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Original Research

Formulation Development, Characterization and Antimicrobial Activity Evaluation of Sugar-Based and Sugar-Free Syrup Prepared with the Ingredients of *Jushanda-Nazla*

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Abstract

Jushanda (decoction), often used in traditional medicine, has many drawbacks like disagreeable taste, bulky dose, cumbersome preparation, and short stability. Therefore, in the present study, *jushanda nazla* (JN) was modified and optimized into a sugar-based (SBS) and sugar-free (SFS) syrup dosage form and evaluated for various physicochemical parameters, microbial contaminations and antimicrobial activities. JN was concentrated and reduced to minimum quantity by heating and twelve batches of SBS and five batches of SFS were prepared for optimization. Out of these, the best batch of SBS and SFS were selected based on consistency and minimum bulk. The best batches were further evaluated for the physicochemical parameter, microbial and heavy metal contamination. The finished products were also evaluated for antimicrobial activity against selected microbes. SBS was optimized with 170 mL JN reduced to 50 mL, sugar 40% (w/v) and consistency of one wire. SFS was optimized with 170 mL JN reduced to 30 mL, and 96 mg of aspartame. Organoleptic characters, ash value, viscosity, specific gravity and pH of finished products were satisfactory. The concentration of total phenolic, flavonoid, tannin, alkaloid and glycyrrhizin and chlorogenic acid were comparative in JN, SBS and SFS. SBS and SFS passed the WHO guideline for microbial and heavy metal contamination. Finished products showed significant antimicrobial activity against *Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae* and *Salmonella*. Analytical data of SBS and SFS showed significant optimistic results hence, generated data can be used for future reference. However, developed formulations should be further evaluated for their stability, safety and clinical efficacy.

Keywords: Antimicrobial agent; Chlorogenic acid; Glycyrrhizin; *Jushanda*; Unani medicine

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Introduction

Drugs rarely occur in nature in their useful form. Most of the time drug substances are required to be converted into a specific dosage form to make it palatable according to patients' requirements and disease conditions. Organoleptic characters of a dosage form and availability of the same drug in the different dosage forms play a very important role in patient compliance and for consistent use of a drug for successful completion of the treatment course [1].

In the traditional system of medicine, especially in the upper and lower respiratory tract disorders and during *munzij wa mushil* (body detoxification) therapy, drugs are given in the form of *jushanda* (decoction). To prepare this dosage form plant materials are soaked in the water overnight *Cordia myxa* L. and then boiled to extract plant material in the water. Though, *jushanda* has some advantages like containing active/required ingredients of plant material in dissolved form in water and quick absorption. On the other hand, it has many drawbacks like it often has a unpleasant taste and has to be taken in large quantity. *Jushanda* has to be freshly prepared every time before use, as it has a very short shelf life and is highly vulnerable to microbial contamination. Furthermore, the raw ingredients of the *jushanda* formulation are bulky and should be dispensed in paper cover or polythene bag. Therefore, patients' compliance with *jushanda* is very low [2].

Jushanda may be modified into *sharbat* (syrup), a dosage form commonly used in Unani medicine. In comparison to *jushanda*, *sharbat* possesses remarkable taste-masking properties for bitter drugs, as it contains sugar as a sweetening agent and flavours. The syrup is readily acceptable by children, old age patients and nauseous persons [3]. It is more portable than *jushanda* due to comparatively lesser bulk and has a higher shelf life.

Sugar-based syrups (SBS) are in use since ancient times in traditional medicine and *jushanda* formulation can be easily modified into SBS. However, this concept is not compatible with the present emerging disease scenario related to diabetes and lifestyle diseases. Current diabetic studies are disappointing in view of the sugar-based syrups dosage form as *sharbat* prepared with classical Unani method usually contains more than 65% sugar. Hence, diabetic patients or patients on restricted calorie intake cannot take this dosage form [4]. Additionally, SBS may cause dental carries and diminish oral hygiene. Furthermore, many people do not like very sweet taste [5].

So, there is an immense need to formulate low calorie or sugar-free syrup (SFS) for the formulations used in the form of *jushanda*.

Jushanda nazla (JN) is one such formulation commonly used in Unani medicine since ancient times to treat upper respiratory tract infections, allergic upper respiratory disorders and influenza. These are the most prevalent upper respiratory tract disorders in the general population [6]. Adults may experience common colds 2-5 times per year, and children may experience 7-10 colds per year [7]. Owing to the high incidence, especially among children, common cold creates a significant economic and social burden and is the most important cause for individuals

missing from work and school [6].

Many over-the-counter and prescription-based medications are available in the conventional system of medicine to treat this disease. However, many drugs are found ineffective or have a strong adverse effect especially in children, including dryness of the mouth due to anticholinergic effects, sedation, paradoxical excitation, respiratory depression, nausea, vomiting and abdominal pain [8].

Therefore, frequently physicians and patients turn towards alternative medicine to treat this common problem.

In the present study, SBS and SFS dosage forms were developed with the JN. Aspartame was used as a sweetening agent in SFS. Physico-chemical standardization of prepared SBS and SFS were carried out and compared with the original dosage form i.e. JN. Also, the antibacterial activity of all three dosage forms was evaluated against gram-positive and negative bacteria.

Materials and Methods

Procurement of raw drugs

The ingredients of formulation were procured from herbalist/raw drug dealer at Bengaluru, Karnataka, India during April and May 2017. Plant materials were identified and authenticated by Pharmacognosist, Dept. of Pharmacognosy, Centre for Repository of Medical Resources (C-RMR), Trans-Disciplinary University (TDU) under FRLHT, Bengaluru. (Accession number 4591, 4592, 4593, 4594). Purchased plant materials were cleaned from impurities and dirt and stored in airtight containers.

Ingredients of jushanda nazla

Baheedana (*Cydonia oblonga* Mill) seeds 3 g, *aslussoos* (*Glycyrrhiza glabra* L.) root 6 g, *unnab* (*Ziziphus jujuba* Mill.) fruits 7 nos. (about 7 g), *sapistan* (*Cordia myxa* L.) fruits 20 nos. (about 10 g), and water 340 mL [9].

Preparation of JN

All the ingredients (in proportion as mentioned above) except *baheedana* were crushed and soaked in sterile water overnight. It was boiled in the morning at low flame until reduced to half. *Baheedana* was soaked in distilled water overnight alone and its mucilage was separated in the morning by manual scrubbing. Mucilage was added to the prepared decoction at the end and mixed rigorously [9].

