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POLYHERBAL FORMULATIONS OF GOKSHURADI CHURNA: ASSESSMENT OF HEAVY METAL, AFLATOXIN, PESTICIDE RESIDUE AND MICROBIAL CONTENT

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Abstract

Aim: Although the safety of the polyherbal ayurvedic remedy Gokshuradi Churna (GC) is unknown, it is useful for inflammatory conditions like arthritis and other joint problems. The examination of heavy metals, the detection of aflatoxin, the presence of pesticide residue, and microbiological counts have not yet been assessed. The parameters above were assessed in the current investigation. The objective of the current study was to assess the safety criteria (heavy metal, aflatoxin, pesticide residue, and microbiological profile) of the formulations GC-I, GC-II, and GC-Marketed Formulation (MF). GC-I and II were developed internally.

Materials and Methods: According to AYUSH [Ayurveda, yoga, unani, siddha, naturopathy] Pharmacopoeial laboratory for Indian medicine (PLIM) guidelines, the formulations were evaluated for its safety parameters at Indian Institute of Chennai (IIT), Chennai and Tamilnadu Test Solutions Private Limited, Chennai was used for testing heavy metals and aflatoxins were tested using thin layer chromatography (TLC). The Pesticide residues content was estimated by Gas Chromatography while microbial count by pour plate method.

Results: Results: According to AYUSH Pharmacopoeial Laboratory for Indian Medicine guidelines, the presence of the heavy metals mercury, arsenic, lead, and cadmium was within the recommended limit. However, aflatoxins, pesticide residues, and microbes were not found in the samples, indicating that the formulation Gokshuradi Churna was toxic-free.

Conclusion: According to the PLIM criteria of AYUSH, GC revealed heavy metal concentration below the allowed limit. Microbes and particular pathogens were not present in the GC samples, and neither were aflatoxins or pesticide residues. Since the formulations were safe for therapeutic use, the current study provides that assurance.

Keywords: Aflatoxin; Heavy metal analysis; Pesticide residue; Microbial content; Gokshuradi Churna

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INTRODUCTION

Traditional medicine is frequently used to boost energy and enhance the immune system as well as to prevent and treat a wide range of ailments [1]. Many people in impoverished countries still rely on herbal treatments for their primary care [2]. Depending on the sample's composition and any pollutants or residues, different amounts of dangerous metals are permitted as impurities [3]. Plants are the main conduit for heavy metals from contaminated soil to people. Heavy metals have the potential to affect people even at extremely low concentrations due to their low renal excretion rates [4]. Mycotoxins known as aflatoxins are primarily produced by *Aspergillus parasiticus, Aspergillus flavus*, and sporadically *Aspergillus nomius* [5]. Aflatoxin is one of the most potent mutagenics and carcinogens. In addition to genotoxicity and teratogenicity, aspartame toxicity also includes immunological suppression. How much of it can be found in food and animal feed is regulated by law [6]. Herbal supplements may contain pesticide residues as a result of improper farming practises and abuse of pesticides during production. Organo chloride pesticide residues were found in many medicinal plants that were produced and sold for commercial purposes [7].

Furthermore, exposure to dust, pollen, mould, and fungi may have detrimental effects [8]. According to a 2011 Hong Kong study [9], the adulteration of Chinese herbal anti-diabetic drugs with unreported pharmaceuticals, including both registered and illegal substances, is a significant and unappreciated problem. Moreover, the United Arab Emirates' natural and imported medicinal plants were unsafe for human ingestion due to high levels of heavy metals [10]. The quality and safety standards for herbal medicines based on the presence of heavy metals and microbiological load are of great interest to health authorities and medical professionals. The contamination of these herbal products both reduces their effectiveness and poses a major threat to the health of customers [11]. Since this could help patients acquire trust in their prospective use as therapeutic medicines and contribute to their acceptability on a worldwide scale, it is imperative to standardise traditional medicines using scientific methodologies to establish their safety and quality. The current investigation was carried out to ensure the safety of the aforementioned objective.

