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Research Article

STANDARDIZATION AND ANALYSIS OF AYURVEDIC FORMULATION

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ABSTRACT

Ayurveda is most commonly used in India which is a natural health care system. The developed countries are mostly using ayurvedic medicines and also cosmetics which are prepared with a your vedic ingredients and nutraceuticals. The ayurvedic plants are identified and properly grown for the preparation use. The physicians and pharmacists should have thorough knowledge in safety and effectivity formulations in patients who are highly using this herbal components. These herbal preparations are not standard and not estimated the quality in the market. In the present investigation, the quality of formulated arista, Kanakarishtam has been accessed. The present findings have shown that all the standard parameters based on which the standardization was carried out, were under acceptable limit as compared to pharmacopoeial specifications. Further, clinical assessment is to be carried out to completely mark the product as "safe and effective".

Keywords: Kanakarishtam, Standardization, Estimation.

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INTRODUCTION

In 5000BC, ayurveda is most commonly used in India which is a natural health care system. The developed countries are mostly using ayurvedic medicines and also cosmetics which are prepared with a your vedic ingredients and nutraceuticals. The ayurvedic plants are identified and properly grown for the preparation use. The physicians and pharmacists should have thorough knowledge in safety and effectivity formulations in patients who are highly using this herbal components. These herbal preparations are not standard and not estimated the quality in the market. The standard process was estimated the safety and effectiveness of the preaparations. The asava, arista formulations are prepared and used for the treatment of diseases and are self generated fermented herbal products. The herbs are boiled in Water to produce decoction. In arista fermentation, sugar and other spices are added to implement Assimilation to give aroma, 12% alcoholic and commonly sweet in taste. The present study is performed to monitor the standard Kanakarishtam. It used for soporific, nervine tonic, cardiotonic, stomachic, carminative, diuretic and also as stimulant. There are different techniques of phytochemical screening, microbiological, physicochemical properties, toxicological and pharmacological studies are used to prepare and estimate the prepared quality of the components.

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METHODOLOGY

Preparation of formulation

The herbs used in the preparation of Kanakarishtam were collected from the local herbal market stores. They were processed properly and authenticated duly by a taxonomist. Kanakarishtam (KA) was prepared according to Table 1 as per the WHO guidelines for quality control following GMP and GLP. The ingredients 1 to 7 were crushed well and boiled in water. The volume of the decoction was reduced to half

the initial volume followed by the addition of the ingredients 8 to 10. Later, coarse powders of the remaining ingredients (11 to 22) were added to the cold filtered decoction. The vessel was sealed and subjected to fermentation for month (Nadakarni, 1993). The fermented product i.e., Kanakarishtam (SA) is filtered, packed in tightly stoppered glass bottle and stored at 10^{o}c. They were further subjected to various standardization techniques.

Characterization

The prepared Kanakarishtam was evaluated for its physical characters like colour, odour, taste and pH. Physicochemical evaluation like total ash, water soluble ash, acid insoluble ash, sulphated ash, water, ether and alcohol soluble extractive value, loss on drying, and determination of foreign matter were carried out (Sandeep et al., 2010).

The prepared formulation was subjected to FTIR analysis to study the nature of functional groups and to report the identity of the chemical consitituents present in the formulations with that of the herbs used. FTIR spectrum of formulation and powder of *Datura metel* were derived and compared for the presence of identical groups.

As the ayurvedic formulations do not limit the heavy metal content the formulation had been tested for the heavy metal content. The heavy metals such as lead, cadmium, mercury and arsenic was determined using the atomic absorption spectroscopy.

HPTLC analysis

The prepared formulation is analyzed using HPTLC fingerprinting to confirm the presence of biomarker compound and to quantify it. The major ingredient in Kanakarishtam is *Datura Metel* belonging to the family Solanaceae. The plant has been reported to contain Hyocyamine, Atropine, Alkaloids, Triterpenes and Tannins (Kokate, 1996). So in consideration to this, atropine is selected as a biomarker compound and the formulation is evaluated for its presence and quantity.

HPLTC is used to identify and quantify the chemical constituents present in an extract. It gives the complete details of the type of active constituents present in a plant extract (Wagner, 1996). In the present work CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS- 4 software was used. All the solvents used were of HPLC grade obtained from MERCK Ltd. All weighing were done on Precisa XB 12A digital balance.

