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Pharmacognostic and Physicochemical Standardization of *Nigella sativa* and *Allium cepa* Seeds

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Abstract

Standardization is the code of conduct in order to ensure the proper identification, authentication and also for the standardization of crude herbal drugs. The quality of herbal drugs is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. Towards authentication and quality assurance of medicinal plants, pharmacognostic, physicochemical studies of *Nigella sativa* and *Allium cepa* seeds were performed. The macroscopic and physicochemical parameters like ash value, loss on drying, foaming index, swelling index, extractive values and fluorescence analysis were carried out as per WHO guidelines. The findings of Pharmacognostic and physicochemical studies can be used as markers in the identification and standardization of *Nigella sativa* and *Allium cepa* seeds as a herbal remedy and also towards monograph development on the plant. Further it assists in validating this raw material for use in herbal formulations in the upcoming era.

1 Introduction

The traditional medicine is widely used for various human ailments. Traditional system of medicines has become significantly more popular all over the globe because of the effective and curative nature for chronic disease with less toxicity. Herbal medicines are not a simple task since many factors influence the biological efficacy and reproducible therapeutic effect. Standardization of herbs is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical, standardization, and *in-vitro, in-vivo* parameters[1,2]. The quality assessment of herbs is of paramount importance in order to justify their acceptability in modern system of medicine.

One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. The task of lying down standard for quality control of herbal crude drug and their formulation involves biological e particular disease area, chemical profiling of the material and lying down specification for the finished product. Therefore, in case of herbal drugs and product, the word “standardization” should encompass entire field of study from cultivation of medicinal plant to its clinical application[3,4]. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compound/s would serve as an additional parameter in assessing the quality of the sample[5].

Nigella sativa (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments.

In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on

regular basis in Tibb-e-Nabwi (Prophetic Medicine). It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders. Extensive studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties, etc. Due to its miraculous power of healing, *Nigella sativa* has got the place among the top ranked evidence based herbal medicines[6-8].

Allium cepa, family: Liliaceae (Alliaceae) is commonly known as garden onion or bulb onion, has been important coniferous plant in ayurvedic and indigenous medicinal systems. Clinical trials and animal research support the use of *Allium cepa* for anti asthmatic, anti diabetic, anti viral, anti thrombotic , hypo cholestremic, anti inflammatory, anti oxidant, aphrodisiacs, cardiotonic, diueretic, expectorant, stimulant, anti cancer, platelet aggregation inhibitor and insecticidal properties. It is also used in osteoporosis treatment. The major biochemical constituents of onion extraction are identified as quercetin, allicin (S-oxodiallyl disulfide), alliin (S-allyl L-cysteine S-oxide), diallyl disulfide (allyl disulfide), S-methyl L-cysteine S-oxide (3-(methyl sulfinyl alanine), propanethial S-oxide (thiopropanal S-oxide) [12] and 3-mercapto-2-methypentan-1-ol[9-12].

Nigella sativa seed, which is well known medicinal herb susceptible to adulteration or substitution due to its great therapeutic value. Adulteration and substitution by morphologically similar seeds are the primary concern in commercially available *Nigella sativa* seed. *Nigella sativa* seeds are adulterated by non-viable and aged *Allium cepa* seeds, because of their high similarity with *Nigella sativa* seeds. In addition, the exhausted *Nigella sativa* seed (seed after the oil extraction) may be adulterated and sold in ground form. Thus, it is advisable to get this seed from a trustworthy supplier.

Despite the medicinal importance of *Nigella sativa* and *Allium cepa* seeds, and its likely danger because of the adulterant, information on the pharmacognostic parameters for identification of the both seeds in whole and powdered form are unavailable.

The present study was aimed to assess macromorphology, pharmacognostic and physiochemical screening of the seeds of *Nigella sativa* and *Allium cepa* towards standardization and monograph development.

2 Materials and Methods

2.1 Collection and identification of plant material

The seeds of *Nigella sativa* and *Allium cepa* were purchased from the local market of Bhopal, Madhya Pradesh, India. The plant material were identified and authenticated by Dr. Shoukat Saeed Khan, Department of Botany, Safia Science College, Bhopal (MP), India. The seeds were reduced to coarse powder and stored in airtight container till further use.

2.2 Morphological examination

The collected seeds were used to study variation in morphological features specially color, shape, size and taste of seeds. For color and shape the seeds were examined under compound binocular microscope (Magnus MLX).

2.3 Pharmacognostic evaluation

Air-dried powdered material was subjected to qualitative physicochemical estimations. The procedures were followed as mentioned earlier.

