RESEARCH ARTICLE

Preliminary Screening of Various Parameters for Design and Development of Kumaryasava Formulation

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ABSTRACT

Ayurveda is the most popular traditional system among these and this indigenous system originated from India. Kumaryasava (KS) is a very popular arista in our state, probably due to their taste, alcoholic content, medicinal uses and physiological importance. The quality assessment of ayurvedic formulation is of prime importance in justifying their acceptability when compared with the modern system of medicine. A study reported that the therapeutic efficacy of herbal formulation always depends on its method of preparation and hence, there is need to develop a formulation with statistical optimization. The constituents essential for preparation of KS involve dravya, dravadravya, sandhaneeya dravya, prakshepa Dravya and madhura dravya. The other ingredients in formulation were selected based on *in silico* molecular docking for the antioxidant and hepatoprotective properties. In this article attempt is made to describe the screening of Kumaryasava formulation for optimization of the concentration of sweetening agent, fermentation duration and fermentation temperature for alcohol generated and selection of various excipients that affect formulation efficacy. According to the preliminary screening study it was observed that the sugar concentration 35 to 40%, fermentation time 30 days for ghataki flowers and fermentation temperature 25°C has reported considerably significant results for alcohol content analyzed by gas chromatography. The preliminary screening parameters of KS formulation will be applied effectively for the development of formulation with a statistical approach.

Keywords: Kumaryasava, *Aloe vera*, Honey, Fermentation, Polyherbal.

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INTRODUCTION

Herbal medicines have a long history of practice for the treatment of diseases along with its prevention. Asavas are good health tonics and have therapeutic value in ayurveda. KS formulations are widely utilized over the counter for the treatment of urinary disorders, as a health tonic for the digestive system, liver disorders and cough.[1]

Kumaryasava (KS) is a unique and valuable ayurvedic polyherbal formulation cited in the Indian ayurvedic formulary. It contains *aloe vera* as the key ingredient along with self-fermented galenical that contains 40-50 crude drugs.[2] *Asavas are* prepared from fresh juices. *Kumaryasava* is a very popular *arishta* in our state, probably due to their taste, alcoholic content, their medicinal uses and physiological importance.[3] KS contains phytochemicals such as saponins, phenolic compounds, tannins and anthraquinone glycosides that contribute to various therapeutic uses. [4] According to the specifications of Ayurvedic Pharmacopoeia of India KS should comprise NLT 5% v/v and NMT 10%v/v of self-generated alcohol.

The attracting factor in the formulation of any *arishta* is *prakshepaka dravyas*. It is a mixture of a ten plant materials and are made into coarse powder form. This *prakshepaka dravyas* remodel the formulations to give an aromatic flavor color and enhance therapeutic actions.[1,5] Hence there is a pre-requisite to select the specific ingredients for formulation of KS. The fermenting vessel is another important criterion. Earthen pots are generally used as fermentation vessels in traditional Ayurvedic preparations and are known as *sandhana paatra*.[3] A study reported that depending upon the number of ingredients required for the preparation, duration of fermentation is extended.[2] An earlier study has reported that during fermentation, there was a gradual decrease of sugar.[5] The most marketed formulation of KS contains about nineteen ingredients. Hence there is need for screening of ingredients for their specific properties in the formulation of KS.[4] Considering the fact an attempt was made for screening of ingredients with their concentrations for the development of KS. The present study performed *in silico* molecular docking of *aloevera*, evaluation of concentration of the sweetening agent,

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temperature for fermentation and duration of fermentation required for the formulation of KS.

MATERIALS AND METHODS

Selection of Drug and Excipients for Asava Preparation

Asava is a traditional Ayurvedic medicine prepared by soaking the medications in a solution of sugar or jaggery for certain period, whether they are in powder or kashaya (decoction) form. By encouraging fermentation and producing alcohol, this process is finished with a final addition of dhataki pushpa (*Woodfordia fruticosa*). The ensuing self-generated alcohol facilitates the hydroalcoholic extraction of the active components from the crude medicine and acts as a preservative. Asava preparation requires a few fundamental ingredients, including medicinal substances (dravya), solvents (drava dravya), sweets (madhura dravya), fermentation agents (sandhna dravya), and additives (prakshepa dravya).[6]

Dravya (Drug substances)

