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Pharmacognostical standardization and preliminary phytochemical explorations on *Salvia hispanica* L. seeds

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ABSTRACT

Salvia hispanica L. famous as 'chia' is an annual herbaceous plant belonging to family Labiatae, is a richest source of alpha linolenic fatty acid. A detailed examination on chia seeds was performed. The microscopic investigationsshowed the presence of lignified fibres, epidermis cells, aleurone grains, fatty oil globules and lignified sclerides etc. Physicochemical assessment of chia seeds revealed foreign organic matter (0.76%), loss on drying (8.09%), pH (4.39), moisture content (8.96%), swelling index (8.36), foaming index (<100), total ash (7.09%), water soluble ash (1.09%), acid insoluble ash (0.48%), sulphated ash (0.94%),relative density (0.892), refractive index (1.462), acid value (1.862), saponification value (194.29), peroxide value (4.680), acetyl value (169.28) and iodine value (74.62). Elemental analysis was performed using atomic absorption spectroscopy various heavy metals (As, Hg, Pb, Cd) and other elements (Cu, Zn, Fe and Mg) all were within limits. Chia seeds crude material as well as its extract showed no microbial contamination. High performance thin layer chromatographyfingerprinting profile of chia seeds fixed oil showed the presence of alpha linolenic acid. Work on standardization assumes imperative consequence. However,wide-ranging literature survey on this plant revealed that, more research work is required to update the standardization parameters. Therefore, findings of this study will facilitate quality control and presence of the various phytoconstituents in plant seed.

Keywords: *Salvia hispanica*, Standardization, Pharmacognostical, Physicochemical, HPTLC fingerprints.

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INTRODUCTION

Pharmacognostical standardization build up quality control parameters of plants. It guarantees the verification of plants and deterrence of adulteration[1-2]. Consequently, every nation has embraced an arrangement of rules quality control of the home grown drug[3]. *Salvia hispanica* L. (family: Labiatae), commonly known as chia, is an annual herbaceous plant. It is native to central and southeren Mexico and Guatemala. It is considered a pseudocereal, mainly cultivated for its edible, hydrophilic chia seed, grown and commonly used as food in several countries of western South America, western Mexico, and the southwestern United States. Plant can grow in a wide range of well drained clay and sandy soils with reasonable salt and acid tolerance. The word chia is derived from a Spanish word chian which means oily, it is oilseed, with a power house of omega-3 fatty acids, superior quality protein, higher extent of dietary fibre, vitamins and minerals[4-5]. The plant seeds showed various pharmacological activities like antioxidant[6], cytotoxic[7], anti-inflammatory[8], antimicrobial[9], anti-tryptic[10], hypolipidemic[11], hypoglycemic[11], anti-proliferate[11] activities. Standardization and quality control of plants are likewise fundamental for the overall acknowledgment of herbal items in present day arrangement of medications.

The present study pharmacognostical standardization and preliminary phytochemical investigations on *S. hispanica* L. seeds will help in further explorations of this nutritive and therapeutic effective seed.

MATERIALS AND METHOD

Collection and identification of plant material

Salvia hispanica L. seeds were procured from Ch. Charan Singh Haryana Agriculture University, Hisar, Haryana, India. The seeds were taxonomically identified by Dr. Sunita Garg, Scientist, CSIR-NISCAIR, New Delhi, India. A voucher specimen was deposited in the herbarium of NISCAIR (*L. sativum*; No. NISCAIR/RHMD/Consult/-2017/3112-61-2). The plant was identified as *Salvia hispanica* L. belonging to family Labiatae.

Morphological studies

The morphological studies were performed by visual examination and with the help of dissection microscope. The

morphological characteristics like colour, odour, taste, shape, size and texture were determined.

Powder microscopy

Small amount of seeds powder was taken on slide. This powder was stained with phloroglucinol and concentrated hydrochloric acid. Then, it was mounted in glycerine and covered with cover slip. The prepared slides were observed under light microscope (Carl Zeiss Primo star, Germany).

Physicochemical parameters

Physicochemical parameters of the seeds were studied using standard procedures[12-13]. These parameters include foreign organic matter, loss on drying, pH studies, moisture content, swelling index, foaming index, extractive values, ash values, relative density, refractive index, acid value, saponification value, peroxide value, acetyl value and iodine value.

