



Research Article

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PHARMACOGNOSTICAL AND PHARMACEUTICAL EVALUATION OF POLYHERBAL FORMULATION TRIPHALADI CAPSULE WITH SEVEN BHAVANA

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ABSTRACT

Haritaki, Bibheetaki, Amalaki, Yastimadhu, Gokshura, Guduchi, Haridra, Daruharidra, Shunthi and Punarnava are the potent Rasayana drugs mentioned in all the ancient classics of Ayurveda which improves the quality of life and delay the senile degenerative disorders. Bhavana (trituration) is an important Samskara (process) mentioned in classics by which even a small dose of a drug may be made very potent to produce a very high result. Triphaladi capsule is a combination of above 11 dried herbs which was triturated seven times. Aim of the study was to screen the differences in pharmacognostical and pharmaceutical profile of Triphaladi capsule prepared with seven Bhavana. The powder of Triphaladi compound was triturated with decoction of the same compound seven times and made into dried powder which was filled in capsules and subjected to pharmacognostical and pharmaceutical evaluation. After Bhavana significant changes were found in the form of disturbed collenchyma cells and cork cells of Guduchi, collapsed epicarp cells of Haritaki, fragmented fibres of Yastimadhu, disturbed pitted vessels of Yastimadhu etc. Also in analytical study changes like increase in water soluble extractive value, increase in ash value, increase in pH, increased number of HPTLC spots etc were noted.

Key words: Bhavana, HPTLC, Triphaladi yoga, Pharmacognosy, pharmaceuticals, trituration.

INTRODUCTION

Eyes are the most important part of life. One can't imagine a life without eyes. Blindness not only causes human suffering but is also a heavy economic burden! Cataract is such leading cause accounting for 51% of blindness and 33% visual impairment worldwide.¹ In developed countries cataracts affect approximately 50 % of people between ages 65 and 74 and 70% over the age of 75. In developing countries, the age factor is reduced to 50 years and above due to improper nutrition, non availability of ophthalmic health care, exposure to UV rays etc. Senile cataract continues to be the main cause of visual impairment and blindness in the world.² The number of blind persons in India in 2000 was estimated to be 18.7 million (95% confidence interval [CI]: 15.2-22.3), of which 9.5 million were cataract-related. If there is no change in the current trend of blindness, the number of blind persons in India would increase to 31.6 million (95% CI: 26.4-36.9) in 2020.³

Any opacity in the lens or its capsule, whether developmental or acquired, is called cataract.⁴ Any factor physical or chemical which disturbs the critical intra and extracellular equilibrium of water and electrolytes or deranges the colloid system within the lens fibers tends to

bring about opacification.⁵ Although significant progress has been made toward identifying risk factors for cataract, there is no proven primary prevention or medical treatment. Surgical removal of cataract remains the only therapy. In many countries surgical services are inadequate, and cataracts remain the leading cause of blindness.

WHO/NPCB (National Programme for Control of Blindness) survey has shown that there is a backlog of over 22 million blind eyes (12 million blind people) in India, and 80.1% of these are blind due to cataract. To clear the backlog of cataract cases by the year 2000 and to tackle the rising incidence, 5-6 million cataract operations annually will have to be performed as against the present rate of 1.7 million per year.⁵ Also with lack of technical hand and equipments visual outcome will be poor.⁶ Risk of complication like posterior capsular opacification (PCO) is the most frequent. Decreased visual acuity induced by PCO is reported to occur in 20% to 40% of patients 2 to 5 years after surgery. The only effective treatment for PCO, Nd:YAG laser capsulotomy carries vision-related complications and risks and puts a significant financial burden on the health care system.⁷

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. Vision 2020, the right to sight is a recent global initiative launched by WHO to eliminate avoidable blindness by the year 2020. To join in the group effort we should contribute our bit. Like all Swabhavabalapravritta (occurring due to natural process/ aging process) disease cataract can be delayed by Rasayana (rejuvenators/which delay the aging process). So Triphaladi compound containing these types of Rasayana drugs was selected to delay the senile changes in the lens.