Preparation of extract

10ml of the formulation was extracted with 10ml of methanol by heating at $50\text{-}55^{0}$C for 10 min. The extract was filtered and used for HPTLC. Accurately 50mg of atropine was weighed and dissolved in distilled water. This was made up to 10ml in order to prepare 5mg/ml concentration and used for spotting. On precoated aluminium TLC plate (20 X 10cm) with silica gel 60 F_{254}, 30µl of methanol extract solution was applied as 11.6mm band using Linomat V applicator with a Hamilton syringe. Applied plate was developed in an overnight saturated twin trough chamber containing Chloroform: Glacial acetic acid: Methanol: Water (6:3.2:1.2:8) as mobile phase. The plate was developed for a migration distance of 76.5mm. It was then scanned under wavelengths, 220nm using Deuterium lamp.

Estimation of alcohol content

25ml of the preparation being examined was accurately measured and transferred to the distillation flask. It was diluted with 150ml of water, distilled and not less than 90ml of distillate was collected into a 100ml volumetric flask. The temperature was adjusted to 24°c-25°C. The relative density was determined at 24 to 25 ^{o}c. The percentage of ethanol contained in the preparation was determined (Anonymous).

Statistical analysis

The means and significance difference between them was calculated by using one way ANOVA followed by Dunnet's T-test.

RESULTS & DISCUSSION

The prepared Kanakarishtam was evaluated for its Physical characters and given as below. It is soluble in Ethanol, methanol and water and insoluble in Pet ether, chloroform, benzene. It had odour similar to that of alcohol and tasted sweet. Kanakarishtam was dark brown in colour and the pH was found to be 3.42 which is acidic in nature. The values for the physicochemical constants such as ash values, loss on drying, alcohol content and extractive values were given in table 2. The alcohol content was found to be 10.07% v/v. All the values are under acceptable limits as per Indian pharmacopoeia. Preliminary phytochemical screening revealed the presence of secondary metabolities like alkaloids, flavonoids, steroids, flavonoids, tannins and saponins. FT-IR spectrum of the sample was taken and the spectrum shows the number of well defined peaks in 400-4000cm^{-1} region along with peaks in higher region. The broad peaks at 3446 cm^{-1}, 3442cm^{-1} is due to OH in both formulation and centella powder revealing the presence of similar functional groups in both of the samples. The broadening of the peak in formulation indicates the presence of multiple functional groups related to other ingredients in formulation along with those found in centella alone. The spectra were shown in figure 1 and 2. The results form AAS revealed the content of heavy metals present in the formulation and their content is given in table 3. HPTLC analysis showed a peak regarding the atropine in the

chromatogram of formulation in suggesting the presence of Atropine. It also showed many other peaks proving the presence of other active principles other than atropine.
The microbial content in the formulation was tested. All the tests showed negative results for the growth of *E.coli, Staphylococcus aureu*s, yeast and mould thus suggesting that the formulations were free from microbial growth and contamination which is a primary requirement for an ayurvedic formulation.

Table 1. Composition of Kanakarishtam for 500 ml

Ingredients	Biological source	Parts used	Quantity in mg
Datura	Datura metel	Leaves	1000
Haritaki	Terminalia chebula	Whole plant	225
Usira	Vetiver zizinaloides	Roots	225
Ardraka	Gingiber zerumbet	Rhizomes	225
Sata puspa	Anithum graveolens	Seeds	225
Madhu	Honey	---	450
Sakara	Sugar	---	1100
Dhataki	Woodfordia floribunda	Flowers	300
Arenuka	Piper aurentiacum	Seeds	30
Pippali	Piper longum	Seeds	13
Vacha	Sweet flag	Plant	12
Kustha	Costus	Roots	14
Asvagandha	Withania somnifera	Root	15
Guduchi	Tinospora cardifolia	Stem and root	12
Vidanga	Emblica officinallis	Fruits	10
Jala	Water	---	12.5

*****all quantities taken in grams and some of them in milliliters whichever relevant.

Table 2: Estimation of physical characteristics of Kanakarishtam

S. No	Tests	SA (% w/w)
1	a) Foreign matter b) Sand & Silica	- -
2	Ash values a)Total ash content b) Acid insoluble ash c) Water insoluble ash d) Sulphated ash value	 2.3 0.62 0.5 0.41
3	Extractive values a) Alcohol soluble extractive b) Water soluble extractive c) Ether soluble extractive	 31.4 29.6 16.1

Table 3: Estimation of trace elements in the formulation

S. No	Element	Concentration in SA (ppm)
1	Lead (Pb)	4.6
2	Mercury (Hg)	0.058
3	Cadmium (Cd)	0.24
4	Arsenic (As)	0.62

CONCLUSION

In the present investigation, the quality of formulated arista, Kanakarishtam has been accessed. The present findings have shown that all the standard parameters based on which the standardization was carried out, were under acceptable limit as compared to pharmacopoeial specifications. Further, clinical assessment is to be carried out to completely mark the product as “safe and effective”.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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