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Comparative analytical study on effect of *Bhavana* on *Karavellaka Churna* (*Momordica charantia* Linn.) and *Bhavita Karavellaka Churna*

Research Article

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Abstract

Bhavana is an Ayurveda process of triturating a powdered drug with herbal juices or decoction. In classical texts this procedure is narrated to have potential to change or initiate positive therapeutic properties in a formulation. Ayurveda literature also shows that if *Bhavana* of drug juice is given to the powder of same drug then the final product will have few fold more activities than simple powder form of that drug. Present work was undertaken to study this concept on analytical ground as analysis provides basic understanding of changes in chemical composition which is actually related with therapeutic activities. *Karavellaka* (*Momordica charantia* Linn.) was chosen as drug for this work as it is well known drug for hyperglycemia. Powder of *M. Charantia* was made in 6 batches each of 1 kg. Three batches were kept in powder form (*Karavellaka Churna*, KC) and remaining three batches were given *Bhavana* of *M. Charantia* juice (*Bhavita Karavellaka Churna,* BKC). Physico-chemical analysis along with nutrition value was done to access the proportion of change. All studied parameters showed higher range of values in BKC than KC. Similar result is observed in nutritional value analysis. All these results conclude that *Bhavana* has synergistic action in increasing concentration of chemical constitutes of the drug. The increase in concentration may reduce the therapeutic dose.

Key Words: *Karavellaka*, *Momordica charantia* Linn., *Bhavana.*

Introduction

Ayurveda specific processing method (*Aushadhi Sanskara*) of herbs as well as metals and minerals is one of the fundamental concept of Ayurveda pharmaceutics. *Shodhana* (purification), *Jarana* (open pan frying) and *Marana* (incineration to create metal/mineral calx) are most common processing's of single metallic and mineral drugs. On the other hand, *Bhavana* (trituration) and preparation of *Ghana Satva* (solidified extracts) are the two methods which can be utilized to enhance therapeutic efficacy of single herb. According to a research giving *Bhavana* to powder of a herb by using juice extracted from the same herb result in significant increase in therapeutic potential. The increased therapeutic potential can be accessed by various analytical testing's.(1,2)

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In recent few years, the market of herbal medicine has grown significantly and there seems great competition to discover cost effective herbal medicine. Modification in dosage form or increasing the potency of available dosage form is also attracting focus of innovative researchers. Giving *Bhavana* to a drug by using juice extracted from the same drug (*Bhavita* herb) is one of the easiest, time saving and inexpensive procedure which has been studied on *Rasayana Churna* and *Guduchi Churna* and has been found positively enhancing the drug action.(3)

According to analytical point of view, *Bhavana* results in changing the chemical profile of *Bhavita* material. However such change need to be studied on analytical ground to know its actual extent as well as to know the change occurred in final product. *Karavellaka* (*Momordica charantia* Linn.) is one of the well known medicinal herbs which has *Pramehaghna* (anti-diabetic) activity and scientific researches has proven its anti-hyperglycaemic efficacy.(4,5) Considering the results of positive effect of *Bhavana* on *Bhavita Dravya*, an assumption can be made that *Karavellaka Swarasa Bhavana* may also result in changing physico-chemical nature of *Bhavita Karavellaka Churna* (BKC). Therefore present work is planned for comparative analytical evaluation of *Karavellaka Churna* (KC) and BKC.

Material & methods:

Materials:

M. charantia was used as a raw material. 9 kg *M. charantia* fruits were procured from local market of Wardha. 3 kg *M. charantia* for preparation of KC, 3 kg for extraction of juice for *Bhavana* and 3 kg for preparation of powder for BKC. Each 3kg batch was again divided into batches of 1 kg for development of standard operating procedure (SOP). (Table 1)

