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Research Article

GC FINGERPRINT DEVELOPMENT OF *TRACHYSPERMUM AMMI* LINN. AND ITS MARKETED POLYHERBAL FORMULATIONS "AJMODADI CHURNA"

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ABSTRACT

Most of the traditional systems of medicines are effective but they lack standardization. So there is a need to develop standardization technique. Standardization of herbal formulations is essential in order to assess the quality, purity, safety, and efficacy of drugs based on the amounts of their active principles. Ajmodadi churna contains eleven herbal ingredients in which *Trachyspermum ammi* is one of the main ingredient and it is used in the treatment of indigestion, constipation and to improve appetite. In this research paper, an attempt has been made to develop standardization method based on the Phytochemical investigation and GC fingerprinting profile of *Trachyspermum ammi* and its marketed polyherbal formulations, Ajmodadi churna of four different companies. The fingerprinting profile of *Trachyspermum ammi* is taken as a reference standard in comparing with four marketed formulations of Ajmodadi churna. The main objective of the present study was focused on identification of *Trachyspermum ammi* present in polyherbal formulations of Ajmodadi churna based on Phytochemical investigation and GC fingerprinting profiles.

Keywords: Ajmodadi churna, Trachyspermum ammi, Gas Chromatography, Fingerprinting profile.

INTRODUCTION

In the last few decades, there has been an increasing interest in and use of traditional treatments in urban areas all over the world. According to WHO, 80 percent rural population in developing countries depends on traditional medicines to meet their primary health care needs.[1] The quality and therapeutic efficacy of herbal drugs is dependent on the active constituents which are present in the plant cell.[2] It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical locations With the growing need for a safer drug, attention has been drawn to the quality, efficacy, and standard of Ayurvedic formulations.[3] Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization.[4] *Trachyspermum ammi* Linn.(Umbelliferae), known in India as Ajowan, is widely distributed in northern part of the India. In India, the fruit are used as remedy for indigestion and colic and also used in poultices to relieve asthma and arthritis. It is also having aphrodisiac properties. It is used in a steeped liquid form against diarrhea and flatulence. It is mostly used for indigestion and dyspepsia. It is the main ingredient of Ajmodadi churna. Ajmodadi churna

is a polyherbal ayurvedic medicine used as a carminative and an antispasmodic, is a strong wormifuge, and helps in all painful conditions like sciatica and stiffness in back and also restores normal digestive functions .[5-10] The main objective of the present study was focused on phytochemical investigation and identification of *Trachyspermum ammi* present in polyherbal formulations of Ajmodadi churna based on GC fingerprinting profiles. In the present research article GC Chromatogram of *Trachyspermum ammi* is taken as standard for comparing its fingerprinting profile with four marketed formulations of Ajmodadi churna. These formulations contain *Trachyspermum ammi* as main ingredient. In this research work qualitative analysis was done based on the fingerprinting profile of standard plant in comparison with its formulations.

MATERIALS AND METHODS

Chemicals and Reagents

The entire chemicals used in the experiment were of analytical grade. All the solvents used in the experiment were procured from RFCL Pvt.LTD, New Delhi, India.

Plant material

Trachyspermum ammi (Figure No.1) was procured from the local market of Jaipur, Rajasthan, India from the ayurvedic store Jagram gangasahay, tripolian bazaar, shop number.362 and their identity was confirmed by correlating their morphological microscopical characters with their literature review.[11]

Preparation of Powder

Crude drug has taken and roasted in a stainless steel pan at low temperature till it becomes free from Moisture. The sample of *Trachyspermum ammi* (seeds) was powdered in a pulverizer and pass through sieve number 80 ≠. It is packed in tightly closed containers to protect from light and moisture.

Marketed formulations

The marketed samples of various brands of Ajmodadi churna i.e., Jamuna pharmaceuticals, Krishna pharmaceuticals, Navjeewan pharmaceuticals & Sadhana chemicals (Figure No.2) were used in the present research work which were purchased from a registered of Ayurvedic Pharmacy in Jaipur, Rajasthan.

Extraction procedure

The powdered churna of *Trachyspermum ammi* and its marketed formulations of Ajmodadi churna was soxhleted with methanol for 72 hrs. After completion of extraction, residue was removed by filtration followed by the evaporation of solvent and extract was concentrated and these extracts were used for Phytochemical and GC analysis.[12]

Phytochemical analysis of extract[13, 14]

Various chemical tests were carried out for the methanolic extracts of *Trachyspermum ammi* and its marketed polyherbal formulations, Ajmodadi churna to identify the presence of various Phytochemical constituents and the results were showed in **Table No.1** in results and discussion.

