PHARMACOGNOSTIC STUDIES ON TRACHYSPERMUM AMMI LINN. A POWDER ANALYSIS

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ABSTRACT
Ajowan (Trachyspermum ammi, Umbelliferae) is one such plant, having been prescribed for digestive, respiratory, renal, dental, a germicide, antispasmodic, antifungal agent and platelet aggregation inhibitory action, antifungal potency and blood pressure lowering action and many other maladies in Asian traditional medicine. This crude drug powder study was aimed to develop characteristics of powder crude methods in order to assess the quality of herbal drugs for therapeutic value. We have developed a simple scheme for quality and authentication of botanical ingredients. Due to very little literature is available for quality evaluation of crude drug powder. Samples subjected to various microscopical characteristics (botanical characterization), physicochemical analyses and Fluorescence test. The set parameter were found to be sufficient to evaluate crude drug powder and can be used as reference standards for the quality control /quality assurance study.

Keywords: Trachyspermum ammi, Quality test, Physicochemical parameter, Crude drug powder, Microscopy.

INTRODUCTION
Trachyspermum ammi (Umbelliferae), known in India as Ajowan, is widely distributed in northern part of the India1. Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the ayurvedic medicines is the lack of standard quality control profiles. The quality of herbal medicine i.e. the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of plant-based drugs, it is difficult to establish quality control parameters. To overcome these problems modern analytical techniques are expected to help in circumventing this problem2. Between 1999-2001 the ayurvedic pharmacopoeia of India was published in three volumes, which gave the botanical identity of plants, composition, analytical procedures etc. In spite the effort made for the standardization of ayurvedic medicine, major problems remain because the formulary lists only 635 whereas the herbal medicines in actual use are believed to be at least 1000 with many regional variations. The absence post market surveillance and paucity of test laboratory facilities also make the qualities control of ayurvedic medicines exceedingly difficult at this time. Therefore an attempt has been made to analyse crude drug powder of Ajowan (Trachyspermum ammi) used in has been reported to be a germicide, antispasmodic, antifungal agent3 and platelet aggregation inhibitory action4, antifungal potency5 and blood pressure lowering action6.

MATERIALS AND METHODS
Chemical and reagents
The entire chemical used in experiment were of analytical grade. All the solvents used in the experiment were procured from merck specialities Pvt.Ltd, Mumbai, India.

Plant material
Yavani - Trachyspermum ammi (fruit,figure1,2) was procured from the local market of Jeypore, Koraput, Odissa, India and the plant material were authenticated by botanist Mr. S.R. Dash, H.O.D Dept of Botany Vikram Dev College Jeypore, Koraput, Odissa.Voucher specimens (DSNCOP15) of the same have been deposited in the museum, Nova college of pharmacy for future reference.
**Preparation of powder**
Crude drug has taken and roasted in a stainless steel pan at a low temperature till it becomes free from moisture. The sample *Trachyspermum ammi* (fruit) was powdered in a pulverizer and pass through sieve number 80#. It is packed in tightly closed containers to protect from light and moisture.

**Organoleptic Evaluation**
Organoleptic evaluation⁷ (Table1) refers to evaluation of formulation by colour, odour, taste, texture etc. Organoleptic characters of the samples were carried out based on the method as described by siddiqui et al⁹.

**Microscopical study**
Microscopic analysis¹⁰ of *Trachyspermum ammi* powder (figure3) were carried out to by classical pharmacognostical methods. Choral Hydrate Solution: Dissolve 50 g of choral hydrate in 20 ml of distilled water. Glycerin: Pure or diluted as required with one or two volumes of distilled water. Used as a general mountant¹¹.

**PHYISCOCHEMICAL PARAMETERS**
Physicochemical investigations of the drug⁸ were carried out and they include determination of moisture, extractive values and ash values.

**Determination of foreign matter**
Drugs should be free from moulds, insects, animal faecal matter and other contamination such as soil, stones and extraneous material. 100g of the drug sample to be examined was weighed and spread out in a thin layer. The foreign matter⁷ (Table1) was detected by visual inspection, separated, weighed and the percentage present calculated.

**Determination loss on drying**
It is important that the portion taken was large enough to be a representative for the sample. About 10g of accurately weighed drug was dried at 105 °C for 5hours, and then weighed again. Percentage was calculated with reference to initial weight (Table1).

**Determination of pH**
Determination of pH (Table1) of crude powder¹² in 1% w/v and 10% w/v in water at 24°C was done.

**Determination of total ash**
The determination of total ash¹³ (Table1) is a method to measure the amount of the inorganic residual substance when the sample is ignited. Total ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and non physiological ash (ash from extraneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material was placed in a suitable tared crucible of silica previously ignited and weighed. The powdered drug was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash.

