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Pharmacognostic and Phytochemical Investigation of *Camellia sinensis* and *Trachyspermum ammi*



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HUMAN

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ABSTRACT

The present study deals with the pharmacognostical and phytochemical screening of ajwain seed (*Trachyspermum ammi*) and green tea leaves (*Camellia Sinensis*) like morphological and phytochemical for its benefits relating to the health problem. For the investigation, organic ajwain seed and green tea leaves was collected from local market and both the sample was extracted with ethanol in a soxhlet extractor. Extract of *Trachyspermum Ammi* was light brown in colour, slightly acrid odour, Nonaromatic taste and powdered microscopy shows presence of endosperm cells, stone cells, parenchymatous cells, protein bodies etc and is found to be of as per API. Extract of *Camellia Sinensis* was Dark green in colour, shooting characteristic odour, bitter astringent taste. The observations obtained after investigation will enable to standardize the botanical individuality of the drug from its crude. The standardization of herbal medicines is completely essential when it serve as a carrier for health benefits.

INTRODUCTION

The use of herbs as a curable and preventable medicine for various illness is as old as human civilization. According to the knowledge based on the traditional, use of medicinal herbs or plants, still play a critical purpose to deal the basic health benefits in the society. Over the last decade, because of the adverse effect associated with the used of synthetic drug, boulevards have been exposed for herbal medicine because on the consideration of scientific research it is found that the green medicine or herbal medicine is safe as comparative to synthetic drug. Some plants have the medicinal ability to synthesize a chemical constituents or substances that delivered a biological effects on human health, which is beneficial for us. Due to the present of some important bioactive chemical constituents of plants, which are mainly responsible for exerts pharmacological affect are glycoside, alkaloids, tannin, flavonoid and phenolic compounds etc. afterward herbs or crude drug is main elements for the manufacturing of various medicinal preparation so precise standardization of plant material screening is very important of its study. The standardization process can be reached by following stepwise phytochemical screening. *Trachyspermum ammi* commonly known as ajwain and *Camellia Sinensis* also known as green tea are one of the important forms of the family Apiaceae and Theaceae. Both the plant are have a various traditional used like stimulant, carminative, antiobesity, abdominal tumors, piles, bronchial problem, asthma etc. The main purpose of the phytochemical investigation was that the testing of plant extracts for their substance of different classes of chemical compounds. ^[1,2,3] Preliminary screening of phytochemical for various types of chemical constituents present in the extract of plant should be followed by quantitative and qualitative chemical tests^[4]. To contribute general estimate or idea about the nature of chemical constituents that is mostly commonly present in the crude sample.

The present investigation deals with the pharmacognostic and phytochemical investigation of the leaf of *Camellia Sinensis* and the fruits of *Trachyspermum ammi* was done in a taxonomic way by extending pharmacognostical evaluation which include organoleptic evaluation, microscopic evaluation, thin layer chromatography phytochemical investigation include all the test for secondary metabolite, and physicochemical investigation include LOD (loss on drying), total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive to absolve its prophylactic use as a therapeutic agent.

MATERIALS AND METHODS

Collection and authentication of plant materials:

The fruits of *Trachyspermum ammi* and the leaves of *Camellia Sinensis* were collected from the lower Assam, District-Udalguri. The botanical identity of the plant specimen of *Camellia Sinensis* and *Trachyspermum Ammi* were confirmed by a taxonomist at Department of Botany, Gauhati University, Guwahati, Assam. The samples were authenticated to be *Camellia Sinensis* (L), family: Theaceae With accession no 18671 and *Trachyspermum Ammi* (L), family: Apiaceae. With accession no 18670.

Methods

Preparation of Extracts:

500gm of powdered drug of *Camellia Sinensis* (green tea) was extracted by using the solvents ethanol respectively by using a continuous hot percolation process or Soxhletation^[5]. After the completion of the process, each solvent extract was then condensed by purifying off the solvent under reduced pressure. After completion of extraction the crude extract was collected and weighed and the percentage yield of ethanol extract 6%. The obtained crude extract was then stored at 4°C for further used. Similarly, 500gm of the dried crude powder of the *Trachyspermum Ammi* (ajwain) was extracted with ethanol by using the same procedure which is used for extraction of green tea for 72 hrs. After that, the menstrum was filtered and dried on water bath. The percentage yield of the ethanolic extract of *Trachyspermum Ammi* was 8 % w/w^[6,7].

Pharmacognostical studies

Organoleptic screening

Different organoleptic parameters of the collected plant materials were screened for primary cognostical evaluation. The various parameters are like color, size, shape, odor, and taste of the *Camellia Sinensis* leaves and fruits of *Trachyspermum ammi*. It admit it the conclusions from studies ensued due to effect of sense organs^[8, 9,10,11].

Macroscopic characteristics

For Macroscopical Studies, morphological characters of the leaf and fruits such as colour, shape, base, apex, margin were characterized as Per the Trease and Evans (1983)^[12] and Wallis (1985). The macroscopic study of *Trachyspermum ammi* and *Camellia Sinensis* were depicted in Figure 1 and Figure 2.

