Pharmacogn. Res., 2024; 16(4):837-853.
https://www.phcogres.com

Original Article

Modification and Physicochemical Evaluation of *Naque Nazla* a Unani Infusion Preparation for Cold and Flu into Soluble Granules

Mohammad Zaigham[1], Hamiduddin[1,*], Mohd Akhtar Ali[1], Mohammed Abdul Waheed[2]

[1]Department of Ilmul Saidla (Unani Pharmacy), National Institute of Unani Medicine (NIUM) Kottigepalya, Magadi Main Road, Bangalore, Karnataka, INDIA.
[2]Research Officer In-charge, CRU-UNANI, (Under Central Council for Research in Unani Medicine (CCRUM), Edathala, Aluva Kochi, Ernakulam Kerala, INDIA.

ABSTRACT

Background: Infusions and decoctions for Nazla/Zukam (Cold and Flu) are time tested in Unani System of Medicine. Naque Nazla (NN) Infusion containing *Viola odorata* Linn. flower, *Zizyphus jujuba* Linn. fruit, *Cordia dichotama* Forst. fruit, *Althea officinalis* Linn. Seed, *Malva sylvestris* Linn. Seed, *Borago offlclnalis* Linn. leaves, *Glycyrhhiza glabra* Linn. root is used as an effective remedy for common cold and related symptoms. This formulation need modification for better drug compliance and portability. Attempt was made to make soluble granule in unit sachet, from NN which may retain the advantages of infusions and also address the problems of quality control, preparation, and administration of infusions/decoctions. **Materials and Methods:** Granules batch was selected on the basis of ease of preparation, Solubility and total solid content by Water-bath method of drying of NN (NNG 1), Granules were also prepared by different initial drying process of NN viz. NNG 2 (Freeze drying) and NNG 3 (Rota evaporator). Batch-NNG 1 was also evaluated for short term preliminary stability study. Physicochemical evaluation of all the batches was carried out with Glycyrrhizin estimation by HPLC and fingerprinting by HPTLC. **Results:** NN dried with 10% maltodextrin was selected due to ease of powdering as a Non-Native Extract (NNE). Three different procedures of drying in NNG1, NNG 2 and NNG 3 batches do not reveal much difference when evaluated, except dry yield of NNE in NNG 2 was more in comparison to NNG 1 and NNG 3 batches. Granules batch with NNE powder (with 10% maltodextrin) 63%, fine powder of NN ingredient (filler) 10%, liquorice powder (sweetening agent) 5%, Starch soluble 20%, SSG 2%, Satte Pudina 0.25%, and Aerosil-200 5% of all the ingredient was selected. Physicochemical parameters of NN and formulated Granules such as organoleptic properties, pH value, total LOD at 105°C, Ash & extractive values, test for mucilage, microbial contamination, HPLC (Glycyrrhizin estimation), HPTLC data etc., was set in. Short term (3 month) stability study of NNG1 predicted 1 year stability at room temperature in sachet packed condition. **Conclusion:** NNG1 method can be a more feasible and economical method. It displayed higher Glycyrrhizin content. Successful formulation of soluble granules was achieved.

Keywords: Physicochemical, Evaluation, Naque-Nazla, Granules, Cold flu, Unani Medicine.

Correspondence:
Dr. Hamiduddin
Associate Professor and HOD, Department of Ilmul Saidla (Unani Pharmacy), National Institute of Unani Medicine (NIUM), Kottigepalya, Magadi Main Road, Bangalore-560091, Karnataka, INDIA.
Email: drhamid2003@rediffmail.com

Received: 25-05-2024;
Revised: 21-08-2024;
Accepted: 04-09-2024.

INTRODUCTION

Unani System of Medicine (USM) is one of the popular traditional systems of medicine, as a formulator in USM we may follow, traditional dosage forms such as Arq (distilled matter), Joshanda (Decoction), Khesanda (Infusion), Sharbat (Syrup), Majun, (Confection), Laooq (Linctus), Safoof (Dried powder drug) etc., or modern dosage form such as tablet, Lozenges, Sachets (lyophilized forms), Soluble granules, Capsule etc.,[1] There is an option and sometime imperative need to convert the traditional formulations into these modern forms. One such preparation is *Joshanda*/*Khesanda* or Naqu (Decoction/Infusion), there disadvantage are difficulties in ensuring quality control of the herbal ingredients, transportation, storage and accurate dosage etc., It needs appropriate dosage form modification to address packaging and preservation issue, to provide better acceptability, drug compliance to fulfill the need of contemporary life style i.e. to avoid consumption of large volume of unpleasant tasting medicines and save inconvenience and time they required to prepare it. Granule formulation may retain the advantages of Infusions/decoctions and can address the problems of quality control, preparation, and administration and can also be useful to group it into low, middle, and high dose ranges to some

DOI: 10.5530/pres.16.4.96

extent. Granules are quickly becoming the most common form of traditional medicine formulations in the world and it is one of the important factors in promoting Chinese traditional medicine. Infusions and decoctions for *Nazla/Zukam* (Cold and Flu) are time tested and commonly used formulations in Unani System of Medicine.[2-4]

An attempt was made to prepare granule dosage form with suitable & permissible excipients from *Naque Nazla* (Infusion) containing Gule Banafsha, Unnab, Sapistan, Tukhme Khatmi, Tukhme Khubbazi, Barge Gaozaban, Aslussoos,[5] formulation with same ingredients is mentioned in Haziq[6] This polyherbal preparation is extensively used by masses as a remedy for treatment of common cold/flu and claimed very effective.[2] Physicochemical analysis of prepared granules from different procedures and stability testing in sachet packing was also carried out for final batch. Only water-based extracts for preparation of formulation were used to replicate a traditional Unani Infusion (*Naqu*). Objectives of the work is formulating soluble granule dosage form of *Naque Nazla (NN)* with its Physico-chemical analysis.

MATERIALS AND METHODS

Granules from *Naqu-e-Nazla* were formulated by preparing multiple batches and final batch was selected by analyzing those batches. Manufacturing procedure of *Naqu-e-Nazla Granules (NNG)* preparation was developed. Granules were also prepared by different initial drying process of NN and one of the batches was evaluated for short term stability. Thereafter physicochemical evaluation of *NN* and modified, *NNG* were completed.

The five ingredients of *Naqu-e-Nazla* were procured from the In-House Pharmacy of the National Institute of Unani Medicine and rest two drugs from the herbal raw drug dealer, A.B. General store Bangalore. Identification and authentication of drugs was conducted by Botanist, Senior Asst. Prof. Noorunnisa Begum, Centre for Repository of Medicinal Resources (C-RMR), Trans-Disciplinary University (TDU), Foundation for Revitalization of Local Health Tradition (FRLHT), Bangalore with accession number 4520 to 4526. All chemicals, reagents and excipients used were of analytical/standard grade.

Preparation of classical formulation (*Naqu*)

All crude drugs were cleaned manually from impurities and the *Naqu* (Infusion) was prepared as per method mentioned in Unani classical text *Alqarabadeen.*[7] Drugs mentioned in number. are converted to their equivalent weight. *Naqu-e-Nazla* one dose contains Gule Banafsha (*Viola odorata* L.) Flowers 7 g, Unnab (*Zizyphus jujuba* L.) Fruits (5 no.) 5 g, Sapistan (*Cordia dichotoma* F.) Fruits (7 no.) 4 g, Tukhme Khatmi (*Althea officinalis* L.) Seeds 7 g, Tukhme Khubbazi (*Malva sylvestris* L.) Seeds 7 g, Gaozaban (*Onosma bracteatum* W.) Leaves 5 g, Aslussoos (*Glycyrrhiza glabra* L.) Roots 5 g.[4,5] Aslussoos, Unnab, Sapistan were first passed through the process of *Neem-Kob*. It is the process by which hard and fibrous drugs (roots, stems, seeds, etc.,) are crushed to small pieces in an iron mortar and softened in order to obtain the maximum efficacy, when used in the preparation of decoction or infusions. The word "*Neem Kofta*" is suffixed to the name of the drug in the formula which has to undergo this process.[8] Now all the drug are mix together and soaked in 360 mL. boiling hot water (RO water), overnight.[8] Total weight of one dose of Naque contain total 40 g of drug. Drug water ratio taken was 1: 9. After soaking overnight, the ingredients of Naqu were meshed manually and strained well with the help of Muslin cloth. Finally, 300 mL of Naqu was obtained.

Formulation of granules of Naqu-e-Nazla

First dry yield was obtained from freshly prepared single dose of Naqu (300 mL) on water-bath. Then Nonnative extract powder of *NN.* was obtained by trial-and-error basis by mixing in different excipients. Batch with 10% maltodextrin was selected due to ease of powdering and drying of extract/Naqu and use of less quantity of excipient Table 1.[9]

Then final batches were prepared by drying on water bath Rota-evaporator and lyophilizer (Freeze drying) and mixing with different excipients. Selected batch was evaluated on the basis of solubility, processing ease and total solids.

Water-bath method (NNG1)

Step 1: Selected non-native extract (extract with excipient) batch was utilized for further preparation of NNG by drying on water-bath and is termed as NNG1. in selected batch prepared Naqu-e-Nazla was mixed with 10% Maltodextrin and stirred well. This extract was then spread on stainless steel tray and dried on water-bath at 50-60°C. Dried extract was scrapped out[9] and this scrapped extract was powdered with the help of grinder and porcelain mortar and pastel and stored in air tight glass jar with silica gel pouch as a desiccant, for further use.

Step 2. (Granulation): Different batches of granules were prepared with different excipients in different proportions with obtained non-native extract on trial-and-error basis. Each excipient was weighed accurately on digital weighing balance. Different excipients used are Lactose, Microcrytalline cellulose, Starch, Adsorbent (Aerosil 200), Super disintegrant (Sodium Starch Glycolate) and Binder (Gum Acacia) with variation in their quantities, summarize in (Table 4). Time and temperature for drying of granules was kept constant i.e. 60 min and 60°C respectively. Total 12 batches of granules were prepared and from these entire batches ideal batch was selected on the basis of ease of preparation, ideal solubility and total solid contents specifications.[10]

Mixing of excipients to the extract

Powder of the ingredients of Naqu-e-Nazla (in same proportion) used as an excipient/filler was passed through No. 100 # sieve,

fine powder of liquorice (*G. glabra*) of No. 100 #, Starch (soluble), Sodium Starch Glycolate (SSG), Fumed silica (Aerosil) and Satte Pudina (Mint) were mixed properly to the extract powder by geometrical method with the help of small mixer jar to simulate R.M.G.[11]

Powder of ingredient with respective ratio of NN formulation used as a filler/excipient was first sterilized at 160°C for 1 hr before mixing.[11,12] 32 mL of distilled water (for the batch of 200 g. of granules) was added to the mixture (extracts and excipients) for wetting with the help of spray and mixture was then blended in mixer until damp mass was obtained.