GC Analysis

2µl of methanolic extracts of *Trachyspermum ammi* and its marketed polyherbal formulations, Ajmodadi churna sample was injected through GC column by using suitable inert gas as mobile phase. During this process the oven was programmed initially at fixed temperature. Thereafter, the temperature was increased gradually at certain level and maintain constant. The column effluent was monitored with detector and chromatograms were obtained based on these peaks we have confirmed the presence or absence of *Trachyspermum ammi* (standard plant) in marketed polyherbal formulations of Ajmodadi churna and they were showed in **Figure No.3-7** in results and discussion.

Chromatographic conditions

Instrument name :Chemito 8610 GC system
Software : Spinchrome
Column : BP_5 capillary column (30 m length, 0.32 mm i.d. and 1 µm film thickness)
Carrier gas : Nitrogen (99.999%)
Fuel gasses : Hydrogen and oxygen
Initial temperature : 40 ºC for 10 min.
Final temperature :Temperature was increased up to 180 ºC at the rate 10ºC min-1, kept constant at 180 ºC for 5 min, and then increased up to 220ºC at the rate 5ºC min^{-1}.
Total run time : 35 min.
Sample volume : 2 µl
Split ratio : 20
Flow rate : 3 µl min-1
Detector : Flame ionization detector

RESULTS AND DISCUSSION

Methanolic extracts of *Trachyspermum ammi* and its marketed polyherbal formulations, Ajmodadi churna of different companies were subjected to preliminary phytochemical screening and the results were showed in **Table No: 1.** According to these report formulations showed presence of Phenols and Phytosterols, alkaloids, glycosides, and flavanoids.

Ajmodadi churna obtained by the various source are not of the similar formula and are not as described by Bhaisajya Ratnavali and therefore differences are huge in this cases. The peak between Rt of 1.22 -1.4 min appears to be most important as it contributes to approximately 97.4% area. The % area of *Trachyspermum ammi* was found to be 97.3 % in Ajmodadi churna manufactured by Jamuna Pharmaceuticals corresponding Rt of 1.3. The % area of *Trachyspermum ammi* was found to be 97.4 in Ajmodadi churna manufactured by Krishna Pharmaceuticals corresponding Rt of 1.3 min. The % area of *Trachyspermum ammi* was found to be 43.66% in Ajmodadi churna manufactured by Navajeevan Pharmaceuticals corresponding Rt of 1.3 and the % area *Trachyspermum ammi* was found to be 97.3% in Ajmodadi churna manufactured by Sadhana Chemicals corresponding Rt 1.2 min. This peak possibly represents the various Phenolic, Polyphenolic and closely related substances that are thymol, carvacrol etc. while the various substances limonene, γ-terpene, myrcene etc. will be showed in the small amount in earlier. The Ajmodadi churna manufactured by Navjeewan Pharmaceuticals shows the very different value as the lowest % area and peak at Rt 1.3 min. Ajmodadi churna manufactured by Navjeewan Pharmaceuticals shows the lowest amount of *Trachyspermum ammi* in it.

Table 1: Phytochemical tests of *Trachyspermum ammi* and its formulations

S. No.	Compounds Tested	Tests applied	*Trachyspermum ammi*	Jamuna	Krishna	Navjeewan	Sadhana
1	Alkaloids	a) Mayer's b)Dragendroff's c) Hager's d) Wagner's	– + + –	– – + +	– – + +	– – + +	– – + +
3	Glycosides	a)Borntrager's b)Legal's	+ +	+ +	+ +	+ +	– +
4	Flavonoids	a) Alkaline reagent b) Lead acetate	+ +	– +	– +	– +	– +
5	Phenols	Ferric chloride	+	+	+	+	+
6	Phytosterols	a)Libermann buchard's b)Salkowski's test	+ +	+ +	+ +	+ +	+ +
8	Amino acids	a) Ninhydrin	–	+	+	–	–
9	Saponins	a)Froth test b)Foam test	– +	– +	+ –	+ –	– –