Elemental analysis

Elemental analysis of the powdered seeds was done using nitric-perchloric acid digestion method by using the procedure recommended by the AOAC (1990). For this analysis, 1 gm of accurately weighed powdered seeds material was boiled gently with 10 ml of concentrated nitric acid for 30-45 min. This mixture was cooled down and 5 ml of 70% perchloric acid was added to it. Subsequently, the mixture was allowed to boil at gentle temperature until the emergence of dense white fumes. This solution was cooled down and distilled water weighed at 20ml was poured to it and boiled to release the white fumes. After cooling, the solution was filtered through Whatman No. 42 filter paper[14-15]. The Atomic Absorption Spectroscopy (AAS) (GBC 932 plus) was used to analyse the gathered samples after filtration. An atomic absorption spectrophotometer with hollow cathode lamp for lead (Pb), cadmium (Cd), copper (Cu), arsenic (As), zinc (Zn), mercury (Hg), iron (Fe) and magnesium (Mg) was used. The standard solutions of As, Hg, Pb, Cd, Cu, Zn, Fe and Mg at various wavelengths 193.7, 253.7, 283.5, 228.8, 324.8, 213.9, 248.3, 242.1 nm respectively were used to calibrate the instrument. Subsequently, the standard calibration curves of these elements were formulated. The optimization of the instrument was done as per the specified requirement while the results were calculated in ppm levels.

Preparation of crude extract

The selected plant materials were pulverized mechanically to coarse powder. The coarse powdered material were subjected for defatting with successive extraction in a soxhlet extractor with hexane, chloroform, ethyl acetate and ethanol respectively. After extraction, the solvent was distilled off and the resulting extract was evaporated to dryness on water bath to a dry residue and kept in a desiccator.

Preliminary phytochemical screening

The prepared n-hexane, chloroform, ethyl acetate, ethanol and aqueous extracts were put through various chemical parameters for the existence of different phytoconstituents like carbohydrates, glycosides, alkaloids, saponins, flavonoids, phenols, steroids and triterpenoids, proteins and amino acids[3-16].

Determination of microbial count

The crude seed powder and extract of *S. hispanica* L. seeds of the plant was evaluated for microbial contamination by various bacteria like Total Bacterial count, Total Enterobactor count, Total Yeast/Mould count, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, according to the Baried method and WHO guidelines[17-18].

Derivatization of fatty acids (DFAs)

2 g of oil sample was saponified by means of 10 ml 1.0 M ethanolic KOH in 250 ml round bottom flaskat 90°C for 1 h. Then, acidified with 0.2 ml of 6 M HCl, and then 10 ml of water was added. Thereafter, extracted with 10 ml of hexane and hexane was evaporated by using rota evaporator apparatus. The resultant material was methylated with 1 ml of 10% BF_3 in methanol at 37°C for 20 min. Water was added to the solution, and then derivatized fatty acids were extracted with 10 ml of hexaneand further used for HPTLC analysis[19].

Procedure

10.0 µl of the standard solution and 20.0 µl of each sample solution was applied with the help of CAMAG Linomat-5, Switzerland; applicator, as bands on the TLC plates. Chromatography was performed on (5x10) aluminum packed silica gel 6.0F_{254}HPTLC plate with 0.2 nm thickness (Merck, Darmstadt, Germany). Before use, the plates were dried in an oven at 105°c for 5 min. Development of the plate with mobile phase was done up to 90.0 mm height with chloroform: ethyl acetate (95:5) (v/v). Hair dryer was used for drying the plate. The plate was sprayed with anisaldehyde – sulphuric acid reagent spraying reagent and dried for 5 to 10 minutes at 105°C in hot air oven. Then the plate was photo documented in visible light and R_f value was calculated.

RESULTS AND DISCUSSION

Morphological studies

The seeds occurred in oval pieces with thickness of 1 to 1.5 mm wide and 2 to 2.5 mm long in size. Seeds were of white, grey, black and brown in colour having smooth texture. It had characteristic odour and bitter in taste. Results are shown in the Figure 1.

Figure 1: Morphology of S*alvia hispanica* L. seeds

Powder microscopy

In powder microscopy, *S. hispanica* seeds showed the presence of parenchyma cells, rounded collenchymatous cells in hypodermis and polygonal epidermis cells filled with mucilage, lignified sclerides, aleurone grains, oil globules, pigmented layer, pitted walls and yellow colouredlignified fibreswhich are shown in Figure 2.

