

Short Communication

# Standardization of Poly-Herbal Compound (Aerosol) Using Raman Spectrometry and Gas Chromatography Mass Spectrometry

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## ABSTRACT

Standardization of drugs refers to the confirmation of its identity and determination of its quality and purity. There are various procedures used for the standardization of drugs as there is increase in the advancement of various technologies and chemical knowledge of the various methods. Botanical, chemical, spectroscopic and biological methods are used for estimating active constituents present in the crude drugs in addition to its physical constants. Ayurveda is the science which deals with a vast variety of plants which are used to cure different types of ailments but the major problem faced by ayurvedic drugs is the standardization. Therefore, it is a need for the researchers in the medical field of Ayurveda to emphasise on evaluation and characterization and standardization of various plants and the formulations. Aerosol is the suspension of solid particles or liquid droplets in the air or any gas. In this study, an ayurvedic aerosol compound of four herbal drugs has been prepared using hot percolation technique and standardization was done using Raman spectroscopy and Gas chromatography mass spectrometry. The study reveals the presence of different phytochemical compounds and 40 types of metabolites in the compound.

**Keywords:** Standardization, Ayurveda, aerosol, Raman spectroscopy, metabolites herbal drugs,

## INTRODUCTION

Herbal drug standardization is a very deep and wide subject. There are various contradictory theories on the subject of herbal medicine and the relationship of drug and human physiology. In Ayurveda, there is a vast knowledge of important herbs for the purpose of standardization. For the production of standardized therapeutically effective ayurvedic formulations. India can stand as one of the major country and can also play lead role. India also needs to explore the medicinally important plants which can only be achieved when the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public

health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety, and efficacy.

Standardization is said to be the best technical application for selection in making appropriate choices for ratification coupled with consistent decisions for maintaining obtained standards. Ayurveda has a vast knowledge of herbal or plant products useful in some of the major ailments. Extraction methods used pharmaceutically involves the separating the active constituents of plant from the inactive components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilise

compounds with similar polarity. The authenticity, quality and purity of herbal drugs are established with the reference mentioned in pharmacopoeia. The pharmacopoeia mainly involves structural, analytical, physical standards for the drugs. Critical examination and identification of crude drugs is required for manufacturing any formulation due to diversity and variability in the chemical characteristics of different plants. In the present study, a herbal aerosol containing four herbal drugs was prepared using hot percolation technique and standardization of the drug was done using Raman spectrometry and Gas chromatography mass spectrometry and thin layer chromatography. Extraction Procedure done in the study is the general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and distillation techniques (water distillation, steam distillation, phytonic extraction (with hydro fluorocarbon solvents). For aromatic plants, hydro water and steam distillation, hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro extraction, protoplast extraction, micro distillation.

According to Ayurveda drug is considered as the one of the most important part in the treatment. There is nothing in this world which cannot be used as medicine. [1,2]

Drug: (Greek – *drogue*, chemical material)

Any substance that, when given to the living organism, may modify one or more of its functions. [3]

Drug is any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the

recipient according to the world health organization. That which is the substratum of action and qualities and coexistent cause is substance (drug). [4] Treatment, disease-remover, corrective, remedy, drug, redress, sedative, restorative, and wholesome agent, - all these, it should be known, are different names of the Medicine. [5] Drug should be characteristics with Usability in multiple forms, worth of quality, applicability. [6] The course of the treatment which cures the original disease but produces some other kind of complication is not the correct line of treatment, the correct one is that which cures but does not provoke. [7] That is the right medicine which is made for health. [8]

## MATERIALS AND METHODS

The drug selected for preparing aerosol are- *Shirisha* (*Albizia lebbek*), *Nagarmotha* (*Cyperus rotundus*), *Kantakari* (*Solanum xanthocarpum*) and *Shyama Tulsi* (*Ocimum basilicum*). These drugs are reported to be given through nebulisation in aerosol form and moreover all are reported for having bronchodilator, anti-inflammatory, antitussive, mucolytic, antibacterial and antioxidant effect on animal as well human research trials. [9]

