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Research Article

PREPARATION AND PHYSICOCHEMICAL ANALYSIS OF *NISHA AMALAKI* - AN AYURVEDIC FORMULATION

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ABSTRACT

Samskara is one of the important processes in preparation of Ayurvedic formulations. It enhances the medicinal property of the formulation and attains maximum potency to cure the diseases. Ayurvedic texts describe many formulations for different ailments. *Nisha amalaki* is reputed for treating diabetes and its complication conditions. This formulation has been considered complementary medicine or alternative to conventional medicines across the globe.

This formulation consisting of two potent herbs namely *Haridra* and *Amalaki* mentioned in *Caraka Chikitsa*. *Amalaki* (*Emblica officinalis*), belongs to family Euphorbiacae is one of the ingredient in *Nishamalaki churna*, This drug is used for the purpose of rejuvenation, aphrodisciac, cures tiredness, bleeding disorder, Diabetes mellitus, eye disorder etc. The other and main ingredient of *Nishamalaki* is *Haridra* (*Curcuma longa*), belong to family Zingebearaceae which is recommended for treating the skin disorder, diabetes mellitus etc. The concept of *Samaskara* has been explained in Caraka samhita vimana sthana for the transmigration of *Gunas* better therapeutic effect of the drugs. The present work deals with preparation of *Nisha Amalaki* by doing *Bhavana samskara* as mentioned in ancient texts including raw drug procurement and preparation. Hence this polyherbal formulation needs science-based approach toward manufacturing process and chemical standardization.

KEYWORDS: *Nisha Amalaki*, Phytochemical analysis, TLC.

INTRODUCTION

India has the rich heritage of traditional medicine and Ayurveda is one of the well developed ancient system of medicine in which many hidden truths are explained. This system of plant based medicines is gaining recognition throughout the world and many herbal drugs are now clinically tested and accepted for manufacturing.[1] According to WHO, about 25per cent of prescribed human medicines are derived from plants and 80 per cent people still depend on traditional system of medicines. The herbal wealth of India and the knowledge of their medicinal properties have a long tradition, as referred in *Rigveda* and other ancient literature. [2]

In spite of the large number of herbal formulations available in the market, standards for their quality are yet to be laid for many of them (Eapen Saumy M.S, et al 2002; Patel K.N, 1996). Standardization of herbal medicines is the process to set a standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. A herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product. Moreover, many dangerous and lethal side effects have recently been reported, including direct toxic effects, allergic reactions, effects from contaminants, and interactions with herbal drugs. It is therefore became need to analyse the herbal drugs to develop effective formulation with the help of fast, sensitive and accurate quality control tests, which will be in alignment with modern technology.[3]

Nisha Amalaki is a formulation consisting of mainly two potent herbs namely, *Nisha* commonly said as *Haridra* (*Curcuma longa* Linn.) and *Amalaki* (*Emblica officinalis* Gaertn). *Haridra* is a spice derived from the rhizomes of *Curcuma longa*, which is a member of the ginger family (Zingiberaceae). The action of *Haridra* in classics is mentioned as *Kaphavata shamaka, Shothahara, Vedanasthapaka, Vishaghna, Krumighna, Raktaprasadana, Pramehaghna, Kushthaghna, Jwaraghna* etc.[4]

Amalaki commonly known as Indian gooseberry or *Amla*, is perhaps the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Several parts of the plant are used to treat a variety of diseases, but the most important is the fruit. Many ailments are treated by the fruit which is used either alone or in combination with other plants. These includes the action like *Tridoshahara, Pramehaghna, Raktapittahara, Rasayana, Vrushya, Garbhasthapana, Medhya, Keshya, Stambhana, Deepana, Rochana, Anulomana, Hrudya, Mutrala, Chakshushya, Daha prashamana* etc.[5]

Hence based on the above rationale the present study is undertaken with an aim to prepare and analyse the formulation *Nisha Amalaki* which is mentioned by many *Acharyas* mainly in the context of *Prameha chikitsa*[6] and other *Mutravaha srotodushti vikaras.*[7] For convenience purpose this *Nisha Amalaki* were made into *Vati* (tablet) form. Further this *Nisha Amalaki vati's* were analysed for its physico chemical properties and TLC for standardization and establishment of chemical profile and quality herbal formulation.

MATERIALS & METHODS

Raw materials

Cultivated *Haridra* rhizome was collected from Munyal (Gokak) and *Amalaki* fruits were collected from KLE's Narashingpur farm. The *Amalaki* fruit and *Haridra* rhizomes were authenticated at AYUSH approved Central Research facility, at Shri B M K Ayurveda Mahavidayalaya, Shahapur, Belgaum and voucher number (CRF/12/708-709) of the drugs given in Central Research Facility.

