Conclusion: Current trend of herbal market and the visible financial growth with evident therapeutic graph has proven its effectiveness. Minimum side effects make the herbal and alternative medication more attractive for the end users. This poly herbal extract based lozenges have proven the quality by both protocols including qualitatively via organoleptic attributes and quantitatively by HPTLC and spectrophotometry.

Keywords: Polyherbal, Lozenges, Sorethroat, HPTLC, Spectrophotometery.

INTRODUCTION

The recognitions and advancements in evidence based herbal treatments have gained popularity along with the allopathic medication. Cough is the most common infection which has been increasing and considered to be an evidence defence mechanism for eradication of foreign material from respiratory tract[1].

The herb in poly herbal lozenges is Adhatodavasica that is utilized for cough treatment in Unani and ayurvedic medicine. The vasicine acetate, 2-acetyl benzyl amine vasicine are present in *Adhatoda vasica* that produce anti-inflammatory activity[2]. Medicinal properties associated with *Adhatoda vasica* are antiseptic, expectorant (used in bronchial, asthmatic and pulmonary infections), cough, pulmonary disease as well as respiratory stimulant[3,4]. The fresh leaves of *Adhatoda vasica* traditionally used for muscular sprains, bronchial congestion, cough and fever. The presence of vasicine in the leaves of *Adhatoda vasica* has been recommended in many complication of respiratory ailment and the key biochemical agents for the relief of bronchial malaise are vasicine and vasicinone[5,6].

The second ingredient of poly herbal lozenges is *Glycyrrhiza glabra* that is used for hoarseness in throat infections and soothes mucous membrane and relieves spasm[7]. *Glycyrrhiza glabra* has antioxidant and anti-inflammatory properties and used widely for the cure of different treatment[8,9]. *Piper longum* root and fruit is the component of lozenges used for cough, asthma, analgesic, counter irritant, bronchitis, muscular pain and other respiratory tract infection[10]. It has been claimed for the remedy in respiratory troubles mentioned in literature citation and possess bronchodilator, strong mucolytic expectorant activity and claimed that bioavailability enhances the therapeutic responses[11]. *Hyssopus*

*officinalis*is used as a remedy for cough, works as an expectorant and provide soothing effects on respiratory tract[12]. Another herb of the polyherbal formulation is *Alpinia galangal* that is known for intermittent fever, cough, cold and fever[13,14]. *Viola Odorata* used to cure bronchitis, asthma, cough and lungs diseases[15,16]. *Mentha piperita* as essential oil is utilized as an antibacterial, antifungal and anti-viral agents that inhibit many microorganisms such as *Pseudomonas solanacerum, Aspergillus niger* etc.[17]. The oil of *Mentha piperita* oil (vapour) an inhalant for respiratory congestion as well as used for the treatment of cough, inflammation oral mucosa, throat and bronchitis[18]. However its leaves are used for sore throat, cough, sore throat, cough, toothache, inflamed gums and as mouth wash[19].

The aim of this study is to develop, design and evaluate polyherbal lozenges via quantitative and qualitative standardization for symptomatic relief. It was the endorsement that the biomarker *vasicine* is present in lozenges and evaluated by HPTLC and spectrophotometry[20].

MATERIALS AND METHODS

Study Design

Experimental study based on development, design and evaluation of extract based polyherbal lozenges. This aqueous extract based lozenges manufactured in a modern dosage form i.e., lozenges[21].

Collection of Herbs

Herbs samples were purchased from insaf kirana store Karachi, dried storage conditions was maintained with respect to light and temperature. The herbs were identified and compared with authentic specimen by a team of taxonomists available at the Karachi University Herbarium (KUH). For minimize the errors, the verification is also carried out by University of Karachi. Similarly it was also made sure that every other material including solvents, reagents and chemicals must be of pure analytical grade and acquired with proper documentation of COA's and MSDS forms through local supplier[22].

Preparation of Extracts

The herbs (excluding *Glycyrrhiza glabra*) grind, crush, weigh accurately and put in extractor with de-ionized water in a set ratio of 1: 8.57. The extractor was heated with continuous Stirring till boiling. The temperature was maintained within 110-120˚C. Then the temperature was reduce and sustained up to 90-100°C for 03 hours. After addition of *Glycyrrhiza glabra* extracts, it was filtered and passed through the mesh No. 100. After the completion of evaporation process the preservatives propyl and methyl paraben were added to the thick aqueous extract. Thereafter 10 minutes of stirring temperature was maintained at 110˚C. It was taken care that aqueous extract was not dried of more than 20% of the total extract weight[23-25].

