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PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF *TRIPHALADI GHANA-VATI* - AN *AYURVEDIC* POLYHERBAL FORMULATION FOR SENILE IMMATURE CATARACT

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ABSTRACT

On comparing signs and symptoms of senile cataract according to *Ayurvedic* texts this condition can be categorised under *Jara Janya Vyadhi* (Senile disorders). *Rasayana* drugs mentioned in *Ayurvedic* classics are said to be possess Rejuvenating and *Chakshushya* properties i. e. Beneficial for eyes. *Triphaladi Ghana-Vati* contains Eleven drugs which are known for their potent psycho-physical rejuvenating action/*Rasayana* properties, Adaptogenic properties, Anti-AGE properties (Anti- Advanced glycation end products), and Immunomodulatory actions. In the present study drug has shown encouraging clinical outcome in senile immature cataract. The polyherbal compound *Triphaladi Ghana vati* was subjected to Pharmacognostical evaluation, Physicochemical properties, phytochemical analysis and HPTLC studies. Sclerides, Silica deposits, Chollenchyma cells, Pitted vessels, Starch grains, Trichomes, Crystal fibres, Stone cells, Oleorasins, Acicular crystals, Annular vessels, Oil globules, Epicarp, Rod shaped crystals were found on Pharmacognostical evaluation. Alkaloids Tannin & Phenolic compounds, Flavonoid, Glycosides, Anthroquinon glycosides, Carbohydrate, Sugar and Steroids were present on carrying Phytocostituents evaluation. Physicochemical constants like pH, Loss on drying, Water soluble Extract, Alcohol soluble extract and Total Ash value were evaluated along with organoleptic characteristics.

KEYWORDS: *Triphaladi Ghana Vati*, Cataract.

INTRODUCTION

In the West, the incidence of cataract in people over 50 years is 15%, while in developing countries it is about 40%.[1] In developed countries Cataracts affect approximately 50 % of people between ages 65 and 74, 70 % over age of 75 years. In developing countries, the age factor is much reduced due to improper nutrition, non availability of ophthalmic health care, exposure to UV rays etc. The World Health Report published in 1998.[2] estimated that there were 19.34 million people who are bilaterally blind (less than 3/60 in the better eye) from age-related cataract. This represented 43% of all blindness. The number of blind people in the world and the proportion due to cataract is increasing due to population growth and increasing longevity. The result of these two factors means that the population aged over 60 years will double during the next 20 years from approximately 400 million now, to around 800 million in 2020. The incidence of new cases of cataract blindness is unknown. Minassian and Mehra estimated that for India alone 3.8 million people become blind from cataract each year.[3] There has been a substantial increase in CSR (Cataract Surgery Rate) in India especially after the inception of the World Bank-supported Cataract Blindness Control Project.[4] Professional interest and technological upgradation of skills and the availability of affordable equipment and intraocular lenses have all fuelled the increase in cataract surgery in India. A CSR of 3000 was targeted under Vision 2020: the right to sight, for India, by the year 2000.[5] Current trends show that this target has been achieved, but still there are regional disparities across the country. The CSR for the year 2002-03 ranges from a high of 8440 per million populations to a low of 130 per million populations,[6] Keeping these things in mind it is important to use surgery judiciously. Clearly, the tremendous burden of blindness due to cataract remains a major challenge for eye care professionals with public health perspective. Surgical treatment of cataract imposes great economic burden on the state and the backlog is perhaps too big to be handled by surgery alone. So it is important to seek other approaches to tackle the problem by seeking to identify factors that might modify the onset or delay the progression of cataract. If such a factor is identified which simply delays the onset of cataract by a period of 10 years, the number of cataract surgeries would drastically decrease by 45% or more.[7] Since the Cataract is three times more common in India than in United States, this will lessen the financial burden on the Indian government.

