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RESEARCH ARTICLE**PRELIMINARY PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF AROGYAVARDHINI COMPOUND- AN EMERGING FORMULATED MEDICINE FOR METABOLIC SYNDROME****Padhar Bharatkumar C^{*1}, Dave Alankruta R², Harisha CR.³, Shukla VJ⁴**¹Ph.D Scholar, Department of Kayachikitsa, I.P.G.T & R.A, Gujarat Ayurved University Jamnagar, India²Associate Prof. & I/C Head of Department of Kayachikitsa, I.P.G.T & R.A, Gujarat Ayurved University Jamnagar, India³Head of Pharmacognosy laboratory, I.P.G.T & R.A, Gujarat Ayurved University Jamnagar, India⁴Head of Pharmaceutical laboratory, I.P.G.T & R.A, Gujarat Ayurved University Jamnagar, India*Corresponding Author's mail: dr.bharat1987@yahoo.in

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ABSTRACT**Context:** *Arogyavardhini* Compound is an emerging formulated herbo-mineral formulation for treatment of metabolic syndrome. Metabolic syndrome is group of risk factors like, increased waist circumference, insulin resistance, increased triglycerides, decreased high density lipoproteins and hypertension for coronary artery diseases and type 2 diabetes mellitus. *Arogyavardhini* compound consist equal quantity of *Arogyavardhini Rasa* and single bulb garlic powder. *Arigyavardhini Rasa* having proven anti dyslipidemic and weight reducing effect and Garlic having antidiabetic, antidyplipidemic, antihypertensive effect, the combination called *Arogyavardhini* Compound has been formulated for management of metabolic syndrome.**Aim:** Authentication of raw drug of *Arogyavardhini* Compound and phytochemical evaluation of finished product.**Materials and methods:** *Arogyavardhini* Compound was evaluated on the basis of powder microscopy and analytical parameters like pH, Ash value, acid insoluble ash, water soluble extract, methanol extract and high performance thin layer chromatography. **Results:** Powder microscopy revealed the presence of Annular vessels of *Musta*, Starch grains of *Vacha*, Stone cells of *Pippali*, Stone cells of *Chitraka*, Oleoresins of *Shunthi*, etc. Physicochemical parameters such as total Ash value (15.91%), water soluble extract (13.5%), methanol soluble extract (17.2%) were assessed in preliminary physicochemical scanning. HPTLC revealed maximum 09 spots in short wave UV 254 nm. & 07 spots were obtained in long wave UV 366 nm.**Conclusion:** Pharmacognostical study revealed genuinity of raw drugs. Physico-chemical and HPTLC studies inferred that the formulation meets the minimum quality standards as reported in the API at a preliminary level. The inference from this study may be used as reference standard in the further quality control researches.**Key Words:** *Arogyavardhini* Compound, HPTLC, Pharmacognosy, Physicochemical analysis.**INTRODUCTION**

Nature is mother of mankind. It blesses us through various minerals and herbs to live a healthy and wealthy long life. Since ancient times, humanity has depended on the mixture of the plant and mineral resources for food clothing, protection and traditional medicine to cure a number of diseases. *Arogyavardhini* Compound is an emerging herbo-mineral formulation of thirteen ingredients (Table No.1) formulated for treatment of metabolic syndrome.¹ In Ayurveda metabolic syndrome can be compared with *Avaranjanya Madhumeha*², which is condition of excessive accumulation of *Meda* (Fatty tissues), *Kleda* and *Kapha* leading *Aavarana* (Obstruction) of *Vata* resulting in excretion of *Ojasa*

(Essence of body tissues) with urine. Drugs of *Arogyavardhini* Compound like *Kajjali* (Combination of mercury and sulphur) have *Yogavahi*³ (increasing potency of formulation and not altering the pharmacological action of contents in combinations), *Tamrabhasma* having *Lekhan* and *Sthaulahar* properties⁴ *Abhraka Bhasma* have *Pramehahar* property⁵ *Lauha Bhasma* have *Sthaulayahara* and *Rasayana* property⁶, *Shilajatu* and *Guggulu* have *Lekhan*, *Rasayan*, *Pramehahar* properties,⁷ *Triphala* have *Pramehahara* and *Rasayan* properties⁸, *Katuki* have *Pramehahar*, *Medahar* properties⁹. *Lasuna* have *Rasayan* and *Avaran har* properties¹⁰. So it can be said

that overall effect of formulation may be *Lekhan*, *Sthaulyahar*, *Pramehahar*, *Avaranhar* and *Rasayan* and thus it will be useful in condition of *Avaranjanya Madhumeha* also called as metabolic syndrome.