Processing of Raw drug

Raw *Haridra* rhizomes were boiled in water at a moderate temperature. Later it was washed and dried under sunlight for 7days and collected in a clean jar. *Haridra* was made into fine powder (in pulveriser) by sieving through 120 no. sieve in KLEU's Ayurveda pharmacy. It was packed in air tight container and sealed by continuous seal packer. Raw *Amalaki* fruits were cleaned manually for their physical impurities like mud, dust, sticks, fibers and ripened *Amalaki* fruits were separated from the remaining fruits and un-ripened and infested are discarded. Then those fruits were stored in clean place.

Figure 1 – Raw *Haridra* Figure 2 - Pulvarized *Haridra Churna* Figure 3 – *Amalaki* ripe fruits

Figure 4 - *Amalaki* fruits **Figure 5 & 6 – Preparation of *Amalaki swarasa***

Method of Preparation of *Nisha Amalaki Vati*

Quantity

Sr. No.	Drug	Latin Name	Family	Part	Quantity
1.	*Haridra*	*Curcuma longa* Linn.	Zingiberaceae	Rhizome	1 part
2.	*Amalaki*	*Emblica officinale* Gaertn.	Euphorbiaceae	Fruit pulp juice	Quantity sufficient

Preparation

Amalaki fruits are made into small pieces and the seeds were removed. *Amalaki* fruits pulp were pounded in *Khalva yantra* (without adding water) till it becomes soft paste (*Kalka*). This paste is squeezed in clean cloth and *Swarasa* was obtained. *Haridra churna* was taken into *Khalva yantra* and *Amalaki swarasa* was added to *Haridra churna* till it soaks completely. *Bhavana* was carried out till *Haridra* powder dries. Same procedure was followed for 3days. *Vati* of 1 gm each were prepared and made to dry for 7 days. These *Vati* were stored in clean glass air tight container.

Figure 7-10: Preparation of *Nisha Amalaki Vati* by *Bhavana* process

Results of Analysis of Prepared Drug

Table 1: Represents the Organoleptic characters of *Nisha Amalaki*

Sl. No.	Parameters	*Nisha amalaki*
1	Colour	Yellowish green
2	Odour	Aromatic
3	Taste	Sour followed by astringent
4	Consistency	Hard

Microbial Limit Test of *Nisha Amalki*

Table 2: Illustrates results of Microbial Results

Sl no	Microbial limit test	Result
1	S. Aureus	Absent
2	P. Aeroginose	Absent
3	E. Coli	Absent
4	S. Abony	Absent

Microbial Load Test

Table 3: Illustrates results of Microbial load test:

Description macroscopic	Limits (As per IP)	Results
Total bacterial count	30-300cfu/ml	106cfu/ml
Total fungal count	10-100cfu/ml	48cfu/ml

Physicochemical Properties of *Nisha Amalaki*

Table 4: Illustrates the results of physicochemical analysis of *Nisha Amalaki*

Sr. No.	Parameters	*Nisha Amalaki*
1	pH at 5% aqueous solution	3.37
2	Loss on Drying at 110^{0}C (% w/w)	3.4%
3	Total Ash (% w/w)	5.33%
4	Acid Insoluble Ash (% w/w)	1.901%
5	Water Soluble Extractive (%w/w)	41.30%
6	Alcohol Soluble Extractive (%w/w)	15.5%
7	Powder microscopic	15-20 micro
8	Hardness test	6.9kg/m^2
9	Disintegration time	39mins

Qualitative Parameters of *Nisha Amalaki*

Test for Inorganic Components

Prepared ash of the drug material was added with 50% of v/v HCl. The filtrate was then subjected to analyze the inorganic elements. The results are tabulated in Table no.5

Table 5: Illustrates the inorganic components present in *Nisha Amalaki*

Sr. No.	Parameters	*Nisha Amalaki*
1	Carbonate	-
2	Calcium	-
3	Magnesium	-
4	Potassium	-
5	Iron	+
6	Sulphate	-
7	Chloride	-
8	Nitrate	-
9	Sodium	-

'+' – Presence/ positive test, '-' – Absence/negative test

Preliminary Phyto chemical Screening

Aqueous and Alcoholic extracts of *Nisha Amalaki* were prepared with cold maceration technique. They were further subjected for qualitative phytochemical screening. The results are mentioned below in Table no: 6

Table 6: Illustrates the results of phytochemicals in *Nisha Amalaki* aq. and alc.ext

Sl.No	Parameters	Reagent / test	*Nisha Amalaki*	
			Aqueous	Alcoholic
1.	Carbohydrates	Molish	-	-
2.	Reducing Sugar	Benedicts	-	-
3.	Monosaccharides	Barfords	-	-
4.	Pentose	Bails	-	-
5.	Hexose	Selwinoffs	-	-
6.	Non-reducing sugar	Benedicts	+	+

