Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (1), 129-136

Synthesis of *Nisha-Amalaki Yoga* (*NAY*) Using Special *Ayurveda* Pharmaceutical Technique: *Bhavana* (Levigation/Wet Milling)

Research Article

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Abstract

Bhavana (Levigation) is a pharmaceutical process, which is preferably useful to nullify the unwanted effects of drugs and to potentiate the drug action by transforming the physical and chemical changes. In this process herbal powders are triturated along with herbal juice, decoction, or any organic liquid media till complete absorption of liquid with trituration process. This article is going to throw light on potentiation, synergistic action, increase in shelf life, and dose reduction of *Nisha-Amalaki Yoga*. In pharmaceutical process of *Nisha-Amalaki Yoga*,3 *Bhavana* of *Emblica officinalis Gaertn.* fruit juice was given to the powder of *Curcuma longa L.*, until complete absorption of juice and dried under sunlight. Due to wet milling techniques, beneficial physicochemcal parameters changed in finished product. Weight of *Choorna* increased in successive manner indicating accumulation of solid particles of *Emblica officinalis Gaertn*. juice in *Curcuma longa L.* powder. Various organoleptic changes such as change of taste, change of color are also noticed. Continuous wet trituration with pressure reduced the particle size (60 to 85 mesh size) and made it compatible. GC-MS (Gas Chromatography-Mass Spectrometry) analysis revealed the presence of the major compounds such as1,1-Difluoro-Tetramethylcyclopropane (1.52%), AR-Tumerone (35.11%), 7- Curlone (55.30%) and Iso-aromadendrene epoxide/Iso-pinocarveol (8.07%). These compounds possess antidiabetic property which will be useful for upcoming clinical trial on prediabetes.

Key Words: Bhavana, GC-MS, Pharmaceutical techniques, Physicochemical changes.

Introduction

Ayurveda has its unique methodology to prepare various drug dosage forms which have been designed according to the nature of drug. The quality of finished drug obtained after process solely depends on the quality of its raw materials and standard operating procedure adopted for its preparation. (1) *Samskara* i.e., different pharmaceutical techniques are being used while preparing the *Ayurvedic* drug into final dosage form. Amongst them, *Bhavana* is one, which is used for either potentiation of the drug or elimination of impurities. (2) It enhances the medicinal properties of the drug and attains maximum potency to treat the diseases. (3)

Nisha-Amalaki Yoga (NAY), an Ayurvedic formulation has been mentioned in many classical texts being used as a herbal remedy for treatment of *Prameha* (Diabetes Mellitus). (4) Different interventional studies also show its mild to moderate hypoglycemic effect specially in newly diagnosed type 2 diabetes mellitus/*Prameha*/*Madhumeha*. (5-8)

In this study, *Curcuma longa L.* was pulverized as mentioned in *Ayurvedic* classics. Quantity sufficient fruit juice of fresh *Amalaki* was prepared and continuous wet milling process was conducted till complete absorption of juice. Then dried under sunlight and prepared fine powder and filled into capsules to increase shelf life. The phytochemical constituents of the NAY are analyzed by GC-MS to identify the biologically active compounds.

Aims and Objectives

1. To enhance the efficacy of drug by successive *Bhavana* as a special Ayurveda pharmaceutical technique.
2. To increase the shelf life and combined drug compatibility of *NAY* on the basis of *Bhavana*.
3. To minimise the dose and increase patient's compliance of *NAY*.
4. To evaluate percentage of bioactive compound present in final product *(NAY)* through GC-MS.

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Materials & Methods

*Nisha-Amalaki Yoga (*NAY*)*

NAY was prepared in a GMP certified pharmacy by taking raw dry rhizome of *Nisha* (*Curcuma longa L.*) and fresh *Amalaki* (*Emblica officinalis Gaertn.*) fruits which were purchased from authentic ayurvedic raw material supplier. To increase the compliance of NAY, finally obtained *Choorna* was filled in Capsule and got packaged in a reputed Pharma company. Dried rhizome of *Curcuma longa L.* (figure 1) was identified by the botanist of Parul Institute of Applied Science and *Dravyaguna* department of PIA [Ref-PU/PIA(DG) Certi-2]. The external impurities/ foreign bodies of the rhizome were removed, then dried under sun and coarse powder was prepared in the disintegrator machine (figure 2). To make it fine, the coarse powder was further ground in a pulveriser, followed by passing it through a sieve of size 60. (Figure 3 & 4).