There are several components of *Arogyavardhini vati* which are known to have hypolipidemic effects, i.e., *Picrorrhiza kurroa*¹¹, *Terminalia chebula*¹², *Terminalia bellerica*¹³, *Emblica officinalis*¹⁴, and *Guggulu*¹⁵. *Lasuna* also possesses anti-hyperlipidemic, anti-diabetic and anti-hypertensive activity.¹⁶ So the combination of *Arogyavardhini Rasa* and *Lasuna* may exert significant effect on metabolic syndrome.

Metabolic syndrome is a disorder of energy utilization and storage, diagnosed by a co-occurrence of three out of five of the following medical conditions: abdominal (central) obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low high-density cholesterol (HDL) levels. Metabolic syndrome increases the risk of developing cardiovascular disease, particularly heart failure, and diabetes. Some studies have shown the prevalence in the USA to be an estimated 34% of the adult population and the prevalence increases with age.¹⁷

During the past decades there has been increasing acceptance and public interest in natural products and

therapies in both developing and developed countries. So, we cannot assure drug industries insulation from adulterations and quality decrement. Therefore, quality control for efficacy and safety of herbal products is of main concern.^{18, 19} Maintaining the quality standards of the formulation is a challenge. The development of this traditional system of medicine with the perspective of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of the natural products in healthcare.^{20, 21} Initial steps in quality standardization of compound formulation is to establish the presence of each ingredient in the finished product,²² followed by the pharmaceutical analysis. In the present study, *Arogyavardhini* Compound was subjected to pharmacognostical (powder microscopy), HPTLC, densitogram and pharmaceutical evaluation for various physicochemical parameters in order to prepare a preliminary profile of formulation for future.

MATERIAL AND METHODS

Collection of raw materials: All the raw drug materials were collected from the pharmacy attached with institute. The ingredients and parts used of the drugs are given in table- 1.

Table 1: Ingredients of *Arogyavardhini* Compound:

Content	Latin name	Part used	Ratio	Form
<i>Suddha Parada</i>	-	-	1 part	Powder
<i>Suddha Gandhaka</i>	-	-	1 part	Powder
<i>Loha Bhasma</i>	-	-	1 part	Bhasma
<i>Abharaka Bhasma</i>	-	-	1 part	Bhasma
<i>Tamra Bhasma</i>	-	-	1part	<i>Bhasma</i>
<i>Haritaki</i>	<i>Terminalia Chebula</i> Linn.	Fruit	2 part	<i>Churna</i>
<i>Amalaki</i>	<i>Emblica officinalis</i> Linn.	Fruit	2 part	<i>Churna</i>
<i>Bibhitaki</i>	<i>Terminalia Bellirica</i> Roxb.	Fruit	2 part	<i>Churna</i>
<i>Suddha Shilajatu</i>	-		3 part	<i>Churna</i>
<i>Suddha Guggulu</i>	<i>Commiphora mukul</i> Hook.	Gum	4 part	<i>Churna</i>
<i>Eranda Moola</i>	<i>Ricinus Communis</i> Linn	Root	4 part	<i>Churna</i>
<i>Katuki</i>	<i>Picrorrhiza Kurrora</i> Roxb.	Root/Rhizome	22 part	<i>Churna</i>
<i>Nimba Patra Svarasa</i>	<i>Azadirachta Indica</i> A.Juss	Leaves Juice	<i>Mardana</i> for 2 days	
Single bulb <i>Lasuna</i>	<i>Allium ascalonicum</i> Linn.	Bulb	44 part	<i>Churna</i>