Therefore the entire world is looking at the other Systems of Medicine to tackle the situation and looking upon preventive Ophthalmology for improving and maintaining vision. In such a scenario, a study on Immature Cataract management gains much importance and therefore this Endeavour. Rasayana refers to the nutrition and its transportation in the body. *Rasayana* drugs mentioned in *Ayurvedic* classics are said to be possess Rejuvenating and *Chakshushya* properties i. e. Beneficial for eyes.[8] Rasayana is actually that which increases the essence of each Dhatu, starting from Rasa. Such a state of improved nutrition is claimed to lead to a series of secondary attributes like longevity, immunity against diseases, enhancing the function of sensual organs, increasing the mental competence and delaying of aging. Immunomodulators are considered now as one of the most potent tools in the management of health and disease. In Ayurvedic practice, the objective of immune enhancement is achieved through the use of the *Rasayana* and *Vajikarana* therapy, following *Achara Rasayana* measures and also by use of *Ojovardhaka* remedies.

Triphaladi Ghana Vati contains eleven drugs (Table-1). These drugs are known for their potent psycho-physical rejuvenating action/ *Rasayana* properties. In Ayurvedic classical texts administration of potent psycho-physical rejuvenator formulations comes under *Rasayana chikitsa* which frees one of diseases, improves quality of life and delays senile degenerative changes. Cataract is also an ageing disorder eleven among the most commonly used anti-oxidant; adaptogenic potent, well established rejuvenator herbs were selected. Adaptogenic properties of *Tulasi.*[9], *Gudoochi, Hareetaki, Amalaki.*[10] ,Anti-AGE properties (Anti-Advanced glycation end products) of *Shunthi* and *Tulasi.*[11], Hypoglycaemic, immunomodulatory, anti-oxidant effect of *Triphala*, Immunomodulatory and Rejuvenating aspects of *Gudoochi*, *Gokshura* and *Amalaki.*[12] free radicals scavenging action of *Punarnava*, increase of bio-availability of bioactive compound effect of *Shunthi.*[13].anti-inflammatory and anti-oxidant properties of *Yashtimadhu*, Adaptogenic, anti- inflammatory, anti – hypercholestraemic action of *Haridra* and *Daru haridra.*[14] are well documented in authorized Research journals.

MATERIAL AND METHODS

Collection of Raw materials for *Triphaladi Ghana Vati*

The ingredients of *Triphaladi Ghana Vati* were procured from the Pharmacy, Gujarat Ayurved University, Jamnagar and *Ghana-Vati* was prepared (Table: 1) in the Pharmacy, Gujarat Ayurved University, Jamnagar, India. Their identities were confirmed in the

Pharmacognosy laboratory of, I.P.G.T. & R.A., Jamnagar by correlating their morphological and microscopical characters with those given in the literature.

Method of preparation of the test drug *Triphaladi Ghana-Vati*

All the eleven drugs were taken in a vessel and eight times water was added and boiled on mild fire until one-eighth liquid is remained. It is then filtered and boiled again until water content is evaporated to the maximum. After preparing *Ghana* in the Pharmacy, Gujarat Ayurved University, Jamnagar and *Ghana-Vati* were prepared.

Organoleptic Characteristics

It was done with the help of *Panchagyanendriya Pariksha* /examination with the help of sense organs. Various parameters such as colour, odour, taste, touch and texture of the finished product were observed and recorded.[15] (Table: 2)

Microscopical Evaluation of *Triphaladi Ghana Vati*

The diagnostic characters of microscopic analysis of *Triphaladi Ghana Vati* showed the presence of tannin content from *Haritaki,* simple starch grains and Trichomes (type) of *Vibheetaki,* Sclerides of *Amalaki, Vibheetaki and Daru haridra,* Annular scalariform vessels and simple starch of *Shunthi,* border pitted vessels and simple and compound starch grains of *Guduchi,* glandular Trichomes of *Tulasi,* groups of stones and stratified fibres of *Gokshura,* oleo-resin, pitted stones and scalariform vessels belongs to *Haridra,* parenchyma cells and prismatic cells from *Yashtimadhu* and acicular crystals belongs to *Punarnava*. Fig 1, 2, 3.

