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PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF TRADITIONAL SIDDHA FORMULATION NUNA ELAI KUDINEER IN ACCORDANCE WITH REGULATORY GUIDELINES

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ABSTRACT

Indian system of traditional medicines like siddha has numerous advantage of translational research. Several novel siddha formulations with power therapeutic and clinical application are of international importance. Popularization of siddha medicine become core aspect on the globalization scenario. According to the regulatory needs siddha formulation need to be standardized and should confirm the quality requirement as expected. Medicinal plants represent the oldest source of pharmacotherapy used by mankind. A considerable number of traditional systems of medicine (folk medicine) have emerged over the last millennia under different cultural conditions. Even nowadays, the majority of people in less developed countries have to rely on herbal remedies as primary health care. Hence the main aim of the present investigation is to systematically standardize the formulation Nuna Elai Kudineer (NEK) as per AYUSH – PLIM guideline and to explore the possible therapeutic phytocomponents present in the formulation that can able to render the maximum biological benefits upon clinical utilization. The results obtained from the physicochemical evaluation reveals that total ash value of NEK was found to 5.46%. In which the acid insoluble ash was 0.76 %. Similarly, loss on drying value at 105oC was found to be 0.7% respectively. The results of water soluble extractive of NEK were 28.79% and similarly the alcohol soluble extractive value of NEK were found to be 18.7% w/w.The result of the phytochemical analysis indicates that the formulation NEK shows the presence of alkaloids, flavonoids, triterpenoids, coumarin, phenols, tannins, saponins and sugars. From the datas obtained from the present investigation it was concluded that the siddha formulation Nuna Ilai Kashayam complies with the prescribed standards and also ascertain the genunity of the preparation.

KEY WORDS: ***Siddha formulation, Nuna Ilai Kashayam, Phytocomponents, Physicochemical evaluation***

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1. Introduction

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds [1]. Siddha system of medicine majorly relies on ancient traditional preparations for treating several infectious and non-communicable diseases. As per the vedic literature it has been provoked that this method of treatment has emerged from southern region of India and progressed though out the world. Contribution of herbs towards siddha formulation is considerably innumerable as its playing a very vital role in healing, rejuvenation and mode of action of the drugs [2].

Herbal medicine has become a popular form of healthcare; even though several differences exist between herbal and conventional pharmacological treatments, herbal medicine needs to be tested for efficacy using conventional trial methodology and several specific herbal extracts have been demonstrated to be efficacious for specific conditions. Nevertheless the public is often misleded to believe that all natural treatments are inherently safe, herbal medicines do carry risks, so research in this area must be intensified. The main question that has not been often answered satisfactorily deal with the triad absorption/metabolism/efficacy of herbs and their extracts and is actually an important unsolved problem in judging their many alleged health effects [3].

Standardization and quality control of herbals is a process involving monitoring of the entire process of bioprosception of natural flora, collection, extraction, bio-activity guided fractionation and formation of herbal drugs [4,5] utilizing existing technical standards [6]. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility [7,8]. The quality of herbal drugs is affected by numerous factors: a) Mixtures of many constituents that make physiological responses complex yet holistic; b) Active principle (s) are generally unknown; c) Non-availability of selective analytical methods or standard reference compounds limits development of standard chemical fingerprint, required to ascertain efficacy among various batches; d) Natural variability associated with plants both in wild & non-wild varieties; e) Differences in spectra of bioactivity in natural vs. chemo-varieties and chemo cultivars and; f) Variability in source and quality of the raw material etc. Nuna Elai Kudineer is one such novel siddha formulation majorly consist of Morinda tinctoria , Cuminum cyminum and Trachyspermum ammi indicated for treating several disease. the main aim of the present investigation is to systematically standardize the formulation Nuna Elai Kudineer (NEK) as per AYUSH – PLIM guideline and to explore the possible therapeutic phytocomponents present in the formulation that can able to render the maximum biological benefits upon clinical utilization.