The fundamental components of asava includes dravyas, which are gathered in the necessary amounts following their authentication in accordance with the guidelines outlined in the Indian Ayurvedic Pharmacopoeia. Pesticides, other residual pollutants, and foreign matter should not be present in the raw materials utilized in the formulation. The three primary sources of the medications used to prepare asava are herbal, mineral, and animal origins. Nine yonis (ingredients)—dhanya (cereals), moola (roots), phala (fruits), kanda (tuber), pushpa (flowers), patra (leaf), twak (stem bark), saara (heart wood), and sarkara dravya (sweetening agents)—are suggested by "Charaka" for the preparation of different asavaristas. Furthermore, "Susruta" suggests using loha churna (iron powder) and bhasma (ash) of specific medications (Palasa and Tilanala bhasmas). Ghee and madhu, which come from animal sources, are typically used to anoint the pot or vessel that will be used as a fermentation container. Typically, asava is made with mrudu (soft), volatile pharmaceutical ingredients made as cold decoction, hot decoction, or swarasa such as chandana, ushira, and karpoora.[6]

Drava Dravya (Solvents)

Medicinal plant parts are extracted using decoction and infusion techniques in asava preparation. Water is the most prevalent liquid described in several classic works that discuss the use of various liquids as solvents. Additional beverages include of fruit juice, plant juice, decoctions, curd water, buttermilk, gomutra, kanji, and dhanyamla, among others. Additionally, it has been noted that in certain formulations, two or three liquids are blended as a solvent. Because the powdered medicines utilized in this formulation are soluble in water and miscible with the resulting alcohol, water was used as the solvent in this formulation.[6]

Sandhana Dravya (Fermentative agents)[7]

The natural carriers of fermenting organisms that start the fermentation process are Dhataki pushpa (*Woodfordia fruticosa*), Madhuca pushpa (*Madhuca longifolia*, Macbr), Kinva (microbial inoculums), yeast, and occasionally puga, Badara twak (bark), and Babbula twak (bark).[7] The qualities of dhataki pushpa have been documented by Acharya Charaka, but Acharya Vagbhatta was the first to employ it in the fermentation process to create sandhana kalpana, a fermented product.[8] Sharangadhara Samhita and Ashtanga Hrudaya as Sandhana Dravya describe madhuka pushpa and dhataki pushpa.[9-11] These flowers' tannin content encourages yeast growth by creating an environment that is favorable to it. In the event that dhataki pushpa is not present, the fermentation process can be started by adding wild yeast found in honey, resins, and Madhuca flowers to the mixture.[12] The dry nectariferous portion of these nectariferous and extremely tanniferous blooms contains yeast spores.[13-15]

The literature claims that when *W. fruticosa* flowers are used as inoculums, the fermentation process proceeds far more slowly than when M. indica and S. cerevisiae are used as inoculums. Based on this, KS was prepared using *W. fruticosa* and M. indica as the fermenting agents, and a comparative evaluation of the amount of alcohol created and antioxidant studies was carried out. Gas chromatography was used to assess the alcohol produced. In order to choose a fermenting agent, the KS produced with a comparable content and DPPH scavenging activity were assessed. The findings showed that the maximum antioxidant activity was found in *W. fruticosa* flowers-based KS, at 95.23 ± 0.36%, compared to M. indica's 82.62 ± 1.23%. The presence of phenolic chemicals, which have strong antioxidant activity, in *W. fruticosa* flowers may be the cause of this elevated antioxidant activity. *W. fruticosa* was therefore, employed in the fermentation process to develop the KS formulation. As a result, it has been determined that the formulation with the highest antioxidant activity was made using the conventional approach described in the Ayurvedic Pharmacopoeia.

The traditional method of making Kumaryasava involves fermenting A. vera leaves. Woodfordia fruticosa (Family: Lythraceae) flowers are introduced as inoculums during preparation to initiate the fermentation of alcohol. The natural yeasts found on *W. fruticosa* flowers are used in this fermentation process.[8,9] It is advised to allow the fermentation process take up to one month because the type of yeast on the blooms can differ according on where the source is. Water-insoluble active principle components of the crude pharmaceuticals are extracted due to the fermentation process's production of alcohol. The Ayurvedic Pharmacopoeia of India states that the amount of self-generated alcohol in Kumaryasava should be between 5% and 10% v/v (40–80 g/l). Formulations based on *W. fruticosa* flowers are helpful for a number of ailments, including rheumatic illnesses, weakness, burning sensation in the stomach, leucorrhoea, and dysfunctional uterine haemorrhage.[10-12]

Madhura Dravya (Sweetening agents)

Madhura dravya is necessary since it provides the fermentation's foundation. As a source of carbohydrates in asava preparations, some madhura dravyas, like guda (jaggery), sharkara (sugar),

phanita (molasses), sitopala (candy sugar), "Matsyandika" & Khanda sita (different types of jaggary & sugar), have special biochemical properties that are used in specific quantities. These sugary compounds stimulate and stimulate bacterial development in the fermentation medium, which ferments the combined mixture. The kind, amount, and calibre of sweetening agents determine the rate of fermentation and the calibre of the finished product. While honey may cause the process to be slower but more persistent, the inclusion of alkaline molasses accelerates the fermentation process.[13-15] The best amount of sugar to use when preparing asavaristas is forty percent.