Triphaladi compound containing Haritaki, Bibheetaki, Amalaki, Yastimadhu, Gokshura, Guduchi, Haridra, Daruharidra, Shunthi and Punarnava is an Anubhoota yoga (experienced formulation) based on the practices of Shalaky department of I.P.G.T. & R.A., Jamnagar, India. Varma Radhika, Rajgopala Manjusha *et al* (2013) had evaluated the drug Triphaladi compound for its anti cataract activity experimentally as well as clinically with encouraging results. In that study it was used in choorna (powder) form and many of the patients were not comfortable to take it so in present study it was planned to make the same compound in capsule form with trituration of the same drugs to potentiate it and by that reducing the required dose. Triphaladi capsule is a combination of above 11 dried herbs which was triturated seven times with the decoction of the same compound then after made dried powder and filled in 500 mg capsules. These all 11 drugs are known for their actions; like adaptogenic properties of Guduchi, Haritaki, Amalaki;⁸ anti cataract activity of Haritaki, Amalaki, Bibheetaki (Triphala)⁹; anti advanced glycated end products activity of Shunthi¹⁰; antioxidants activity of Haridra, Amalaki, Yashtimadhu, Tulsi, Bibheetaki, Guduchi, Sunthi¹¹; adaptogenic, immunomodulatory and anti inflammatory properties of Punarnava¹²; immunomodulatory and anti inflammatory properties of Gokshura¹³; adaptogenic, anti inflammatory, anti cataract effect of Haridra¹⁴ and anti inflammatory activity of Daruharidra¹⁵.

MATERIALS AND METHODS

Collection of the drugs

Ingredients of Triphaladi compound viz. fruits of Haritaki (*Terminalia chebula* Retz), Bibheetaki (*Terminalia bellerica* Roxb), Amalaki (*Emblica officinalis* Gaertn), roots and rhizomes of Yastimadhu (*Glycyrriza glabra* Linn), fruits of Gokshura (*Tribulus terrestris* Linn), stem of Guduchi (*Tinospora cordifolia* Meirs), rhizome of Haridra (*Curcuma longa* Linn), Daruharidra (*Berberis aristata* DC), Rhizome of Shunthi (*Zingiber officinale* Rose), whole plant of Punarnava (*Boerhavia diffusa* Linn) were procured from the institutional pharmacy and leaves of Tulasi (*Ocimum sanctum* Linn) were collected from local area of Jamnagar, India. (Table 1) Their characteristics were confirmed by correlating their morphological and microscopical features with relevant literature.

Preparation of the drug

Equal quantities of the obtained fruits, stems, roots/rhizomes, leaves, whole plant were shade dried and made into fine powder separately with the help of mechanical grinder, sieved through 85# and mixed together mechanically to get homogenous mixture.

Importance of Bhavana

Bhavana, the trituration means mixing the solid matter with the liquid media for a particular time period with the sufficient pressure.

- It brings minute particles of the material in contact with the liquid media and impregnates properties of media to the material.
- It transforms coarse powder to finer state
- It leads to unique and suitable physiochemical changes.

Preparation of Triphaladi capsule with seven Bhavana

The prepared powder of Triphaladi compound was triturated with decoction of the same compound seven times in end runner. In each Bhavana sufficient amount of decoction made from Triphaladi compound was added to the powder of Triphaladi compound as it is very well soaked and then triturated for 6-8 hours daily till the Bhavana given to the powder was completely absorbed. On completing the seventh Bhavana, the obtained powder was dried and filtered through 120# sieve mesh. Then the material was filled in 500mg gelatine capsules and stored in air-tight container.

Pharmacognostical evaluation Organoleptic evaluation

Various characters like colour, odour, taste and touch are recorded by using sensory organs.¹⁶

Powder microscopy of the finished product (deposited specimen voucher no. PHM6141/2014-15) was done without stain and after staining with Phloroglucinol+HCl. Micro photographs were taken under Carl Zeiss Trinocular microscope attached with camera.¹⁷ By Powder microscopy observed the characters, determined the chemical nature of the cell wall along with the form and chemical nature of the content of the cells.

