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The Structural Analysis Of Plant Constituents Of Trachyspermum Ammi

Dr. Suman Lata Pandey

Assistant Professor, Department of Chemistry, DAV (PG) College, Dehradun, India

Abstract – India, with a rich history & legacy of Ayurveda, has been using this ancient repository of plants and their extracts for their medicinal properties. The objective of this study is to study the structural constituents of Trachyspermum ammi seeds for their potential medicinal applications. T. ammi is a native of Egypt and has been cultivated in different regions in South & West Asia such as Iraq, Afghanistan, and India.

The results of our analysis showed the presence of flavonoids, saponins, alkaloids, phenols, glycosides, & carbohydrates in T. ammi. Further four compounds were isolated, and their structural analysis revealed the presence of flavones & flavanones.

These constituents have been known to show different medicinal properties for humans and their presence clearly indicates that extracts from T. ammi can be leveraged for them.

Keywords: Trachyspermum ammi; plant extracts; phytochemicals; medicinal plants

1. Introduction

The history of medicine in India can be traced as back as to vedic times. In Rigveda (4500 B.C. to 1600 B.C.) perhaps the oldest repository of human knowledge and Atharvaveda, we find the mention of different types of medicines prepared from the extracts of different plants. In other works of later vedic period particularly in Ayurveda, the proportion of various drugs have been given in detail. Susruta – Samhita written before 1000 B.C. Contains a comprehensive chapter on therapeutics and Charak – Samhita of the same period gives a remarkable description of the materia–medica as it was known to Ancient Hindus.

Plant chemistry is an important branch of chemistry which deals with the isolation of organic constituents in pure form and their studies from structural and physiological point of view. The chemistry of plant products has now taken a new shape depending upon the recent highly developed techniques like chromatography, infrared, ultraviolet, nuclear electron spin resonance spectroscopies, mass spectroscopy, polarography, and counter current separation. The great diversity and complexity of the chemical nature of natural products & their presence sometimes in very poor amount in plants had been a major obstacle in the progress of phytochemistry. With the availability of modern physio-chemical techniques, as described above, it has become possible for an organic chemist to investigate successfully various biologically active compounds occurring in very small amount.

Medicinal properties of plants depend upon the presence of one or more physiologically active compounds because in addition to physiologically active constituents, it might also contain some toxic substances injurious to the body. It therefore becomes necessary to isolate the physiologically active principles from plants in pure form and to study their exact composition and structure by chemical examination and then subject them to physiological tests.

The Plant *Trachyspermum Ammi* belongs to the natural order Linneaceae, commonly known as "Ajowan". It is an annual herbaceous plant bearing greyish brown seeds. It is grown in Iran, Egypt, Afghanistan, and chiefly in India. Ajowan seeds are used in small quantities for flavouring numerous foods, as anti-oxidants, as preservative in medicine or for manufacture of essential oil for ultimate use in perfumery, essences and in medicine, etc. Oil of Ajowan is an almost colourless to brown liquid possessing characteristic odour and a sharp burning taste.

2. Materials & Methodology

Ajwain (*T. ammi*) was purchased from the local market in Dehradun, Uttarakhand. The seeds were dried in an oven and powdered in an electric grinder. The plant morphology was determined through simple physical observation and the shape, size, color, and odor were noted.

Dried fruits of *Trachyspermum Ammi* were first extracted with petroleum ether (60-80) by refluxing it in a round bottomed flask 6 hours daily for 4 days over an electric bath in order to defat it. The extract was filtered off. The plant material after extraction with petroleum ether was further extracted with ethanol in a flask under reflux 8 hours daily for 15 days over an electric water bath. The extract was filtered while hot and was kept overnight at room temperature. A light brown deposit settled down at the bottom of the flask. This deposit was filtered off and was kept aside for further analysis. The filtrate obtained after the separation of light brown deposit was concentrated to half of its volume and was kept in a refrigerator for 2 days. A similar light brown deposit appeared which was filtered and mixed with first deposit.

The filtrate obtained, after the separation of the deposit in the previous operations, was concentrated to half its volume and poured into excess of distilled water with continuous stirring using a mechanical stirrer. After a few hours, the resulting precipitate was filtered. Thus three portions – the light brown coloured water insoluble deposit, brown coloured water insoluble portion and a water soluble portion were obtained. They were handled separately.

