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PHYSICO-CHEMICAL CHARACTERIZATION OF KANTAKARYADI GRANULES -AN APPROACH TO ITS STANDARDIZATION

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ABSTRACT

Introduction- Granules (*khanda kalpana*) are the modified dosage form of *Avaleha kalpana* (Confections) where the ratio of sugar is altered in content and final product as well with reducing moisture content in the final product. A standard granular dosage form is very easy for dispensing and has more self-life with uncompromising therapeutic potency, in comparison to the *avaleha* dosage form of the same ingredients. **Aim and Objective:** In the current research work, it is tried to characterize the said granule in terms of its physico-chemical analysis for developing fingerprint data for its rational, safe clinical use. **Methodology:** The current research at first the *Kantakaryadi granules* is manufactured by modifying the pharmaceutical processing of *Kantakaryadi avaleha*, by following the working principles of *Avaleha kalpana* and *khanda kalpana*, thereafter. Then the prepared *Kantakaryadi granules* are analyzed by following the modern scientific analytical techniques like HPTLC study, Phytochemical analysis, Total ash Value, pH value etc. for generating the fingerprint data. **Observation and Discussion:** In the said research the analytical findings reveal that it contains alkaloids, glycosides, tannins and carbohydrates in significant amounts whereas

flavonoids, steroids and terpenoids are also found in the sample. It is lacking saponins, proteins and starch, having pH- 5.99 and 38.74% sugar. The analytical data suggests its comfortable rational use in human beings with additional benefit of enhanced self-life, appropriate palatability, easy storing and transportation along with convenience of drug delivery and drug dispensing system, in comparison to the same avaleha. **Summary and Conclusion:** *Kantakaryadi granule* is a noble solid dosage form and physico-chemically suitable for rational therapeutic use. All the analytical data observed are within the normal range of human use for therapeutic purposes. The data generated for Physico-chemical characterization could be the finger print standard for *Kantakaryadi granules* for further research in academics and industries.

KEYWORDS: *Kantakaryadi avaleha*, HPTLC, Phytochemicals.

INTRODUCTION

Ayurveda, the science of life, developed in the Indian Sub-continent is dealing with the holistic approach of health care. Many dosage forms are there in Ayurvedic practice of medicines that includes decoctions, confections, granules, oleaginous preparations, metallic/mineral ashes, herbo-mineral tablets, powders etc. Granule dosage form came into the scenario of Ayurvedic practice in about the 19th Cen. AD. Granules (*khanda kalpana*) are the modified, tertiary dosage form prepared out of *avaleha*, the secondary dosage form which is prepared out of decoctions, the primary dosage form; by further decreasing the moisture content significantly to convert the product into solid dosage form, from the semisolid dosage form. The current research material i.e. *Kantakaryadi granule* is the modified dosage form of *Kantakaryadi avaleha*, a potential medicine described in *Sharangdhar Samhita* and is being used to cure hiccough, breathlessness, dyspnoea, cough[1][2][3] with high efficacy. In current practice of Ayurvedic medicines, the subject *Rasa shastra* and *Bhaishajya kalpana* continues to play a significant role in Ayurveda, albeit with some adaptations to meet contemporary demand and supply with nobility of dosage form.

There has been an emphasis on drug designing, new drug development, and modifying the dosage form according to contemporary need and it's implementation in the entire drug delivery system. From Vedic era, through Samhita period and medieval period till the 21st century, the pharmaceutics of herbo-mineral medicines and their drug delivery system have been changed enormously. The conversion of *avaleha* dosage form into granules, facilitates

the easy drug delivery system, that is packing, dispensing and administration along with increasing shelf life as the dosage form necessarily reduces the moisture contents.

The characterization of granules is based on the features of *avaleha kalpana* as mentioned in ancient literatures[6][7] with certain extended observations, such as observing the increasing the number of strings (*tantumatvam*) in the final product. In contemporary quality assessment and product characterization method, phytochemical analysis, HPTLC study and ash values etc. are in practice to standardize the product for it's rational therapeutic use. Modern research techniques are being employed to validate the Ayurvedic medicines and their therapeutic potential along with understanding of their mechanisms of therapeutic action on the basis of chemicals/phytochemicals present in the final product, that shall facilitate the global acceptance of Ayurvedic formulations and in turn shall augment the health tourism and economic values.