The liquid was filtered through muslin cotton cloth and allowed to cool. After cooling at room temperature decoction (JN) was stored in an airtight amber colour glass bottle in the fridge below 4 ºC temperature and used for the preparation of various batches of SBS and SFS and their analytical tests. Prepared JN was used within 3 days period.

Preparation of sugar-based syrup

To prepare the SBS, JN was concentrated and reduced to a minimum quantity by heating and evaporation on low flame. Sugar was added after the specific volume of JN was achieved. The consistency of SBS was maintained as mentioned in classical Unani literature [10]. Sodium benzoate 0.2% and citric acid 0.2% were added

at the end of the process as a preservative [11]. The finished SBS was stored in amber colour, dry air-tight and sterile glass bottle at room temperature.

Preparation of sugar-free syrup

To prepare the SFS, JN was concentrated and reduced to the minimum possible quantity till it approaches the *sharbat* like consistency by heating and evaporation at low flame. As soon as the specific consistency of *sharbat* was achieved, aspartame was added considering its potency 1:200 in comparison to sugar/sucrose [12]. Sodium benzoate 0.2% and citric acid 0.2% were added at the end of the process as a preservative. After cooling at room temperature finished SFS was stored in an amber colour, dry sterile and air-tight glass bottle at room temperature.

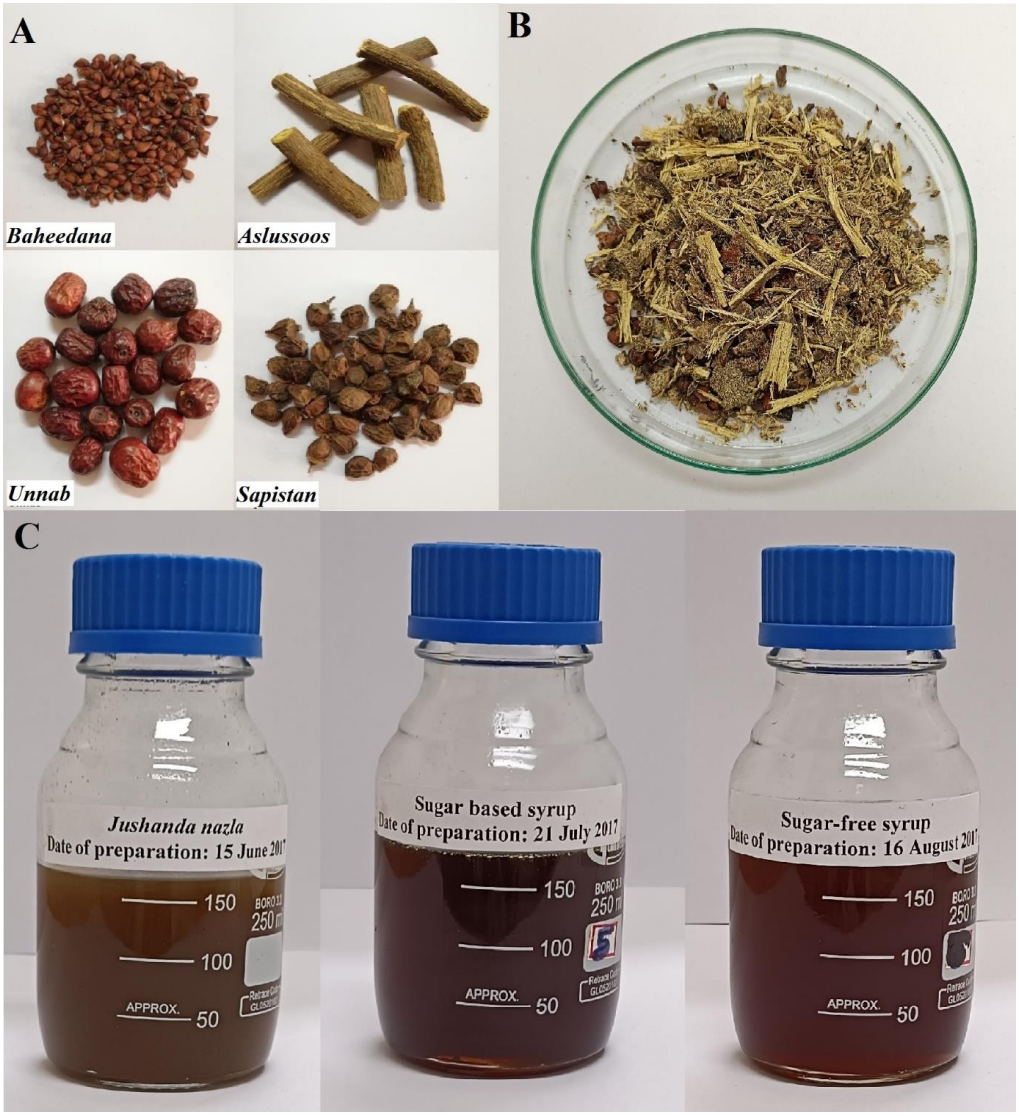

Figure 1. Preparation of *jushanda nazla* and its syrup; A) Ingredients of *jushanda nazla*, B) Ingredients crushed for the preparation of *jushanda nazla*, C) Prepared *jushanda nazla*, sugar based syrup and sugar free syrup

Optimization of the finished product

SBS and SFS were optimized for: 1) minimum dosage unit by reducing it to minimum bulk and 2) viscosity. The bulk was reduced and viscosity was increased in geometric progression by heating and evaporation and multiple batches were prepared. Out of these batches, the best batch was selected based on minimum bulk, suitable consistency and viscosity. Consistency and viscosity were fixed by the classical parameter and Brookfield viscometer. Syrup giving one wire when a drop of syrup kept between thumb and index finger than compressed and removed or at the end of ladling; and drop of *sharbat* should not spread when kept on the smooth surface and maintain spherical shape were taken as a classical parameter to assess the consistency of SBS and SFS as per the classical method.

Evaluation parameters

Organoleptic characters

The appearance was recorded according to consistency, whether it was semisolid, liquid, homogenous, crystallization, sedimentation. The colour of the finished product was decided by using a Pantone colour chart and a specific number/code was assigned along with the colour name [13].

