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Research Article

STANDARDIZATION AND TOXICOLOGICAL EVALUATION FOR EFFICACY AND SAFETY OF *PIMPINELLA ANISUM* L.

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ABSTRACT
The demand of herbal medicine is increasing day by day due to their efficacy, safety (rare chances of side effects) in the treatment and good faith of society on herbal medicine and also their products. Therefore standardization and toxicological study of herbal medicine is quite necessary to check quality, Purity, strength and safety of the drugs. The present work is an attempt to evaluate toxicological parameters for safety of an herbal drug *Pimpinella anisum* L. such as microbial contamination, aflatoxins, pesticide residues and heavy metals. Additional standardization parameters like, physical constants, ash content, solvent residues, and microscopical analysis were also carried out and reported. The results obtained may be helpful to the regulatory authorities, scientific organizations and manufacturers for developing standards and maintaining the quality of drugs.
Keyword – Standardization, WHO, *Pimpinella anisum* L, Toxicological parameters

INTRODUCTION

Pimpinella anisum Linn. (syn Aniseed) is an annual herb with pinnatified leaves and umbel of white flowers belongs to family Apiaceae. It is native to Mediterranean region and cultivated throughout the world. In India, it is cultivated in North-West India, Uttar Pradesh, Punjab and Orissa[1.] It contains mainly trans-anethole, much smaller amounts of estragole, cis-anethole, p-anisaldehyde and pseudoisoeugenyl-2-methylbutyrate [2-3.] In literature review it was found that the plant is antimicrobial [4]. It has antiviral and immunostimulant effects[5]. It is reported that it has antioxidant, spasmolytic, estrogenic and anti-estrogenic, local anaesthetic and sedative action[6.] India has a vast heritage of traditional systems of medicine for various ailments. The quality control standards of various medicinal plants used in indigenous system of medicine becoming more relevant today in view of commercialization based on medicinal plants [7]. Due to lack of quality control measures, people are unable to utilize the benefit of the traditional systems of medicine. The quality of a plant product is determined by the prevailing conditions during growth which includes seed selection, growth conditions, use of fertilizers, harvesting, drying and storage hence, they are capable of adulterated. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals, and pesticides can alter the quality, safety, and efficacy of herbal drugs. Due to this scientific awareness a scenario has created to undertake the research activities like standardization of traditional medicines and to develop the scientific methods for the manufacture of quality medicines of *Pimpinella anisum* L.

MATERIAL AND METHODS

Preparation of powders

Pimpinella anisum (fruit) were bought from a local supermarket (Khari Bowli) in Delhi and authenticated by DR. H.B. Singh (NISCAIR/RHMD/Consult/2011-12/1823/123).The drug was powdered and passed through sieve no.80.

Analytical parameters

The sample was subjected for analytical parameters such as physico-chemical studies like total ash, acid insoluble ash, and water soluble ash, extractive value, loss on drying 105 º, foreign matter and *pH* values for 1% aqueous solution was carried out as per the WHO guidelines [8].

Microbial load and heavy metal were carried out as per the Haque et al procedure [9]. Aflatoxin, pesticide residues, was carried out by standard methods [10].

Thin Layer Chromatography

Preparation of extract

4 gm of drug dissolved in 40 ml of ethanol and sonicate for half an hour. The solution was then concentrated on water bath for analysis.

Development of TLC

The ethanolic extract was applied on precoated silica gel 60 F254 TLC plate (E. merck) as absorbent and developed the plate using solvent systems toluene: ethyl acetate: formic acid (9.3 0.7: 0.5 *v/v)* as mobile phase.. After developing, the plates were dried and observed the colour spots at UV-254 and anisaldehyde- sulphuric acid spraying reagent.

RESULTS AND DISCUSSION

Pimpinella anisum fruit has brown coloured, aromatic odour and sweet in taste.

Microscopically observation

The epicarp composed of a layer of colourless cells with unevenly thickened and pitted walls; in surface view the cells are seen to be arranged with three or four rows straight-walled, somewhat elongated cells alternating with wider areas of irregularly shaped cells with slightly sinuous walls. The covering trichomes, which are nearly always found detached from the epicarp. They are conical, slightly curved and usually unicellular. The very numerous brown fragments of the vittae composed of thin-walled cells, polygonal to elongate in surface view. The salaried of the mesocarp, which are usually found in groups in a single layer, often associated with thinner-walled undignified parenchymatous cells. The testa composed of a single layer of brown cells, with thin, sometimes slightly beaded, walls. The fragments of lignified

fibro-vascular tissue composed of small, thin- walled fibres and vessels with spiral and annular thickening (Fig 1-7).