Table 1: Detail of ingredient and quantity

Sr.	Batch and Code	Quantity of fresh material	Purpose
1	*Karavellaka Churna* (KC)	3 kg (1 kg in each sub batch)	For *Churna* preparation (KC)
2	*Bhavita Karavellaka Churna* (BKC)	3 kg (1 kg in each sub batch)	For preparation of 3 times BKC
3	*M. charantia* Fruits	3 kg (1 kg in each sub batch)	For extraction of *Swarasa* (juice) for *Bhavana*

Methods

All collected *M. charantia* fruits were first washed and shade dried for 3 hours. Vertical section of *M. charantia* fruits was taken and seeds were removed from the interior part of fruit. (Fig.1 to 3) Then the seeds were made (excluding 3 kg *M. charantia* fruits required for *Bhavana*) into small pieces and fine paste was made by grinding in mixer. (Fig. 4 and 5) The paste was allowed to dry in hot air oven for 6 hrs per day at 60 ^{0}C. It took 3 days for complete drying of the paste. The obtained dried powder for KC batches was sieved through mesh no.80 and then stored in airtight plastic bottles. The dried powder of BKC batches was further subjected for 3 times *Bhavana* with *M. charantia* fruit juice. The juice was extracted by making paste of *Karavellaka* pieces and pressured filtering through cotton cloth. The obtained juice was utilized for 3 *Bhavana* to prepare BKC. (Fig. 6 to 8)

Analytical study

Botanical identification of *Karavellaka* species was done from National Botanical centre, Bangalore. In analytical study, KC and BKC were tested by organoleptic and physico-chemical parameters. The organoleptic characters involved the testing of samples using sensory organs. These are four subjective parameters are colour, odour, taste and touch. Physico-chemical parameters such as total ash, acid insoluble ash, alcohol-soluble extractive, water-soluble extractive, foreign matter and microbial contamination were analyzed as per pharmacopeia of India. Nutritional value of both the samples was also tested to understand the differences in their chemical components caused due to effect of *Bhavana*. The methods of analysis are as per given below.

Determination of Protein(6)

The pellet of sample tissue remaining after extraction of chlorophyll (ERT/REAC SOP #2030, Chlorophyll Determination) is treated to remove lipids, then allowed to dissolve in 0.1 N Sodium Hydroxide (NaOH) for 15 minutes to generate a crude protein extract. The solution is centrifuged, and duplicate 0.1 milliliters (mL) aliquots are pipetted into separate test tubes. The protein content is determined on these duplicates using the Bicinchoninic Acid (BCA) method. This involves mixing the sample with reagents which react to form a product with a colour intensity that is proportional to the amount of protein in the sample. The colour is quantified as an absorbance reading in a spectrophotometer at a wavelength of 562 nanometers (nm).

Determination of Total Fat(7)

5.00 g sample was weighted on a torsion balance in a 50 ml beaker. 0.5 ml of 95 percent alcohol was added and sample was stirred until it is homogeneous. 0.5 ml of 95 percent alcohol was added again and stirred thoroughly. Finally 1.0 ml of 95 percent alcohol was added and stirred again. The contents of the beaker were washed into a Babcock 9 gm cream bottle, using a minimum quantity of ethyl alcohol, 50 per cent by volume. Centrifuged for 10 minutes at approximately 1200 r.p.m. More 50 percent of alcohol was added to bring the layer of fat just below the neck of the flask and again centrifuged for 5 minutes. Sufficient 50 percent alcohol was again added to bring the column of fat well within the neck of the flask and again centrifuged for 5 minutes. The flask was removed from the centrifuge and reading was noted for percentage of fat directly. The reading was multiplied by 1.8 to obtain the percentage of fat in the sample, since the bottle is calibrated for a 9 g sample.

Determination of Carbohydrate(8)

Total Carbohydrate content was determined by subtraction of the sum of the crude protein, total fat, moisture, and ash from the total weight of the sample.

Determination of Total Mineral(9)

Dried plant samples were ashed in a furnace by nitric (AR) and hydrochloric acid.(10) Afterwards, distilled water (50 ml) was added to samples in a volumetric flask. Atomic Absorption Spectrometry was used for determination of mineral contents.

