



Research Article

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AJWAIN (*Trachyspermum ammi*) FROM NORTH-WEST IRAN

Hossein Mostafavi*, Sakha Pezhhanfar

Department of Organic Chemistry & Biochemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran

*Corresponding Author Email: hmostafavi@tabrizu.ac

Article Received on: 01/07/15 Revised on: 19/08/15 Approved for publication: 26/08/15

DOI: 10.7897/2230-8407.069119

ABSTRACT

Most spices, owe their individual properties to the pharmacologically active secondary metabolites that they contain. In this study plant Ajwain (*Trachyspermum ammi*) from North-West Iran was screened for the presence of major phytochemical groups. Three solvent extracts (water, ethanol, ethylacetate) obtained from Ajwain (*Trachyspermum ammi*) traditionally used in the past and even now for various therapeutic effects including fatigue, diarrhea, bloating, abdominal tumors and especially in local area of Tabriz to cause to heal up the joint pain and loss of appetite. Phytochemical screening of the plant Ajwain (*Trachyspermum ammi*) showed the presence of carbohydrates, alkaloids, flavonoids, teriterpenoids, steroids, tannins, phenolic compounds, coumarins, resins, saponins, oil and fat, inorganic acids and ascorbic acid as major phytochemical groups. Ajwain (*Trachyspermum ammi*) tested negative for the presence of glycosides, starch, amino acids, protein, phelobotanin and free antraquinones and organic acids.

Keywords: *Trachyspermum ammi*, Ajwain, Qualitative phytochemical analysis, bioactive constituents.

INTRODUCTION

Herbal remedies from plants continue to provide a popular alternative for treating known and emerging disease that have defiled many orthodox medical treatments. Herbal medicines are also in great demand in the developing world for primary health care because of their efficiency, safety and lesser side effects. According to World Health Organization (WHO) traditional medicines are relied upon 60-80% of the world's population for their primary health care needs¹. Most of these herbal remedies have stood the test of time, particularly for the treatment of allergic, metabolic and cardio-vascular diseases².

Ajwain, *Trachyspermum ammi*, (L.) Sprague ex Turill belonging to the family Apiaceae is an important seed spice. It is known as bishop's weed, carum seed or carum ajowan. The common synonyms are *Trachyspermum copticum* Linn, *Carum copticum* Benth and Hook, *Ammi copticum* Linn, *Ptychotis coptica* DC and *Lingusticum ajowain*, Roxb. The correct generic position of this spice is very uncertain. Boissier considers it to belong to the genus Ammi, where Linnaeus originally put it, and as per Genera Plantarum it has been referred to Carum. In the recent past it was placed in the section *Trachyspermum*, which includes about 14 species³. Ajowan is indigenous to India and Egypt⁴.

Ajwain is annual, aromatic and herbaceous plant. It is profusely branched with a height of 60-90 cm small, erect with soft fine hair. It has many branched leafy stems, feather-like leaves 2-3 pinnately divided, segments linear with flowers terminal and compound. The fruits are small, ovoid, muricate, around cremocarps, 2-3 mm long, with greyish-brown compressed mericarps with distinct five ridges and tubercular surface. The fruits are the size and shape of parsley. The fruits have a very pungent aromatic taste and, when rubbed, they evolve a strong aromatic odour resembling that of thyme (*Thymus vulgaris*).

The corp belongs to family Apiaceae and order Apiales. As per conventional classification of spices, out of the five types, ajowan is

classified as aromatic spice, mostly dried fruits of which are used as spices. *Trachyspermum* is a cross-pollinated crop and has a somatic chromosome number of 2n=18. The flowers are self-fertile but cross-pollination occurs through insects.

The aim of present study was to investigate the bioactive constituents of Ajwain (*Trachyspermum ammi*) from North-west Iran and correlate their bioactive constituents with their pharmacological activity^{5,6}.

MATERIAL AND METHODS

Plant Material

Ajwain (*Trachyspermum ammi*) was obtained from field and local market of Tabriz Iran. The plant materials was authenticated by botanist in the Department of pharmacy, University of Tabriz, shade dried and powdered by an electrical grinder.

Preparation of crude extracts

Electrical grinder was used to crush the adulterant free plant material into coarse powder. Each of the dried and powdered samples was Soxhlet with ethanol 90%, water and ethylacetate for 80 hours.

The extracts were concentrated using rotary evaporator (Rotavap, Heidolph Labortechnik VV 2000) with water bath set at 60° C. After that, the respective extracts were weighed and percentage extractive value were determined. The dried extracts were transferred to separated amber glass jars and stored at 4 °C in a refrigerator.