Physico- Chemical analysis:

Quantitative standards revealed that total ash content was 4.4366 % and 0.9966% of acid insoluble siliceous matter was detected in the drug. The water soluble extractive value (24.9812%) indicates the presence of inorganic content. The alcohol soluble extractive (21.3211%) value indicates the extraction of polar constituents. The LOD and foreign matter were found 8.6206% and 1.84 %. The drug contain slightly acidic compound, because PH value of aqueous solution was 6.4 (Table-I).

Microbial contamination

Study revealed that total bacterial count and total fungal count were found to be within the permissible limit. *Pimpinella anisum* fuits contains volatile oil which has antimicrobial action. That is why total bacterial count and total fungal count were found very less in number (Table 2).

Aflatoxins analysis

Aflatoxins are toxic mycotoxins produced by several species of *Aspergillus* moulds. Four compounds produced by these moulds are aflatoxins B_1, B_2, G_1, and G_2. Of these, aflatoxin B_1 is the most carcinogenic and the most commonly occurring variety. Aflatoxins are a group of highly oxygenated heterocyclic compounds with closely related structures. Monitoring of a variety of foods is necessary to ensure consumer safety. Scientists identified at least four related compounds that caused acute toxicity and liver carcinogenicity in duckling feeding trials. Aflatoxins were characterized as B (blue fluorescence) and G (green fluorescence). Four aflatoxins, B1, B2, G1, and G2, are synthesized by *A. flavus*. In cases of contamination, aflatoxin B1, the most toxic and most carcinogenic, is almost always present[9]. Aflatoxins such as B_1, B_2 and G_1, G_2 were not detected in the entire drugs (Table 3).

Pesticide residue

The toxic pesticide residue such as o, p-DDD, p, p'- DDD, o, p-DDE, p, p'-DDE, o, p '-DDT, p, p'-DDT, Endosulfan, α-HCH, β-HCH, γ-HCH, δ-HCH was absent in the entire drug (Table 4).

Toxic heavy metals

Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) are natural constituents of the environment like air, water and soil. Furthermore, they are produced by technical and industrial processes and thus have gained importance as contaminants. Medicinal plants growing in nature can accumulate heavy metals to a certain extent depending on their individual properties and the concentration of heavy metals in soil, air and water. As heavy metals pose a hazard to human and animal health, their content in plants used for consumption or medicinal purposes must be limited[9]. The toxic heavy metal like cadmium, arsenic and mercury were not detected in the drug and lead under acceptable limit also indicates the maintenances of quality of the drug (Table 5)..

TLC analysis

Thin layer chromatography studies of ethanolic extract of the sample were showed number of spots in UV - 254 nm, and after spraying with anisaldehyde-sulphuric acid reagent. The R_f values of the were shown in figure- 8 and 9. The plates were visualized using anisaldehyde-sulphuric acid reagent and heated at 105º C till appears colored spots.

CONCLUSION

Hence, the microscopic features, analytical parameters, TLC profiles together may be used for quality evaluation and the standardization of the *pimpinella anisum*. Thus, the data generated in this analysis will help in setting up regulatory limit, to ensure the quality of in Indian medicine.

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Result of Physico-Chemical parameters

Table – 1. Result of Physico-Chemical parameters

Parameters	
Total ash (% w/w)	4.4366 %
Acid insoluble ash (% w/w)	0.9966 %
Alcohol soluble matter (% w/w)	21.3211
Water soluble matter (% w/w)	24.9812
Loss of weight on drying at 105^0 c	8.6206%
pH of 1% Aqueous solution	6.4
Foreign matter analysis	1.84 %

Table No. 2. Observations of Microbial

S. No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	<10 CFU/gm	10^5 CFU /gm
2	Total Fungal Count	<10 CFU/gm	10^3 CFU /gm

Table No. 3. Observations of Aflatoxins Residues

S. No.	Test Parameter	Test Method	Result
1.	Aflatoxin B_1	AOAC 990.33	Not Detected
2.	Aflatoxin B_2	AOAC 990.33	Not Detected
3.	Aflatoxin G_1	AOAC 990.33	Not Detected
4.	Aflatoxin G_2	AOAC 990.33	Not Detected

