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Preparation and Evaluation of Classical Ayurvedic Formulation Kantkari Avaleha

Dr. Satish V Kilaje[1], Shubham Patil[1], Pranil Toraskar[1], Pratik Ugare[1], Dr. G. Krishnamurthy[2]

[1]Dr. J J Magdum Pharmacy College, Jaysingpur, Dist. Kolhapur, 416101, Maharashtra, India.
[2]Department of Pharmacognosy, Al Ameen College of Pharmacy, Bengaluru, Karnataka, India.

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ABSTRACT
Objective: The objective of this study was to prepare and evaluate **Kantkari Avaleha**.
Methods: The selected formulation **Kantkari Avaleha** was prepared as per the methodology described in Ayurvedic texts. The prepared and marketed formulations were analyzed for their physico-chemical constants using established methods in accordance with WHO guidelines, the Indian Pharmacopoeia, and standard protocols from the Ayurvedic Pharmacopoeia of India, Herbal Pharmacopoeia, and other relevant sources.
Results: A profile consisting of distinguishing parameters was developed to provide means of correct identification. Some important organoleptic features of **Kantkari Avaleha** laboratory and market formulations like color, odour, taste, appearance, touch, etc. were documented. Physico-chemical constants were determined and documented. The formulations were tested for the presence of heavy metals, microbial content, pesticide residues and aflatoxin, which were also found within the limits of WHO specifications. Preliminary phytochemical studies of **Kantkari Avaleha** revealed the presence of terpenoids and phenolics as chief constituents. High performance thin-layer chromatography (HPTLC) method was developed and reported for the quantification of Oleanolic acid and Lupeolacid as marker constituents to ensure identity and quality of **Kantkari Avaleha**.
Conclusion: The studies carried out on **Kantkari Avaleha** can serve as a valuable tool and provide suitable standards for its identification. Phytochemical screening and the quantitative HPTLC assay method developed for quantification of Oleanolic acid and Lupeolin **Kantkari Avaleha** can serve for the routine quality control of commercial samples and help in justifying the traditional claims endowed upon this formulation in a scientifically accepted manner.

Keywords: Kantkari Avaleha, Ayurvedic Formulation, Oleanolic Acid and Lupeol, Phytochemical Evaluation.

INTRODUCTION

Avaleha is one of the ayurvedic polyherbal formulation mentioned in ayurvedic literatures, which is recommended for internal administration. These medicines are prepared from aqueous solutions (like swarasa, kwatha and hima) in addition with some other substances like sharkara, madhu as prakshepaka dravyas until a semisolid form is achieved[2]. Kantakari avaleha is one type of avaleha preparation that consists of 13 ayurvedic medicinal plants in which kantakari (Solanum xanthocarpum) is the major ingredients[2]. It is commonly used for the treatment of Hikka (Hiccup), Kasa (Cough), Swasha (Dyspnoea), Sula (Colicky Pain)[2]. The common etiology of above diseases is inflammation.

Kantkari Avaleha contains panchang of kantakari (Solanum xanthocarpum), stem of guduchi (Tinospora cordifolia), stem of chavya (Piper chaba), root of chitraka (Plumbago zeylanica), rhizome of musta (Cyperus rotundus), gall of karkatashrungi (Pistacia integerrima), mixture of trikatuchurna (Zingiber officinale, Piper nigrum, Piper longum), panchanga of dhanvayasa (Fagonia cretica Linn.), root of bharngi (Clerodendrum serratum), root of rasna (Pluchea lanceolata), rhizome of Karchura (Curcuma zedoaria), fruit of pippali (Piper longum), extract of vamshalochana (Bambusa bamboos), sharkara, ghrita, madhu, til tail, and water for decoction.

The aim of the present study was to explore Kantkari Avalehaformulations appearing in Ayurvedic traditional medicine for establishing the scientific data pertaining to quality assessment.

MATERIALS AND METHODS

Materials

Procurement of Selected Formulations and Crude Drugs

The selected formulations, Kantkari Avaleha, the crude drugs required for the preparation of laboratory formulation were procured from Soham Ayurved Rasashala, Solapur, Maharashtra.

Reagents and Chemicals

Chemicals, solvents and reagents used for the study were of analytical grade.