Each nation has a unique national limit for dangerous metals and microbial contamination in various herbal products, depending on the type of plant and whether it is a raw material or finished product [3]. The AYUSH Pharmacopoeia Laboratory for Indian Medicine (PLIM) criteria were used to evaluate the formulation's safety attributes.

MATERIALS AND METHODS

Gokshuradi Churna I and II formulations includes six herbal drugs fruit of *Tribulus terrestris*, family - Zygophyllaceae, fruit of *Terminalia chebula*, family - Combretaceae, root of *Boerhaavia diffusa*, family - Nyctaginaceae, wood of *Cedrus deodara*, family - Pinaceae, rhizhome of *Zingiber officinale*, family - Zingiberaceae, bulb of *Allium sativum*, family - Liliaceae equal quantity in powder form.

Preparation of Churna

The raw materials such as Gokshura fruit (1part), Haritaki fruit (1part), Punarnava root (1part) Devadaru wood (1part), Ginger rhizome (1part), and Garlic bulb (1part) were used for the preparation of GC- I and II. For GC-I and GC – II, the raw materials were collected at various season of the year from the local market of Coimbatore and authenticated. Each herbal component was cleaned, dried, and ground separately. The powders passed completely through sieve number 44 and at least partially through sieve number 80. Each powdered ingredient was weighed out separately to ensure a homogenous composition, which was then blended and placed through sieve number 44.

Marketed Sample

The marketed sample of Gokshuradi Churna (GC-MF) was procured from Paramanand Ayurveda Pvt ltd, Delhi.

The formulations was evaluated for heavy metal content at IIT, Chennai. Aflatoxins, pesticide residues, microbial content at Tamilnadu Test Solutions Private Limited, Chennai.

Heavy Metal Analysis

One gram of powder was used for each sample. All of the chemicals used were of the analytical kind. Samples were gradually cooked on a hot plate using a wet digesting procedure until a white residue developed inside the fume hood chamber. To digest the materials, nitric acid and perchloric acid (HNO3: HClO4, 6:1) were utilised. Each residue was dissolved in 0.1 N nitric acid and diluted to a volume of 10 mL. The digested samples were analysed on an Inductively Coupled Plasma Emission Spectrophotometer (ICP-OES). In order to develop a calibration curve and validate the metal content of each analytical sample, standard reference samples of As, Hg, Pb, Cd, and Co were used [12,13].

Aflatoxins

Aflatoxin B1 and G1 each had concentrations of 0.5 g per ml, whereas Aflatoxin B2 and Aflatoxin G2 each had concentrations of 0.1 g per ml. Standard samples were dissolved in a combination of chloroform and acetonitrile (9.8:0.2). The concentration of the test solution was 1 g/ml. Standard aflatoxin was administered in volumes of 2.5L, 5L, 7.5L, and 10L to the surface of a pre-coated TLC plate. Similar to this, the test sample was positioned, the spots were left to dry, and the chromatogram was developed in an unsaturated chamber with a solvent system made up of a chloroform, acetone, and isopropyl alcohol (85: 10: 5) mixture until the solvent front had moved at least 15 cm from the origin. The plate was removed from the developing chamber, the solvent was marked and the plate was allowed to air-dry. The spots were located on the plate by examination under UV light at 365nm [14].

Pesticide Residues

The pesticide residues were examined using gas chromatography. Around 10 g of the test sample were extracted with 100 ml of acetone and homogenised for a brief period of time. Acetone was added with another test batch after more filtration was permitted. The test sample was heated in a rotary evaporator until virtually all of the solvent had evaporated at a temperature that didn't exceed 40 °C. The residue was mixed with a tiny quantity of toluene R and heated once more to completely evaporate the acetone. The resultant residue was subsequently processed through a membrane and broken down with toluene [15].

