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SYSTEMATIC APPROACH ON STANDARDIZATION AND PHYTOCHEMICAL INVESTIGATION OF SIDDHA TRADITIONAL MEDICINE KARAPPAN NEI

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ABSTRACT

Siddha is an art of healing that ultimately stays a head on global traditional therapies. Herbs essentially becomes an innumerable part of siddha formulations; it was strongly believed that Siddhars are ancient physicians who had carefully suggested the herbs for each preparation based on the presence of healing bioactive components. At this context being an multi components formulations most often siddha drug requires crucial validations to ensure the quality and genunity of the formulated medicines. Present study aimed at evaluating the siddha formulation Karappan Nei by systematic physicochemical and phytochemical investigation in accordance with regulatory guidelines. Organoleptic evaluation of the formulation KRN indicated with yellowish greasy dense viscous liquid and also possess strong characteristic odor which confirms the genunity of the drug. The results obtained from the physicochemical evaluation reveals that the viscosity value of KRN was 34.64 and Refractive index was 1.36. Further Weight per ml, Iodine and Saponification value were identified as 0.159 (gm/ml), 76.835 (mg I2/g) and 238.3 (mg of KOH). Acid and Peroxidase value of KRN was found to be 0.6732 (mg KOH/g) and 4.18 (mEq/kg). The result of the qualitative phytochemical analysis indicates that the formulation KRN shows the presence of biologically significant phytochemicals such as flavonoids, glycosides, steroids, triterpenoids, coumarin, and saponins. From the result analysis of the present investigation it was evident that the formulation KRN possess versatile bioactive phytocomponents and the physicochemical data reveals the quality and genunity of the formulation that compiles with the regulatory standards.

KEY WORDS: ***Siddha formulation, Physicochemical, Phytochemical Standardization, Karappan Nei, Regulatory guideline***

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1. Introduction

Siddha medicine have been potentially substantiating the treatment of humans for several dreadful diseases and disorders. It was predicted that combination of herbs may act synergistic in exerting the recurrent pharmacological action. Ghee based siddha drugs are versatile in revealing its potency due to quicker onset of action and also fatty vehicle like ghee facilitates wide range of absorption across the biological barriers. Traditionally medicinal herbs provide certain potential bioactive phytochemicals that contribute to health promotion and disease prevention in humans [1]. While the macro and micronutrients in plants were long thought to be one of the essential components for human health, phytochemicals have recently emerged as modulators of key cellular signaling pathways [2]. Phytochemicals, often referred as secondary metabolites, are non-nutritive chemical compounds produced by plants via several chemical pathways. Recent studies have demonstrated that a large number of phytochemicals can be highly beneficial to the human disease and for general ailment [3-5]. With several studies indicating the effects of phytochemical-rich foods on health, it is strongly suggested that administering phytochemicals can help to improve health [6-8]. Based on such evidence, many researchers have already proven the roles of phytochemicals in health improvements.

Herbs signifies as a primary source of traditional medicines being used in treating different ailments. More than fifty thousand herbs are of medicinal importance that are being used for pharmaceutical purposes [9]. About 80% of worldwide populations rely on traditional medicines for primary health-care needs [10]. The national drug regulatory authority must ensure issue of license for importers, wholesalers, manufacturers and assemblers of herbal medicinal [11]. As of now, the dealers of imported herbal medicinal products need to apply for one or more of the licenses depending on the type of business involved, such as license of importers, wholesalers, manufacturers, and assemblers.

Karappan Nei is a ghee based polyherbal formulation that comprises of the ingredients such as Andrographis paniculata , Pterocarpus santalinus, Terminalia chebula, Cyperus rotundus, Cedrus deodara, Murraya koenigii, Strychnos potaotrum, Curcuma longa , Piper longum along with milk and ghee. This formulation widely explored for treating wide range of disease. The main objective of the present investigation is to systematically standardize the formulation Karappan Nei (KRN) as per regulatory guideline to document the quality and phyto-active components present in the formulation.

2.Materials and Methods

2.1. Ingredients

The major ingredient present in the formulation Karappan Nei are Andrographis paniculata, Pterocarpus santalinus, Terminalia chebula, Cyperus rotundus, Cedrus deodara, Murraya koenigii, Strychnos potaotrum, Curcuma longa , Piper longum along with milk and ghee.

2.2. Source and authentication of raw drug

Raw drugs were bought from Indigenous authentic country drug shop at Chennai, Tamil Nadu, India. Herb were identified and authenticated by the Botanist of Central Council for Research in Siddha, Tamil Nadu, India.

2.3. Method of preparation

The above mentioned raw drug were purified as per the standard operating procedure and subjected for formulation.

Figure 1: Siddha formulation Karappan Nei

2.4. Physicochemical Evaluation [12-17]

Organoleptic evaluation of the drug was made with respect to state, appearance, nature and odor etc.

2.4.1. Determination of Iodine value

About 20 gm of test sample was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 mins and refrigerated for an hour. About 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the

appearance of yellow colour. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.

2.4.2. Determination of saponification value

About 2 gm of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeatthe same procedure without taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

2.4.3. Determination of Viscosity value

Viscosity determination were been carried out using Ostwald viscometers. Measurement of viscosity involves the determination of the time required for a given volume of liquid to flow through a capillary. The liquid is added to the viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back into the lower reservoir. The time that it takes for the liquid to pass between two etched marks, one above and one bellow the upper reservoir, is measured.

2.4.4. Determination of Refractive Index

Determination of RL was carried out using Refractometer.

