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DETAILED COMPARATIVE PHARMACOGNOSTICAL AND PHYSICO-CHEMICAL EVALUATION OF *TRIPHALADI YOGA* W.S.R IMPORTANCE OF *BHAVANA* (TRITURATION)

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ABSTRACT

Triphalaadi yoga is a combination of eleven dried herbs i.e. *Haritaki*, *Bibhitaki*, *Amalaki*, *Yastimadhu*, *Gokshura*, *Guduchi*, *Haridra*, *Daruharidra*, *Shunthi* and *Punarnava* in powder form which is administered as a *rasayana* therapy in patients to delay senile changes. These eleven drugs chosen for this study is an *Anubhoota yoga* from the *Shalakya Tantra* Department of I.P.G.T& R.A, Jamnagar and has the solid backing of previous researches for their most important individual activities. *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 yoga* is a combination of above 11 dried herbs which was triturated seven times. Present study was to screen the differences in pharmacognostical and pharmaceutical profile of *Triphaladi yoga* prepared with seven *Bhavana*. The powder of *Triphaladi yoga* was triturated with *Kwatha* (decoction) of the same compound seven times and made into dried powder and subjected to pharmacognostical and pharmaceutical evaluation. After *Bhavana* significant changes were found in the form of Broken scleride of *Bibhitaki*, Broken stone cell of *Haritaki*, Fragment of trichome of *Tulsi*, Broken cork cells of *Guduchi*, Ruptured cell wall of Cholenchyma of *Gudhuchi*. Analytical study changes like increase in water soluble extractive value, Increase in pH, Decrease ash value, increased number of HPTLC spots etc were noted.

KEYWORDS: *Bhavana*, *Triphaladi yoga*, Pharmacognosy, Trituration.

INTRODUCTION

Conversion of undesired properties of a raw material into desirable properties is an important skill of a physician. *Samskara* is a process in which the nature of raw drug is converted according to the requirement of the formulation. *Bhavana* is one among such *Samskara* which is defined in different literatures of *Rasashastra*. In general, the raw material will be augmented with specific liquids for specific duration with specific method. It leads to particle size reduction of molecules subjected for the *Bhavana* by repeated movement and pressure of pestle. *Bhavana* with organic juices improves the bioavailability of the drugs thereby enhances their rate of absorption.[1]

Triphaladi yoga is a combination of eleven dried herbs i.e. *Haritaki*, *Bibhitaki*, *Amalaki*, *Yastimadhu*, *Gokshura*, *Guduchi*, *Haridra*, *Daruharidra*, *Shunthi* and *Punarnava*

in powder form which is administered as a *rasayana* therapy in patients to delay senile changes. These eleven drugs chosen for this study is an *anubhoota yoga* from the *Shalakya Tantra* Department of I.P.G.T& R.A, Jamnagar and has the solid backing of previous researches for their most important individual activities viz. Adaptogenic properties of *Guduchi*, *Haritaki*, *Amalaki*[2], anti cataract activity of *Haritaki*, *Amalaki*, *Bibhitaki* (*Triphala*)[3]; anti advanced glycated end products activity of *Shunthi*[4]; antioxidants activity of *Haridra*, *Amalaki*, *Yashtimadhu*, *Tulsi*, *Bibhitaki*, *Guduchi*, *Sunthi*[5]; adaptogenic, immunomodulatory and anti inflammatory properties of Punarnava.[6];

immunomodulatory and anti inflammatory properties of Gokshura[7]; adaptogenic, anti inflammatory, anti cataract effect of *Haridra*[8] and anti inflammatory activity of *Daruharidra*.[9] The powder of *Triphaladi yoga* was triturated with decoction of the same compound seven times and made into dried powder.

Importance of *Bhavana*

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

On triturating the particle size of the powder become smaller.
It will also incorporate the property of liquid media.
It transforms coarse powder to finer state.
It leads to unique and suitable physiochemical changes.
It will also enhance the palatability of the product.

MATERIALS AND METHODS

Collection of the drug

Ingredients of Triphaladi compound viz. fruits of *Haritaki* (*Terminalia chebula* Retz), *Bibhitaki* (*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* Rosc), 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.

Preparation of *Triphaladi yoga* with seven *bhavana*

The prepared powder of *Triphaladi yoga* was triturated with decoction of the same compound seven times in end runner. In each *bhavana* sufficient amount of decoction made from *Triphaladi yoga* was added to the powder of *Triphaladi yoga* 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.