This damp mass, was then passed through sieve no. 16 in the granulator (Cemach Granulator, oscillating type 8 inch, GMP Model, M/C No.1417) and wet granules were collected in stainless steel tray for further processing. Obtained granules were dried in hot air oven (Labline Mod. No. HO 6.7) on 60°C for 1 hr. and after cooling granules were stored in airtight glass jar with silica pouch for evaluation Table 2.[13]

Packing of granules

Dried granules were accurately weighed and packed in to Triplex foil sachet (purchased from Sambhav foils and flexible, Bengaluru) with the help of Paddle sealing machine, model Pack-o-matic, PACTEC INDIA. Each triplex foil sachet was a multi layered laminated pouch (Alu Foil pouch) of size, 85 mm×130 mm. which was made up of two different plastic layers and one aluminium sheet in between the two plastic layers. The innermost plastic layer was photo resistant, thus preventing the harmful rays to the material during transportation.[14]

Freeze drying (Lyophilizer) method

In this method proportion of excipient same as batch no 12 was used but initial drying of NN (for making non-native extract) was done by Lyophilizer. Freshly prepared Naqu-e-Nazla was mixed with 10% Maltodextrin and stirred well. This extract was filled in flasks (200 mL *Naqu* in the flask of 500 mL capacity) and these flasks were freezed at the temperature of -45°C overnight. Then flasks were attached with the nozzles of lyophilizer and vacuum pump was switched on with freezer. After three days and 18 hr *Naqu* was completely converted in to dry powder, this powder was collected and stored in air tight glass jar with silica gel pouch, for further use to prepare NNG2 Batch.[15]

Rota-evaporator method

In this method also proportion of excipient same as batch no 12 was used but initial drying of NN. Was done by the help of Rota-evaporator. Freshly prepared *Naqu-e-Nazla* was mixed with 10% Maltodextrin and stirred well. This extract was filled in the flask of the rota-evaporator (200 mL *Naqu* in the flask of 1000 mL capacity). Temperature was kept at 60°C and rotation of flask was kept on 60 rpm. After 1 hr. And 20 min a thick *Naqu* was obtained, which was further dried in a steel tray on water bath at the temperature of 60°C.[13]

Finally, the dried extract was scrapped out and stored in air tight glass jar with silica gel pouch, to prepare granules termed as NNG3.[16] All the three batches of granules prepared by different method of drying i e. by Water bath, freeze drying (Lyophilizer) and Rota-evaporator was subject to evaluation by various physicochemical parameters. Batch prepared by water bath method (NNG1) was subjected for short term stability testing for 3 month for preliminary comment on the stability of formulated granules. Each 6 g granules, were packed in Triplex Sachets and marked/labelled properly. The procedure was carried with extreme care to avoid contamination.

Methodology of accelerated stability testing

Accelerated short term Photo stability studies were done on granules with appropriate packaging/container (XXX sachet) to simulate marketed formulation as per ICH guidelines. Formed finished product was analyzed for previously described physicochemical parameters including microbial analysis. Prepared sachets with proper marking of formulation name, date of preparation, date of commencement of thermal/humidity or photo-challenges and date of withdrawal were put inside the stability chamber. Thermal/humidity challenge was carried out for a period of three months. Prepared batch (First pack) NNG1 was tested for various analytical parameters at the time of manufacture i.e. zero month (Baseline) and at 3 months, and the same batch (NNG1) subjected to photo Analysis was kept in stability chamber for accelerated stability study and named as NNGS. For thermal/humidity stress regulated at 40±2°C temperature and relative humidity at 75±5%. The last pack (Sachet Batch) NNGS was removed from stability chamber at the completion of the three months and is studied for various parameters.[17]

Photostability testing of *Naqu-e-Nazla* granules was done initially at illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 Watt/hours per square meter. Option 2 was used in this study. A cool and white fluorescent lamp designed to produce an output similar to that specified in ISO 10977 (1993) and near UV fluorescent lamp having a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm and significant proportion of UV in both bands of 320 to 360 nm and 360 to 400 nm was used.[18]

Calculation of time is doe as per photostability chamber Model-Osworld photostability chamber OPSH G-41258. NNG (sachet) was exposed to fluorescent light for 4 days 12 hr and UV light for 22 hr 13 min to achieve 1.2 million lux hour fluorescent light and 200-Watt hours/square m^2 UV light. During the photostability study stability chamber was run at 40±2°C temperature and relative humidity at 75±5%.

Physicochemical evaluation

Analytical parameters for freshly prepared Naqu

Organoleptic properties

Colour, odour, taste and touch of *Naqu* were evaluated.[19] Total solid content: 10 mL of *Naqu* was taken in a tarred porcelain evaporating dish and was heated on water bath at 60-70°C and then it was kept in oven at 105°C until constant weight of residue was obtained.[20]

Mucilage content

Freshly prepared *Naqu* was taken as per classical *Unani* text. Acetone was added in the quantity of three times the volume of the *Naqu* to precipitate the mucilage. Then the mucilage was separated and collected in a tarred petri dish and dried in an hot air oven at a temperature not more than 50°C. Weighed and the quantity of mucilage was calculated.[21] pH value, Specific gravity and viscosity measurement with U tube viscometer was done as per method mentioned in Unani Pharmacopoeia of India.[22]

Viscosity measurement with Brookfield viscometer

Brookfield viscometer, DV-II+Pro and RV spindle set was used for the measurement of viscosity. Instrument was levelled by adjusting the levelling screws in respect of the viscometer bubble level. Spindle was selected and attached to the spindle coupling nut. Then spindle was inserted and centered in the test material until the fluid's level reached at the immersion groove on the spindle shaft. Gaurd leg was mounted. The speed of particular spindle at room temperature was set at different rpm. The reading of the viscometer was allowed to stabilized and viscosity was measured in terms of Centipoise.[23]

Ash value

Total Ash, water soluble ash was determined according to the method mentioned in the protocol for testing Ayurvedic, siddha & Unani medicines.[24] Acid insoluble ash: The acid insoluble ash was determined according to the National formulary of Unani medicine. Part–II.[25]

Analytical Parameters for Granules

Organoleptic properties

Colour, odour, taste and general appearance of Granules were evaluated.[19] pH value of 1% and 10% solution and total solid content was determined according to the method mentioned in the Unani Pharmacopoeia of India. Part II Vol. II, First edition and the same in Indian Pharmacopoeia.[22,26]

Granules/Powder characterization

Angle of repose: Angle of repose was calculated according to the method adopted by G. Lumay et al. by using formula.[27]

$$\text{Tan } Ø = 2h/D$$

h = Height of powder (from graph paper to tip of funnel), D = Mean diameter of the powder.

Bulk density, tapped density, Compressibility index and Hausner's ratio was also (Table 3) Scale of Flow Ability (for powder and granules) as per japanese pharmacopoeia.[28,29]

Loss of weight on drying at 105°C

Loss of weight on drying at 105°C was determined according to the method mentioned in the National formulary of Unani medicine. Part–II.[25]

Extractive Values

Alcohol soluble extractive value and Water-soluble extractive value was determined according to the method mentioned in the Protocol for testing Ayurvedic, siddha & Unani medicines.[24] **Successive extractive value** With the help of Soxhlet apparatus drug was subjected to continuous hot extraction using different solvent in increasing order of polarity successively. That is Petroleum ether → benzene→ chloroform→ ethanol. 10 g granules was subjected to extraction with each solvent for 63 hr successively. The extracts were filtered by passing through Whatman's filter paper No. 1 and dried on water bath and extractive values were determined with reference to the weight of the drug (w/w). Mean extractive value was calculated after repeating the process three times.[30]

Non successive extractive value

Soxhlet apparatus was used for non-successive extraction of drug. Water, ethyl alcohol and petroleum ether were used as solvents separately each for 10 g of drug. The extracts were filtered using filter paper (whatman No. 1) and evaporated on water bath. Mean extractive values were determined with reference to drug taken (w/w) after repeating the process for three times.[31]

Test for qualitative analysis of constituents of granules

Test for Tannin, Terpenoide, Saponins, Alkaloids (Dragendroff's, Hagers, Mayer's and Wagners test), Glycoside, Flavonoids, Phenols (Ferric chloride test), Carbohydrates (Fehling's test), Anthrone test: Benedict's test was done according to the method adopted in the research paper by Venkatesan D *et al.* and Physicochemical standardization of Unani formulations. Part IV.[32-34] **Test for Mucilage:** A specimen was prepared in Ruthenium red and was examined under a low power microscope. Then mucilage was appeared as pinkish-red or yellow coloured mass.[24] **Sugar estimation:** Sugar estimation (total sugar and reducing sugar) was done according to the total carbohydrate protocol and Baskan KS. *et al.*[35,36] **Microbial contamination tests:** Microbial contamination test was done according to the method mentioned in the Ayurvedic Pharmacopoeia of India; Part II, Vol. II.[37]

Table 1: Batches of Non-native extracts prepared with different excipients.

Sl. No.	Naqu (mL)	Excipients according to the dry yield of Naqu i. e. 6 g from 300 mL							
		g/%	Starch	Lactose	Cellulose	Gum Arab	Silicon dioxide	Aslussoos (powder)	Malto dextrin
1	300	g	1.5	-	-	-	-	-	-
		%	25						
2	300	g	-	1.5	-	-	-	-	-
		%		25					
3	300	g	-	-	1.5	-	-	-	-
		%			25				
4	300	g	-	-	-	1.5	-	-	-
		%				25			
5	300	g	-	-	-	-	1.5	-	-
		%					25		
6	300	g	-	-	-	-	-	1.5	-
		%						25	
7	300	g	-	-	-	1.5	-	1.5	-
		%				25		25	
8	300	g	-	-	-	-	-	-	1.5
		%							25
9	300	g	-	-	-	-	-	-	0.6
		%							10

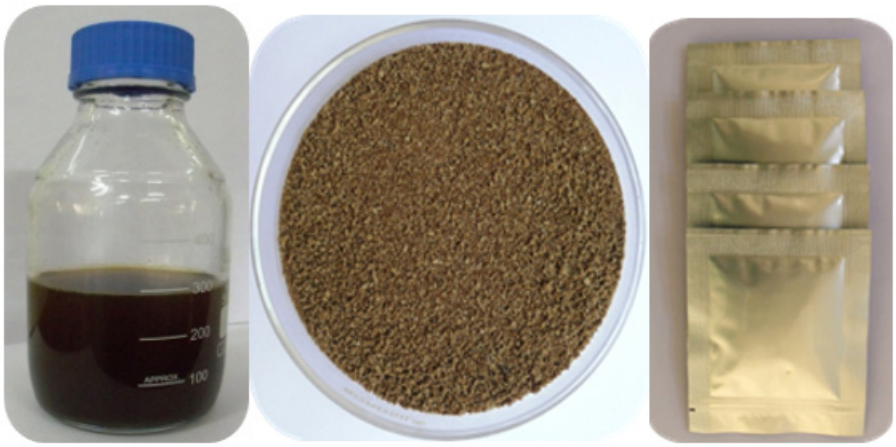

Figure 1: Images of Naque Nazla (NN) and formulated granules NNG1.

