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Evaluation of anti-hypertensive activity of *trachyspermum ammi* seeds *extract on* albino rats

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Abstract

The study evaluates the antihypertensive activity of *Trachyspermum ammi* seed extract in albino rats. A preliminary phytochemical analysis identified the presence of steroids, alkaloids, glycosides, carbohydrates, flavonoids, and saponins in the extract. No mortality or behavioral abnormalities were observed during the experiment, confirming the extract's safety at administered doses.Nifedipine, a standard antihypertensive drug, significantly reduced blood pressure parameters, serving as a positive control. Hypertension was induced in DDS model rats using an 8% NaCl method. The ethanolic extract of *T. ammi* (EET) at a high dose significantly reduced systolic, mean, and diastolic blood pressure, indicating a dose-dependent antihypertensive effect.Phytochemicals such as alkaloids, glycosides, flavonoids, and saponins, known for their antihypertensive properties, are likely responsible for this activity. These compounds may act through mechanisms like vasodilation, diuretic effects, or modulation of vascular resistance.The findings highlight the potential of *T. ammi* as a natural antihypertensive agent. Further research focusing on isolating and characterizing its active constituents is needed to elucidate their mechanisms and therapeutic applications. Such studies could contribute to the development of plant-based treatments for hypertension, providing an alternative or complementary option to conventional drug therapies.

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INTRODUCTION

Trachyspermumammi is a recent immigrant like Egypt and, therefore, is cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. It is cultured in Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar, and West Bengal, India. trachyspermumammi belongs to the family Apiaceae, a highly regarded pharmaceutically essential inoculum hot sauce. It and its root systems were also anti-diabetic through the environment, as well as the seedling's excellent presentation of addictive stimulant characteristics. It and its seeds also contain 2–4.4% caramel-

colored influenced oil, generally known as ajwain oil [1]. The most crucial element of this oil is thymol, which can be used to diagnose gastrointestinal diseases, total absence like insatiable hunger, and pulmonary troubles. This same petroleum exhibition halls fungistatic, antimicrobial, and anti-aggregatory effects on human beings. Ajwain is a conventional possibilities thyme widely used to cure infectious illnesses in animals and humans. This same pineapple possesses psychoactive drugs antispasmodic as well as demulcent characteristics. That is a significant vital operative such as flatus, altered mental status, stomach pain, and diarrhea. Like ajwain, its seed seems harsh and foul smelling, so it behaves like antiparasitic, purgative, purgative, and antidiarrheal. This also helps cure abdomen tumor cells, retroperitoneal discomfort, and pile caps. Seeds also contain an essential oil that usually contains 50% thymol, a robust demand, and supply, an antispasmodic but systemic insecticide. carbopol is sometimes used through oral care products but also fragrance [2].

PHARMACOLOGICAL ACTIVITY:

Ajwain, including its distinctive feature of fragrant odor and foul-smelling texture and flavor, has been widely used as a hot sauce through soups and stews. Its seedlings have been used in small amounts, such as flavor enhancers in innumerable types of food, just like artificial ingredients, through treatments, and for the manufacturing process, such as volatile oils through perfumery. Through the Indian system of education, ajwain is run regarding trying to cure abdomen disorders in children; one drag and drop like smashed fresh fruit seems to be externally applied and designed to relieve gastrointestinal problems and discomfort, as well as dry and hot methods and techniques of both the fresh fruit seems to be implemented through breast such as bronchitis [3]. T. Ammi has also been shown to have the antimicrobial, based algorithm, gastrointestinal psychoactive drug, antihypertensive, cardioprotective, antispasmodic, broncho-dilating, anti-lithiasis, hypotensive, emergency contraceptive, galactagogue, antiplatelet-aggregatory, potent anti-inflammatory, anti - inflammatory, anti filarial, gastroprotective, antiplasmodial, antiparasitic, biotransformation like aflatoxin, as well as socially beneficial consequences. Medicinal utilizes like t. ammi fresh fruit include purgative, emmenagogue, cough suppressant, and antibacterial, but also factors considered, such as antimicrobial. Seedlings immersed in water through lime juice as beta vulgaris works more efficiently (badam) were also specified through trying to cure amenorrhoea. Still, it is also used as an antipyretic, febrifugal, and even in remedy like typhoid [4].