In present study combination of guda (jaggery) and madhu (honey) was utilized that enhances fermentation rate and promotes antioxidant potential of *KS* formulation. Acharya Sharangadhara states that if one Drona (12.288 liters) is equal to one Tula (4.8 kg) of jaggery, then half of a Tula should be used for honey. It becomes difficult to dissolve sweet ingredients, thickens the liquid, and reduces the likelihood of fermentation if the ratio above equals or doubles the amount of Dravyas. Since yeast can withstand high sugar concentrations, it grows best in a solution of 30–40% sugar.[13]

Prakshepa Dravya (Additives)

Lavanga, ela, twakpatra, nagakesara, trikatu, and other prakshepa dravyas are added to the formulation after the decoction is prepared. Aside from adding flavor, color, and perfume to the formulation, their tikshana guna (antifungal, antiseptic, and bactericidal qualities) prevent contamination. These are combined in the form of yavakuta (coarse powder) or fine powder with the sandhana patra. Some asava formulations list metals (Loha, Tamra, Swarna) as components.Add a few thin gold leaves to Sarasvataristhta. In Sandhana Dravya, metals do not dissolve; instead, they improve the constituents' qualities.[16] Various texts recommend adding finely ground metal powders on certain days, including the first, fourth, or fifth day during or after the fermentation process is finished. Admixtures are sometimes added to or spread over wort in the form of a paste and then thoroughly mixed in order to encourage air or oxygen exchange at the beginning of fermentation and so limit the growth of microbes for appropriate fermentation. [17-19]

The *KS* formulation different about additives added as mentioned in composition table in coarse powder form. The marketed formulation composed of nineteen ingredient formulation of *KS* that was considered as control batch. However, in developed *KS* formulation additives Haritaki, Jatiphala and Jatamansi was added to enhance the antioxidant and hepatoprotective potential.[16]

Sandhana Patra (Vessels or Containers)

The containers found in "Brihattrayee" are referred to by a number of terms, including Bhanda, Kalasa, Kumbha, Ghata, Bhajana Patra, Ghrta Bhavita Patra, Madhulipta Patra, Lohapatra, Tamrabhajana, Sucibhajana, etc. These receptacles are composed of wood, soil, or metals like copper and iron. These ought to be robust enough to endure extended processing times. Because earthenware is readily available, inexpensive, has a controlled temperature, and is inert by nature, ancient literature generally advise using it for asavarista preparation. However, the possibility of breakage and water seeping out, which reduces yield and thickens the formulation, are two of the main drawbacks of earthenware pots. These days, the pharmaceutical sector uses steel and plastic containers for fermentation.[10] According to literature, porcelain pots yield more than earthen pots, as evidenced by the creation of Arjunarista. For the preparation of Sandhana kalpana, Swarna (Saraswatarista) and other metallic containers are also advised.[13] certain classic books recommend using wooden pots for fermentation, however there are certain limitations. First, because wood affects liquid absorption, wooden pots require pre-treatments. Other containers are also employed, such as stone for Kumariasava and iron for Madhvasva.[14]

The KS formulation was kept in glass and plastic containers for ten days in order to choose the ideal storage container. It was discovered that the KS formulation kept in a plastic container encourages the growth of fungi. Conversely, because glass does not react with other formulation components, the formulation kept in the glass container was inert and did not grow fungus. It was so chosen to keep the formulation in a glass container for storage.

Sandhana Kriya (Fermentation process)

During the fermentation process, precisely measured amounts of dravyas are introduced to the dravadravya. During the fermentation process, precisely measured amounts of dravyas are introduced to the dravadravya. The alcohol is then allowed to ferment for the predetermined amount of time in a suitable location after being put into the prepared and suggested container together with the other necessary ingredients. Before adding liquid medium or wort to the fermenting vessel (Purana), head space must be left empty. This head space allows the liquid to foam, flash, and aerate. It occupies at least a quarter to a fifth of the fermenter's overall volume. After the fermentation starts, the patra needs to be sealed (sandhi bandhana) by encircling a long piece of cloth with one side covered with clay. The clay side of the ribbon should stay external during the sealing process, and the blank side should line the vessel's rim and lid. Subsequently, the container is kept in a dimly lit area with minimal air movement. Tests are performed to determine if fermentation is finished or continuing ongoing after it has finished.[16,17] Following the establishment of the required organoleptic character in the formulation, the fermentation is halted after a predetermined amount of time, and the fermented liquid is filtered and left for a while to allow the sediments to sink to the bottom. After collecting the clear supernatant liquid, the fermented liquid is ready to be used.[8]