To evaluate the odour 50 mL sample was kept in a beaker. Samples were examined by slow and repeated inhalation of air over the material after taking a few deep breaths in the fresh open air. First, the strength (graded as none, weak, moderate distinct and strong) and then quality (assigned as aromatic, fruity, musty, mouldy, rusty, pungent) of the odour was determined. The taste was evaluated after cleaning the mouth a few times with plain drinking water. The sample was placed on the tongue and first its strength (graded as weak, distinct, strong) and then quality like sweet, bitter, pungent, oily was evaluated [14].

Physico-chemical evaluation

All test samples were evaluated for ash value (total ash, water-soluble ash, and acid insoluble ash), viscosity, specific gravity, pH (in pH of 1% and 10% solution) [15]. Under preliminary phytochemical tests for organic constituents, total alkaloids, proteins, glycosides flavonoids, tannins, phenols, carbohydrate [16], phytosterols/terpenes, diterpenes [17], quinones, anthraquinones and coumarins [18] were carried out.

Quantitative analysis

Total sugar: Spectrophotometric method using anthron reagent was employed for estimation of total sugar present in decoction, SBS, and SFS free syrup. H2SO4 solution (75%) was prepared a day before the experiment (500 mL volumetric flask containing 100 mL of DDW placed in an ice bath on magnetic stir and adding 390 mL of 95-97% H2SO4 in volumetric flask slowly and carefully, after cooling down the volume was adjusted to 500 mL with DDW). Glucose standard solution was prepared by dissolving 0.05 g glucose with 500 mL DDW (stock solution). The final stock concentration was 100 mg glucose/litre.

Different aliquots of standard glucose (200 µg/mL) were taken in different test tubes and the

volume was made up to 1 mL with distilled water in all the test tubes. Later, 4 mL of anthrone reagent (200 mg of anthrone in 100 mL of 95% sulphuric acid in ice cold condition) was added to all the tubes and incubated in a boiling water bath for 8 minutes and cooled rapidly. The absorbance was read at 630 nm. The test samples were also processed similarly by taking 100 µL of the sample. The standard graph was plotted and the amount of carbohydrates in each sample was calculated [19].

Reducing sugar: Spectrophotometric method was used for the estimation of reducing sugar of the finished product. Dinitro salicylic acid (DNS) (1 g) was dissolved in 50 mL of 2N NaOH and 40 g of sodium-potassium tartrate was dissolved in 100 mL of distilled water. Both solutions were mixed well and the volume was increased up to 250 mL using distilled water. The stock solution was prepared with 100 mg of glucose dissolved in 100 mL of distilled water and made up to the volume of 1000 mL. The concentration of glucose in the stock solution was 1 mg/mL. In one test-tube blank sample was prepared, and in other test-tube working standard solution was taken in 0.2, 0.4,0.6, 0.8, and 1 mL and made up to 1 mL by adding the required amount of distilled water. To prepare the test sample, 1 g SBS was taken into a 200 mL flask and made up to the volume using DDW. The test sample of SFS was prepared by taking 1 g syrup in a 20 mL flask and made up to the volume using DDW. Samples were dissolved, filtered and 0.2 mL of filtrate was taken in the 7th test tube. Then 3 mL DNS reagent was added to all test tubes to react and 5 mL of distilled water was added to all the test tubes. It was boiled in the water bath for 15 minutes and cooled. Absorbance was measured at 540 nm. The concentration of reducing sugar was calculated by the graph plotted against the concentration of glucose and optical density at the X and Y axis respectively [20].

Total phenolics: The amount of phenolic compound in the test samples was determined by Folin-Ciocalteu reagent method with some modifications. 2.5 mL of 10% Folin Ciocalteu reagent and 2 mL of 2% solution of Na_2CO_3 was added to 1 mL of the test drug. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid was used as standard (1 mg/mL). The results were determined from the standard curve and were expressed as gallic acid equivalent mg/g of test sample [21].

Total flavonoids: The aluminium chloride colorimetric assay was used for total flavonoids determination. The test drug (100 µL) was mixed with 2.5 mL of distilled water and 300 µL of 5% sodium nitrate. Then, it was incubated at room temperature for 5 minutes and 300 µL of 10% aluminium chloride, 2 mL of 1 M sodium hydroxide and 1 mL of distilled water were added. Then, the absorbance of the reaction mixture was measured at 512 nm, along with the standard quercetin and blank. The total flavonoids content was determined by comparing the test drug and standard quercetin curve prepared from a reference solution containing quercetin in different concentrations. Values were expressed as microgram (quercetin equivalent)

per mL [21].

Total tannin: Estimation of total tannin content in decoction, SBS and SFS was carried out by the Folin Ciocalteu method. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) was pipetted into different test tubes. Distilled water (7.5 mL) was added to all the test tubes. Further, 0.5 mL of Folin Ciocalteu phenol reagent and 1mL of 35% Na_2CO_3 solution was added and made up the volume to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV-Visible spectrophotometer. The tannin content was expressed in terms of µg of GAE/g of test drug [22].

Total alkaloid: Total alkaloids were determined by the spectrophotometric method using bromocresol green. Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 mL of 2N NaOH and 5 mL distilled water until completely dissolved and the solution was diluted to 1000 mL with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (7.16 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Caffeine standard solution was made by dissolving 1 mg caffeine in 10 mL distilled water. To prepare the standard curve, accurately measured aliquots (0.2, 0.4, 0.6, 0.8 and 1 mL) of caffeine standard solution was taken and transferred each to different separatory funnels. Then, 5 mL of pH 4.7 phosphate buffers and 5 mL BCG solution was taken and shake a mixture with 1, 2, 3 and 4 mL of chloroform. The test drugs were collected in a 10 mL volumetric flask and diluted to adjust volume with chloroform. Further phosphate buffers and BCG solution were added as per the method. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without caffeine [23].