Microbial Count

Pour plate procedures were utilised to evaluate the product's sterility. An organism grows more quickly when a contaminated or unsterile sample (formulation) comes into contact with a nutrient-rich media. The unique pattern of colonies emerges when the permitted period of time has elapsed, allowing for the differentiation of the organism's growth. The colonies are referred to as colony-forming units (CFUs). A test sample and 15 mL of molten agar that had been cooked to 45°C in a sterile petri dish were contaminated. The dish was tilted and swirled to completely blend the sample and agar. Agar was not handled during the whole gelling process. (About ten minutes.)The plates were then turned over and kept at 37 degrees Celsius for 24-48 hours before being left there for a further 72 hours to observe fungal development. Then, grown colonies of the organism were counted and their CFU values computed.

Test for Specific Pathogen

The test sample was instantly injected into the proper pathogen medium using the pour plate technique (EMB, DCC, Mannitol, Cetrimide). After 24 to 72 hours, the plates were incubated at 37°C for observation. According to the pattern of colony formation in various media, a certain pathogen's presence was identified by its distinctive colour.

RESULTS

Table 1.Heavy metal content

PERKIN ELMER OPTIMA 5300 DV ICP-OES				
Sample code (GokshuradiChurna)	**Element symbol and Wavelength (nm)**	**Weight of sample in gms / Volume in ml**	**Dilution Factor**	**Concn.in ppm µg/ml (or) mg/litre**
Sample 1 (GC-I)	As 188.979	0.0504g/50ml	1	BDL
	Cd 228.802	0.0504g/50ml	1	0.001 mg/L
	Co 228.616	0.0504g/50ml	1	0.004 mg/L
	Hg 253.652	0.0504g/50ml	1	BDL
	Pb 220.353	0.0504g/50ml	1	BDL
Sample 2 (GC-II)	As 188.979	0.0508g/50ml	1	BDL
	Cd 228.802	0.0508g/50ml	1	BDL
	Co 228.616	0.0508g/50ml	1	0.002 mg/L
	Hg 253.652	0.0508g/50ml	1	BDL
	Pb 220.353	0.0508g/50ml	1	BDL
Sample 3 (GC-MF)	As 188.979	0.0508g/50ml	1	BDL
	Cd 228.802	0.0508g/50ml	1	BDL
	Co 228.616	0.0508g/50ml	1	0.002 mg/L
	Hg 253.652	0.0508g/50ml	1	BDL
	Pb 220.353	0.0508g/50ml	1	BDL

Table 2.

Aflatoxins						
PARAMETERS	**METHOD**	**UNITS**	**SPECIFICATION**	**GC-I**	**GC=II**	**GC-MF**
AflatoxinB1	Ayurvedic Pharmacopoeia	Ppm	LT 0.5 ppm	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
AflatoxinG1		Ppm	LT 0.5 ppm	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
AflatoxinB2		Ppm	LT 0.1 ppm	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
AflatoxinG2		Ppm	LT 0.1 ppm	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
ppm – parts per million. LOQ – Limit of quantification						

Table 3.

Microbial parameters						
PARAMETERS	**METHOD**	**UNITS**	**SPECIFICATION**	**GC-I**	**GC=II**	**GC-MF**
TotalBacterialcount	Ayurvedic Pharmacopoeia	cfu/ml	NMT 100000	<10	<10	<10
TotalFungalCount		cfu/ml	NMT 1000	<10	<10	<10
E.coli		Per 10 ml	Absent	Absent	Absent	Absent
Salmonella		Per 25ml	Absent	Absent	Absent	Absent
Staphylococcus *aureus*		Per ml	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa		Per ml	Absent	Absent	Absent	Absent
CFU/g: colony-forming units per gram						

Table 4. PESTICIDE RESIDUE:

METHOD: EPA 8081B				
PARAMETERS	**UNITS**	**GC -I RESULTS**	**GC -II RESULTS**	**GC –MF RESULTS**
OC –Pesticides				
2,4'–DDE	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
2,4'–DDT	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
2,4'–DDD	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
4,4'–DDD	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
4,4'–DDE	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
4,4'–DDT	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Aldrin	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
alpha–Endosulfan	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)

