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Pharmaceutical and Pharmacological Evaluation of *Kantakari Avaleha*

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ABSTRACT

Avaleha is one of the ayurvedic polyherbal formulation mentioned in ayurvedic text. It is predominately used for internal administration for the management of Hikka (Hiccup), Kasa (Cough), Swasha (Dyspnoea), Sula (Colicky Pain). In this study, kantakari avaleha was prepared by a method described previously in Ayurvedic Pharmacopeia of India, part-1. The prepared sample was standardized by employing various parameters such as organoleptic, physicochemical, qualitative, and quantitative analysis. The major secondary metabolite like total phenolic content (11.23 mg/gm of avaleha with respect to gallic acid equivalent) was quantitatively estimated, which indicates the therapeutic value of avaleha. Further, anti-inflammatory activity of kantakari avaleha was evaluated using formalin-induced paw edema method. The results showed a significant reduction in paw edema at 1.25 g/kg per oral dose similar to indomethacin 10 mg/kg, p.o.

KEYWORDS

Avaleha, Kantakari, Total Phenolic Content, Anti-Inflammatory Activity

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INTRODUCTION

Avaleha is one of the ayurvedic polyherbal formulation mentioned in ayurvedic literatures, which is recommended for internal administration[1]. 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[3,4]. Literature survey on kantakari avaleha revealed that standardization of kantakari avaleha and the effect of kantakari avaleha in inflammation are not evaluated experimentally. Therefore, the present study was designed to prepare, standardize, and evaluate the anti-inflammatory activity of kantakari avaleha in experimental model.

Materials and Methods

Collection of materials:

The ingredients of kantakari avaleha were purchased from DinanathGula market, Varanasi and authenticated by Prof. Anil Kumar Singh, Dept. of Dravyaguna, Faculty of Ayurveda, Banaras Hindu University, Varanasi. The ingredients purchased for kantakari avaleha were panchang of kantakari (*Solanum xanthocarpum*), stem of guduchi (*Tinospora cordifolia*), stem of chavya (*Piper chaba*), root of chitraka (*Plumbago zeylanica*), rizome 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 shati (*Hedychium spicatum*), fruit of pippali (*Piper longum*), extract of vamshalochana (*Bambusa bamboos*), sarkara, ghrita, madhu, tila tail, and water for decoction.

Preparation of Kantakariavaleha:

Kantakari avaleha was prepared by a method described previously[5]. Kantakari (4.8kg) was mixed with 12.288 L of water in an iron pot and boiled until reduced to 3.072 L of decoction or kwath. The kwath was filtered and mixed with guduchi, chavya, chitraka, musta, karkatshrungi, trikatu churna, dhanvayasa, bharngi, basna, shati, sugar, ghee, and tila taila. Then, the mixture was heated until avaleha pakasidha lakshna was observed[6]. Then, the mixture was cooled and fine powders of

banshalochana and pippali (prakshepdravya) were added[2].

Standardization of finished product:

The prepared sample was 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[7].The phytochemical screening was performed using different qualitative assay methods described previously[8].Total reducing sugar content was determined using a titration method as described previously[9]. Quantitative estimation of total polyphenolic content was performed using spectroscopic method and was expressed in equivalent of Gallic acid[10].

Anti-inflammatory study

Animals:

Adult male albino rats weighing 150–200 g were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Rats were acclimatized to standard laboratory conditions for one week. The animals were housed in large polypropylene cages, maintained at 25±2°C, humidity 55±10%, and a 12 hr light/dark cycle. The animals were maintained on standard pellet diet and water ad libitum. All experiments were carried out as per the guidelines of committee for the Purpose of Control and Supervision of Experiments on Animals (Dean/2016/CAEC/637).

Dose calculation:

The human dose of kantakari avaleha has been reported to vary from 6 to 12g[5]. The highest dose has been considered for the experimental purpose. The animal dose of kantakari avaleha was calculated using approved dose conversion guide and 1.25 gm/kg was estimated as the animal dose based on body surface area ratio[11].

Preparation of dosage form:

Suspension of kantakari avaleha was prepared by triturating 1.25 gm of kantakari avaleha in 3 mL of 0.5% tween 80. Indomethacin suspension was prepared by triturating 10 mg of indomethacin dissolved in 1 mL of 0.5% tween 80.