The linkus lozenges herbal ingredient such as herbs, ratio and quantity are delineated in Tables 1 and 2.

ANALYTICAL STUDIES

Qualitative Identification

Take 4 lozenges and dissolve in distilled water to make 100 ml solution filter the obtained solution through a folded ashless filter paper (Solution A).

Add 5 drops of 3% solution of ferric chloride to 3 ml of solution A; yellowish green color will appear (Tanning agents).

Put 5 ml of solution A in a test tube and add 1.5 ml of concentrated sulphuric acid, red-orange color will gradually appear at the bottom **(**Glycyrrhizic acid)[26].

Vasicine: The reddish pink colour spot referred vasicine alkaloid presented in the chromatogram.

Quantitative Determination

Quantitative determination for flavonoids: The quantitative determination was performed by spectrophotometry for *flavonoids.*

Sample preparation: For the determination, the test solution was prepared with 20 number of lozenges crushed and then take 10 g into 200 ml flat bottomed flask, added 50 ml of 30% ethanol solution. To complete the disintegration process, it was kept for 2 hours and then filtered.

Method: The 10 ml of the tested solution were taken in two, 25 ml volumetric flasks each. After that 2 ml of 3% aluminium chloride solution in 30% ethanol into the 1st flask and 2 ml of 0.1 M of hydrochloric acid solution in the 2nd flask were added. Then the volume was made up to the mark with 30% ethanol[27].

The optical density of the 1st flask solution within 40 minutes in a cuvette with 10 mm layer at the wavelength of 395 nm was determined, and 2nd flask solution was uses for a comparison[28-30].

Aggregate flavonoids in 1 tablet substance in mg (x) as luteolin-7-glucoside is figured as per the accompanying recipe:

$$X=\frac{10\times 25\times 50\times D\times M_0}{10\times M\times 401}=\frac{1250\times D\times M_0}{401\times M}$$

Where,

D- Optical thickness of arrangement

401- Specific ingestion file of aluminum complex of luteolin - 7 –glucoside at the wavelength of 395 nm

M0- Average capsule weight

M- Preparation weight

Aggregate flavonoids substance as luteolin-7-glucoside ought to be not less than 0.02 mg.

Quantitative determination of vasicine (Adhatoda vasica) by HPTLC: CAMAG Linomat 5, CAMAG Scanner III equipment was used for the quantitative determination in which HPTLC silica gel $G_{60}F_{254}$was utilized with solvent system EtOAc: $CHCl_3$: EtOH: NH_3 (6:3:1:1) system and observed at 254 nm UV/ Wave length[31].

Sample preparation: Take 20 tablets (exact weight) dissolve in 50 ml of water and transferred it in a 250 ml dividing funnel. Add 3 ml of hydrous ammonia. Add 25 ml of chloroform to the obtained solution in the funnel. Shake carefully during 3 minutes. After full division of layers filter lower chloroformic layer through the paper filter with anhydrous sodium sulphate (about 10 g) in 500 ml round bottom flask. Repeat the extraction process 4 times combined chloroformed extraction steamed to dryness on a water bath under vacuum. Dissolve the dry residue in 5 ml of methanol. Solution was used as a sample[32].

Vasicine standard solution preparation: Place about 1.4 mg of vasicine in a 10 ml volumetric flask and dissolve in methanol. Bring the solution's volume to the mark with methanol.

After TLC preparation and development the plate was scanned in the densitometer by linear scanning at 256 nm by use of a TLC Scanner III CAMAG with D_2 and W absorption, and integrates the area of the spots corresponding to Vasicine standard[33].

Vasicine content in Linkus lozenges is calculated by the following formula:

$$X=\frac{A_{SMP}\times W_{STD}\times \text{Sample dilution}\times P}{A_{STD}\times \text{Standard dilution}\times W_{SMP}\times 100}M$$

The contents of the vasicine were found not less than 0.02 mg per lozenges.

RESULTS

The poly herbal linkus lozenges for sore throat and cough contain extracts of *Adhatoda vasica Nees*. (Bansa), *Glycyrrhiza glabra L.*(Mulethi), *Piper longum L.* (FilfilDaraz),*Alpinia galangal (Khulanjan)*, *Hyssopusofficinalis (Zufa)*, *Viola odorata (Banafshan)* and *Cordia latifolia (Lasorda)*. Bioactive components of the herbs used in the poly-Herbal formulation biomarker was identified and estimated for standardization. The determination in appearance, weight, uniformity, diameter and thickness was analysed organoleptically. Assay accomplished by HPTLC for the determination of vasicinecontents was not less than 0.02 mg per lozenges.While finished product specification included blister pack, leak test and microbiological testing.