Pharmacognostical evaluation organoleptic evaluation

Various characters like colour, odour, taste and touch are recorded by using sensory organs.[10] Powder microscopy of the finished product was done without stain and after staining with Phloroglucinol+HCl. Micro photographs were taken under Carl Zeiss Trinocular microscope attached with camera.[11] 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 physicochemical 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, tannin and phenolic compounds, flavonoids, saponin and anthraquinone glycosides.[12]

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.[13]

RESULTS

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, Plate A.

Acicular crystal of *Punarnava*, cork cells in surface view of *Punarnava*, Fibre passing through medullary ray of *Punarnava*,. Annular vessel of *Sunthi*, Starch grain of *Sunthi*, Stone cell of *Daruharidra*, Boarder pitted vessels of *Bhibhitaki*, Epicarp cells of *Bhibhitaki*, Rosette crystal of *Bhibhitaki*, Tannin content of *Haritaki*, Epicarp cells of *Haritaki*, Simple trichome of *Haritaki*, Sillica depotion of *Amalaki*, Mesocarp cells of *Amalaki*, Border pitted vessels of *Guduchi*, Chollenchymatous cells of *Guduchi*, Cork cells of *Guduchi* in surface view, Idioblast of *Yastimadhu*, Pitted vessel of *Yastimadhu*, Rhomboidal crystal of *Yastimadhu*, Group of Scleride in *Yastimadhu*, A cut fragment of Pitted vessel in *Daruharidra*,Pitted stone cell of *Daruharidra*, A cut fragment of scleriform vessel in *Haridra*, Parenchymatous cell with yellow content in *Haridra*, .

Simple trichome of *Tulsi*, Spiral vessel of *Tulsi*, A Group of Stone cells in *Gokshura*, Lignified fibre of *Gokshura*, Striated ligniged cprk cells in surface view of *Gokshura*, Striatified fibre of *Gokshura*.Plate B (1-31)

Rosette crystal of *Bibhitaki*, Broken scleride of *Bibhitaki*, Trichome of *Bibhitaki*, Broken stone cell of *Haritaki*,

Silica depotion of *Amalaki,* Fragment of trichome of *Tulsi,* Broken cork cells of *Guduchi,* Ruptured cell wall of Cholenchyma of *Guduchi,* Cut fragment of piited vessel of *Guduchi,* Ruptured Starch grain of *Haridra,* Cut fragment of Scleriform vessel of *Haridra,* Cut fragment of fibre of *Yastimadhu,* Opened cut fragment of pitted vessel of *Yastimadhu,* Stone cell with disturbed wall of *Yastimadhu,* Stone cell covering a layer of *Daruharidra,* Pitted stone cells of *Daruharidra,* Scleride with ruptured and smotthen wall of *Daruharidra,* Fibre with opened Stratification of *Gokshura,* Rosette crystal of *Gokshura.*Plate C (1-19)

Physicochemical analysis

Results of physicochemical analysis like loss on drying, ash value, water soluble extract, alcohol soluble extract, ash value etc are shown in Table 3.

High performance thin layer chromatography (HPTLC)

The colour and Rf values of resolved spots of HPTLC were noted. (Table-4) (Plate no. D)

Table 1: Ingredients of *Triphaladi yoga*

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

Table 2: Organoleptic characters of *Triphaladi yoga*

Sr. No	Various parameters	Results	
		Triphaladi yoga powder with seven Bhavana	Triphaladi yoga without seven 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: Physico-chemical parameters

Sr No	Parameters	Results	
		Triphaladi yoga* powder with 7 *Bhavana	***Triphaladi yoga* powder without *Bhavana***
1	pH	6.0	5.5
2	Loss on drying	6.46 %	6.20 %
3	Ash value	5.24 %	5.35%
4	Water soluble extractive	28.5%	26.46%
5	Alcohol soluble extractive	22.13%	21.3 %

❖ **HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)**

Table 4: R_f values obtained by HPTLC

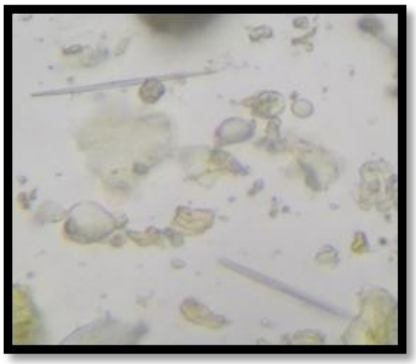
Sample	Visualize under short UV (254 nm)		Visualize under short UV (366 nm)	
	No. of spots	R_f value	No. of spots	R_f value
Triphaladi Yoga* 7 *bhavana	10	0.00, 0.07, 0.12,0.20, 0.26, 0.38, 0.44, 0.63, 0.76, 0.84	06	0.01,0.14, 0.28, 0.35, 0.38, 0.46
Triphaladi yoga* without *bhavana	09	0.01, 0.06, 0.21, 0.33, 0.39, 0.45,0.62, 0.66, 0.79	05	0.01, 0.11, 0.23, 0.31, 0.48