MATERIAL AND METHODOLOGY

At first *Kantakaryadi granules* were prepared by following the working principles of *Avaleha kalpana* then modifying it through *khanda kalpana* with the ingredients described for *Kantakaryadi avaleha*.[1][4] Then the prepared granules were assessed for their physico-chemical characters to generate the finger print data.

Preparation of *Kantakaryadi granules*

For preparing the research drug candidate, the genuine raw materials were collected, and authenticated by the experts and by following acceptable features mentioned in the reference texts.

At the first step the decoction of *Kantakari Panchanga* was prepared by following the 'SOP' of *kwath kalpana* and the reference of *Kantakari avaleha*.[1][5] Followed by the decoction preparation, preparation of fine powder of *Prakshepa Dravya* such as *vansalochana, trikatu* etc. was done.

After preparing decoction *Kantakaryadi* granule was prepared by following the 'SOP' for *Avaleha kalpana* requiring 01 to 02 thread consistency (*tantumatvam*) of chasni (sugar solution with decoction), was modified by extending the cooking process up to 03 to 04 thread consistency. The powdered *Prakshepa dravyas* were added during mild heating condition followed by the addition of sesame oil and ghee. The product was assessed for

ancient quality control parameters like *Tantumatvam* (appearance of strings/threads), *Apsumajjnam* (sinking in still water), *Gandha-varana-rasotapatti* (odor, color and taste), *Bhajate- pidite-mudra* (fingerprint impression) and observed compatible with the rule.

At last honey was added on cooling, mixed well, and filtered through 10 No. sieve, followed by air drying to complete remove the moisture and convert the product into solid granular and crystalline form.

Characterization of prepared granules

The *Kantakaryadi* granule prepared was characterized by following both ancient quality control parameters of *Khanda kalpana* mentioned in Ayurvedic literatures as well as by following HPTLC study, Phytochemical analysis and other modern analytical technique used for standardization and for quality evaluation of current herbal medicine (*Khanda kalpana*).

Before complete cooling and sieving (Analysis of the product just after cooking process)[6][7]

***Tantumatvam* (appearance of strings/threads):** Some materials at it's hot phase taken in between index and thumb, then pressed and released. The same procedure was repeated for 03 times and the number of strings appeared was counted each time. It was observed that 03 to 04 strings were appeared in each time.

***Apsumajjnam* (sinking in still water):** At the end point of cooking some of the material was taken by spatula and put into the still water taken in a clean beaker. It was observed that the material was sink to the bottom of the beaker.

Gandha-varana-rasotapatti (odor, color and taste): At the end of the cooking process significant odor of the ingredient materials was observed with dark brown color and bitter taste.

Bhajate- pidite-mudra (fingerprint impression): Some of the materials was taken in a stainless-steel plate and on cooling thumb was pressed over the material. It was observed that the appearance of fingerprint was clear.

After complete cooling and sieving (Analysis of the final product)

Consistency: - The product obtained after air dry and devoid of moisture, was solid and moderately hard in nature. It was free flow granular in shape.

Color and odor: - The prepared granules were dark brown in color with sweet and light pungent odor. The taste of the final product was little bitter following little sweet and pungent.

Characterization of granules by following the contemporary methods

The prepared *Kantakaryadi* granule was subject to Phytochemical analysis, HPTLC finger print analysis, with other physico-chemical analysis like pH value, Toal Ash value, Acid Insoluble Ash value, and Total sugar percentage. All analysis were done at VASU Research Center, Vadodara, Gujrat.

MATERIALS AND METHODS

MATERIALS

Sample: Prepared *Kantakaryadi* granules

Chemicals: - Ethyl Acetate, Toluene, Acetic Acid, Vanillin sulphuric acid reagent etc.

Tools and Equipment's

HPTLC apparatus with all accessories, MERCK - TLC / HPTLC Silica gel 60 F254, separating funnel, CAMAG Linomat 5 – Applicator, CAMAG TLC Twin Trough Chamber, Heating device, silica crucible, filter paper, glass rod, Borosil beaker, spatula etc.