Simultaneously the freshly collected *Emblica officinalis Gaertn.* fruits (figure 6) were cleaned, washed and made into small pieces with the help of knife (figure 7). Those pieces were added in the fruit juicer to express the juice. The obtained fibrous residues were discarded (figure 8&9). While preparing juice by juicer, small pieces of *Emblica officinalis Gaertn.* fruit were coming out as a residue. That residue was further taken in a mixture pot and juice was expressed by adding sufficient quantity of water. Then *Curcuma longa L.* powder was taken in end runner and *Emblica officinalis Gaertn.* fruit juice was added there, till it became wet like mud and trituration process was continued till mixture became very soft wet mass (figure 10). The *bhavita* material i.e., finally obtained paste was transferred to the stainless-steel tray and dried under sunlight for 5-7 days (figure 11). As mentioned above, *Bhavana* process was repeated for 2 more times and dried NAY obtained in average 0.5-1cmgranular size (figure 13). To fill fine powder of *NAY* in capsule, granules were pulverized into fine powder (85 No) form and filled in hard gelatine capsule having strength of 500 mg (figure 15). Finally, 60 capsules were filled in plastic container for further use.

Figures showing step-wise preparation of *Nisha-Amalaki Yoga*

Figure 1: Raw rhizome of *Curcuma longa L.*

Figure 2: Disintegration of Raw *Curcuma longa L.*

Figure 3: Sieve shifter

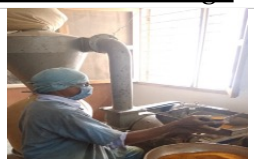


Figure 4: Pulverization of *Curcuma longa L.* coarse powder

Figure 5: Fine powder of *Curcuma longa L.*

Figure 6: Fresh *Emblica officinalis Gaertn.* Fruits

Figure 7: Pieces of *Emblica officinalis Gaertn.* Fruits

Figure 8: Preparation *Amalaki* of juice

Figure 9: *Emblica officinalis Gaertn.* fruit juice

Figure 10: *Bhavana* process in end runner

Figure 11: Shifted in ss tray for drying

Figure 12: Drying in sunlight

Figure 13: Drying process

Figure 14: Fine powder of *NAY*

Figure 15: *NAY* capsules

Observations

Three batches each weighing 30 kgs of *Emblica officinalis Gaertn*. were ground separately for the three *bhavana* process which resulted in extraction *of* 27.3 litres, 27.1 litres and 28 litres of juice respectively. (Table-4) Quantity sufficient water was added while grinding of *Amalaki* pulp. Average time required for each *Bhavana* was 3 hours. (Table 5) While preparation of *NAY*, various organoleptic changes were noticed. Organoleptic test showed that, the bright yellow colour of *Curcuma longa L.* powder turns into greenish dark yellow after 3rd *Bhavana*. Bitter and spicy taste of *Curcuma longa L.* turned into bitter, astringent and somewhat sour after processing with juice of *Emblica officinalis Gaertn*. (Table 6,7,8) Throughout the process, *Curcuma longa L.* powder had absorbed approximately 1.9 litre/kg juice of *Emblica officinalis Gaertn*. in first *Bhavana*, 1.6 litre/kg in second *Bhavana* and 1.5 litre/kg in third *Bhavana*. (1st *Bhavana*-1.9x14=26.6 lit, 2nd *Bhavana*- 1.6x16.7=26.4 lit, 3rd *Bhavana*- 1.5x18.6=27.9 lit of *Emblica officinalis Gaertn.* juice was used) Initial weight (14 kilogram) of *Curcuma longa L.* powder was increased to 21 kilogram after three *Bhavana* of *Emblica officinalis Gaertn*. juice. (Table 9) After *Bhavana* process final product obtained with combined characters. Stickiness of wet mixture

was increased in successive *Bhavana*. The compactness of the drug also increased which is indicative of significant reduction in particle size, resulting in the formation of very hard pieces after complete drying. (Graph 1) Initial weight of *Curcuma longa L.* powder was increased with successive *Bhavana*, so we can say that *Emblica officinalis Gaertn.* solid particles added in *Curcuma longa L.* powder. (Graph 2) Continuous trituration process removes moisture from *Emblica officinalis Gaertn.* juice and solid particles accumulated in *NAY*. Finally obtained granules of *NAY* with size of 1-2 cm were very hard, so for the purpose of capsule filling, the fine powder was prepared (85 mess size). The fine powder of *NAY* was filled in capsules. From16 kgs of *NAY* fine powder, approximately 31,995 hard gelatine capsules with 500mg strength were prepared and packed in plastic box with capacity of 60 capsules.

Table 1: Showing Ingredients of *NAY*

Sr. No.	Drug name	Botanical name/ Family	Part used	Quantity
1	*Haridra*	*Curcuma longa L.*/ Zingiberacae	Rhizome	1 part
2	*Amalaki*	*Emblica officinalis Gaertn.*/ Euphorbiacea	Fruit	Q.S.