2.Materials and Methods

2.1. Ingredients

The major ingredients present in the formulation Nuna Elai Kudineer (NEK) are as follows

1.Morinda tinctoria

2.Cuminum cyminum

3.Trachyspermum ammi

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 purified as per the standard procedure followed by this partial frying of all three drug were made till it turns black and blended together as coarse powder. 5 gm of the coarse powder were extracted with 160 ml of purified water and were allowed to boil for reduction in to 1/8 parts to get final formulation.

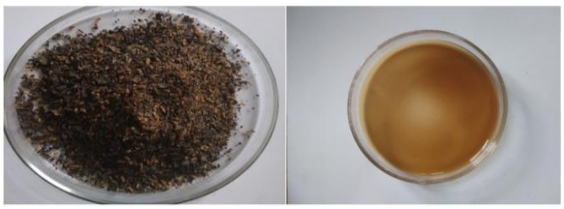

Figure 1: Crude Drug and Decoction form of Nuna Elai Kudineer (NEK)

2.4. Physicochemical Evaluation [9,10]

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

2.4.1. Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105oC for 5 hours and then weighed.

2.4.2.Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

2.4.3.Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

2.4.4.Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.4.5.Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

2.5. Preliminary Phytochemical Investigation [11]

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 100ºC. 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 NEK

Organoleptic evaluation of the crude formulation NEK indicates Solid crude material with dark brownish black color and also with characteristic odor which confirms the genunity of the drug. Similarly, decoction complies with moderately viscous reddish brown colored liquid possess strong aromatic odour. The results obtained from the physicochemical evaluation reveals that total ash value of NEK was found to 5.46%. In which the acid insoluble ash was 0.76 %. Similarly, loss on drying value at 105oC was found to be 0.7% respectively. The results of water soluble extractive of NEK were 28.79% and similarly the alcohol soluble extractive value of NEK were found to be 18.7% w/w. As shown Table 1.

Table 1: physicochemical evaluation of *Nuna Elai Kudineer*

S.No	Parameter	Mean (n=3) SD
1.	***Loss on Drying at 105 °C (%)***	0.7 ± 0.2
2.	***Total Ash (%)***	5.467 ± 0.3055
3.	***Acid insoluble Ash (%)***	0.7 ± 0.06
5.	***Alcohol Soluble Extractive (%)***	18.7 ± 2.884
6.	***Water soluble Extractive (%)***	28.79 ± 4.805

3.2. Qualitative Phytochemical evaluation of NEK

It is evident that the biological activities of the most of the medicinal herbs are majorly due to the presence of various active principles or phytoconstituents. The result of the qualitative phytochemical analysis indicates that the formulation NEK shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, triterpenoids, coumarin, phenols, tannins, saponins and sugars. The results were tabulated in Table 02 and shown in figure 2.

Table 2: Preliminary phytochemical analysis of *Nuna Elai Kudineer*

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

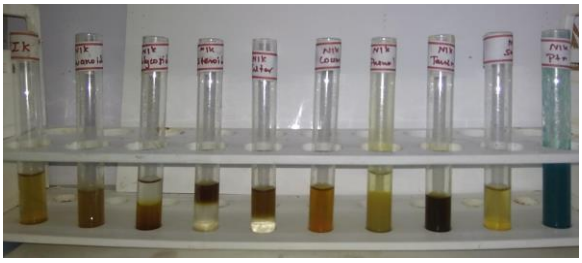

Figure 2: Preliminary phytochemical observation of Nuna Elai Kudineer

4.Discussion

Siddha formulation gaining public popularity because of its safety and high range of efficacy. Most of the times siddha drug acts by prophylactic and therapeutic as well. Herbs plays very vital role in mediating the mechanism of several siddha formulations. This system has unique SOP for standardizing the raw drug and finished formulations. Physiochemical analysis offers wide range of therapeutic information on organic and inorganic particulates with reference to aqueous and alcohol soluble constituents [12]. Extractive value advocates the index of maximum range of soluble therapeutic in the present form of the formulation. Organoleptic evaluation of the crude formulation NEK indicates Solid crude material with dark brownish black color and also with characteristic odor which confirms the genunity of the drug. Similarly, decoction complies with moderately viscous reddish brown colored liquid possess strong aromatic odour. The results obtained from the physicochemical evaluation reveals that total ash value of NEK was found to 5.46%. In which the acid insoluble ash was 0.76 %. Similarly, loss on drying value at 105oC was found to be 0.7% respectively. The results of water soluble extractive of NEK were 28.79% and similarly the alcohol soluble extractive value of NEK were found to be 18.7% w/w.