2.3.1 Ash values

2.3.1.1 Determination of total ash value

Accurately weighed about 3 gms of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

2.3.1.2 Determination of acid insoluble ash value

The ash obtained as directed under total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

2.3.1.3 Determination of water soluble ash value

The total ash obtained was boiled with 25 ml. of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

2.3.1.4 Determination of sulphated ash value

Accurately weighed about 3 gms of air dried powdered drug and moistened with sulphuric acid and thereafter heated gently at a temperature as low as practicable until the sample was thoroughly charred. After cooling the residue was moistened with small amount of sulphuric acid (usually 1 mL), heated gently until white fumes were no longer evolved and ignited. Care was taken to ensure that flames were not produced at any time during the procedure. The crucible was cooled in the desiccators, weighed accurately and percentage residue was calculated.

2.3.2 Loss on drying

About 1.5 gm. of powdered drug was weighed accurately in a tared porcelein dish which was previously dried at 105 °C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

2.3.3 Determination of foaming index

Weighed accurately about 1 g of coarsely powdered seeds and transferred to 500 ml conical flask containing 100 ml of boiling water maintained at moderate boiling at 80- 900 C for about 30 mins. Then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100 ml (V1). Cleaned 10 stopper test tubes were taken and marked with 1 to 10. The successive portions of 1, 2 ml up to 10 ml drug was taken in separate tubes and adjusted remaining volume with the liquid up to 10 ml in each. After closing the tubes with stoppers, Shook them for 15 seconds and allowed to stand for 15 mins then measured the height. If the height of the foam in each tube is less than 1cm, the foaming index is less than 100(not significant).

Here, if the foam is more than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube was the index sought. If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the plant material needs to be measured and transferred to a 100ml volumetric flask (V2) and volume is to be maintained up to 100ml and follow the same procedure. Foaming Index was calculated by using this formula:

Foaming Index = 1000/a in case of V1

Foaming Index = 1000 × 10/a in case of V2

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

2.3.4 Determination of swelling index

It was carried out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed 1g of plant material into a 25 ml glass-stopper measuring cylinder. The internal diameter of the cylinder was about 16 mm, the length of the graduated portion about 125mm, marked in 0.2 ml divisions from 0-25 ml in an upwards direction. 25 ml of water was added and shake the mixture thoroughly every 10 minutes for 1 h. Allowed to stand for 3 h at room temperature. Measured the volume in ml occupied by the plant material, including any sticky mucilage.

2.3.5 Extractive values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means.

2.3.5.1 Determination of alcohol soluble extractive value

5 gms of the air-dried coarse powder of the plant material was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

2.3.5.2 Determination of water soluble extractive value

Weigh accurately the 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug[13-17].

2.3.6 Fluorescence analysis

Fluorescence characteristics of the powdered plant material of *Nigella sativa* and *Allium cepa* were observed in daylight and UV light. Also the fluorescent study was performed on treating the drug powder with different chemical reagents[18,19].

3 Results and Discussions

For the Commercialization of plant based formulations, quality and identity of starting material is utmost essential which can be best established by Pharmacognostical Standardization. Pharmacognostical characters are helpful in confirming the identity and in the determination of purity, quality of crude drug[20]. As per WHO microscopic characters help in establishing identity and purity and hence the present Pharmacognostical research was aimed at establishing the Pharmacognostical standards for *Nigella sativa* and *Allium cepa*.

3.1 Morphological examination

The macroscopic characteristics of *Nigella sativa* and *Allium cepa* are presented in table 1 and table 2, respectively. Such morphological features may be used to identify germplasm tentatively as the same have been found significant for the study of seeds and their properties.

3.2 Pharmacognostical studies

The safety and efficacy of herbal products are dependent upon the standardization of the herbs used. The physicochemical characters of all plant have been done for standardization of crude drug. The physicochemical characters of *Nigella sativa* and *Allium cepa* were studied, and the results are presented in tables 3.

Table 1: Macroscopic characteristics of *Nigella sativa* seeds

Particulars	Observation
Shape	Pear-shaped with slightly curved tapered ends. One side is flat and the other is convex. The surface is slightly and regularly embossed
Colour	Black with hints of light grey
Size (mean of 25 samples)	Length 3.9 cm, Width 2.3 cm
Flavour and evolution of taste	Metallic taste when the seed comes into contact with dental enamel. After crushing, taste of lead pencil, followed by sharp, aromatic peppery taste, becoming irritant at the base of the throat and leaving a very persistent bitterness on the palate.