MATERIALS & METHODS

Drugs & Chemicals

- 8% NaCl solution
- Methanol
- Nifedipine
- Distilled water
- surgical spirit
- Carboxymethylcellulose
- Formaldehyde
- Ethanol

PLANT MATERIAL :

Ajwain (trachyspermumammi) had been obtained from the field and native industries like Tabriziran. This same plant raw material had been authenticated by botanists in the pharmacy department of the University of Tabriz, shade-dried and powdered by such an electronic grinder.

PREPARATION OF CRUDE EXTRACT:

An electric grinder crushes this adulterant-free plant material into the coarse nanoparticle. Each of the dried and powdered samples collected had been soxhlet for ethanol 90%, water, and ethyl acetate for 80 hours.

The help in extracting has been primarily focused on using a rotating evaporator, as for water baths established sometimes when 60° c. After that, these same various methods of extracting have been weighed. Still, the percentage resource extraction valuation has been decided. This same dried help in extracting has been transmitted wholly kept in separate amber bottle containers and stored there at four °c inside a fridge [5].

EXTRACTION PROCEDURE :

This same powder-form drug was extracted, and the solvent was 70% ethanol utilizing soxhlet equipment. This same separation was carried out

until after the extricate had become colorless. This same solvent has been obliterated that once marc if before another separation was carried out. This same extricate had been dried beside a rotating evaporator, sometimes at 400 c. These same cleaning agents have been far away from this same extrication while evaporating inadequate pressure drops. This same hardened extricate, thereby procured, had been managed to be kept in a desicator after that for further research [6].

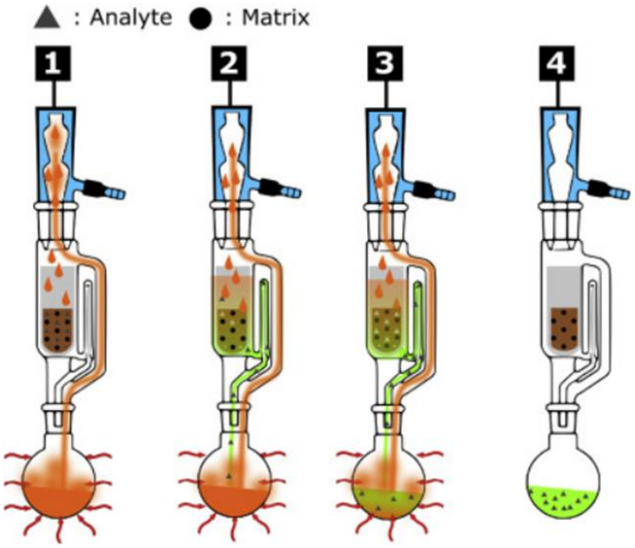

Figure 1 Soxhlet Apparatus

PHYTOCHEMICAL SCREENING

This same ethanol extract obtained, besides succeeding extraction methods, has been made the subject of preliminary phytochemical analysis to identify the character-like components present inside the ajwain obtained from the field and native industry like Tabriziran [7],[8].

This same detailed examination about phytoconstituents testing process like continues to follow:

Mentioned at the below:

Alkaloids test:

Dragendorff's test:

0.5 ml of plant extracts and Dragendorf's reagent were added. A reddish-brown precipitate validates a specific experiment just like a positive.

Hager's reagent:

A few drops of Hager's reagent are applied to 0.5 ml plant extract. The appearance of yellow PPT confirms the presence of alkaloids.

Wagner's test :

Add 0.5 ml plant extract, and then Wagner's reagent is added. The reddish brown indicates the test is positive.

Mayer's test

Add 0.5 ml plant extract, then Mayer's reagent is added, and the white creamy PPT indicates the positive test.

Tests for carbohydrates

Anthrone test :

With 2.5 ml of water and 0.5 mg of plant extract added, the filtrate was agitated to concentrate it. Add 0.5 ml of the anthrone reagent mixture. Green or blue color production suggested the existence of carbohydrate molecules.