High-Performance Liquid Chromatography condition

Quantitative analysis of glycyrrhizin and chlorogenic acid was carried out using HPLC analysis. The HPLC apparatus consisted of Waters HPLC systems with 510 series pump, an autosampler, and a diode array detector linked to an Empower chromatography data system, The separation of the glycyrrhizin and chlorogenic were carried out on a Hiber, prepacked column, Li Chrospher 100, RP-18e (i.d.5 µm, Size 125 × 3 mm, Merck Germany), secure with another guard column of the same material (4 × 4 mm). A combination of two solvent systems was used as a mobile phase. To prepare the solvent systems A, anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) (0.136 g) was dissolved in 900 mL of distilled water and orthophosphoric acid (0.5 mL) was added. Further, the mixture was made up to 1000 mL with distilled water. Acetonitrile was used as solvent B. Both solvents systems were filtered through a 0.45 µm membrane and degassed in a sonicator for 3 minutes before use. The gradient condition employed for solvent A and solvent B in HPLC is elaborated in Table 1. The standard glycyrrhizin sample (CAS Number: 1405-86-3 Merck) in

concentration of 0.15 mg/mL and chlorogenic acid (CAS Number: 327-97-9 Merck) in concentration of 0.13 mg/mL were prepared using HPLC-grade water. Test samples of decoction, SBS and SFS were prepared by dissolving 500 mg drug in 100 mL distilled water. Glycyrrhizin and chlorogenic acid were analysed at 254 nm and 330 nm wavelengths, respectively, with the injection volume of 20 µL, a flow rate of 1.5 mL/min [24].

Calculations:

Quantitiy of molecule=(Area of sample)/(Area of the standard)×(Weight of the standard)/(Standard dilution)×(Sample dilution)/(Weight of the sample)×Purity of standard

Table 1. Gradient condition employed for solvent A and solvent B in HPLC

Sr. No.	Time (min)	Buffer concentration (solvent A)	Acetonitrile concentration (Solvent B)
1.	0.01	95.0	5.0
2.	18.0	55.0	45.0
3.	25.0	20.0	80.0
4.	28.0	20.0	80.0
5.	35.0	55.0	45.0
6.	40.0	95.0	5.0
7.	45.0	95.0	5.0

Tests for heavy metals: The presence of heavy metals (Pb, As, Cd and Hg) was tested only for the decoction using Inductively Couple Plasma Optical Emission Spectrometry (ICP-OES) [25].

Microbial contamination

All three samples were tested for the presence or absence of total viable aerobic count, yeast count and specific pathogens i.e. *Escherichia coli, Salmonella, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Microbial analysis was carried out as per the USP guidelines [26].

Antibacterial activity

Resazurin assay utilising microtitre plate (96 wells) was used to check the minimum inhibitory concentration (MIC) of test samples (JN, SBS and SFS) against 24 h cultured *Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus mutans, Staphylococcus aureus, Bacillus cereus, Haemophilus influenzae, Salmonella,* and *Escherichia coli*. All used microbes were clinical isolates.

Preparation of bacterial culture: Using the aseptic method a colony of each microorganism was added into a 100 mL bottle of iso-sensitest broth, tightly covered with the cape and kept in an incubator for 12 h at 35 °C. Then, under aseptic precaution clean sample of bacteria was obtained using a centrifuge at 4000 rpm for 5 min. The supernatant was discarded and sediment was again suspended in 20 mL sterile normal saline and recentrifuged at 4000 rpm for 5 min. This procedure was repeated until the supernatant was transparent. Now sediment was suspended in 20 mL of sterile normal saline, and optical density was recorded at 500 nm. Further, the optical density was adjusted in the range of 0.5 to 1.0 employing serial dilutions with the microbial concentration of 5 × 10 cfu/mL. Iso-sensitest broth was used as culture media in this essay [27].

Preparation of resazurin solution: The resazurin

solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water using a vortex mixer.

Preparation of the plates: Under aseptic conditions rows on the plate were labelled numerically and columns were numbered alphabetically. A volume of 100 µL of the test material was pipetted into the first row of the plate. Nutrient broth (50 µL) was added to all other wells and serial dilutions were performed in decreasing concentrations. Resazurin 0.1% (30 µL) solution was added to each well. Further, 30 µL iso-sensitised broth was added to each well. Finally, 10 µL of prepared bacterial culture was inoculated to each well to achieve a concentration of 5×10 cfu/mL. Each microbe was inoculated in a separate column. A column was used as a control with all solutions except bacterial inoculation and instead 10 µL of nutrient broth was added to maintain the volume. One column was inoculated with all solutions except the test or standard drug. Each plate was wrapped loosely with a cling sheath to avoid bacteria from dehydration. Plates were placed in a BOD incubator at 37 °C for 24 h. After completion of 24 h, colour change was assessed visually. The appearance of colour change in any column from purple to pink was recorded. The presence of blue and pink colours was recorded as no growth and growth respectively. The minimum concentration at which the purple colour appeared was taken as the MIC value. Tests were carried out in triplicate and an average of three values was calculated as the MIC for the test sample [28,29].

Results

Optimization of sharbat and sugar-free syrup of JN

In the present study for the optimization of SBS, in total 16 batches were prepared. Out of which, formulation prepared with JN 170 mL reduced to 50 mL and sugar 40% (w/v) added gave one *tar* (wire) consistency. This formulation was exactly as viscous as the consistency of *sharbat* mentioned in classical Unani text. Hence, this batch was finalized as an ideal batch (Table 1). To prepare SFS in total five batches were tried. Out of which a batch prepared with JN (170 mL) reduce to 30 mL, added 96 mg aspartame and gave viscosity of classical *sharbat* were finalised as an ideal batch (Table 1).

Table 2. Optimization of sugar-based and sugar-free syrup prepared with the ingredients of *Jushanda nazla*

Sr. No	Water used (mL)	Decoction used (mL)	Volume of prepared syrup (mL)	Sugar (%) or Aspartame (mg)	Consistency	Remark
Optimization of sugar-based syrup						
1.	340	170	60	70	Viscous	Rejected
2.	340	170	50	70	Highly viscous	Rejected
3.	340	170	40	70	Viscous as *laooq*	Rejected
4.	340	170	30	70	More viscous than *laooq*	Rejected
5.	340	170	60	60	Viscous	Rejected
6.	340	170	50	60	More viscous	Rejected
7.	340	170	40	60	Highly viscous	Rejected
8.	340	170	30	60	Viscous as *laooq*	Rejected

9.	340	170	60	50	Highly viscous	Rejected
10.	340	170	50	50	Highly viscous	Rejected
11.	340	170	40	50	Highly viscous	Rejected
12.	340	170	30	50	Viscous as *laooq*	Rejected
13.	340	170	60	40	Viscous	Rejected
14.	**340**	**170**	**50**	**40**	**One tar exactly viscous as *sharbat***	**Accepted**
15.	340	170	40	40	Viscous	Rejected
16.	340	170	30	40	Highly viscous	Rejected
Optimization of sugar-free syrup						
1.	340	170	10	32	Highly viscous	Reject
2.	340	170	15	48	Highly viscous	Reject
3.	340	170	20	64	More viscous	Reject
4.	340	170	25	80	viscous	Reject
5.	**340**	**170**	**30**	**96**	**Viscous as a syrup**	**Accepted**

Physico-chemical and microbial contamination evaluation of SBS and SFS

Prepared SBS was dark brown, thick viscid liquid with a moderate sweet pleasant smell and sweet test. SFS had the almost same organoleptic character except it has a sweet taste with a slight bitterness at the end.