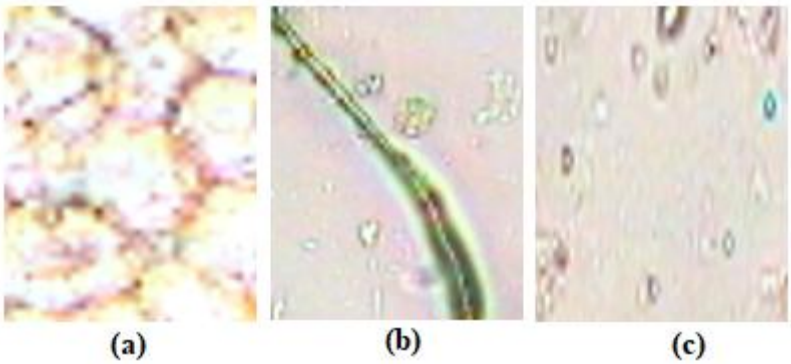

Figure 2: Powder microscopy of *S. hispanica* seed (a) fragment of epidermal parenchyma cells (b) lignified fibres (c) oil globules

Physicochemical analysis

The results of different standardization parameters such asforeign organic matter, loss on drying, pH, moisture content swelling index, foaming index, extractive values, ash values, relative density, refractive index, acid value, saponification value, peroxide value, acetyl value and iodine value are given in the Table 1. These physicochemical parameters will be useful in identification of the plant seeds even in its powdered form.

Table 1: Physicochemical characters of *Salvia hispanica* L. seeds

S.No.	Parameter	Value
1.	Foreign organic matter	0.76± 0.09 % w/w
2.	Loss on drying	8.09± 0.61 % w/w
3.	pH	4.39
4.	Moisture content	8.96± 0.37% w/w
5.	Swelling index	8.76ml/g
6.	Foaming index	Less than 100
7.	**Ash values (% w/w)**	
	Total ash	7.09 ± 0.33
	Water soluble ash	1.09 ± 0.71
	Acid insoluble ash	0.48 ± 0.61
	Sulphated ash	0.94 ± 0.37
8.	**Extractive Value (% w/w)**	
	Hexane extract (pale yellow colour) Hot extraction method Cold maceration method	 32.08 ± 0.44 29.15 ± 0.35
	Chloroform extract (brownish yellow colour) Hot extraction method Cold maceration method	 26.86 ± 0.47 22.63 ± 0.11
	Ethyl acetate extract (yellowish brown colour) Hot extraction method Cold maceration method	 21.02 ± 1.37 16.09 ± 0.51
	Ethanol extract (reddish brown colour) Hot extraction method Cold maceration method	 11.23 ± 0.54 9.13 ± 0.62
	Aqueous extract (brown colour) Hot extraction method Cold maceration method	 15.2 ± 0.62 12.26 ± 0.33
9.	**Oil parameters**	
	Relative density	0.892± 0.08 % wt./ml
	Refractive index	1.462
	Acid value	1.862± 0.31
	Saponification value	194.29± 0.19
	Peroxide value	4.68± 0.23
	Iodine value	169.28 ± 0.59
	Acetyl value	74.62± 0.42

Values are expressed as Mean±S.E.M.; n=3

Determination of microbial count

The crude seed powder and extract of *S. hispanica* L. seeds of the plant was evaluated for microbial contamination by various bacteria like Total Bacterial count, Total Enterobactercount, Total Yeast/Mould count, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and results are shown in table no. 2.

Table 2: Microbial count of *Salvia hispanica* L. seeds

Sample name	Total Bacterial Count (cfu/g)	Total yeast and mould count (cfu/g)	Total Enterobacter count (cfu/g)	E.coli (cfu/g)	Salmonella sp. (cfu/g)	P. aeruginosa (cfu/g)	S. aureus (cfu/g)
Limits for crude raw material*	$1X10^7$	$1X10^5$	$1X10^4$	100	Absent	_	_
Raw material	$181X10^3$	$51X10^2$	<100	Absent	Absent	-	-
Limits for extracts**	1000	100	100	Absent	Absent	Absent	Absent
Hexane extract	Absent	Absent	Absent	Absent	Absent	Absent	Absent

*Quality control methods for Medicinal Plant Materials 1992, WHO/PHARMA/92.559,37

**In house specifications provided by Dabur Research Laboratories, Herbal Drug Research, Sahibabad, Ghaziabad.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of *S. hispanica* L. seeds showed the presence of glycosides, carbohydrates, phenols, saponins, tannins, flavonoids, steroids and terpenoids in different extracts. The results of preliminary phytochemical screening of seed extracts are given in Table 3.