Microscopical Investigation:

The preliminary microscopical screening of *Camellia sinensis* was determined by using the transverse section of fresh leaf. The sample was first treated with chloral hydrate solution and different staining reagents and chemicals were used to detect the lignified cells in the cross sections^[13]. The section was mounted on slides and studied under Trinocular Research Microscope [Figure 3] and the powder Microscopical investigation of *T. Ammi* was accomplished by using standard procedure (Johansen D A, 1940) ^[14]. In this method, hand sections of the seeds was used and filtered with safranin and climbed in glycerine. The drug powder was then treated with phloroglucinol + HCL and Jeffrey's reagent for the purpose of clearing the tissues. Studied using with the microscope [Figure 4].

Qualitative Phytochemical Screening

The qualitative phytochemical screening of extract of both the samples *Trachyspermum ammi* and *Camellia sinensis* were performed by using the standard methods, which are described in the Indian Pharmacopoeia ^[15]. The ethanolic extracts of *Trachyspermum Ammi* and *Camellia sinensis* were studied for various phytochemicals properties like Phenols, alkaloids, carbohydrates, flavonoids, glycosides, Steroids tannins and terpenoids by using precipitation and coloration reactions^[16].

Quantitative Phytochemical Screening

The quantitative phytochemical screening is also done for both the sample *Trachyspermum ammi* and *Camellia sinensis*. For the quantitative estimation various processes like Thin layer chromatography, Loss on drying, P^H value, total ash value, acid insoluble ash, extractive values were determined by following the methods, which are described in the Indian Pharmacopoeia ^[15].

RESULTS AND DISCUSSION

Organoleptic evaluation

Various organoleptic parameters of the plant species, such as color, odour, touch and taste were described in Table no. 1.

Table No. 1: Organoleptic study results

CHARACTERS	<i>Camellia sinensis</i> (Green tea)		<i>Trachyspermum Ammi</i> (Ajwain)	
	Fresh leaves	Dried leaves	Fresh fruits	Dried fruits
Colour	Green	Greyish black	Olive green	Light brown
Odour	Soothing characteristic	Characteristic	Characteristic	Slightly acrid
Taste	Bitter astringent	Bitter astringent	Aeromatic	Non aeromatic
Touch	Slightly Rough	Rough	Soft	Rough

Macroscopic characteristics

In the study of macroscopical characters of the leaves of *Camellia sinensis*(L.) and the fruits of *Trachyspermum Ammi* (L.) showed in Figure 1 and Figure 2 and the macroscopic parameters are described in Table no. 2.



Figure No. 1: Leaves of green tea



Figure No. 2: Fruits of ajwain

Table No. 2: The macroscopic study of fresh leaves *Camellia sinensis* and fruits of *Trachyspermum Ammi*

CHARACTERS	<i>Camellia Sinensis</i> (Green tea)	<i>Trachyspermum Ammi</i> (Ajwain)
Colour	Dark green	Pale brown
Shape	symmetric, Longley oblong or oblong	symmetric, Longley oblong or oblong
Base	Rounded or cuneate	Rounded or cuneate
Margin	Regular	Regular

Microscopical Characteristics

Leaf microscopy of the respective sample like leaves of *Camellia sinensis* (L.) was presented in [Figure3], which shows the presence of anomocytic stomata located on the abaxial surface [Figure 3.1], typical anatomical markers [3.2] and presence of unicellular and thick trichomes [Figure 3.3] of the species as per the references and confirming the identity of the plant material and the powder microscopy of the sample of *Trachyspermum Ammi* presented [Figure 4] shows the presence of endosperm cells.



Figure No. 3.1: Anomocytic stomata



Figure No. 3.2: Typical anatomical mark fragment

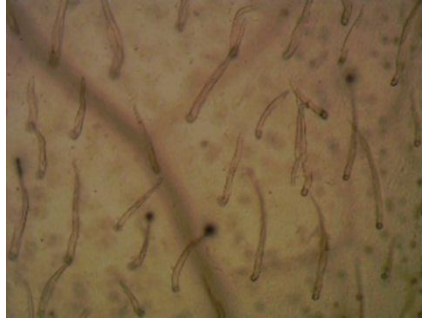


Figure No. 3.3: Unicellular and thick trichomes



Figure No. 4: Endosperm cells

Physicochemical Investigation

The various results of qualitative chemical tests for extracts of *Camellia sinensis* and *Trachyspermum Ammi* were mentioned in Table no. 3 and Table no. 4 respectively.