Plate A: *Triphaladi yoga* powder

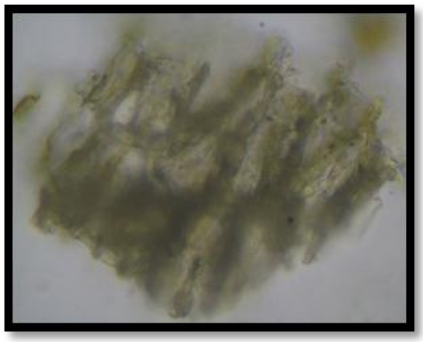
With seven *Bhavana*

Without *Bhavana*

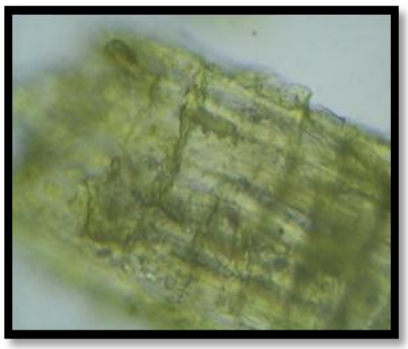
Plate A: Powder characters Before Bhavana.



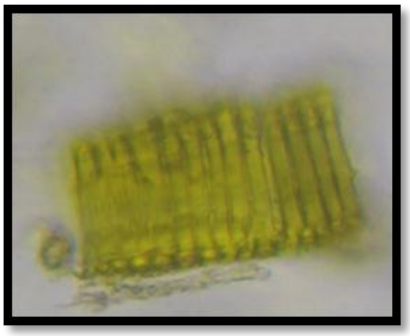
1.Acicular crystal of *Punarnava*



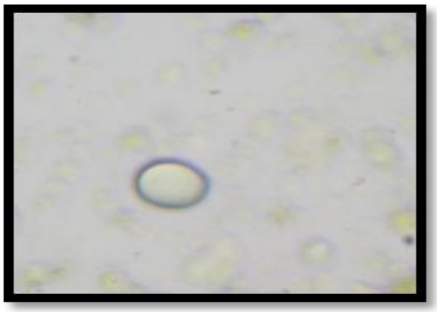
2. cork cells in surface view of *Punarnava*



3.Fibre passing through medullary ray of *Punarnava*

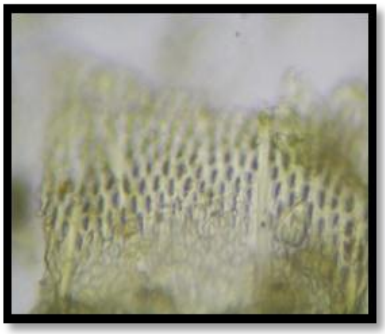


4. Annular vessel of *Sunthi*

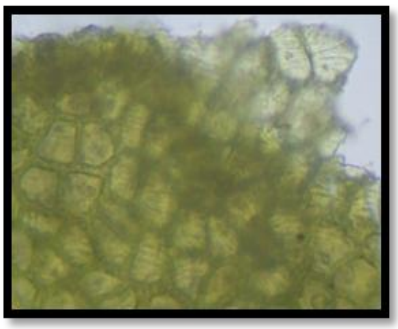


5. Starch grain of *Sunthi*

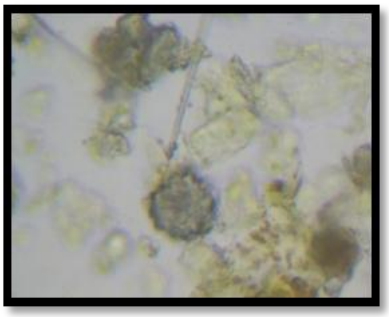
6. Stone cell of *Daruharidra*



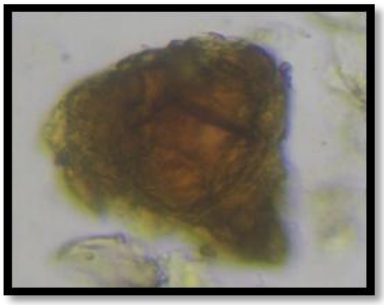
7. Boarder pitted vessels of *Bhibhitaki*



