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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 asses 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.

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INTRODUCTION

Trachyspermum ammi (Umbelliferae), known in India as Ajowan, is widely distributed in northern part of the India[1]. 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 problem[2]. Between 1999-2001 the ayurvedic pharmacopeia 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 agent[3] and platelet aggregation inhibitory action[4], antifungal potency [5] and blood pressure lowering action[6].

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 evaluation[7] (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 al[9].

Microscopical study

Microscopic analysis[10] of *Trachyspermum ammi* powder (figure3) were carried out to by classical pharmacognostical methods. Chloral Hydrate Solution: Dissolve 50 g of chloral 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 mountant[11].

PHYSICOCHEMICAL PARAMETERS

Physicochemical investigations of the drug[8] 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 matter[7] (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 powder[12] in 1% w/v and 10% w/v in water at24^{0}C was done.

Determination of total ash

The determination of total ash[13] (Table1) is a method to measure the amount of the inorganic residual substance when the drug sample is ignited. Total ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and non physiological ash (ash from extrageneous 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 extractive[13] (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 fluorescent[7] 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 and the slide observed after a few minutes in daylight, under UV 254 and UV 366.One mg of the powdered drug was placed on a micro slide and treated with 1 N NaOH in methanol and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug

was placed on a micro slide and treated with 50% KOH and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with 50% H_2SO_4 and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with Conc. H_2SO_4 and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with 50% HNO_3 and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with conc. HNO_3 and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with iodine water and observed under UV 366 and UV 254 and in daylight while still wet.

Determination of physical characteristics

Bulk density and Tap density

The term bulk density (Table1) refers to a measure used to describe a packing of particles or granules. The equation[14, 15] for determining bulk density (Db) is Db=M/Vb, 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 (Table1).

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 α = H/ R or α = arc tan H/R, Where α 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 (Table1). The equation for measuring the Hausner ratio is: Df / Do Where, Df = Tapped density, Do = Bulk density

Carr's index

Another indirect method of measuring the powder flow from bulk density is Carr's index (Table1). The equation for measuring Carr's index is% compressibility = Df-Do X 100/ Df_Where, Df = Tapped density, Do = 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[16]. 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 figure3. 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 table1. The ash values[17] 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 table2. 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 table3. 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 table3. The physical characteristics of the crude drug powder (average values along with standard deviation) are shown in table1.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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Table.1-Organoleptic and Physicochemical characteristics of the drug powder (*Trachyspermum ammi)*

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

Table.2- Results of phytochemical investigation for phytocontituents present in crude drug powder (*Trachyspermum ammi)*

Test Sample	Tannin	Alkaloids	Glycosides	Fixed oils	Gums/ Resins	Mucilage	Protein	Steroids	Terp-enes
T. ammi	-	-	+	+	-	-	-	+	+

(+) = present, (-) = absent

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

Sr. No.	Material	*Trachyspermum ammi*		
		Day light	UV254 nm	UV 366 nm
1.	Powder as such	L.Br.	BR.	F.Y.
2.	In NaOH(1N) in H_2O	YB.	G.Y.	Y.
3.	P + In HCl (1N)	BR	G.Y.	BR
4.	P + In NaOH (1N) in MeOH	YB	BR.	PG
5.	P + 50% KOH	YB	G.Y.	FB
6.	P + 50% H_2SO_4	BR	Y.	LG
7.	P + 50% HNO_3	BR	Y.	FB
8.	P + Conc. HNO_3	ReB	Y.	FB
9.	P+ Conc. H_2SO_4	BR	G.Y.	LG
10.	P + Iodine in water	BL	BR.	FB

BL: Black, BR: brown, PY: Pale yellow,Y: yellow, G: Green, LG: Light green, LY: Light yellow, GY: Greyish yellow, LBr: Light brown, FY: Fluorescencent yellow. F.B.: Fluorescencent blue, , Re: red, YB:Yellowish brown, ReB:Reddish brown, ReY:Reddish yellow, PG:Pale green, P:Powder.

Figure1. *Trachyspermum ammi* (fruit)

Figure2. *Trachyspermum*[18] *ammi* (fruit

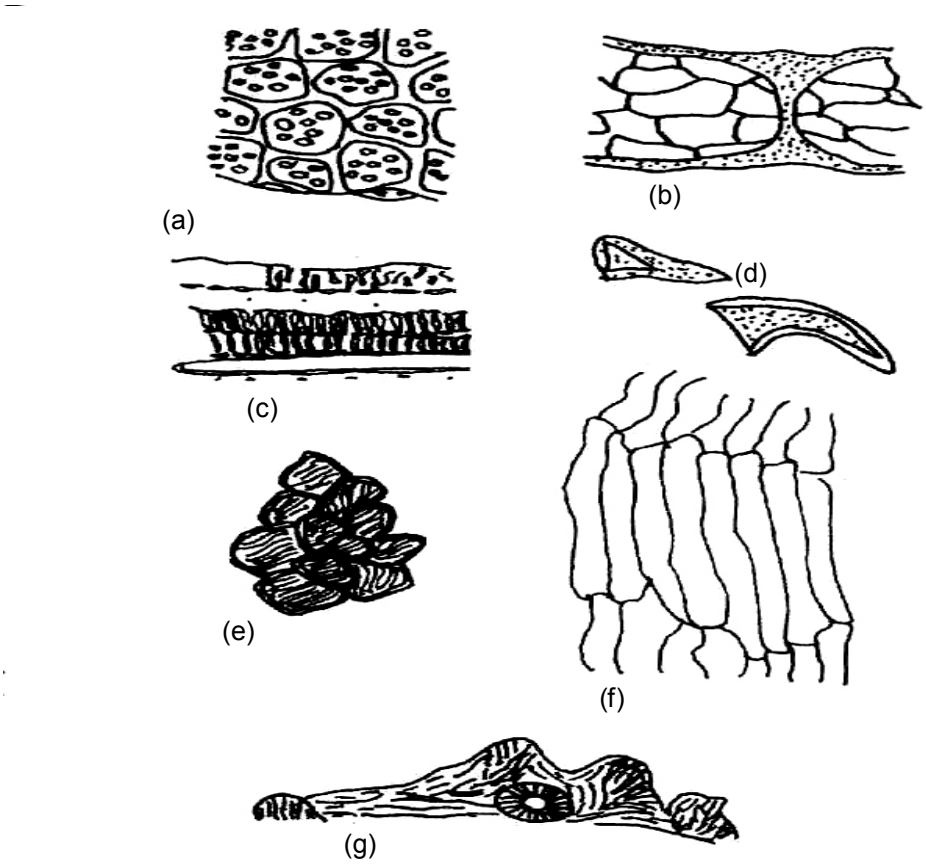

Figure3. *Trachyspermum ammi* (fruit) powder microscopy
(a) Endosperm, (b) Vittae, (c) Vascular strand, (d) unicellular warty trichomes, (e) striated cuticle in surface view, (f) Endodermis, (g) Striated cuticle.

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