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Quantitative Determination of Chemical Markers in Poly Herbal Cough Syrups: A Systemic Review

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Abstract: Polyherbal medicines are not a simple task as many traits impact the biological efficacy and reproducible therapeutic effect. Polyherbal Cough preparations used for its antitussive activity from an ancient time. Pharmacological properties of an herbal formulation depend on Phyto constituents present. An overview covering various techniques employed in extraction and characterization of different herbal cough syrups. An overview covering various analytical techniques employed in quantification of markers in different marketed herbal cough syrups. The regulatory approvals to ascertain consistent chemical profile and biological activity of future drug candidate include reproducibility by repetitive testing using different batches to control batch-to-batch variation and development of standard assay markers. New approaches enable analysts to separate and determine biomarkers in complex mixtures of herbal formulations. Various marketed herbal cough syrups like Adulsa syrup, Honitus syrup, Herbal cough syrup, Linkus Syrup, Poly herbal Cough syrup, Echinopsechinatus Roxb. Herbal Cough Syrup and Zeal syrup are studied for their plant part used therapeutic activity and phytochemical quantification.

Keywords: Phytoconstituent, HPLC, HPTLC, Poly Herbal Formulation.

INTRODUCTION

Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization. Development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analysis of marker/bioactive compounds and other major constituents, is a major challenge to scientists. Many Validated Chromatographic Methods are used for the Quantification of Phytoconstituents. According to regulatory guidelines and pharmacopoeias macroscopic and microscopic evaluation and chemical profiling of the botanical materials is used for quality control and standardization. High performance liquid chromatography (HPLC) and High Performance Thin Layer Chromatography (HPTLC) are valuable tools for quantitative determination of Phytoconstituents.

CHALLENGES IN ANALYSIS OF HERBAL DRUGS

Confirming consistent marker content is an important aspect of standardization, it does not in itself associate to a standardized product. Standardization requires careful control of both raw material quality and manufacturing processes. It is obvious that the content is the most difficult one to assess, since in most herbal drugs the active constituents are unknown.

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Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not.

To prove identity and purity, criteria such as type of preparation sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. Purity is closely linked with the safe use of drugs and deals with factors such ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis (UV, IR, MS, and NMR), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias. The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses. A special form of assay is the determination of essential oils by steam distillation. When the active constituents (e.g. sennosides in Senna) or markers (e.g. alkydamides in Echinacea) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, GC, mass spectrometry (MS), or a combination of GC and MS (GC/MS), can be employed.

ESTIMATION METHODS

Various marketed herbal cough syrups like Adulsa syrup, Honitus syrup, Herbal cough syrup, Linkus Syrup, Poly herbal Cough syrup, Echinopsechinatus Roxb. Herbal Cough Syrup and Zeal syrup are studied for their plant part used, therapeutic activity and phytochemical quantification. Many Validated Chromatographic methods are used for the Quantification of Phytoconstituents.

A brief summary of the general approaches in extraction, isolation and quantification of bioactive compound from Herbal formulations.

- Extraction (sonication, heating under reflux, soxhlet extraction and hyphenated techniques like super critical fluid extraction, microwave assisted extraction are used for the extraction purposes).
- Isolation of Phytoconstituents from the plant extract by using different techniques like column chromatography, preparative chromatography, sublimation, distillation, fractional crystallization.

Quantification of the separated constituents is done by using different methods like, High performance thin layer chromatography (HPTLC), high pressure liquid chromatography (HPLC), gas chromatography-mass spectrometry, ultra high performance liquid chromatography-mass spectrometry.

All developed methods for quantitative estimation of Phytoconstituents like Ursolic acid, Glycyrrhizin, Piperine, Vasicine, Eugenol, Cineol, Thymol, 6-Shogaol, 8-Shogaol and 10-Shogaol from plants are reported to be validated for different parameters like accuracy, precision, linearity, limit of detection and limit of quantitation.

Table 1: Quantification of Herbal Cough Syrups

Types of Dosage form	**Plant used**	**Therapeutic activity**	**Phyto constituents**	**Analytical method**
Herbal cough syrup	Piper nigrum (Fruit) Piper longum (Fruit)	Potent Anti-tussive and Bronchodilator	Piperine	**HPTLC** **S.P**. : Silica gel 60F_{254} **M.P**.: Ethyl acetate: Hexane (60:40 v/v) **UV detection** :330nm **R_f Value**:0.37
Pelargonium Syrup SANA Pharmaceutical Research Co. (Amman, Jordan)	Pelargonium sidoides (Root)	Cough associated with acute bronchitis or common cold	Umckalin	**HPLC** **S.P. :** C_{18} Column (250 × 4.6mm,5µm) **M.P.:** Acetonitrile : Phosphoric acid (pH-2.5) (25:75, v/v) **Flow rate:** 1.0 ml/min **UV detection:** 310 nm **Retention time:**11.13 min
Adulsa Syrup	Adhatoda vasica (leaves)	Expectorant and bronchodilator	Vasicine	**HPTLC** **S.P**. : Silica gel 60F_{254} **M.P**.: Methanol: Toluene: Dioxane: Ammonia (2:2:5:1, v/v/v). **UV detection** :254nm **R_f Value**:
	Ocimum sanctum (leaves)	Stimulant, aromatic, spasmolytic, stomachic and is a good immune-modulatory agent		
	Glycyrrhiza Glabra (Root)	Anti-inflammatory and it reduces inflammation of the respiratory		

