ORIGINAL RESEARCH PAPER

Volume-8 | Issue-3 | March-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANALYSIS OF PHYTOCHEMICAL PROFILE AND OTHER SAFETY PARAMETERS FOR A POLYHERBAL FORMULATION (DERMOSHINE) BY USING ADVANCED INSTRUMENTATION

Ayurveda

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ABSTRACT

India is a country with rich traditional medicine knowledge and is a frontier to the entire world with a larger documentation of medicinal plants. The herbs and their medicinal uses attract a large number of researchers globally. A larger population of India relies heavily on the herbal medicines for their primary health care needs. Medicinal plants play a key role in achieving this object of WHO. Pharmaceutical research mainly focuses on the screening of active constituents of plants and their isolation. Both WHO and AYUSH encourage and design the quality analysis of herbal raw materials and finished products in order to ensure their quality and safety. The main objective of the present study was to determine the phytochemical profile in the selected polyherbal formulation Dermoshine (DSE) by using GC-MS technique. Totally 16 compounds were identified and safety parameters such as microbial load and heavy metals are found within the limits. Other parameters like pesticide residues and aflatoxins are found below detectable limits. The results give a positive indication on the safety and quality of DSE. Further, study is focused on the other advanced instrumentation like LC-MS analysis, Toxicological and Pharmacological studies are under process.

KEYWORDS

Phytochemical profile, Pesticide residues, Heavy metals, Aflatoxins.

INTRODUCTION

The Indian Ayurvedic system as per the recorded literature has included herbs as one of the most powerful healing ingredient[1]. Due to the scientific advancement, more and more pharmacologically active ingredients of Ayurvedic medicines as well as their usefulness in drug therapy have been identified. Basically, it is the phytochemical constituents in the herbs such as saponins, tannins, alkaloids, alkenyl phenols, flavonoids, terpenoids, phorbol esters, sesquiterpenes and lactones which lead to the desired healing effect. A single herb may even contain more than one of the aforementioned phytochemical constituents, which works synergistically with each other in producing enhanced pharmacological action. Scientific studies have revealed that phytochemical constituents of individual plants are insufficient to achieve desired therapeutic effect and hence plants of varying potency when combined may produce a greater therapeutic effect.

In recent years the advancement in Dermatology and skin care attracts the population globally as dermatological problems leads to a cosmetic impact on people. The need for a dermo-care product not only as an external application but also as an internal medicine without any side effects and improved cosmetic care can be achieved by a suitable polyherbal formulation. Even though herbal medicines have become popular and effective in the treatment of several ailments, they are still unacceptable in the treatment modalities due to lack of standardization, lack of identification of active ingredients, lack of clinical trial outcomes and lack of toxicological evaluation. The advanced instrumentation techniques help us to identify the phytochemicals by using GC-MS, heavy metals and pesticides in order to ensure safety of the drug. In this research work, a marketed dermo-protective polyherbal ayurvedic formulation 'Dermoshine' has been taken for the thorough analysis. The formulation is subjected to the screening further safety parameters such as microbial load, Aflatoxins by using appropriate protocol specified in WHO[2], AYUSH, (API[3] and SPI[4]) guidelines. The drug is manufactured by Rumi Herbals Pvt. Ltd, a GMP certified, 22 years old company and is marketed by Rohini Global Marketing Pvt. Ltd. Chennai.

MATERIALS AND METHODS

Sample

The present sample for study is as a gift sample received from Rumi Herbals Pvt. Ltd, Chennai. The sample is tested for all the quality control parameters. The parameters assessed were reported in the previously published research article by the same authors.[5]

Preparation of sample for GC-MS analysis

One gram of the drug Dermoshine (DSE) is weighed and soaked it overnight in 10ml of ethanol solution. This sample was then filtered using Whatman No. 1 filter paper and the extract was passed through anhydrous sodium sulfate. Take 2ml of extract and injected in to GC-MS instrument and performed the sample analysis by Triple Quadrupole Acquisition Method by using Agilent Mass Hunter Workstation software -7000 Series.

Sample Preparation for screening pesticides

2g of the sample was weighed and placed in the centrifuge. To this 20ml of dichloromethane is added and shaken well. After 10 min vortex, remove the organic layer. This process of extraction is repeated twice. Removed the organic layer and passed through anhydrous sodium sulfate in order to remove the moisture. The sample is then evaporated to dryness by using nitrogen evaporator at 40° C. The residue is re-dispersed in 1ml of Acetonitrile and used for GC-MS and LC-MS analysis.

Sample Preparation for screening of Aflatoxins

2 g of accurately weighed sample in Petri dish is transferred to a Blender jar.