Table No. 4. Observations of Pesticides Residues

S. No.	Pesticides	Test Method	Result
1.	α-BHC	AOAC 970.52/EPA 525.2	Not Detected
2	β-BHC	AOAC 970.52/EPA 525.2	Not Detected
3	γ-BHC (Lindane)	AOAC 970.52/EPA 525.2	Not Detected
4	δ-BHC	AOAC 970.52/EPA 525.2	Not Detected
5	Heptachlor	AOAC 970.52/EPA 525.2	Not Detected
6	Heptachlor Epoxide	AOAC 970.52/EPA 525.2	Not Detected
7	α-Chlordane	AOAC 970.52/EPA 525.2	Not Detected
8	α-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
9	β-Chlordance	AOAC 970.52/EPA 525.2	Not Detected
10	Endrin	AOAC 970.52/EPA 525.2	Not Detected
11	Total DDE	AOAC 970.52/EPA 525.2	Not Detected
12	Total DDD	AOAC 970.52/EPA 525.2	Not Detected
13	Total DDT	AOAC 970.52/EPA 525.2	Not Detected
14	β-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
15	Endrin Aldehyde	AOAC 970.52/EPA 525.2	Not Detected
16	Endoulfan Sulfate	AOAC 970.52/EPA 525.2	Not Detected
17	Aldrin	AOAC 970.52/EPA 525.2	Not Detected
18	Endrin Ketone	AOAC 970.52/EPA 525.2	Not Detected
19	Methoxychlor	AOAC 970.52/EPA 525.2	Not Detected
20	Dieldrin	AOAC 970.52/EPA 525.2	Not Detected
21	Alachlor	AOAC 970.52/EPA 525.2	Not Detected
22	Butachlor	AOAC 970.52/EPA 525.2	Not Detected
23	Monocrotophos	AOAC 970.52/EPA 525.2	Not Detected
24	Phorate	AOAC 970.52/EPA 525.2	Not Detected
25	Mevinphos	AOAC 970.52/EPA 525.2	Not Detected
26	Dimethoate	AOAC 970.52/EPA 525.2	Not Detected
27	Malathion	AOAC 970.52/EPA 525.2	Not Detected
28	Methyl parathion	AOAC 970.52/EPA 525.2	Not Detected
29	Chlorpyrifos	AOAC 970.52/EPA 525.2	Not Detected
30	Ethion	AOAC 970.52/EPA 525.2	Not Detected
31	Atrazine	AOAC 970.52/EPA 525.2	Not Detected
32	Simazine	AOAC 970.52/EPA 525.2	Not Detected
33	Diazinon	AOAC 970.52/EPA 525.2	Not Detected
34	Phosphamidon	AOAC 970.52/EPA 525.2	Not Detected
35	Fenitrothion	AOAC 970.52/EPA 525.2	Not Detected
36	Fenthion	AOAC 970.52/EPA 525.2	Not Detected
37	Phosalone	AOAC 970.52/EPA 525.2	Not Detected
38	Quinalphos	AOAC 970.52/EPA 525.2	Not Detected
39	Coumaphos	AOAC 970.52/EPA 525.2	Not Detected
40	Parathion	AOAC 970.52/EPA 525.2	Not Detected
41	Malaoxon	AOAC 970.52/EPA 525.2	Not Detected
42	Dichlorvos	AOAC 970.52/EPA 525.2	Not Detected
43	2,4-D	PAM Vol I / EPA 515.3	Not Detected

Table No. 5. Observations of Heavy Metal Residues

S. No.	Test Parameter	Test Method	Result
1.	Cadmium (Cd)	ICP-OES	Not Detected
2.	Lead (Pb)	ICP-OES	0.40 mg/kg
3.	Arsenic (As)	ICP-OES	Not Detected
4.	Mercury (Hg)	ICP-OES	Not Detected

Fig- 1: T.s of *Pimpinella anisum fruit*

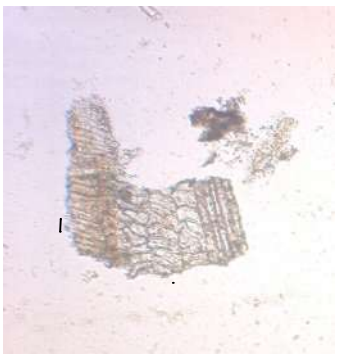

Fig-4: Fibro-vascular tissue and trichome

Fig-7: Raphe

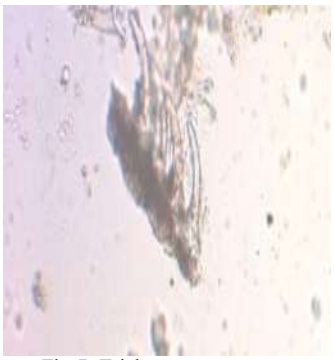

Fig-7: Trichome

Rf Value	Bands
0.10	Green
0.13	Green
0.18	Green
0.40	Green
0.46	Green
0.52	Green

Fig-8: TLC plate of ethanolic extract in duplicate and Scanned at 254 nm

Rf Value	Bands
0.14	Green
0.18	Green
0.28	Pink
0.40	Violet
0.46	Violet
0.52	Violet
0.58	Purple
0.76	Violet
0.87	Violet

Fig-9: TLC plate of ethanolic extract in duplicate and Scanned after spraying with anisaldehyde reagent

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