See Table 3 for details of evaluation and Figure 1 for TLC plate of HPTLC Vasicine.

The Sample TLC plate and standard TLC were prepared with Methanol. Multiple dilutions have prepared with herbal lozenges for test for less chances of error.

Chromatograms of Vasicine by HPTLC are shown in Figure 2.

DISCUSSION

Polyherbal extract based lozenges has been designed with the help of 7 multiple herbs. Development of lozenges includes heating and evaporation process. The overall measures taken for the analysis of qualitative, quantitative and physical parameters at bulk and finished stage expose the adherence with the processes developed and adopted are in compliance with the standard of GMP guidelines and requirements consequently supporting the impression that poly herbal lozenges can compete with the good standard products. In another study it was cited and concluded that herbal Linkus syrup was analysed by the quantitative and qualitative manner for determination of biomarker and used for the validation of vasicine. Study of the manufacturing process of Vasu syrup for cough was validated which used the poly herbal formulation with adherence to reproducibility.

The herbal lozenges were designed and developed after extensive study of herbs, formulation dosage optimization, manufacturing techniques of lozenges and evaluation of qualitative-quantitative parameters by precise and modern analytical instruments and methods for assessment.

CONCLUSION

The 'Linkus' polyherbal extract based lozenges have active ingredient like vasicine. The quantity determination of vasicine and flavonoids has been

analysed by HPTLC for the quality assurance. The study has endorsed the quality and effectiveness of the poly herbal extract lozenges with its active indigents and specifications. This study revealed that linkus lozenges are suitable dosage form in symptomatic relief. The standardization provides a specific and rapid tool to set the quality standard identity, specificity and reproducibility in linkus lozenges.

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Table 1. Linkus lozenge's herbs parts, ratio and quantity

Name of thick aqueous extracts	Local name	Parts Used	Quantity of extract
*Adhatodavasica*Nees.	Bansa, ArusaMalabar, Behkar	Leaves	12.727 mg
Piper longum L.	FilfilDaraz, Piplamol	Fruit and root	2.121 mg
Glycyrrhizaglabra L.	Mulethi, Mulaithi	Roots	0.208 mg
Alpiniagalanga (L.) Wild	Khulanjan, galangal	Rhizome	1.062 mg
Hyssopusofficinalis L.	Zufa	Leaves	1.062 mg
Cordialatifolia	Sapistan, Lasorda	Fruit	2.121 mg
Viola odorata L.	Banafshan	Flowers	2.677 mg

Table 2. Pharmacological actions of herb and quantity per lozenge

S.No	Herbs	Part Used	Vernacular name	Quantity of Herb/lozenge	Pharmacological Action
01	Adhatodavasica	Dry leaves	Bansa, ArusaMalabar	109.1 mg	Dhuley report the Bronchodilator and expectorant effect[23], Anti-asthmatic and used in respiratory disorder[24] used as respiratory ailments in asthma, bronchitis, whooping cough and common cough[25]
02	Glycyrrhyzaglabra	Dry root	Mulethi, Mulaithi	1.78 mg	Anti-inflammatory, reduction in respiratory tract inflammation, spasmodic cough relieve[26]
03	Piper longum	Dry fruit	FilfilDaraz, Piplamol	18.18 mg	Prevents recurrent attacks of bronchial asthma[27] prevent bronchospasm and anti-asthmatic[28]
04	Viola odorata	Dry Leaves and flowers	Banafsha	4.55 mg	Flowers is effective in fever, flu, cough, pneumonia and body pain[29]
05	Hyssopusofficinalis	Flowering tops	Zufa	9.1 mg	Reliever for cough, expectorant, also anti- inflammatory[30]
06	Cordialatifolia	Dry fruit	Sapistan, Lasorda	18.18 mg	Against H. Influenza[31]
07	Alpiniagalanga	Dry rhizome	Khulanjan galangal	9.1 mg	Used for cold, chronic cough, asthma and lung diseases[32] Respiratory diseases[33] Anti-inflammation, antimicrobial activity[34]

