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ANALYSIS OF HEAVY METAL, AFLATOXIN, PESTICIDE RESIDUE, MICROBIAL CONTAMINATION AND PHYTOCHEMICAL ANALYSIS OF SIDDHA HERBAL DRUG KANDUPARANGI VER CHOORANAM (KVC)

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ABSTRACT

Aim: The aim of the study was to evaluate the presence of Heavy metal, Aflatoxin, Pesiticide residue and Microbial contamination of Siddha herbal formulation Kanduparangi ver Chooranam (KVC). **Place of study:** Heavy metal analysis, Aflatoxin assay, Pesiticide residue, Microbial contamination analysis and phytochemical evaluation were conducted at Noble Research Solutions, Kolathur, Chennai -99. **Methodology:** The Siddha formulation Kanduparangi ver Chooranam was prepared as per Good Manufacturing Practices (GMP) guidelines and the Heavy metal analysis, Aflatoxin assay, Pesiticide residue, Microbial contamination analysis and phytochemical evaluation were conducted at Noble Research Solutions, Kolathur, Chennai -99. **Results:** The results of Heavy metal analysis of Kanduparangi ver Chooranam (KVC) shown the presence of Lead at 2.017 PPM and Arsenic, Cadmium, Mercury at Below Detection Limit (BDL). Aflatoxin assay of KVC shown the absence of Aflatoxin B1,

Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. Pesticide residue analysis showed that there were no traces of Pesticide residues such as Organo chlorine, Organo phosphorus, Organo carbamates and Pyrethroids. In Microbial contamination analysis, Test for Specific Pathogen shown the absence of Organisms E-coli, Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa and No Bacterial and fungal growth or colonies were observed in the Sterility test of KVC as per the methods of AYUSH specifications. The Phytochemical screening of KVC shown the presence of Alkaloids, Carbohydrates, Saponins, Flavanoids, Diterpenes, Gum and Mucilage. **Conclusion:** From the results, it is concluded that the study medicine KVC has Heavy metal content below the permissible limit as per PLIM guidelines of AYUSH, and the sample were free from Aflatoxins, Pesiticides, Microbes and Specific Pathogens and it shown the compendious understanding of presence of Phytochemical components which ensures that the study medicine Kanduparangi ver Chooranam was safe therapeutically.

KEYWORDS: Kanduparangi ver Chooranam, Siddha, Sinusitis, Heavy metal, Aflatoxins, pesticide, phytochemicals.

Index Terms – Kanduparangi ver chooranam (kvc), Sinusitis, Heavy metal.

INTRODUCTION

A drug is a substance used as a medicine, which are used either directly as crude drug or after undergone some processes. Siddha system of Medicine uses drugs of plant or animal or metal or mineral origin. To deprive of its impurities, crude raw drugs of either origin should under gone respective standard purification processes and after the actual medicine preparation should be done. This gives better results therapeutically and also helps in detoxification and assures safety. Since Siddha system has multiple combinations of medicines and various preparatory processes like powdering, heating, boiling, drying, grinding, calcinations, sublimation, filtration and so on, chances for impurities, mishandling, is possible which could affect the global acceptance scientifically. This can be rectified by employing proper procedures and can be analysed by standardization techniques through various parameters to get the reproducible standards.[1] Heavy metal tests are performed to look for potentially dangerous levels of metals at certain concentrations and some of them includes, Lead, Arsenic, Cadmium, Mercury and Chromium, which are extremely toxic. Pesticide residues which could present in medicines due to usage of pesticides in cultivation process and for economic return.[2] Pesticides and heavy metals can accumulate in the body through biological chains while being persistent and not biodegradable. Thus, it is important to

monitor their concentration.[3] Aflatoxins are a class of hazardous mycotoxin compounds produced by Aspergillus flavus and Aspergillus parasiticus that have structural similarities. The aflatoxin group contains about 16 compounds out of which only aflatoxin B1, B2, G1 and G2 are regularly monitored. High moisture content and temperature leads to occurrence of mycotoxins and have adverse health effects on humans.[4] According to research, approximately 80% of people in developing countries use traditional herbal remedies as their primary form of healthcare. Contamination by microorganisms of various types that may be adherent to the leaves, stems, blossoms, seeds, and roots from which herbal medicines are manufactured. Microorganisms can also be added throughout the processes of harvesting, handling, open-air drying, preserving, and manufacturing. Due to consumers uncompromising conditions and microbial infections, the presence of microbial contaminants in herbal products might negatively impact their health status, posing a global health issue. Therefore, it is essential to ensure that users of herbal products are safe.[5] Thus, the present study deals with the analysis of Heavy metal, Aflatoxin, Pesticide Residue, Microbial Contamination and phytochemical evaluation of Siddha Herbal Formulation Kanduparangi ver chooranam, indicated for Sinusitis.