### Pharmacodynamics:

#### A. SHIRISHA:

Botanical Name: *Albizzia lebbek*

Natural Order : Mimosaceae

Classical Names : *Shirisha*, *Shukapriya*, *Mridupushpaka*, *Lomapushpaka*

#### IDENTITY, PURITY AND STRENGTH:

Foreign matter: Not more than 1 Per cent

Total ash: Not more than 8 Per cent

Acid-insoluble ash: Not more than 1 Per cent

Alcohol-soluble extractive: Not less than 12 Per cent

Water-soluble extractive: Not less than 6 Per cent

#### Mode of action:

*Shirisha* has a significant cromoglycate-like action on the mast cells. In addition, it appears that it inhibits the early processes of sensitization and synthesis of reaginic-type antibodies. [10,11]

### BRONCHODILATOR ACTIVITY

Aqueous extract of both stem bark and flowers significantly reduced ( $p < 0.01$ ) bronchospasm induced by micro-aerosols of histamine acid phosphate (1% solution) and acetylcholine chloride (1% solution) in guinea pig bronchi. In another study, Sirisha has shown a significant disodium cromoglycate like activity on the mast cells. [12]

### ANTI- INFLAMMATORY ACTIVITY

The anti-inflammatory activity of *Albizia lebbek* was studied on rat models. [13] The extracts obtained using petroleum ether; chloroform and ethanol were administered at the concentrations of 100, 200 and 400 mg/kg body weight. [13] The petroleum ether and ethanol extracts at 400 mg/kg, showed maximum inhibition of inflammation induced. [14]

### ANTI ALLERGIC ACTIVITY

*Albizia lebbek* at different concentrations has got potent mast cell stabilizing property and the  $IC_{50}$  value of *Albizia lebbek* was found to be 85  $\mu\text{g/ml}$ . This inhibitory potential of catechin from *Albizia lebbek* is perhaps due to modulation of two important effector's functions, histamine release and cytokine expression of antigen -IgE activated mast cells. [13]

### ANTI-FUNGAL ACTIVITY

The anti-fungal activity of lebeckalysin was screened with agar diffusion assay. 200  $\mu\text{g}$  of lebeckalysin were added to test its inhibitory effect on different fungi. The pathogenic species used to test its activity are *Mycosphaerella arachidicola*, *Fusarium oxysporum*, *Helminthosporium maydis*, *Valsa mali* and *Rhizoctonia solani*. Nystatin was used as a positive control. The  $IC_{50}$  value for the anti-fungal activity of lebeckalysin against *R.solani* was determined. [12]

### ANTI MICROBIAL ACTIVITY

Antimicrobial screening of active principle(s) isolated from stem bark of *Albizia lebbek* showed that the total glycosides, cardenolide glycosides and

anthraquinone glycosides/anthraquinones were active against the test cultures selected for study. Study of the mode of action of the active principles against aerobes showed that the glycosides caused leakage of cytoplasmic constituents. Electron micrographs of *Staphylococcus aureus* cells treated with the minimum inhibitory concentration of anthraquinones revealed coarse granulation of the cytoplasmic matrix, vacuolation of the cells, and in a few cases, disruption of the cell surface. [15]

### Previous research on shirisha:

- Kajaria D, Tripathi J, Tiwari S. Anti-asthmatic effect of Shirishadi compound through nasal spray actuation. AYU. 2014 Jul;35(3):261.
- Kajaria D, Tripathi J, Tripathi YB, Tiwari S. In-vitro  $\alpha$  amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug-Shirishadi. Journal of advanced pharmaceutical technology & research. 2013 Oct 1;4(4):206.
- Kajaria D, Tripathi JS, Pandey BL. The analgesic and anti-inflammatory activities of the hydroalcoholic extract of "Shirishadi compound" in animal model. Journal of Applied Pharmaceutical Science. 2011 Dec 1;1(10):98.
- Divya K, Tripathi JS, Tiwari SK, Pandey BL. Evaluation of anti-cholinergic and anti-anaphylactic activity of shirishadipolyherbal compound. International Journal of Pharmacy. 2012;3:1-4.
- Kajaria DK, Gangwar M, Kumar D, Sharma AK, Tilak R, Nath G, Tripathi YB, Tripathi JS, Tiwari SK. Evaluation of antimicrobial activity and bronchodilator effect of a polyherbal drug-Shirishadi. Asian Pacific journal of tropical biomedicine. 2012 Nov 30;2(11):905-9.
- Kajaria D. Study of Antiasthmatic effect of standardized extract of Ayurvedic Compounds via Nasal Spray Actuation (in aerosol form) in rodents and its comparative clinical study.