Phytochemical Analysis

The phytochemical tests were carried out for the above mentioned plant extract using the standard procedures to identify the components Mentioned at the below^{7, 8, 9,10}.

Tests for alkaloids

Dragendorff's test

To 0.5 ml of plant extracts the Dragendorff's reagent was added. (Potassium bismuth iodide solution). A reddish brown precipitate confirms that test as positive.

Hager's test

To 0.5 ml of plant extracts, a few drops of Hager's reagent was added. Formation of yellow precipitates confirms the presence of alkaloids.

Wagner's test

To 0.5 ml of plant extracts the Wagner's reagent was added. (Solution of Iodine in potassium Iodide). A reddish brown precipitate confirms that test as positive.

Mayer's test

To 0.5 ml of plant extracts the Mayer's reagent was added. (Potassium mercuric iodide solution). A white creamy precipitate confirms that test as positive.

Tests for carbohydrates

Anthrone test

0.5 mg of plant extracts was shaken with 2.5ml of water, filtered and the filtrate was concentrated. To this 0.5ml of anthrone reagent solution was added. Formation of green or blue colour indicated the presence of carbohydrates.

Benedict's test

0.5 mg of plant extracts was shaken with 2.5 ml of water, filtered and the filtrate was concentrated. To this 1.25 ml of Benedict's solution was added and boiled for 5 minutes. Brick red precipitate indicated the presence of carbohydrates.

Fehling's test (free reducing sugars)

At the first step equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartarate and sodium hydroxide in distilled water) reagents are mixed carefully. Then few drops plant extracts was added and boiled. Brick red precipitate of cuprous oxide indicated the presence of free reducing sugars.

Molisch's test

To 0.5 ml of plant extracts few drops of alcoholic α -naphthol was added.

Then 0.2 ml of concentrated sulphuric acid was added slowly along the sides of test tubes. Reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Barfoed's test

About 0.5 mg of plant extracts was dissolved in distilled water and filtered. 1 ml of the filtrate was then mixed with 1 ml of Barfoed's reagent in a test tube and then heated on a water bath for a period of two minutes. A reddish precipitate of cuprous oxide confirms that test as a positive.

Fehling's test (combined reducing sugars)

0.5 ml of plant extracts was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralised with sodium hydroxide solution. Then few drops of Fehling's solution was added and heated on a water bath for 2 minutes. Reddish-brown precipitate indicated the presence of combined reducing sugars.

Tests for flavonoids

Shinoda's test

To 0.5 ml of plant extracts a piece of metallic magnesium was added, followed by addition of 2 drops of concentrated hydrochloric acid. Presence of deep red colouration indicated the presence of flavonoids in the extract.

Ferric chloride test

To 0.5 ml of plant extracts a few drops ferric chloride solution was added. The presence of green colouration indicated the presence of flavonoids.

Lead ethanoate test

To 0.5 ml of plant extracts 0.3 ml of lead ethanoate solution was added. A buff-coloured precipitate indicated the presence of flavonoids.

Alkaline reagent test

To 0.5 ml of plant extracts few drops of sodium hydroxide solution was added. A yellow colouration which turns to colorless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Tests for glycosides

Borntrager's test (Anthraquinone Glycosides)

1 ml of benzene and 0.5 ml of dilute ammonia solution were added to the plant extracts. A reddish pink colour indicated the presence of glycosides.

Keller killaini's test (Cardiac glycosides)

0.4 ml of glacial acetic acid containing traces of ferric chloride and 0.5 ml of concentrated sulphuric acid were added to the plant extracts carefully. A reddish-brown colour formed at the junction of the two layers and the upper layer turned bluish green indicating the presence of glycosides.

Test for resins

0.5 ml of plant extracts were treated with a few drops of acetic anhydride solution followed by one ml of concentrated sulphuric acid. Resins give colouration ranging from orange to yellow.

Test for saponins

Froth test

A pinch of the dried powdered plant was added to 3 ml of distilled water. The mixture was shaken vigorously. Formation of a foam indicated the presence of saponin.

Tests for steroids and triterpenoids

Liebermann - Burchard Test

0.5 ml of plant extracts was treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added along the sides of the test tube, shows brown ring at the junction of two layers and the upper layer turns green that shows the presence of sterols and formation of deep red colour indicated the presence of triterpenoids.

Salkowski Test

0.5 ml of each extract was treated in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, red colour at the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicates the presence of triterpenoids.