Sinusitis is an infection of the sinuses. These infections usually happen after a cold or with allergies. Frontal sinus begins to grow after birth as nasofrontal ducts whereas maxillary, ethmoidal and sphenoidal sinuses are present right at birth. Sinusitis can be classified as either acute or chronic. The ethmoid and maxillary sinuses are the earliest to develop in paediatric sinusitis. In recent times, among people, there has been a positive ray of hope in getting treatment from Siddha for sinusitis also. With that importance, one such herbal formulation Kanduparangi ver chooranam which is mentioned in Gunapadam part -1, K.S.Murugesa Muthaliyar[6] for sinusitis, has been analysed for heavy metal, aflatoxin, pesticide residue, microbial contamination and phytochemical evaluation in order to promote its credibility through scientific way.

MATERIALS AND METHODS

The herbal preparation, Kanduparangi ver chooranam, was identified in the canonical text "Gunapadam part -1, K.S.Murugesa Muthaliyar". The ingredients for this formulation are included in Table -1.[7]

Table 1: Ingredients of Kvc.

INGREDIENTS	BOTANICAL NAME
Kanduparangi ver	*Cleodendrum serratum*

COLLECTION, IDENTIFICATION AND AUTHENTICATION OF THE DRUG

The plant materials were procured from a raw drug shop located at Parry's Corner in Chennai, Tamil Nadu. These materials were subsequently verified and confirmed by botanical and pharmacological experts at the Government Siddha Medical College Hospital in Arumbakkam, Chennai – 106.

PURIFICATION OF THE DRUGS

The drug mentioned here was purified as per the Siddha literature. All impurities such as sand and dust have been removed.

PREPARATION OF THE DRUG PROCEDURE

- The purified raw drug listed in Table 1 was meticulously ground into a fine powder using a mortar and pestle.
- This powder, named Kanduparangi ver chooranam, was then stored in an airtight container for safekeeping.

RESULTS AND DISCUSSION

1. HEAVY METAL ANALYSIS OF KVC

Heavy metal screening of MRC shown that it contains Arsenic, Cadmium, Mercury were BDL (Below Detection Limit), and Lead was 2.017 PPM, whose maximum limit was upto10PPM.[8] However, its lower limit indicating the safety of the drug.

Table 2: Test report of Heavy metal analysis of KVC.

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	2.017	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

2. AFLATOXIN ASSAY OF KVC

The results of Aflatoxin assay of KVC by TLC shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which

indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.[9]

Table 3: Test report of Aflatoxin assay of KVC.

Aflatoxin	Sample KVC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

3. PESTICIDE RESIDUE ANALYSIS OF KVC

Pesticide residue analysis of MRC with the parameters Organochlorine pesticides, Organophosphorus pesticides, Organo carbamates, Pyrethroids were found to be that there were no traces of pesticides residues and the results were given below.[10]

Table 4: Test report of Pesticide residue of KVC.

Pesticide Residue I. Organo Chlorine Pesticides	Sample KVC	AYUSH Limit (mg/kg)
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II. Organo Phosphorus Pesticides		
Malathion	100 µg/kg	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

4. MICROBIAL CONTAMINATION ANALYSIS OF KVC

Microbial contamination analysis of KVC by test for specific pathogen shown that there were No growth was observed after incubation period, reveals the absence of specific pathogen. Results were given below.[11]

Table 5: Test report of Specific pathogen of KVC.