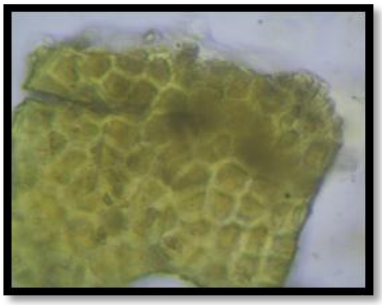
8. Epicarp cells of *Bhibhitaki*



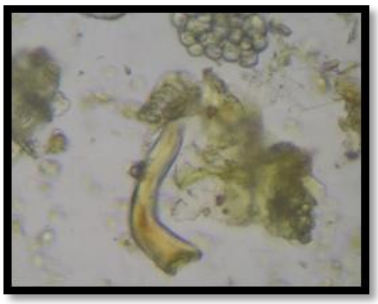
9. Rosette crystal of *Bhibhitaki*



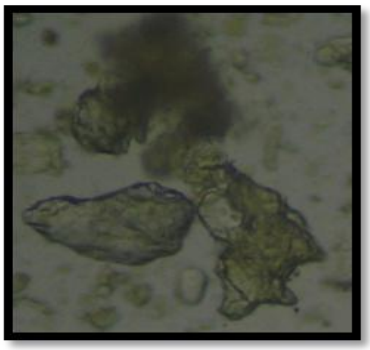
10. Tannin content of *Haritaki*



11. Epicarp cells of *Haritaki*

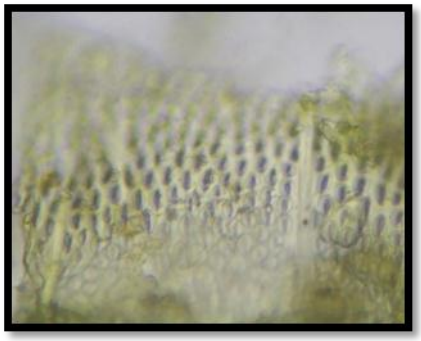


12. Simple trichome of *Haritaki*



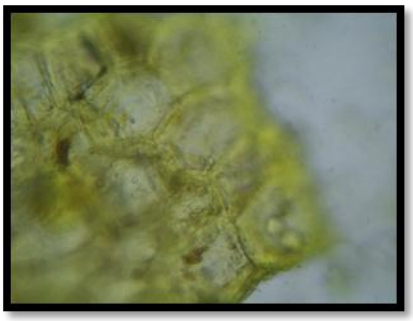
13. Sillica depotion of *Amlaki*

14. Mesocarp cells of *Amlaki*

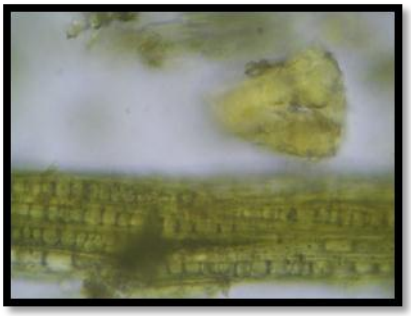


15. Border pitted vessels of *Guduchi*

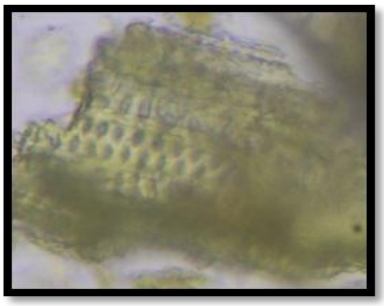
16. Chollenchymatous cells of *Guduchi*



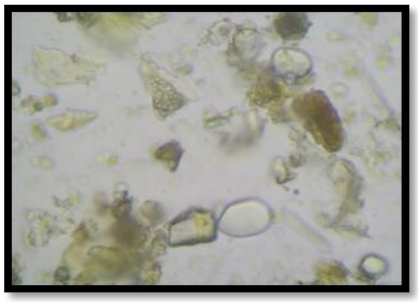
17. Cork cells of Guduchi in surface view



