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Research Article

PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF TRADITIONAL HERBAL DRUG *NIGELLA SATIVA* SEED

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ABSTRACT

Plants have been used for medicine from time immemorial because they have fitted the immediate personal need and are easily accessible and inexpensive. In recent years more people throughout world are turning to use medicinal plant products in healthcare system. The Indian herbal industry is growing in a tremendous rate. More number of herbal products is arrived in the market. The safety and efficacy of herbal products are dependent upon the standardization of these herbal drugs. Nigella satiua L. (Ranunculaceae) is used as a spice and for the treatment of various diseases. Recent pharmacological investigations of the seed extract revealed that a wide spectrum of biological activities including anti-inflammatory, analgesic, antibacterial, antifungal, anti-helmintic, bronchodilatory, hypertensive and immunoprotecting activities. For standardization and quality assurance, three attributes viz. authenticity, purity and assay are desirable. Authenticity relates to proving that the material is true i.e., it corresponds to the right identity. Physicochemical parameters of N. sativa seed powder were determined and reported as total ash, water-soluble ash and acid-insoluble ash. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content and pH was also determined. The quality control of plant material is a general requirement to be fulfilled. Good quality assurance is necessary when dealing with the plant products. The present study declares that standardization of Nigella sativa seed might be useful to supplement information in regard to its identification parameters and quality control of herbal drugs.

KEYWORDS: *Standardization, Herbal drugs, Medicinal plant, Quality Control, Physicochemical parameters.*

INTRODUCTION

Herbal drugs constitute a major part in all the tradition systems of medicine. Plants have been used for medicine from time immemorial because they have fitted the immediate personal need and are easily accessible and inexpensive. With the passage of time, there has been more demand of herbal medicines due to a general disillusionment with conventional medicine. The desire for a "natural" life style has resulted in an increasing utilization of alternative or complementary therapies with the natural products in general and herbal medicines in particular. The demand is high but there is a shortage of supply of genuine herbal medicines.

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Herbal products represent a number of unique problems when quality control aspects are considered. These are because of the nature of the herbal ingredients present therein, which are complex mixtures of different secondary metabolites that can vary considerably depending on environment and genetic factors. In almost all the traditional systems of medicine, the quality control aspects have been considered from its inception itself by the Rishis and later by the Vaidyas and Hakims. However, in modern concept, it requires necessary changes in their approach. In recent years more people throughout world are turning to use medicinal plant products in healthcare system [1].

For the quality control of traditional medicines, the traditional methods are procured, studied, documented and then the traditional information about identification and quality assessment is interpreted properly in terms of modern assessment. Quality assurance is an integral part of traditional medicine, which ensures that it delivers the required quantity of quality medicament. The finger printing and marker compound analyses are nowadays getting momentum for the standardization of traditional medicinal formulations. Here, the concentration of the secondary metabolites, which are the major constituents of herbal drugs, is studied, which provides valued

scientific standardization procedures. This technique not only helps in establishing the correct botanical identity but also helps in regulating the chemical sanctity of the herbs. For standardization and quality assurance, three attributes viz. authenticity, purity and assay are desirable. Authenticity relates to proving that the material is true i.e., it corresponds to the right identity. Authentication itself involves many parameters including gross morphology, microscopy, chemical analysis etc.

In English, *Nigella sativa* seed is variously called fennel flower, nutmeg flower, Roman coriander, blackseed or black caraway. *Nigella satiua* L. (Ranunculaceae) is a herbaceous plant which grows in Mediterranean countries. It is used as a spice and for the treatment of various diseases [2]. Recent pharmacological investigations of the seed extract revealed that a wide spectrum of biological activities including anti-inflammatory, analgesic, antibacterial, antifungal, anti-helmintic, bronchodilatory, hypertensive and immunoprotecting activities [3]. Thymoquinone is the major active principle of *N. sativa* and constitutes about 30% of its volatile oil or ether extract. Many of the pharmacodynamic effects reported above for *N. sativa* are due to thymoquinone [4]. The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. For the research work on standardization of herbal formulations and neutraceuticals a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance.