### **B.KANTAKARI:**

*Kantakari* consists of mature, dried whole plant of *Solanum surattense*

Botanical name: *Solanum xanthocarpum*

Family: Solanaceae

It is perennial, very prickly diffused herb of waste land, found throughout India.

Sanskrit : *Vyaghree, Nidigdika, Kshudra, Kantakarika, Dhavani, Nidigdha.*

#### **IDENTITY, PURITY AND STRENGTH:**

Foreign matter: Not more than 2%

Total Ash: Not more than 9%

Acid-insoluble ash: Not more than 3%

Alcohol-soluble extractive: Not less than 6%

Water-soluble extractive: Not less than 16%

**Anti asthmatic property:** Glycoalkaloid and fatty acid fractions of the *Solanum xanthocarpum* extract cause liberation of histamine from chopped lung tissue. The effect of the drug on bronchial asthma may be attributed to the depletion of histamine from bronchial and lung-tissue. The expectorant action is due to inorganic nitrate content. [16]

Mast cell stabilization activity: *Kantakari* showed that ethanol extract of *Solanum xanthocarpum* shown a significant antihistaminic activity in histamine induced contraction in goat tracheal chain preparation. [17]

**Antiallergy Activity:** Apigenin is present in *Solanum xanthocarpum* shown anti-allergic effect on ovalbumin (OVA)-induced asthma model mice. OVA-induced mice showed allergic airway reactions and included an increase in number of eosinophils in bronchoalveolar lavage. [18]

#### **Previous study done:**

- PatilAmol. Aziz Arbar. Deepti A.K.Evaluation of bronchodilator effect of *kantakari* extract nebulization in wheezing children.IJRAP.2014 June 5(3).
- Evaluation of anti-inflammatory activity of *kantakari*.
- Kajaria D, Tripathi J, Tiwari S. Anti-asthmatic effect of *Shirishadi* compound

through nasal spray actuation. AYU. 2014 Jul;35(3):261.

- Kajaria D, Tripathi J, Tripathi YB, Tiwari S. In-vitro  $\alpha$  amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug-*Shirishadi*. Journal of advanced pharmaceutical technology & research. 2013 Oct 1;4(4):206.
- Kajaria D, Tripathi JS, Pandey BL. The analgesic and anti-inflammatory activities of the hydroalcoholic extract of "*Shirishadi* compound" in animal model. Journal of Applied Pharmaceutical Science. 2011 Dec 1;1(10):98.
- Divya K, Tripathi JS, Tiwari SK, Pandely BL. Evaluation of anticholinergic and anti-anaphylactic activity of *shirishadipolyherbal* compound. International Journal of Pharmacy. 2012;3:1-4.
- Kajaria DK, Gangwar M, Kumar D, Sharma AK, Tilak R, Nath G, Tripathi YB, Tripathi JS, Tiwari SK. Evaluation of antimicrobial activity and bronchodilator effect of a polyherbal drug-*Shrishadi*. Asian Pacific journal of tropical biomedicine. 2012 Nov 30;2(11):905-9.
- Kajaria D. Study of Antiasthmatic effect of standardized extract of Ayurvedic Compounds via Nasal Spray Actuation (in aerosol form) in rodents and its comparative clinical study.

### **C.TULSI (SHYAMA):**

*Krishna Tulsi* is also known as *Shyama Tulsi* or *Kali Tulsi* or *Purple Leaf Tulsi*, it looks purple it does have an aroma and peppery flavor.