Qualitative analysis showed the presence of alkaloids, glycosides, flavonoids, tannins, phenols, carbohydrate, phytosterols/ terpenes, diterpenes, quinones and coumarins whereas, anthraquinone and proteins were absent in both SBS and SFS. Quantitative analysis values for secondary plant metabolites are presented in table 2.

Evaluation of heavy metals in JN using Inductively coupled plasma - optical emission spectrometry (ICP-OES) exhibited the lead, arsenic, cadmium and mercury within the permissible limit (Table 4). Microbial contamination analysis for SBS and SFS qualified the WHO limits (Table 2).

Table 3. Characterization of *jushanda nazla*, sugar-based syrup and sugar-free syrup

Sr. no.	Test	Jushanda nazla	Sugar-based syrup	Sugar-free syrup
1.	Organoleptic character			
	• Appearance	Thinly viscid liquid	Thinly viscid liquid	Moderately viscid liquid
	• Colour	Light brown Panton 464 C	Dark brown Panton 476 C	Slightly dark brown Panton 4695 C
	• Smell	Moderate, pleasant,	Thinly viscid liquid	Moderate, pleasant
	• Taste	Bitter and slightly sweet	Dark brown Panton 476 C	Sweet and slightly bitter
2.	Ash value (%)			
	• Total ash	0.26 ± 0.01	0.44 ± 0.01	0.34 ± 0.01
	•Acid insoluble	0.14 ± 0.01	0.22 ± 0.01	0.16 ± 0.01
	• Water soluble	0.06 ± 0.00	0.12 ± 0.01	0.10 ± 0.001

3.	Viscosity (using spindle 5)	113	1050	493
4.	Specific gravity	1.01 ± 0.05	1.14 ± 0.01	1.09 ± 0.01
5.	pH			
	• 1% solution	6.24	5.16	5.91
	• 10% solution	5.97	5.84	5.83
6.	Quantitative analysis			
	• Total sugar (µg/µL)	279.89	413.96	329.40
	• Reducing sugar (µg/µL)	76.294	120.419	109.946
	• Total phenolic (gallic acid equivalent µg/mL)	8.54	19.853	32.446
	• Total flavonoid (quercetin equivalent µg/mL)	0.107	0.246	0.279
	•Total tannin (gallic acid equivalent µg/mL)	0.58	1.5	2.9
	•Total alkaloid (mg/mL)	0.157	0.260	0.283
	•Glycyrrhizin (µg/µL)	0.13	0.24	0.32
	• Chlorogenic acid (µg/µL)	0.0001	0.0003	0.0006
7.	Microbial contamination analysis			
	• *Total bacterial count (Cfu/gm/mL)			
	• #Total fungal count (Cfu/gm/mL)			
	$Pathogenic bacteria			
	• Escherichia coli	Absent	Absent	Absent
	• *Salmonella*	Absent	Absent	Absent
	• Staph. aureus	Absent	Absent	Absent
	• P. aeruginosa	Absent	Absent	Absent

*WHO limit for total bacterial count: 10^5 cfu/gm/mL; # WHO limit for total fungal count: 10^3 cfu/gm/mL; $WHO limit for pathogenic bacteria: absent

Table 4. Evaluation of heavy metals in *jushanda nazla* using ICP-OES

Sr. No.	Metals	Results (in ppm)	Permissible limit (in ppm)
1.	Lead (Pb)	0.116	10
2.	Arsenic (As)	0.026	03
3.	Cadmium (Cd)	0.073	0.3
4.	Mercury (Hg)	0.013	01