Physicochemical analysis

In phytochemical analysis loss on drying, ash value, water soluble extract, alcohol soluble extract etc were assessed. Preliminary tests were carried out on methanolic extract of test drugs for the presence or absence of phytoconstituents like alkaloids, tannins and phenolic compounds, flavonoids, saponin and anthraquinone glycosides.¹⁸

High performance thin layer chromatography (HPTLC)

HPTLC was performed as per the guideline provided by API. Methanolic extract of drug sample was used for the spotting. HPTLC was performed using Toluene+ Ethyl acetate+ Acetic acid (7:2:1) solvent system and observed under visible light. The colour and Rf values of resolved spots were noted.¹⁹

RESULTS AND DISCUSSION

Pharmacognostical evaluation

Organoleptic evaluation

Results of various parameters such as colour, odour, taste, touch and texture of the finished products (powder) are shown in Table 2. The colour of Triphaladi compound of previous study was golden yellow while the same of Triphaladi compound with seven Bhavana of present study was dark greenish. The colour change owes to prolong trituration of the compound. As it is well-known that during trituration, mild heat is generated due to friction which darkens the grinding matter. Triphaladi compound with seven Bhavana possesses Kashaya (astringent), Madhura (sweet) and Tikta (bitter) rasa (taste). Bitter taste is decreased and sweet taste is also noted in Triphaladi compound with seven Bhavana in comparison to Triphaladi compound without Bhavana. The alteration in rasa is might be due to the effect of Vibhaga (elimination) process carried out during Bhavana Samskara of the drugs. The more water soluble components like that of Yastimadhu are increased in the compound by seven times trituration which is also responsible for the sweet taste. Touch and texture of the Triphaladi compound with seven Bhavana are very fine and soft compared to Triphaladi compound without Bhavana might be due to breakdown of the hard cellular structures and the exposed cellular contents by prolonged trituration of the drugs.

Powder microscopy

The diagnostic characters of microscopic analysis of Triphaladi compound with seven Bhavana shows the presence of disturbed collenchyma cells of Guduchi, disturbed cork cells of Guduchi, collapsed epicarp cells of Haritaki, pitted stone cells of Haritaki, tannin content of Haritaki, fragmented fibres of Yastimadhu, disturbed pitted vessels of Yastimadhu, disturbed fibres' walls of Yastimadhu, crystal fibres of Yastimadhu, starch grains of Yastimadhu, scleroids of Yastimadhu, stone cells of Yastimadhu, microcrystal of Punarnava, oil globule of Tulasi, olioeresin content of Shunthi, simple starch grains of Shunthi, pitted stone cells of Daruharidra with white lumen, prismatic crystals of Daruharidra, stratified fibers of Gokshura, scleroids of Bibheetaki, Tannin content of Bibheetaki, olioeresin content of Haridra, annular vessels of Haridra and silica deposition of Amalakai (Photo plate 1)

Powder microscopy of Triphaladi compound with seven Bhavana showed disturbed collenchyma cells and

disturbed cork cells of Guduchi as well collapsed epicarp cells of Haritaki were found. Similarly fragmented fibres of Yastimadhu, disturbed pitted vessels of Yastimadhu, disturbed fibres' walls of Yastimadhu were also noted which the result of prolonged trituration are. As an outcome locked contents in the cellular compartment are freed which might results in increased and quick absorption as well increased assimilation and bioavailability of the drugs. Thus trituration process might potentiate the medicine and therefore reduces the required dose. Other structures as mentioned earlier are similar with previous study of Triphaladi compound powder.