Observation and Results

On the basis of observations, it is clearly noted that average 92 % weight loss occurs during making dry powder from wet *M. charantia* fruits including the loss

due to removal of seeds. Average 1 kg *M. charantia* fruits has yield average 74.26 and 74.53 gm dried powder in KC and BKC batches respectively. (Table 2) The 3 kg *M. charantia* fruits which were kept for extraction of juice yield average 336.33 ml juice which was found sufficient for 3 times *Bhavana* to dried powder as the average weight of dried powder was 74.53 gm only. (Table 3) *Bhavana* was given for three consecutive days and the average duration for each day was 2.40 hours. Average 110 ml *M. charantia* juice was found sufficient for *Bhavana* to taken quantity of powder. (Table 4) Observations of organolepic characters of KC and BKC showed nearly same characteristics. The organoleptic parameters have very mild difference but it is not classifiable such as the colour of BKC is slightly more greenish brown than KC. [(Table 5) (Fig.9 and 10)] Analysis of KC and BKC revealed considerable difference in all parameters except in absence of foreign matter and microbial load. The observations indicate that every parameter have higher value in BKC compared to KC. (Table 6) Similar observations are detected in nutritional value analysis which also support the results obtained in physico-chemical analysis. Highest difference is detected in Carbohydrate and Total energy. In both the parameters BKC showed much more proportion of carbohydrate and total energy compared to KC. (Table 7).

Table 2: Weight changes during preparation of *M. charantia* powder

Batch	Sub batches	Initial Quantity Of *Karavellaka* Fruit (gm)	Weight changes after removing seeds			Weight of paste (gm)	Weight after Drying	Powder weight after sieving (gm)	% weight loss compared to initial quantity
			Obtained quantity (gm)	Loss (gm)	% weight loss				
KC	1	1000	854	146	14.6	845	81.1	76.2	92.38
	2	1000	850	150	15	840	80.5	74.1	92.59
	3	1000	840	160	16	829	79.3	72.5	92.75
Average	**1000**	**848**	**152**	**15.20**	**838**	**80.30**	**74.26**	**92.57**	
BKC	4	1000	850	150	15	841	80.8	74.9	92.51
	5	1000	852	148	14.8	842	81.4	75.3	92.47
	6	1000	846	154	15.4	837	82.2	73.4	92.66
Average	**1000**	**849.33**	**150.66**	**15.06**	**840**	**81.46**	**74.53**	**92.54**	

Table 3: Extraction of juice of *M. charantia*

Sr.	Weight of *Karavellaka* fruits (gm)	Weight of paste (gm)	Obtained quantity of juice (ml)
1	1000	847	345
2	1000	844	344
3	1000	832	320
Average	**1000**	**841**	**336.33**

Table 4: Observations of preparation of BKC

Batch	Quantity of powder (gm)	Number & Quantity of juice for *Bhavana* (ml)			Average quantity of juice for *Bhavana*	Average duration of *Bhavana* (for 3 days)	Weight of obtained BKC (gm)	% weight gain
		1st	2nd	3rd				
BKC-1	74.9	120	115	105	113.33	2.40 hours/ day	82	9.48
BKC-2	75.3	120	110	100	110.00	2.40 hours/ day	86	14.21
BKC-3	73.4	120	100	100	106.67	2.40 hours/ day	80	8.99
Average	**74.53**	**120**	**108.33**	**101.66**	**110.00**	**3.40 hours/ day**	**82.66**	**10.89**

Table 5: Observations of organoleptic characters of KC and BKC

Sr.	Test	KC	BKC
1	Colour	Greenish brown	Greenish brown
2	Odour	Non specific	Non specific
3	Taste	Bitter	Bitter
4	Touch	Smooth powder	Smooth powder