18. Idioblast of *Yastimadhu.*

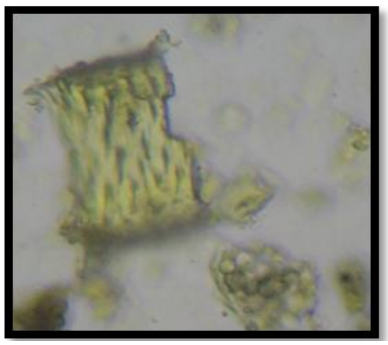


19. Pitted vessel of *Yastimadhu*



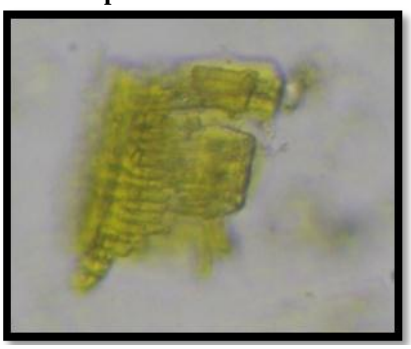
20. Rhomboidal crystal of *Yastimadhu*

21. Group of Scleride in *Yastimadhu*

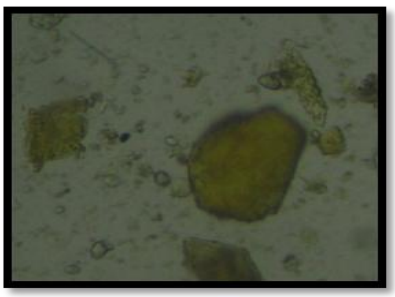


22. A cut fragment of Pitted vessel in *Daruharidra*

23. Pitted stone cell of *Daruharidra*



24. A cut fragment of scleriform vessel in *Haridra*

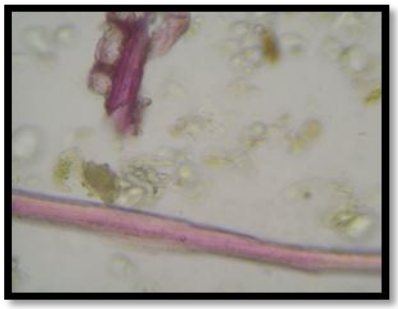


25. Parenchymatous cell with yellow content in Haridra

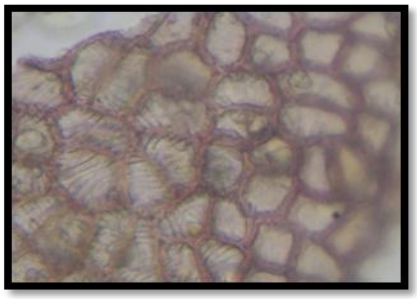
26. Simple trichome of *Tulsi*

27. Spiral vessel of *Tulsi*

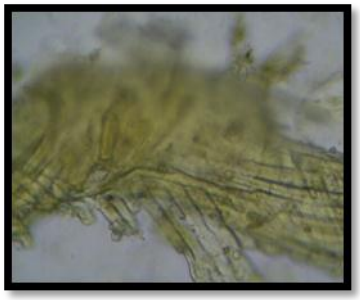
28. A Group of Stone cells in *Gokshura*