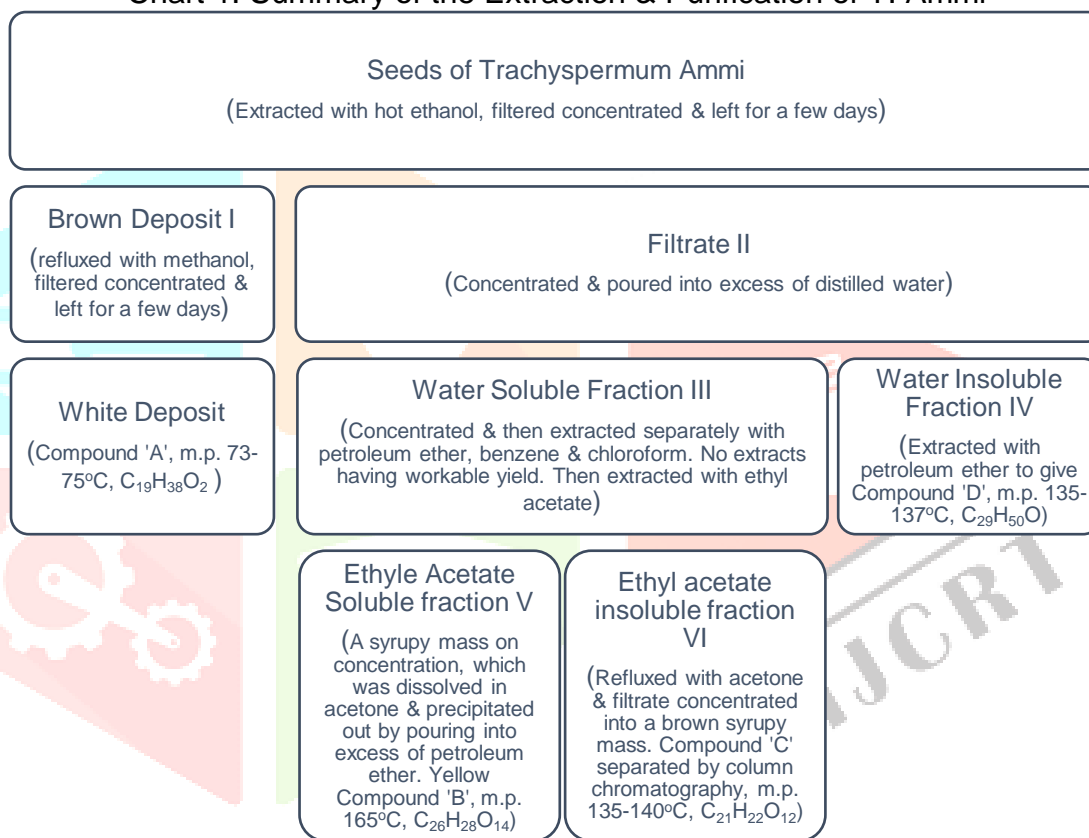
The light brown colored deposit from the ethanolic extract of the plant was subjected to further purification by refluxing it with methanol. Only a small portion of the deposit was found soluble. The rest on examination was found to be mainly inorganic and hence rejected. The methanol soluble portion when examined chromatographically on silica gel G. thin layer chromatoplates was found to contain a mixture of several organic compounds. On concentration of the methanolic extract, a white amorphous mass was deposited which was filtered. It was found to give a single spot on paper and thin layer chromatoplates. The compound was purified by column chromatography using silica-gel. The purity and homogeneity of the compound was checked by thin layer chromatography as well as paper chromatography. It was designated as compound 'A'.

The filtrate obtained after filtering off the precipitate was concentrated and successively extracted with several organic solvents using a continuous liquid-liquid extractor. It was extracted with petroleum ether, benzene and chloroform separately. The extracts revealed several compounds, which because of their small amounts could not be separated into pure compounds and hence were rejected. The remaining filtrate was then extracted successively with ether and ethyl acetate in liquid-liquid extractor. The ether extract on distillation yielded an orange colored syrupy mass, which was dissolved in acetone and precipitated out by pouring into excess of petroleum ether. The precipitate when examined chromatographically on thin layer chromatoplates of silica gel G revealed the presence of one compound. It was designated as compound 'B'. Ethyl acetate insoluble fraction was dried by evaporation and was refluxed with acetone in a round bottomed flask. The acetone extract revealed the

presence of several constituents. Only one component was separated by column chromatography over the column of silica gel using mixture of organic solvent in order of their increasing polarities. Elution with ethyl acetate: acetone mixture (1:1 v/v) gave a compound designated as compound 'C'.

The water insoluble fraction of the ethanolic extract was examined over the chromatoplates of silica gel. It showed many spots showing thereby the presence of many components associated with a large quantity of chlorophyll and fat. The water insoluble fraction was transferred to a 250ml ground-joint round bottomed flask and was extracted with petroleum ether. The petroleum ether extract was concentrated and left overnight in a refrigerator. A white deposit along with some chlorophyll was obtained. The deposit was examined chromatographically on thin layer chromatoplates of silica gel 'G'. It was found to be a mixture of many components. The deposit was chromatographed over silica gel. Elution with petroleum ether gave a white compound designated as compound 'D'. The compound was crystallized from benzene: chloroform mixture (1/1 v/v) and was found to be a sterol by its color reactions.

Chart 1: Summary of the Extraction & Purification of T. Ammi



3. Results & Discussion

The 4 compounds were purified through column, thin layer, & paper chromatography processes. Further, standard reagent tests to identify functional groups were done to these extracted compounds. Additionally, Rast's method was used to determine the elemental composition of the compounds, followed by UV & IR spectroscopies (NMR spectroscopy as well, wherever required) to further analyze the functional groups.

The results are summarized below:

	Compound 'A'	Compound 'B'	Compound 'C'	Compound 'D'
Test Observations	-Keto-alcohol [1]	-Aglycone with flavone nucleus [2-7] -3 hydroxyl groups identified [8-10] -Reducing group of sugar has a glycosidic linkage	- β -linkage between aglycone & sugar molecule -3 hydroxyl groups identified [11-13] -Reducing group of sugar has a glycosidic linkage	-Sterol derivative [14-22]
Identified Structure	Nonadecane-7-ol-2-one	5,3' dihydroxy flavone 7-o- β -D. galactopyranosyl α -D-xylopyranoside	3,5,7,5' tetrahydroxy-3'-methoxyflavanone 4'-o- β -L-xylopyranoside	β - sitosterol

The study revealed the 4 key compounds of T. ammi & these can be potentially considered for further evaluation of their applications. Combined with the several active phytochemical components, T. ammi can be taken up as a candidate for further scientific exploration for its hidden curative & therapeutic potential.

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