MATERIALS AND METHODS

The plant specimens for the study were collected from the local market and were positively identified and authenticated by the National Botanical Research Institute (Council of Scientific and Industrial Research), Rana Pratap Marg, Lucknow. A voucher specimen no. (NBRI-SOP-202), Reference no. (NBRI/CIF/296/2012), dated 10/04/2012.

Standardization Parameters of Nigella Sativa Seeds:

1. Extractive Value:

The amount of an extractive value that obtained from a drug in a particular solvent is often an approximate measure of the amount of certain constituents that the drug contains. The drug should be extracted with different solvents in order of their increasing polarity to get the correct and dependable values. Generally petroleum ether, alcohol and water extractives are taken into consideration for fixing the standard of a drug. The petroleum ether extract contains fixed oil, resins and volatile substances, but when the extract is heated at 105^{0}C until constant weight, the volatile substances are volatilized leaving only resin, coloring matter and fixed oil. Alcohol can dissolve almost all the substances, but is generally used for determining the extractive index for those drugs which contain glycosides, resins, alkaloids etc. Water is used for the drugs containing water-soluble substances as chief constituents. The extractive values were determined according to the method described in Pharmacopoeia [5].

Hot Extraction: The dried and coarsely powdered drug (10 g) was packed in a Soxhlet apparatus and was subjected to extraction with different solvents like ethanol, petroleum ether, chloroform, acetone, methanol and water over 72 hours. The total volume of extract was readjusted with the same solvent to 100 ml. The extract was divided into 4 parts each of 25 ml. Then each part of 25 ml of extract was transferred to a tared bottom dish and was evaporated to dryness on a water bath, then weighed without delay and their constant extractive values with different solvents were calculated.

$$\% \text{ Extractive matter} = \frac{\text{Weight of extractable matter}}{\text{Weight of Drug}} \text{ X } 100$$

2. Ash Values:

Ash value is an important parameter for the purpose of determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating of material causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The ash remained after ignition of medicinal plant materials is estimated by different methods which measure total ash value, water soluble ash value and acid insoluble ash value. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly material. The water soluble ash is used to estimate the amount the amount of inorganic elements [6].

$$\% \text{ Ash content} = \frac{\text{Weight of Ash}}{\text{Weight of extract}} \text{ X } 100$$

a) Total ash: The total ash is designed to measure the total amount of material remaining after ignition. Indian Pharmacopoeia 1996 and WHO prescribes suitable methods for determination of ash values. The extract (1 g) was placed in the tared platinum or silica crucible and was incinerated at a temperature not exceeding 450^{o}C until free from carbon. It is then cooled and weighed to get the total ash content.

b) Acid insoluble ash: Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid (HCl) and igniting the remaining insoluble matter. This gives the amount of silica present in sample, especially as sand and siliceous earth. Ash was boiled with 25 ml of dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450^{o}C to a constant weight.

c) Water soluble ash: Water soluble ash value is the difference in weight between the total ash value and the residue after treatment of total ash with water. It is a good indicator of either previous extraction of water soluble salts in the extracts.

Ash was dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450^{o}C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash is obtained.

3. Determination of pH:

An accurately weighed 1 g of the drug was dissolved in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode [7].

4. Preliminary Phytochemical Screening:

The preliminary phytochemical screening was carried out using plant extracts for their content of different classes of compounds. The various extracts obtained then subjected to

qualitative chemical tests for identification of different plant constituents present in the crude drug [8, 9]. The extracts should be subjected to preliminary phytochemical investigation for detection of following [10].

A. Alkaloids
B. Carbohydrates
C. Cardiac Glycosides
D. Phenolic Compounds
E. Flavonoids
F. Protein
G. Saponins
H. Sterols
I. Resins
J. Lipids/Fats

Test for carbohydrates and glycosides:

A small quantity of the plant extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to the following testes to detect the presence of Carbohydrate and glycosides [11].

(a) Molisch's test: The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H2SO4 was added along the sides of the test tube. Formation of brown ring at the interface of two liquids shows the presence of carbohydrates.

(b) Legal's test: To the hydrolysate 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink color shows the presence of glycosides.

(c) Fehling's test: The filtrate was treated with 1 ml of Fehling's solution A and B and heated on the water bath. A reddish precipitate shows the presence of carbohydrate.