During fermentation the formulation was analysed for alcohol generated on 10th day and after completion of fermentation i.e. 21st day by Gas chromatography. The precaution was taken to avoid the recurrent opening of formulation vessel as it may interfere the fermentation process and affect the amount of alcohol generated.

Sandhana Sthala (fermentation place/location)

Classical wisdom states that a fermentative vessel should be stored outdoors or inside of a Dhanyarashi (paddy heap), Dhanya Madhya (céréales heap), Yavapalla, Sugupta sthana (an isolated location), "Tusa madhya," etc.[14,15] Sandhana Kriya, or fermentation, is kept at a constant temperature in Dhanyarashi because its constant temperature is higher than room temperature. Sodhala has illustrated a number of new places, such Koshthasara, Bhugarbha (underground), Suryatrapa (under the sun), etc60. Yogendra Chintamani gave Jambirdrava a new sandhana location, Ashwashala (the horse shed). Generally speaking, 25 to 30°C is thought to be the perfect temperature for healthy fermentation 10. In today's world, most commercial production setups employ air conditioners to maintain consistent temperature. Since fermentation bacteria are light-sensitive, direct light and air contact is avoided while they are being stored. The created KS formulation was kept at room temperature in an airtight container.

Sandhana Avadhi (Duration of fermentation)

The factors that determine the fermentation are Desha (location or nation), Ritu (season), and Dravya (drug type). Due to variations in component types, sweetener amounts, location, time of year, and usage of fermentation initiators, among other factors, the fermentation period can range from seven days to six months. Various classics emphasize the maintenance of minimum and maximum fermentation times.[13] For example, in preparations that contain iron as the metal source, the maximum recommended time restriction is until the metal dissolves fully in the solution. Vasa is a key component of Vasarishta, which can be prepared quickly. In contrast, Gugguluasava contains a lot of elements, and since guggul is a resin, it could revenue lengthier to turn into Asava. Therefore, variations in the length of fermentation are noted in various formulations based on the type of the constituents.[20,21]

Screening of KS formulation

The standardized ayurvedic preparations of uniform quality are prerequisite for the therapeutic efficacy; otherwise variations in formulation batches develop. Hence, statistical approach is considered for the formulation of KS to gratify the standard quality product with maximum efficacy. The formulation was inspected for the effect of three ingredients that are core part for KS as concentration of sweetening agent (madhu: honey), fermentation duration, fermentation temperature individual at three levels with vision for application of statistical designs.

***In-silico* Molecular Docking Study**

Our comprehension of the interactions between proteins and ligands is facilitated by the molecular docking technique, which involves inserting the ligand hooked on the binding pocket. The graphical user interface molecular docking software iGEMDOCK facilitates molecular docking, virtual screening, and post-screening analysis. Using iGEMDOCK facilitates pretreatment of the protein and ligands as well as present docking data with the GUI interface9. The goal of the current work is to determine the pharmacological potential of a group of phytocompounds discovered to be present in KS formulation through molecular docking experiments utilizing iGEMDOCK. For the hepatopreventive and antioxidant properties of aloe, haritaki, and honey, a molecular docking research was conducted.[22,23]

Data and Databases

The Protein Databank (http://www.rcsb.org/) was used to determine the three-dimensional structure of a protein.[24] Using Discovery Studio Visualizer 3.5, the binding site for the downloaded protein, 1G5M, was predicted (Fig. 1).The evident binding pocket contains the ligand binding site, which is represented as a group of dots inside the highlighted circle. The phytocompounds listed in Table 8.1 were the ligands employed in this investigation. with the use of the in silico research tool PASS, available online.[25] ChemSketch was used to generate the structures of these ligands (ACD/ ChemSketch v. 01, 2010). The primary structure of the protein was obtained from UniProt (http://www.uniprot.org).[26] The protein's various physicochemical properties were calculated by ProtParam.[15]

Ligand and Protein Preparation

Using Discovery Studio Visualizer 35, the 3D structures of ligands and the protein Bcl-2 were displayed. They were made ready by removing water molecules from the protein, applying the proper shape, adding hydrogen, and attaching the right charges.