29. Lignified fibre of *Gokshura*

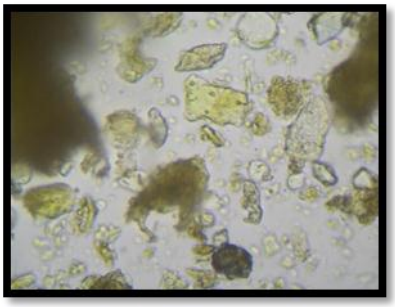


30. Striated ligniged cork cells in surface view of *Gokshura*



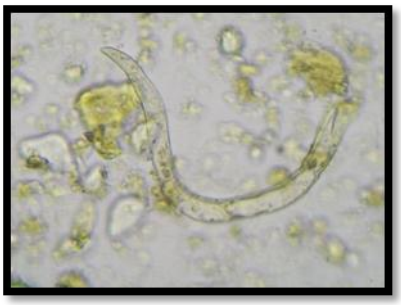
31. Striatified fibre of *Gokshura*

Plate B: Powder characters After Bhavana

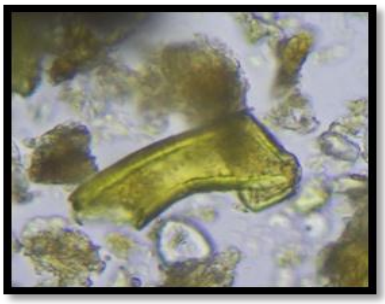


1. Rosette crystal if *Bibhitaki*

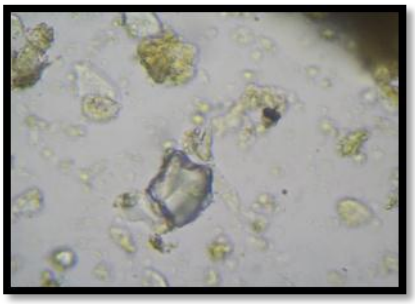
2. Broken scleride of *Bibhitaki*



3. Trichome of *Bibhitaki*

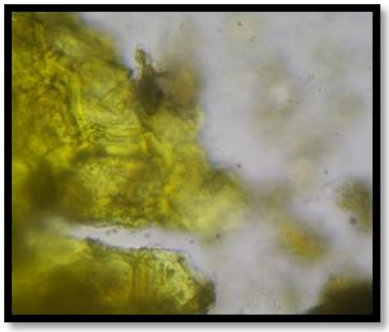


4. Broken stone cell of *Haritaki*

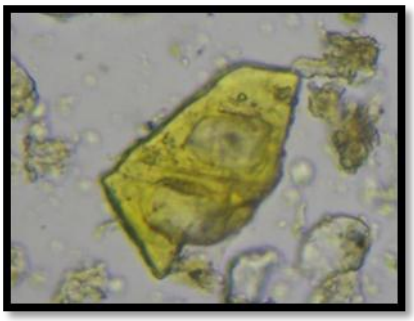


5. Silica depotion of *Amalki*

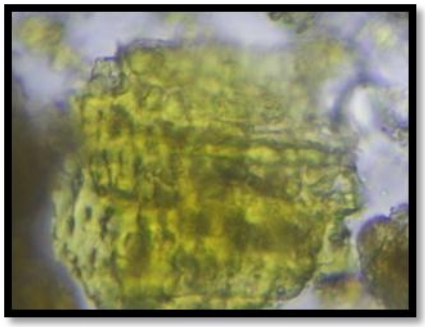
6. Fragment of trichome of *Tulsi*



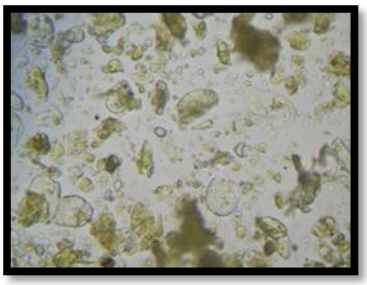
7. Broken cork cells of *Guduchi*



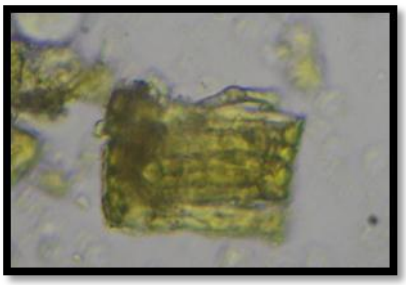
8. Ruptured cell wall of Cholenchyma of *Gudhuchi*



9. Cut fragment of piited vessel of *Guduchi*

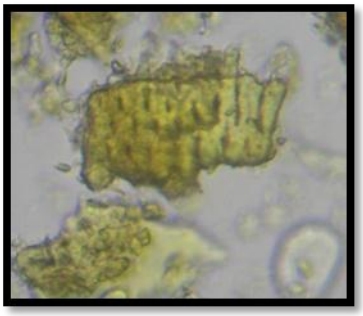


10. Ruptured Starch grain of *Haridra*

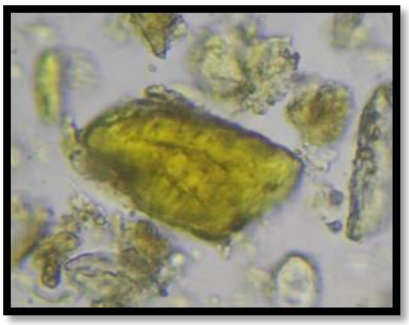


11. A cut fragment of Scleriform vessel of *Haridra*

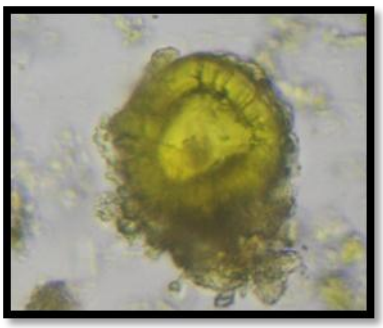
12. A cut fragment of fibre of *Yastimadhu*



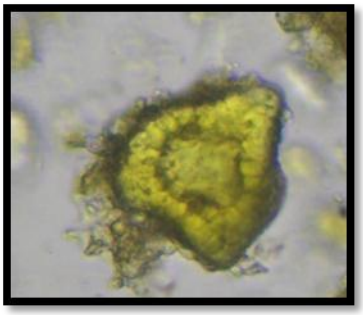
13. A opened cut fragment of pitted vessel of *Yastimadhu*



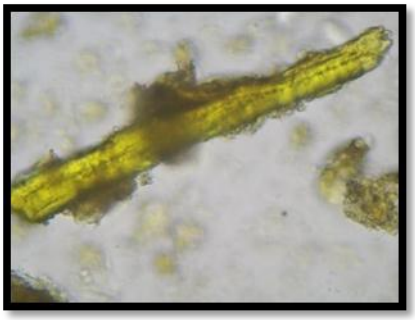
14. Stone cell with disturbed wall of *Yastimadhu*