Animal grouping and drug treatment:

Rats were randomly allocated into three groups namely control group, kantakari avaleha group, and indomethacin group. The control group received vehicle (0.5% tween 80 suspension) and the kantakari

avaleha group received kantakari avaleha (1.25 gm/kg) per orally for 14 consecutive days. Animals in the indomethacin group received per oral administration of the vehicle for 13 days and indomethacin suspension (10 mg/kg) on day 14. Experiments were performed during the day time.

Formalin induced hind paw edema model:

On day 14, one hr after vehicle or drug administration, all the animals of different groups received 0.05 m L of 2.5% formalin (40% formaldehyde) subcutaneously injected to the sub-plantar surface of the right hind paw. Then, the right hind paw volume was measured just before formalin administration (0 min), and at 30, 60,120, 180, and 240min after formalin administration[12]. The paw edema was estimated for each time point as the difference in paw volume before and after formalin administration.

Statistical analysis:

Data were represented as mean + SEM. Statistical analysis was performed using GraphPad Prism version 7.03 software (GraphPad Software Inc., USA). Data from total phenolic content and reducing sugar content were analyzed using unpaired t-test. Paw edema data were analyzed using two-way analysis of variance followed by Bonferroni's multiple comparison test. $P < 0.05$ was considered statistically significant.

Table 1 Organoleptic parameters of kantakari avaleha

Sl. No.	Organoleptic Parameters	Laboratory Sample	Commercial sample
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

RESULTS AND DISCUSSION

Kantakari avaleha is an ayurvedic formulation recommended for various diseases like Hikka (Hiccup), Kasa (Cough), Swasha (Dyspnoea/Asthma), Sula (Colicky Pain)[2]. In this study, kantakari avaleha was prepared as per classical text using authenticated raw drugs and the formulation was standardized by evaluating organoleptic parameters, physico-chemical parameters, phytochemical screening, and quantitative estimation of phenolic and reducing sugar content. Further, the formulation was evaluated for anti-inflammatory activity in a rat model of paw edema. Organoleptic study revealed that kantakari avaleha of laboratory sample was blackish-brown in color while commercial sample was brown which may be due to difference in manufacturing procedure and raw material quality (Table 1). Laboratory

sample was thick semisolid mass and commercial sample was thinner consistency, which may be due to higher moisture content (Table 1).

The taste and appearance was quite similar, but laboratory sample was more astringent and bitter comparison to commercial sample, reflecting higher content of sweetening agent or less in kasay dravyas (Table 1).

Table 2 Physicochemical evaluation of kantakari avaleha

Sl.No.	Physicochemical Parameters	Laboratory Sample	Commercial Sample
1	**Loss on drying 110 °C (% w/w)**	16.78	19.25
2	**Total ash value (% w/w)**	5.72	4.81
3	**Acid-insoluble ash (% w/w)**	0.137	0.112
4	**Water-soluble extractive (%w/w)**	61.88	53.38
5	**Alcohol (95%)-soluble extractive (%w/w)**	44.09	50.78
6	**Petroleum ether-soluble extractive (% w/w)**	25.87	18.52
7	**pH value of 1% aqueous preparation**	5.24	5.89
8	**Acid value**	11.07	10.13

Organoleptic screening was undertaken to establish the profile of polyherbal formulation to its identification, adulteration, and substitution. WHO recommended organoleptic study should be proposed as a practice for the analysis of polyherbal drugs. Physico-chemical evaluation of both samples of kantakari avaleha were done using various parameters like loss on drying, ash value, extractive value, pH, acid value and physiochemical parameters evaluation are mention in Table 2.

The present study revealed that loss on drying in the sample is attributed to moisture content. Loss on drying value of commercial sample was 19.25% comparison to 16.78% in laboratory sample. Loss on drying gives information about possibility of enzyme degradation, so proper care and packing of formulation is highly essential[13]. The ash value represents inorganic salt occurrence naturally or due to adulteration propose[14]. A high ash value is representation of contamination, substitution, adulteration, or carelessness in preparation of formulation. The total ash value and acid insoluble ash value of laboratory sample were 5.72% and 0.137%, respectively as compared to 4.81% and 0.112% in commercial sample. Extractive value indicate the solubility of various class of compound in a particular solvent, it reveals that the formulation is rich with which type phytoconstituents and its nature. The extractive value revealed that both formulations have more water-soluble (polar) phytochemical constituents than petroleum ether (non-polar), further extractive value for both polar and non-polar solvent of laboratory sample is high

comparative to commercial sample. Similarly, other physico-chemical parameters acid value and pH are high in laboratory sample compared with commercial sample.