Molecular Docking Study

A freeware docking program with an interactive graphical user interface (GUI) for virtual screening, docking, and post-screening is called iGEMDOCK.[3,4] The commercial Kumaryasava formulation constitutes about more than 15 ingredients and there is need to screen the ingredients corresponding to antioxidant and hepatoprotective features. Hence, docking study was performed for herbs aloe Vera, dhataki, haritaki and honey against specific proteins related to antioxidant, hepatoprotective and anticancer characteristics as mentioned in Table 1. Docking parameters were established using the following factors: 10 solutions, 80 generations, 800 population size, and accurate docking, which is a very precise and slow method of docking. Post-docking analyses were performed for the best-docked postures using the features, interaction profile, interaction analysis, and interaction profile cluster. [25,26]

Evaluation of Formulation

Organoleptic examination

Organoleptic characters of the optimized *KS* formulation were observed i.e. by examining the appearance, colour, taste and odour.

Table 1: Molecular docking study details of ligand and proteins

Name of plant	*Ligand*	*Protein*
Aloe babbadensis	Kameferol Pinobanskin-3-o-butyrate	Human Carbonyl Reductase-1

Physicochemical investigation

Quality assessment of ayurvedic products is relevant in the present era. These tests are used to evaluate the ayurvedic preparations in order to ensure its quality. The major tests used for routine analysis of *asava* samples are physico – chemical parameters like total solids, pH, colour, taste, alcohol content, total reducing and non-reducing sugar, specific gravity etc were performed as per pharmacopiea. The alcohol content was evaluated by specific gravity and gas chromatography. In the present study the standardization procedure on the basis of macroscopic or visual examination (colour, texture, odour, taste etc,), physico - chemical properties (quantity determination of sugar, alcohol, pH etc.,)

RESULT AND DISCUSSION

In-silico Molecular Docking Study of *Aloe barbadensis*

In-silico molecular docking was performed for *Aloe barbadensis* that is used as a chief ingredient in formulation of Kumaryasava. The docking study was performed for the antioxidant and anticancer property of *aloe barbadensis* .[27]

- Kameferol (PubChem ID- 5280863) is docked with the binding pocket of Human Carbonyl Reductase-1 (PDB ID- 4z3d) with the binding affinity (-8.9 Kcal/mol) as shown in Figure 1. It formed conventional hydrogen bonds with ASA A: 89, TYR C:193, LYS C:197, GLY C:228. Also exhibit van der Waals interactions with GLY C:15, GLY C:17, GLY C:91, GLY C:11, LYS C:14, ALA C:90, VAL C:137, SER C:138, TRO C:229, VAL C:230, AA C:235, THR C:232.
- Pinobanskin-3-o-butyrate is docked with the binding pocket of Human Carbonyl Reductase-1 (PDB ID- 4z3d) with the binding affinity (-7.8 Kcal/mol) as shown in Figure 2. It formed van der Waal bond interactions with PHE B:28, LEU B:27, PRO C:257, CYS B:25, GLY B:53, LEU B:54, SER B:55, LYS C:271, TYR C:252, GLY B:30 and also Pi alkyl interactions with ARG B:26, LEU C:27, LEU C:255.[7,8]

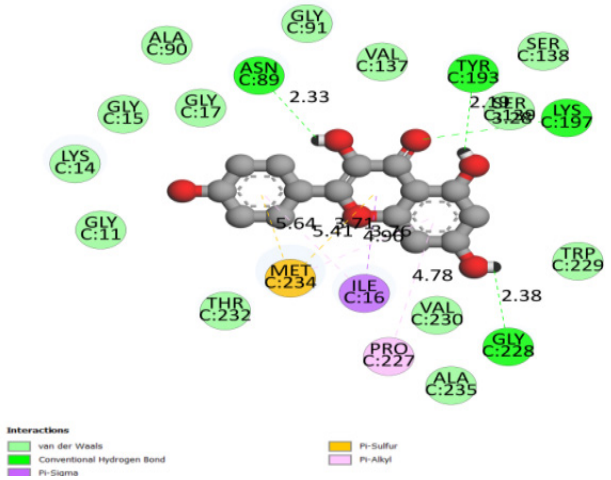

Figure 1: Molecular docking of Kameferol Human Carbonyl Reductase-1

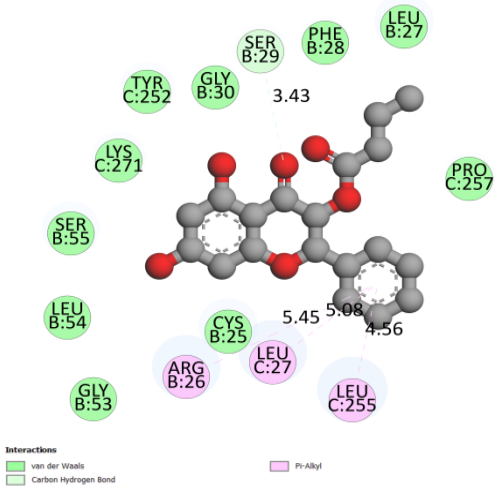

Figure 2: Molecular docking of Pinobanskin-3-o-butyrate Human Carbonyl Reductase1