Fig. 1: Seeds of *Trachyspermum ammi*

Jamuna pharmaceuticals

Krishna pharmaceuticals

Navjeewan pharmaceuticals

Sadhana chemicals

Fig. 2: Marketed formulations of Ajmodadi churna

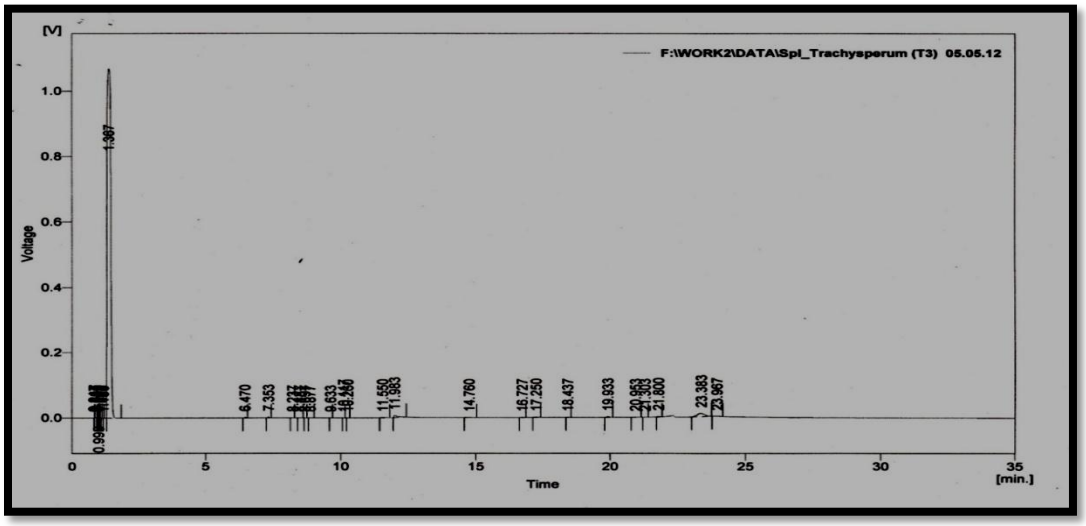

Fig. 3: GC Chromatogram of *Trachyspermum ammi*

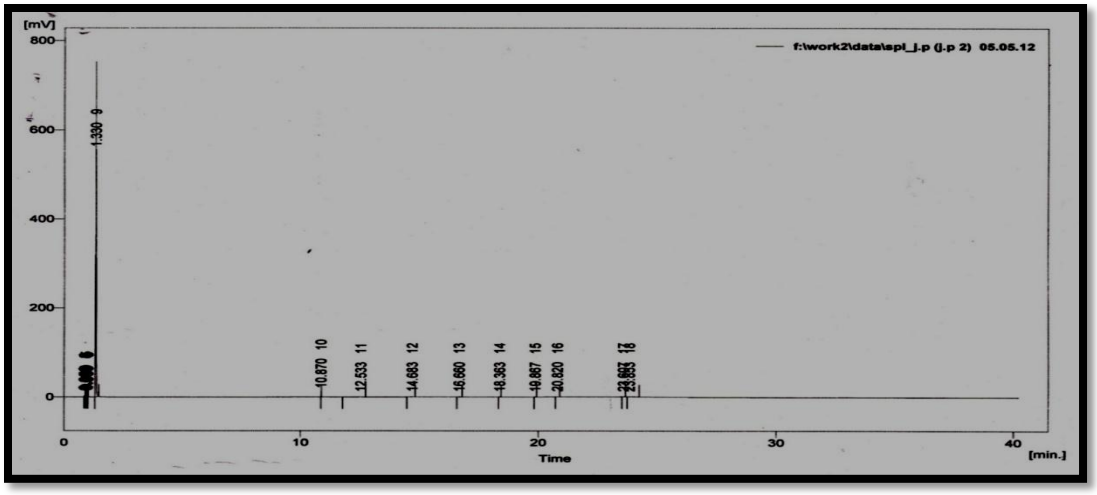

Fig. 4: GC Chromatogram of Ajmodadi churna (Jamuna Pharmaceuticals)