Physico-chemical Constants

In physical evaluation foreign matter, loss on drying, total ash value, acid alcohol soluble extractive value, water soluble extractive value as well as pH value was determined. (Table: 3)

Phyto-chemical Analysis

Preliminary tests were carried out on methanolic extract for the presence or absence of phytoconstituents like alkaloids, tannins & phenolic compounds, flavonoids, saponins and anthraquinone glycosides. (Table: 4)

High performance thin layer chromatography:.[16]

The HPTLC profiles of methanolic extract of the polyherbal formulation *Triphaladi Ghana Vati*

Stationary Phase – Silica Gel GF 254

Mobile Phase – Toluene: Ethyl Acetate: acetic acid (7: 2 : 1) V/V

Detection – Short UV (254 nm), Long UV (366 nm)

The chromatograph was performed by spotting metahanolic extract of '*Triphaladi Ghana Vati* on pre coated silica gel aluminium plate GF-254 (250 µm thickness) using Camag Linomat V sample applicator and 100µl Hamilton syringe. The 05 µl of samples, in the form of bands of length 05 mm, were spotted 15 mm from the bottom, 10 mm apart, using nitrogen aspirator. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the remmision mode.

Chromatographic conditions

Details of Chromatographic conditions are given in table-5.The developed plate was scanned to obtain densitogram in visible range from 600nm to 800 nm with 100 nm interval.Chromatograms of the samples were developed by using the mobile phase. Then all tracks were scanned under 254 nm and 366 nm. (Table: 6)

RESULT AND DISCUSSION

This study shows physichochemical analysis followed by phytochemical screening of *Triphaladi Ghana Vati*. Further metahanolic extract basically contained moderately polar & polar secondary metabolite (flavonoids, steroids, terpenodial etc) due to solvent polarity. Again HPTLC fingerprinting result shown some uv-vis sensitive chromophore, auxochrome, conjugated double bond which indirectly represent the secondary metabolite. HPTLC fingerprinting is the most versatile tool to identify the natural polyherbal formulation with appropriate chromatogram, densitogram and retardation factor.

