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PRELIMINARY PHARMACOGNISTIC EVALUATION OF SIDDHA HERBAL FORMULATION *DEVA CHOORANAM*

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ABSTRACT
The Siddha system of medicine is considered to be an ancient traditional system of medicine with meticulous documentation and practiced by a large population of South India. Since recent times, herbal medicines have been focused as global interest due to its long term safety and efficacy. Therefore the need to standardize these medicinal formulations and ensure the quality and safety through the application of modern techniques has become an arena of paramount importance. Deva Chooranam is a formulated combination of herbs which can be used for the treatment of Acquired immune deficiency syndrome and its complications. This study was done as a preliminary physico chemical analysis and was subjected to analysis such as total ash, loss on drying, total sugar, reducing sugar,fat content, microbial load per the Pharmacopeial laboratory standards of Indian medicine. The results revealed that the formulated drug was free of microbial contamination and Aflatoxins and Pesticide Residues.

KEYWORDS: Herbal formulation, Acquired immune deficiency syndrome, HIV, Physico chemical Analysis, Siddha.

INTRODUCTION
Herbal medicines have richest bio source of phytochemicals, micro and macro nutrients for the treatment and management of several chronic diseases. World health organization (WHO) recommends the use of herbal based traditional medciines due to its safety, efficacy, less expensive and easily availability. *Deva Chooranam* is a herbal formulation consisting of three medicinal herbs, *Cedrus deodara* (Devadaru), *Alpinia galanga* (Arathai), *Cinnamomum tamala* (Lavanga pathiri). The source of these three herbs have been taken from Agathiyar gunavagadam of classical *Siddha* literature based on the indications mentioned such as Chronic fever, diarrhea, dysentery, oral ulcers, respiratory ailments, skin diseases and tumours that can be found in the co morbid clinical conditions of HIV infected individuals.[1]

Therapeutic efficacy of medicinal plants depends on the quality and quantity of chemical constituents. Hence it is very essential to evaluate the pharmacognostical specifications of medicinal formulations to meet the growing demand for natural drugs. Pharmacognosy is the study of naturtally derived medicines and basically deals with the standardization, authentication and analysis of these drugs through morphological, phytochemical and physic chemical analysis. These pharmacognostic parameters ensures the identity and purity of drug which will help to prevent adulteration. Therefore in this study, the herbal formulation Deva chooranam (DC) was subjected to various steps of pharmacognostic analysis and the results were obtained and analysed.

MATERIALS AND METHODS
Collection and Identification of plant materials: The herbal ingredients were collected from local markets of Chennai and authenticated by botanist, Department of National Institute of Siddha, Chennai.

Preparation of *Deva Chooranam*
All the ingredients *Cedrus deodara* (Devadaru), *Alpinia galanga* (Arathai),*Cinnamomum tamala* (Lavanga pathiri)*)* are taken in equal amounts and were purified according to Siddha literatuyre. Then they were ground into fine powder and filtered using a mesh cloth and stored in a sterile container.

Dosage: 5-10 gms twice daily in Milk.

The prepared test drug Deva Chooranam was subjected to various pharmacological evaluation at Noble research solutions, Chennai and also at Bureau veritas, Guindy, Chennai.

Organoleptic evaluation
The test drug DC was subjected to visualization for size, colour, texture, taste and smell and texture by the perception through five senses. The texture is best examined by taking a small quantity of material and rubbing it between the thumb and forefinger and was described as smooth rough or gritty determining its softness or hardness.

Physico-chemical evaluations of raw materials
All the physic-chemical parameters were carried as specified in WHO and AYUSH PLIM guidelines. The selected drug sample DC will be subjected for the determination of physico-chemical properties *viz.*, Loss on drying, ash values and extractive values for water soluble and alcohol soluble extractives by physical methods. The particle size was determined by dry sieve method.

Percentage Loss on Drying: 10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

Determination of Total Ash
3 g of 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 was calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

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

Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

Determination of Water Soluble Ash
The ash obtained by total ash test was boiled with 25 ml of water for 5 mins. The insoluble matter was collected in crucible and will be washed with hot water, and ignited for 15mins at a temperature not exceeding 450ºC. Weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated as.

Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

Determination of Alcohol Soluble Extractive
About 5 g of test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105ºC, to constant weight and weighed. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated as.

Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

Determination of Water Soluble Extractive
About 5 g of the 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 to stand and for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105ºC, to constant weight and weighed. The percentage of water-soluble extractive with reference to the air-dried drug.

Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

Determination of pH: About 5 g of test sample was dissolved in 25ml of distilled water and filtered. The resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation.[2]

Fluorescence analysis in dried powder
Sample DC was subjected to fluorescence analysis under visible light and UV – Light at 365 nm under closed circuit cabinet. Each fluorescence characteristic of the treated sample was observed under ordinary light and then under UV light of wave lengths 365 nm. The drug was treated with acids viz., Conc. HCl, Conc. H2SO4, Conc. HNO3 and glacial acetic acid. The drug was treated with alkaline solutions viz., aqueous NaOH and ferric chloride. They were subjected to fluorescence analysis in visible light and in short UV- light (254 nm) and long UV- light (365 nm).[3]

Preliminary phytochemical studies
The following preliminary tests was carried out for the identification of the nature of chemical constituents.[4,5]

Test for phenolic compounds - Ferric chloride test
Substance in water was added with 5% alcoholic ferric chloride. Dark blue or green colour indicates the presence of phenol.

Test for Tannin: Few ml of substance was shaken with water and added with lead acetate solution. White precipitate indicates the presence of tannin.

Test for steroids (Lieberman Burchard Test)
The test substance was dissolved in a few drops of chloroform. 3ml of acetic anhydride was added followed by drops of conc. H_2SO_4. Appearance of bluish green colour indicates the presence of sterols.

Test for flavonoids (Shinoda test)
A small quantity of the substance was dissolved in alcohol, to which magnesium bits and concentrated HCl were added. On heating this mixed solution over a water bath, the appearance of majenta colour shows the presence of flavonoids.

Triterpenoids (Noller's Test)
To a few mg of the extract, tin and thionyl chloride was added and heated in water bath. Purple colour indicates the presence of tritepenoids.

Test for Proteins (Biuret test): To the sample solution in a test tube, sodium hydroxide solution and a few drops of very dilute (1 %) copper II sulphate solution was added and mixed gently. Appearance of purple colour indicates the presence of protein.

Test for Glycosides: Substance was treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside.

Test for Reducing sugar (Fehling's Test)
To the sample solution, Fehling's reagent is added. The appearance of brick red precipitate or colouration indicates the presence of reducing sugar.

Test for anthraquinones
Few milligram of the crude powder was shaken with 10 ml of benzene and filtered. To this filtrate, 0.5 ml of 10 % ammonia solution was added and the mixture was shaken well and the presence of the violet colour in the layer phase indicates the presence of the anthraquinone.

Test for Quinones: To few mg of the sample, few drops of concentrated sulphuric acid was added. Appearance of red colour shows the presence of quinone.

Test for alkaloids (Dragendorff's Test)
A few mg of the sample was warmed with 2% H_2SO_4 for 2 min and was filtered in a separate test tube and added to a few drops of Dragendorff's reagent. The presence of orange red precipitates indicates the presence of alkaloids.

Test for saponins
To a few mg of the extract, distilled water was added and shaken well. The formation of foam indicates the presence of saponin of compounds.

Test for heavy metals
Tests for heavy metals such as lead, cadmium, arsenic and mercury were done for the study drugs by using AYUSH approved procedures. Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detectingmetals and metalloids in environmental samples. The total heavy metal content of the sample DC was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arenic, lead and cadmium concentrations in the test sample DC it is mandatory for the sample to proceed with sample digestion.Test sample DC digested with 1mol/L HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.[6]

Microbial load
Microbial assays refer to the biological assays specially performed with micro-organism like bacteria and fungi for determination of potency of antibiotics, antimicrobial and antifungal drugs.Total bacterial count and total fungal counts will be done for both formulations as per the procedure mentioned in WHO report (QSA/05.131.)