7.	Polysaccharide	Iodine test	-	-
8.	Proteins	Millons test	-	-
9.	Amino Acids	Ninhydrin test	-	-
10.	Steroids		-	-
11.	Glycosides	Cardiac Glycosides	-	-
		Coumarin	-	-
		Anthraquinie	-	-
12.	Saponins		-	-
13.	Flavonoids		+	-
14.	Alkaloids	Dragandroff's	+	+
15.	Tannins & phenolic		+	-
16.	Test for vitamins Vitamin A Vitamin B Vitamin C		 - - +	 - - +
17.	Test for organic acid Oxalic acid Citric acid Tartaric acid		 - + -	 - + -

'+' – Presence/ positive test, '-' – Absence/negative test result

Fluorescence Analysis of *Nisha Amalaki*

The powder of the *Nisha Amalaki* was made and subjected to various reagents. It was then observed under normal light, 254nm and 366nm.The results are mentioned below in table no:7

Table 7: Illustrates the results of Fluorescence analysis of *Nisha Amalaki*

Sr. No.	Materials	*Nisha Amalaki*		
		DL	UV 254nm	UV 366nm
1	Powder As such	B	G	G
2	P + 1N. NaOH	B	BR	BR
3	P + Picric Acid	B	G	Y
4	P + Acetic Acid	BR	G	Y
5	P + 1N. HCL	BR	B	BR
6	P + 1N. HNO_3	B	G	Y
7	P + Iodine 5%	B	BR	B
8	P + 5% $FeCl_3$	B	B	B
9	P + 50% HNO_3	B	G	Y
10	P + Methanol	B	G	BR
11	P + Methanol + NaOH	B	G	BR

B: black **BR :** brown **G:** green **Y:** yellow

Thin Layer Chromatography (TLC)

Thin layer chromatography was performed for the normal phase separation of components of alcoholic extract of *Nisha Amalaki*.

Many trial and error methods are performed to fix the solvent system for separation of maximum number of chemical compounds present in the drug. Finally Ethyl Acetate : Toluene : Acetone (4.5:4.5:1) is taken as a solvent for *Nisha Amalaki* extract. The spots obtained from *Nisha Amalaki* extract were examined under ultra violet light.

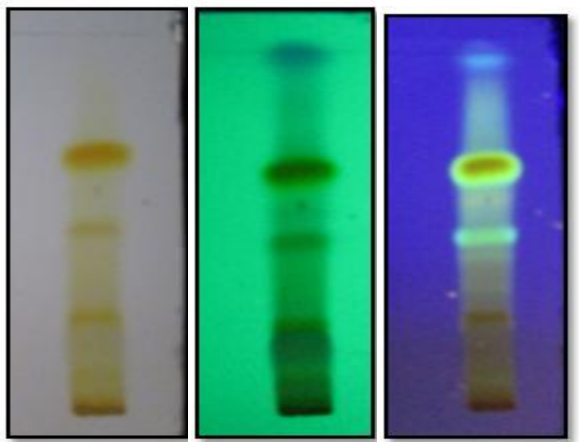

Figure 11 : Showing TLC of *Nisha Amalaki* with different spots in Visible light, Short wavelength (UV 254 nm) and Long wavelength(UV 366 nm) respectively.

The resolution factor was calculated by using the formula Rf = distance travelled by solute/ distance travelled by solvent. The results are reported in the Table no.8.

Table 8: Illustrates Rf values of phytochemicals separated during TLC from alcoholic extract of *Nisha Amalaki*

Spots	Visible light	Spots at UV 254 nm	Spots at UV 366 nm
1st	0.025	0.037	0.025
2nd	0.0875	0.1	0.075
3rd	0.2375	0.1875	0.2
4th	0.475	0.2375	0.225
5th	0.65	0.2875	0.2875
6th		0.4625	0.345
7th		0.55	0.4625
8th		0.63	0.6375
9th		0.75	0.725
10th		0.825	0.95
11th		0.9375	
12th		0.975	

DISCUSSION

Haridra and *Amalaki* are the two raw drugs used in the preparation of *Nisha Amalaki vati*. Curcumin, the principal curcuminoid found in *Haridra*, is generally considered its most active constituent. It has been proved for Antioxidant Effects, Hepatoprotective Effects, Antimicrobial Effects, Cardiovascular Effects, immunity enhancing property, More recently, evidence that curcumin may have anti-inflammatory and anticancer activities has renewed scientific interest in its potential to prevent and treat the disease. [8] And the action of *Amalaki* include antipyretic, analgesic, anti-inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive. *E. officinalis* possesses, antitussive, anti atherogenic, adaptogenic, cardioprotective, gastro protective, anti anemic, antihypercholesterolemic, wound healing, anti diarrheal, anti atherosclerotic, hepato protective, nephroprotective, and neuro protective properties as demonstrated in numerous preclinical studies. Along with this, *E. officinalis* also reported to possess anti carcinogenic property, radio modulatory, chemo modulatory, chemo preventive, free radical scavenging, antioxidant, anti-inflammatory, anti mutagenic and immune modulatory activities.[9]