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

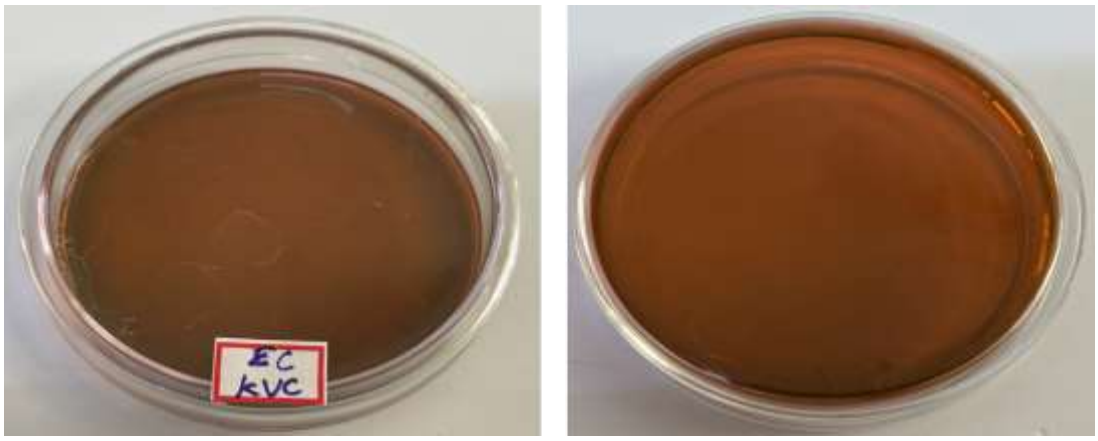

Figure 1: Culture plate with E-coli (EC) specific medium.

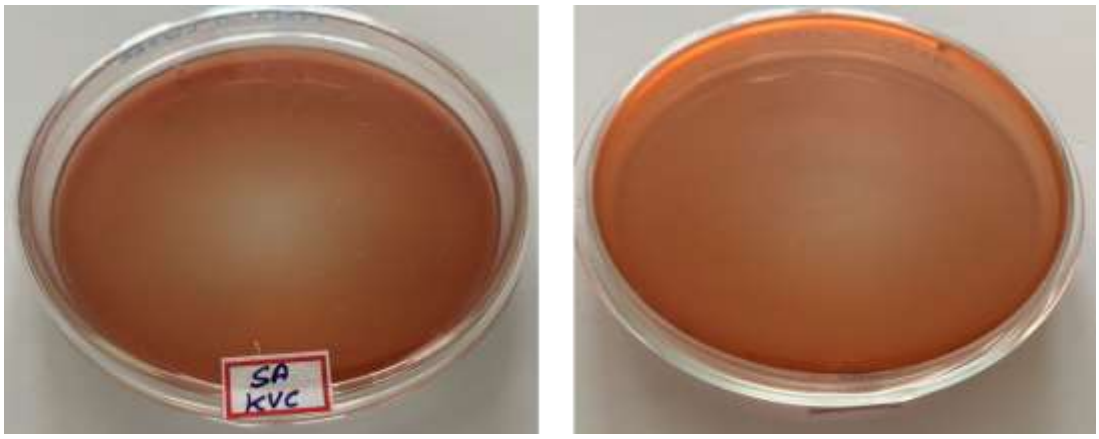

Figure 2: Culture plate with Salmonella (SA) specific medium.

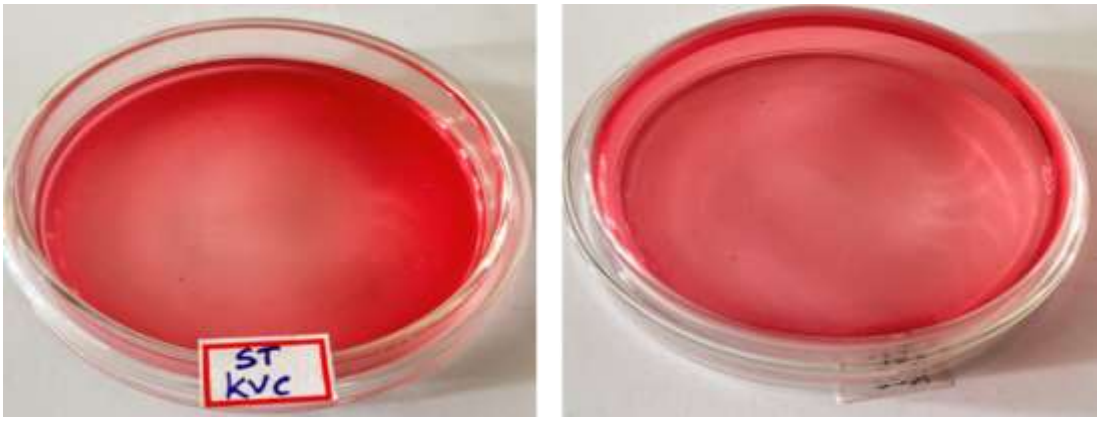

Figure 3: Culture plate with Staphylococcus Aureus (ST) specific medium.