METHOD

5g of sample was weighed in a beaker and 10 mL of water was added. Then it was subjected to sonicate for 15 minutes then was filtered with help of simple filter paper. After this, the filtrate was transferred to a separating funnel and partition with 20 mL of Ethyl Acetate. Then Ethyl acetate layer was collected in a separate beaker. The procedure was repeated twice with 15 mL of Ethyl Acetate. All the Ethyl acetate layers were pooled in an evaporating dish and was evaporated to dryness and allow to cool. Thereafter, the sample was reconstituted with 2 mL Ethyl Acetate and was filtered with 0.22 µm syringe filter. The test solution thus obtained was used for HPTLC fingerprinting.

The test sample solution was applied in 10 µl in the form bands with the programming of linomats applicator in previously prepared HPTLC plate by following SOP. The solvent was allowed to be evaporated and placed the plate in the saturated tank, vertical so that spots or bands are above the level of mobile phase. The tank was closed and allowed standing at room temperature until the mobile phase ascended to the marked line. The plate was removed, dried and visualized as in UV-V is light at 254nm, 366nm and 540nm. After development, the

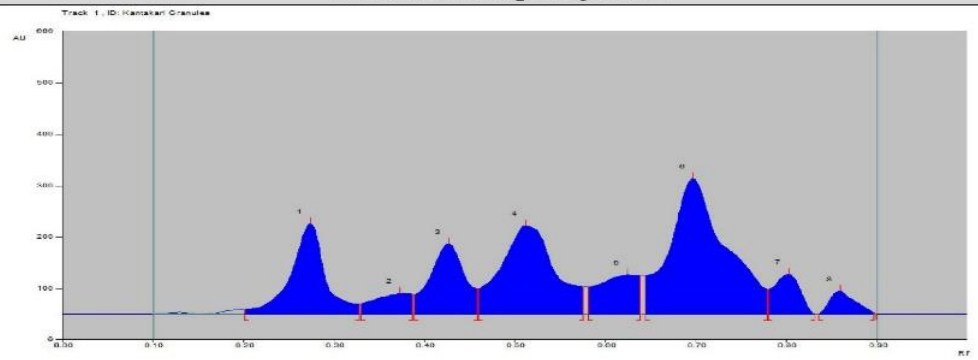
plate was allowed to dry in air, then was sprayed with 5% sulphuric acid in methanol reagent followed by heating at 1050C for about 03 min and was scanned at wavelength of 254nm, 366nm and 540nm. All the data of chromatographic spot, Rf value and graphical data of the scanning chromatograms was observed and recorded.

Experimental findings of HPTLC identified on chemical compounds separation and visualization at different UV-Vis. (254, 366 and 540nm). Below table shows the main Rf values identified from major spots at every detection point.

Rf Value @ 254 nm		**Rf Value @ 360 nm**		**Rf Value @ 540 nm**	
Spot No.	**Rf Value**	**Spot No.**	**Rf Value**	**Spot No.**	**Rf Value**
1	0.21	1	0.21	1	0.21
2	0.31	2	0.24	2	0.31
3	0.39	3	0.31	3	0.39
4	0.60	4	0.43	4	0.60
5	0.65	5	0.51	5	0.65
6	0.68	6	0.68	6	0.68
7	0.77	7	0.87	7	0.77
8	0.84			8	0.84

When the HPTLC fingerprint data was analyzed at different wavelength, it is revealed from the obtained Rf values, sample is the mixture of various phytochemicals and may contain different Alkaloids, Glycosides, Tannins and Carbohydrate as major components whereas different Steroids, Terpenoids and Flavonoids are as minor components.