(d) Borntrager's test: Hydrolysate was mixed with chloroform and the chloroform layer is separated. To this layer equal quantity of dilute ammonia solution was added. Ammonia layer becomes pink color, indicating the presence of glycosides.

(e) Baljet test: Hydrolysate was treated with sodium picrate solution. Yellow color shows the presence of cardiac glycosides.

(f) Froth formation test: Hydrolysate was treated with water. Forth formation shows the presence of saponin glycosides.

Test for fixed oils and fats:

(a) Spot test: Small quantity of extract was pressed between two filter papers. Spots of oil stain on the paper indicate the presence of fixed oil.

(b) Saponification test: Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap or neutralization of alkali shows the presence of fixed oils and fats.

Test for proteins and free amino acid:

A Small amount of the extract was dissolved in few ml of distilled water and treated with following reagents.

(a) Millon's test: Appearance of red color shows the presence of proteins and free amino acids.

(b) Ninhydrin reagent: Appearance of purple color shows the presence of proteins and free amino acids.

(c) Biuret test: Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

Test for saponins:

Foam test: The plant extract was diluted with 20 ml of distilled water and shake vigorously in a graduated cylinder for 15 minutes. The formation of foam layer shows the presence of saponins.

Test for phenolic compounds and tannins:

Small quantity of the extract mixed with distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

(a) Dilute ferric chloride solution (5% w/v): Violet color.

(b) 1% solution of gelatin containing 10% sodium chloride: White precipitate.

(c) 10% lead aceatte solution: White precipitate.

Test for phytosterols:

Small quantity of the plant extract was dissolved in 5 ml of chloroform and this chloroform solution was performed to the following tests to detect the presence of phytosteroles.

(a) Libermann-Burchard's test: The above preapared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3 ml of acetic anhydride. Formation of bluish green color indicates the presence of phytosterols.

(b) Salkowski reaction: To 1 ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

Test for Alkaloids:

Small portion of the extract was mixed with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the following tests.

(a) Mayer's reagent: Cream precipitate

(b) Dragendroff's reagent: Orange brown precipitate

(c) Hager's test: Yellow precipitate

(d) Wagner's test: Reddish brown precipitate

Test for flavonoids:

(a) With aqueous NaOH solution: Small quantity of the extract was dissolved in aqueous sodium hydroxide. Appearance of yellow colour shows the presence of flavonoids.

(b) With conc. sulphuric acid: To a small amount of extract, concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

(c) Shinoda's test: Small amount of extract was dissolved in alcohol then pieces of magnesium and concentrated hydrochloric acid was added drop by wise and heated.

Appearance of magenta color indicates positive result for presence of flavonoids.

RESULTS AND DISCUSSION

Macroscopical Study: Seeds were flattened, oblong, angular, rugose tubercular, small, funnel shaped, 0.2 cm long and 0.1 cm wide. It had blackish color, slightly aromatic odor and taste bitter.

Physicochemical evaluations: Physicochemical parameters of *N. sativa* seed powder were determined and reported as total ash, water-soluble ash and acid-insoluble ash. Alcohol soluble and water soluble extractive values were determined. This gave the amount of water and alcohol soluble components. The moisture content and pH was also determined (table 1).

Seed powder of Nigella sativa showed the presence of total ash 4.69 % w/w, acid insoluble ash 0.13 % w/w, water soluble ash 1.65 % w/w, water soluble extractive 11.47 % w/w, alcohol soluble extractive 9.08 % w/w, moisture content- 3.01 % and pH- 6.9.

Preliminary phytochemical Screening:

The extraction with various solvents was done by soxhlet apparatus using seed powder of *N. sativa* (25g). Extracts obtained were concentrated and tested for various chemical tests to detect the presence of different phytoconstituents. Phytochemical testing showed the positive result for steroid in chloroform extract. Alcohol extract gave positive response for alkaloids, glycosides and sugars (table 2).