The Many scientific studies have been performed on medicinal properties of *tulsi* on vitro, animal as well as on humans. The studies reveal that *tulsi* has a inimitable fusion of actions, below are some of the medicinal value of *tulsi*. [19]

Antibacterial, Antiviral, Antifungal, Antimalarial, Anthelmintic, Anti-ulcer, Anti-spasmodic, Anti-asthmatic, Anti-tussive, Anti-diabetic, Anti-

hypercholesterolemia, Anti-hypertensive, Anti-pyretic, Anti-allergic, Anti-oxidant, Anti-inflammatory, Hepato-protective, Immunomodulatory

Tulsi has vitamin C and other anti-oxidants such as "Eugenol" that can protect the heart from harmful effects of free radicals. It also reduces the total cholesterol levels so it can be useful for heart disease patients. [19]

**Anti-asthmatic activity:** A 50% hydro alcoholic extract and the volatile oil extracted from fresh leaves were evaluated against histamine and ach induced pre-convulsive dyspnea in pigs. Both the extract and the oil exhibited a significant dose dependent anti-asthmatic activity. The volatile constituents of the fresh leaves were thought to be the main factor responsible for the activity. [19]

Previous study done:

A Comparative PharmaceuticoPharmaco Clinical Study Of Effect Of Different Formulations Of Tulsi Churna (Tablet), Tulsi Arka And Tulsi Aerosol On Shwasa.

#### **D.NAGARMOTHA:**

It is a dried rhizome

**Botanical name:** Cyperus scariosus Linn.

**Family:** Cyperaceae

It grows throughout the country, common in waste grounds, gardens and roadsides, upto an elevation of 1800 m.

Sanskrit name : *Mustaka, Vaarida*

#### **IDENTITY, PURITY AND STRENGTH**

Foreign matter: Not more than 2%

Total ash: Not more than 8%

Acid-insoluble ash: Not more than 4%

Alcohol-soluble extractive: Not less than 5%

Water-soluble extractive: Not less than 11%

Volatile oil: Not less than 1%

#### **Antimicrobial activity**

The essential oil (0.2%) was extracted by hydrodistillation from the tubers of *C. rotundus* collected from Dehradun, Uttarakhand. The hydrodistilled oil of *C. rotundus* was subjected to GC-MS analysis. The oil was found to be effective against various bacterial and fungal strains. [20]

#### **Antimutagenic and radical scavenging activity**

This study evaluates mutagenic and antimutagenic effects of aqueous, total oligomers flavonoids (TOF), ethyl acetate and methanol extracts from aerial parts of *C. rotundus*. [21]

#### **Antispasmodic activity**

An aqueous extract of rhizomes of *C. rotundus* (ACR) was tested for its anti-diarrheal and anti-spasmodic activity. Anti-diarrheal effect of ACR was evaluated in castor oil induced diarrhea in mice and antispasmodic effect was evaluated by charcoal meal test in mice at a dose of 125, 250, 500 mg/kg. [22]

#### **Anticonvulsant and antioxidant activity**

Regarding high incidence of epilepsy in human society and with respect to insufficient therapies, in the present study, anticonvulsant effect of *C. rotundus* extract was experimentally examined. [23]

#### **Antibacterial activity**

The Antibacterial activity of *Cyperus* oil was studied for various microorganisms (*S. aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes*, *E. coli* and *P. aeruginosa*) using inhibition zone method (Aromatogram). The MIC and MBC for each microbe were estimated. [24]

#### **Antiplatelet activity**

*C. rotundus*, a well-known oriental traditional medicine, has been reported to exhibit wide spectrum activity in biological systems including the circulatory system, however, little information is available on its antiplatelet activity. [25]

#### **Previous study done:**

- Kajaria D, Tripathi J, Tiwari S. Anti-asthmatic effect of Shirishadi compound through nasal spray actuation. AYU. 2014 Jul;35(3):261.
- Kajaria D, Tripathi J, Tripathi YB, Tiwari S. In-vitro  $\alpha$  amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug-Shirishadi. Journal of advanced pharmaceutical

technology & research. 2013 Oct 1;4(4):206.