HPTLC Chromatogram @ 254 nm



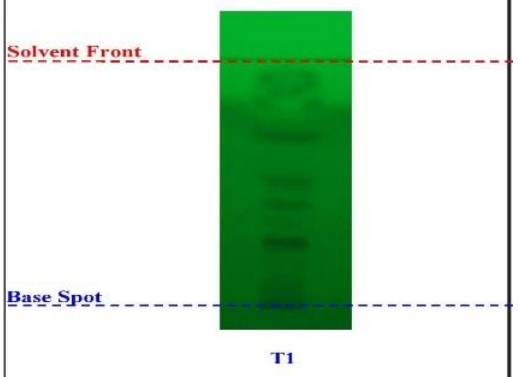
2D chromatogram

R_f value @ 254 nm

Spot No.	Track T1
1	0.21
2	0.31
3	0.39
4	0.60
5	0.65
6	0.68
7	0.77
8	0.84

Track T1: Kantakaryadi Granules

HPTLC Plate @ 254 nm



HPTLC FINGERPRINTING REPORT

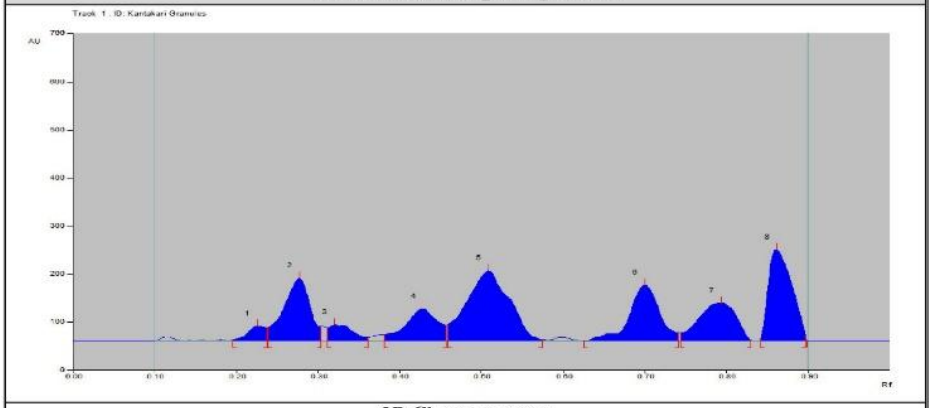
Preparation of Test solution: Weigh 5 g of sample in a beaker and to it add 10 mL of Water. Sonicate for 15 minutes and filter with help of simple filter paper. Transfer the filtrate to a separating funnel and partition with 20 mL of Ethyl Acetate. Collect the Ethyl acetate layer in a separate beaker. Repeat the procedure twice with 15 mL of Ethyl Acetate. Pool all the Ethyl acetate layers in an evaporating dish and evaporate to dryness and allow to cool. Thereafter, reconstitute the sample with 2 mL Ethyl Acetate and filter with 0.22 µm syringe filter. Use the Test solution thus obtained for HPTLC fingerprinting.

Preparation of Spray reagent [5 % Sulphuric acid in Methanol reagent]: 5 mL Sulphuric acid is cautiously mixed with 100 mL Methanol.

Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F_{254} on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	10.0 µL
Distance Between Tracks	8 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl acetate : Acetic acid (7 : 2 : 1 v/v)
Pre-chromatographic derivatization	@ 254 nm, @ 366 nm and @ 540 nm (after derivatization)
Visualization	Vanillin sulphuric acid reagent
Spray reagent	CAMAG – Dip tank for about 1 minute
Derivatization mode	TLC Plate Heater Preheated at 100 ± 5^{0}C for 3 minutes
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 5^{0}C for 3 minutes

HPTLC Chromatogram @ 540 nm



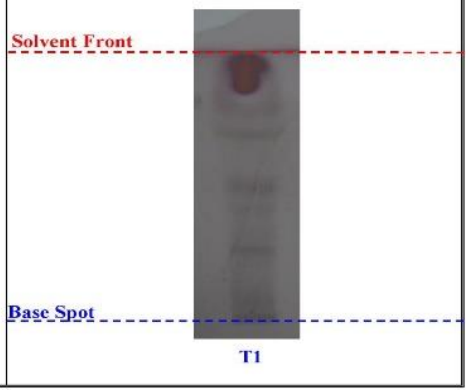
2D Chromatogram

R_f value @ 540 nm

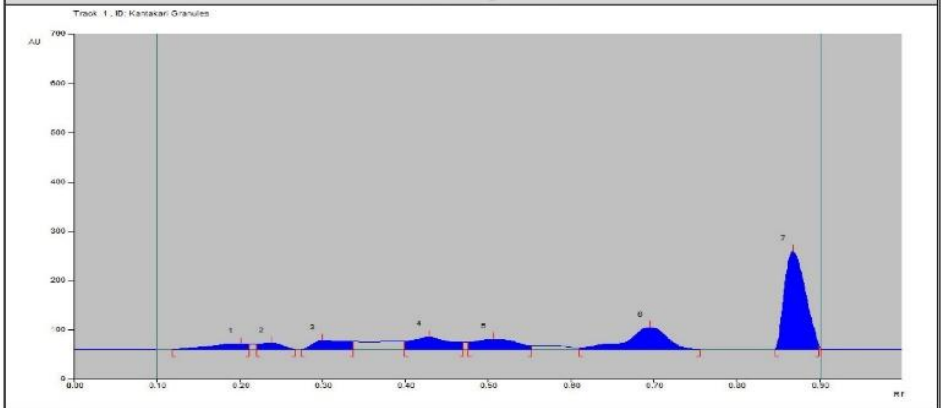
Spot No.	Track T1
1	0.21
2	0.31
3	0.39
4	0.60
5	0.65
6	0.68
7	0.77
8	0.84