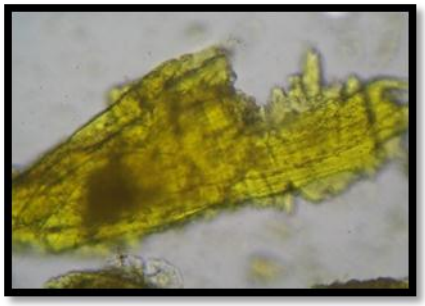
15. Stone cell covering a layer of *Daruharidra*



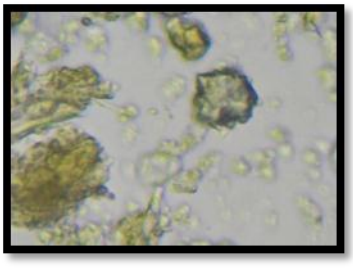
16. Pitted stone cells of *Daruharidra*



17. Scleride with ruptured and smotthen wall of *Daruharidra*

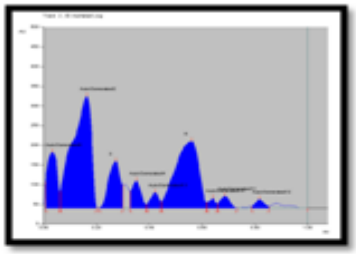


18. Fibre with opened Stratification of *Gokshura*

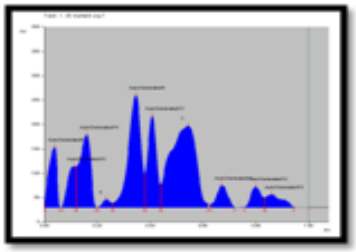


19. Rosette crystal of *Gokshura*

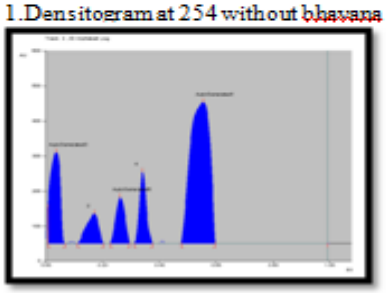
Plate C: HPTLC plate of methanolic extract of Triphaladi yoga compound



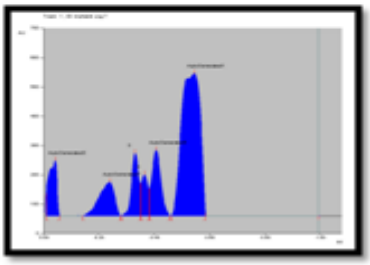
1.Densitogram at 254 without bhavana



2.Densitogram at 254 with seven bhavana



3.Densitogram at 366 without bhavana



4.Densitogram at 366 with seven bhavana

DISCUSSION

There was a slight color and taste variation between *Triphaladi yoga* without *Bhavana* and with *Bhavana*. The colour of *Triphaladi yoga* without *Bhavana* was golden yellow while the same of *Triphaladi yoga* with seven *bhavana* 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 yoga* 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 yoga* with seven *bhavana* in comparison to *Triphaladi yoga* without *bhavana*. The alteration in *rasa* is might be due to the effect of 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[xv]. Touch and texture of the *Triphaladi yoga* with seven *bhavana* are very fine and soft compared to *Triphaladi yoga* without *bhavana* might be due to breakdown of the hard cellular structures and the exposed cellular contents by prolonged trituration of the drugs. Broken cork cells of *Guduchi*, Ruptured cell wall of Cholenchyma of *Gudhuchi*, Cut fragment of piited vessel of *Guduchi*, Ruptured Starch grain of *Haridra*, Cut fragment of Scleriform vessel of *Haridra*, Cut fragment of fibre of *Yastimadhu*, Opened cut fragment of pitted vessel of *Yastimadhu*, Stone cell with disturbed wall of *Yastimadhu*, Stone cell covering a layer of *Daruharidra*, Scleride with ruptured and smoothen wall of *Daruharidra*, Fibre with opened Stratification of *Gokshura* 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.

A considerable difference was found in the values of some of the physicochemical parameters of *Triphaladi yoga* with seven *bhavana*. Both the water and the methanol soluble extractive increased in the *Triphaladi yoga* with seven *bhavana* sample. There was not much variation in the pH, which ranged from 5.5 t0 6.0. This reveals that a more concentrated form or more water and methanol soluble content may not affect the pH of the samples. Results of physicochemical analysis like loss on drying at 110^0 c is the major factor for the stability of the drugs. The results of loss on drying of *Triphaladi yoga* with seven *bhavana* showed lower limits than prescribed in API but Loss on drying in *Triphaladi yoga* with seven *Bhavana* was more than that of the *Triphaladi yoga* without *Bhavana*, indicating the presence of more moisture in *Triphaladi yoga* with seven *Bhavana*. The ash value indicates the presence of inorganic contents in the sample. There was not much variation in the ash value between two samples.