Table 3: Preliminary phytochemical screening of *Salvia hispanica* L. seedsextracts

Test	n-hexane extract	Chloroform extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
Carbohydrates					
Molish test	-	-	-	+	+
Benedict's test	-	-	-	+	+
Fehling's test	-	-	-	+	+
Barfoed test	-	-	-	+	+
Test for pentose sugar	-	-	-	+	+
Test for hexose sugar	-	-	-	-	+
Alkaloids					
Dragondroff's test	-	+	-	+	+
Meyer's test	-	+	+	+	+
Wagner's test	-	+	+	+	+
Hager's test	-	+	-	+	+
Glycosides					
Modified Borntrager's test	-	-	-	+	+
Keller Killiani test	-	-	-	+	+
Legal test	-	-	-	+	+
Sodium picrate test	-	-	-	+	+
Fluorescence test	-	-	-	+	+
Saponins					
Foam test	-	-	-	+	+
Flavonoids					
Vanillin HCl test	-	-	+	+	+
Ammonia test	-	-	-	+	+
Shinoda test	-	-	+	+	+
Phenols					
Ferric chloride test	-	-	+	+	+
Lead acetate test	-	-	+	+	+
Steroids and triterpenoids					
Salkovaski Test	+	-	+	+	-
Libermann Burchard's test	+	-	+	+	-
Fixed oils and fats					
Spot Test	+	+	-	-	-
Saponification test	+	+	-	-	-

+ denotes present and – denotes absent

Elemental analysis

The elemental contents Pb, As, Zn, Cd, Cu, Hg, Ni, Co, Fe and Mg were analysed in the powdered seeds. The results are shown as under Table 4.

Table 4: Elemental analysis of *Salvia hispanica* L. seeds

Metal	Concentration (ppm)
Lead	0.159
Arsenic	0.000
Copper	6.85
Cadmium	0.004
Mercury	0.769
Magnesium	8.927
Nickel	0.059
Cobalt	0.072
Zinc	0.802
Iron	5.028

HPTLC fingerprinting

HPTLC fingerprinting of alpha linolenic acid from *S. hispanica* L. seeds oil shown in Figure no.3.

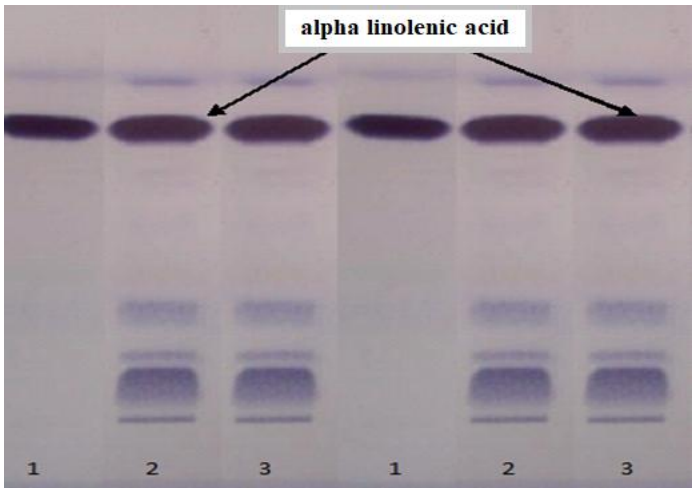

Figure 3: HPTLC fingerprinting of alpha linolenic acid from *S. hispanica* L. seeds oil

Table 5: Rf value of alpha linolenic acid in *Salvia hispanica* L. seeds

S. No.	Sample	Distance travelled by alpha linolenic acid (mm)	Rf value of alpha linolenic acid
1	alpha linolenic acid standard	71	0.861
2	CSO spot 1	71.1	0.862
3	CSO spot 2	71.1	0.862

CONCLUSION

The investigation was performed to build up the quality control parameters of *S. hispanica* L. seeds. The outcomes of pharmacognostical studies about what's more, preliminaryphytochemical screening can be utilized as an analytic device for the standardization of the *S. hispanica* L. seedsto encourage quality control and distinguishing proof of the plant and to limit the adulteration.