Methods

In order to develop the methods to assess the quality control parameters of the selected formulations, selected formulations were subjected to determination of physico-chemical constants using reported methods as per WHO guidelines (WHO, 1998) and Indian Pharmacopoeia, 1996.[6,7]

The selected formulationKantkari Avalehawas prepared as per the methodology described in Ayurvedic texts.

Kantkari Avaleha

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Method of Preparation of Kantakari Avaleha

The laboratory formulation of Kantakari avaleha was prepared as per the Sharangdhar Samhita.

Ingredients of Kantkari Avleha

The ingredients were purchased as per the Pharmacopoeial standards for Kantakari avaleha preparation listed under table 1 as panchang of kantakari (Solanum xanthocarpum), stem of guduchi (Tinospora cordifolia), stem of chavya (Piper chaba), root of chitraka (Plumbago zeylanica), rhizome of musta (Cyperus rotundus), gall of karkatashrungi (Pistacia integerrima), mixture of trikatuchurna (Zingiber officinale, Piper nigrum, Piper longum), panchanga of dhanvayasa (Fagonia cretica Linn.), root of bharngi (Clerodendrum serratum), root of rasna (Pluchea lanceolata), rhizome of Karchura (Curcuma zedoaria), fruit of pippali (Piper longum), extract of vamshalochana (Bambusa bamboos), sharkara, ghrita, madhu, til tail, and water for decoction.

Table 1. Ingredients of Kantkari avleha

Sr.No.	Ingredient	Botanical name	Part used	Quantity
1	Kantakari	Solanum xanthocarpum	Panchang	Q.S.
2	Guduchi	Tinospora cordifolia	Stem	1.47%
3	Chavya	Piper chaba	Stem	1.47%
4	Chitrak	Plumbago zeylanica	Root	1.47%
5	Musta	Cyperus rotundus	Rhizome	1.47%
6	Karkatshrungi	Pistacia integerrima	Galls	1.47%
7	Sunthi	Zingiber officinale	Rhizome	1.47%
8	Marich	Piper nigrum	Fruits	1.47%
9	Pippali	Piper longum	Fruits	1.47%
10	Dhanvayas	Fagonia cretica	Panchanga	1.47%
11	Bharangi	Clerodendrum serratum	Root	1.47%
12	Rasna	Pluchea lanceolata	Root	1.47%
13	Karchura	Curcuma zedoaria	Rhizome	1.47%
14	Sharkara	---	---	29.41%
15	Goghritam	---	---	11.77%
16	Til Tail	Sesamum Indicum	Oil	11.77%
17	Madhu	---	---	11.77%
18	Vanshlochan	Bambusa arundinacea	Stem Extract	5.88%
19	Pippali	Piper longum	Fruit	11.77%

Preparation of Kantakari Avaleha Laboratory Formulation(KALF)

The specific classical method mentioned for Kantakari avaleha in Sarangadhara Samhita was followed for the preparation of Kantakari avaleha. According to that; added Sita (sugar candy) in Kantakari avaleha and stirred over heating until dissolve the sugar for 10 minutes. Temperature of homogenous mixture was around 80ºC. Then the mixture of sugar and Kwatha was filtered through double cloth to remove physical impurities of sugar candy. Then added the powdered ingredients named as Churna Dravya (Sr.no.2-13 in Table 5.19) with Ghrita and Taila together to the filtrate.

The mixture was heated between 90- 95ºC with stirring, till it attained the consistency of Leha as confirmed by the formation of soft bolus (avleha pariksha), which did not disperse in water, which was latter kept for self-cooling up to around 60ºC temperature.

The fine powders of Vansh lochana, Pippali were added and stirred properly to get uniform mixture and allowed to cool to room temperature. Madhu was added at 30ºC temperature and mixed thoroughly to obtain homogeneous blend. Final product was stored in airtight containers.

Standardization of Kantakari Avaleha Laboratory and Marketed Formulation

The Laboratory and Marketed formulations of Kantakari avaleha were standardized as per WHO guidelines under the various headings using standard protocol obtained from Ayurvedic Pharamacopoeia of India, Herbal Pharmacopoeia, Herbal compendium, reference books and official agency guidelines listed under table 2.