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against invasion by many microorganisms, insects and other herbivores [13]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection [14]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [15]. Tannins bind to proline rich proteins and interfere with the protein synthesis [16]. The medicinal properties and pharmacological actions of these phytocomponents are well-known to Indian traditional medicine. Medicinal herbs are known to contain various active principle of therapeutic value and possess biological activity against a number of diseases [17].

It is evident that the biological activities of the most of the medicinal herbs are majorly due to the presence of various active principles or phytoconstituents. The result of the qualitative phytochemical analysis indicates that the formulation NEK shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, triterpenoids, coumarin, phenols, tannins, saponins and sugars.

5.Conclusion

Herbal-derived remedies need a powerful and deep assessment of their pharmacological qualities and safety issues due to the large and growing use of natural-derived substances all over the world, which cannot rely only on the tradition or supposed millenarian beliefs; explanatory and pragmatic studies are useful and complementary in the acquisition of reliable data both for health caregiver and patients. Outcome of the present investigation clearly indicates that the siddha formulation NEK shows the presence of alkaloids, flavonoids, triterpenoids, coumarin, phenols, tannins, saponins and sugars and also Complies with the prescribed standards which in turn ascertain the genunity of the preparation.

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6. References

1. S Sasidharan. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. Afr J Tradit Complement Altern Med. 2011; 8(1): 1–10.
2. D.Sivaraman, N.Anbu, N.Kabilan, M.Pitchiah Kumar, P.Shanmugapriya , G.J.Christian. Exploration of Anti-Urolithiasis Potential of Traditional Siddha formulations Amukkara Chooranam and Karisalai Karpam Chooranam by Struvite Crystal Growth Inhibition Assay. Pharmacognosy Journal.2019;11(4) :683-688
3. Firenzuoli F, Gori L, Crupi A, Neri D. Flavonoids: risks or therapeutic opportunities? Recenti Prog Med. 2004;95:345–51.
4. National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. Geneva: World Health Organization, “Introduction”; 2005. p. 143.
5. Diabetes action now”. Booklet- Designed by WHO; 2004. p. 20.
6. Folashade KO, Omoregie EH, Ochogu AP. Standardization of herbal medicines. Int J Biodivers Conserv. 2012;4(3):101–112.
7. Bhutani KK. Herbal medicines an enigma and challenge to science and directions for new initiatives. Indian J Nat Prod. 2003;19(1):3–8.
8. Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmaconsy. Pharmacognosy. 2005. pp. 1–99.
9. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
10. Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH. Ministry of Health & Family Welfare, Govt. of India
11. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol:Wright-Scientechnica; 1975:36-45
12. D.Sivaraman. Evaluation of AChE enzyme inhibition potential of Indian Medicinal Herbs Ficus hispida, Morinda tinctoria, Sapindus emarginatus and their Biological significance in Alzheimer’s Disease Therapy. Research Journal of Biotechnology. 2018;13 (8):110-115

13. Bonjar GH, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biol Sci. 2004;4:405–12.
14. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564–82.
15. Zablotowicz RM, Hoagland RE, Wagner SC. Effect of saponins on the growth and activity of rhizosphere bacteria. Adv Exp Med Biol. 1996;405:83–95.
16. Shimada T. Salivary proteins as a defense against dietary tannins. J Chem Ecol. 2006;32:1149–63.
17. Ayyanar M, Ignacimuthu S. Pharmacological actions of Cassia auriculata L. and Cissus quadrangularis Wall: A short review. J Pharmacol Toxicol. 2008;3:213–21.