Track T1: Kantakaryadi Granules

HPTLC Plate @ 540 nm



HPTLC Chromatogram @ 366 nm



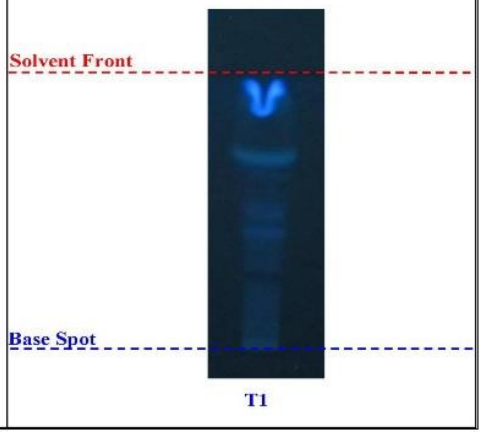
2D chromatogram

R_f value @ 366 nm

Spot No.	Track T1
1	0.21
2	0.24
3	0.31
4	0.43
5	0.51
6	0.68
7	0.87

Track T1: Kantakaryadi Granules

HPTLC Plate @ 366 nm



Phytochemical Analysis

MATERIALS

Drug (Sample): - *Kantakaryadi Granules*

Chemicals: - Sulphuric acid (H_2SO_4), Hydrochloric acid (HCL), Potassium Iodide (KI), Mayer's reagent, Dragendorff's reagent, Ferric chloride ($FeCl_3$), Sodium hydroxide (NaOH), Ninhydrin solution, alcoholic KOH, Molisch's reagent and Vanillin solution**,** Lead acetate solution.

METHOD

At first aqueous extract and alcoholic extract of the sample was prepared as test solution for different phytochemical analysis.

Sl. No.	Name of Phytochemicals	Test Method
	Alkaloids	Aqueous solution of the sample gave pale yellow color precipitation with Mayer's reagent indicating present of alkaloids of the purine groups. Orange color precipitation appeared in Dragendorff's reagent indicating presence of alkaloids.
	Glycoside	To an aqueous extract of the sample, Glacial Acetic Acid, a few drops of $FeCl_3$ and conc. H_2SO_4 were added. A reddish-brown color at the junction of two layers and changing of the upper layer into Bluish Green indicated presence of Glycoside in the sample.
	Flavonoids	02 ml HCL was added to the alcoholic extract of the sample, in a test tube. Red color was developed indicating presence of flavonoids in the sample.
	Tannin	Aqueous extract of the sample is treated with Vanillin- HCl-Alcohol reagent (Vanillin 1gm + 10 ml conc. HCl +10 ml Alcohol) and brick red color is formed indicating the presence of tannin.
	Steroid	02 ml of aqueous and alcoholic extract are refluxed separately with solution of alcoholic KOH till complete saponification process occurred. The saponified mixture is then diluted with distilled water and extracted with ether. The ethereal extract thus obtained is evaporated and the residue is subjected to Liebermann burchard's test. The appearance of blue-green color confirms the presence of steroids in the sample.
	Terpenoids	05 ml of aqueous extract of sample was mixed with 02 ml of chloroform in a test tube, then 03 ml of conc. H_2SO_4 was added carefully from side of the test tube slowly, a reddish-brown color interface was observed indicating presence of terpenoids in the sample.
	Saponin	01 ml of Aqueous extract of the sample was added to 1% of lead acetate solution in a test tube. White color precipitation was

		observed indicating the presence of saponins in the sample.
	Carbohydrate	2 ml of the aqueous extract of the sample was taken in a test tube and 2 ml of the Molisch's reagent was added, shaken carefully, followed by pouring of 1 ml. of conc. H_2SO_4 from side of the test tube slowly. After some time, a red brown ring at the junction of the two layers is observed, indicating the presence of carbohydrate in the sample.
	Protein	Alcoholic solution of Ninhydrin was added to the aqueous extract of the sample. Absence of formation of violet color indicates absence of proteins in the sample.
	Starch	When aqueous solution of the sample is mixed with Iodine solution, non-appearance of blue-black color confirms the absence of starch**.**