Table 2: Parameters for Kantakari Avaleha

Sr.No.	Test
1	Description
	Colour
	Odour
	Taste
2	Loss on drying at 105°C
3	Total ash
4	Acid insoluble ash
5	pH
6	Total solid
7	Fat content
8	Reducing sugar
9	Total sugar
10	Total Phenolic content
11	Identifications, TLC/HPTLC
12	**Test for heavy metals** Lead Cadmium Mercury Arsenic
13	**Microbial contamination** Total bacterial count Total fungal count
14	**Test for specific Pathogen** E. coli Salmonella spp. S. aureus Pseudomonas aeruginosa
15	**Pesticide residue** Organochlorine pesticides Organophosphorus pesticides Pyrethroids
16	Test for Aflatoxins (B1,B2,G1,G2)
17	Phytochemical Evaluation

Determination of Phenolic Content: (AP part – I, 2001, API part – I-1999, AFI part – I -2003)

The total phenolic content of the formulations was determined by the Folin-Ciocalteau method with some modifications. Five grams of KALF and KAMF was mixed in 50 ml water and then filtered with Whatman No.1 paper. 0.5 ml of the filtrate was added to 2.5 ml of 0.2 N Folin Ciocalteau reagent and placed for 5 minutes. Two

ml of 75 g/L of Na_2CO_3 was then added and the total volume made upto 25 ml using distilled water. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760 nm using 1 cm cuvette in a Perkin-Elmer UV-VIS lambda 25 spectrophotometer. Tannic acid (0 - 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Tannic acid equivalents (TAE) / g of extract.

Considering wide therapeutic applications of **Kantakari avaleha**and presence of the marker constituents, to ensure identity and quality of **Kantakari avaleha**, a simple, sensitive, specific and reproducible High performance thin-layer chromatography (HPTLC) method was developed for the quantification of markers in the formulation.

RESULTS

Standardization of Finished Product

The laboratory formulation of Kantakari avaleha and marketed formulation of Kantakari avaleha were standardized by employing various parameters such as organoleptic, physicochemical, qualitative, and quantitative analysis. The data were also compared with commercially available product. The organoleptic characteristics of both prepared and the commercially available product of Kantakari avaleha were analyzed on the basis of observational criteria (appearance, color, taste, and odor). The physicochemical parameters such as loss on drying, extractive value in different solvent, pH, and acid value were determined as per the guidelines mentioned in Ayurvedic Pharmacopeia of India. The phytochemical screening was performed using different qualitative assay methods described previously. Total reducing sugar content was determined using a titration method as described previously. Quantitative estimation of total polyphenolic content was performed using spectroscopic method and was expressed in equivalent of Gallic acid.

Assessment of heavy metal contamination, microbial contaminants, pesticides, and aflatoxins are mentioned in Tables 6,7,8,9 and 10.

Table 3. Results of organoleptic parameters of Kantakari avaleha KALF and KAMF

Sl. No.	Organoleptic Parameters	Laboratory Sample (KALF)	Marketed sample (KAMF)
1	Color	Blackish-brown	Brown
2	Odor	Characteristics	Characteristics
3	Taste	Pungent-bitter	Bitter-astringent
4	Appearance	Thick Semisolid	Semisolid
5	Touch	Soft and viscous	Viscous

Table 4. Results of Physico-Chemical Analysis of Kantakari avalehaKALF and KAMF

Sr. No.	Physicochemical Parameters	Laboratory Sample (KALF)	Marketed sample (KAMF)
1	Loss on drying 110 °C (% w/w)	12.31	12.10
2	Total ash value (% w/w)	00.98	01.33
3	Acid-insoluble ash (% w/w)	<0.01	<0.01
4	pH value (1% aqueous preparation)	6.00	6.02
5	Total Solids (%)	87.69	87.90
6	Fat content (%)	00.33	00.11
7	Reducing sugar (%)	60.52	31.91
8	Total sugar (%)	67.33	62.08
9	Phenolic content (mg/100g)	1389.36	344.26

Table 5. Results of Qualitative Phytochemical screening of Kantakari avaleha KALF and KAMF

Sr. No.	Phytoconstituent Screening Test	Laboratory Sample (KALF)			Marketed sample (KAMF)		
		Aq. Ext.	Me. Ext.	Chl. Ext.	Aq. Ext.	Me. Ext.	Chl. Ext.
1	**Carbohydrates**						
	Molisch's reagent	+	+	+	+	+	–
	Fehling test	+	+	–	+	+	–
	Reducing sugar test	+	+	–	+	+	–
2	**Alkaloids**						
	Dragendorff's test	+	+	+	+	+	+
	Mayer's test	+	+	+	+	+	+