Table 2: Showing particle size reduction of raw *Curcuma longa L.*

Size of Raw *Curcuma longa L.*	Particle Size of coarse *Curcuma longa L.* powder	Particle size of fine *Curcuma longa L.* powder	Particle size of *NAY*
3 cm to 7 cm length	22 to 44 mesh size	60 mesh size	85 mesh size

Table 3: Showing weight of *Curcuma longa L.* powder during pharmaceutical process

Initial weight of Raw *Curcuma longa L.*	Weight of coarse *Curcuma longa L.* powder	Weight officinalis *Curcuma longa L.* powder	Residue
17 kg	15250 gm	14100 gm	210 gm

Table 4: Showing Initial and Final Weight While Processing of *Emblica officinalis Gaertn.* During Each *Bhavana*

Initial weight of Raw *Emblica officinalis Gaertn.*	Weight of *Emblica officinalis Gaertn.* Pieces	Final weight of Juice of *Emblica officinalis Gaertn.*	Weight of wet residue
1st*Bhavana*- 30 kg	27.5 kg	27.3 litres	12 kg
2nd*Bhavana*- 30 kg	27.7 kg	27.1 litres	12.4 kg
3rd*Bhavana*- 30 kg	27.6 kg	28 litres	11.9 kg

Table 5: Table Showing Duration Required for Trituration in *Bhavana* Process

Number of *Bhavana*	Average duration for trituration
1st*Bhavana*	2 hours, 30 minutes
2nd*Bhavana*	3 hours
3rd*Bhavana*	3 hours

Table 6: Showing Organoleptic Tests of *Curcuma longa L.* Powder

Organoleptic Test	Observations
Colour	Brownish yellow
Odour	Slightly spicy
Taste	Bitter and Spicy
Consistency	Solid
Touch	Smooth

Table 7: Showing Organoleptic Tests of *Emblica officinalis Gaertn.* juice

Organoleptic Test	Observations
Colour	Pale green
Odour	Sour
Taste	Sour, Astringent and somewhat sweet
Consistency	Watery/liquid
Touch	Cold

Table 8: Showing Organoleptic Tests of Juice of *Emblica officinalis Gaertn. Bhavita Curcuma longa L.* Powder

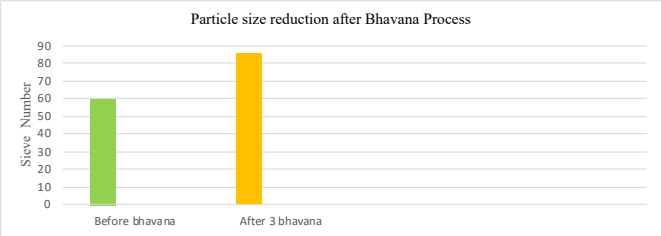
Organoleptic Test	Observations
Colour	Dark greenish yellow
Odour	Slightly spicy and sour
Taste	Bitter, Astringent and somewhat sour
Consistency	Solid
Touch	Smooth

Table 9: Showing Initial and Final Weight of Juice of *Emblica officinalis Gaertn. Bhavita Curcuma longa L.* Powder

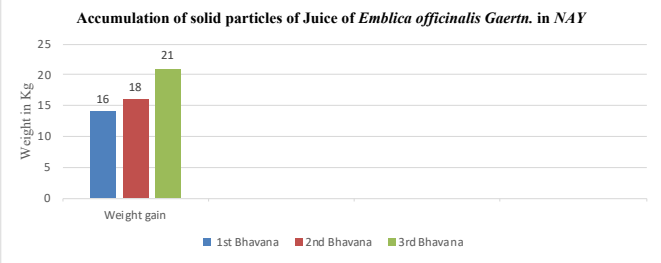
Number of *Bhavana* process	Total *Curcuma longa L.* powder	Total Juice of *Emblica officinalis*	Weight of each *Bhavana* finished product
1St Process	14 kg	27.3 litres	16.700 kg
2nd Process	16.5 kg	27.1 litres	18.900 kg
3rd Process	18.6 kg	28 litres	21.300 kg

Note: Quantity sufficient water was added while grinding of *Emblica officinalis Gaertn.* pulp. (During each bhavana, the quantity of fresh juice extracted from juicer was 13-14 lit. Further, *Emblica officinalis Gaertn.* pulp was grinded in mixture with addition of sufficient water.)