		tract, relieve a spasmodic cough		**Vasicine** :0.74
	Zingiber officinale (Rhizome)	Stimulant, aromatic stomachic		
	Piper Longum (Fruit)	Prevents recurrent attacks of bronchial asthma, immune-modulator activity, anti-allergic		
	Mentha arvencis (leaves)	Expectorant and bronchodilator		
	Foeniculum vulgare (Fruit)	flavouring agent		
Honitus syrup	Ocimum sanctum	Stimulant, aromatic, spasmolytic, stomachic and is a good immune-modulatory agent		**HPLC** **S.P. :** C_{18} Column (150 × 4.6mm, 5µm) **M.P.:** Acetonitrile and (0.05%) Ortho-phosphoric acid (85:15, v/v) **Flow rate:** 1.0 ml/min **UV detection:** 227 nm **Retention time:** 6-Shogaol:4.4 min 8-Shogaol: 5.4 min 10-Shogaol:7.2 min
	Glycyrrhiza Glabra	Anti-inflammatory and it reduces inflammation of the respiratory tract, relieve a spasmodic cough		
	Viola odorata	cough, asthma, fever with burning sensation, body ache		
	Solanum xanthocarpum	anti-tussive and expectorant property		
	Zingiber officinale	**stimulant, aromatic stomachic**	6-Shogaol, 8-Shogaol, 10-Shogaol	
	Piper Longum	Prevents recurrent attacks of bronchial asthma, immune-modulator activity, anti-allergic		
	Adhatoda vasica	Expectorant and bronchodilator		
	Curcuma zedoaria	Stomach disorders like flatulent colic and ulcers.		
	Mentha piperita	Expectorant and bronchodilator		
	Apis Mellifica	Relieves Cough and Throat irritation		
Herbal cough syrup	Adhatoda vasica (Leaves)	Expectorant, Anti-tussive	**Vasicine**	**HPTLC** **S.P**. : Silica gel 60F_{254} **M.P**.: n-Hexane: Ethyl acetate: Glacial acetic acid (8.5: 1: 0.5 v/v/v). **UV detection** :300nm **R_f Value**: Vasicine:0.5 Glycyrrhizin:0.44 Eugenol:0.75 Cineole:0.77
	Glycyrrhiza glabra (Root)	Anti-inflammatory and it reduces inflammation of the respiratory tract, relieve a spasmodic cough	**Glycyrrhizin**	
	Ocimum sanctum (Leaves)	Stimulant, aromatic, spasmolytic, stomachic and is a good immune-modulatory agent	**Eugenol**	
	Zingiber officinale (Rhizome)	Stimulant, Aromatic stomachic	**Cineole**	
Linkus Syrup	Adhatoda vasica (Leaves)	Bronchodilator and expectorant, use in breathlessness	**Vasicine**	**HPLC** **S.P. :** C_{18} Column (300 × 3.9 mm, 10µm) **M.P.:** Acetonitrile: 0.1 M Phosphate buffer: Glacial acetic acid (15: 85: 1 v/v) **Flow rate:** 0.7 ml/min **Detection:** 300 nm
	Glycyrrhiza glabra (Roots)	Anti-inflammatory and it reduces inflammation of the respiratory tract, relieve a spasmodic cough		
	Piper longum (Fruits)	Prevents recurrent attacks of bronchial asthma, immune-modulator activity, anti-allergic		
	Viola odorata (Dried leaves)	Expectorant		
	Hyssopus officinalis (Flowering tops)	antiseptic, cough reliever, and expectorant, soothing and cough Suppressant properties. antibacterial activity against S. aureus		
	Alpinia galangal (Dried rhizome)	Cough and respiratory troubles, cold, flu, lung diseases,		
	Cordia latifolia (Dried fruit)	gastrointestinal, respiratory and dermatological disorders		
	Althea officinalis (Dried seeds)	cough suppression, bronchodilator activity		
	Zizyphus jujube (Dried fruit)	colds and coughs, antibacterial, antiseptic properties, produced sound sleep		
	Onosma bracteatum (Dried leaves and stem)	bronchitis and asthma		