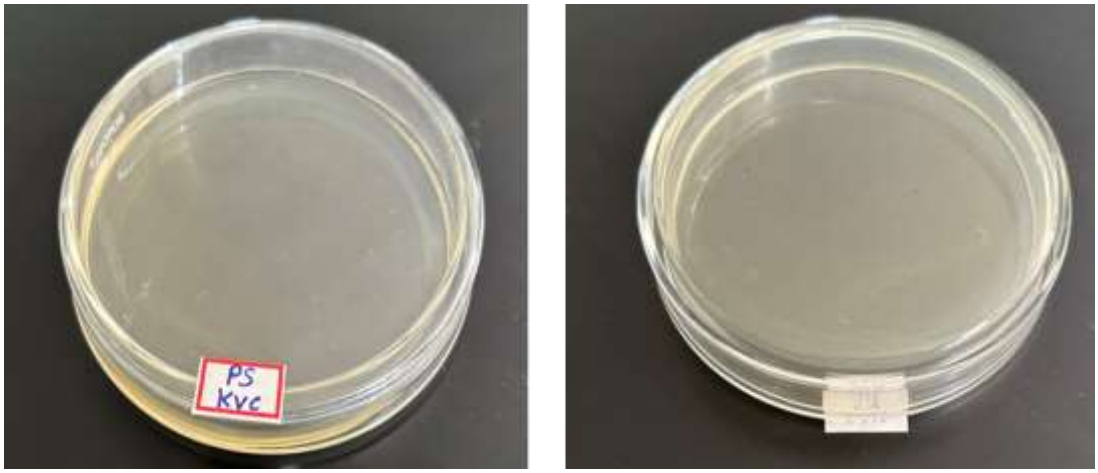

Figure 4: Culture plate with Pseudomonas Aeruginosa (PS) specific medium.

Sterility test of KVC also found to be that there were No growth / colonies was observed in any of the plates inoculates with the test sample which ensures that the sample is devoid of microbial contamination in both the tests.[12]

Table 6: Sterility test report of KVC.

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

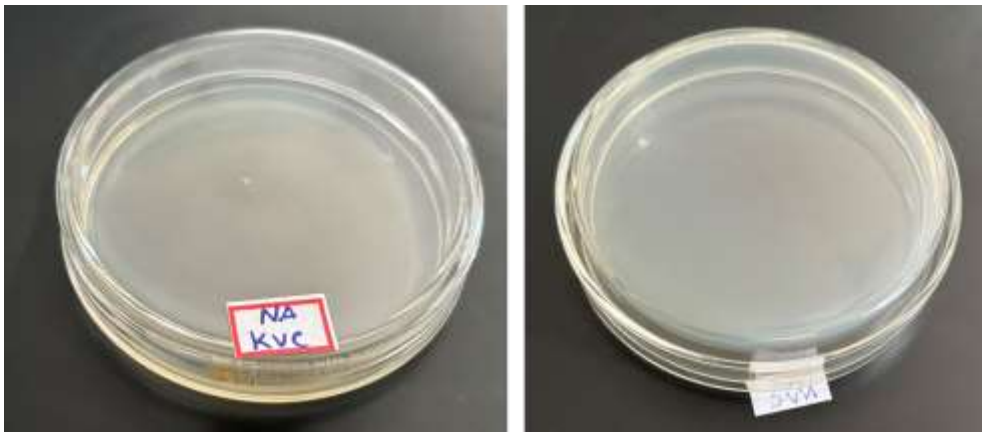

Figure 5: Colony plate for Total bacterial and fungal count of KVC.

5. PHYTOCHEMICAL ANALYSIS OF KVC

The Phytochemical screening of KVC shown the presence of Alkaloids in Wagner test, Carbohydrates in Molisch's test, Benedict test, Saponin in Foam test, Flavanoids in lead acetate test, Diterpenes in Copper acetate test, Gum and Mucilage in Gum and mucilage test of the study sample. Also, shown the absence of Tannins, Phenols and Quinones.[13]

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 coumarins

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

Alkaline reagent test. Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

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

A. 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.

Figure 6: Qualitative Phytochemical analysis of KVC.

CONCLUSION

Through the present study, it is concluded that the sample Kanduparangi ver Chooranam (KVC) was found to be safe with the presence of heavy metals below the detection limit, Devoid of aflatoxins, pesiticide residues, and microbial contamination in specific pathogens, as well as bacterial and fungal counts and it shown the compendious understanding of

presence of Phytochemical components. This ensures the quality profile of KVC in terms of contamination from biological chains. This preliminary standardisation study would assist in further research and clinical trials with the basic quality sustain.

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