Graph 1



Graph 2



GC-MS analysis of *NAY*

Instruments used for GC-MS:

Gas chromatograph with mass spectrometer

Specifications:

Analyzer: Quadrupole with prefilter, Mass Range: 20-620 Daltons (amu), Mass Stability: ± 0.1 m/z mass accuracy over 48 hours, Ionization Modes: EI/CI Ionization, Vacuum Pump: Turbo molecular pump 250L/Sec, Software: Turbo Mass

Method adopted for GC-MS:

Preparation of extract

About 20 gm *NAY* powder soaked in 50 ml of methanol in covered jar. The content of the jar was macerated intermittently for proper mixing and left to stand for about 72 hours. Then the sample was filtered and concentrated using a rotary evaporator at 45oC. Separate semi-liquid extract was obtained, labeled, and stored at a temperature below 4°C until ready for analysis. Analysis was done within 72 hours of extraction.

Results of GC-MS analysis:

Total twenty compounds were identified in the methanolic extract of the *NAY* following GC-MS analysis: The names of compounds, retention times (RT), peak areas (in percentage), molecular formula and molecular weights of identified components are presented in Table 1. GC-MS analysis of *NAY* powder exhibits presence of various bioactive compounds which are shown in (Graph 3) and (Fig16 -19). 1-difluoro-tetramethylcyclopropane, 5,10-pentadecadiyn-1-ol, 3-methyl-6-hepten-1-yn-3-ol, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, bicyclo [3.1.1]heptane, 6-methyl-2-methylene-6-(4-methyl-3-pentenyl)-,[1 benzamide, n-(5-chloro-1,4,6-trimethyl-1h-pyrazolo[3,4-b]pyridin-3-yl)-3-1-p-tolyl-2-(4h-[1,2,4] triazol-3-lsulfanyl)-ethanone (1r,5r)-2-methyl-5-((r)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene 1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (z,e)- benzamide, 3-methyl-n-(1,4,6-trimethyl-1h-pyrazolo[3,4-b]pyridin-3-yl)- m-toluic acid, oct-3-en-2-yl ester, 1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (z,e)- 6-octen-1-yn-3-ol,3,7-dimethyl-trans-.alpha.-bergamotene,(z,z)-.alpha.-farnesene(e)-1-(6, 10-dimethylundec-5-en-2-yl)-4-methylbenzene,s,z)-2-methyl-6-(p-tolyl) hept-2-en-1-ol (r)-1-methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene,(e)-1-(6,10-dimethylundeca-5,9-dien-2-yl)-4-methylbenzene ar- tumerone 3-methyl-2-butenoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester, butyric acid, 2-methyl-4-(2,5-xylyl)-, 2-methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one, 1h-indene, 2,3,4,7-tetrahydro-,3-methyl-2-butenoic acid, 2-methyloct-5-yn-4-yl ester, 1,3-pentadiene,5-(2,2-dimethylcyclopropyl)-2,4-dimethyl-,(z or e)-1-pentene, 5-(2,2-dimethylcyclopropyl)-2-methyl-4-methylene. Comprehensive traditional knowledge on *NAY* can be validated by modern pharmacological studies emphasizing the chemical nature of *NAY*, its effects on various parameters and detailed studies of the mechanisms of the observed biological actions and molecular study. Keeping in view the before mentioned biological properties of *Curcuma longa L.* and *Emblica officinalis Gaertn.*, it is quite clear that combination of *Curcuma longa L.* and *Emblica officinalis Gaertn.* being available in natural form, being nontoxic with a wide spectrum of biological activity, may find its application in the formation of various medicinal preparation which can help in the treatment of various diseases in coming future such as diabetes and cancer. (10,11).

Area Percent Report

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Last Modified : Wednesday, November 10, 2021 3:53:03 PM
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2	2	16.020	3,330,169.5	14,412,125	MM	0.00		0.00	TIC	35.11
3	3	16.350	5,246,018.5	11,630,229	MM	0.00		0.00	TIC	55.30
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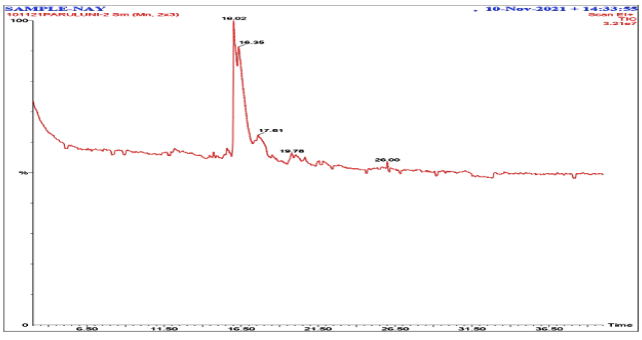