alpha–HCH	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
beta–Endosulfan	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
beta–HCH	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Chlordane(cis&trans)	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Cholorthalonil	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
delta–HCH	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Dicofol	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Dieldrin	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
EndosulfanSulfate	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Ebdrin	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Fenvalerate&Esfenvalerate	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Heptachlor	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Heptachlorepoxide	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Hexachlorobenzene	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Hexachlorocyclohexane	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Lindane(gamma-HCH)	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Methoxychlor	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Pyrethrins	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
OP Pesticides–G				
4-Brono -2- Chlorphenol	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Chlorfenvinphos (cis & trans)	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Chlorpyrifos methyl	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Diazinon	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Dichlorvos	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Ethion	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Fenitrothion	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Fenthion	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Formothion	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Parathion Methyl	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Quinaphos	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Disulfoton	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Fenamiphos	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
Methidathion	mg/kg	BQL(LOQ 0.001)	BQL(LOQ 0.001)	BQL(LOQ 0.001)
OP PESTICIDES-L				
Acephate	mg/kg	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
Azinphos ethyl	mg/kg	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
Azinphos methyl	mg/kg	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
Coumaphos	mg/kg	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)
Dimethoate	mg/kg	BQL (LOQ 0.1)	BQL (LOQ 0.1)	BQL (LOQ 0.1)

DISCUSSION

Globally, numerous research have been conducted to assess the microbiological contamination and heavy metal content of herbal products. Studies carried out in a number of nations, including Japan, Nigeria, Brazil, South Africa, Saudi Arabia, and Egypt, revealed that some of the most widely used medicinal plants and formulations contained metals like Lead, Cadmium, Aluminium, Mercury, and Arsenic above the established standard [16,17,18,19]. Beyond-permissible levels of heavy metals in a medicine would have substantial adverse effects on the brain, kidney, developing foetus, vascular system, and immune system [20]. Similarly, aflatoxins which have a number of harmful side effects like hepatotoxicity, carcinogenicity, and immune suppression linked with them, aflatoxins have become a significant concern to human health. A permitted limit for their content in the plant has thus been established by WHO [21].

Acceptable limit has been established by AYUSH in India to examine safety characteristics for all types of herbal

formulations. As GC-I, GC-II, and GC-MF were powder formulations, the results were evaluated, and it was discovered that all of the formulations' heavy metal content for arsenic, cadmium, cobalt, mercury, and lead was within the allowed limit as per the PLIM criteria stated in Table 1 [22]. According to the restrictions specified by AYUSH, Aflotoxins B1, B2. G1, G2 shown in Table 2 were not detected. The bacteria and fungi that were found to be absent are listed in Table 3 as a microbiological count. Test results for the pathogens E. coli, Salmonella, Staphylococcus aureus, and Pseudomonas aeruginosa, which are presented in table 3, confirmed that none of the formulations included them [21,22]. Organo chlorine, organo phosphorous, and pyrethroid pesticide residues were not found in the GC formulations shown in Table 4.

Nonetheless, there are still possibilities for product contamination during subsequent steps like drying, storage, etc. Hence, standardisation through established scientific methods is necessary.

CONCLUSION

According to the results of the current investigation, all four safety parameters tested on GC-I, GC-II, and GC-MF were determined to be within the acceptable range as per the PLIM recommendations established by AYUSH. The amount of heavy metals was found to be below the allowable limit. Microbes and particular pathogens were not present in any of the three GC samples, and neither were aflatoxins or pesticide residue. The current investigation confirms that the formulation was safe for therapeutic use as a result. This analytical data can be utilised to create standards and as a benchmark for assessing the GC's level of quality.

The amount of hazardous metals, mycotoxins, pesticide residues, and microbiological contaminations varies depending on the source, season, and location of the ingredients, therefore the results point to the safety of all three GC formulations for therapeutic use.

CONFLICT OF INTEREST

There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE

Traditional medicine is renowned for its multicomponent therapies and polyherbal composition for controlling a variety of health issues. In order to ensure safety, the current research is focused on standardising a polyherbal formulation by comparing residue analyses such as heavy metal concentration, microbiological load, pesticide analysis, and aflotoxin determination. This ancient idea needs to be thoroughly examined in the context of current medical knowledge and, if appropriate, put to use.

COMPETING INTERESTS

There are no competing interests, according to the authors.

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