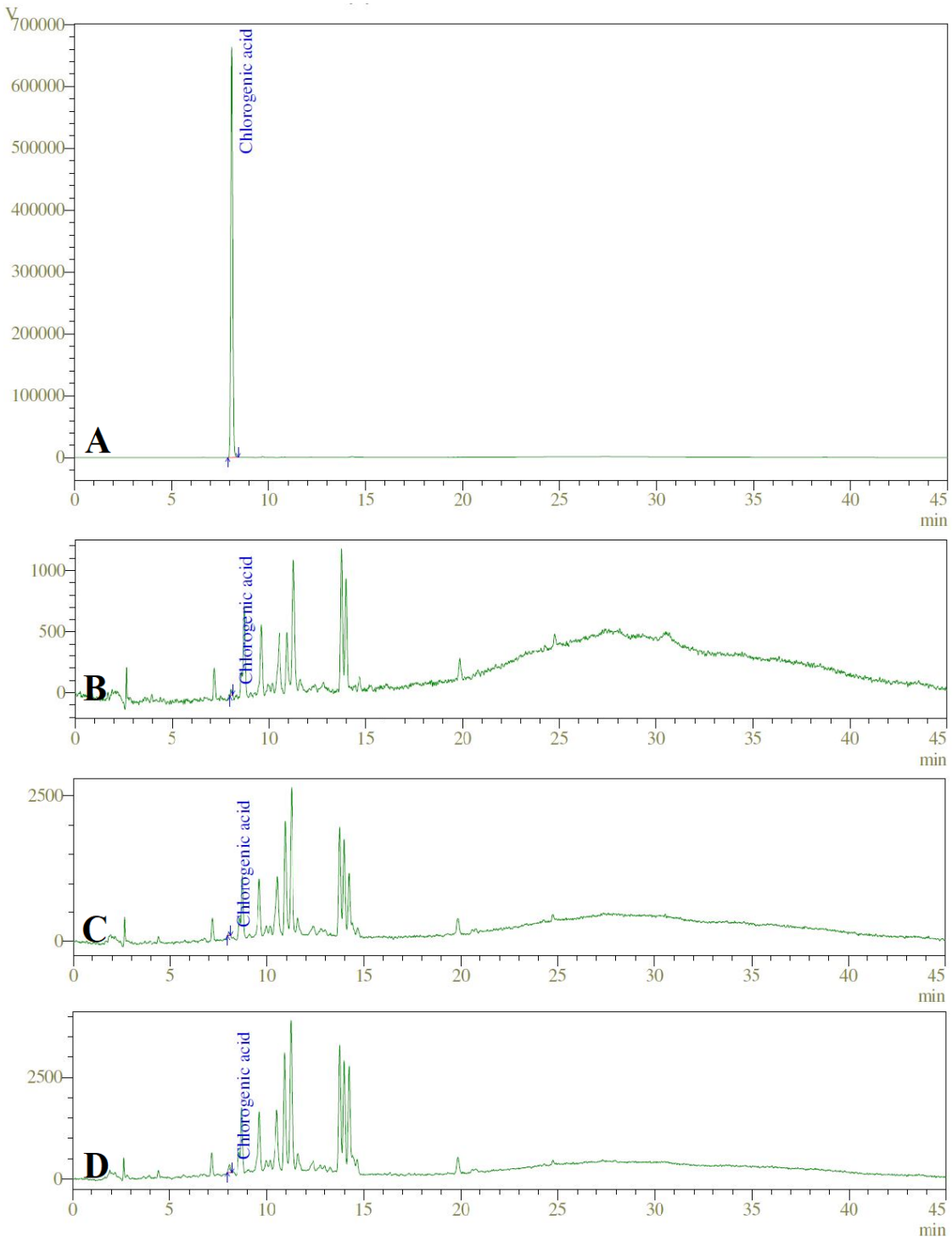

Figure 2. HPLC Chromatogram of A) standard chlorogenic acid, B) chlorogenic acid in *jushanda nazla*, C) chlorogenic acid in sugar-based syrup of *jushanda nazla*, and D) chlorogenic acid in sugar-free syrup of *jushanda nazla*

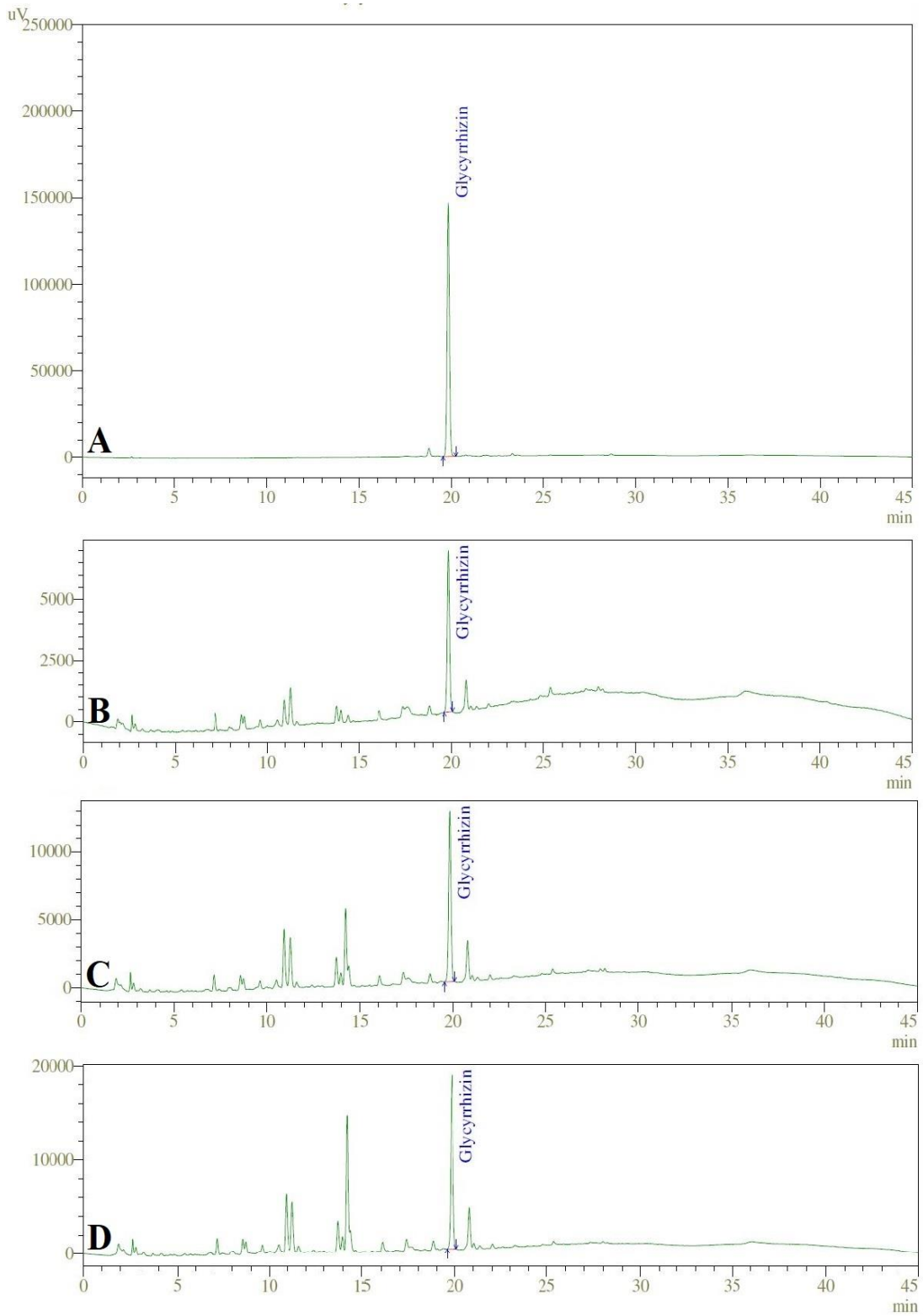

Figure 3. HPLC Chromatogram of A) standard glycyrrhizin, B) glycyrrhizin in *jushanda nazla*, C) glycyrrhizin in sugar-based syrup of *jushanda nazla*, and D) glycyrrhizin in sugar-free syrup of *jushanda nazla*

Antimicrobial effects of sharbat and sugar-free syrup

SFS showed a potent effect against *S. pyogenes*, *S. pneumonia* and *H. influenzae* (2.5 µg/µL) followed by *Salmonella* (3.125 µg/µL). However, JN and SBS [except SFS against *H. influenzae* (2.5 µg/µL)] exhibited a weak or very weak antimicrobial effect on selected micro-organisms. SFS also exhibited a very weak antimicrobial effect against *S. mutans* (13 µg/µL) and B. cereus (25 µg/µL). Very weak and similar antimicrobial effect in JN, SBS and SFS was observed against *S. aureu*s and *E. coli* (Table 5).