HPLC (high-performance liquid chromatography) and HPTLC (high-performance thin layer chromatography) analysis

HPLC: HPLC for estimation of Glycerrhizin was done at Natural remedies Bangalore.

Method of Analysis: Quantity of Glycyrrhizin in NNG1, NNG2, NNG3 and in infusion sample (NN) was estimated. **Preparation of solvent system Solvent A:** 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in 900 mL of HPLC grade water and 0.5 mL of orthophosphoric acid was added. The solution was made up to 1000 mL with water and filtered through 0.45 µ membrane and made it degas in a sonicator for 3 min. **Solvent B:** Acetonitrile**.** Estimation of glycerrhizin was done with appropriate reference detail of glycerrhizine used. Gradient conditions including Time and Buffer concentration (Solvent A) and Acetonitrile concentration (Solvent B). Column: Hibar, Prepacked column, Li Chrospher 100, RP-18e (5µm) (Merck) Phenomenex-Luna 5µ C-18(2) Size: 250x 4.60 mm, Detector: Photo diode array detector or UV Detector; Wave length: 254 nm; Flow rate: 1.5 mL/min; Injection volume: 20 µ; Standard

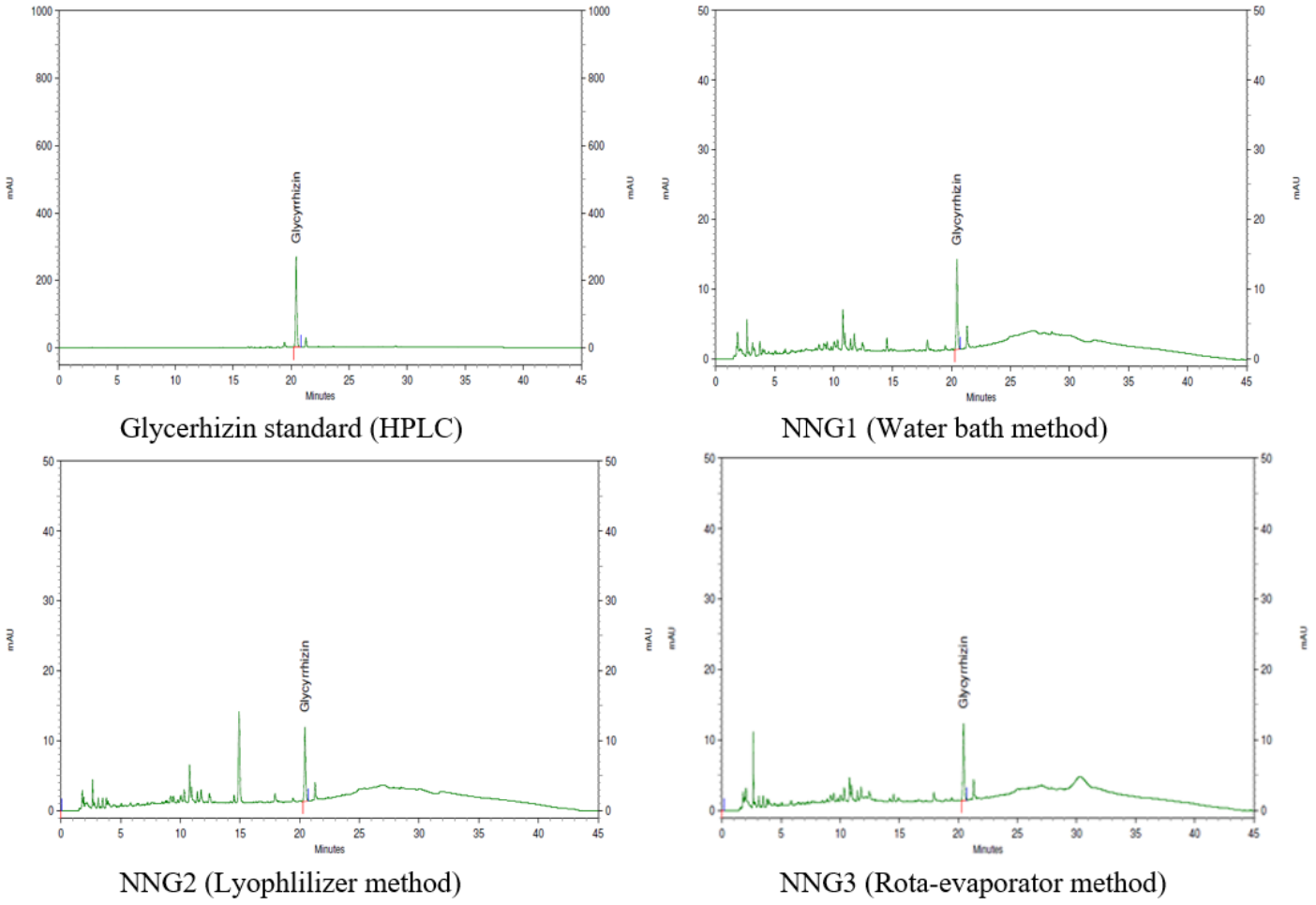

Figure 2: Glycerhizin estimation in NNG1, NNG2 and NNG3 by HPLC.

preparation: 0.1 mg/mL of Glycyrrhizin in 0.25% Ammonia solution. Sample preparation: 10 mg/mL of sample (NNG1, NNG2 & NNG3, Infusion sample) in 0.25% Ammonia solution. **Procedure:** Instrument had set as per the chromatographic condition as prescribed above and 20 µL of standard preparation was injected and the chromatogram was recorded, this volume was injected 3 times and the mean area and the RSD were calculated. RSD should not be more than 2.0%. 20 µL of sample preparation was injected and the chromatogram was recorded. **Calculation:** The quantity of Glycyrrhizin was calculated using following formula

$$\frac{\text{Area of the sample}}{\text{Area of the standard}} \times \frac{\text{Weight of standard in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution x Purity of standard (\%)}}{\text{Sample weight in mg}}$$

HPTLC: HPTLC work was done at Natural remedies Bangalore.

An attempt has been made to evolve preliminary chromatographic physico-chemical profile of NNG and also for comment on stability tested batch (NNG1, NNG2, NNG3, NN, NNGS). **Method of analysis:** Sample preparation: 25 mg per mL of given sample in HPLC grade methanol; Stationary phase: Pre-coated silica gel on aluminium plate; Mobile phase:n-butanol: Water: Glacial acetic acid (7:2:1); Application:10µl of sample preparations mentioned above were applied using TLC applicator (Linomat V, Camag). HPTLC Scanning: 234 nm, 366 nm and 430 nm (for stability study); Derivatization agent: Anisaldehyde sulphuric acid.

Statistics: All the results are depicted in Mean± SEM.

RESULTS

Physicochemical studies of *Naqu-e-Nazla* (Infusion)

One dose of prepared *Naqu-e-Nazla* (*NN.*), contains 300 mL of infusion of the drugs. Colour of *Naqu-e Nazla* (*NN.*) was found to be Amber colour; The odour of *Naqu* was found to be characteristic/aromatic and faint; he taste of *NN.* was found to be sweetish and mucilaginous; The touch of *Naqu* was found to be Glutinous/mucilaginous/, slippery; The mean pH value of *NN.*, 1% and 10% solution were found.6.92 ±.0.05, 6.84 ±0.02, 6.74±0.02 respectively.

Total solid content in % was 4.76±0.06, & mucilage content in % was 11.125±0.548, Values of Specific gravity was 1.592±0.002 and viscosity Naqu by U tube method 7.613±0.006 & and Brook Field viscometer was 40.4 (C.P.) in specification [RPM 100, Spindle

Table 2: All batches of *Naque Nazla* granules (NNG1).

Batch		Ext.	Asl.p.	Starch	Acacia	Lactose	PVP	SSG	Mint	Mix pd	Aerosil	Soluble (min)/Insoluble	TSC g%
1	g	5	2.5	2.5	-	-	-	-	-	-	1	5	23.52
	%	50	25	25	-	-	-	-	-	-	10		
2	g	5	1.25	2.5	1.25	-	-	-	-	-	1	Insol.	-
	%	50	12.5	25	12.5	-	-	-	-	-	10		
3	g	5	2.5	1.25	-	1.25	-	-	-	-	1	Insol.	-
	%	50	25	12.5	-	12.5	-	-	-	-	10		
4	g	5	2.5	1.25	1.25	-	-	-	-	-	1	Insol.	-
	%	50	25	12.5	12.5	-	-	-	-	-	10		
5	g	5	2.5	2.5	-	-	0.05	-	-	-	1	3	28.28
	%	50	25	25	-	-	0.5	-	-	-	10		
6	g	5	2.5	2.5	-	-	-	0.05	-	-	1	3	29.28
	%	50	25	25	-	-	-	0.5	-	-	10		
7	g	7.50	2.5	-	-	-	-	0.05	-	-	1	4	28.08
	%	75	25	-	-	-	-	0.5	-	-	10		
8	g	5	-	2.5	-	-	-	0.05	-	2.5	0.5	3	28.08
	%	50	-	25	-	-	-	0.5	-	25	5		
9	g	6	0.5	2	-	-	-	0.05	-	1.5	0.5	3	28.28
	%	60	5	20	-	-	-	0.5	-	15	5		
10	g	6.3	0.5	2	-	-	-	0.2	-	1	0.5	1	30.08
	%	63	5	20	-	-	-	2	-	10	5		
11	g	6.3	0.5	2	-	-	-	0.2	0.1	1	0.5	1	30.12
	%	63	5	20	-	-	-	2	1	10	5		
12	g	6.3	0.5	2	-	-	-	0.2	0.025	1	0.5	1	30.32
	%	63	5	20	-	-	-	2	0.25	10	5		

ASl.P.- Aslussoos, 12 =Final Batch, Ext.= extract, Mix pdr. = mix powder.

No.02, Torque 10.1%, and Temp. (°C) 25.6], Ash value for Naqu was Total ash (%) 0.53±0.02, Acid insoluble ash (%) 0.16±0.005 and Water soluble ash (%) 0.48±0.02. Yield of Naqu-e-Nazla: Yield of Naque nazla by three different methods of drying viz. Waterbath (%) Was 6.08±0.02, Lyophilizer (%) was 9.02±0.02 and Rota evaporator (%) was 6.04±0.03. freez drying (Lyophilizer) method displayed highest yield.

Formulation of NNG1

Out of 12 different batches prepared of formulated granules with different excipient batch No. 12 (NNG1) was selected as final ideal batch depicted in Table 2.

Physicochemical studies of formulated Granules

Organoleptic properties

The appearance of granules of Waterbath (NNG1), lyophilizer (NNG2) and rotatory evaporator (NNG3) batches were found to be amorphous in each batch. The colour of granules of NNG1, NNG2 and NNG3 batches were found to be brown in each. batch. The odour of granules of NNG1, NNG2 and NNG3 batches were found to be faint and characteristic/aromatic in each batch. The taste of granules of NNG1, NNG2 and NNG3 batches were found to be sweetish, mucilaginous in each batch (Figure 1).