- Pinobanskin-3-o-propanoate is docked with the binding pocket of Human Carbonyl Reductase-1 (PDB ID- 4z3d) with the binding affinity (-7.7 Kcal/mol) shown in Figure 3. It made conventional hydrogen bonds with ALA B:254, SER B:29, ASP B:84 and also give van der Waals interactions with LYS B:129, GLY B:132, GLN B:131, VAL B:85, ARG B:133, LEU B:255, LEU B:27.[27]

Preliminary Screening for Optimization of Process Parameters

The Kumaryasava formulation was prepared with selection of *Aloevera* as chief ingredient including other additives madhu: honey as sweetening agent and dhataki flowers for fermentation. The levels of concentration of sweetening agent (madhu: honey), fermentation duration and fermentation temperature was recognized based on the preliminary screening.

Concentration of Sweetening Agent (madhu: honey)[17]

The concentration of sweetening agent has significant impact on fermentation process and alcohol generation. The higher

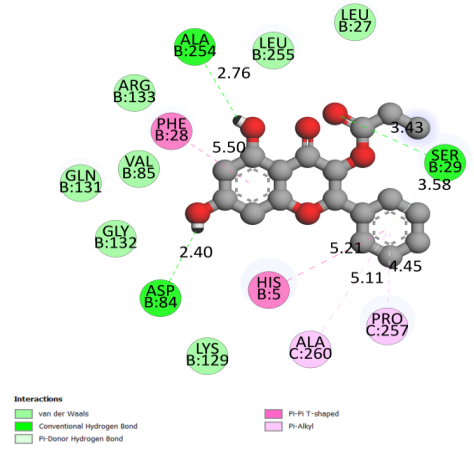

Figure 3: Molecular docking of Pinobanskin-3- o-propanoate Human Carbonyl Reductase-1

concentration of sweetening agent than the desired standard limit promotes the bacterial growth, increases viscosity that slows down the fermentation process and ultimately the alcohol generation reduced as illustrated in Table 2. Hence the considerable attention should be given to select the sweetening agent concentration.

According to the reported literature 40% sugar is the most ideal quantity for preparation of asava. However, we were using the combination of sweetening agent i.e madhu and honey in proportionate ratio. Hence, the combination of sweetening agent in concentration 30, 35, 40, 45 and 50% was evaluated for the viscosity and based on the results three levels was selected. The viscosity of formulation was determined by Brookfield Viscometer, according to the results as mentioned in Table. The sweetening agent with a concentration 30% has comparatively low viscosity and low sugar content that causes reduction in fermentation and alcohol content. However, on contrary 50% concentration, the solution was too viscos that, promotes bacterial growth hence it was not recommended for the formulation of *KS*. Hence, concentration 35, 40 and 45% was selected for the development of *KS* formulation applying factorial design with three levels.

Fermentation Duration

The time it takes for fermentation to complete varies depending on the season. According to the literature, fermentation takes place over the course of six days in the fall and summer, ten days in the winter, and eight days in the wet and spring. Thirty days is enough in a cold temperature, but 7 to 10 days is sufficient in a hot tropical climate.[15] Depending on the formulation, fermentation periods could range from 7 to 180 days. The results showed that with time, the specific gravity, total solid content, and sugar content all decreased. There was a six-month increase in the quantity of alcohol consumed. The pH level does not change.