Table No. 1: Physiochemical Parameters of Nigella Sativa Seeds

Physicochemical parameters	Value Mean
Total Ash	4.69 % w/w
Acid insoluble ash	0.13 % w/w
Water soluble extract	11.47 % w/w
Water soluble ash	1.65 % w/w
Ethyl alcohol soluble extract	9.08 % w/w
Moisture content	3.01 %
Ph	6.9

Table No. 2: Phytochemical Analysis of Nigella Sativa Seeds

Test for constituents	Petroleum ether extract	Chloroform extract	Ethyl alcohol extract
Color &Consistency	Nearly Colourless oily	Very light yellow oil	Yellow gummy
Alkaloid	Negative	Negative	Positive
Glycoside	Negative	Negative	Positive
Steroid	Negative	Positive	Negative
Tannin	Negative	Negative	Negative
Flavonoid	Negative	Negative	Negative
Terpene	Negative	Negative	Negative
Sugars	Negative	Negative	Positive
Saponin	Negative	Negative	Negative

**** Positive: present, Negative: absent***

Total ash was approximately, 36 (thirty six) times and 3 (three) times more than acid insoluble ash and water soluble ash respectively. Ethanol soluble extractive value was less than Water soluble extractive value. Phytochemical investigation of seed powder was found to contain alkaloids, glycosides, steroids and sugars.

The various physical parameters for evaluation of the herbal drugs are important in detecting adulteration or improper handling of drugs. The total ash value indicates us about the presence or absence of foreign inorganic matter such as metallic salts or silica information. The moisture content of the drug is optimal, thus it could not encourage bacterial, fungi or yeast growth, as the general standard of moisture content in crude drug is not more than 14 % w/w. The ash values, extractive values and moisture content of seeds were determined. Pharmacognostic parameters including physico-chemical evaluation is used for quality of herbals, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs. Purity pertains to evaluating that there are no other substances present in the plant material. Purity depends upon the absence of foreign matter, whether organic or inorganic, while quality refers essentially to the amount of the main constituents or components, Based on the concentration and nature of the secondary plant metabolites though, crude drug may conform to all the official standards for quality control [12].

The quality control of herbal products is a general requirement to be fulfilled. Good quality assurance is necessary when dealing with the plant products, intended to be released in market as drug constituents or as test substances in basic pharmacological experiments. Therefore, efforts should be made to obtain and maintain the high quality of these plant products. Quality refers to the inherent value of the drug i.e. the amount of medicinal components or active constituents present. These constituents are classified into groups of non-protoplasmic cell contents. These groups include carbohydrates, glycosides, neutral principles, acids alkaloids, volatile oils, lipids, oleoresins, balsam, steroids, amino acids, hormones etc. Solubility profile, starting from petroleum ether to water, shows the wide range of non-polar and that it may be a good source of

active ingredients for pharmacological evaluation. Ash values, on the other hand, are one of the best physicochemical parameters for the evaluation of plant drug for its purity, quality and strength. To check any adulteration or non-deliberate mixing in the commercial batches specifications must be laid down for each her. To check any adulteration or non-deliberate mixing in the commercial batches specification must be laid down for each herb.

CONCLUSION

The present study on standardization, physicochemical and phytochemical evaluation of *Nigella sativa* seed would be helpful to supplement information in its identification parameters assumed significantly in acceptability of herbal drugs in present scenario of major regulatory laws to control quality of herbal drugs. India has potential to become a major player in the world production of standardized and therapeutically effective ayurvedic herbal formulation. India needs to explore the medicinally important plants for standardization and therapeutic evaluation. The Indian herbal drug industry is growing many folds in recent years. More number of herbal medicinal products is introduced in the market. The efficacy of herbal medicinal products is dependent upon the standardization of these herbal products. The traditional approach towards quality control is insufficient for current herbal market and hence there is need for more advanced techniques for quality control and standardization. The quality of plant product is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. With the advancement in the chemical knowledge of herbal drugs various methods like organoleptic, physical, chemical, spectroscopic and biological methods are used for estimating active constituents present in the herbal drugs. Standardization of plant material methods should take into consideration various aspects contributing to the quality of the herbal drugs. It is also important to study the influencing factors like effect of the climatic conditions, and condition of the storage on the potency of a crude drug or the formulation prepared by herbals using it as a whole or as extract or the constituent isolated. It is also important to standardize excipients and additives incorporated in the formulation along with the main drug constituent.

It is recommended that various government agencies should follow a more universal approach to herbal quality by adopting the WHO guidelines and also developing monographs using the various quality parameters outlined above. Thus standardization will strengthen the regulatory process and quality control.

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