2 g of NaCl and 25ml of extraction solvent, which is a mixture of 80:20 Methanol and water were added and washed the petri jar. Blend for 2 min at high speed and centrifuge at 6000rpm for 10 min. The pH is adjusted to 7.4 using 2M NaOH; 10 ml of this filtrate is pipetted out and diluted with 100ml of Phosphate Buffer saline solution (PBS) and mixed thoroughly. IAC columns are adjusted to room temperature prior to conditioning. Removed the cap from the top of the column and fixed in the vacuum manifold. The whole 100 ml of diluted filtrate was passed through the column at a flow rate of 2 - 3 ml per min. A slow, steady flow rate is essential for the capture of the toxin by the antibody. Washed the column by passing 10 ml of water at a flow rate of 6 ml per min and repeated with another 10 ml PBS. The water washings were discarded. Air was passed through the column to remove residual liquid. Eluted the bounded aflatoxins from the column at a flow rate of 1 drop per sec using 1.0 ml of 100% methanol and following elution, pass 1.0 ml of millipore water through the column and collected in an amber glass vial. Then the extract was analyzed by HPLC-FLD system.

Preparation of samples by acid digestion method for analysis of heavy metals

2g of sample was weighed in a Kjeldahl flask, a mixture of nitric acid :

perchloric acid (4:1) was added in the flask and heated continuously till the solution became colorless. The sample was then transferred to a 25 ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead, Cadmium, Arsenic and Mercury were prepared as per the protocol in the manual. The samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic Absorbance Spectrophotometer (AAS) (Shimadzu).[6]

Microbial safety profile

Microbial contamination in herbs has an adverse effect on human health. Microbial screening was carried out to estimate the number of viable microorganism present in the formulation. Various differential and selective medias are utilized for screening microbial contamination. For Total viable count (Casein soyabean digest agar), Total *yeast* and *moulds* (Sabouraud's dextrose agar with antibiotics), *E.coli* (MacConkey agar and EMB agar), *Salmonella typhi* (Brilliant Green agar), *Staphylococcus* sp. (Mannitol salt agar) *Pseudomonas aeruginosa*, (Cetrimide agar) were used to screen the organisms as per the AYUSH guidelines.

RESULTS AND DISCUSSION

The present plant drug was formulated as dermo-protective agent by using traditional medicinal plants. This formulation was prescribed by Ayurvedic and Siddha Physicians for several skin diseases. This drug is used as an internal medicine and the phytochemical interpretation of the formulation is given below.

Identification of phytoconstituents

Interpretation of mass spectra of GC-MS was performed utilizing the database of National Institute of Standard and Technology (NIST) having more than 62,000 designs. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight (MW), and structure of the components of the test materials were ascertained.

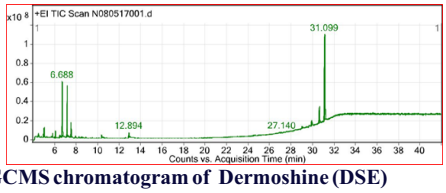

Fig 1. GCMS chromatogram of Dermoshine (DSE)

Screening of Pesticides

The presence of pesticidal residue causes a major threat to the safety and quality of the products. Herbal drugs are accountable for the pesticide remainders, which gather from agricultural practice, such as spraying, handling of soils during farming and administering fumigants throughout the storage. The residues of pesticides including their metabolites and/or degradation products will remain in plants or in the soil that become a notable source of contamination for herbal medicines. In the present work both GC-MS and LC-MS/MS was used to screen the pesticide residues.

GC-MS analysis of pesticides

The pesticide residues were analyzed in the formulation by using Agilent Technologies, 5977 single Quadrupole GC/MS.

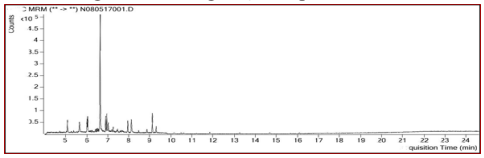

Fig 2: GC-MS chromatogram of pesticide residues

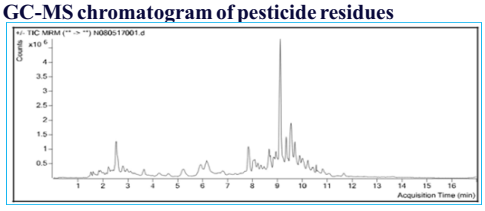

Fig 3: LC-MS/MS chromatogram of pesticides residues

Table 1: Phytoconstituents present in DERMOSHINE (DSE)