	Wagner's test	+	+	+	+	+	−
3	**Glycosides**						
	Borntrager's test	+	+	−	+	+	−
	Legal test	+	+	−	+	+	+
4	**Phenolic compounds& tannin**						
	Ferric chloride test	+	+	+	+	+	+
5	**Flavanoids**						
	Shinoda/Pew test	+	+	−	+	+	−
	Lead acetate test	+	+	−	+	+	−
6	**Proteins & amino acids**						
	Millon's reagent	−	+	−	+	+	−
	Ninhydrin reagent	−	+	−	−	+	−
7	**Saponins**						
	Foam test	+	+	−	+	+	−
	Sodium bicarbonate test	+	+	−	+	+	−
8	**Triterpenoids**						
	Salkowski test	+	+	+	+	+	+
	Liebermann-Burchard's test	−	+	+	+	+	+
+ (positive) represents presence of the Phytoconstituent − (negative) represents absence of the Phytoconstituent Aq.: Aqueous; Me.: Methanolic; Chl.: Chloroform; and Ext.: Extract							

Table 6. Results for heavy metal contamination of KALF and KAMF

Sr. No.	Heavy Metal	Observation (ppm)		Permissible limits (ppm) As per (API)
		KALF	KAMF	
1	Mercury	<0.1	<0.1	1
2	Cadmium	<1.0	<1.0	0.3
3	Lead	<1.0	<1.0	10
4	Arsenic	<1.0	<1.0	3

Table 7. Results for Total bacterial count and Total fungal count of KALF and KAMF

Sr. No.	Microbial contamination	Observation (cfu/g)		Permissible limits As per (API)
		KALF	KAMF	
1	Total bacterial count	32×10^2	60×10^2	NMT 10^7 cfu/g
2	Total fungal count	7×10^1	1×10^1	NMT 10^4 cfu/g

Table 8. Results for Test for specific Pathogen of KALF and KAMF

Sr. No.	Pathogen	Observation		Permissible limits As per API
		KALF	KAMF	
1	Escherichia coli (cfu/25g)	Absent	Absent	NMT 10^2 cfu/g
2	Salmonella spp. (cfu/25g)	Absent	Absent	Absence Per Gram
3	Pseudomonas aeruginosa (cfu/g)	Absent	Absent	Absence Per Gram
4	Staphylococcus aureus (cfu/g)	Absent	Absent	Absence Per Gram

Table 9. Results for Test for Pesticide residue of KALF and KAMF

Sr. No.	Pesticide	Observation (ppm)	
		KALF	KAMF
1	Organochlorine pesticides	Not detected	Not detected
2	Organophosphorus pesticides	Not detected	Not detected
3	Pyrethroids	Not detected	Not detected

Table 10. Result for the estimation of Aflatoxins of KALF and KAMF

Sr. No.	Aflatoxins	Observation (ppb)		Permissible Limit (ppb) As per (API)
		KALF	KAMF	
1	B1	< 10	< 10	0.5
2	G1	< 10	< 10	0.5

3	B2	< 10	< 10	0.1
4	G2	< 10	< 10	0.1

DISCUSSION

Some important organoleptic features of Kantkari Avaleha laboratory and market formulations like color, odour, taste, appearance, touch, etc were documented.

Determination of physico-chemical constants indicated a Loss on drying of 12.31 % & 12.10%, total ash value 0.98 and 01.33%w/w, acid insoluble ash was <0.01, pH 6.00 , total solids 87.69 and 87.79%, fat content 00.33 and 00.11%, Reducing sugar 60.52 and 31.91, Total sugar 67.33 and 62.08 Phenolic content 389.36 and 344.26 respectively for Laboratory formulation and marketed formulation The Kantakari avaleha Laboratory formulation and marketed formulation were tested for the presence of heavy metals, Microbial content, pesticide residues and aflatoxin which were also found which arewithinthelimitsofWHOspecifications.

Preliminary phytochemical studies of Kantakari avaleha revealed the presence of carbohydrates, alkaloids, phenolic compounds and tannins, flavonoids, steroids and terpenoids as main constituents.