Benedict's test :

2.5 ml of water and 0.5 mg of plant extract were added, agitated, and filtered to produce a concentrated filtrate. Benedict's solution, 1.25 ml, has been added to this and heated for 5 minutes. A brick-red precipitate detected carbohydrates.

Fehling's test (free reducing sugars) :

In the initial step, carefully mixed equal volumes of Fehling's A and B reagents (potassium tartrate and sodium hydroxide in distilled water and copper sulfate, respectively) were added to the reaction. A small amount of plant extract was then added and heated. A brick-red cuprous oxide precipitate suggested the existence of free-reducing carbohydrates.

Molisch's test:

A few drops of alcoholic α-naphthol were added to 0.5 ml of plant extract. After that, 0.2 cc of sulphuric acid concentration was injected progressively along the sidewalls of the test tubes. A reddish-violet ring at the junction of the two layers indicated the presence of carbs.

Barfoed's test:

0.5 mg of plant extract was dissolved in filtered water. The test tube was heated in a water bath for two minutes after mixing one milliliter of filtrate and one milliliter of Barfoed's reagent.

That test was positive, according to a reddish ppt of cuprous oxide.

Fehling's test :

To hydrolyze 0.5 ml of plant extract, 5 ml of diluted Hcl was used in a boiling process. The resultant solution was then neutralized with sodium hydroxide solution. Fehling's solution was diluted slightly and cooked in a water bath for two minutes. The existence of reddish-brown ppt detected a combination of reducing sugars.

Tests for flavonoids :

Shinoda's test :

Two drops of strong hydrochloric acid were added after adding 0.5 ml of plant extract with a piece of metallic magnesium. The solid red color indicated the presence of flavonoids in the extract.

Ferric chloride test :

0.5 milliliters of plant extract were mixed with a few drops of ferric chloride solution. The presence of flavonoids is indicated by green coloration.

Lead ethanoate test:

Include 0.5 ml of plant extract. A solution of lead ethanoate in 0.3 ml was added.

A buff-colored PowerPoint presentation has demonstrated the existence of flavonoids.

Alkaline reagent test :

A few drops of sodium hydroxide solution were added to 0.5 ml plant extract.

Flavonoids were detected by a golden coloration that became colorless when a few drops of diluted acetic acid were added.

Tests for glycosides :

Borntrager'stest :

One milliliter of benzene and half a milliliter of diluted ammonia solution were combined with the plant extracts.

A reddish-pink coloration identified glycosides.

Keller Killaini's test :

Carefully added to the plant extracts were 0.4 ml of glacial acetic acid with traces of ferric chloride and 0.5 ml of concentrated sulphuric acid.

To show the presence of glycosides, the top layer turned bluish-green, and the intersection of the two layers took on a reddish-brown tint.

Test for resins:

After 0.5 ml of plant extracts were treated with a few drops of acetic anhydride solution, one milliliter of strong sulphuric acid was added. Resins provide orange to yellow coloration.

Test for saponin:

Froth test :

3 ml of distilled water should contain a pinch of a dried powdered herb. The mixture was vigorously shaken. The foam's formation revealed the existence of saponin.

Triterpene and Steroid tests:

Liebermann - Burchard Test:

Concentrated sulphuric acid is added along the test tube's sides after adding 0.5 ml of plant extracts, first treated with a few drops of acetic anhydride. This caused the upper layer to turn green, indicating the presence of sterols, and to form a deep red, indicating the presence of triterpenoids.

Salkowski Test:

Shake well and add 0.5 ml of each extract processed in chloroform and a few drops of strong sulfuric acid. After allowing the mixture to stand for a while, the lower layer will turn red, signifying the presence of sterols, and the lower layer will turn yellow, indicating the existence of triterpenoids.

Tests for tannins:

Lead acetate Test:

A few drops of 10% lead acetate and 0.5 ml of plant extracts were added. The residue's development showed the presence of tannins.

Ferric chloride Test:

0.5 ml of plant extracts and a few drops of a 0.1% ferric chloride solution were added. Tannins were present when a brownish-green or blue-black coloration formed.

Test for Starch:

Plant extracts, 0.5 ml, are added. Iodine was added as a reagent.