Peak		Start	RT	End	Height	Area	Area %	Chemical name
	1	1.466	1.612	1.759	161297467	1230947400	12.25	m-Guaiacol
	2	1.759	2.037	2.314	299565588.7	3661705126	36.43	Benzaldehyde, 2-hydroxy-4-methoxy-
	3	2.461	2.607	2.87	160834116.7	1141870233	11.36	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
	4	8.503	8.971	9.278	82656713.27	2127512803	21.17	2,3-Diazabicyclo[2.2.1]hept-2-ene, 5-ethenyl-4,7,7-trimethyl-, (1.alpha.,4.alpha.,5.beta.)-
	5	9.336	9.775	9.907	73944225.5	1264604901	12.58	Propylparaben
	6	9.936	10.097	10.273	93005168.61	953245922.2	9.48	Ar-tumerone
	7	10.609	10.917	11.151	677360059.8	8436359836	83.93	.beta.-bisabolol
	8	11.604	11.765	11.926	307556906	2488736482	24.76	Curlone
	9	12.146	12.307	12.409	63260212.16	460267887.3	4.58	7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-
	10	12.424	12.526	12.833	68593866.01	811489774.2	8.07	l-Gala-l-ido-octose
	11	12.848	13.097	13.272	505189577	7372828712	73.35	9,12-Octadecadienoyl chloride, (Z,Z)-
	12	13.287	13.521	13.857	639200192.3	10051441382	100	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
	13	15.247	15.452	15.642	314757255.4	3730189728	37.11	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
	14	16.93	17.193	17.427	107011668.3	1520950896	15.13	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-
	15	18.32	18.627	18.978	135983012.4	2413247127	24.01	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
	16	19.388	19.636	19.929	63017903.47	942441290.5	9.38	Alpha amyrin

Identification of Mycotoxins (Aflatoxins)

Most drugs are prone to bio deterioration by moulds and other fungi during post-harvest processing, transport and storage; thus they are responsible for considerable economic loss. The 4 major aflatoxins are B1, B2, G1 and G2. Approximately 40% of the productivity loss to diseases in developing countries is due to diseases exacerbated by aflatoxins. Aflatoxins are extremely dangerous to both animal and human being, causing morbidity as well as mortality and therefore their control in human food and animal feed is essentially important. The aflatoxins were screened in the formulation by using HPLC- FLD, Agilent 1260 Infinity system, Column: Zorbax Eclipse Plus C18.

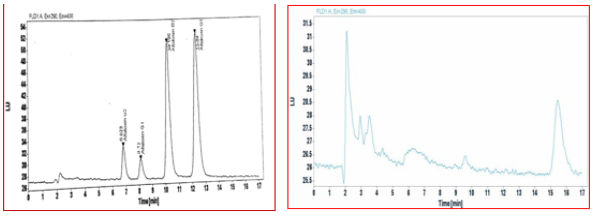

Fig 4: HPLC-FLD chromatogram of Aflatoxins (A. Standard, B. Sample)

Table 2: List of standard Aflatoxins and their RT by HPLC-FLD

Name	RT (min)	Area	Height	Amount (ng/µl)
Aflatoxin G2	6.832	101.9671	5.9167	10.192
Aflatoxin G1	8.12	71.8558	3.6322	10.169
Aflatoxin B2	10.198	527.1737	24.4125	10.001
Aflatoxin B1	12.34	626.3959	25.9039	10.043

Table 3: List of pesticides Analysis of Dermoshine (DSE)

Compound	RT	Resp	Final Conc	Units
Monochrotophos	3.400	0	ND	ng/ml
Methyl Paraoxon	8.525	0	ND	ng/ml
PhorateSulfoxide	9.347	0	ND	ng/ml
PhorateSulfone	9.396	0	ND	ng/ml
Isoproturon	9.756	7017	ND	ng/ml
Methyl Parathion	-	-	ND	ng/ml
2,4-D	9.760	603	ND	ng/ml
Phorate	10.935	0	ND	ng/ml

SUMMARY AND CONCLUSION

The present review provides an overview of the extremely diverse phytochemicals present in the polyherbal formulation Dermoshine (DSE). The drug DSE had shown results for various phytoconsituents on analysis by using hyphenated instruments like GC-MS and LC-MS/MS. The analysis by the above techniques has not recorded any pesticide residues. HPLC-FLD system also not reported the presence of any of aflatoxins. Heavy metals were screened by using Atomic Absorption Spectroscopy and found within the limits.

Microbial screening was done as per AYUSH guidelines, found Total Bacterial Count (TBC) and total Yeast Mould Count (TYM C) within the limits. The analysis reports documented in the present study has established the scientific evidence for the safety aspects of the Dermo-protective polyherbal formulation Dermoshine (DSE). Toxicological and pharmacological studies are in progress.

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