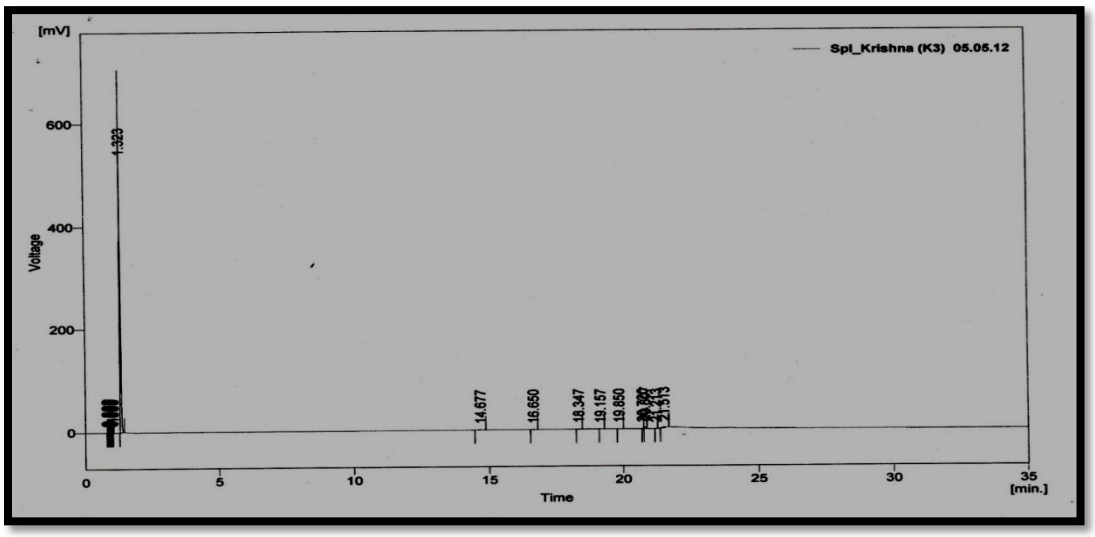

Fig. 5: GC Chromatogram of Ajmodadi churna (Krishna Pharmacueticals)

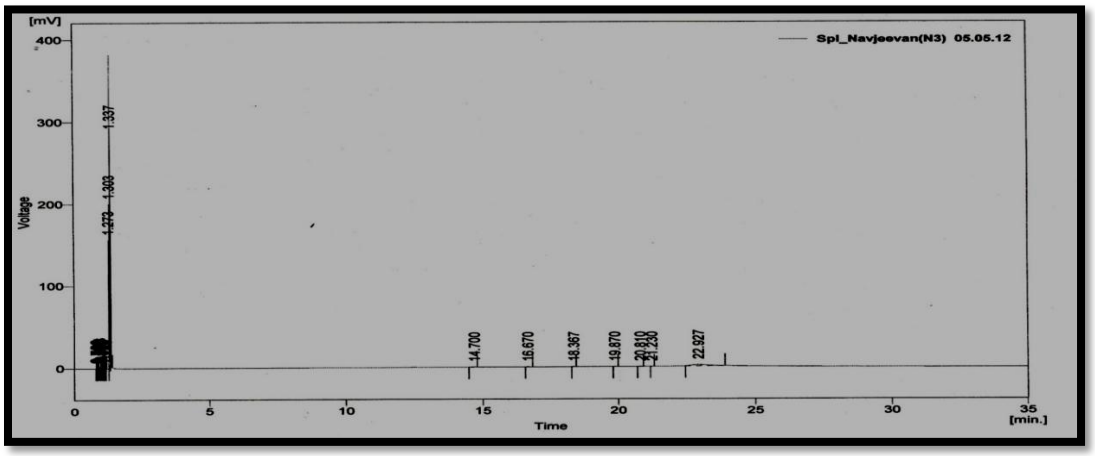

Fig. 6: GC Chromatogram of Ajmodadi churna (Navjeewan Pharmaceuticals)