OBSERVATION

Phytochemical Analysis of the sample *Kantakaryadi* granules was done and the presence of following phytochemicals were revealed.

Table Showing the Presence of different Phytochemicals in *Kantakaryadi* Granules.

Sl. No.	Name of Phytochemicals	Presence/Absence	Limits
1	Alkaloids	+++	NA
2	Glycoside	+++	NA
3	Flavonoids	++	NA
4	Tannin	+++	NA
5	Steroid	+	NA
6	Terpenoids	+	NA
7	Saponin	-	NA
8	Carbohydrate	+++	NA
9	Protein	-	NA
10	Starch	-	NA

Determination of Total Ash: 02 gm of sample was accurately weighed. Then the grounded sample was incinerated after taking in a tared silica crucible at temperature not exceeding 450^0c until free from carbon. Then it was cooled and the ash of the sample was weighed. The percentage of ash with reference to the air-dried sample was calculated.

OBSERVATION: - 10.61% total ash was observed

Determination of Acid insoluble ash: 02 gm. Of sample was accurately weighed. Then the grounded sample was incinerated after taking in a tared silica crucible at temperature not exceeding 450^0c until free from carbon. Then it was cooled and the ash of the sample was weighed.

Ash was boiled for 05 minutes with 25 ml of dil. HCl. The insoluble matter was collected in Gooch crucible, was washed with hot water and ignite at a temperature not exceeding 450^0c to constant weight. Then the percentage of acid insoluble ash was calculated with reference to the air-dried sample.

Observation: - 09.33% acid insoluble ash was observed.

Analysis of pH value

METHOD

pH meter was calibrated by using standard buffers of known pH 4.0 and 9.2 at 30^0C, the reference electrode was thoroughly washed with distilled water every time and water was drained by using filter paper. The sample was Shaked well and homogenized just before taking pH reading. The oil or grease free electrode was dipped into the sample in such a way the glass bulb of the electrode is fully immersed in the solution. Reading was observed on the digital meter.

It was observed that the pH of the sample (*Kantakaryadi* granule was 5.99

Determination of Size of the granules

To determine the size of the *Kantakaryadi* granules sieving method was followed. At first the samples were sieved mechanically through 10 No. sieve. Then the sample was made pass through 20 and 40 No. sieve.

OBSERVATION

Table Showing Size of *Kantakaryadi* granules.

% Retain on 10 No. sieve	30.36 %
% Passes through 10 No. sieve	63.51 %
% Retain on 20 No. sieve	3.95 %
% Retain on 40 No. sieve	0.95 %

DISCUSSION

With change in time and socio-economic scenario of the Globe a larger population are attracted towards the herbal medicines with it's changed, easy and convenient dosage form. Along with the convenient dosage form, quality control of the medicine and quality assurance to the consumer are the important factors for dissemination of traditional medicines in global market. Now a days share of herbal medicines in Global market is 8.5%. Herbal medicine market contributes 8.5%. of GDP in India.

Kantakaryadi avaleha is a potential medicine used to treat different diseases like hiccough, breathlessness, dyspnoea, cough since hundreds years. It contains Kantakari, G*uduchi, Chavya, Chitrak-mool, Trikatu* etc, *Kantakari* is potential bronchodilator, Trikatu is used as appetizer and digestive agent.

Avaleha is prepared by heating the decoction with sugar, till the consistency appears enough thick to exhibit 01 to 02 string appearance (*tantumatvam*) of *chasni* (sugar solution with decoction), following the addition of *prakesha Dravya*, then honey, on complete cooling. Different quality control techniques are described in literatures for *avaleha* paka like *Tantumatvam, apsumajjnam, bhajate pidite mudra*[6][7] etc. Granules are the modified tertiary dosage form, prepared out of *avaleha* by reducing the moisture content to convert the semisolid *avaleha* into solid granules.