Tests for tanins

Lead acetate Test

To 0.5 ml of plant extracts, a few drops of 10 % lead acetate was added. Precipitate was formed, indicated the presence of tannins.

Ferric chloride Test

To 0.5 ml of plant extracts, few drops of 0.1% ferric chloride solution was added. Formation of brownish green or a blue black colouration indicated the presence of tannins.

Test for starch

To 0.5 ml of plant extracts Iodine as reagent was added. Appearance of dark blue colour which disappeared on heating and reappears on cooling indicated presence of starch.

Tests for inorganic acids

Sulphate Test

To 0.5 ml of plant extracts, the lead acetate reagent was added. White precipitate which is soluble in NaOH, indicated the presence of sulphate.

Carbonate Test

To 0.5 ml of plant extracts, dilute HCl solution was added. Libration of CO₂ gas, indicated the presence of carbonates.

Tests for organic acids

Malic acid Test

To 0.5 ml of plant extracts few drops of 40% FeCl₃ solution was added. Formation of yellowish colour indicated the presence of malic acid

Oxalic acid Test

To 0.5 ml of plant extracts, few drops of 1% KMnO₄ and dilute H₂SO₄ were added. Disappearance of colour indicated the presence of oxalic acid.

Test for ascorbic acid

To 0.5 ml of plant extracts, 2 ml water, 0.1 gram sodium bicarbonate and about 20 mg ferrous sulphate were added, shaken and allowed to stand. A deep violet colour was produced. To this 5 ml of 1M sulphuric acid was added, the colour disappeared showing the presence of ascorbic acid

Tests for phenolic compounds

Lead acetate Test

To 0.5 ml of plant extracts few drops of 10% lead acetate solution was added. White precipitate indicated the presence of phenolic compounds.

Ferric chloride Test

To 0.5 ml of plant extracts, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Tests for amino acids

Millons Test

To 0.5 ml of plant extracts 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added. White precipitate appears which turns red when gentle heating.

Ninhydrin Test

To 0.5 ml of plant extracts few drops of 5% ninhydrin was added and then boiled. Appearance of violet colour indicated the presence of amino acids.

Tests for protein

Biuret Test

To 0.5 ml of plant extracts, 4% NaOH solution and few drops of 1% CuSO₄ solution were added. Violet colour appears, indicated the presence of protein.

Millon's Test

To 0.5 ml of plant extracts 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added. White precipitate appears which turns red when gentle heating.

Test for oils and fats

A small quantity of the dried plant was pressed between the two filter papers. Oil stain on the filter papers indicated the presence of oils and fats.

Test for coumarins

To 0.5 ml of plant extracts, the solution of 10% NaOH was added. The appearance of yellow colour indicated the presence of coumarins.

Test for phlobatanins

To 0.5 ml of plant extracts, the solution of 10% ammonia solution was added. Appearance of pink colour indicated the presence of phlobatanins.

Test for anthraquinones

To 0.5 ml of plant extracts few drops of 2% HCl was added. Red colour precipitate indicated the presence of anthraquinones.

Table 1: Phytochemical constituents of *Trachyspermum ammi*

S.NO	Phytoconstituents	Water	Ethanol	Ethylacetate
1.	Alkaloids a. Dragendorff's test b. Hager's test c. Wagner's test d. Mayer's test	 + + + -	 + + + -	 + + + -
2.	Carbohydrates a. Anthrone test b. Benedict's test c. Fehling's test (free reducing sugars) d. Molisch's test e. Barfoed test (monosaccharides) f. Fehling's test (reducing sugars)	 - + + + + + +	 + + + + + + +	 - - - + - - +
3.	Flavonoids a. Shinoda test b. Ferric chloride test c. Lead ethanoate test d. Alkaline reagent test	 + + + +	 + + - +	 + + - +
4.	Glycosides a. Borntrager's test (Antraquinone glycosides) b. Keller killaini (Cardiac glycosides)	 - - -	 - - -	 - - -
5.	Resins	+	+	+
6.	Steroids a. Liebermann-Burchard's test Terpenoids b. Salkowski test	 + + +	 + + +	 + + +
7.	Tanins a. Lead acetate test b. Ferric chloride test	 + +	 + +	 + +
8.	Starch	-	-	-
9.	Inorganic acids a. Sulphate test b. Carbonate test	 + +	 - -	 + +
10.	Organic acids a. Malic acid test b. Oxalic acid test	 - -	 - -	 - -
11.	Ascorbic acid	+	+	+
12.	Phenolic compounds a. Lead acetate test b. Ferric chloride test	 + +	 + +	 - +
13.	Amino acids a. Millons test b. Ninhydrin test	 - -	 - -	 - -
14.	Protein a. Biuret test b. Millons test	 - -	 - -	 - -
15.	Coumarins	+	+	+
16.	Phlobotanins	-	-	-
17.	Antraquinones	-	-	-