Pharmacognostical study: Raw drugs were identified and authenticated by the Pharmacognosy laboratory. The identification was carried out based on organoleptic characters of powder (*Churna*),²³ later pharmacognostical evaluation of the powder (*Churna*) was carried out. Powder (*Churna*) was dissolved in small quantity of distilled water, filtered through filter paper, studied under the Corlzeiss trinocular microscope attached with camera, with stain and without stain. The microphotographs were also taken under the microscope.^{24, 25}

Method of preparation of *Arogyavardhini* Compound: *Arogyavardhini rasa* was prepared by standard method mentioned in *Ayurveda*. *Kajjali* (black

mercury sulphide, *Loha bhasma* (incinerated iron) *Abhraka Bhasma* (incinerated mica), *Tamrabhasma* (incinerated copper), *Haritaki* powder, *Bibhitaka powder*, *Amalaki* powder, *Shuddh Shilajita* (Black Bitmin), *Erandmula*, *Suddha Guggulu* and *Katuki* powder were weighed accurately. First *Kajjali* and *Bhasma* placed in *Khalva* and mixed properly, than remaining powder added in this mixture and mixed thoroughly. *Nimbpatra Svarasa* was added till the mixture immersed completely and trituration was carried out till the mixture get semisolid form and dried. Same procedure was followed for second *Bhavana* also.²⁶

Single bulb *Lasuna* was collected from green vegetable grocer market of local area. *Lasuna* was first made into

paste and then dried in oven at 60 °C temperature for 4-5 days.

Dried paste of *Lasuna* was made into fine powder and sieved in mesh no.80. The equal quantity of *Lasuna* powder was mixed well with *Arogyavardhini Rasa* in mass mixing machine till the homogeneous mixture was obtained.

Pharmaceutical evaluation: *Arogyavardhini* Compound was analyzed by using qualitative and quantitative parameters at pharmaceutical laboratory. The common parameters mentioned for *Churna* in Ayurved pharmacopeia of India and C.C.R.A.S guidelines are total Ash value, pH value, water and methanol soluble extracts.²⁷ On its base the parameters were selected. Presence of more moisture contents in a sample can create preservative problems of *Churna*. Hence loss on drying was also selected as one of the parameter.^{28, 29}

High performance thin layer chromatography: Methanol extract of *Arogyavardhini* Compound was spotted on pre-coated silica gel GF CO₂₅₄ aluminum plate as 5 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of camag, linomate V sample applicator fitted with a 100 µL. Hamilton syringe was used as the mobile phase. After development, densitometry scanning was performed with a camage TLC scanner III reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 manufactured by CAMAGE Switzerland).The slit dimensions were 6.00 x 0.45 mm and the scanning speed was 20 mm per second.³⁰

OBSERVATIONS AND RESULTS

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Arogyavardhini* Compound. For this, coarse powder of all the ingredients was subjected to organoleptic and microscopic evaluation separately to confirm the genuineness of all the raw drugs. Later after the preparation of formulation, pharmacognostical evaluation was carried out.

Organoleptic evaluation: Organoleptic features like color; odor and taste of the *Arogyavardhini* Compound were recorded and are placed in table no 2.

Table 2: organoleptic characters of *Arogyavardhini* Compound:

Parameter	Results
Color	Dark grayish
Odor	Like garlic odor
Test	Bitter, astringent
Consistency	Fine course powder

Microscopic evaluation: Microscopic evaluation was conducted by dissolving powder of *Arogyavardhini*

Compound in the distilled water and studied under microscope for the presence of characteristics of ingredient drugs. The diagnostic characters are Scleroids of *Haritaki* (Image:01), Parenchyma cells of *Lasuna* (Image:02), Rhomboidal crystals of *Katukai* (Image:03), Stone cells with brown contents of *Katukai* (Image:04), Elongated parenchyma cells of *Lasuna* (Image:05), Stone cells of *Haritaki* (Image:06), Pitted scleroid of *Bibhitaka* (Image:07), Parenchyma cells with prismatic crystals of *Lasuna*(Image:08), Parenchyma cells with rhomboidal crystals of *Katuki*(Image:09), Spindle shaped fibers of single bulb *Lasuna* (Image:10), Silica deposition of *Amalaki* (Image:11), Mesocarp cells of *Amalaki* (Image:12), Fragment of annular vessels of *Lasuna* (Image:13), Simple fibers of *Amalaki* (Image:14), Trichomes of *Bibhitaka* (Image:15), Parenchyma cells and brown contents of *Erandmula* (Image:16), Pitted vessels of *Katuki* (Image:17), Lignified fibers of *Erandmula* (Image:18) Simple trichome of *Nimbpatra* (Image:19) and Tannin contents of *Haritaki*(Image:20).