- Kajaria D, Tripathi JS, Pandey BL. The analgesic and anti-inflammatory activities of the hydroalcoholic extract of "Shirishadi compound" in animal model. *Journal of Applied Pharmaceutical Science*. 2011 Dec 1;1(10):98.
- Divya K, Tripathi JS, Tiwari SK, Pandey BL. Evaluation of anti-cholinergic and anti-anaphylactic activity of shirishadipolyherbal compound. *International Journal of Pharmacy*. 2012;3:1-4.
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- Kajaria D. Study of Antiasthmatic effect of standardized extract of Ayurvedic Compounds via Nasal Spray Actuation (in aerosol form) in rodents and its comparative clinical study.

#### **Preparation and authentication of the drugs:**

##### **Herbal aerosol compound preparation:**

All the four drugs were collected from the local market of Delhi and Identification and authentication was done by NISCAIR Institute, New Delhi (certificate attached in annexure)

Aerosol was prepared by the hot percolation method through soxhlet apparatus.

Soxhlet is used for separating component that are soluble in solvent. It is a continuous solid-liquid extraction process of transferring the partially soluble component of solid to liquid phase. Aerosol: a suspension of liquid and solid particles produced by an aerosol generator such as the small-volume nebulizer (SVN), the pressurized metered-dose inhaler (pMDI),

or the dry-powder inhaler (DPI). Aerosol therapy: delivery of solid or liquid aerosol particles to the respiratory tract for therapeutic purposes.

##### **Procedure**

Soxhlet process in which small volume of hot menstrum was passed over the drug by the time, as gain and again to dissolve out the active constituents until the drug was exhausted.

*Shirisha twak*/bark powder: 25 grams

*Nagarmotha* rhizome powder: 25 grams

*Tulsi* leaves (fresh): 25 grams

*Kantakari panchanga* powder: 25 grams

As mentioned above the total quantity of crude drugs powder are taken in extraction chamber of soxhlet and moistened with water & alcohol in the ratio of 2:1 (distilled water 250 ml and ethanol 150ml) for few hours until the drug was totally moistened. filter paper thimble was made along with some cotton which was placed into the wider part of the extractor. Thimble is used to prevent choking of the lower part of the extractor by drug particles. Soxhlet apparatus was switch on so that the vapours are allowed to pass through the side tube to the condenser where they are condensed and fall on to the packed drug through which it percolates and extract out the active constituents of the drug. Level of the liquid in the siphon increases with the volume of the menstrum and when it reaches the maximum point, it is siphoned out into the flask. The menstrum vaporizes when further heating occurs and the dissolved active constituents remain behind in the flask. The filling and emptying of the body of the extractor alternately goes on continuously till the drug is exhausted. Percolation of the same quantity of menstrum was done repeatedly for 14 to 15 through the drug and the active constituent were collected in the flask. The extract is then reduced in the hot oven which again dissolved in 1000ml of solvent. [26]

##### **Standardization of drug:**

Herbal aerosol compound:

**Table no.1: Analysis of Phytochemical constituent in herbal drugs used in Herbal compound (+) indicates present, (-) indicates absent**

Plants Phytochemical	Albizia lebbeck	Cyperus rotundus/scariosus	Solanum xanthocarpum	Ocimum basilicum
Alkaloids	+	-ve in Aq. + in EtoH	+	+
Flavonoids	+	+	+	+
Saponin	+ in Aq. - ve in EtoH	-ve	+	+
Tannin	+	+	+	+
Glycosoides	-ve	+	+	+
Steroids	-	+	+	+
Carbohydrates	+	+	+	+
Proteins	+	-ve	+	+
Amino acid	+	+	+	-
Cardiac glycoside	+	-ve in Aq + in EtoH	+	+
Phlobatannis	+	+	+	-
Reducing sugar	+	+	+	-
Anthraquinone	+	+	+	-

**STANDARDIZATION OF DRUG done by following procedures:**

1. Thin Layer Chromatography
2. Gas Chromatography and Mass Spectrometry
3. Raman spectroscopy

**Protocol for Standardization of Prepared drug:-**

1. Color of sample.
2. Refractive index.
3. Specific gravity.
4. Thin layer chromatography.
5. Gas chromatography mass spectrometry(GC-MS)
6. Raman spectroscopy.