Table 3. Formulation evaluation details

Parameter	Sampling Plan	Specification	Testing Method
Bulk Product			
Appearance	One sample from beginning in each lot than from middle and end.	Brown colour lozenges with characteristic odour	Organoleptic
Average Weight	One sample from beginning in each lot than from middle and end.	From 2.375 g to 2.625 g	British Pharmacopoeia

Weight Uniformity	20 lozenges from beginning in each lot than middle and end.	2.5 g ± 5%	British Pharmacopoeia
Thickness and Diameter	10 lozenges samples from each lot from beginning than middle and end.	Thickness7 mm ± 1 mm Diameter17 mm ± 1 mm	Verniercalliper
Assay	10 lozenges samples each from beginning, Middle and end from each batch	Total Alkaloids as Vasicine NLT 0.080	Spectrophotometer
Finished Product			
Blister Appearance	10 lozenges samples from each lot at beginning than middle and end.	As per standard	Visual
Leak test	10 lozenges samples in each lot taken from beginning than middle and end of blistering process.	No leakage	Vacuum desiccator

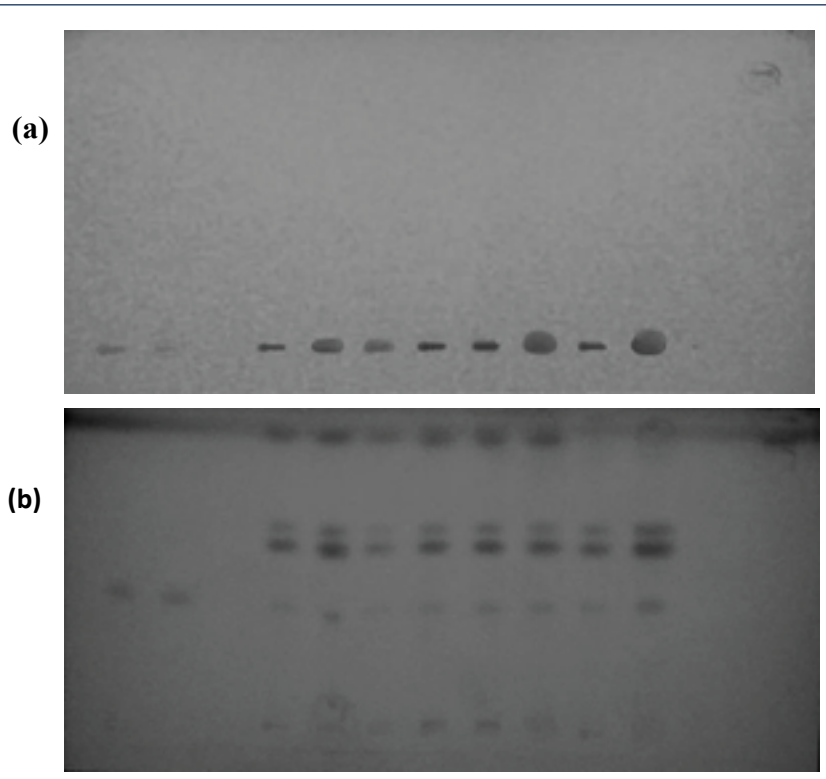
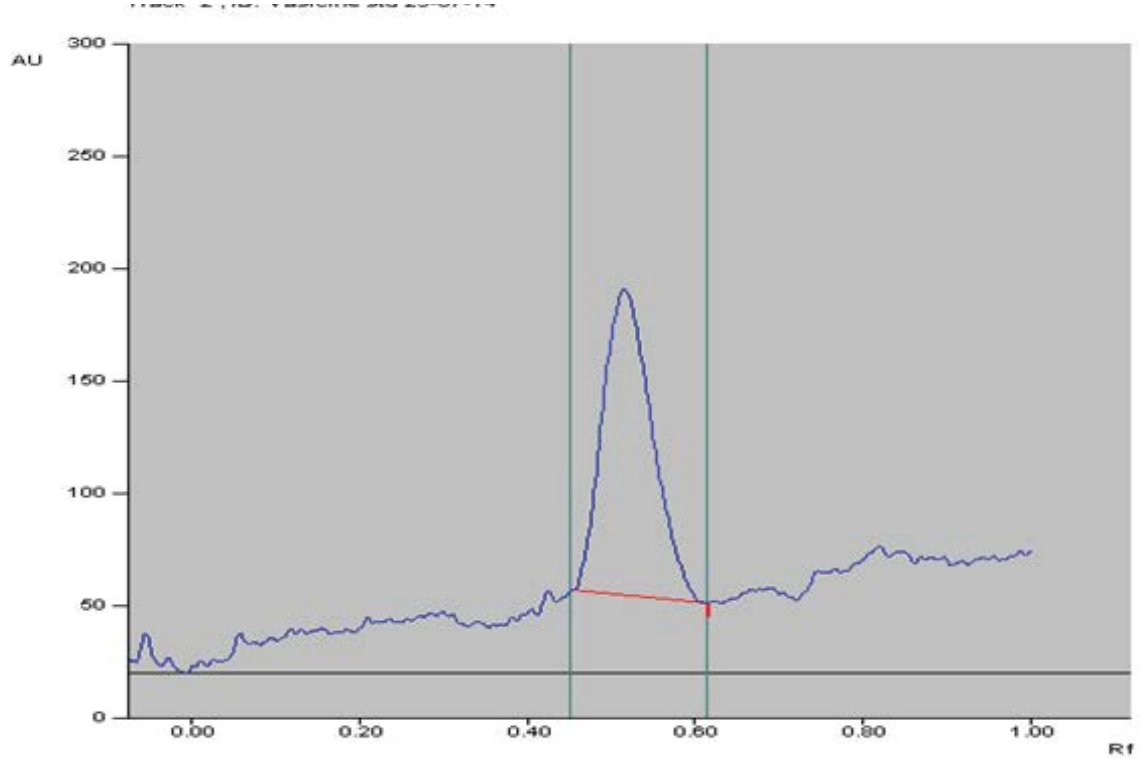
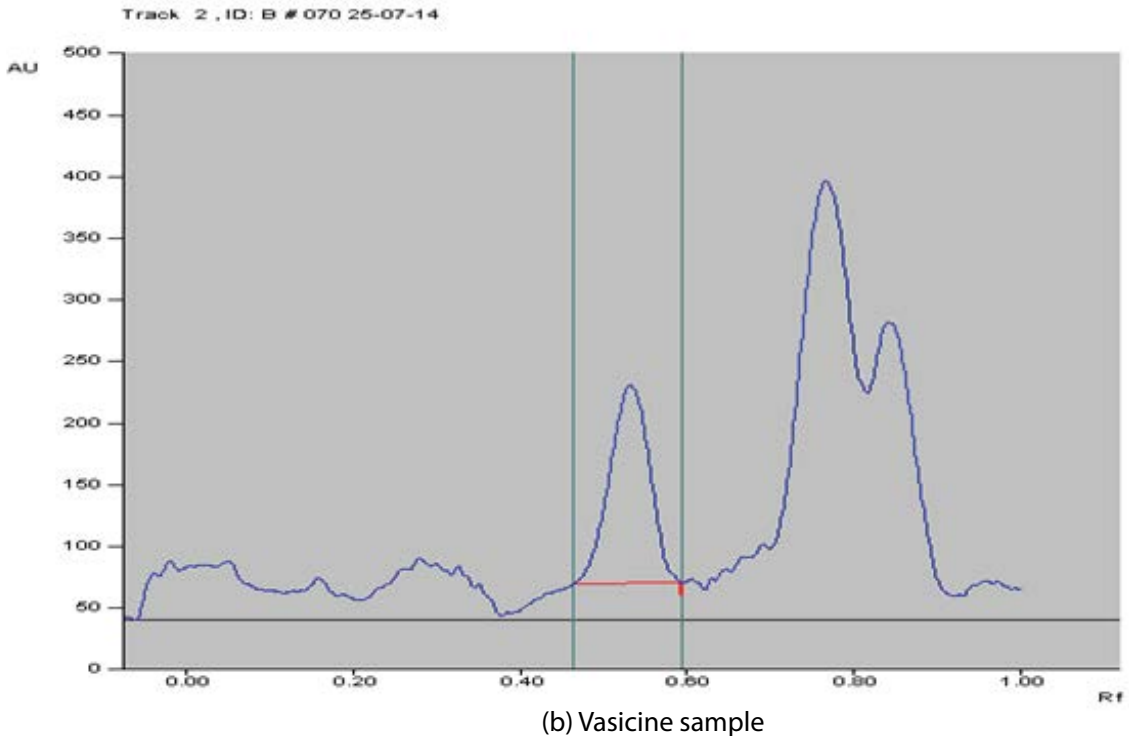


Figure 1. TLC plate for HPTLC vasicine (a) TLC plate with standard and sample spots (b) Plate with standard and sample spots developed



(a) Vasicine standard



(b) Vasicine sample

Figure 2. Chromatograms of vasicine by HPTLC