Fermentation in Kumaryasava began on the 5th day and ended on the 30th.[13, 14] The duration of fermentation has a significant impact on alcohol generated. Around 30 days duration was considered as desired for significant amount of alcohol content for the developed formulation.[25] Preliminary screening was carried out for the developed KS formulation for fermentation duration 10, 20, 30 and 40 days and formulation was evaluated for sugar content as previous procedure mentioned earlier as well as the alcohol content by gas chromatography. In addition the changes in organoleptic characters like colour, smell, touch and taste during fermentation were evaluated. Organoleptic shifts in KS from light brown to dark brown suggested that phytochemicals were probably extracted from the composition's herbal components. The first preparation's herbal nature indicated the presence of herbal extractives. The aroma began to become herbal on the tenth day of fermentation and became slightly alcoholic, indicating the beginning of the fermentation process and the production of alcohol. For the next twenty to forty days of fermentation, there was a persistent alcoholic odour. Throughout the entire fermentation process, the touch was kept wet. Due to the presence of a large volume of sweetening agents (jaggery and honey) during fermentation, the preparation's initial taste was sweet and slightly bitter. This changed to an astringent bitter taste, which finally turned to an astringent taste due to the use of sugar by microorganisms and potential phytochemical extraction. The results revealed that as the fermentation process initiated on 5th day; the alcohol generated on 10th day was very low that indicates the progress of fermentation process. The alcohol generated for above fermentation duration illustrated in Table 3. According to the results it was noted that for 40 days fermentation process amount of alcohol generated was slightly reduced and maintained and the formulation started developing bacterial growth. Hence, the fermentation duration 10 days, 20 days and 30 days was considered for the development of formulation using statistical approach.

Table 2: Effect of Concentration of Sweetening agent on Viscosity

Formulation code	*Concentration of sweetening agent (% w/v)*	*Viscosity (cp)*
KS1	30	2.56 ± 1.81
KS2	35	5.48 ± 0.32
KS3	40	5.68 ± 0.15
KS4	45	6.10 ± 0.42
KS5	50	9.36 ± 1.23

It was noted that fermentation altered the biochemical composition of the mixture in terms of pH, sugar, ethanol, specific gravity, and total solids as shown in Table 4. All things considered, these studies demonstrate that while there are little to no variations in the specific gravity and solid content, the pH steadily drops, the amount of sugar decreases, and the concentration of ethanol gradually rises. During the first 10 days of fermentation in Kansas, the pH decreased significantly from 5.2 to 3.61 and remained there until the end. The level of total solid showed a remarkable change in just 10 days of fermentation (`), increasing from 201.23 mg/ml to 95.14 mg/ml and then gradually reducing to 33.10 mg/ml.[28]

After 10 days of fermentation, the total sugar content in Kansas abruptly decreased, which is associated with a rise in ethanol levels during this time. The development and metabolic activity of the microorganisms included in this mixture can also be linked to these alterations. Up to 20 days of fermentation, the

Table 3: Changes in organoleptic characters of Kumaryasava during fermentation

Fermentation duration	*Color*	*Smell*	*Touch*	*Taste*
0	Light brown	Herbal	Watery	Sweet, Slightly bitter
10	Brown	Slightly alcoholic	Watery	Astringent, bitter
20	Dark brown	Alcoholic	Watery	Astringent, bitter
30	Dark brown	Alcoholic	Watery	Astringent
40	Dark brown-black	Alcoholic	Watery	Astringent

Table 4: Changes in biochemical composition of Kumaryasava during fermentation

Fermentation Duration	*pH*	*Total sugar content*	*Ethanol content*	*Specific gravity*	*Total solid content*
0	5.2	21.30	0	1.23	112.9 ± 0.61
10	4.3	15.69	2.21 ± 0.31	1.19	75.21 ± 0.08
20	3.3	13.02	4.11 ± 0.29	1.13	50.74 ± 0.86
30	3.6	12.30	6.20 ± 0.06	1.09	33.10 ± 0.37
40	3.5	12.33	5.21 ± 1.59	1.20	32.62 ± 2.14

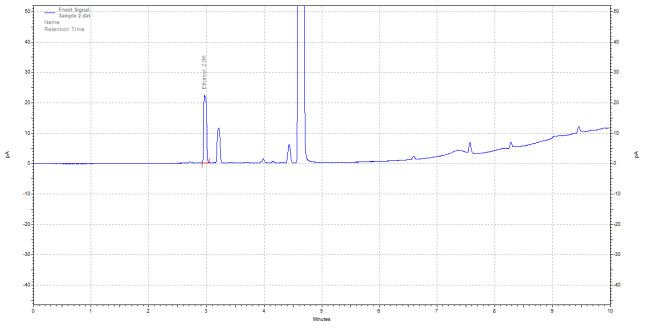

Figure 4: Gas chromatogram of KS formulation at fermentation duration 10 days

concentration of ethanol was raised; after that, it was gradually lowered and maintained. In the KS formulation, the kinetics of the steady decline or elimination of sugar and simultaneous rise in ethanol level were documented. Throughout the last stages of fermentation, the specific gravity remained around 1.1, reflecting how watery the preparation was. As a result, the tactile sense also registers as watery.