Table 2: Macroscopic characteristics of *Allium cepa* seeds

Particulars	Observation
Shape	Generally angular; most often with four walls. The seed outline was very variable – elliptic, roundish, obovate, or wide ovate.
Colour	Black with slightly sinning
Size (mean of 25 samples)	Length 2.7 cm, Width 1.6 cm
Flavour and evolution of taste	Slight sweet taste when the seed comes into contact with dental enamel

Table 3: Physical parameters of plant powder

Studied parameters	Observations (% w/w)	
	Nigella sativa	***Allium cepa***
Loss on drying	9.2±1.12	12.6±0.42
Total ash value	5.8±0.23	6.1±0.58
Acid insoluble ash value	1.2±0.18	1.7±0.09
Water soluble ash value	2.6±0.04	2.1±0.15
Sulphated ash value	5.1±0.78	4.8±0.49
Foaming index	Less than 100	Less than 100
Swelling index	0.5±0.03	0.6±0.07
Alcohol extractive value	12.3±0.24	10.8±0.38
Water extractive value	16.2±0.51	12.7±0.29

Values are mean ± SEM of three determinations

The ash value is used for the determination of inorganic materials, such as carbonate, silicates, oxalates, and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. The value obtained for the *Nigella sativa* and *Allium cepa* were around 5.8±0.23% and 6.1±0.58%, respectively as total ash.

The acid insoluble ash determines the acid insoluble material present in the drug material and the water soluble ash determines the water soluble material specifically the water soluble inorganic salts. The acid insoluble values of *Nigella sativa* and *Allium cepa* were 1.2±0.18% and 1.7±0.09%, respectively. The water soluble ash values of *Nigella sativa* and *Allium cepa* were 2.6±0.04% and 2.1±0.15% respectively. The sulphated ash values of *Nigella sativa* and *Allium cepa* were 5.1±0.78% and 4.8±0.49%, respectively. The loss on drying of the *Nigella sativa* and *Allium cepa* powder were 9.2±1.12% and 12.6%, respectively. The swelling index of the *Nigella sativa* and *Allium cepa* powder were 0.5±0.03% and 0.6±0.07%, respectively.

The alcohol extractive values of *Nigella sativa* and *Allium cepa* were 12.3±0.24% and 10.8±0.38%, respectively. The water extractive values of *Nigella sativa* and *Allium cepa* were 16.2±0.51% and 12.7±0.29%, respectively. The findings of extractive values indicate that both plants contains higher amount of highly water soluble phytoconstituents.

The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration.

Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter.

The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash which is insoluble in dilute hydrochloric acid. A higher limit of acid insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some analysts favour mixing of sulphuric acid with the powdered crude drug before ashing and this sulphated ash value is normally less fusible than ordinary ash.

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. The powder drugs exhibit different fluorescence character in the presence of different chemical reagents under ultra-violet light due to presence of different functional groups in drug. The results of fluorescence characteristics of plant are displayed in table 4. The fluorescence analysis is a tool for the qualitative analysis of crude drug.

Table 4: Fluorescence analysis of *Nigella sativa* and *Allium cepa* powder

Chemical Treatment	Day light		UV light	
	Nigella sativa	***Allium cepa***	***Nigella sativa***	***Allium cepa***
Powder as such	Black	Yellow	Black	Yellow
Powder + 1 N HCl	Black	Yellow	Blackish Green	Fluorescence
Powder + aqueous 1 N NaOH	Greenish	Yellowish Green	Dark Green	Fluorescence Yellowish Green
Powder + alcoholic 1 N NaOH	Greenish	Yellowish	Dark Green	Fluorescence Green
Powder + 50% HNO_3	Yellowish orange	Yellowish orange	Dark Green	Green
Powder + 50% H_2SO_4	Yellowish Green	Yellowish orange	Blackish Green	Fluorescence Yellowish Green
Powder + Methanol	Blackish	Yellowish	Blackish Green	Greenish yellow
Powder + Water	Blackish	Yellowish	Yellowish Green	Green

Information's generated in this work are empirical in terms of standardization of the drug and also to fetch the attention of pharmacologist to explore *Nigella sativa* and *Allium cepa* in the line of scientific research.

4 Conclusion

The present work helps in identification and authentication of the *Nigella sativa* and *Allium cepa* seeds. The findings of pharmacognostic and physicochemical act as reference information for correct identification of *Nigella sativa* and *Allium cepa* seeds and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.

5 Conflicts of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

6 Author's contributions

AAR carried out literature review and experimental work of the present study. KJ was responsible for statistical work and calculations in addition to manuscript proofing. Both authors read and approved the final manuscript.

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