Plate-1 Powder microscopy of Triphaladi Ghana Vati

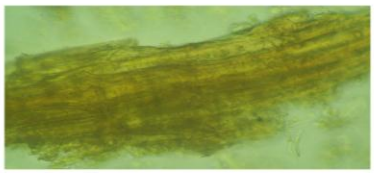


Fig no 1.1 Sclerides-Amalaki

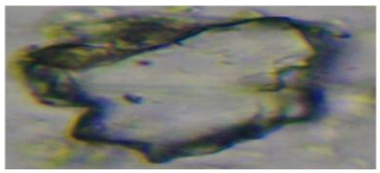


Fig no 1.2 Silica Deposits-Amalki

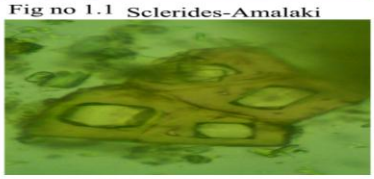


Fig no.1.3 Chollenchyma-Guduchi

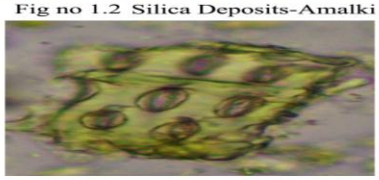


Fig no 1.4 Pitted Vessel Guduchi

Fig no 1.5 Starch Grain--Shunthi

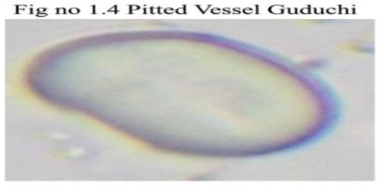


Fig no 1.6 Starch grain Shunthi

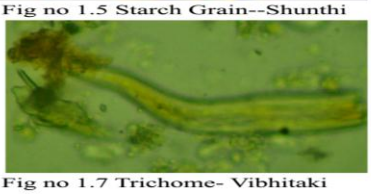


Fig no 1.7 Trichome- Vibhitaki

Fig no 1.8 Warty Trichome- Tulsi

Plate 2 :Powder microscopy of Triphaladi Ghana Vati

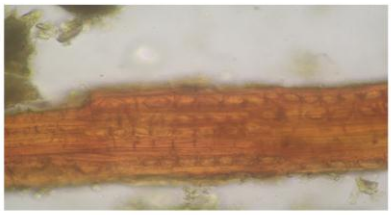


Fig no 2.1 Crystal Fibres Yashti

Fig no 2.2 Stone cells Yashti

Fig no 2.3 Stone cell-Gokshura

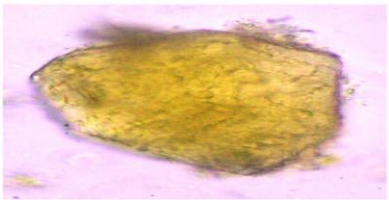


Fig no 2.4 Oleorasin-Haridra

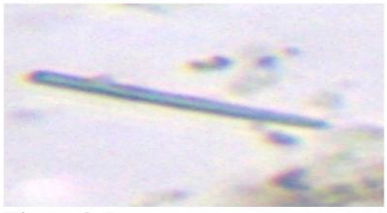


Fig no 2.5 Acicular Crystal Punarn.