Starch was present because of the appearance of a dark blue tint that occurred after heating and then returned after cooling.

Tests for inorganic acids :

Sulphate Test :

Add 0.5 ml of plant extracts before adding the lead acetate reagent. The existence of sulfate was revealed by a white residue soluble in NaOH.

Carbonate Test:

0.5 ml of plant extracts were added after the diluted HCl solution. The existence of carbonates was revealed by the CO2 gas libration.

Tests for organic acids :

Malic acid Test :

0.5 ml of plant extract has been added, followed by a few drops of a 40% FeCl3 solution. Malic Acid was present, as shown by the formation of a yellowish tint.

Oxalic acid Test :

A few drops of 1% KMnO4 and diluted H2SO4 were added along with 0.5 ml of plant extracts. Oxalic acid was present because the color disappeared.

Test for ascorbic acid:

The following ingredients were added, agitated, and left to stand: 0.5 ml of plant extracts, 2 ml of water, 0.1 gram of sodium bicarbonate, and around 20 mg of ferrous sulfate. A rich violet hue was created. The color vanished when adding five cc of 1M sulfuric acid, indicating that ascorbic acid was detected.

Tests for phenolic compounds:

Lead acetate Test:

A few drops of a 10% lead acetate solution were added after adding 0.5 ml of plant extracts. White PPT showed that phenolic chemicals were present.

Ferric chloride Test :

0.5 ml of plant extracts and a few drops of a neutral 5% ferric chloride solution were added. There were phenolic chemicals present because of the dark green coloration.

Tests for amino acids :

MillonsTest :

Plant extracts, 0.5 ml, are added. Millon's reagent, mercuric nitrate in nitric acid with traces of nitrous acid, has been included in a volume of 2 ml. White precipitate first forms, then when it is gently heated, it turns red.

Ninhydrin Test :

After adding a few drops of 5% ninhydrin, 0.5 ml of plant extract was added before boiling. The violet appearance suggested the existence of amino acids.

Tests for protein :

Biuret Test :

Add 0.5 ml of plant extracts, a few drops of a 1% CuSO4 solution, and a 4% NaOH solution. The appearance of violet color suggested the existence of glycoprotein.

Millon's Test

Plant extracts, 0.5 ml, are added. Millon's reagent, 2 ml, was added. White precipitate first forms, then when it is gently heated, it turns red.

Test for oils and fats :

The dried plant was lightly compressed between the two filter papers. Oil stains detected the presence of oils and fats on the filter papers.

Test for coumarins :

Add 0.5 ml of plant extracts before adding the 10% NaOH solution. The emergence of yellow color revealed the existence of coumarins.

Test for phlobatanins :

0.5 ml of plant extracts were added before the 10% ammonia solution. Phlobatanins were identified because of the pink coloration.

Test for anthraquinones :

A few drops of 2% HCl were added after adding 0.5 ml of plant extracts. Anthraquinones were present, as evidenced by the red color of the residue.

PHARMACOLOGICAL STUDIES :

Experimental animals:

Inbreed Swiss albino rats (150-200 g) of male gender have been acquired from the veterinary department of Sicra Labs Pvt Ltd Hyderabad. These rats have been maintained in a well-ventilated chamber for 12:12 hour light/dark rotation through polypropylene enclosures. Standard pellet feeds Hindustan Lever Limited., Bangalore, and drinking the water had been made

available libitum throughout the experiment. Rats have been acclimatized to laboratory situations 1 week before the start, like experimental studies. Ethics review committee approval has been acquired through the AEC animal ethics committee,suchasCPCSEAreg.no.769/2011/CPCSE committee, for supervision and control, such as experimental animal studies[9].

Induction of Hypertension:

DSS method is where salt is given in the diet or water.

Diet:

8% NaCl diet high salt. Treating rats with a diet that includes 8% NaCl is the primary method to stimulate high blood pressure inside the DSS model. It has been used for a few years and is acknowledged by many of the study's authors. Previous Research demonstrates a particular love has been recognized 5 weeks now since trying to put these same DSS rats on such a diet that includes 8% class rats have been fed a steady diet as well as the 8% nacl eating plan just after the age of six weeks, as well as the left side of the heart handle a variety had been labeled just at 15-20 weeks. Through the latter phase, these same DSS rats were shown to work diligently in breathing and hypokinesis on the left side of the heart worldwide. All of the DSS rats ended up dead within one week after humongous respiratory congestion.