Table 5. Minimum inhibitory concentration (MIC) of *jushanda nazla*, sugar-based syrup of *jushanda nazla* and sugar-free syrup of *jushanda nazla* against micro-organisms

Sr. No.	Test organism	Minimum inhibitory concentration (MIC) in µg/µL		
		Jushanda nazla	Sugar-based syrup of *jushanda nazla*	Sugar-free syrup of *jushanda nazla*
1.	*Streptococcus pyogenes*	13	8	2.5
2.	*Streptococcus pneumoniae*	10	8	2.5
3.	*Streptococcus mutans*	50	25	13
4.	*Staphylococcus aureus*	50	50	50
5.	*Bacillus cereus*	25	25	25
6.	*Haemophilus influenzae*	12	5	2.5
7.	*Salmonella*	8	6	3.125
8.	*Escherichia coli*	50	50	50

Discussion

In this study *jushanda nazla* a decoction formulation was modified into the sugar-based liquid dosage form. In total sixteen batches were tried and a batch with a minimum bulk of a unit dosage with the consistency of one *tar*' (wire) was selected as a final batch. The final batch of *sharbat* was prepared with the 170 mL decoction reduced to 50 mL and only 40% (w/v) of sugar was added to achieve one tar consistency. Whereas, sugar-free liquid dosage form was prepared with 170 mL decoction reduced to 30 mL and 96 mg of aspartame was added to improve the taste.

While preparing *sharbat* as per the method mentioned in the classical Unani texts, it is advised to incorporate the sugar in the liquid, in the ratio of 65-85:100 w/v [10,30]. In this ratio, the finished product of liquid dosage form generally gives one tar (wire) consistency.

However, it is not an incontrovertible rule. In many texts, it is also mentioned that precaution should be taken while the syrup is prepared with the mucilage or mucilaginous plant materials as

mucilage develops thick consistency earlier and negligence may spoil the syrup [10,30].

Present test drug, *jushanda nazla* contains *baheedana*, *sapistan, unnab* and *aslusoos*, out of them *baheedana* and *sapistan* have a significant quantity of mucilage, that considerably alters the consistency of liquid while preparing syrup. The present study was taken up with a goal to convert the decoction into syrup dosage form along with the maximum reduction in bulk of the unite dose. The goal was successfully achieved; however, as only 40% sugar was added to the syrup, stability of the syrup was unconvinced as sugar syrup are stable only when prepared with 65% or higher sugar concentration. Hence to keep the finished product stable, sodium benzoate and citric acid (0.2% w/v each) were added as preservatives.

While optimizing the sugar-free syrup from JN, instead of sugar, aspartame was used. The absence of sugar gave thin consistency to the liquid that allowed more reduction of the bulk volume of the formulation. It was attained by further heating and evaporation. As SFS was prepared without sugar syrup there were ample chances of microbial growth. Hence to avoid microbial contamination sodium benzoate and citric acid (0.2% w/v each) were also added as a preservative.

To assess the quality of the finished product, various tests were carried out and data were set as standards for quality control. Qualitative analysis showed that secondary plant metabolites were present in both finished product as it was present in decoction. Likewise, the quantitative analysis also showed the presence of secondary plant metabolites total phenolic, flavonoid, tannin, alkaloid. Further, the concentration of these groups of molecules increases in the SBS and SFS as their bulk is reduced. JN showed the minimum concentration of plant metabolites, which was higher in SBS whereas, SFS showed maximum concentration.

However, it was observed that the concentration of secondary plant metabolites was not increased in proportion to the reducing volume of the bulk. This finding may be attributed to the degradation of some plant metabolites due to continuous heat application.

In the present study, JN contained glycyrrhizin 0.13 µg/µL which was increased to 0.24 µg/µL in SBS and 0.32 µg/µL in SFS that is 1.8 times more in SBS and 2.46 times higher in SFS from JN. Chlorogenic acid was 0.001 µg/µL in JN which increased to 0.003 µg/µL and 0.006 µg/µL in SBS and SFS that is 3 times and 6 times higher in comparison to JN, respectively. Whereas, the volume of SBS and SFS was reduced 3.4 and 5.66 times from decoction, respectively. It was revealed that secondary plant metabolites present in decoction were not heat-stable and degraded to some extent during the heat-based evaporation process while reducing the volume to prepare SBS and SFS.

Heavy metals in decoction and microbial contamination in decoction and both finished products were within allowable limit; hence, the product was fit for human use regarding the heavy metals and microbial contamination [31].

JN, SBS and SFS showed antimicrobial effects against *S. pyogenes*, *S. pneumoniae*, *H. influenzae* which justifies the use of JN in upper and

lower respiratory tract infection since ancient time. These findings also correlate with the previous studies carried out to evaluate the antimicrobial effect of plant materials included in JN [32,33,34,35].

The antimicrobial effect of SFS was found more potent when compared with JN and SBS. This effect may be attributed to the presence of excess secondary plant metabolites in SFS. Also, as the finished product of SFS was more effective against *S. pyogenes, S. pneumoniae* and *H. influenzae*, it may be more successfully employed in respiratory tract infections caused by these organisms in comparison to JN. Furthermore, decoction and its finished products were also found effective against *Salmonella*. To date this, formulation (JN) was not used in the treatment of *Salmonella* infection; however, some previous studies showed that the plant materials used in this formulation have some antimicrobial effects on *salmonella* [36]. This finding may open a new door for further research.

Though the present study exhibited very interesting findings, there were some limitations, that may be addressed with further research. In this study, HPLC was carried out using only two markers from *aslusoos* (glycyrrhizin) and *bahidana* (chlorogenic acid); however, *unnab* and sapistan should also be quantified by using their marker molecules. Further, the developed syrup should be evaluated for its safety, efficacy and stability through preclinical and clinical research studies and comparisons should be made with the classical formulation.