After procurement and standardization of raw ingredients, drugs were pulverized and sieved through 120 sieve. The *Amalaki swarasa bhavana* was given for three times to *Nisha churna*. The quantity of *Swarasa* should be sufficient to soak the *Churna* completely. The powdering of the *Nisha* in pulverizer was essential because it is hard and when it is taken in large quantity, it becomes difficult to convert it into fine form and also it is time consuming. Again after pulverizing it was given three times *Bhavana* with *Amalaki swarasa*. However, there is no reference as how many times *Bhavana* is to be given, but the *Bhavana* reduces the particle size of the formulations and that intern helps for fast absorption and speedy action. Because of smaller particle size, absorption rate will be greater.

Nisha Amalaki was found to have 6.9 kg/m^2 hardness and 39 minutes disintegration time which was noticed within acceptable limit of *Vati*. Excess of water in the raw drug or final product responsible for microbial growth, fungi and detoriation following hydrolysis, therefore moisture content should be less. In *Nisha amalaki* moisture content is less that is 3.4% which helps for prevention of degradation of *Vati*.

Prepared *Nisha* A*malaki* was subjected for all the preliminary phyto-chemical studies, inorganic and organic tests. The results revealed that *Nisha Amalaki* is free from unwanted organic and inorganic compounds. The water soluble extractive of *Nisha* A*malaki* is 41.30% and alcohol soluble extractive is 15.5%, which shows that it is having good solubility in water. Organic tests revealed presence of non-reducing sugars, alkaloids and Vitamin C in both Alcoholic and Water extracts. *Amlaki* fresh juice is one of the richest natural sources of vitamin C which containing nearly twenty times as much vitamin C as orange juice. [10] These vitamin C or ascorbate may act as an antioxidant against oxidative stress in the body. In 2013, researchers discovered that Vitamin C alone can kill drug-resistant Mycobacterium tuberculosis by producing oxidative radicals that damage DNA[11] also it helps for absorption of iron in the body. [12]

Tannins, phenols and flavenoids were also present in water extracts. Study have shown that these tannins from *Amalaki* are responsible for delaying development of diabetic cataract in rats along with antioxidant property, Which means these formulation may also helpful in diabetic complications. Also presence of tannins, alkaloids, phenolic compounds, amino acids and carbohydrates in *Amalaki* might be responsible for potent anti-pyretic and analgesic activities of the formulation. [13]

Flavonoids in this formulation plays an important role and proved to be responsible for antiplatlet, [14] inflammatory activity, antioxidant, anti microbial and anti cancerous activity of *Nisha Amalaki*. [15]

Also this flavonoids present *Amalaki* reduce the levels of lipid in serum and tissues of rats induced hyperlipidemia by eliciting highly potent hypolipidaemic and hypoglycaemic activity. It was concluded that *Amalaki* may be effective for hypercholesterolemia and prevention of atherosclerosis due to presence of these phyto-constituents. In addition to this, flavonoids were also found to be effective in elevating the haemoglobin levels and also act as antitumor. [16]

Presence of tannins shows the astringent & bitter property of the formulation.[17] Inorganic test showed presence of Iron which is an important supplementary component in day today life which can prevent anaemia.

TLC of *Nisha Amalaki* shows 3 major spots in visible light, 5 major spots in UV 254 nm and 4 major spots in UV 366 nm which indicates possible constitute responsible for its therapeutic effect. When compared to standard spot of *Amalaki* & *Haridra* separately it revealed that spot at 0.34 and 0.63 shows presence of Gallic acid and Ascorbic acid respectively in the formulation which is major phytochemical present in *Amalaki.* [18] Another dark yellow colour spot at 0.75 shows presence of Curcuminoid, a chief component of *Haridra*[19] which is responsible for enhancing the cellular resistance to oxidative damage also for other activities like anti arthritis, anti carcinogenic, hepatoprotective activity[20] of this formulation.

Therefore the results of present analysis shows that *Nisha Amalaki vati* is beneficial for humans in various pathological conditions due to presence of various metabolites.

CONCLUSION

After analysis of *Nisha Amalaki vati* by different parameters such as loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractives and thin layer chromatography shows good co-relation between them. The results obtained would be used to lay down a set of new pharmacopeal standards for the preparation of *Nisha Amalaki vati* to obtain optimal efficacy of the medicine. So it can be concluded that these parameters helps for further evaluation of *Nisha Amalaki*. For the further purity and potency the formulation procedure could be performed under QA/QC laboratory of pharmaceutical house.

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