The phytoconstituents present in an ayurvedic polyherbal formulation is related to its biological activity. Therefore, standardization of formulation based on phytochemical aspect is highly essential to justify its therapeutic activity experimentally[15]. In the present investigation, preliminary phytochemical screening of both samples of kantakari avaleha showed presence of major phytochemicals like carbohydrate, alkaloids, glycosides, tannins, flavonoid, saponin, phenolics, protein, and amino acid present in all three extracts and the phytochemical constituents' evaluation are mention in Table 3.

Table 3: Qualitative phytochemical screening evaluation of kantakari avaleha

Sl. No.	Phytoconstituent Screening Test	Laboratory Sample			Commercial Sample		
		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						
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

The respective solvent like water, alcohol, and chloroform were used to distinguish the chemical nature of phytochemical present in avaleha. The phytochemical screening was monitored with TLC study. The study revealed that both the samples contain major secondary metabolites, which are responsible for biological activity.

The quantitative estimation of primary and secondary metabolite like total phenolic content and reducing sugar are helpful in standardization and design of ayurvedic formulation like kantakari avaleha[16]. The quantitative estimation of major secondary metabolite like total phenolic content and primary metabolite like total reducing sugar justified therapeutic effect of avaleha. The study showed that the avaleha contains good quantity of phenolic content (Figure 1).

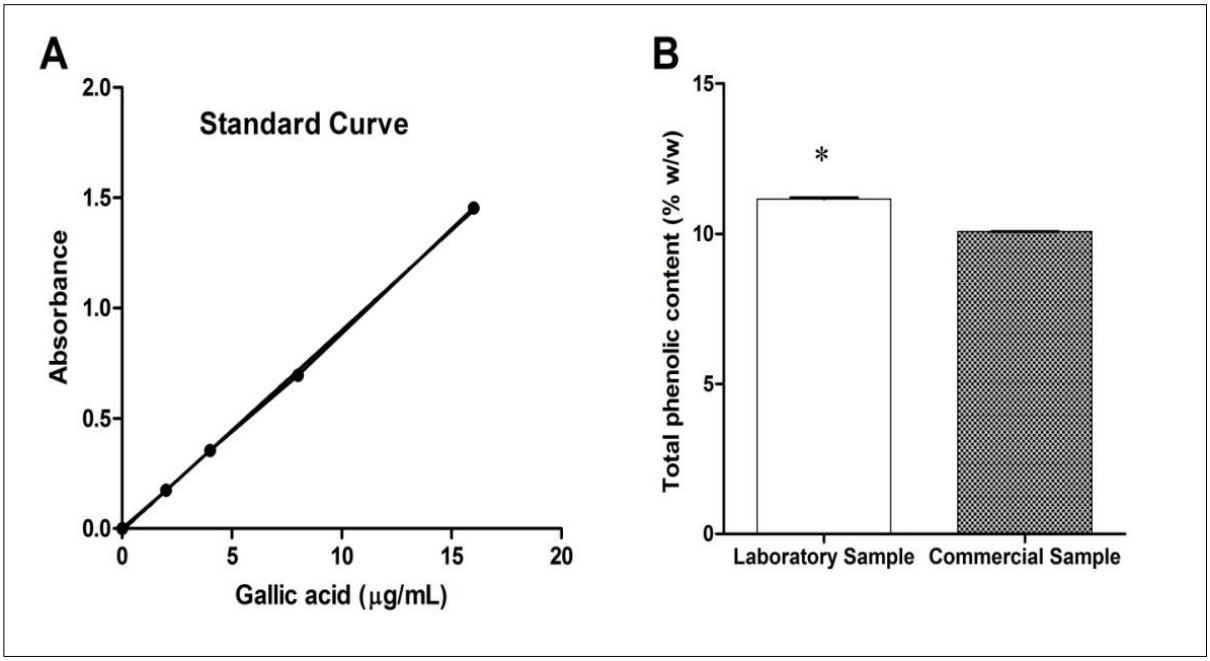

Figure 1 Quantitative estimation of total phenolic content in laboratory and commercial samples of kantakari avaleha**.** Gallic acid content in the samples was interpolated from standard curve of gallic acid (A). A significantly higher ($P < 0.05$) level of total phenolic content was observed in laboratory sample of kantakari avaleha compared with commercial sample (B)