In current research, the process is modified and the cooking process is further continued to remove more water content till the appearance of 03 to 04 thread consistency followed by addition of other materials in fine powder form (80 mesh) then addition of sesame oil and ghee. At last honey was added on colling, mixed well, and filtered through 10 No. sieve, followed by air drying to complete remove the moisture and convert the product into solid granular and crystalline form.

Granulation is the process of forming or crystalizing the medicaments into granules. In Ayurvedic pharmaceutics, *Avaleha kalpana* (confections) and *guda-kalpana* are further processed to *khanda kalpana* for providing a more stable and suitable dosage form. In this process water content is reduced further to prevent the segregation of the constituent particles and facilitates the aggregation of the medicaments into a comfortable size, shape where the drug distribution maintains maximum homogeneity in a single It causes the tight bonding and good adherence between the particles hence symmetric dosage form is form is achieved and shelf life is enhanced.

Objective parameters described in literatures to assess the product for it’s proper consistency are like *Tantumatvam, apsumajjnam, bhajate pidite mudra* etc. are amazing techniques, well enough to authenticate the prepared medicine.

Tantumatvam (appearance of strings/threads) signifies that the product is having minimum moisture content hence could be stored for longer duration. Thus, increasing the shelf life.

Apsumajjnam (sinking in still water) quality of the product helps to maintain the homogeneity of drug distribution in dosage form and to calculate accurate dose. ***Gandha-varana-rasotapatti*** (odor, color and taste) like organoleptic features signify the proper quality of the product. ***Bhajate- pidite-mudra*** (fingerprint impression) signifies the tightness among the particles of the dosage form that provides a symmetric shape and size.

HPTLC study and Phytochemical analysis reveals the sample contains alkaloids, glycosides, tannins and carbohydrates in significant amount whereas flavonoids, steroids and terpenoids are also found in the sample. The prepared *Kantakaryadi* granule lack of saponins, proteins and starch. The presence of alkaloids suggests the sample is having immune improving potentiality and boost up the defense mechanism of the body. Alkaloids are naturally occurring nitrogen containing compounds which exhibits significant antimicrobial activities.[8] Glycosides play an important role in growth regulation and defense mechanisms. Natural steroids cause anti-inflammatory activities. Natural steroids, alkaloids may cause anti-inflammatory action, Tannins causes antioxidant, antimicrobial and antiparasitic effects.[9] The study reveals that the combining effect of phytochemicals present in *Kantakaryadi* granule may be responsible for it's therapeutic effect. The study also agrees with the fact that after modifying the dose, the presence of essential required phytochemicals are also there in the prepared final dosage form. pH of the research drug is observed 5.99. The result suggests, it is slightly acidic in nature. It may be due to the different composition of the raw materials used. The prepared medicine contains 38.74% sugar, which is within the limit, hence it may be used in broader population of patients. Total ash value and acid insoluble ash value are found insignificant in research drug candidate.

Kantakaryadi granules is having size between 10 mesh to 20 mesh, crystalline in nature with free-flowing granule having brown color and characteristic odor.

Kantakaryadi granules are having suitable Physico-chemical characteristics for human use. It is having significant no. of therapeutically active phytochemicals. The HPTLC data suggests the presence of various Alkaloids, glycosides, tannins, steroids and terpenoids those may be responsible for rational therapeutic use.

CONCLUSION

The demand of time is to develop the new dosage form of therapeutic potential medications, that would have more self-life with good palatability and having easy drug delivery and drug

dispensing system. Granules are the modified tertiary dosage form, prepared out of *avaleha* by reducing the moisture content to convert the semisolid *avaleha* into solid granules.

Kantakaryadi granules are having suitable Physico-chemical characteristics for human use. It is having significant no. of therapeutically active phytochemicals. The HPTLC data suggests the presence of various Alkaloids, glycosides, tannins, steroids and terpenoids those may be responsible for rational therapeutic use.

The modified dosage form is suitable for human use in place of *Kantakaryadi avaleha* with additional benefit of enhanced self-life, appropriate palatability, easy storing and transportation along with convenience of drug delivery and drug dispensing system. The data generated for Physico-chemical characterization could be the finger print standard for *Kantakaryadi* granule for further research in academics and industries.

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