CONCLUSION

Thus, from the present study, it can be concluded that the pharmacognostical studies carried out on Kantakari avaleha can serve as a valuable tool and provide suitable standards for the identification of Ayurvedic formulations. Phytochemical screening and the quantitative HPTLC assay method developed for quantification of Lupeol and Oleanolic acid in Kantakari avaleha can serve for the routine quality control of commercial samples and help in justifying the traditional claims endowed upon the formulations in a scientifically acceptable manner.

REFERENCES

1. Brahma SK., Therapeutic importance of Rasayana Drugs with a special reference to their multi dimensional actions. Aryavaidyan. 2003:16(3):160-163.
2. Patwardhan, B. and Hooper, M., Ayurveda and future drug development. Int. J. Alternative Complement. Med., 1992:10: 9–11.
3. Panossian A., Wikman G. and H. Wagner, Plant adaptogens III. Earlier and more recent aspectsand concepts on their mode of action. Phytomedicine, 1999:6(4):287-300.
4. Mishra V, Agrawal M, Onasanwo SA, Madhur G, Rastogi P, et al. Antisecretory and cyto-protective effects of chebulinic acid isolated from the fruits of Terminalia chebula on gastric ulcers. Phytomedicine. 2013; 20: 506-511.
5. Baliga MS, Meera S, Mathai B, Rai MP, Pawar V, et al. Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: a review. Chin J Integr Med. 2012; 18: 946-954.
6. Gautam MK, Goel S, Ghatule RR, Singh A, Nath G, et al. Curative effect of Terminalia chebula extract on acetic acid-induced experimental colitis: role of antioxidants, free radicals and acute inflammatory marker.Inflammopharmacology. Inflammopharmacology. 2012; 21(5), DOI:10.1007/s10787-012-0147-3
7. Sharangadhara, Sharangadhara Samhita, Pandit ParashuramaShastri, Varanasi: Chaukhambha Orientalia Publications; Uttarakhanda 13/2: 379.
8. Vagbhatta, Ashtang Hridaya, Sutra Sthana, 5/39, Harishastri Paradkar Vaidya, editor. Reprinted ed. Rashtriya Sanskrit Samsthana, New Delhi, 2002; pp. 73-4.
9. Mukherjee PK, Rai S, Bhattacharyya S, Debnath PK, Tuhin KB, et al. (2006) Clinical study of Triphala – A well known phytomedicine from india. Iranian journal of Pharamacology & Therapeutics 5: 51-54.
10. Sushruta Sushruta Samhita Dalhana Comm - Nibandhasangraha, Chowkhambha Orientalia Varanasi, sutrasthana. 2002; 46/143: 227.
11. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C (2006) Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of Emblica officinalis Gaertn. J Ethnopharmacol 104: 113-118.
12. Rehman H, Yasin KA, Choudhary MA, Khaliq N, Rahman A, ChoudharyMI, Malik S, Studies on the chemical constituents of Phyllanthus emblica, Natural Product Research. 2007; 21(9): 77581.
13. Jain SK, Khurdiya DS, Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry (Emblica officinalisGaertn.) juice, Plant Foods for Human Nutrition. 2004; 59(2): 63-6.
14. Santoshkumar J, Manjunath S, Pranavkumar MS, A study of antihyperlipidemia, hypolipedimic and antiatherogenic activity of fruit of Emblica officinalis (Amla) in high fat fed Albino Rats, International Journal of Medical Research and Health Sciences. 2013;2(1): 70-77.

15. Baliga MS, Prabhu AN, Prabhu DA, Shivashankara AR, Abraham A, Palatty PL, Antidiabetic and Cardio protective Effects of Amla (Emblica officinalis Gaertn) and its Phytochemicals: Preclinical Observations, Bioactive Food as Dietary Interventions for Diabetes. 2013; 583-600.
16. Chatterjee A, Chattopadhyay S, Sandip K, Bandyopadhyay, Biphasic Effect of Phyllanthus emblica L. Extract on NSAID-Induced Ulcer: An Anti-oxidative Trail Weaved with Immunomodulatory Effect, Evidence Based Complementary and Alternative Medicine. 2011; 1-13.
17. Yokozawa T, Kim HY, Kim HJ, Tanaka T, Sugino H, Okubo T, Chu D, Juneja LR, Amla (Emblica officinalis Gaertn.) Attenuates Age Related Renal Dysfunction by Oxidative Stress, Journal of Agricultural and Food Chemistry. 2007; 55: 7744-52.