Table No. 3: Qualitative chemical tests for *Camellia sinensis*

Sl. no	Chemical test	Results	
1.	Tests for Alkaloids	Mayer's Test	Present
		Wagner's Test	Present
		Hager's Test	Present
		Dragendroff's Test	Present
2.	Tests for Glycosides	Borntrager's Test	Absent
		Legal's Test	Absent
		Keller Killiani test	Absent
		Legal's test	Absent
3.	Test for Phenols	Ferric chloride test	Present
4.	Test for Flavonoids		Present
5.	Test for Steroids	Salkowski test	Present
6.	Test for Tannins	Lead acetate test	Present
		Shinoda test	Present
7.	Test for Terpenoids	Liebermann - Burchard's	Absent
8.	Test for carbohydrate	Molisch's test	Absent
9.	Test for reducing sugar	Fehling's test	Present
10.	Test for protein	Copper sulphate test	Present
11.	Test for amino acid	Ninhydrin	Absent

Table No. 4: Qualitative chemical tests for *Trachyspermum Ammi*

Sl no	Chemical test	Results	
1.	Tests for Alkaloids	Mayer's Test	Present
		Wagner's Test	Present
		Hager's Test	Present
		Dragendroff's Test	Present
2.	Tests for Glycosides	Borntrager's Test	Present
		Legal's Test	Present
		Keller Killiani test	Present
		Legal's test	Present
3.	Test for Phenols	Ferric chloride test	Present
4.	Test for Flavanoids		Present
5.	Test for Steroids	Salkowski test	Present
6.	Test for Tannins	Lead acetate test	Present
		Shinoda test	Present
7.	Test for Terpenoids	Libermann - Burchard's	Present
8.	Test for carbohydrate	Molisch's test	Present
9.	Test for reducing sugar	Fehling's test	Present
10.	Test for protein	Copper sulphate test	Absent
11.	Test for amino acid	Ninhydrin	Absent

Determination of Ash Values

In the determination of ash value, it was found that the total ash values, acid insoluble ash, water soluble ash, and sulphated ash showed a higher percentage value in green tea leaves as compared to ajwain fruits, which are described in Table no. 5.

Table No. 5: Ash value of *Camellia sinensis* and *Trachyspermum Ammi*

Ash	<i>Camellia sinensis</i> (green tea)	<i>Trachyspermum ammi</i> (Ajwain)
Total ash	4.30%	4.80%
Acid insoluble ash	0.80%	0.94%
Water soluble ash	41.20%	11.20%
Sulphated ash	50 %	21.34 %

Determination of Loss on Drying

The parameter loss of drying (LOD) was found to be 7.90% for *Camellia sinensis* and 7.14% for *Trachyspermum ammi*, which indicating that the presence of moisture or any other volatile compound is more in the dry powder of green tea as compare to ajwain, represented in Table no. 6.

Table No. 6: LOD of *Camellia sinensis* and *Trachyspermum Ammi*

LOD	<i>Camellia sinensis</i> (green tea)	<i>Trachyspermum ammi</i> (Ajwain)
Loss on drying	7.90%	7.14%

Determination of Extractive Values:

The extractive values of the dry powder of the leaves and fruits of the *Camellia sinensis* and *Trachyspermum ammi* exhibited the higher in alcoholic and water extractive content in green tea in compared to ajwain.

**Table No. 7: Extractive value of *Camellia sinensis* and *Trachyspermum Ammi*
Chromatography Study of the extracts**

Extractive values	<i>Camellia sinensis</i> (green tea)	<i>Trachyspermum Ammi</i> (Ajwain)
Alcohol Soluble Extractive	41.20%	11.20%
Water Soluble Extractive	50 %	21.34 %

Thin layer chromatography

TLC was performed for the both the extracts of fruits of *T. Ammi* and leaves *C.sinensis*. Four major spots were identified in toluene and ethyl acetate at (9:1) & R_f value were determined, which are shown in Table no. 8 and in the Hexane: Diethyl ether: Acetone (5:4:2) solvent system shown three spot & R_f value were determined, shown in Table no. 9.

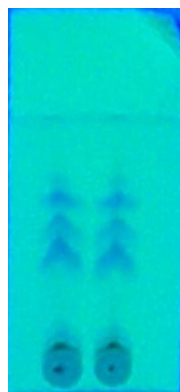


Figure No. 5: The separation of crude extract of green tea by TLC using silica gel GF254

Table No. 8: Solvent system and R_f values of green tea.

Extract	Solvent System	No of Spot	R_f Value
Ethanol	Hexane: Diethyl ether: Acetone (5:4:2)	A	0.93
		B	0.59
		C	0.41
		D	0.35

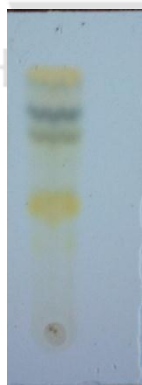


Figure No. 6: The separation of crude extract of ajwain by TLC

Table No. 9: Solvent system and R_f values of ajwain

Extract	Solvent System	No of Spot	R_f Value
Ethanol	Toluene and ethyl acetate at (9:1)	A	0.76
		B	0.58
		C	0.47
		D	0.41

CONCLUSION

The basic objective of this present work is to complete pharmacognostical and phytochemical investigation of the leaves of *Camellia sinensis* (L.) and fruits of *Trachyspermum ammi* (L.). Physicochemical properties such as Thin layer chromatography, Loss on drying, P^H value, total ash value, acid insoluble ash, extractive value and preliminary phytochemical investigation were very important parameters for standardizations of the drug. The present study also revealed that the result of macroscopic and microscopic characterization of the anatomical structures of plant metabolite storage.

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