REFERENCES

1. Chanda S. Importance of pharmacognostic study of medicinal plants: an overview, Journal of Pharmacognosy and Phytochemistry, 2014; 2(5):69-73.
2. Amponsah IK, Mensah AY, Otoo A, Mensah MLK, Jonathan J. Pharmacognostic standardisation of *Hillerialatifolia*(Lam.) H. Walt. (Phytolaccaceae), Asian Pacific Journal of Tropical Biomedicine, 2014; 4(12):941-946.
3. Sharma N, Singh S, Singh SK. Pharmacognostical standardization and preliminary phytochemical investigations on Acacia auriculiformis A. Cunn. Ex. Benth stem bark. Journal of Medicinal Plants. 2017; 5(1):398-402.
4. Ullah R, Nadeem M, Khalique A, Imran M, Mehmood S, Javid A, Hussain J. Nutritional and therapeutic perspectives of Chia (Salvia hispanica L.): a review. Journal of food science and technology. 2016; 53(4):1750-1758.
5. Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. Physicochemical characterization of chia (*Salvia hispanica*) seed oil from Yucatán, Méxic,. Agricultural Sciences, 2014; 5(03):220.
6. Scapin G, Schmidt MM, Prestes RC, Rosa CS. Phenolics compounds, flavonoids and antioxidant activity of chia seed extracts (*Salvia hispanica*) obtained by different extraction conditions, International Food Research Journal, 2016; 23:2341-2346.
7. Dugganaboyana G, Palanisamy C, Kiran K, Sridhar M, Velliyur K. Dietary evaluation, antioxidant and cytotoxic activity of crude extract from chia seeds (*Salvia hispanica* L.) against human prostate cancer cell line (PC-3), International Journal of Pharmacognosy and Phytochemical Research, 2016; 8:1358-1362.
8. Gazem RA, Puneeth HR, Madhu CS, Sharada AC. Physicochemical Properties and in Vitro Anti-Inflammatory Effects of Indian Chia (*Salvia hispanica* L.) Seed Oil, Journal of Pharmacy and Biological Science, 2016; 11:1-8.
9. DivyapriyaGK, Veeresh DJ, Yavagal PC. Evaluation of antibacterial efficacy of chia (*Salvia hispanica*) seeds extract against porphyromonasgingivalis, fusobacterium nucleatum and aggregatibacteractinomycetemcomitans-an in-vitro study, International Journal of Ayurveda and Pharma Research, 2016; 4:22-26.
10. de Souza AP, Nascimento LM, de Lima VC, de Carvalho FM, dos Santos EA, de Araújo Morais AH. Anti-tryptic activity in seed and food product of chia (*Salvia hispanica* L.), DEMETRA: Food, Nutrition & Health, 2017; 12:319-331.
11. Gazem RA, Puneeth HR, Sharada AC. Hypolipidemic, hypoglycemic and antiproliferateproperties of chia seed oil and its blends with selected vegetable oils-An in-vitro study, IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 2016; 2: 33-39.
12. The Ayurvedic Pharmacopoeia of India. New Delhi: Department of yoga and Naturopathy, Unani and Siddha, Ministry of Health and Family Welfare, Govt. of India. 1996; 1st edition: 137.
13. Indian Pharmacopoeia. Ghaziabad, India: The Indian Pharmacopoeia Commission, Government of India. 2010; 6th edition: 82-171.
14. AOAC. AOAC official methods of analysis. Association of Official Analytical Chemists. Arlington: Virginia. 1990: 15th edition: 84-85.
15. Hseu ZY. Evaluating heavy metal contents in nine composts using four digestion methods. Bioresource Technology. 2004; 5:53-59.
16. Sajitha MK, Thoppil JE. Phytochemical evaluation and in vitro antioxidant studies of selected species of Gomphostemma Wall. ex Benth. from Western Ghats. Journal of Drug Delivery and Therapeutics. 2018; 8:32-37.
17. Baired RM, Hodge NA, Denyer SP. Hand book of microbial quality control. 2000: 22-36.
18. WHO guidelines quality control methods for medicinal plant materials. WHO Geneva, New Delhi: A.I.T.B.S. Publishers and Distributors; 1998: 60-70.
19. Ichihara KI, Fukubayashi Y. Preparation of fatty acid methyl esters for gas-liquid chromatography, Journal of lipid research, 2010; 51(3):635-640.