Physicochemical studies of Granules (NNG)

Granules characterization, LoD at 105°, Ash values, mucilage content, pH at 1% & 10% sol. of NNG 1, NNG 2 & NNG 3 is depicted in Table 3, Total solid contents of granules NNG1 and NNG1-S (stability batch at 3 month) are displayed in Table 7.

Qualitative Phytochemical analysis of constituents for NNG1, NNG2 and NNG3 viz. Saponins, Terpenoids, Flavonoids, Phenols, Alkaloids, Glycosides, Tannins, Carbohydrates, Mucilage was found positive. Extract values of NNG1, NNG2 & NNG3 are depicted in Table 4.

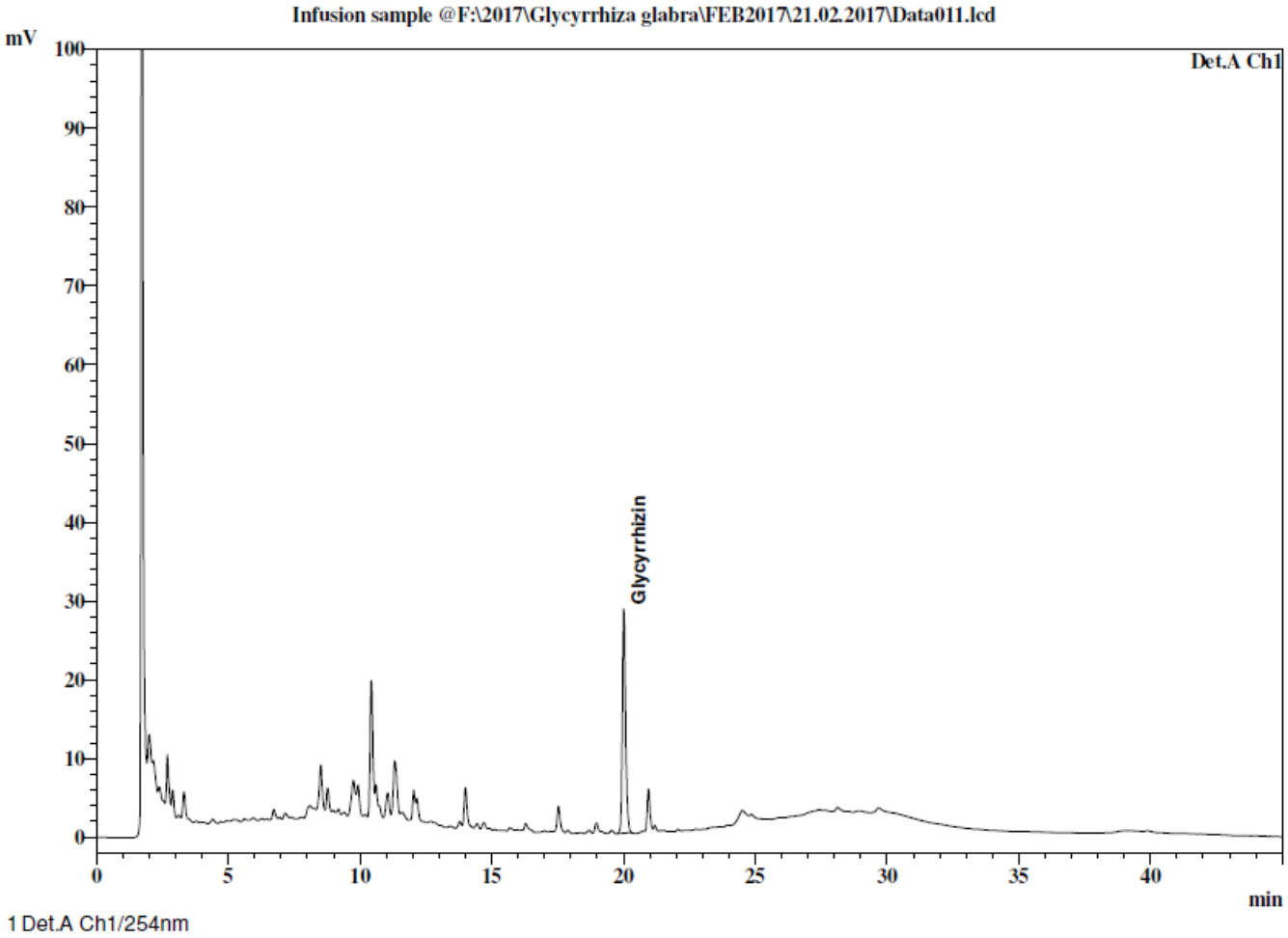

Figure 3: Glycerhizin estimation by HPLC in Infusion (NN).

Sugar estimation of granules reveals Total sugar in NNG1: 2.58±0.05, NNG2: 3.2±0.05 and NNG3: 2.64±0.04. Reducing sugar NNG 1: 2.28±0.05, NNG 2: 2.99±0.01 and NNG 3: 2.52±0.05. Alcohol soluble and water-soluble extractive (%) value of granules (NNG1) was found to be 19.30±0.32 and 71.486±0.86 respectively. Non-successive extractive values of granules prepared by Waterbath (NNG 1) in (%) was found to be Pet. Ether 0.05±0.01, Benzene 0.62±0.20, Chloroform 0.68±0.02, Ethanol 35.52±1.94 and Water was 76.50±0.32.

HPLC Quantification of Glycrrhizin in Naque Nazla and formulated granules is depicted in Table 5, Figures 2 and 3 and for NNG1 and NNGS stability batch is depicted in Figure 9. HPLC profile of NNGS and NNG1 showing almost comparable peaks, is displayed in Figure 9, Peak detail of HPLC profile for NNG1 and NNGS (0 and 3month) is displayed in Table 8.

In HPTLC analysis no. of peaks in infusion, NNG1, NNG2 and NNG3 at different wavelengths (254, 366, 430 nm) and Rf Value is displayed in Table 6 and Figure 8, HPTLC Fingerprinting (TLC Profile) and Densitogram at 430 nm is displayed in Figures 4 and 5 and HPTLC 3D overlay of Infusion, NNG1, NNG2 and NNG3 at 430 nm is displayed in Figure 6 and at 254 nm and 366 nm is displayed in Figure 7, HPTLC fingerprinting overlay of NNG1 and NNGS stability study batch at 254 nm is displayed in Figure 10.

Shelf-life study

Waterbath (0 month) NNG1 and Waterbath (3 month) NNGS both were same both display Appearance: Amorphous, Colour: Brown, Odour: Faint and Characteristic/aromatic, Taste: Sweetish/mucilaginous. Bulk density, Tapped density, Car's index, Hausner's ratio and Angle of Repose, pH, total solid, mucilage content, LoD and Ash values of granules NNG1 at base line (Day o) and NNGS (s) i.e. at 3 months is depicted in Table 7.

Qualitative Phytochemical analysis of constituents for NNG1 (0), NNGS 3month viz. Saponins, Terpenoids, Flavonoids, Phenols, Alkaloids, Glycosides, Carbohydrates, Mucilage was found positive in both samples except tannin was present in NNG1 (0) whereas was absent in NNGS 3 months batch. Sugar estimation of granules reveals Total sugar in NNG1(0 month) 2.58±0.05, and NNGS (3 month): 2,53±0.03 and Reducing sugar in NNG1(0 month) was 2.28±0.05, and in NNGS (3 month) was 2.22±0.06.

HPLC Quantification of Glycrrhizin is depicted in Table 7, Total microbial count in NNG1 (day 0) and NNGS (at 3 months) was found within limit, detail is depicted in Table 7.

DISCUSSION

Review of the drugs and formulation NN subjected to modification revealed several pharmacological activities such as Azmi AA. et. al. reported antibacterial activity in ingredients of *Joshanda*, *Zizyphus jujube* (*Unnab*), *Onosma bracteatum* (*Gaozaban*) and *Glycyrrhiza glabra* (*Mulethi*) it displays significant degree of *invitro* activity against Staph. aureus while *Cordia latifolia* (*Sapistan*) against *H. Influenzae*.[6] Abdullah *et al.* reported antimicrobial, phytotoxic activities in decoction of 100 g of *Joshanda* containing 3 g of *Althaea officinalis*, 9 g of *Cordia latifolia*, 5 g of *Glycyrrhiza glabra,* 3 g of *Malva sylvestris,* 5 g of *Onosma bracteatum* 5 g of *Viola odorata* and 5 g of *Zizyphus Sativa*., it displayed profound activity against Gram positive *S. aureus*.[38] Khan H. *et al.* Reported Anti-inflammatory and antioxidant activity of *Joshanda* in carrageenan induced paw oedema and cotton pellets induced granuloma and DDPH free radical scavenging test.[39] Review of toxicity showed that these ingredients are safe. Muralidharan P. *et al.* reported that *Glycyrrhiza glabra* extract did not produce any toxic symptoms of mortality up to the dose level of 2000 mg/kg body weight in rats, and hence the drugs were considered safe.[40] Vispute *et al.* reported Acute toxicity of Glycyrrhizin (crude extract 48-58%): LD_{50} values in rats and mice LD_{50} s.c. 4-4.4 g/kg LD_{50} i.p. 1.42-1.70 g/kg LD_{50} oral 14.2-18.0g/kg[41] Vishal A. *et al.* studied toxicity of all the extracts of Banafsha (*Viola odorata* aerial parts), on rats using extracts at dose of 2000 mg/kg body weight by fixed dose method showed it to be safe.[42]

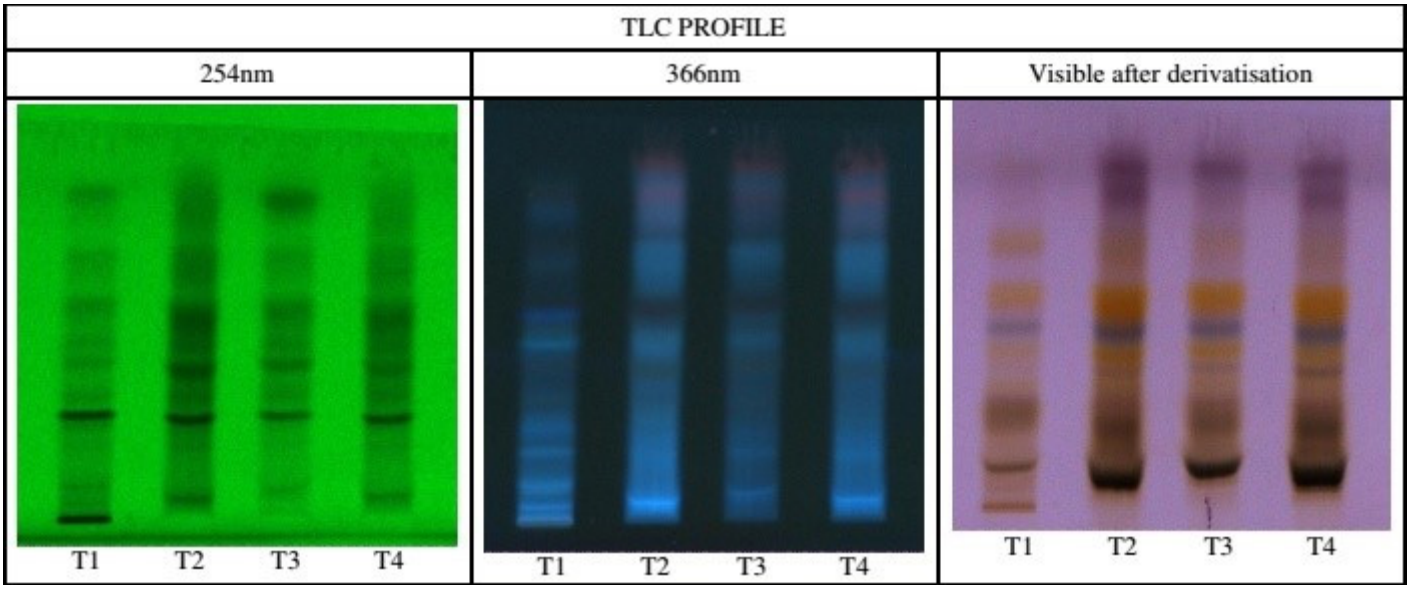

Figure 4: HPTLC Fingerprinting (TLC Profile), T1-Infusion sample, T2-NNG1, T3-NNG2, T4-NNG3.