SAMPLE-NAY **101121PARULUNI-2**

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	726	517	1,1-DIFLUORO-TETRAMETHYLCYCLOPROPANE	134	C7H12F2	823-25-6
2	721	378	5,10-PENTADECADIYN-1-OL	220	C15H24O	64275-50-9
3	713	435	3-METHYL-6-HEPTEN-1-YN-3-OL	124	C8H12O	51193-99-8
4	705	589	BENZENE, 1-(1,5-DIMETHYL-4-HEXENYL)-4-METHYL-	202	C15H22	644-30-4
5	703	568	BICYCLO[3.1.1]HEPTANE, 6-METHYL-2-METHYLENE-6-(4-METHYL-3-PENTENYL)-, [1	204	C15H24	55123-21-2
6	702	333	BENZAMIDE, N-(5-CHLORO-1,4,6-TRIMETHYL-1H-PYRAZOLO[3,4-B]PYRIDIN-3-YL)-3-	328	C17H17ON4Cl	900319-26-0
7	700	430	1-P-TOLYL-2-(4H-[1,2,4]TRIAZOL-3-YLSULFANYL)-ETHANONE	233	C11H11ON3S	326888-02-2
8	689	514	(1R,5R)-2-METHYL-5-((R)-6-METHYLHEPT-5-EN-2-YL)BICYCLO[3.1.0]HEX-2-ENE	204	C15H24	58319-06-5
9	686	564	1,3,6,10-DODECATETRAENE, 3,7,11-TRIMETHYL-, (Z,E)-	204	C15H24	26560-14-5
10	684	406	BENZAMIDE, 3-METHYL-N-(1,4,6-TRIMETHYL-1H-PYRAZOLO[3,4-B]PYRIDIN-3-YL)-	294	C17H18ON4	900319-24-8
11	683	460	M-TOLUIC ACID, OCT-3-EN-2-YL ESTER	246	C16H22O2	900292-61-2
12	678	530	1,3,6,10-DODECATETRAENE, 3,7,11-TRIMETHYL-, (Z,E)-	204	C15H24	26560-14-5
13	677	514	6-OCTEN-1-YN-3-OL, 3,7-DIMETHYL-	152	C10H16O	29171-20-8
14	669	537	TRANS-.ALPHA.-BERGAMOTENE	204	C15H24	13474-59-4
15	665	541	(Z,Z)-.ALPHA.-FARNESENE	204	C15H24	900293-03-1
16	662	476	(E)-1-(6,10-DIMETHYLUNDEC-5-EN-2-YL)-4-METHYLBENZENE	272	C20H32	900413-12-5
17	657	371	1,9-DICHLORONONANE	196	C9H18Cl2	821-99-8
18	656	419	(S,Z)-2-METHYL-6-(P-TOLYL)HEPT-2-EN-1-OL	218	C15H22O	78339-53-4
19	655	498	(R)-1-METHYL-4-(6-METHYLHEPT-5-EN-2-YL)CYCLOHEXA-1,4-DIENE	204	C15H24	28976-67-2
20	654	439	(E)-1-(6,10-DIMETHYLUNDECA-5,9-DIEN-2-YL)-4-METHYLBENZENE	270	C20H30	55968-43-9

SAMPLE-NAY **101121PARULUNI-2**

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	829	643	CARVYL TIGLATE, CIS-	234	C15H22O2	900383-34-6
2	826	714	AR-TUMERONE	216	C15H20O	900292-71-0
3	824	683	(E)-.GAMMA.-ATLANTONE	218	C15H22O	108549-47-9
4	815	705	AR-TURMERONE	216	C15H20O	532-65-0
5	810	610	CARVYL TIGLATE, TRANS-	234	C15H22O2	900383-67-6
6	802	634	CARVYL ANGELATE, CIS-	234	C15H22O2	900383-30-1
7	783	674	(Z)-.GAMMA.-ATLANTONE	218	C15H22O	108549-48-0
8	760	612	CINNAMYL ANGELATE, E-	216	C14H16O2	900383-67-5
9	754	623	3-METHYL-2-BUTENOIC ACID, 2,7-DIMETHYLOCT-7-EN-5-YN-4-YL ESTER	234	C15H22O2	900299-31-4
10	750	378	BENZENEBUTANAL, .GAMMA.,4-DIMETHYL-	176	C12H16O	4895-19-6
11	735	383	3,4-NONADIEN-6-YNE, 5-ETHYL-3-METHYL-	162	C12H18	61227-88-1
12	733	619	TUMERONE	218	C15H22O	180315-67-7
13	725	581	MYRTENYL TIGLATE	234	C15H22O2	900383-62-3
14	706	502	3-METHYL-2-BUTENOIC ACID, 2,6-DIMETHYLNON-1-EN-3-YN-5-YL ESTER	248	C16H24O2	900299-31-5
15	705	355	BENZENEBUTANOIC ACID, 2,5-DIMETHYL-	192	C12H16O2	1453-06-1
16	703	561	MYRTENYL ANGELATE	234	C15H22O2	138530-45-7
17	673	436	BENZENE, 1-(3-CYCLOPENTYLPROPYL)-2,4-DIMETHYL-	216	C16H24	54815-16-6
18	667	331	BUTYRIC ACID, 2-METHYL-4-(2,5-XYLYL)-	206	C13H18O2	30316-14-4
19	663	529	TUMERONE	218	C15H22O	180315-67-7
20	658	327	BUTYRIC ACID, 3-METHYL-4-(2,5-XYLYL)-	206	C13H18O2	30275-76-4