Physicochemical analysis

Results of phytochemical analysis like loss on drying, ash value, water soluble extract, alcohol soluble extract, ash value etc are shown in Table 3. Loss on drying at 110 °C is the major factor for the stability of the drugs. The results of loss on drying of Triphaladi compound with seven Bhavana showed lower limits than prescribed in API but it is slightly higher than the previous study which might be due to trituration process with the decoction of the same compound. High water and alcohol soluble extractive values are important for evaluation of crude drugs. The water extractive value of the drug is high while alcohol extractive value is slight low than the previous study which indicates that load of total polar extractive components are increased while that of non-polar extractive component of the drugs are decreased might be due to trituration process. Total Ash value was slightly on higher side indicates increase in inorganic component of drug, which is obvious as the Bhavana was given by decoction, the more water soluble component of the drug was added and it is also consistent with the previous researches.²⁰ pH of both the samples indicate towards acidic nature of samples but the pH of Triphaladi compound with seven Bhavana is slightly on higher side also in previous researches it was proven.²⁰ Observation on preliminary tests of test drugs for the presence or absence of phyto-constituents are shown in Table no.4 Preliminary quantitative analysis of the drug showed the presence of the alkaloids, tannin and phenolic compounds, flavonoids, anthraquinone and saponin glycosides etc similar to previous study indicating the active compounds of the drug were not disturbed during the process.

High performance thin layer chromatography (HPTLC)

The colour and Rf values of resolved spots of HPTLC were noted. (Table-5) (Plate no. 2 & 3) In HPTLC profile of the methanolic extract of the drug 4 spots at Rf 0.01, 0.10, 0.35, 0.67 were observed in 256nm UV light spectrum while 6 spots at Rf 0.01, 0.13, 0.30, 0.39, 0.61, 0.67 were observed in 366nm UV light spectrum which was higher than the previous study. The explanation behind this is seven times trituration process of the compound resulting in breakdown of the hard cellular wall and release of individual intracellular moieties of the drugs which are responsible for the higher peaks in

HPTLC. This HPTLC profile can also be used for standardization and fingerprinting of the Triphaladi compound with seven Bhavana in future references.

Table 1: Ingredients of Triphaladi capsule

Sr. No	Name of ingredients	Botanical name	Proportion
1	Haritaki	<i>Terminalia chebula</i> Retz	1part
2	Bibheetaki	<i>Terminalia belerica</i> Roxb	1part
3	Amalaki	<i>Emblica officinalis</i> Gaertn	1part
4	Yastimadhu	<i>Glycyrriza glabra</i> Linn	1part
5	Gokshura	<i>Tribulus terrestris</i> Linn	1part
6	Guduchi	<i>Tinospora cordifolia</i> Meirs	1part
7	Haridra	<i>Curcuma longa</i> Linn	1part
8	Daruharidra	<i>Berberis aristata</i> DC	1part
9	Shunthi	<i>Zingiber officinale</i> Rosc	1part
10	Punarnava	<i>Boerhavia diffusa</i> Linn	1part
11	Tulasi	<i>Ocimum sanctum</i> Linn	1part

Table 2: Organoleptic characters of Triphaladi compound

Sr. No	Various parameters	Results	
		Triphaladi compound capsule with seven Bhavana	Triphaladi compound powder without Bhavana
1	Colour	Dark greenish	Golden yellow
2	Odour	Aromatic	Aromatic
3	Taste	Kashaya, Madhura, Tikta	Kashaya, Tikta
4	Touch	Fine	Smooth
5	Texture	Soft	Soft

Table 3: Results of physicochemical analysis of Triphaladi compound

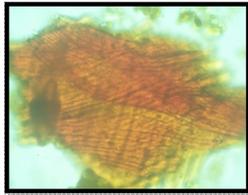
Sr. No	Parameters	Results	
		Triphaladi compound capsule with seven Bhavana	Triphaladi compound powder without Bhavana
1	pH	5.5	4.73
2	Weight variation	Maximum 572mg Minimum 536mg	-
3	Loss on drying	7.29%	3.99%
4	Water soluble extract	29.55%	26.19%
5	Alcohol soluble extract	12.79%	26.79%
6	Ash value	10.05%	8.5%