Table 6: Observations of analysis of KC and BKC

Sr.	Test	API standard	KC (values in % w/w)				BKC (values in % w/w)			
			1	2	3	Average	1	2	3	Average
1	Total ash	Not more than 8.5	6.7	6.4	6.5	6.53	7.4	7.6	7.2	7.40
2	Acid insoluble ash	Not more than 0.6	0.24	0.22	0.30	0.25	0.31	0.33	0.30	0.31
3	Water soluble ash	Not more than 4	3.54	3.48	3.51	3.51	3.7	3.6	3.7	3.67
4	Alcohol-soluble extractive	Not less than 6	18	20	19	19.00	21	24	23	22.67
5	Water-soluble extractive	Not less than 28	36.8	37.2	36.7	36.90	37.6	37.9	38.2	37.90
6	Foreign matter	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
7	Moisture content	-	3.20	3.18	3.20		3.21	3.21	3.20	
8	Microbial contamination	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 7: Analysis of nutritional value of KC and BKC

Sr.	Parameter	KC (%)		BKC (%)	
1	Protein	1.54		5.98	
2	Total Fat	0.0		0.0	
3	Carbohydrate	25.43		73.10	
4	Total Minerals	10.26		13.30	
		Calcium	0.79	Calcium	1.02
		Copper	1.58	Copper	2.05
		Iron	1.97	Iron	2.56
		Magnesium	1.58	Magnesium	2.05
		Manganese	1.58	Manganese	2.05
		Zinc	2.76	Zinc	3.58
5	Crude fibers	4.35		5.71	
6	Total energy	260.64 Kcal/100 gm		316.32 Kcal/100 gm	

Discussion:

The classical texts of Ayurveda as well as the development in Ayurveda pharmaceutics clearly represents that the ancient seers of Ayurveda has discovered and successfully practiced few pharmaceutical techniques which has significant role in changing pharmacokinetic properties of medicinal substances. *Bhavana* is one among such pharmaceutical procedures in which juice, decoction or liquid prepared from medicinal herbs is used to triturate one or more herbal or herbo-mineral combinations. Studies indicates that process of *Bhavana* results in impregnation of active molecules from the liquid utilized for *Bhavana* to the substance which is triturated.(11,12,13) In another words, *Bhavana* process can be used to enhance medicinal properties of a drug by triturating it with the liquid extracted from another drug which has similar potential. Here it can be claimed that this synergistic way of enhancing potential can be used in definite way by triturating a drug with liquid extracted from the same drug. The changes occurred after such procedure can be well accessed on analytical ground. Analytical studies has its own role in pre-clinical evaluation of possible potential in a medicinal product and thus analytical study serve as a creator of solid base for further researches on clinical ground. Considering these facts,

present study was done on comparative analytical evaluation of KC and BKC.

Fruits like *M. Charantia* have high proportion of liquid in fruit cells which result is significant weight loss after drying. In pilot study, attempt was made to make powder of *M. Charantia* fruit by making tiny pieces and then allowing them for shade drying followed by grinding in mixer. However it was noted that the tiny pieces of *M. Charantia* become much hard which made grinding difficult. Therefore instead of making small pieces, pulp of wet fruit was made in to paste in grinder and then the pulp was dried on hot air oven. This procedure is easy compared to making pieces. Still weight loss detected is about 92% which may indicates that only 8 % portion of *M. Charantia* fruit includes fibers, proteins, carbohydrates, vitamins and minerals. Dried powder of *M. Charantia* was stored in airtight container to avoid microbial contaminations as well as to interaction with atmospheric changes. Powder prepared for BKC was subjected for *Bhavana* in wet grinder machine. Utilization of grinder helps in applying uniform pressure and friction which plays role in impregnation of chemical constitutes from liquid to the drug. Average 10.89% weight gain is detected after *Bhavana* in three batches of BKC. This observation itself represents addition of components of liquid in the dried powder. However as the juice contain cells and fibers of *M. Charantia* hence analysis was a must step to decide whether there is only increase in mass or actual increase in chemical constitutes too.