Phenolic derivates are largest group of secondary metabolite, gaining attention due to their good anti-oxidant property and their marked effect in oxidative stress associated diseases like inflammation[17]. The phenolic rich formulation counters free radical species and reactive species under oxidative condition[18]. Also, previous study reported that high phenolic content of medicinal plant have good effect in countering inflammation[19].The study also evaluated the total reducing sugar content in both sample and it was observed that laboratory sample contain higher amount of reducing

sugar than the commercial sample (Figure 2) Anti-inflammatory activity of kantakari avaleha was evaluated using a rat model of formalin-induced paw edema.

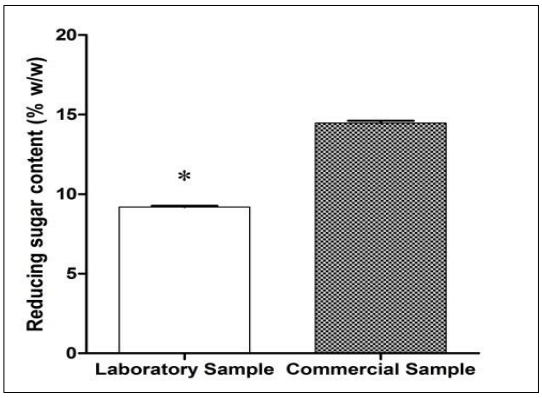

Figure 2 Quantitative estimation of reducing sugar content in laboratory and commercial samples of kantakari avaleha. A significantly lower ($P < 0.05$) level of reducing sugar content was observed in laboratory sample of kantakari avaleha compared with commercial sample

The results showed a significant reduction in paw edema at 1.25 g/kg per oral dose (Figure 3).

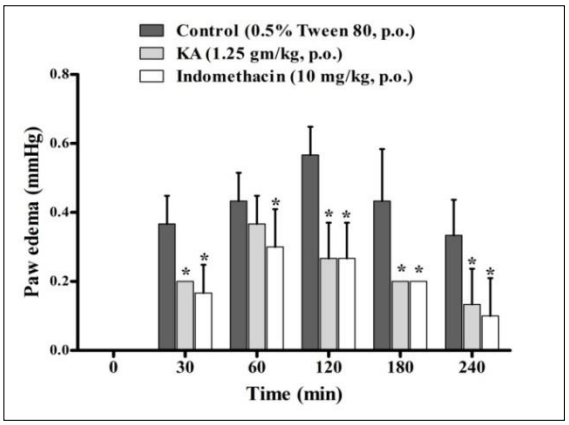

Figure 3 Effect of kantakari avaleha on formalin-induced paw edema in rats. Data represent mean ± SEM, n = 6. Per oral (p.o.) administration of Kantakari avaleha showed statistically significant ($P < 0.05$) reduction in paw edema at 30, 120, 180, and 240 min after formalin administration. Kantakari avaleha showed anti-inflammatory activity similar to indomethacin (10 mg/kg, p.o.).*$P < 0.05$ compared to control group.

Formalin-induced paw edema is a primary test to confirm the anti-inflammatory activity novel agents[20].The result of this study indicates that the kantakari avaleha may be used as an anti-inflammatory agent after further study. The anti-inflammatory activity of kantakari avaleha may the reason behind benefits against its current indications such as asthma, pain, and cough.

CONCLUSION

Kantakari avaleha is an important polyherbal formulation mentioned in the Ayurvedic Pharmacopeia of India, Part- I. The literature study revealed that kantakari avaleha is effective against Asthma (inflammatory condition of bronchial tubes)[21]. This study evaluated and fixed certain quality control parameters for kantakari avaleha. For comparison of analytical data, one commercial product was procured and analyzed. Further, kantakari avaleha was evaluated for anti-inflammatory activity and it showed significant anti-inflammatory activity against formalin-induced hind paw edema model at 1.25 g/kg. Further study on kantakari avaleha is necessary to consider it as an effective anti-inflammatory agent against inflammatory diseases.

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