Section: Research Paper
ISSN 2063-5346

Evaluation of Synergistic Effect of *Camellia Sinensis* and *Trachyspermum Ammi* for Anti-Inflammatory

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Abstract: In medicine, plant offer a great range of natural component belongs to different molecular families which have interesting biological activities which enchant various researchers to their illumination to give knowledge that will lead to advances in medicines, The physical and botanical characteristics of the leaves are authenticated by an expert botanist. 15 g of dried green tea leaves is mixed with 150 ml of acetone or ethanol (75%) or aqueous for 1 min using an Ultra Turax mixer (13,000 rpm), Phytochemical tests for confirming the presence of carbohydrates, reducing sugars, tannins, flavonoids, saponins, phytosterols and fixed oils and fats in aqueous and alcoholic extracts of *Camellia Sinensis. Camellia sinensis* and *Trachyspermum ammi* may be beneficial as adjuvants in addition to conventional anti-inflammatory drugs since they potentiated the anti-inflammatory action of ibuprofen in the relevant inflammatory models.

Keywords: Anti-inflammatory, herbal plants

1. Introduction

From the very ancient time to till now the plants have been the basis of more traditional medicines systems throughout the world and further continued to furnish mankind with new remedies. The several varieties of medicinal plants and their purified components and natural products from the medicinal plants give immense opportunities for new drug development due to the unparalleled convenience of diverse chemical compounds [1]. The enhancing the

awareness about the adverse effects of drugs had made the western pharmaceutical industries to revolve regarding the plant based Indian and Chinese medicine [2]. From the natural sources, nature has been a source of medicinal plants for past few years and an imposing number of modern drugs have been isolated from the natural sources. The several medicinal plants have been used for years in daily life to treat the numerous diseases all over the world. They have used as a remedy and for health care preparations [3].

In medicine, plant offer a great range of natural component belongs to different molecular families which have interesting biological activities which enchant various researchers to their illumination to give knowledge that will lead to advances in medicines [4]. It is crucial that any crude drug for pharmacological and pharmaceutical use required to be subjected to scrutiny for botanical identity. The role of phytochemical analysis are look for at this juncture to give a set of diagnostic features of the drug which will assist to a substantial extent to ascertain the botanical specification of the drug [5]. The phytochemicals are non-nutritive plant chemicals that show the protective and disease preventive properties. To protect itself plants produce these chemicals but the current research revealed that many phytochemicals can protect the humans against diseases [6].

Medicinal plants are another medicine for treatment of several diseases because of their presume acceptability, effectiveness, affordability, safety and low a cost. Currently, there is enhanced utilization of herbal formulations by the people due to the strong belief that these products are natural and safe for the treatment of ailments [7]. Indian government and other institutes throughout the world carry clinical and laboratory research on herbal medicine within the context of the eastern belief system but herbal medicines is not generally studied as part of conventional [8]. Observation is mostly fascinated on the consideration of efficacy of plant-based drugs used in the traditional medicine because they are economical and have less side effects. According to W.H.O about 80% of the world population relies mostly upon herbal remedies.

Utilization of herbs and plants for restorative uses has a long practice. In India and China, these foundations go back a huge number of years. When thought of as "traditional medication" utilized by local or old societies, herbal medication has risen as a well-known other option or supplement to current medication. As indicated by the World Health Organization, 4 billion individuals, just about 70 % of the total populace, utilize herbal

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medication for some part of essential human services (Abramov V, 1996). It is assessed that in the United States alone, herbal dietary enhancements surpass $3 billion every year (The U.S. Food and Drug Administration, 1999).

Ayurveda, the ancient curative system of India, succeeded in the Vedic Era in India. As per historical evidences, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were composed around 1000 B.C. The Ayurvedic Materia Medica includes 600 herbal plants along with therapeutics. Herbs like turmeric, fenugreek, ginger, garlic and holy basil are essential portion of Ayurvedic preparations. These Ayurvedic preparations formulations include one herb or more than one herb (poly-herbal preparation).

Medicinal herb is a collection of various phytochemicals like alkaloids, saponins, glycosides, sesquiterpene lactones, resins, oleoresins and oils (essential and fixed). Now a day there is rising in attention to produce formulations make up of plant based. Researchers are regularly identifying chemical constituents from herbs, isolated them and detecting their pharmacological actions.

The World Health Organization has currently defined traditional medicine (involving herbal drugs) as contain therapeutic practices that have been in existent, for hundreds of years, before the development and spread of modern medicine and still in use today [9]. In India, around 20,000 medicinal plants have been recorded although traditional communities are using only 7,000 – 7,500 plants for the treatment of several function diseases [10]. The art of herbal medicine is to dissect pharmacologically and therapeutically active herbal drugs from harmful and toxic plants and to develop combinations of several medicinal plants compounds as safe and effective herbal remedies. Standardization and strict control measures are necessary to monitor viable high quality of herbal products and to prohibit contaminations that imperfectly affect patients consuming herbal [11, 12].

2. Materials & Methods

Collection and Extraction Method of *Camellia Sinensis*

The study is conducted after obtaining ethical clearance from PRES, Pravara Rural College of Pharmacy, Pravaranagar. C. sinensis leaves are procured from local market. The leaves will verify by Botanical Survey of India, Pune. The physical and botanical characteristics of the leaves are authenticated by an expert botanist. 15 g of dried green

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tea leaves is mixed with 150 ml of acetone or ethanol (75%) or aqueous for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature and filtered using Whatman No. 1 paper. The filtered solution are evaporated under vacuum at 40°C to a constant weight and then dissolved in respective solvents- Aqueous Extract, Alcoholic Extract & Ethanolic Extract (14).