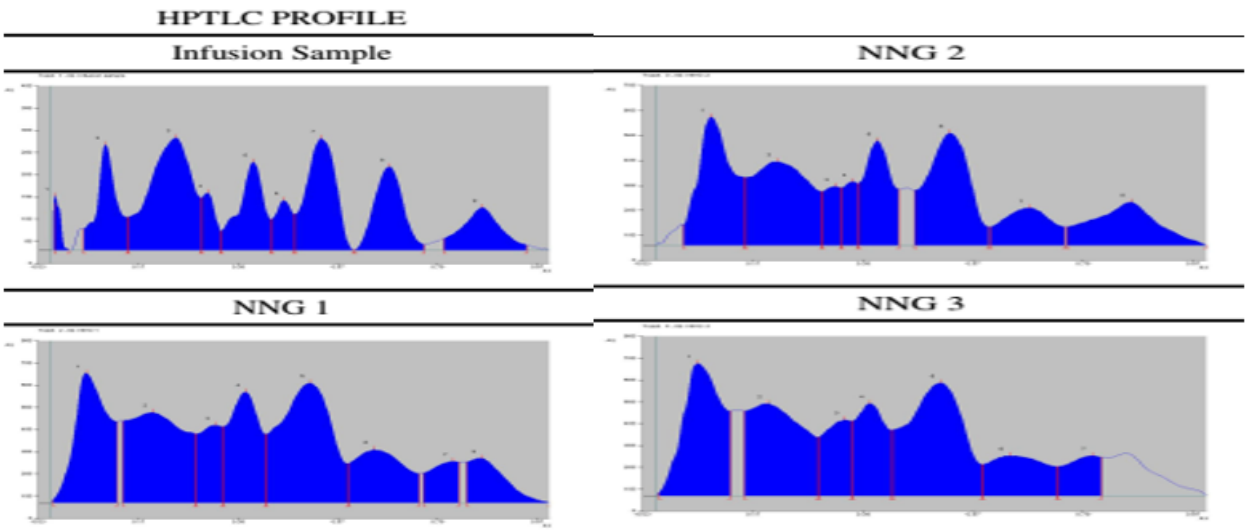

Figure 5: HPTLC profile (Densitogram) NN and formulated granules at 430 nm.

Table 3: Granules characterization, and physicochemical parameters of NNG1, NNG2 & NNG3.

Parameters	NNG1	NNG2	NNG3
Bulk density (g/mL)	0.5166±0.01	0.5198±0.003	0.5106±0.005
Tapped density (g/mL)	0.6218±0.008	0.6870±0.005	0.6294±0.004
Car's index (%)	7.0714±0.53	6.2534±0.10	7.4114± 0.28
Hausn. Ratio	1.2485±0.004	1.3283±0.01	1.1750±0.07
Angle of Repose (θ)	34.106±0.26	37.248±0.91	34.273±0.54
LoD at 105°	2.30±0.005	2.65±0.15	2.29±0.014
Total ash (%)	17.16±0.39	13.70±0.22	17.23±0.44
Acid insoluble ash (%)	15.95±1.583	08.01±0.14	11.78±0.11
Water soluble ash (%)	3.80±0.17	2.45±0.05	2.26±0.01
Mucilage content (%)	29.746±0.70	30.900±0.08	29.666±0.87
pH at 1% Sol.	7.24± 0.006	7.28±0.04	7.26±0.01
pH at 10% Sol.	6.92±0.02	6.93±0.02	6.90 ± 0.006

Table 4: Successive extractive values of formulated granules.

Solvents	NNG1	NNG2	NNG3
Pet. Ether (%)	0.05±0.01	0.08±0.02	0.06±0.01
Benzene (%)	0.33±0.02	0.36±0.04	0.32±0.03
Chloroform (%)	0.45±0.09	0.46±0.09	0.42±0.08
Ethanol (%)	16.95±0.15	17.00±0.15	16.89±0.11

Dry yield of *Naqu-e-Nazla* (NN) from one dose of 40 g of total crude drugs combinations, was found to be 6.08±0.02 by Hot plate, 6.04±0.03 by Rota-evaporator and 9.02±0.02 g. by Lyophilizer. Yield was more in case of lyophilized batch. It was not possible to prepare the native dried extract of *Naqu* due to large amount of mucilage without any excipient. Maltodextrin played an important role in the preparation of dried extract (Non-Native extract) of NN. Granulation was done with ease after the preparation of non-native extract (Table 1) (Figure 1). Mixing of NNE and excipient was done in small jar of mixer/grinder which simulates industrial model RMG (Rapid mixer granulator) and act as a high shear mixer. Out of 12 different batches prepared of formulated granules with different excipient batch No. 12 (NNG1) was selected as final ideal batch. This batch was selected after analysis of all the batches on the parameters mainly solubility (soluble or insoluble) and ease of preparation, and it displayed more total solid content (30.33), Higher the total solid lower will be the drying time and better will be the drying efficiency. (Tables 1 and 2).

Granules of batch 1, 2 and 3 were insoluble after continuous stirring till the 3 min, rendering it as insoluble. After mixing super-disintegrants (PVP, SSG) granules became soluble. Granules of the batch of SSG were more soluble than the granules of the batch in which PVP was used. Granules formulated with super-disintegrants, SSG in batch no 12 were soluble completely in 1 min, rendering no granule remaining insoluble except suspending mucilaginous bodies. Proper solubility might also be achieved by mixing other fillers such as soluble starch and mixed powder of the formulation which reduces stickiness of the formulation and aid in releasing the drug. Solubility was tested on observation basis (mixing in luke warm water) which was 1 min by stirring. The movement drug get dispersed in water the process of absorption will start and thereby increase the bioavailability of drug. Mixed powder of the formulation was used as a filler to reduce the quantity of other filler and to increase the potency of the formulation by increasing the drug quantity present in it. Starch used in the formulation is also a multipurpose excipient, used primarily as a filler after wetting also act as binder and disintegrant.[43] NNG1 batch was prepared in water bath which simulates jacketed vessels in industrial model, batch NNG2 was prepared by freeze-drying and NNG3 batch was prepared in Rota-evaporator. In Batch NNG3 initially Rota-evaporator was used for drying further drying was done in hot air oven (Table 2).[44]

Physicochemical studies of formulated Granules

The granules were amorphous with appreciating colour and odour, which is very tough in the preparation of herbal drug formulation. The appearance of granules of NNG1 (Waterbath), NNG2 (lyophilizer) and NNG3 (Rotatory evaporator) batches were found to be amorphous in each batch and are of uniform appearance with brown in colour and faint and characteristic/aromatic odour in each batch. The taste of granules of all three batches was found to be sweetish, mucilaginous in all batches.

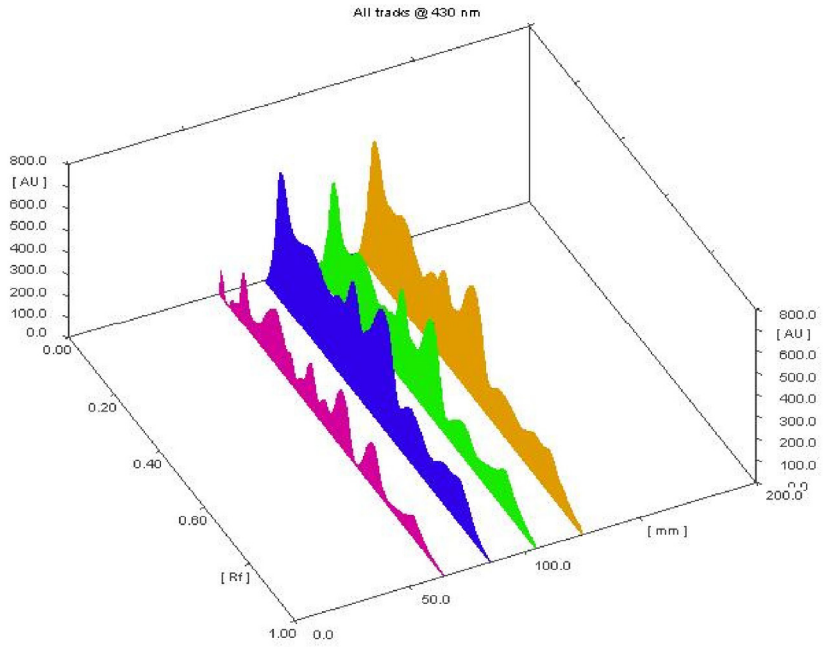

Figure 6: HPTLC 3D overlay of Infusion, NNG1, NNG2 and NNG3 at 430 nm.

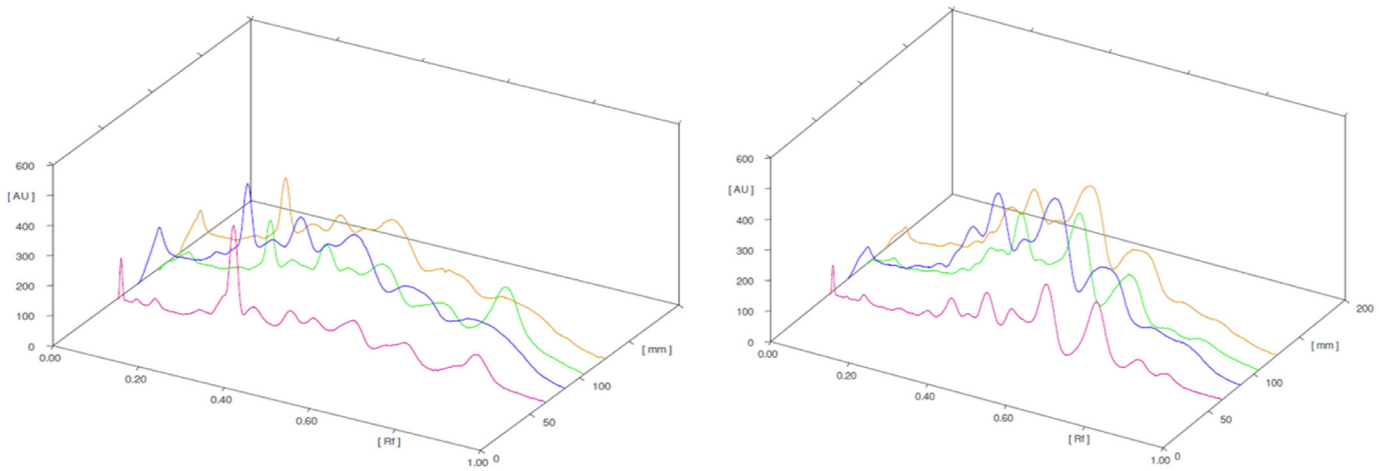

Figure 7: HPTLC, 3D overlay of Infusion, NNG1, NNG2 and NNG3 at 254 nm and 366 nm.