Table 4: Results of phytochemical analysis of Triphaladi compound

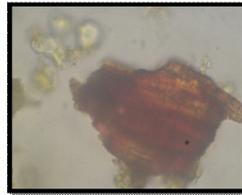
Sr No	Components	Results	
		Triphaladi compound capsule with seven Bhavana	Triphaladi compound powder without Bhavana
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannin and Phenolic compounds	+	+
4	Saponin glycosides	+	+
5	Anthraquinone glycosides	+	+

Table 5: Results of HPTLC study of Triphaladi compound

No of spots	Triphaladi compound capsule with seven Bhavana		Triphaladi compound powder without Bhavana	
	254nm	366nm	254nm	366nm
1	0.01	0.01	0.35	0.23
2	0.10	0.13	0.73	
3	0.35	0.30		
4	0.64	0.39		
5		0.61		
6		0.67		



Stratified fibres of Gokshura



Scleroids of Bibheetaki



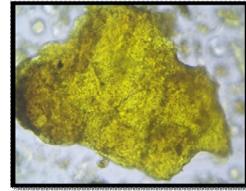
Pitted stone cells of Haritaki



Disturbed pitted vessels of Yastimadhu



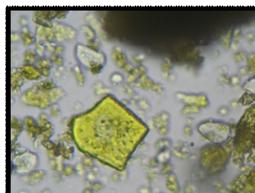
Collenchyma cells of Guduchi



Collapsed epicarp cells of Haritaki



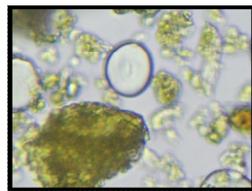
Disturbed walls of fibres of Yastimadhu



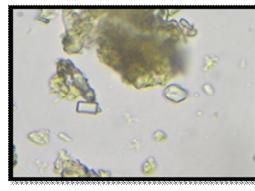
oil-resin content of Shunthi



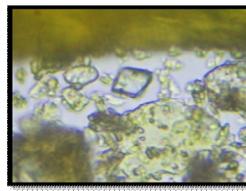
Fibres of Yastimadhu



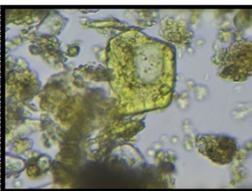
Starch grains of Yastimadhu



Microcrystal of Punarnava



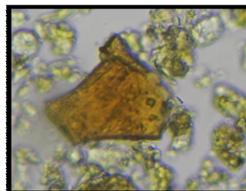
Prismatic crystal of Daruharidra and Oil globule of Tulsi



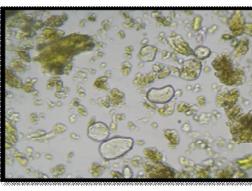
Disturbed collenchyma cells of guduchi



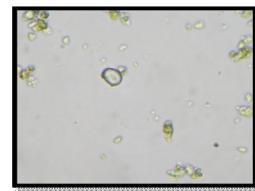
Pitted stone cells of Daruharidra with white lumen