Poly herbal Cough syrup,	Zingiber officinale (Rhizome)	Stimulant, Aromatic stomachic		**HPTLC** **S.P**. : Silica gel $60F_{254}$ **M.P**.: Ethyl acetate: Methanol: Ammonia (8.0: 2.0: 0.2 v/v/v) **UV detection** :300nm **R_f Valu**e: Vasicine:0.54
	Terminalia belerica (Fruit)	Anti-asthmatic, Anti spasmodic, expectorant and Anti-tussive		
	Piper longum (Fruit)	Cough and cold. The antibacterial properties of black pepper are used for treating respiratory disorders		
	Adhatoda vasica	**Expectorant, Anti-tussive**	**Vasicine**	
	Cordia myxa	Cough and cold		
	Glycyrrhiza glabra	Anti-inflammatory and it reduces inflammation of the respiratory tract, relieve a spasmodic cough		
	Curcuma longa	Cough, bronchitis		
	Piper cubeba	Cough		
	Aloe vera	Cough and cold		
Echinopsechinatus Roxb. Herbal Cough Syrup	Glycyrrhiza glabra (Powdered extract)	Anti-inflammatory and it reduces inflammation of the respiratory tract, relieve a spasmodic cough		GLC **Retention time:** Eugenol:5.57 min Thymol:18.55min Zingiberenol:10.36 min
	Piper longum (Powdered extract)	Cough and cold. The antibacterial properties of black pepper are used for treating respiratory disorders		
	Curcuma longa Linn (Powdered extract)	Cough, bronchitis		
	Mentha Piperita (Powdered extract)	Expectorant and bronchodilator		
	Eugenia caryophyllus (Clove Oil)	Expectorant	Eugenol	
	Trachyspermum ammi (Ajwan Oil)	Cough and Cold	Thymol	
	Zingiber officinale (Ginger Oil)	Stimulant, Aromatic stomachic	Zingiberenol	
Zeal syrup	Ocimum sanctum	Antitussive effect	**Ursolic acid**	**<u>Estimation of Ursolic acid</u>** **HPLC** **S.P. :** C_{18} Column (150 × 4.6mm, 5µm) **M.P.:** Methanol: Acetonitrile (30:70) v/v) **Flow rate:** 0.6 ml/min **UV detection:** 210 nm **<u>Estimati0n of Glycyrrhizin</u>** **HPLC** **S.P. :** C_{18} Column (150 × 4.6mm, 5µm) **M.P.:** Buffer: acetonitrile (60:40, v/v) **Flow rate: 1.0 ml/min** **UV detection:** 254nm **<u>HPTLC</u>** **S.P**. : Silica gel $60F_{254}$ **M.P**.: n-Hexane: Ethyl acetate: Glacial acetic acid (8.5: 1: 0.5 v/v/v). **UV detection** :254nm and 366 nm Visible (after spray of Anisaldehyde Sulphuric acid reagent) **<u>R_f Valu</u>**e: **Ocimum sanctum extract** :0.32(366 nm) **Adhatoda vasica extract**:0.61(254 nm) **Glycyrrhiza glabra extract** :0.39(254 nm) **Solanum xanthocarpumextract**:0.20 (366 nm) **Zingiber officinale extract :** 0.57(366 nm) **Trikatu Churna**: 0.44 (366 nm)
	Glycyrrhiza glabra	Expectorant and anti-tussive activity	**Glycyrrhizin**	
	Adhatoda vasica	Bronchodilator		
	Solanum Xanthocarpum	Anti-tussive and Expectorant property		
	Zingiber officinale	Stimulant, Aromatic stomachic		
	Trikatu Churna	Bio enhancer, Expectorant		

SUMMARY

Sr. No.	Phytoconstituent	HPLC (single estimation)	HPLC (combination with other Phytoconstituents)	HPTLC (single estimation)	HPTLC (combination with other Phytoconstituents)	GLC
1	Ursolic acid	----------	1 (Ursolic acid Glycyrrhizin)	---------	----------	-------
2	Glycyrrhizin	----------	1 (Ursolic acid Glycyrrhizin)	---------	1 (Vasicine, Glycyrrhizin, Eugenol, Cineol)	-------
3	Curcumin	----------	----------	---------	----------	-------
4	Solasodine	----------	----------	---------	----------	-------
5	Piperine	----------	----------	1	----------	-------
6	Vasicine	**1**	----------	2	1 (Vasicine, Glycyrrhizin, Eugenol, Cineol)	-------
7	Gingerol	----------	----------	---------	----------	-------
	Zingiberenol	----------	----------	---------	----------	**1** (Zingiberenol, Eugenol, Thymol)
	6-Shogaol, 8-Shogaol, 10-Shogaol	**1**	----------	---------	----------	-------
8	Oleanolic acid	----------	----------	----------	----------	----------

CONCLUSION

For the quality assured herbal product, phytochemical quantification is required. There are various Phytoconstituents like Ursolic acid, Glycyrrhizin, Piperine, Vasicine, Eugenol, Cineol, Thymol, 6-Shogaol, 8-Shogaol and 10-Shogaol are estimated by chromatographic methods. Marker based quantification for the purpose of quality control of herbal medicines can only address to the problem of comparing the integrated sameness and/or difference and controlling their stability of the available herbal products. Thus, the researches concerning the relationship between the chromatographic quantification and efficacy of the herbal medicines are urgent requirements for the quality control of herbal medicines.

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