It was giving soothing effects begin from the oral cavity and pharynx which may be due to presence of menthol/ mint in a very small quality which was added as taste enhancing exicipient. The sweet taste of the granules was due to the presence of natural (Non caloric) sugar substitute glycyrrhizin, which was present in higher concentration in the formulation, which makes it palatable for even children also. pH value is of greater importance in respect of absorption of drug in different parts of the gut. Such as acidic drugs are absorbed in the part of the gut of acidic ph and basic drugs are absorbed in the alkaline medium. So, the better absorbance of the drug correlates to the ph value of the drug.[45] (Tables 3 and 4).

In three batches (NNG1, NNG2, NNG3) neutral and slightly basic pH may be due to presence of Saponin in the formulation. High mucilage content was due to mucilage containing drug in the formulation such as Total solid content and mucilage content of granules of NNG1, NNG2 and NNG3 batches were found to be 30.33±0.16, 30.77± 0.21, 30.10±0.07 and 29.746±0.70, 30.900±0.08, 29.666±0.87 respectively. Mucilage the solid content of granules is near about in same amount which is very interesting. NNG can provide more soothing effect on mucous membrane then NN due to higher mucilage content in it. Stem, leaves and flowers contain mucilage in Viola species (Banafsha).[46] As market sample of Banafsha contain whole herb in the name of *Gule-e-Banafsha*, mucilage is present in the whole plant (Stem, leaves and flowers), beside this drug Sapistan (*Cordia dichotoma* Forst.),[47] Tukhm-e-Khatmi (*Althea officinalis* Linn.),[48] and Barg-e-Gaozaban (*Borago officinalis* Linn.)[47,49] also contains mucilage, mucilage content may provide more soothing effect to the respiratory membrane. No any binder is used binding property is achieved due to the presence of mucilage in the drugs

of the formulation. Studies shows that mucilage of Sapistan is used as a binder for the process of tableting.[47]

Granule characterization parameters play an important role in storage and transportation because these are the priorities after the manufacture of a formulation and before reaching to the patient.[50] According to the scale of flow ability, the granule characterization parameters (angle of repose, compressibility index and Hausner's ratio), shows good flow properties of granules resulting in no need for any aid criteria for the concerned parameters.[28,29] Rota evaporator batch show better flow property than other batches. Good flow property was observed in NNG1 and NNG3 batches in comparison to NNG2 (Table 3).

Low LoD shows that granules have very less moisture (Table 3). This can lead to the longer shelf life of granules and minimum deterioration of the chemical constituents of the formulation. Less moisture is required for proper handling and storage. Dry granules are easily soluble than moistened one. Dry granules also maintain flow of granules at the time of pouring the granules from sachet in to the warm water by the patient providing better acceptability and compliance of new drug dosage form. Low moisture in the granule is better to hold its stability and crispiness for longer time. People prefer granules as a dosage form which are crispy and tasty. After stability test also the moisture content was minimum and within allowable limit in NNGS. This was further confirmed through microbial test which is directly related to the moisture content, because water acts as a potent catalyst for the degradation of the product.[51] Extractive values of granules of Naque Nazla are higher in water indicating the highest solubility especially mucilage which may provide maximum benefit of the formulation to the patient. Successive extractive value of NNG2 Batch was found to be more in comparison to NNG1 and NNG3 which may be due to less degradation of organic constituent particularly heat labile matter in lyophilized one. Qualitative analysis reveals presence of Alkaloids, Saponins, Terpenoids, Flavonoids, Phenols, glycosides, Tannins, Carbohydrates, Mucilage etc. in the NN. and granules of *Naqu-e-Nazla* (NNG1) and also NNGS sample. During analysis Saponins test was found to be strongly positive may be due to main constituent of the formulation in the form of glycyrrhizine and other saponins. Mucilage test was also strongly positive showing the higher concentration of the mucilage in the formulations. The quantity of sugar detected in the granules is very less, make it acceptable for calorie restriction group. Formulated granules are sweet and palatable due to inclusion of non-sugar natural sweetener G. glabra apart from maltodextrin which is only 6% in the formulation.

HPLC Quantification of Glycyrrhizin reveals Glycyrrhizin (%w/w) in 100 g of formulations NNG1: 1.0, NNG2: 0.8, NNG3: 0.8 and in NN (Infusion) 0.04. Marker Glycyrrhizin was selected for the study due to *Aslussoos* (*Glycyrrhiza glabra* Linn.) drug as a part of formulation, and as an excipient for taste, and as a filler in the mix powder of formulation. Quantification of Glycyrrhizin was done to assess degradation of organic constituent during three types of drying process adopted in formulating of granules NNG1 (Water-bath), NNG2 (lyophilizer) and NNG3 (rotatory evaporator) and also to set the standards for formulated granules. Glycyrrhizin peak displayed maximum Area in all the sample for example in case of NNG1, Ret. Time was 20.522 and area was 752968. Glycrrhizin content was slightly higher in NNG1, it might be due to more heat exposure and hydrolysis of glycosides saponins. It is a main sweet tasting constituent of G glabra.[52] (Tables 5 and 8), (Figures 2, 3, 9) When comparing other peaks all the batches displayed same pattern. Only NNG 2 is slightly differ from Infusion sample based on HPLC profile. One Extra peak was found in NNG 2 HPLC Profile (In retention time around

Table 5: HPLC Quantification of Glycrrhizin in Naque Nazla and formulated granules.

Glycrrhizin content	NNG 1 (water bath)	NNG 2 (Lyophilizer)	NNG 3 (Rotatory evaporator)	NN (Infusion)
Glycyrrhizin (%w/w) in 100 g of formulations	1.0	0.8	0.8	0.04
Glycyrrhizin (%w/w) in one sachet (6 g) of formulations	0.06	0.048	0.048	0.0024

Table 6: HPTLC peaks in NNG1, NNG2 and NNG3 at different wavelengths (254, 366, 430 nm).

Samples	No. of peaks at different wavelength		
	254 nm	366 nm	430 nm
Infusion	11	12	09
NNG1	09	09	08
NNG2	09	11	08
NNG3	10	10	07

Rf Value				
Rf Value	Infusion sample	NNG 1	NNG 2	NNG 3
0.1	Brown band	Dark brown band	Dark brown band	Dark brown band
0.2	Brown band	Brown band	Brown band	Brown band
0.3	Light yellow band	Light yellow band	Light yellow band	Light yellow band
0.35	-	Brown band	Brown band	Brown band
0.4	yellow band	yellow band	yellow band	yellow band
0.45	Brown band	Brown band	Brown band	Brown band
0.5	yellow band	yellow band	yellow band	yellow band
0.65	yellow band	yellow band	yellow band	yellow band
0.8	-	Purple band	Purple band	Purple band

Figure 8: R_f Value of NN, NNG1, NNG2 and NNG3.

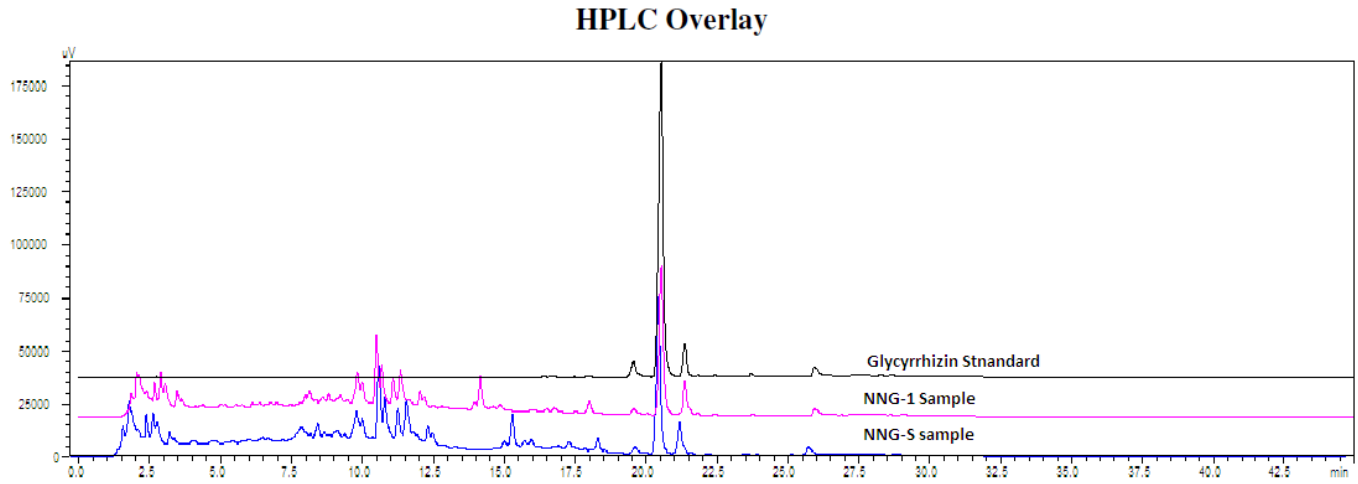

Figure 9: HPLC profile of NNGS and NNG 1 showing almost comparable peaks.

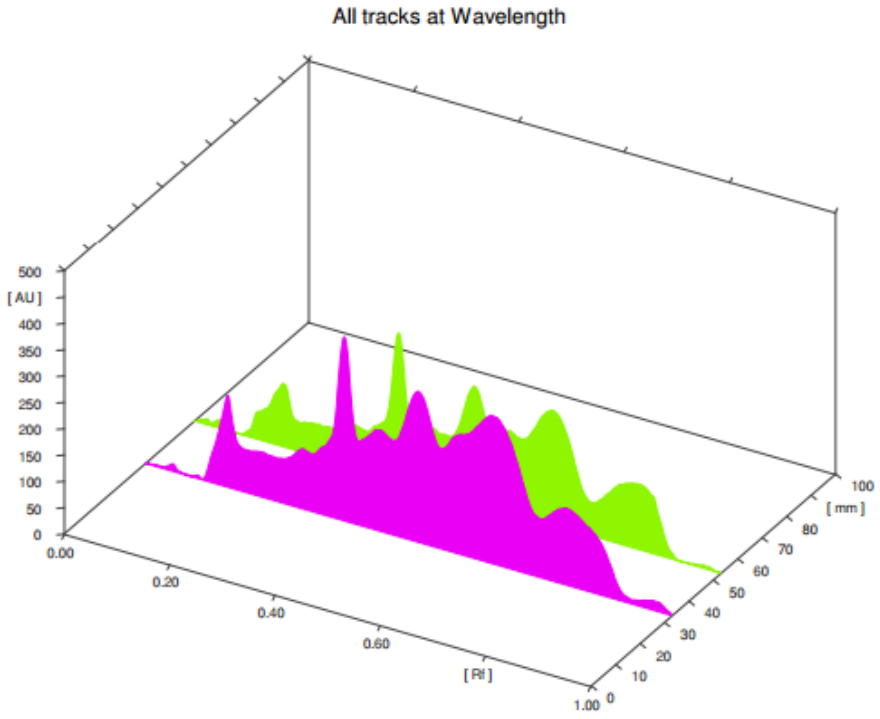

Figure 10: HPTLC fingerprinting overlay of NNG1 and NNGS at 254 nm.