The Polyherbal drug was used as Herbals Nebulizer drug and was administered in the form of Aerosol. Pharmaceutical aerosol may be defined as the preparation containing the active medicament dissolved, suspended or emulsified in a propellant or in a mixture of solvent and propellant and packed in a pressurized aerosol container. When little pressure is applied on the valve attached to the pressurized container, the medicament is

released in the form of mist or aerosol spray.

**Herbal aerosol Compound**

- Color of the drug : Light brown colored suspension
- pH : 5.5
- Specific gravity : 0.8567
- TLC : Stationary Phase : TLC silicate gel 60 F254
- Applying : Directly spotting
- Visualization : Examine under U.V. light, 254 nm

**Thin layer chromatography-(TLC):**

Thin layer chromatography (TLC) was used to separate the Herbal aerosol compound extract into different spots on the chromatate plate. The chromatograms developed on the microscope slide, were dried and observed visually for the various different part of poly-herbal extract components. The developing solvent used in different extract are hexane: ethyl acetate (9:1) and chloroform:methanol(9.5:5).

**The retention factor (Rf) was calculated using:**

$$R_f = \frac{\text{Distance move by the substance (cm)}}{\text{Distance moved by solvent (cm)}}$$

**Table No.2 : TLC Result of hydro-alcoholic extracts of poly-herbal drugs**

Extracts	Solvent system	Number of components	Distance of spots (cm)	Solvent front (cm)	Rf value
H-4 aerosol compound	Hexane:ethyl acetate (9:1)	3	11.5, 9.4, 7.3	13	0.88, 0.77, 0.56
	chloroform: methanol (9.5:5)	6	11.2, 9.2, 5.7, 3.9, 2.9, 2.4	13	0.86, 0.70, 0.43, 0.30, 0.22, 0.18

### Composition of Herbal aerosol compound as analyzed by Gas Chromatography and Mass Spectrometry:

The Polyherbal compound was subjected to GC-MS analysis by using Shimadzu GC-MS (Model QP-2010, Shimadzu Co.) in EI (electron impact) mode. This was carried out at Nanoscale research center (NRF) Indian Institute of Technology (IIT), New Delhi.

**Table no.3: The results indicates the presence of various secondary metabolites in varying concentration-**

S.No.	Name
1.	Dimethyl sulfone
2.	1,2-Cyclopentanedione
3.	Phenol
4.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-
5.	Glycerin
6.	1,3-Dioxol-2-one,4,5-dimethyl-
7.	Pyridine, 3-fluoro-5-amino-
8.	S-Methyl methanethiosulphonate
9.	Mequinol
10.	Cyclohexanamine, N-3-butenyl-N-methyl-
11.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy
12.	2,4,5,6,7-Pentamethoxyheptanoic acid, meth
13.	1,2-Benzenediol
14.	Methane, (methylsulfinyl)(methylthio)-
15.	Benzofuran, 2,3-dihydro-
16.	2-Furancarboxaldehyde, 5-(hydroxymethyl)
17.	Ethanone, 1-(2-hydroxy-5-methylphenyl)-
18.	1,E-6,Z-10-Hexadecatriene
19.	Phenol, 2,6-dimethoxy-
20.	9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-d
21.	Vanillin
22.	Cycloheptasiloxane, tetradecamethyl-
23.	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahyd
24.	10s,11s-Himachala-3(12),4-diene
25.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp
26.	2H-Cyclopentacyclooctene, 4,5,6,7,8,9-hex
27.	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahyd
28.	3-n-Heptyl-7-methyl-9-(2,6,6-trimethylcycl
29.	Spiro[2.5]octane, 5,5-dimethyl-4-(3-oxobut
30.	2-(5-Isopropyl-2-methylphenyl)ethanol
31.	Corymbolone
32.	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodeca
33.	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1
34.	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic
35.	Isolongifolene, 7,8-dehydro-8a-hydroxy-
36.	l-(+)-Ascorbic acid 2,6-dihexadecanoate
37.	9,12-Octadecadienoic acid (Z,Z)-
38.	Octadec-9-enoic acid
39.	Octadecanoic acid
40.	Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl e






### Raman spectroscopy of H-4 herbal aerosol compound:

It is a technique which is mainly based on inelastic scattering of monochromatic light from a laser source usually. Inelastic scattering refers to the change in the frequencies of the photon of monochromatic light after interacting with the sample. Raman spectroscopy commonly used to provide structural fingerprints for identification of the molecules.