**Determination of acid insoluble ash**
The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid; the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash (Table1) with reference to the air-dried drug was calculated.

**Determination of solvent extractive values**

**Alcohol soluble extractive**
5g of coarsely powdered air-dried drug was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive¹³ (Table1) was calculated with reference to the air-dried drug & is represented as %.

**Water soluble extractive**
5g of coarsely powdered air-dried drug was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive (Table1) was calculated with reference to the air-dried drug & is represented as %.

**Fluorescence test**
The powders of different plants of the formulation were examined under visible and UV light. Various powders were treated with different reagents as described by the Chase and Pratt (1949) one mg of powdered drug was placed on a micro slide and observed under UV 366, UV 254 and in daylight to observe the fluorescent⁷ characteristics of the powder, if any. One mg of the powdered drug was placed on a micro slide and treated with 1N HCl and observed under UV 366, UV 254 and in daylight while wet. One mg of the powdered drug was placed on a micro slide and treated with 1N NaOH in methanol and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug...
Determination of physical characteristics

Bulk density and Tap density

The term bulk density (Table 1) refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density (Db) is

\[ \text{Db} = \frac{M}{V_b} \]

Where M is the mass of the particles and Vb is the total volume of the packing.

The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device that has a specially cut rotating can. 100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density.

Angle of Repose

Angle of Repose has been used as an indirect method of quantifying powder flowability; because of its relationship with interparticle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties (Table 1).

Method: The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder or granulation was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. Thus, with R being the radius of the conical pile \( \tan \alpha = \frac{H}{R} \) or \( \alpha = \arctan \frac{H}{R} \), Where \( \alpha \) is the angle of repose.

Hausner ratio

It is related to interparticle friction and as such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres, have a ratio of approximately 1.2, whereas more cohesive, less Flowable powders such as flakes have a Hausner ratio greater than 1.6 (Table 1). The equation for measuring the Hausner ratio is: \( D_f / D_o \) Where, \( D_f = \) Tapped density, \( D_o = \) Bulk density

Carr’s index

Another indirect method of measuring the powder flow from bulk density is Carr’s index (Table 1). The equation for measuring Carr’s index is % compressibility = \( \frac{D_f-D_o}{D_f} \times 100 \) / Df Where, \( D_f = \) Tapped density, \( D_o = \) Bulk density

RESULTS AND DISCUSSION

In The present study microscopic examination, physicochemical, phytochemical studies were performed. The Trachyspermum ammi (fruit) studied for the presence of foreign matter is mentioned in Table 1. The foreign matter was removed and the powder was prepared. A part of the pure powder was kept aside to study the various parameters. Quality test for crude drug powder was performed for moisture content, ash content, water soluble extractive, methanol soluble extractive, acid insoluble ash and water insoluble ash were found to be within standard range. The powder has brown in colour, possessing pungent/salty taste, having characteristic odour, smooth powder. It was observed that more than 50% of samples passed through 80 mesh sieve. The Microscopic observation of the fruit powder shows the following structures in figure 3. The following structures were observed: Endosperm, Vittae, Vascular strand, unicellular warty trichomes and striated cuticle in surface view, Endodermis, Striated cuticle. pH of drug powder was acidic. Extractive values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other method. Further, these values indicate the nature of the constituents present in the drug and the most effective solvent to be used for the preparation of its extract. The percentage alcohol soluble extractive and percentage water soluble extractive are reported in table 1. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. Total ash reflects the care taken in its preparation as all traces of organic matter are removed in ashing and usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. A higher limit of acid insoluble ash reflects the cases where silica may be present or when the calcium oxalate content of the drug is very high. Ash values of the drug powder was
studied and mentioned in table 1. The ash values of the sample were carried out based on the method as described by world health organization (WHO) guidelines for medicinal plant materials. Phytochemical tests performed using standard test procedure with specific reagents, results of the phytochemical investigation indicates the presence of glycosides, fixed oils, steroids, terpenes which shown in table 2. In the table 3 the fluorescent analysis of powder drug has been reported. Behavior of the drug powder with different chemical reagent and solvent: the crude drug powder was subjected to reaction with 10 different chemical reagent and color changes were observed in table 3. Observation under daylight and UV: The crude drug powder as such and with different were observed under daylight and UV (366 nm) for the fluorescence analysis. Powder shown different colour changes were observed in table 3. The physical characteristics of the crude drug powder (average values along with standard deviation) are shown in table 1. The flowability of the formulation was found to be poor in powder, which was further confirmed by high values of Angle of repose, Hausner ratio and Carr’s index. Microscopical characters and physicochemical profile have been suggested to play important role in establishing the authenticity of various ingredients used in any particular formulation, the behaviour of the drug powder with different chemical reagent will also be helpful in characterization of the crude drug.