15 min one extra peak was observed). But no high variation in Glycyrrhizin content of all the three samples was noted. This extra peak in NNG2 which was prepared by freeze drying might be due to retention/preservation of some highly heat labile organic constituent. (Tables 5 and 8), (Figures 2, 3, 9).

HPTLC fingerprinting profile is also set in for this work and future reference. NN sample displayed one extra peak at different Rf value at 430 nm in comparison to formulated granules, it might be due to presence of some unknown organic constituent which may be not detected in granules sample and fingerprinting pattern of formulated granules also displaying the excipient in peaks used in the formulation. Higher area percentage in granules batch is displaying higher concentration of constituent. Peak and pattern of HPTLC fingerprinting displayed in NNG1, NNG2 and NNG 3 are almost similar displaying no major change in the formulated granules by different method of drying (Table 6, Figures 4-8).

In short term preliminary accelerated Stability study of water batch NNG1 at 0 and 3 months (denoted as NNGS after three month) was accessed for less than 5% variation.[17] Organoleptic properties at 0 month and at 3 months (NNGS) were found to be similar (Tables 7, and 8), (Figure 10) The retention of sweet taste shows lesser degradation during the storage. In classical Unani text stability of drugs states that until the organoleotic characteristics are in equilibrium drugs are assumed to be stable and any deviation in the Organoleptic characteristics indicates the loss in shelf life of the drug.[53] NNG1 retained stability under accelerated storage conditions. The variation in the difference in HPLC Quantification of Glycrrhizin in NNG1 (day 0) and NNGS (at 3 month) in (%w/w)/100 g of formulations was minimal (less than 5%). Suggest stability of the sample. (Table 7) HPLC profile of NNGS and NNG1 displayed almost similar peaks, suggests stability in respect of other unknown constituents. Recommendation of W. Grimm was followed for this work with modification for predictive factor of 3.3 for zone 4 which include India, for estimation stability condition of 40°C/75% for the period of six months, but with modification this preliminary study was performed for 3 month using same method and formula i. e. 3 x 3.3 =9.9 = 1 year.[54] Formulated granules was stable for 1 year at room temperature in packed condition with this method.

Though Formulated granules are subject to different process of drying and inert and safe permissible excipient are been used in formulating, still as a new modification in combination it should be evaluated for its safety, Mostly reported side effects of glycyrrhizin present in the formulated granules arefluid retention. These effects are related to the inhibition ofcortisol metabolism within the kidney, and the subsequent stimulation of themineralocorticoid receptors.[55] This formulation may need further toxicity evaluations though ADR was reported with very higher glycyrrhizin content and rest of the drug used in the formulation is used traditionally since many decades with no any such reports. Efficacy evaluation is also needed for this

Table 7: Stability study of granules of Water-bath batch (NNG 1).

Parameters	Water-bath (0 month) NNG1	Water-bath (3 month) NNG1 (S)
Bulk density (g/mL)	0.5166±0.01	0.6123±0.01
Tapped density (g/mL)	0.6218±0.008	0.6594±0.007
Car's index (%)	7.0714±0.53	7.1338±0.98
Hausn. Ratio	1.2485±0.004	1.0770±0.01
Angle of Repose(θ)	34.106±0.26	34.447±0.22
LoD at 105°	2.30±0.005	2.32±0.02
Total solid content	30.33±0.16	30.09±0.07
Mucilage content	29.746±0.70	29.166±0.79
pH at 1% Sol.	7.24± 0.01	7.08±0.06
pH at 10% Sol.	6.92± 0.02	6.85±0.11
Total ash (%)	17.16±0.39	16.75±0.19
Acid insoluble ash (%)	12.16±0.39	11.89±0.22
Water soluble ash (%)	3.80±0.17	3.95±0.25
Alcohol soluble extractives (%)	19.30±0.32	18.63±0.16
Water soluble extractives (%)	71.486±0.86	72.463±0.76
Successive extractive values in Solvents		
Pet. Ether (%)	0.05±0.01	0.07±0.01
Benzene (%)	0.33±0.02	0.31±0.01
Chloroform (%)	0.45±0.09	0.55±0.09
Ethanol (%)	16.95±0.15	17.04±0.10
Non-Successive extractive values in solvents		
Pet. Ether (%)	0.05±0.01	0.06±0.01
Benzene (%)	0.62±0.20	0.66±0.07
Chloroform (%)	0.68±0.02	0.71±0.04
Ethanol (%)	35.52±1.9	34.16±0.15
Water (%)	76.50±0.32	76.47±0.23
HPLC Quantification of Glycrrhizin		
Glycyrrhizin content (% w/w) in 100 g of formulations	1.007	1.005
Glycyrrhizin in one sachet (6 g) of formulations	0.060 g (60 mg)	0.060 g (60 mg)
Total microbial count		
Total bacterial count (CFU/g/mL) [Limits as per API 10^5 (100000)]	32500	1200
Total Yeast and Mould count (CFU/g) [Limits as per API 10^3 (1000)]	950	<10

Table 8: Peak detail of HPLC for NNG1 and NNGS (0 and 3 months).

NNG1 at 254nm			NNGS at 254nm		
Peak#	Ret. Time	Area	Peak#	Ret. Time	Area
1	2.662	40722	1	2.668	58378
2	2.885	90308	2	2.906	77451
3	3.042	77701	3	3.043	78697
4	3.458	50777	4	3.488	41404
5	3.628	28479	5	3.650	20670
6	7.978	20310	6	8.153	132601
7	8.119	49563	7	8.456	21922
8	8.349	9417	8	8.715	75861
9	8.590	19114	9	8.942	32844
10	8.795	19298	10	9.142	14852
11	9.215	32258	11	9.406	64850
12	9.439	10801	12	9.657	22822
13	9.812	80305	13	10.077	161158
14	9.984	26583	14	10.281	77422
15	10.290	2124	15	10.633	4058
16	10.483	238928	16	10.854	231862
17	10.676	135248	17	11.076	78836
18	11.076	81078	18	11.836	153189
19	11.337	112492	19	12.075	29750
20	12.023	39693	20	15.296	15330
21	12.166	30755	21	15.569	101702
22	13.951	12126	22	16.004	10823
23	14.145	100035	23	16.235	16159
24	14.610	10311	24	16.754	928
25	14.841	23492	25	17.044	4398
26	16.492	16903	26	17.193	8480
27	16.758	30176	27	17.747	1675
28	17.995	51056	28	18.134	5461
29	19.558	39700	29	18.585	45851
30	20.522	752968	30	19.330	5621
31	21.352	178476	31	19.903	42964
32	21.799	10203	32	20.309	7476
33	25.931	40786	33	20.728	761797
Total		2462184	34	21.472	175812
			35	21.887	9436
			36	25.994	48505
			Total		2641045

formulation which can be done by subjective parameters as a clinical study and it can also be evaluated pre-clinically by *in vitro* and *in vivo* method for related activity of the formulation such as immune assays, anti-viral, in cold and flu etc.

Present study is conducted on commonly used formulation indicated for most common ailment i. e. cold/flu and cough but still is not been standardized and modified in respect of its dosage form which needs further assessment in present scenario. Review of literature suggest that drug of this formulation contain several phytoconstituents such as Saponin, Mucilage, phenolics etc. and various related reported pharmacological activities. Glycyrrhizin (Saponin) present in the formulation inhibits the enzyme 11beta-hydroxysteroid dehydrogenase, which likely contributes to its anti-inflammatory and mineralocorticoid activity.[56] Efficacy of preparation containing these drugs in respiratory diseases for example famous market preparation *Joshina* and *joshandae Nazla* has been validated on experimental animals and exhibit significant antihistaminic, anti-tussive, expectorant, demulcent and anti-inflammatory activity etc.,[57] Formulated granules are a very low-calorie preparation and can be given to cases with calorie restriction and in diabetes which is very rampant in present era. It can be a good alternate when compared to modification of Infusion/decoction in Syrup (*Sharbat*) and *Majoon* (confectionary) which are very high in sugar content.

CONCLUSION

Successful formulation of soluble granules was achieved. Granules prepared by NNG1 method (made by initially drying on water bath) can be a more feasible and economical method. It displayed higher Glycyrrhizin content. Modified soluble granules can provide better acceptability and compliance and can help in improving the drawback of conventional formulation.

ACKNOWLEDGEMENT

The authors are thankful to the Director/authorities of National Institute of Unani Medicine, Bangalore for providing financial assistance and facilities for this study. We also thanks, Natural remedies Bangalore, for the chromatography test.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NN: Naque Nazla; **NNG:** Naque Nazla granules; **NNG 1:** Naque Nazla granules (Waterbath batch); **NNG 2:** Naque Nazla granules (Lyophilizer batch); **NNG 3:** Naque Nazla granules (Rotatory evaporator batch); **NNGS:** Naque Nazla granules (stability); **RMG:** Rapid mixer granulator; **CCRUM:** Central Council for Research in Unani Medicine; **NIUM:** National Institute of Unani Medicine; **R_f:** Retention Time; **RH:** Relative Humidity; **rpm:** Rotation per minute; **SEM:** Standard Error Mean; **SOP:** Standard Operating Procedure; **SSG:** Sodium Starch Glycolate; **TLC:** Thin Layer Chromatography; **USM:** Unani System of Medicine.

AUTHORS' CONTRIBUTIONS

MZ: Writing, Data curation, Investigation, H: Editing, Investigation, Supervision, Methodology, MAA: Investigation, MAW: Writing, Editing.