EXPERIMENTAL PROCEDURE:

ANTIHYPERTENSIVE ACTIVITY

Evaluation of Anti-hypertensive activity of leaf extract of Trachyspermum ammi in hypertensive induced rats by Dahl Salt-Sensitive (DSS)Modal [10].

DSS induced modal:

Wistar albino rats weighing 150–200 g were separated into five groups, with six rats in each group. Before the experimental investigations, the rats were starved for the entire night.

Protocols:

Salt supplementation's impact on DSS rats' blood pressure: Two groups of DSS rats (n=6 each) underwent twice-weekly blood pressure and heart rate checks.

GROUPING OF ANIMALS :

Group 1: Standard control (a 12-day supply of distilled water) Group 2: Positive Control (12 days of 2% NaCl water)

Group 3: Standard medication (10 mg/kg of nifedipine for 12 days)

Group 4: 12 days of EET at a low dose of 250 mg/kg

Group 5: 500 mg/kg of EET at a high dose for 12 days

EVALUATION OF PARAMETERS :

Effect of a single injection of *EET* on hypertensive rats:

For two weeks, two groups of DSS rats got 2% NaCl water to raise their blood pressure. After the second week, one group received an injection of 250mg/kg of EET, while the other received an infusion of normal saline. After the injection treatment, heart rates, both diastolic and systolic blood pressure, were tracked for 150 minutes [11].

Effect of chronic treatment with *EET:*

Forty-eight DSS rats have been subdivided into five groups. Each team received regular water from the tap, and another received 2% NaCl with water for drinking. A 3rd company received 2% NaCl + extract in drinking the water. To ensure that this same animal received the right recommended dose of both extracts, two different living creatures have been housed in each enclosure.

The typical water volume ingested even by rats for each day was evaluated twice weekly to be able to modify this same nifedipine information within water unless essential. Animals also were decided to weigh 2 weeks. Group 5 received water that contained 2% NaCl and got 10 mg/kg of nifedipine for every rat a day.

During the first week, group six received 2% NaCl in their drinking water and 2% NaCl + extract. Nifedipine was given in blocks containing gelatine and jelly while drinking water during the 2nd week. Heart and blood pressure rates have been supervised before the procedure and 2 times per week for 2 weeks.

RESULTS & REPORTS:

PRELIMINARY PHYTOCHEMICAL STUDIES:

Table 1 Phytochemical components of Trachyspermum ammi

S. No	Phytoconstituents	Water	Ethanol
1.	Alkaloids		
	The Dragendorff test	+	+
	The Hager test	+	+
	Wagner's examination	+	+
	Mayer's examination	-	-
2.	Carbohydrates		
	Anthrone examination	-	+
	Benedict's examination	+	+
	The Fehling test	+	+
	The Molisch test	+	+
	The Barfoed test	+	+
	The Fehling test	+	+
3.	Flavonoids		
	Shinoda test,	+	+
	ferric chloride test,	+	+
	lead ethanoate test,	+	-
	and alkaline reagent test	+	+
4.	Glycosides	-	-
	The test of Borntrager	-	-
	The Keller killer		
5.	Resins	+	+
6.	Steroids		
	Liebermann-Burchard's test	+	+
	Terpenoids		
	b. Salkowski test	+	+
7.	Tannins		
	Test for lead acetate	+	+
	Test for ferric chloride	+	+
8.	Starch	-	-
9.	Inorganic acids		
	Sulphate examination	+	-
	Test for carbonate	+	-
10.	organic acid		
	Test for malic acid	-	-
	Test for oxalic acid	-	-
11.	Ascorbic acid	+	+
12.	Phenolic compounds		
	Test for lead acetate	+	+
	Test for ferric chloride	+	+
13.	Amino acids		
	Ninhydrin test	-	-
	Millons test	-	-
14.	Protein		
	Millon's test	-	-
	Biuret test	-	-
15.	Coumarins	+	+
16.	Phelobotanins	-	-
17.	Anthraquinones	-	-