CONCLUSION
Pharmacognostical characters established for the raw material could be employed as Q.C. standards for evaluating its identity and can be used for routine analysis. Purity and potency of the raw materials following the procedure given could be performed in QC/QA laboratory of the pharmaceutical house.

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12. Anonymous, Indian Pharmacopoeia, (Govt. of India, New Delhi, 1966).
### Table 1: Organoleptic and Physicochemical characteristics of the drug powder (*Trachyspermum ammi*)

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>Trachyspermum ammi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Powder</td>
</tr>
<tr>
<td>Colour</td>
<td>Light brown</td>
</tr>
<tr>
<td>Taste</td>
<td>Pleasant</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Foreign matter (% w/w)</td>
<td>2.4</td>
</tr>
<tr>
<td>Loss on drying (% w/w) mean (n=3)± SD</td>
<td>4.7±0.29</td>
</tr>
<tr>
<td>pH of 1% w/v solution mean (n=3)± SD</td>
<td>3.23±0.09</td>
</tr>
<tr>
<td>pH of 10% w/v solution mean (n=3)± SD</td>
<td>3.35±0.317</td>
</tr>
<tr>
<td>Total ash (%) mean (n=6)± SD</td>
<td>8.6±0.29</td>
</tr>
<tr>
<td>Acid-insoluble ash (%) mean (n=6)± SD</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>Water-soluble extractive mean (n=6)± SD</td>
<td>42±0.32</td>
</tr>
<tr>
<td>Alcohol-soluble extractive mean (n=6)± SD</td>
<td>17.9±0.80</td>
</tr>
<tr>
<td>Tap density mean (n=3)± SD</td>
<td>0.45±0.005</td>
</tr>
<tr>
<td>Bulk density mean (n=3)± SD</td>
<td>0.32±0.015</td>
</tr>
<tr>
<td>Angle of repose mean (n=3)± SD</td>
<td>50±0.1126</td>
</tr>
<tr>
<td>Hausner ratio mean (n=3)± SD</td>
<td>1.28±0.0611</td>
</tr>
<tr>
<td>Carr’s index mean (n=3)± SD</td>
<td>27.39±1.724</td>
</tr>
</tbody>
</table>

### Table 2: Results of phytochemical investigation for phytoconstituents present in crude drug powder (*Trachyspermum ammi*)

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>Tannin</th>
<th>Alkaloids</th>
<th>Glycosides</th>
<th>Fixed oils</th>
<th>Gums/Resins</th>
<th>Mucilage</th>
<th>Protein</th>
<th>Steroids</th>
<th>Terpenes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. ammi</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) = present, (-) = absent

### Table 3: Fluorescent analysis and behaviour of the drug powder (*Trachyspermum ammi*) with different chemical reagents/solvents

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Material</th>
<th><em>Trachyspermum ammi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>L, Br.</td>
</tr>
<tr>
<td>2</td>
<td>In NaOH (1N) in H₂O</td>
<td>YB, G.Y.</td>
</tr>
<tr>
<td>3</td>
<td>P + In HCl (1N)</td>
<td>BR</td>
</tr>
<tr>
<td>4</td>
<td>P + In NaOH (1N) in MeOH</td>
<td>YB, BR</td>
</tr>
<tr>
<td>5</td>
<td>P + 50% KOH</td>
<td>YB, G.Y.</td>
</tr>
<tr>
<td>6</td>
<td>P + 50% H₂SO₄</td>
<td>BR</td>
</tr>
<tr>
<td>7</td>
<td>P + 50% HNO₃</td>
<td>BR, Y.</td>
</tr>
<tr>
<td>8</td>
<td>P + Conc. HNO₃</td>
<td>ReB</td>
</tr>
<tr>
<td>9</td>
<td>P + Conc. H₂SO₄</td>
<td>BR</td>
</tr>
<tr>
<td>10</td>
<td>P + Iodine in water</td>
<td>BL, BR, FB</td>
</tr>
</tbody>
</table>

Figure 1. *Trachyspermum ammi* (fruit)

Figure 2. *Trachyspermum ammi* fruit

Figure 3. *Trachyspermum ammi* (fruit) powder microscopy
(a) Endosperm, (b) Vittae, (c) Vascular strand, (d) unicellular warty trichomes, (e) striated cuticle in surface view, (f) Endodermis, (g) Striated cuticle.

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