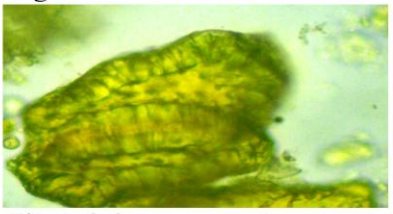


Fig no 2.6 Stone cells-Haridra

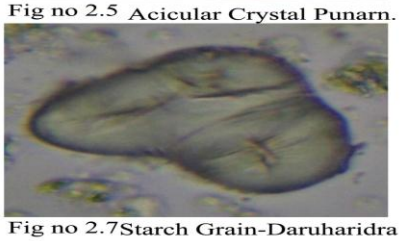


Fig no 2.7Starch Grain-Daruharidra

Fig no 2.8 Trichome Daruharidra

Plate 3 :
Poeder microscopy of Triphaladi Ghana Vati

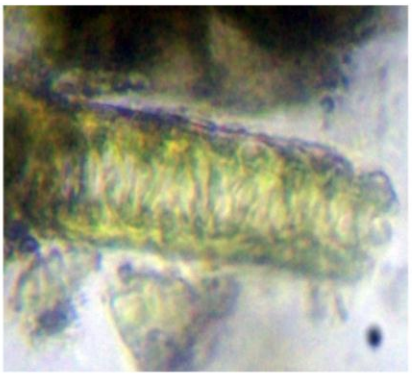


Fig no 3.1 Annular Vessel-Haridra

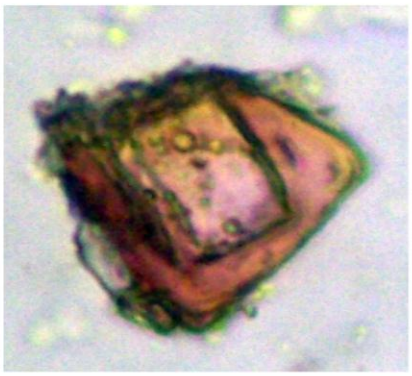


Fig no 3.2 Chollenchyma-Guduchi

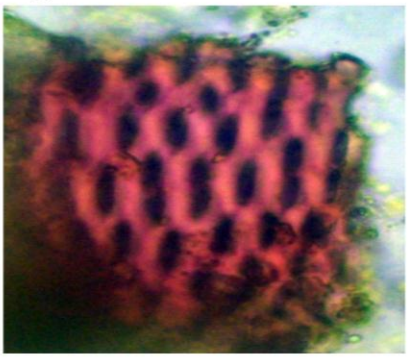


Fig no 3.3 Pitted vessel-Guduchi

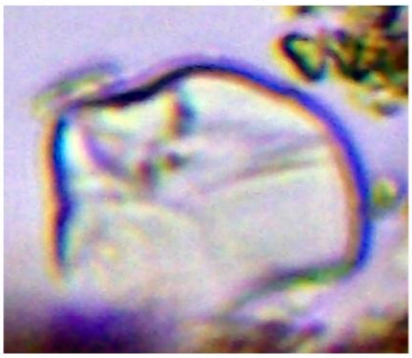


Fig no 3.4 Oil Globule-Tulsi

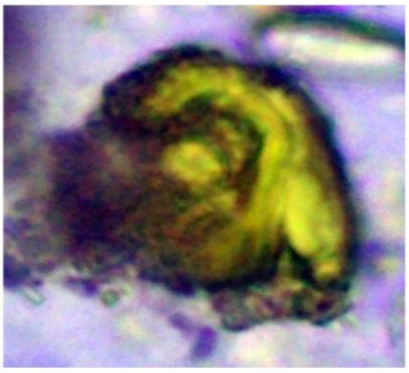


Fig no 3.5 Oleorasin-Shunthi

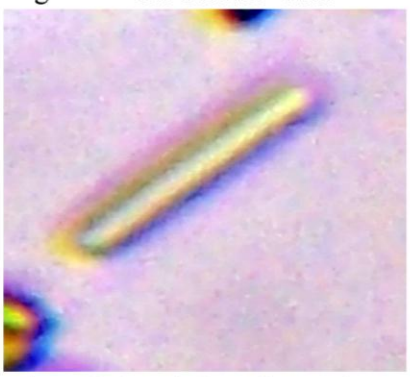


Fig no 3.6 Rod shaped crystal Punarnava

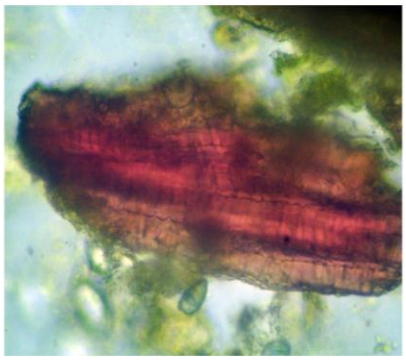


Fig no 3.7 Slerides-Yashtimadhu

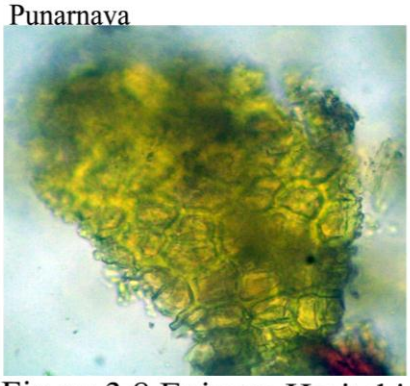
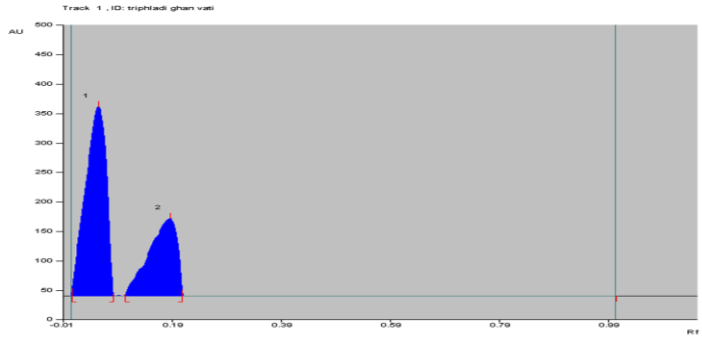
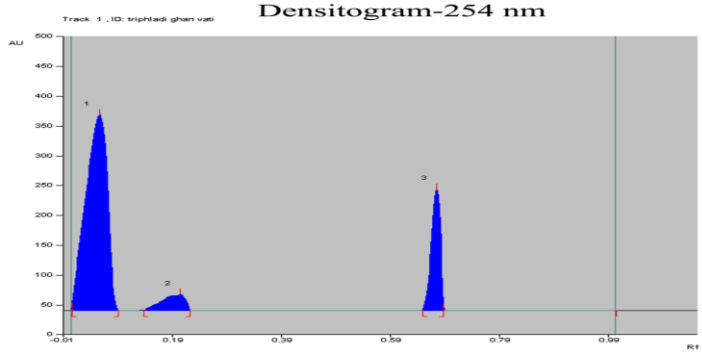