Raman spectroscopy for H-4 aerosol compound was done at Indian Institute of Technology, New Delhi.



**Table no.4: Brief description of H-4 compound drugs**

Plants	Part used	Extract/ Active principle	Probable mechanism of action
Albizia lebbek	Stem bark	Aqueous extract M.C.-Catechin	Mast cell stabilizing effect, antiallergic & antioxidant activity. 
Cyprus scariosus	Root	Aqueous extract/ alcoholic extract M.C.-Sesquiterpenes	Anti-Inflammatory, Antimutagens and Radical scavengers, Antioxidant activities.  
Solanum xanthocarpum	Whole herb	Aqueous/alcoholic extract M.C.-Salasodin, Apigenin, Stigmasterol, Carpesterol, Diosegenin	Bronchodilator, Antiallergic property, Anti-inflammatory 
Ocimum basilicum	Leaves	Aqueous/alcoholic extract M.C.- methyl eugenol and methyl chavicol	Anti-asthmatic, Antitussive, Antimicrobial, Anti allergic properties. 

### Characteristics of the spectrometer used:

Laser- 785 nm  
Laser Power=100 mW  
System- Horiba LabRAM HR

### Raman spectrum graph of H-4 Aerosol compound:

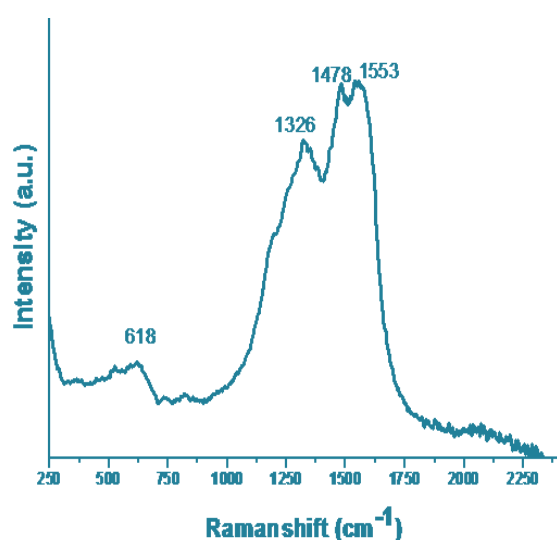


Figure 1. Showing peaks of compounds found in the drug (H-4 aerosol).

### Assessment of peaks:

On comparing the peaks positions found here with standard peak positions and their assignment, the component which are found in the H-4 compound are as:

- With reference to the figure 1  
Peak 618: Phenylalanine  
Peak 1328: Cytosine, Guanine  
Peak 1478: Adenine  
Peak 1553: Pyrimidine

### CONCLUSION

The physicochemical standardization of Herbal aerosol compound was carried out. All the raw drugs were authenticated and standardized with the mentioned constituents of each drug as mentioned in Ayurvedic pharmacopeia of India. The herbal aerosol compound was prepared and studied for various physicochemical

properties. The result indicates the need for the standardization of the drugs in Ayurveda which helps to establish the probable mode of action of the drug.

#### ACKNOWLEDGEMENT

Authors gratefully acknowledge Mrs Sunita from NISCAIR Institute, New Delhi for the valuable support in the authentication of raw drugs and Mr. Shashank Gahlot from IIT, New Delhi for the valuable support in performing Raman spectroscopy for the drug.

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How to cite this article: Nasreen A, Divya K. Standardization of Poly-herbal compound (aerosol) using Raman spectrometry and gas chromatography mass spectrometry. *Int J Health Sci Res.* 2019; 9(10):247-257.

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