SAMPLE-NAY **101121PARULUNI-2**

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	800	588	1-HEPTYNE, 3-METHOXY-3,4-DIMETHYL-	154	C10H18O	54244-92-7
2	752	483	1H-3A,7-METHANOAZULENE-6-METHANOL, 2,3,4,7,8,8A-HEXAHYDRO-3,8,8-TRIMET	220	C15H24O	21441-72-5
3	744	523	BICYCLO[5.1.0]OCTANE, 8-(1-METHYLETHYLIDENE)-	150	C11H18	54166-47-1
4	731	492	1,3-BIS-(2-CYCLOPROPYL,2-METHYLCYCLOPROPYL)-BUT-2-EN-1-ONE	258	C18H26O	900222-08-6
5	721	660	BICYCLO[3.1.1]HEPTANE, 2,6,6-TRIMETHYL-3-(2-PROPENYL)-, (1.ALPHA.,2.BETA.,3.	178	C13H22	50746-55-9
6	717	508	CHAMIGRAN-9-ONE, 2,10-DIBROMO-3-CHLORO-	412	C15H23OClBr2	900139-04-2
7	709	486	.ALPHA.-FARNESENE	204	C15H24	502-61-4
8	705	474	1,3,6,10-DODECATETRAENE, 3,7,11-TRIMETHYL-, (Z,E)-	204	C15H24	26560-14-5
9	705	468	.ALPHA.-FARNESENE	204	C15H24	502-61-4
10	705	476	1-PENTENE, 5-(2,2-DIMETHYLCYCLOPROPYL)-2-METHYL-4-METHYLENE-	164	C12H20	900150-39-5
11	699	618	ISOPINOCARVEOL	152	C10H16O	6712-79-4
12	695	436	1,3,6,10-DODECATETRAENE, 3,7,11-TRIMETHYL-, (Z,E)-	204	C15H24	26560-14-5
13	692	314	4-(4-METHYLPENT-3-ENYL)-3,6-DIHYDRO-1,2-DITHIIN	200	C10H16S2	73188-23-5
14	689	425	ISOAROMADENDRENE EPOXIDE	220	C15H24O	900159-36-6
15	688	530	3-METHYL-2-BUTENOIC ACID, 2-METHYLOCT-5-YN-4-YL ESTER	222	C14H22O2	900299-31-3
16	686	403	(2S,4R)-P-MENTHA-[1(7),8]-DIENE 2-HYDROPEROXIDE	168	C10H16O2	900292-74-4
17	683	616	(Z)-2,6-DIMETHYLOCTA-2,5,7-TRIEN-4-ONE	150	C10H14O	33746-71-3
18	683	428	(Z,Z)-.ALPHA.-FARNESENE	204	C15H24	900293-03-1
19	681	389	ADAMANTANE-1-CARBOXAMIDE, N-(4-ISOPROPYLPHENYL)-	297	C20H27ON	306743-34-0
20	680	453	1,3-PENTADIENE, 5-(2,2-DIMETHYLCYCLOPROPYL)-2,4-DIMETHYL-, (Z OR E)-	164	C12H20	900150-39-6