Fig no 3.8 Epicarp-Haritaki

Plate-4 Densitometric Analysis of Triphaladi Ghana Vati

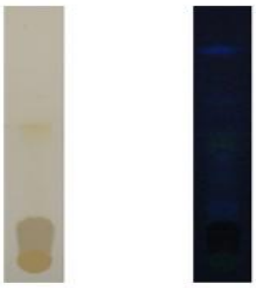


Densitogram-254 nm



Densitogram-366nm

254 nm



366 nm

Table-1 Ingredients of *Triphaladi Ghana-Vati*

Sr No	Name of Ingredients	Botanical name	Parts used	Ratio
1	*Haritaki*	*Terminalia chebula* Retz	Pericarp	1 part
2	*Vibheetaki*	*Terminalia bellerica* Roxb	Pericarp	1 part
3	*Amalaki*	*Emblica officinalis* Linn	Pericarp	1 part
4	*Yashtimadhu*	*Glycyrrhiza glabra* Linn	Roots & stolons	1 part
5	*Gokshura*	*Tribulus terrestris* Linn	Fruits	1 part
6	*Guduchi*	*Tinospora cordifolia* Miers	Stem	1 part
7	*Haridra*	*Curcuma longa* Linn	Rhizomes	1 part
8	*Daruharidra*	*Berberis aristata* DC	Stem	1 part
9	*Tulasi*	*Ocimum sanctum* Linn.	Whole plant	1 part
10	*Punarnava*	*Boerhavia diffusa* Linn	Whole plant	1 part
11	*Shunthi*	*Zingiber officinale* Rosc	Rhizomes	1 part

Table-2 Organoleptic Characteristics of *Triphaladi Ghana-Vati*

S. No.	Parameters	Results
1	Colour	Yellowish Green
2	Odour :	Astringent
3	Taste	Sour followed by Astringent
4	Touch	Hard
5	Texture	*Vati*

Table-3 Physico-chemical Constants

Sr. No	Parameters	Results of *Triphaladi Ghana-Vati*
1	pH	4
2	Loss on drying	9.13%
3	Water soluble Extract	47.46%
4	Alcohol soluble extract	26.54%
5	Total Ash value	10.5%

Table-4 Phyto-chemical Analysis

Sr No	Components	Results
1	Alkaloids	+
2	Tannin & Phenolic compounds	+
3	Flavonoid	+
4	Glycosides	+
5	Anthroquinon glycosides	+
6	Carbohydrate	+
7	Sugar	+
8	Steroids	+

Table-5 Chromatographic conditions

Development Chamber	CAMAG Twin Trough Chamber (20×10 cm^2)
Chamber saturation	30 min
Development time	30 min
Developmenyt distance	80 mm
Detection	Deuterium lamp, Mercury lamp
Photo Documentation	CAMAG reprostar
DATA system	WIN CATS software (Ver. 3.17)
Drying Device	Oven
U.V. spectrum	200 nm to 700 nm
Solvent system	Ethyl acetate: Water: Acetic acid (8:1:1)
Solvent front	18 cm

Table-6 Results of HPTLC Study

No of spots	Rf values of metahanolic extract of *Triphaladi Ghana Vati*	
	254 nm	**366 nm**
1	0.05	0.05
2	0.18	0.20
3	Nil	0.67

CONCLUSION

Analysis of *Triphaladi Ghana Vati* by different parameters such as organoleptic characteristics, pH, Loss on drying, Alcohol soluble extract, Water soluble Extract, Total Ash value and HPTLC densitogram shows good correlation between them. Microscopic characteristics of present formulation shows the presence of diagnostic identifying character of ingredient which are used. These parameters can be used for the evaluation and standardization of *Triphaladi Ghana Vati.* The present study can serve as a reference for further work on *Triphaladi Ghana Vati.*

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