Key: + = Present and - = Absent

RESULTS AND DISCUSSION

The phytochemical test of the crude water, ethanol, and ethylacetate extracts of *Trachyspermum ammi* occurring in North-West Iran showed the presence of carbohydrates, alkaloids, flavonoids, terpenoids, steroids, tannins, phenolic compounds, coumarins, resins, saponins, oils and fats, inorganic acids and ascorbic acid (Table 1). The active principles identified in the water, ethanol and ethylacetate extracts were alkaloids (in Wagner's, Hager's and Dragendorff's reagents). Surprisingly the Mayer's test for alkaloids was negative. This observation for alkaloids also occurred in all three extracts (water, ethanol and ethylacetate). The extracts in the three solvents water, ethanol and ethylacetate show no presence (negative results) for the following active principles: glycosides, starch, amino acids, protein, free antraquinones, phlobatanins and organic acids.

This positive observation helps in providing chemotaxonomic evidence for the classification of the species since *Trachyspermum ammi* belongs to the Apiaceae family, which has been reported to contain these compounds¹¹. The identification of these family of compounds further supports claims of the use of this plant as a traditional medicine as these compounds have valuable amoebicidal, antifungal and anti-inflammatory properties^{12,13}. They have stimulating and tonifying effects on the muscles when consumed and this probably accounts for their use in enhancing male potency. Furthermore, the metabolites: alkaloids, steroids, saponins and tannins found in extracts are known to have curative activity against several pathogens and therefore *Ajwain* is used traditionally in the Indian system of medicine to treat amoebiasis, febrile conditions, stomach disorders, dyspepsia and disorder of inflammation¹⁴.

Probably the constituents of Ajwain (*Trachyspermum ammi*) from which its male potency enhancing properties emanated are based upon the actions of certain steroidal alkaloids. A study has implicated saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property¹⁵. Saponins present in the water and ethanol extracts of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of Leutinizing Hormone (LH). The LH release normally by the pituitary gland helps to maintain testosterone levels, as LH increase so does the testosterone. The increase in testosterone seemed to have translated into the male sexual competence.

Furthermore, this study suggests that the aphrodisiac action may be mediated through a change in the blood testosterone level¹⁶.

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers, anti-inflammatory and anti-carcinogenic. Due to presence of phenolic compounds these might play role in the prevention of several chronic diseases such as cardiovascular disease, cancer, diabetes, bacterial and parasitic infections¹⁷.

Flavonoids present in the all three extracts of this plant can also inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide synthase, which are supposed to be involved in free radical generation, thereby resulting in decreased oxidative damage of macromolecules¹⁸. Research at the Linus Pauling Institute and the European Food Safety Authority shows that flavonoids are poorly absorbed in the human body (less than 5%), with most of what is absorbed being quickly metabolized and excreted. These findings suggest that flavonoids have negligible systemic antioxidant activity, and that the increase in antioxidant capacity of blood seen after consumption of flavonoid-rich foods is not caused directly by flavonoids, but is due to production of uric acid resulting from flavonoid depolymerization and excretion¹⁸.

The presence ascorbic acid in plant species has shown high total antioxidant properties of plants. Due to presence of triterpenoids these might be act as cardio protective and antioxidant¹⁹. Steroids are frequently used signaling molecules biologically and decrease fluidity of membranes²⁰.

This data can help us to choose the superior race of this valuable plant with greater quantity of medically and therapeutically important phytochemicals.

CONCLUSION

The results of this study shows that the three extracts of Ajwain (*Trachyspermum ammi*) from North-West Iran indicates the presence of carbohydrates, alkaloids, flavonoids, teriterpenoids, steroids, tannins, phenolic compounds, coumarins, resins, saponins, oil and fat, inorganic acids and ascorbic acid as major phytochemical groups.

The plant parts studied here has also been seen as a potential source of useful drugs. Further studies are going on in order to isolate, identify characteristics activities and elucidate the structure of the bioactive compounds. Further studies are needed to explore the potential bioactive compounds from this plant and *in vivo* studies are needed for better understanding their mechanism of action.