Table 2 Diastolic Blood Pressure

S.NO	TREATMENT	0 day	6th day	9th day	12th day
1.	Normal untreated	78.13± 2.00	78.0± 0.63	78 ± 0.031	75.4 ± 0.078
2.	Hypertensive untreated	78±0.1.00	82.8±1.49	87.6 ± 02.90**	91.6 ± 1.33**
3.	Hypertensive treated (nifedipine)	75.8±1.74	76.8±1.24	75.4 ± 0.063	86.4 ± 2.81**
4.	EETP 250mg/kg	76.0±2.87	81.2±1.8	83.0 ± 0.1.9**	86.8 ±1.131**
5.	EETP 500mg/kg	76±0.07	78.5±0.65	84.2 ± 0.175**	84.4 ± 0.87**

The mean + SEM values are compared to the control group using Dunnet's t-test after a one-way ANOVA with *P<0.05, **P<0.01, and ***P<0.001 values.

Table 3 Systolic Blood Pressure

S.NO	TREATMENT	0 day	6th day	9th day	12th day
1.	Normal untreated	123.0 ±0.63	124.0 ± 1.05	119 ± 2.03	123 ± 0.078
2.	Hypertensive untreated	124 ± 0.2.52	131.8 ± 1.49**	135.6 ± 0.2.90**	135 ± 1.33**
3.	Hypertensive treated (nifedipine)	124.9 ± 1.74	131.8 ±1.24	136.4 ± 0.063**	136.4 ± 2.81**
4.	EETP 250mg/kg	124.0 ± 2.57	127.2 ± 1.8	135.0 ± 0.1.9**	135.8 ± 1.131**
5.	EETP 500mg/kg	126 ± 0.07	125.5 ± 0.65	134.2 ± 0.175**	135.4 ± 0.87**

The mean + SEM values are compared to the control group using Dunnet's t-test after a one-way ANOVA with *P<0.05, **P<0.01, and ***P<0.001 values.

Table 4 Mean Pulse Rate Blood Pressure

S. NO	TREATMENT	0 day	6th day	9th day	12th day
1.	Normal untreated	168.13 ± 2.00	197.0± 0.73	277.5 ± 0.051	198.4 ± 0.088
2.	Hypertensive untreated	289.0±0.1.0	265.8±1.40*	389.6±0.2.90**	209.6±1.83**
3.	Hypertensive treated (nifedipine)	182.8±1.73	288.8±1.27	243.4±0.064	200.5±2.83**
4.	EET 250mg/kg	175.0±2.87	298.2±1.9	226.0±0.1.9**	225.9±1.123**
5.	EET 500mg/kg	168±0.08	225.4±0.65	209.2±0.177**	258.4±0.89**

The mean + SEM values are compared to the control group using Dunnet's t-test after a one-way ANOVA with *P<0.05, **P<0.01, and ***P<0.001 values.

The high dosage demonstrated a notable anti-hypertensive effect by lowering metrics like systolic, diastolic, and mean blood pressure

STATISTICAL ANALYSIS:

This same statistical significance was evaluated using one path analysis, such as variance ANOVA, and then Dunnet's comparison test, using prism graph software. All data are presented as mean values + SEM, and p<0.05 was considered significant [12].

CONCLUSION:

Preliminary phytochemical analyses were observed in the presence of carbohydrates, flavonoids, saponins, steroids, alkaloids, and glycosides. During the experiment, no rat deaths or abnormalities in behavior were noted. A notable anti-hypertensive effect was demonstrated by nifedipine. In the 8% NaCl approach to generate Hypertension in the DSS model rats, the high dose showed a substantial anti-hypertensive impact by lowering systolic, diastolic, and mean blood pressure. Flavonoids, saponins, glycosides, and alkaloids are phytochemical elements with anti-hypertensive properties. The EET was found to possess anti-hypertensive activities. The active constituent isolation and further research on its activity can be helpful for the prospective study of the treatment of hypertension.

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