SAMPLE-NAY **101121PARULUNI-2**

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	858	428	CYCLOHEXENE, 1-(1-PROPYNYL)-	120	C9H12	1655-05-6
2	857	744	CURLONE	218	C15H22O	87440-60-6
3	818	653	2-METHYL-6-(4-METHYLENECYCLOHEX-2-EN-1-YL)HEPT-2-EN-4-ONE	218	C15H22O	82508-14-3
4	810	489	CARVYL ANGELATE, TRANS-	234	C15H22O2	900383-67-7
5	804	605	MEGASTIGMA-3,7(E),9-TRIENE	176	C13H20	900069-81-9
6	803	673	(E)-.GAMMA.-ATLANTONE	218	C15H22O	108549-47-9
7	801	706	(Z)-.GAMMA.-ATLANTONE	218	C15H22O	108549-48-0
8	786	665	TUMERONE	218	C15H22O	180315-67-7
9	769	562	SPIRO[4.4]NONA-1,6-DIENE, (S)-	120	C9H12	39746-39-9
10	765	375	OXIRANEMETHANOL, 2-PHENYL-	150	C9H10O2	141248-89-7
11	756	450	PENTALENE, 1,2,4,5,6,6A-HEXAHYDRO-2-METHYLENE-	120	C9H12	113679-72-4
12	742	530	CYCLOHEXANE, 1,2,4-TRIS(METHYLENE)-	120	C9H12	14296-81-2
13	739	599	VERBENYL ANGELATE, CIS-	234	C15H22O2	900383-56-3
14	730	577	CARVYL TIGLATE, TRANS-	234	C15H22O2	900383-67-6
15	730	581	CARVYL TIGLATE, CIS-	234	C15H22O2	900383-34-6
16	728	519	CYCLONONA-1,2,6-TRIENE	120	C9H12	900196-99-9
17	726	604	TUMERONE	218	C15H22O	180315-67-7
18	720	592	CARVYL ANGELATE, CIS-	234	C15H22O2	900383-30-1
19	719	429	1H-INDENE, 2,3,4,7-TETRAHYDRO-	120	C9H12	7603-37-4
20	710	579	PERILLA ALCOHOL TIGLATE	234	C15H22O2	900383-58-5

Discussion

Although the reference of NAY has been found in different authoritative books of Ayurveda, no author has mentioned any clear-cut methodology to prepare the drug. Traditionally it has been advised to mix *Curcuma longa L.* powder with juice of *Emblica officinalis Gaertn.* and used when required. But due to frequent use of the drug and unavailability of the fresh raw *Emblica officinalis Gaertn.* in all seasons, it is not feasible to prepare the juice on daily basis. So, to overcome such difficulties, the methodology may be modified by adopting the technique of Bhavana to achieve a suitable dosage form with a better compliance and shelf life. (12) Keeping this concept in mind, *Bhavana* method is adopted to prepare *NAY,* which is further modified in capsule form to increase palatability and compliance to the patient.

Commonly, process of *Bhavana* involves two methodologies. In one method, the drug (in powder form) is completely soaked in *Bhavana dravya* i.e., organic liquid media (juice, decoction, cow urine etc) and is exposed to sunlight to incorporate their properties, whereas the other method involves trituration of powder along with the media till complete absorption of liquid media. For the preparation of NAY, 2nd method was adopted where *Curcuma longa L.* powder was triturated with juice of *Emblica officinalis Gaertn.* in end runner machine until the powder absorbs all the moisture. The quantity of the *Bhavana dravya* should be added so that the powder becomes completely wet and submerged in liquid. After complete trituration process, obtained product was dried in the sunlight. The process was repeated for three times i.e., the powder was subjected to three numbers of Bhavna. To keep Thus *Bhavana* helps us to achieve few things.

Processing of *Bhavana* is performed in different steps with a goal to attain:

Reduced Particle size: **Graph 1:**

Particle size reduction (*Choorna* form) of initial material. Prime motto behind selection of wet trituration is, it facilitates particle size reduction and homogenization leading to modification of properties (*Gunantatradhana*) of the end product which helps in easy assimilation to reach the target tissue and thereby increasing the therapeutic efficacy. (13)

Enhance Potency: **Fig 16-19:**

Addition (*Samyoga*) of specific organic liquid to increase the potency. (14) For adding potency to the formulation, proper impregnation (*Bhavana*) with a juice or decoction of same or other drugs even in a small quantity should be implemented. (15) The impregnation of properties of the media to the material leads to unique and suitable physio-chemical changes i.e., induction of trace element from specific media to potentiate the biological efficacy of the material and to facilitate the absorption. (16) It also helps in increasing the bioavailability of the principal drug, and hence the same doses of the drugs bring about more effective and potent action. (17) Thus, the process of *Bhavana* should logically be considered as an important measure for enhancing bioavailability. (18)

Omission of toxic/unwanted material

Liquid media also helps in neutralizing the unwanted/harmful material either by dissolving or chelation. The quantity of *Bhavana dravya* (Juice of *Emblica officinalis Gaertn.*) must completely moisturize and dip the *Curcuma longa L.* powder. (19) Heat is generated while trituration due to continuous friction and at the same time, atmospheric heat helps in drying of materials quickly which leads to change of chemical and physical bonding and conversion of properties from its previous form (transformation). (20-22)