REFERENCES

1. Zhang X., WHO Traditional Medicine Strategy 2000-2005. World Health Organization. Geneva; 2002. p.1, 16.
2. Igoli JO, Ogaji OG, Tor-Anyiin TA and Igoli NP. Traditional medicine practices amongst the igede people of Nigeria. Afr.J.Trad.CAM, 2005;2:134-152.
3. Kokate CK., A text book of practical pharmacognosy. Vallabh prakashan 5th edn, 2005;107-111.
4. Sayre JK., Ancient Herbs and Modern Herbs, Bottelbrush Press, Sancarlos, CA. 2001.
5. Chauhan B., Kumar G., Mohammad A. A Review on Phytochemical Constituents and Activities of *Trachyspermum Ammi* (L.) Sprague fruits. Am. J. PharmTech. Res., 2012;2(4): 329-340.
6. Gurinder Jeet, K. and Daljit Singh A. Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae - Current status. Journal of Medicinal Plants Research, 2010;4(2): 87-94.
7. Mameta S., Jyoti S. Phytochemical screening of *Acorus Calamus* and *Lantana Camara*. Int Res J Pharm, 2012;3(5): 324-326.
8. Sanjay Parihar, Kartik D. Virani, E. A. Pithawala, M. D. Shukla, S. K. Lahiri, N. K. Jain and H. A. Modi. Phytochemical screening, total phenolic content, antibacterial and antioxidant activity of wild edible mushroom *Pleurotus ostreatus*. Int. Res. J. Pharm. 2015; 6(1):65-69 <http://dx.doi.org/10.7897/2230-8407.06115>
9. Mumtaz, F., Shahid Massod R., Zubair A., Iftikhar A. and Musaddique H. Qualitative phytochemical analysis of some selected medicinal plants in local area of Faisalabad Pakistan. Journal of Pharmacy and Alternative Medicine, 2014;3(3):17- 23.
10. Reddy S., Ammani, Ch., Rose Mary K., Nikhil Rajesh T., Aravind G. and Bala Sekaran Ch. Phytochemical and GC-MS analysis of *Commiphora caudata* (Wt&Arn.) Eng. Bark, Indian Journal of Advances in Plant Research, 2014;1(5):24-29.
11. Meena, S., Kakani RK., Singh, B., Meena, RS., and Meena RD. AA 93: An early maturing variety of Ajwain developed at NRCSS for all Ajwain growing areas, International J. Seed Spices, 2014;4(2):91-93.
12. Hassanshahian M., Bayat Z., Saeidi S., Shiri Y. Antimicrobial activity of *Trachyspermum ammi* essential oil against human bacterial. International journal of Advanced Biological and Biomedical Research, 2014; 2(1): 18-24.
13. Gilani AH., Jabeen Q., Ghayur MN., Janbaz KH., Akhtar MS. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. J. Ethnopharmacology, 2005; 98: 127-135.
14. Sreemoyee Ch., Nandi G., Pradeep Bh. Estimation of Phenolic Components and *in vitro* Antioxidant Activity of Fennel (*Foeniculum vulgare*) and Ajwain (*Trachyspermum ammi*) seeds. Advances in Bioresearch, 2012 ; 3(2): 109-118.
15. Gauthaman K., Adaikan PG. and Prasad RN. Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rates. Life Sci. 2002; 71: 1385-1396.
16. Yakubu MT., Akanji MA. and Oladiji AT. Aphrodisiac potensials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats, Asian Journal Andrology 2005; 7: 399-404.
17. Canini, A., Alesiani, D., D'Arcangelo, G., Tagliatesta, P. Gas chromatography – mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. Journal of food composition and analysis, 2007; 20: 584-590.
18. Cazarolli LH., Zanatta L., Alberton EH., Figueiredo MS., Folador P. and Silva FR. "Flavonoids: Prospective Drug Candidates". Mini Reviews in Medicinal Chemistry. 2008; 3(13):1429-1440.
19. Kusmik, C., Basta G., Lazzerini, G., Vesentini, N., Barsacchi, R. The effect of Ginkgo biloba in isolated ischemic /reperfused rat heart a link between vitamin E preservation and prostaglandin biosynthesis. J. cardiovascular pharmacol. 2004 ;44: 356.

20. Sadava D., Hillis DM., Heller HC. and Berenbaum MR. Life: The science of biology 9th edition, San Francisco, Freeman . 2011 p.105-114.

Cite this article as:

Hossein Mostafavi, Sakha Pezhhanfar. Qualitative phytochemical analysis of Ajwain (*Trachyspermum ammi*) from north-west Iran. Int. Res. J. Pharm. 2015; 6(9):610-615 <http://dx.doi.org/10.7897/2230-8407.069119>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.