2.4.5. Determination of Weight per ml

Weight per ml was determined using the comparative weight calibration method, in which the weight of 1ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation were been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

2.4.6. Acid Value

Accurately 5 g of test sample was weighed and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink color indicated the end point. The volume of consumed KOH solution was determined and the titration of test sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression.

Acid value = Titter Value X 0.00561X 1000 / Wt of test sample (g)

2.4.7. Peroxide value

5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide soluton. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

$$\text{Peroxide value} = 10\ (a - b)/w$$

2.5. Preliminary Phytochemical Investigation [18]

Test for alkaloids

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarin

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is

added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

Aanthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

3.Results

3.1. Results of Physicochemical Analysis of KRN

Organoleptic evaluation of the formulation KRN indicated with yellowish greasy dense viscous liquid and also possess strong characteristic odor which confirms the genunity of the drug. The results obtained from the physicochemical evaluation reveals that Viscosity at 50oC (34.64 Pa s), Refractive index (1.36), Weight per ml (0.159 gm/ml), Iodoine value (76.83 mg I2/g), Saponification Value (238.3), Acid Value mg (0.67 KOH/g) and Peroxidase Value (4.18 mEq/kg). As shown Table 1.

Table 1: physicochemical evaluation of *Karappan Nei*

S.No	*Parameter*	*Karappan Nei*
1	***Viscosity at 50°C (Pa s)***	*34.64*
2	***Refractive index***	*1.36*
3	***Weight per ml (gm/ml)***	*0.159*
4	***Iodine value (mg I2/g)***	*76.835*
5	***Saponification Value (mg of KOH to saponify 1gm of fat)***	*238.3*
6	***Acid Value mg KOH/g***	*0.6732*
7	***Peroxidase Value mEq/kg***	*4.18*

3.2. Qualitative Phytochemical evaluation of KRN

The result of the qualitative phytochemical analysis indicates that the formulation KRN shows the presence of biologically significant phytochemicals such as flavonoids, glycosides, steroids, triterpenoids, coumarin, and saponins. The results were tabulated in Table 02 and shown in figure 2.

Table 2: Preliminary phytochemical analysis of Karappan Nei

S.NO	TEST	OBSERVATION
1	ALKALOIDS	-
2	FLAVANOIDS	+
3	GLYCOSIDES	+
4	STEROIDS	+
5	TRITERPENOIDS	+
6	COUMARIN	+
7	PHENOL	-
8	TANIN	-
9	PROTEIN	-
10	SAPONINS	+
11	SUGAR	-
12	ANTHOCYANIN	-
13	BETACYANIN	-

+ -> Indicates Positive and - -> Indicates Negative

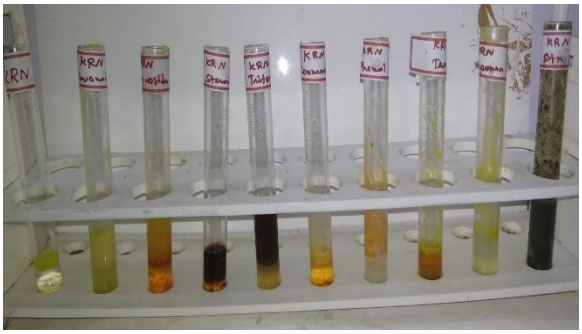

Figure 2: Preliminary phytochemical observation of Karappan Nei

4.Discussion

Plants are considered not only as dietary supplement to living organisms but also traditionally used for treating many health problems and the medicinal value of many plants still remains unexplored investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents [19]. Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes [20].

Recent research demonstrates that many phytochemicals can able to protect humans against several dreadful diseases and disorders. There are many phytochemicals in fruits and herbs and each works differently [21]. Many plant extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defense reactions of plants against infections by pathogenic microorganisms [22]. The result of the qualitative phytochemical analysis indicates that the formulation KRN shows the presence of biologically significant phytochemicals such as flavonoids, glycosides, steroids, triterpenoids, coumarin, and saponins.

Herbal drug standardization is a dynamic phenomenon requires inputs from various branches of life sciences including botanists, plant physiologists, pharmacology, pharmacognosy, chemi- informatics, biochemistry, toxicology, biotechnology, drug development, natural medicine (Ayurveda, Unani, Siddha etc.) and industrial economic/regulatory affairs. An in depth analysis of each step/stage during natural drug development is necessary to ascertain quality, safety and reproducibility. The complexity of chronic diabetes or lack of awareness leads to sudden onset of diabetes poses a significant risk of occurrence of ketoacidosis and diabetic coma, if untreated/unnoticed respectively [23]. In the present investigation organoleptic evaluation of the formulation KRN indicated with yellowish greasy dense viscous liquid and also possess strong characteristic odor which confirms the genunity of the drug. The results obtained from the physicochemical evaluation reveals that Viscosity at 50oC (34.64 Pa s), Refractive index (1.36), Weight per ml (0.159 gm/ml), Iodoine value (76.83 mg I2/g), Saponification Value (238.3), Acid Value mg (0.67 KOH/g) and Peroxidase Value (4.18 mEq/kg).

5.Conclusion

Currently traditional therapy popularizes around the globe due to its safety and high range of efficacy against array of dreadful disease. In order to retrograde the global standard, it become essential that siddha formulation must ensure the quality and standard as required by the regulatory needs. It was well concluded form the present study that the siddha formulation KRN possess biologically active phytocomponents such as flavonoids, glycosides, steroids, triterpenoids, coumarin and saponins further results on physicochemical analysis evident that the formulation KRN satisfies the explorate the recommended regulatory requirements

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