REFERENCES

1. Zaigham, Hamiduddin, Tauheed A, Ali A. Recent trend in traditional medicine dosage form and present status of Unani and Ayurvedic medicine. Int J Pharm Sci Res. 2019;10(4):1640-49.
2. Azmi AA, Jamali S. Rafatmurad and Zaidi AH., Antibacterial activity of joshanda: A polyherbal therapeutic agent used in common cold. Pak J Pharmacol. 2010; 27(1, January):25-8.
3. Ansari AP, Ahmed ZN, Rather SA, Rafeeqi TA, Beigh BS. Immune boosting and anti-influenza effects of an Unani decoction in influenza like illness and COVID-19 likeepidemics: a rationale approach. Int J Res Med Sci. 2020;8(12):4544-52.
4. Brand E. Global Trends in Concentrated Extracts. Available from: http://www.legendaryherbs.com/.../downloadget. php, [accessed on 11-3-2015].
5. Kabeeruddin H. Shara Asbaab (*Tarjumae Kabeer*). part 1 [reprint]. Delhi: Idara Kitabul Shifa; 2009p. p. 166.
6. Khan A. Haziq. Karachi: Madeena Publishing Company, first published in January 1983. p. 75, 76.
7. Kabiruddin HM, Alqarabadeen. 2006 central council for research. In: Unānī Medicine. New Delhi: GOI, pp.1097,1263.
8. Kabeeruddin M, kabeer B. Part III (Dawasazi), New Delhi, YNM: 23,51, 52.
9. Augsburger LL, Hoag SW. Pharmaceutical dosage forms: tablets. 3rd ed. Vol. II, Rational Design and Formulation. Informa healthcare; 2008, pp. 337,338.
10. Parikh DM. Handbook of pharmaceutical granulation technology. 2nd ed. Vol. 1(2),3. Boca Raton: Taylor & Francis Group; 2005. p. 365-83,513,595,603,896-899.
11. Beringer P, *et al.* Remington the Science and Practice of pharmacy. 21st ed. Vol. I. Lippincott; 2005. pp. 717,785,786,896-899.
12. Sahu SN. The Technology of Preparation and distribution of Drugs and Cosmetics, Kislay Book House. Muzaffarpur, India; 1990. p. 123.
13. Rasheed NM, Gupta VC. Standardization of a compound Unani herbal formulation "Qurs-e Luk" with modern techniques. Pharmacogn Res. 2010;2(4):237-41. doi: 10.4103/0974-8490.69115, PMID 21808574.
14. Winfield AJ, Rees JA, Smith I. Pharmaceutical practice. 4th ed. Churchill Livingstone (Elsevier); 2009. p. 215.
15. Rey L, May JC, editors. Freeze-drying/lyophilization of pharmaceutical and biological products. CRC Press; 2010 June 15. p. 2-30.
16. https://en.wikipedia.org/wiki/Rotary_evaporator, this page was modified on 30 august, 2016 at 07: 52.
17. Anonymous. ICH harmonised tripartite guideline stability testing of new drug substances and products_Products/Guidelines/Quality/Q1A_R2/tep 4/Q1A_R2__Guideline.pdf. 2003;Q1A(R2):1. http://www.ich.org /fileadmin/ Public_ Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/tep 4/Q1A_R2__Guideline.pdf.
18. Anonymous. Stability testing: photostability testing of new drug substances and products, Q1B, 1996http: [cited 19-3-2013]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002647.pdf.
19. Anonymous. Physicochemical standardization of Unani formulations. Part VI. New Delhi: CCRUM. Ministry Of H & FW, Government of India; 2006. p. 142-5.
20. Khan TA, Mallya R, Gohel A. Standardization of marketed Ayurvedic formulation, Balaguloochyadi kashayam-physicochemical, microbial evaluation and ephedrine content. J Appl Pharm Sci; 2016;6(12):184-9. doi: 10.7324/JAPS.2016.601226.
21. Ameena K, Dilip C, Saraswathi R, Krishnan PN, Sankar C, Simi SP. Isolation of the mucilages from *Hibiscus Rosa sinensis* Linn. Asian Pac J Trop Med. 2010;3(7):539-43. doi: 10.1016/S1995-7645(10)60130-7.
22. Anonymous. The Unani pharmacopoeia of India. Part II. 1st ed. Vol. II. New Delhi: GOI Ministry of Health and Family Welfare, Department of AYUSH; 2010:42,120,222,224, 209,217.
23. [cited 31-1-2017]Available from: http://www.brookfieldengineering.com. Vol. 13, P. 9.
24. Anonymous. Protocol for testing Ayurvedic, siddha & Unani medicines, Ghaziabad, Ministery of H & FW, Dept of Ayush Govt. of India, YNM;49-50: 122, 36.
25. Anonymous. National formulary of Unani medicine. Part–II. New Delhi: Ministry of Health and Family Welfare, Govt of India; 2011: pp. 17,33,54,136,139, 146-152, 185.
26. Anonymous. Indian pharmacopoeia. Vol. I. New Delhi: Government of India, Ministry of Health and Family Welfare; 2010. p. 201.

27. Lumay G, Boschini F, Traina K, Bontempi S, Remy JC, Cloots R, *et al.* Powder technology, Measuring the flowing properties of powders and grains, journal homepage. 2012:19-27. Available from: http://www.elsevier.com/locate/powtec.
28. Anonymous. The Japanese pharmacopoeia. Japan: Ministry of Health, Labour and Welfare; 2006.
29. Anonymous. The United States pharmacopoeia 30-NF. Vol. 25 (e-book); 2007.
30. Sharma A, Shanker C, Tyagi LK, Singh M, Rao CV. Herbal medicine for market potential in India: an overview. Acad J Plant Sci. 2008;1(2):26-36.
31. Agrawal SS, Paridhavi M. Herbal drug technology, Part II. Hyderabad Universities Press; 2007. p. 320.
32. Venkatesan D, Karrunakarn CM, Kumar SS, Swamy P. Identification of phytochemical constituents of Aegle marmelos responsible for antimicrobial activity against selected pathogenic organisms. Ethnobotanical Leafl. 2009;13:1362-72.
33. Anonymous. Physicochemical standardization of Unani formulations. Part IV. New Delhi: CCRUM. Ministry Of H & FW, Government of India; 2006. p. 160.
34. Gautam SS, Navneet S, Kumar S. The antibacterial and phytochemical aspects of *Viola odorata* Linn. extracts against respiratory tract pathogens. Proc Natl Acad Sci India Sect B Biol Sci. 2012 December 1;82(4):567-72. doi: 10.1007/s40011-012-0064-7.
35. Total carbohydrate protocol. Ebru Dulekgurgen. UIUC04 [cited 12-1-2016]. Available from: http://web.itu.edu.tr/~dulekgurgen/Carbs.pdf.
36. Başkan KS, Tütem E, Akyüz E, Özen S, Apak R. Spectrophotometric total reducing sugars assay based on cupric reduction. Talanta. 2016;147:162-8. doi: 10.1016/j.talanta.2015.09.049, PMID 26592591.
37. Anonymous. The ayurvedic pharmacopoeia of India; Part II. 1st ed. Vol. II. New Delhi: Department of AYUSH; 2008:(Appendices 1 to 5). p. 193.
38. Abdullah IH, Inayat H, Khan H, Khan L, Khan MI, Hassan S, et al. *In vitro* biological activity of decoction of Joshanda. Pak J Pharm Sci. 2014;27:239-43. PMID 24577908.
39. Khan H, Khan MA, Muhammad N, Ashraf N, Gul F, Tariq SA. Anti-inflammatory and antioxidant activity of joshanda Partially mediated through inhibition of lipoxygenase. Phytopharmacology. 2012; 3(1),p.p:19-28.
40. Muralidharan P, Balamurugan G, Babu V. Cerebroprotective effect of *Glycyrrhiza glabra* Linn. root extract on hypoxic rats. Bangladesh J Pharmacol. 2009;4:60-4.
41. Vispute S, Khopade A, '*Glycyrrhiza glabra* Linn. - "klitaka": a review' International Journal of Pharma and Bio Sciences 2011;2:49.
42. Vishal A, Parveena k, Pooja S, Kannappan N, Kumar S. Diuretic, laxative and toxicity studies of *Viola odorata* aerial Parts'. Pharmacologyonline. 2009;1:739-48.
43. Rowe RC, Sheskey PJ, Quinn ME. Handbook of pharmaceutical excipients. 6th ed. Vols. 364-65, 418-20,663, 664. Pharmaceutical Press; 2009. p. 1, 2,3,136, 665, 685-90.
44. Craig LC, Gregory JD, Hausmann W. Versatile laboratory concentration device. Anal Chem. 1950;22(11):1462. Cited in: http://en. wikipedia.org/wiki/Rotary evaporator. Wikipedia (accessed on 14-4-2015].
45. Abba D, Inabo HI, Yakubu SE, Olonitola OS. Contamination of herbal medicinal products marketed in Kaduna Metropolis with selected pathogenic bacteria. Afr J Trad CAM. 2009;6(1):70-7.
46. Malviya R, Srivastava P, Kulkarni GT. Applications of mucilages in drug delivery-a review. Adv Biol Res. 2011;5(1):1-7.
47. Anonymous. The wealth of India. Vol. II. New Delhi: COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH; 2002: pp.187,201).
48. Kirtikar KR, Basu BD. Indian medicinal plants. Second edi. Vol. I. Dehradun: International Book Distribuors; 2008: pp.207,208,296.
49. Khare CP. Indian medicinal plants. Vol. 289(290),395,706,736. New Delhi: Springer (India) Privet Limited; 2007. p. 40,97,173.
50. Sandhya S, Gowthami G, Vinod KR, Sravanthi EV, Saikumar P, Rao KN, *et al.* Formulation and evaluation of herbal effervescent granules incorporated with *Limnophila indica* extract for bacillary dysentery. Annals Biol Res. 2012;3(1):63-72.
51. Bharate SS, Bharate SB, Bajaj AN. Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: A comprehensive review. J Excipients Food Chem. 2010;1(3):3-26.
52. Available from: https://en.wikipedia.org/wiki/Glycyrrhizin#cite_note-12, accessed on0 6/03/2017. Wikipedia.
53. Lateef A. Tauzihat-e-Kulliyat-e-Advia, Ibn-e-Sina academy of medieval medicine and sciences. Aligarh; 2002. p. 251.
54. Grimm W. Extension of the International Conference on Harmonization Tripartite Guideline for Stability Testing of New Drug Substances and Products to countries of climatic zones III and IV. Drug Dev Ind Pharm Extension of international conference on harmonisation tripartite. 1998;24(4):313-25. doi: 10.3109/03639049809085626, PMID 9876591.
55. Ferrari P, Sansonnens A, Dick B, Frey FJ. *In vivo* 11beta-HSD-2 activity: variability, salt-sensitivity, and effect of licorice. Hypertension. 2001;38(6):1330-6. doi: 10.1161/hy1101.096112, PMID 11751713.
56. Asl MN, Hosseinzadeh H. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. Phytother Res. 2008 Published online in Wiley InterScience; 22(6):709-24. doi: 10.1002/ptr.2362, p.709. PMID 18446848.
57. Ahuja VK, Gulati M, Lakhanpal S, Goswami M, Ahmad S. Joshanda: a traditional herbal approach for treatment of respiratory catarrh. Curr Respir Med Rev. 2009;5(2):81-9. doi: 10.2174/157339809788190011.

Cite this article: Zaigham M, Hamiduddin, Ali MA, Waheed MA. Modification and Physicochemical Evaluation of *Naque Nazla* a Unani Infusion Preparation for Cold and Flu into Soluble Granules. Pharmacog Res. 2024;16(4):837-53.