Fermentation Temperature

In ancient times, to avoid temperature changes, containers for Asavarista preparation were placed in Dhanya Rashi (eg-Kanakbindu arista), Bhugarbha (eg-Kharjurasava), Koshthasara (eg-Kumaryasava), and other locations. Temperature affects KS formulations' fermentation process.[28] The literature states that in cold asava, jaggery was added to the herbal infusion, thoroughly mixed, and heated for two minutes; in hot asava, however, the hot parameters of cold asava were found to be lower than those of hot asava. The alcohol asava jaggery was added after the decoction had cooled to 40°C and kept aside for fermentation. The physico-chemical content was evaluated by gas chromatography (Figure 5). The reported literature revealed that on the day of filtration, cold arista had a 7.64 percent alcohol level, whereas hot Arista had no alcohol formation. Tannin concentration in both formulations was determined to be the same. According to literature review it was noted that for fermentation to begin, the temperature should be between 20-35 °C. Hence, the developed KS formulation was evaluated at various fermentation temperatures 25, 35 and 45°C for alcohol content, pH and acid value as given in Table 5.

The temperature at 20°C very slow fermentation process as the temperature condition was insufficient to promote bacterial growth for fermentation. On contrary, fermentation temperature above 50°C as a result of the high temperature (Figure 6), yeast cells were killed while the formulation

Table 5: Effect of fermentation temperature on different parameters

Fermentation Temperature °C	*pH*	*Acid value*	*Ethanol content %(v/v)*
20	5.2	0.12 ± 1.36	1.34 ± 0.85
25	3.4	0.23 ± 0.80	6.01 ± 1.63
35	3.6	0.32 ± 0.34	5.60 ± 1.15
45	3.8	0.45 ± 0.69	5.32 ± 0.95
50	4.7	1.56 ± 1.61	1.85 ± 0.92

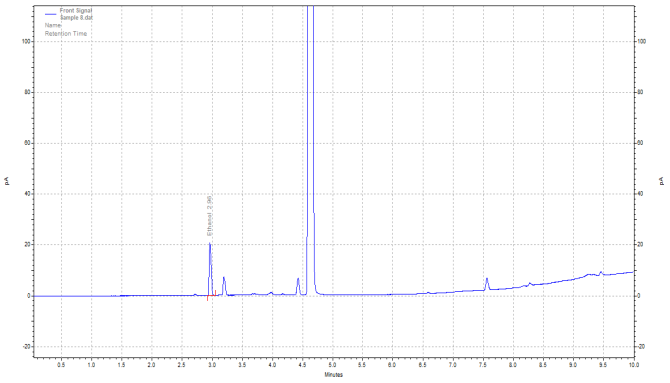

Figure 5: Gas chromatogram of KS formulation at fermentation temperature 20°C

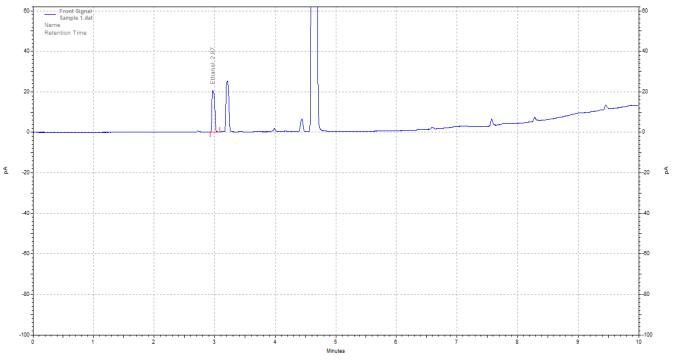

Figure 6: Gas chromatogram of KS formulation at fermentation temperature 50°C

was hot.[29] As a result, the fermentation process was hampered. Yeast cells are not killed in cold decoction, which aids the fermentation process.[30,31] Therefore, the fermentation temperature 25, 35 and 45°C were selected for the development of KS formulation applying statistical design.

CONCLUSION

Kumaryasava formulation includes *Aloevera* as chief ingredient and other constituents that will aid the medicinal benefits. The molecular docking study performed for the *aloevera*, honey and haritaki demonstrated the antioxidant, hepatoprotective and hepatic cancer properties for specific proteins and hence selected for the development of KS. Traditionally, *W. fruticosa* is believed to provide inoculum for fermentation and hence utilized for this study. The preliminary screening was performed and revealed 40% concentration of the sweetening agent, 25°C fermentation temperature and incubation for 30 days. Parameters were recognized as ideal that generated desired self-generated alcohol.

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