The authors concluded that the formulation used in the form of decoction in the alternative system of medicine can be easily converted into a sugar-based or sugar-free liquid dosage form while preserving its phytochemical characteristics and pharmacological activities. By converting JN into sugar-based dosage form, various drawbacks associated with decoction such as unpleasant taste, a large quantity of unit dosage, time-consuming method of preparation, and stability issues can be successfully addressed. Further, the sugar-free liquid dosage form will also be more acceptable for patients with diabetes or calorie-restricted diets. Future studies for optimization and reformulation of other traditional decoctions to a modern dosage form such as sugar-based and sugar-free syrups are recommended.

Conflict of Interests

The authors report no conflict of interest

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None.

References

[1] Menditto E, Orlando V, De Rosa G, Minghetti P, Musazzi UM, et al. Patient centric pharmaceutical drug product design-the impact on medication adherence. Pharmaceutics 2020;12:44.

[2] Yang Y, Ross J. Theories and concepts in the composition of Chinese herbal formulas. In: Chinese Herbal Formulas. Churchill Livingstone, Edinburgh, 2010.

[3] Abraham JI, Mathew FL. Taste masking of paediatric formulation: A review on technologies, recent trends and regulatory aspects. Int J Pharm Pharm Sci 2014;6:12-19.

[4] Goran MI, Ulijaszek SJ, Ventura EE. High fructose corn syrup and diabetes prevalence: A global perspective. Global Public Health 2013;8:55-64.

[5] Winfield J, Rees J, Smith I. Pharmaceutical Practices. 4th Edition, Churchill Livingstone. Elsevier. Philadelphia 2009.

[6] Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the United States. Arch Intern Med 2003;

163:487-494.
[7] Eccles R. Understanding the symptoms of the common cold and influenza. Lancet Infect Dis 2005;5:718-725.
[8] Fashner J, Ericson K, Werner S. Treatment of the common cold in children and adults. Am Fam Physician 2012;86:153-159.
[9] Ghani N. Qarabadeen Najmul Ghani. Central Council for Research in Unani Medicine. New Delhi 2010.
[10] Kabeeruddin M. Al Qarabadeen. Central Council for Research in Unani Medicine. New Delhi 2006.
[11] Rowe RC, Sheskey PJ, Quinn ME. Handbook of pharmaceutical excipients. 6th Edition. Pharmaceutical Press. RPS Publishing. UK 2009.
[12] Tandel KR. Sugar substitutes: Health controversy over perceived benefits. J Pharmacol Pharmacother 2011;2:236-243.
[13] Pantone color chart. Available at URL:https://www.pantone.com/color-finder [Assessed on 05-07-2020].
[14] Pawar HA, Joshi PR. Development and evaluation of taste-masked granular formulation of satranidazole by melt granulation technique. Journal of pharmaceutics 2014;789676.
[15] The Unani Pharmacopoeia of India. part II. Volume II. 1st Edition. Department of AYUSH, Ministry of Health and Family Welfare, Government of India. New Delhi 2010.
[16] Jyothiprabha V, Venkatachalam P. Preliminary phytochemical screening of different solvent extracts of selected Indian spices. Int J Curr Microbiol App Sci 2016;5:116-122.
[17] Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem 2014;2:115-119.
[18] Sanmugarajah V, Thabrew I, Sivapalan SR. Phyto, physicochemical standardization of medicinal plant Enicostemma littorale Blume. IOSR J Pharm 2013;3:52-58.
[19] Miao S, Nie Q, Miao H, Zhang W, Mai K. Effects of dietary carbohydrate-to-lipid ratio on the growth performance and feed utilization of juvenile turbot (Scophthalmus maximus). J Ocean Univ China 2016;15:660-666.
[20] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-428.
[21] Jamuna SS, Karthika PK. Screening of in vitro antioxidant activity of methanolic leaf and root extracts of Hypochaeris radicata L. (Asteraceae). J Appl Pharm Sci 2012;2:149-154.
[22] Kavitha CCI, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes kunthiana (Neelakurinji). J Med Plants 2016; 4:282-286.
[23] John B, Sulaiman CT, George S, Reddy VR. Spectrophotometric estimation of total alkaloids in selected Justicia species. International journal of pharmacy and pharmaceutical sciences 2014;6:647-648.
[24] Sethi PD. HPLC High-Performance Thin Layer Chromatography. CBS Publisher and Distributor Pvt. Ltd, New Delhi 2001.
[25] Garbarino JR, Taylor HE. Inductively Coupled Plasma-Mass Spectrometric method for the determination of dissolved trace elements in natural water. U.S. Geological Survey, U.S Government 1996. Available at URL https://wwwbrr.cr.usgs.gov/projects/SW_inorganic/download/MSMethod.pdf Accessed on 15-10-2017.
[26] Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests (Chapter 61). USP 41, NF 36. Vol 4. US 2018.
[27] Koeth LM, King A, Knight H, May J, Miller LA, et al. Comparison of cation-adjusted Mueller-Hinton broth with Iso-Sensitest broth for the NCCLS broth microdilution method. J Antimicrob Chemother 2000;46:369-376.
[28] Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007;42:321-324.
[29] Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, et al. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol Lett 2016;38:1015-1019.
[30] Raqeequddin HM. Minhajus Saidla wal Kimiya. Idara Kitabul Shifa. New Dehli 2001.
[31] Lohar DR. Protocol for Testing. Ayurvedic, Siddha, Unani Medicines Pharmacopoeial Laboratory for Indian Medicines. Depertment of AYUSH, Ministry of Health & Family Welfare, Government of India. Ghaziabad 2007.
[32] Hossain MA. A phytopharmacological review on the Omani medicinal plant: Ziziphus jujube. J King Saud Univ Sci 2019;31:1352-1357.
[33] Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MB. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother Res 2018;32:2323-2339.
[34] Oza MJ, Kulkarni YA. Traditional uses, phytochemistry and pharmacology of the medicinal species of the genus Cordia (Boraginaceae). J Pharm Pharmacol 2017;69:755-789.
[35] Ashraf MU, Muhammad G, Hussain MA, Bukhari SN. Cydonia oblonga M., a medicinal plant rich in phytonutrients for pharmaceuticals. Front Pharmacol 2016;7:163.
[36] Gupta A, Maheshwari DK, Khandelwal G. Antibacterial activity of Glycyrrhiza glabra roots against certain gram-positive and gram-negative bacterial strains. J Applied Nat Sci 2013;5:459-464.