In successive *Bhavana*, accumulation of *Bhavana* dravya results in increasing the potency of drug. Quantity of *Bhavana dravya* depends on the nature of drug used for *Bhavana*. *Bhavana dravya* should be added in optimum quantity to form very soft paste while continuous trituration. Quantity of liquid media used for *Bhavana* should fulfil the observational parameters such as *Ardrata* (Wetness), *Kardamabha* (Mud consistency), *Samplavana* (*Bhavita dravya* deepen in *Bhavanadravya*) and *Ekibhoot* (Homogenization of *choorna* and *Bhavana dravya*). The process is carried out till attainment of *Subhavit Lakshana* (Test of perfectness of *Bhavana* process). (23)

Physicochemical transformations also occur in product depending upon the type of *Bhavana dravya* (juice, decoction, milk, cow urine) and duration for which *Bhavana* process is adopted. In wet trituration process, *samyoga* of mixer is properly carried out, mixer becomes very soft, smooth in touch, and so sticky, which facilitates better binding (*Samyoga*) of material and thus, facilitating further processing. Liquid media helps in proper trituration, without creating spillage of material in dust form. Wet trituration facilitates particle size reduction and homogenization leading to transformation of properties of the formulation. (24)

In modern pharmacology, mechanical technique to decrease the particle of solids are classified into three categories: dry milling, wet milling and high pressure homogenization. In order to produce particles in the semi micron range and further increase solubility, wet media milling have been developed. Milling involves the application of mechanical energy to physically break down coarse particles to finer ones and is regarded as a "top–down" approach in the production of fine particles. The mechanisms by which milling enhances drug dissolution and solubility include alterations in the size, specific surface area and shape of the drug particles as well as it leads to liberation of reaction components due to cell breakage. (25) This mechanism is same as that to the ancient concept of *Bhavana*. As moisture holding capacity of the drug increases, particle size also reduces. (26) The shelf life of *choorna* as per *Acharya Sharangdhara* is 2 months only. (27) This is a major drawback of this *kalpana* due to which many times *choorna* get wasted. Hence to give solution for this problem, the Drug and cosmetic act has given some precautionary measures and increased the shelf-life period up to 2 years. (28) Some trials have shown the role

of *Bhavana* in enhancing the shelf life beyond 2 years also. (29)

According to *Sharngadhara*, the therapeutic dose of *Choorna Kalpana* (Powders) is 1 *Karsha* (12 gm) and *Anagnisiddha swarasa* (Freshly expressed juice) is ½ pala (24 ml). (30,31) While preparing *NAY*, three *Bhavana* of freshly expressed juice (Potent dosage form) of *Emblica officinalis Gaertn.* was given to *Curcuma longa L.* powder, which resulted into dose reduction of final product. Previous studies on herbal formulations proves the dose reduction and enhanced the effectiveness. (32,33)

Conclusion

Ultimate pharmacotherapeutic changes obtained in *NAY* due to *Bhavana* process are as follows:

1. Combined materials (*Curcuma longa L.* and *Emblica officinalis Gaertn.*) are mixed homogenously.
2. Used material converted into very fine powder form.
3. Changes in materials like softness, smoothness and stickiness which facilitates better binding of material.
4. Enhances the therapeutic property of *bhavita dravya* (Powders of drugs).
5. It can also exhibit synergistic action, that is combined effect of formulation.

Core significances of *Bhavana* (wet milling) process are the potentiation of the drug, reduction in dose, bioavailability enhancer, reduction in particle size, nullify the toxic effects of *bhavita dravya*, dose reduction and change in organoleptic/physicochemical characters. These characteristics plays a vital role to enhance the therapeutic effects of finished product. *Bhavana* helps in the formation of desired compound and converts heterogeneous into homogenous form, macro to micro and rough to smooth form of drugs. It has multi-dimensional pharmaceutical and therapeutic implications. Added liquids act as media for extraction of components of various ingredients as well as media for their chemical interaction. It may act as a catalyst and, also play a role of buffering agent by maintaining of specific pH. Percentage of constituents obtained in final product depends on the duration and numbers of wet milling. Further studies with more numbers of *Bhavana* are required to increase efficacy of the formulation.

Acknowledgment

The authors thank PIA & PIAR, Faculty of Ayurved, Parul University, Vadodara for providing resources and support for conducting pharmaceutical study and Library for literary search.

Conflict of interest

The authors declare no conflict of interest.

Author(s) contributions

NM: Conceptualization; SD: Project monitoring and supervision; RB, NM: Writing–original draft; SD, HB: Writing–review and editing.

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