Physico-chemical analysis showed that all parameters are in range prescribed in Ayurvedic pharmacopoeia of India. Total ash of drug represents presence of inorganic or mineral content.(14) Observed Total ash in BKC is higher than KC which is also confirmed by nutritional analysis in which total mineral content in BKC is 13.30 and in KC it is 10.26 percent. This may also be reason behind higher value of acid insoluble ash in BKC compared to KC. Few components of herbal drug are soluble in water while few are soluble in alcohol. The extent solubility describes which media is suitable solvent for extraction drug constitutes.(15) Alcohol and water-soluble extractives of both sample indicates better solubility in alcohol. However which soluble portion contain which constitutes is not clear and thus claim can not be made regarding which extractive can be used for therapeutic purpose. In both the test, KBC has higher solubility than KC which may be due to higher concentration of phytoconstitutes compared to KC. Both samples were devoid of any microbial contamination or presence of any foreign matter which indicates proper care taken during preparation of KC and BKC.

In nutritional analysis, presence of fat is not detected in both samples. Research indicates that total fat in *M. Charantia* fruit range from 2.9 to 6.4%.(16) The reason behind absence of fat in both sample is unknown, however it can be interpreted that it may be due to geographical differences in the selected sample. The result may need to be revalidated by further repetition of similar research. Carbohydrates are the source of energy which even helps in maintaining energy while fasting.(17)

The proportion of carbohydrate in KBC is nearly three times higher than KC. This finding clearly shows the higher nutritional value of KBC which is actually caused as effect of *Bhavana*. Similar observation is also found in total energy of the test drugs. Elements such as Calcium, Copper, Iron, Magnesium, Manganese and Zinc are proven to have major role in human physiology. Calcium helps in building bones and teeth; it activates various enzymes throughout body; also helps in regulating blood pressure; work on nerves for sending messages, and also helps in clotting of blood. Copper assists as metabolic fuel, plays role in making RBC (red blood cells), act as regulator for neurotransmitters, and for mopping up the free radicals. Iron is well known to help in making hemoglobin as well as myoglobin. Iron is also essential for activation of certain enzymes, collagen, making amino acids, hormones and neurotransmitters. Like calcium, Magnesium also participates in building bones and teeth. It also helps in regulating blood pressure, blood sugar, contraction of muscles, production of various enzymes, and clotting of blood. Manganese helps to metabolize amino acids, carbohydrates and cholesterol. Zinc act on blood clotting, production of proteins and DNA, as immunomodulator, and also helps in healing of wound. (18) More proportion of all these elements in BKC is an evidence of impregnation of constitutes of *M. Charantia* juice into its powder which further highlights significance of *Bhavana* in increasing therapeutic potential of the drug as well as its synergistic action.

Conclusions

Bhavana of *M. Charantia* juice plays vital role in increasing concentration of various constitutes present in its powder form. Therapeutic potential of a drug can be significantly increased by *Bhavana* to that drug by its own juice. Present study also proves the Ayurveda claim of changes in drug attributes by applying *Bhavana*. The analytical profile of BKC indicates further need of research on clinical ground to know its dose and intensity of action as an anti-diabetic agent as well as in other systemic disorders.

Figures:

Fig. 1: Procured *M. Charantia* fruits

Fig.2: Removing of seeds

Fig.3: *M. Charantia* after removing seeds

Fig. 4: Small pieces of *M. Charantia*

Fig.5: *M. Charantia* Pulp for drying to prepare KC

Fig.6: *M. Charantia* Pulp for *Bhavana* to prepare BKC

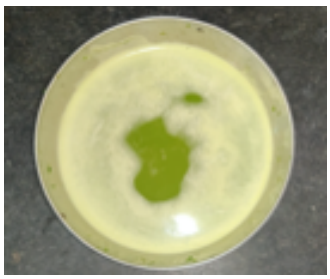

Fig.7: Extracted juice of *M. Charantia*

Fig. 8: *Bhavana* procedure

Fig.9: Prepared KC

Fig.10: Prepared BKC

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