In HPTLC profile of the methanolic extract of the drug 10 spots at Rf 0.00, 0.07, 0.12,0.20, 0.26, 0.38, 0.44, 0.63, 0.76, 0.84 were observed in 256nm UV light spectrum while 6 spots at Rf 0.01,0.14, 0.28, 0.35, 0.38, 0.46 were observed in 366nm UV light spectrum was observed. 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. In the

previous studies by Radhika K Verma. et. al.[14] and Kundan patel et. al.[15] the colour and taste of *Triphaladi yoga* are same but other parameters are showing some differences which may be due to the seasonal variation during collection period of raw material and drug preparation.

CONCLUSION

The result of the present study concluded that in comparison of both the sample there is a marked difference in pharmacognostical and Physicochemical study of both the samples. Thus, *Bhavana* (trituration) is an important process during drug preparation, affecting the physicochemical and biological properties of a dosage form and increase the bioavailability of drug. The seven *bhavana* of *Triphaladi yoga* profile can also be used for standardization and in future references.

REFERENCES

1. Madhulika Priya, Critical review on importance of bhavana in rasoushadh , IAMJ., July - August-2014; 2(4).
2. Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six Rasayana herbs used in Ayurvedic medicine. Phytother Res., 1999; 13(4): 275-91.
3. http://dx.doi.org/10.1002/(SICI)1099-1573(199906)13:4<275::AID-PTR510>3.3.CO;2-J.
4. Suresh Kumar Gupta, V. Kalaiselvan, Sushma Srivastava, Shyam S. Agrawal, Rohit Saxena. Evaluation of anticataract potential of Triphala in selenite induced cataract: In vitro and in vivo studies. J Ayurveda Integr Med., 2010; 1(4): 280-286. http://dx.doi.org/10.4103/0975-9476.74425.
5. Megha Saraswat, Palla Suryanarayana and Geereddy Bhanuprakash Reddy. Antiglycating potential of Zingiber officinalis and delay of diabetic cataract in rats. Mol Vis., 2010; 16: 1525-1537.
6. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in Human Health: Current status and future prospects. J Assoc Physicians India., 2004; 52: 794-803.
7. Agrawal B, Das S, Pandey A. *Boerhaavia diffusa* Linn.: A review on its phytochemical and pharmacological profile. Asian J Appl Sci., 2011; 4: 663-84. http://dx.doi.org/10.3923/ajaps.2011.663.684.
8. Saurabh Chhatre, Tanuja Nesari, Gauresh Somani, Divya Kanchan, Sadhana Sathaye. Phytopharmacological overview of Tribulus terrestris. Pharmacogn Rev., 2014; 8(15): 45-51. http://dx.doi.org/10.4103/0973-7847.125530.
9. Seher Cimen Ozgen, Dikmen Dokmeci, Meryem Akpolat, Cetin Hakan Karadag1, Ozgur Gunduz, Hakan Erbaş et al. The Protective Effect of Curcumin on Ionizing Radiation induced Cataractogenesis in Rats. Balkan Med J., 2012; 29: 358-63.
10. Bharat B. Aggarwal, Sahdeo Prasad, Simone Reuter. Identification of Novel Antiinflammatory Agents from Ayurvedic Medicine for Prevention of Chronic Diseases: "Reverse Pharmacology" and "Bedside to Bench" Approach. Curr Drug Targets., 2011; 12(11): 1595-1653. http://dx.doi.org/10.2174/138945011798109464.
11. Wallis TE. Text book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers & Distributors., 2002; 123-32: 210-5.
12. Trease GE, Evans WC. Pharmacognosy. 12th ed. Eastbourne, U.K: Bailliere Tindall., 1983; 95-99: 512-47.
13. Anonymous. The Ayurvedic Pharmacopoeia of India. part 2, vol 2, Appendices 1st ed. New Delhi: Govt. of India Publication., 2008; 233-5.
14. Patel Kundan R., R Manjusha, C.R. Harisha, Shukla V.J., Pharmacognostical and pharmaceutical evaluation of polyherbal formulation triphaladi capsule with seven bhavana. Int. J. Res. Ayurveda Pharm., 2015; 6(4): 413-419.
15. Radhika K Varma, Manjusha R, Harisha CR, Shukla VJ, Pharmacognostical and physicochemical analysis of triphaladi yoga: an ayurvedic poly-herbal formulation, JPSI., Nov - Dec 2012; 1(6): 9-12.