Test for specific pathogens
Limit tests for specific pathogens for *E. coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacteriaceae* was done for each study drug separately as per the WHO QSA/05.131 procedure.[7,8]

Tests for pesticide residue: Various pesticide residues of organo chlorine and organo phosphorous viz., Aldrin, Dieldrin, trans Chlordane, cis Chlordane, Chlorth alonil, DDT (all isomers), Endrin, Endosulfan(all isomers), Heptachlor, hch (alpha and beta), lindane, Dichlorvas, 4-Bromo,2-Chlorophenol, Acephate, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos methyl, Diazinon, Dimethoate, Omethoate, Ethion, Etrimphos, Fenitrothion, Edifenphos, Iprobenphos, Hexachlorobenzene, Malathion, Malathion. were checked by followingAOAC 2007.01 by GCMSMS /LCMSMS methods.[9]

Test for aflatoxins: The study drugs will be tested for aflatoxins B1, B2, G1 and G2. Standard sample of DC dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2. Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. The spots were

located on the plate by examination under UV light at 365 nm.[10]

Determination of total bacterial count

About 1ml of the test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37° C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU.

RESULTS

Organoleptic evaluation of DC indicated it as Greenish brown fine powder, aromatic odourand bitter in taste.

Physicochemical evaluation of *Deva Chooranam (DC)*

S. No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	7.86 ± 3.03
2.	Total Ash (%)	0.98 ± 0.18
3.	Acid insoluble Ash (%)	0.12 ± 0.03
4.	Water Soluble Ash (%)	7.93 ± 0.80
5.	Alcohol Soluble Extractive (%)	17.74 ± 0.67
6.	Water soluble Extractive (%)	13.77 ± 3.12
7.	PH	4.8
8.	Particle size (100mesh)	18.8%
9.	Pass through	81.1%

Fluorescence analysis in dried powder

S. No	Experiment	Visible light	Short UV – Light 254 nm	Long UV – Light
1.	Sample + Conc. Hcl	Yellowish orange	Greenish yellow	Reddish
2.	Sample + Conc. SulphuricAcid	Brownish Black	Dark reddish brown	Brown
3.	Sample + Conc. Nitric acid	Orange	Florescent green	Orange
4.	Sample + Sodium hydroxide in water	Pale yellow	Fluorescent yellow	Fluorescent yellow
5.	Sample + Ferric chloride	Greenish Yellow	Fluorescent green	Orange
6.	Sample + glacial acetic acid	Yellow	Fluorescent yellow	Crimson red
7.	Sample + Water	Milky White	Lime Yellow	Milky White