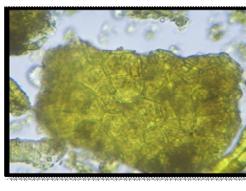
Tannin content of Haritaki



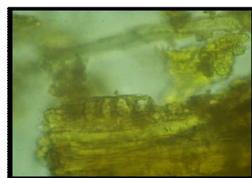
Simple starch grains of Shunthi



Starch grains of Yastimadhu



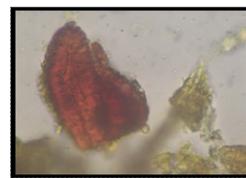
Disturbed cork cells of Guduchi



Crystal fibres of Yastimadhu



Scleroids of Yastimadhu



Stone cells of Bheebitaki

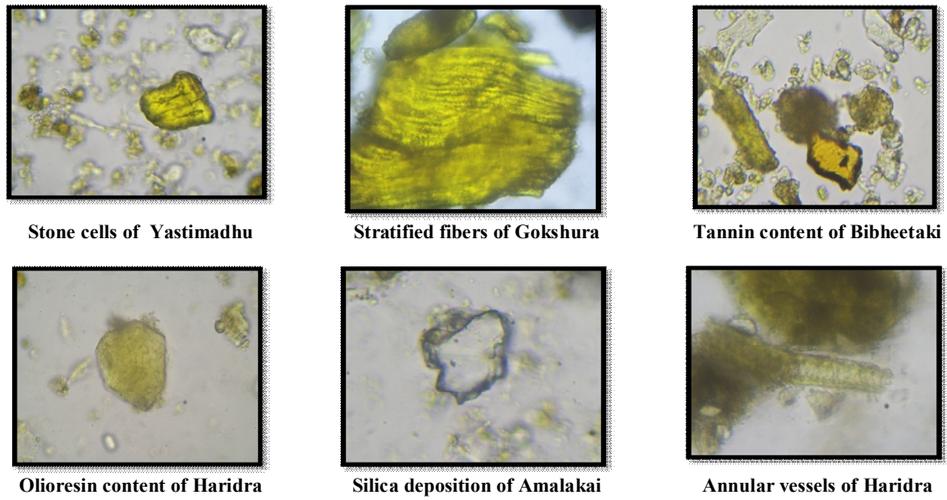


Plate 1: Microphotographs of Triphaladi compound with seven Bhavana



Plate 2: HPTLC plate of methanolic extract of Triphaladi compound

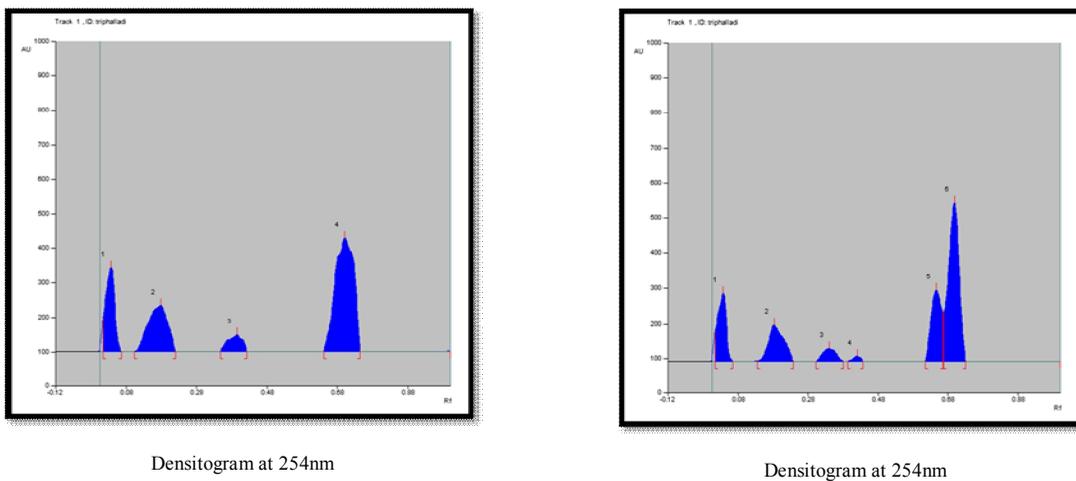


Plate 3: Densitograms of methanolic extract of Triphaladi compound

CONCLUSION

Analysis of Triphaladi compound with seven Bhavana by different parameters and HPTLC densitograms showed good co-relation between them. The study of microscopic characters of present formulation recorded the presence of diagnostic identifying characters of ingredients which are used. It is also evident from the pharmacognostical evaluation that the cellular constituents changes found are due to the Bhavana Samskara which probably increases the bioavailability and potency of the drug in various disease conditions. However, this fact needs to be validated through clinical study and with the help of higher pharmaceutical analytical techniques.

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