Physico-chemical parameters: Physico-chemical parameters of the *Churna* like loss on drying, pH values were found within the normal range. Methanol and water soluble extractive values were found to be 17.2% and 13.5% respectively. Details is shown in table no.3

Table 3: Physico-chemical analysis of *Arogyavardhini* Compound

Parameter	Value
Loss on drying at 110 °C	3.00 %
Ash Value	13.50%
Acid insoluble Ash	0.077%
Water soluble extract	26.72%
Methanol Soluble extract	14.96%
pH (By pH indicator paper)	6.5

High performance thin layer chromatography:

Densitometry scanning of the HPTLC pattern showed 09 spots at corresponding R_f values 0.01, 0.03, 0.11, 0.16, 0.23, 0.28, 0.37, 0.50, 0.88, in short wave UV 254 nm. and 07 spots at corresponding R_f values 0.01, 0.03, 0.07, 0.11, 0.06, 0.24, 0.36, obtained in long wave UV 366 nm (Table no.4). Though it cannot be possible to identify particular chemical constituent from the spot obtained, the pattern may be used as a reference standard for further quality control researches. (Images: 21-23)

Table 4: R_f Values of *Arogyavardhini* Compound:

	R _f Values at 254 nm	R _f Values at 366 nm.
HPTLC	0.01, 0.03, 0.11, 0.16, 0.23, 0.28, 0.37, 0.50, 0.88.	0.01, 0.03, 0.07, 0.11, 0.06, 0.24, 0.36.