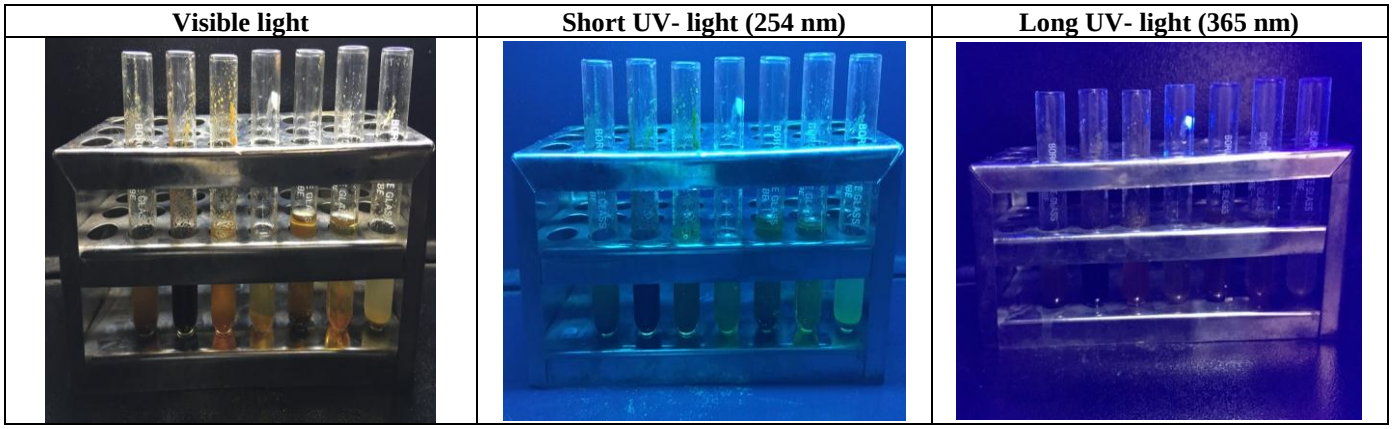

Figure. 2. Fluorescence analysis in dried powder

Phytochemical evaluation of *Deva Chooranam (DC)*

S. No	Parameters	Result
1.	Alkaloids	Present
2.	Flavanoids	Present
3.	Glycosides	Absent
4.	Steroids	Present
5.	Terpenoids	Present
6.	Coumarins	Present
7.	Phenols	Present
8.	Tanins	Present
9.	Proteins	Absent
10.	Saponins	Present
11.	Sugar	Present

Heavy metal analysis of *Deva Chooranam (DC)*

S. No.	Name of the Heavy Metal	Absorption Max Λ max	Result Analysis (in ppm)	WHO Maximum limit
1.	Mercury	253.7 nm	0.0013 ppm	1 ppm
2.	Lead	217.0 nm	0.24 ppm	10 ppm
3.	Arsenic	193.7 nm	0.0025 ppm	3 ppm
4.	Cadmium	228.8 nm	BDL	0.3 ppm

Test for Aflatoxin

All the four aflatoxin B1, B2, G1,G2 were not detected in the drug. As the total fungal count was within the permissible limit, toxins were not promoted in the drug and is free from these aflatoxins.

Test for Microbial contamination

S. No	Parameter	Value (g/100g)
1.	Total *Bacterial* count:	Absent
2.	Total Fungal count:	Absent
3.	*E.coli*:	Absent/g
4.	*Salmonella*:	Absent/g
5.	*Pseudomonas aeruginosa*:	Absent/g
6.	*Staphyloccus aureus*:	Absent/g

Pesticidal residues

All the tested organochlorine pesticides organophosphorus pesticides were found to be lower than the limit of quantification, ie.,0.01 ppm and hence safe as internal medicine.

DISCUSSION

The formulation Deva chooranam was tested for various pharmacognistic parameters like organoleptic, physicochemical parameters, phytochemical and microbiological screening. Organoleptic parameters revealed that Deva Chooranam was Greenish brown, fine powder, with aromatic odour and bitter taste. The results of physicochemical properties for Loss on drying at 105º C, pH, Total ash, Acid insoluble ash, Water soluble ash, and were calculated and results were shown in (Table 3). Ash value is useful in determining purity and quality of drug and indicates the presence of various impurities like carbonate, oxalate and silicate. Percent weight loss on drying or moisture content was found to be 7.86 ± 3.03 w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth.

Fluorescence analysis is an important parameter for qualitative analysis of crude drugs as some of the constituent show fluorescence in visible daylight or in UV light. The Phytochemical screening revealed the presence of alkaloids, flavanoids, Coumarines, Sugars, tannins, proteins, saponins, phenols and absence of glycosides and proteins as shown in (Table 4).Results of the Heavy metal analysis has clearly shows that the sample DC reveals the presence of heavy metals such has mercury, lead and arsenic whereas the heavy metal Cadmium was absent in the present sample. The moisture content is found to be less than ten which indicate the dryness of the drug.The level of mercury and lead was found to be 0.0013 ppm and 0.24 ppm. Similarly the level of arsenic was found to be 0.0025 ppm. All three reported heavy metals seems very less when compare to the allowed recommended limit. The results of Aflatoxin analysis showed that there was no spots were been identified in the test sample loaded TLC plated when compare to the standard indicates that he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.Also the tested sample was free from microbial or pesticidal contamination.[11]

CONCLUSION

Quality control of herbal medicines has been emphasized by WHO using modern techniques and by applying suitable parameters and standards. The results of physicochemical and phytochemical characteristics confirmed the stability and effectiveness of the formulation. The results also showed that the medium of extraction should be aqueous. Hence this preliminary pharmacognistric study would facilitate to set a standard for this traditional medicine.

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