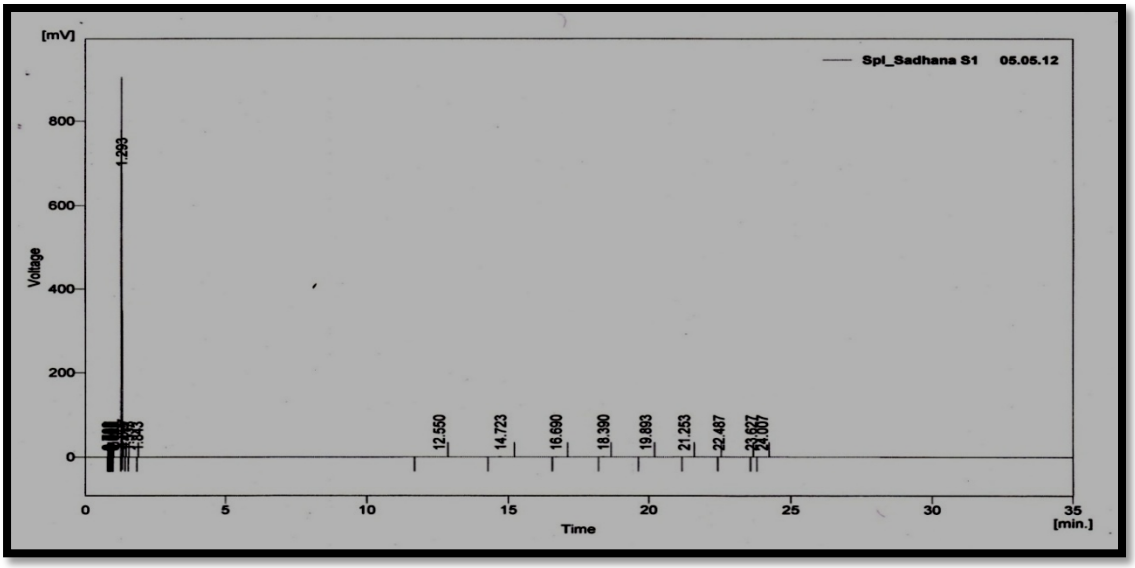

Fig. 7: GC Chromatogram of Ajmodadi churna (Sadhana Chemicals)

CONCLUSION

The results indicate the slight variations in the amount of phytoconstituents in the formulations. A preliminary phytochemical test was performed on all samples and shows the results of the desired lines. Standardization of herb *Trachyspermum ammi* in formulations can be done on the basis of GC chromatographic patterns of plant (*Trachyspermum ammi)* in comparing with its marketed formulations of Ajmodadi churna. Ajmodadi churna manufactured by Navjeewan Pharmaceuticals shows the lowest amount of *Trachyspermum ammi* in it. Therefore it can be concluded that the constituents are altered and hence Navjeewan shows different types of peaks. The characteristic peak was observed in the previous statement lowest amount of *Trachyspermum ammi* in the Navjeewan which leads to doubt on quality of drug which is may be due to the variations in geographical conditions of various plants in Ajmodadi churna manufactured by Navjeewan pharmaceuticals during at the time of collection. The other formulations of Ajmodadi churna manufactured by Jamuna pharmaceuticals, Krishna pharmaceuticals and Sadhna chemicals showed the sufficient amount of *Trachyspermum ammi* in it.

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REFERENCES

1. N.P Yadhav, V.K and Dixit,. Recent approaches in Herbal Drug standardization. International journal of Integrative Biology. 2008;2(3):195-203..
2. Cragg GM, Newman DJ, Weiss RB. Coral reefs, forests, and thermals vents: The worldwide exploration of nature for novel antitumor agents. Semin Oncol. 1997;24:156-63.
3. Agarwal S and Singh RH. Processing of International Congress. Ayurveda. 2002:209-12.
4. S. S. Shukla, Swarnlata Saraf and S Saraf. J Res Educ Indian Med. 2009;15(1):25-32.
5. Chopra RN. Chopra's Indigenous Drug of India. Academic Publishers. 1982: 2nd ed. Calcutta: 93-94.
6. De M, Krishna De A and Banerjee AB. Antimicrobial screening of some Indian spices. Phytother Res. 1999;13:616-18.
7. Singh G, *"et al"*. Studies on essential oils: part 10; Antibacterial activity of volatile oils of some spices. Phytother Res. 2002;16:680-82.
8. Tripathi S, *"et al"*. Studies on antifungal properties of essential oil of *Trachyspermum ammi* (L.) Sprague. J Phytopathol. 1986;116:113-20.
9. Hussein G, *"et al"*. Inhibitory effects of Sudanese medicinal plant extracts on

hepatitis C virus (HCV) protease. Phytother Res. 2000;14:510-16.
10. Nariman F, *"et al"*. Anti- Helicobacter pylori activities of six Iranian plants. Helicobacter 2004;9:146-51.
11. Anonymous. The Ayurvedic Pharmacopoeia of India Govt. of India M.H & F.W Dept. of Health.1990; 1:170-71.
12. Gupta A.K. Introduction to Pharmaceutics – 1; CBS Publications: 148-155.
13. Evans WC. General methods associated with phytochemical investigation of herbal products. Pharmacognosy. 138.
14. Agarwal OP. Advance practical organic chemistry. Geol. publishing house 2000;17th edition Meerut. 43-59.