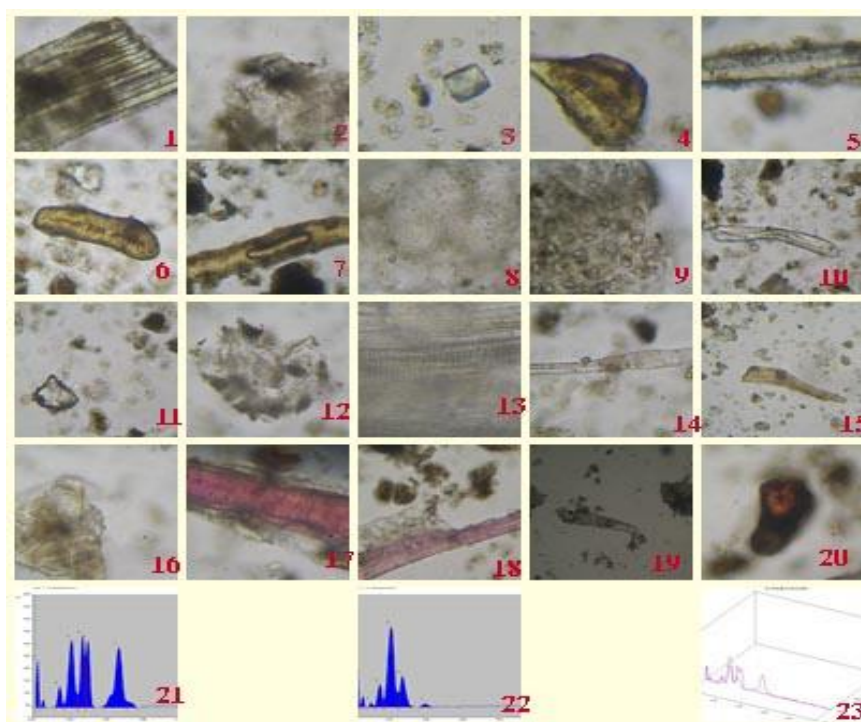


Figure 1: Pharmacognostical and HPTLC images

Legends:Image: 1- Scleroids of *Haritaki*Image: 2- Parenchyma cells of *Lasuna*Image: 3- Rhomboidal crystals of *Katukai*Image: 4- Stone cells with brown contents of *Katukai*Image: 5- Elongated parenchyma cells of *Lasuna*Image: 6- Stone cells of *Haritaki*Image: 7- Pitted scleroid of *Bibhitaka*Image: 8- Parenchyma cells with prismatic crystals of *Lasuna*Image: 9- Parenchyma cells with rhomboidal crystals of *Katuki*Image: 10- Spindle shaped fibers of single bulb *Lasuna*Image: 11- Silica deposition of *Amalaki*Image: 12- Mesocarp cells of *Amalaki*Image: 13- Fragment of annular vessels of *Lasuna*Image: 14- Simple fibers of *Amalaki*Image: 15- Trichomes of *Bibhitaka*Image: 16- Parenchyma cells and brown contents of *Erandmula*Image: 17- Pitted vessels of *Katuki*Image: 18- Lignified fibers of *Erandmula*Image: 19- Simple trichome of *Nimbatra*Image: 20- Tannin contents of *Haritaki*Image: 21- Densitogram of methanolic extract of *Arogyavardhini* Compound at 254nm.Image: 22- Densitogram of methanolic extract of *Arogyavardhini* Compound at 366nm.Image: 23- 3D MWL of methanolic extract of *Arogyavardhini* Compound**DISCUSSION**

Powder microscopy of *Arogyavardhini* Compound revealed the diagnostic characters like Scleroids of *Haritaki*, Parenchyma cells of *Lasuna*, Rhomboidal crystals of *Katukai*, Stone cells with brown contents of *Katukai*, Elongated parenchyma cells of *Lasuna*, Stone cells of *Haritaki*, Pitted scleroid of *Bibhitaka*, Parenchyma cells with prismatic crystals of *Lasuna*, Parenchyma cells with rhomboidal crystals of *Katuki*, Spindle shaped fibers of single bulb *Lasuna*, Silica deposition of *Amalaki*, Mesocarp cells of *Amalaki*, Fragment of annular vessels of *Lasuna*, Simple fibers of *Amalaki*, Trichomes of *Bibhitaka*, Parenchyma cells and brown contents of *Erandmula*, Pitted vessels of *Katuki*, Lignified fibers of *Erandmula*, Simple trichome of *Nimbatra* and Tannin contents of *Haritaki*

which authenticate genuineness of the raw drugs of *Arogyavardhini* Compound.

Taste of *Arogyavardhini* Compound was *Tikta* (bitter), because *Katuki* is in maximum quantity in *Arogyavardhini Rasa* and having strong bitter taste results in bitterness of formulation. Garlic is in equal quantity of *Arogyavardhini Rasa* in this formulation may resulted in garlic like odor of formulation.

Moisture contents should be minimum to prevent degradation of product. Excess of water in formulation encourage microbial growth, presence of fungi or insects and deterioration following hydrolysis. *Arogyavardhini* Compound contains 5.58% w/w moisture, showing that the *Churna* should be protected

from humid atmosphere. Ash values are the criteria to judge the identity and purity of crude drugs were total ash, water soluble and acid insoluble ashes are considered. *Arogyavardhini* Compound contained 15.91% w/w total ash and 0.077% w/w acid insoluble ash. The results revealed that *Arogyavardhini* Compound is free from unwanted organic compounds and production site was good enough keeping sample free from dust and other solid matters. The 13.5% w/w of water soluble extractives and 17.2% w/w methanol soluble extractives were present in *Arogyavardhini* Compound indicating that the drug is having good solubility in water.^[31]

In HPTLC study 9 spots at 254 nm and 7spots at 366 nm were obtained, indicating its possible components of matrix which may possess its therapeutic effect.

CONCLUSION

The ingredients were identified and authenticated pharmacognostically and were used for the preparation. The formulation was subjected to pharmacognostical study reveal genuineness as that all the ingredient microscopical characters were observed. Physico-chemical and HPTLC studies inferred that the formulation meets the minimum quality standards as reported in the API at a preliminary level. The inference from this study may be used as reference standard in the further quality control researches.

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