For hot water extraction, the green tea powder (0.50 g) and 85 ◦C water (18 mL) is mixed in a 50 mL centrifuge tube with a certain liquid/solid ratio, and the extraction is carried out at room temperature (25◦C) for a certain time. For ethanol extraction, the green tea powder (0.50 g) and ethanol (18mL) are mixed in a 50mL centrifuge tube with a certain liquid/solid ratio, and the extraction of green tea polyphenols is carried out at room temperature (25◦C) shaking for 24 h (17).

Collection and Extraction Method of *Trachyspermum ammi*

Ajwain (T. ammi) will obtain from the local market. The seeds will verified by Botanical Survey of India, Pune. The seeds are dried using an oven and powdered using an electric grinder. The study of plant morphology is done using a simple determination technique, the shape, size, color, and odor (13).

The dried ajwain seeds are added to ethanol (1:10 w/v). The seeds are shaken in a water bath for 30 min at 45◦C. The mixture is sonicated with an ultrasonic probe system at 40 kHz for 15 min at 40◦C. The extracts are filter through Whatman No.1. The organic solvent is concentrated by using rotary evaporator under reduced pressure (15). 40 gram of Trachyspermum ammi seed powder is dissolved in 200 ml of distilled water (16). Alcoholic extract of ajwain plant is made by maceration process by dissolving 250 g of dried shaded coarse powder in 600 ml ethanol (95%). After 5 days, the extract is filtered, concentrated, evaporated under vacuum (yield 15 g), the yield powder stored at 2-8 °C for the uses in further experiments (18).

Percentage Yield of Extract

Accurately about 500gm of powdered leaves was subjected to the extraction as per the methodology described and percentage yield was calculated.

Extraction yield (%) = [Weight of the dry extract (g)/ Weight of the sample used for the extraction (g)] x 100

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3. Phytochemical study

Phytochemical tests for confirming the presence of carbohydrates, reducing sugars, tannins, flavonoids, saponins, phytosterols and fixed oils and fats in aqueous and alcoholic extracts of *Camellia Sinensis.*

4. *In-vivo* study

Materials and Methods:

Animals

Healthy Wistar Rat (150-250gm) of either sex, procured from the Pravara Rural College of Pharmacy, Pravaranagar are used for the study.

Animal housing conditions

The animals are housed in a group of 5 per cage at a temperature of 25°C±1°C and relative humidity of 45 to 55 % and under standard environmental conditions (12 hr light and 12 hr dark cycle). The animals are free access to standard pellet rodent diet (M/s. Pranav Agro, Sangali) and water is provided ad libitum throughout the study.

Drugs and chemicals

All the chemicals used for the study are of analytical grade. The drug and chemicals are purchased from local vendors of Pune

Anti-inflammatory model

a) Paw edema:

Material: Carrageenan (1%), Plethysmometer, Extract. Ibuprofen (50mg/kg p.o)

Animals: 48 (8 groups)

Procedure: Male or female Wistar rats with a body weight between 150 and 250 g are used. The animals are starved overnight. To insure uniform hydration, the rats receive 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, again 3 and 6 h, and eventually 24 h after challenge.

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Evaluation: The increase of paw volume after 3 or 6 h is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals gives some hints for the duration of the anti-inflammatory effect. A dose-response curve is run for active drugs and *ED*50 values can be determined (19).

Results

Percentage Yield of Extract

The percentage yield was found to be:

i. Methanolic seed extract of *Trachyspermum ammi* (Ajwain) = 31.02 %w/w
ii. Aqueous seed extract of *Trachyspermum ammi* (Ajwain) = 32.48 %w/w
iii. Methanolic extract of *Camellia sinensis* (Green tea leaves) = 29.95 %w/w
iv. Aqueous extract of *Camellia sinensis* (Green tea leaves) = 31.64 %w/w

Phytochemical study

In this study the extracts of plants were subjected to phytochemical screening study and the results are represented in Table. The phytochemical test performed showed positive results for flavonoids in alcoholic and aqueous extract of *Camellia sinensis* (Green tea).

Table: Phytochemical screening of methanolic and aqueous extract of *Camellia sinensis* (Green tea)

Sr. No	Constituents	Alcoholic Extract	Aqueous Extract
1	**Alkaloids**		
	i. Dragondroff's test	-	-
	ii. Wagner's test	-	-
	iii. Mayer's test	-	-
	iv. Hager's test	-	-
2	**Carbohydrates**		
	i. Molisch test	-	-

	ii. Benedicts test	-	-
3	**Glycosides**		
	i. Legal test	-	-
	ii. Baljet test	-	-
4	**Steroids**		
	Liberman Burchard Test	-	-
5	**Proteins & Amino acids**		
	i. Biuret test	-	-
	ii. Xanthoproteic test	-	-
	iii. Lead Acetate test	-	-
6	**Saponins**		
	Foam test	-	-
7	**Flavonoids**		
	Shinoda test	+	+

- = Neagtive (absent); + = Positive (present)

Table: Phytochemical screening of methanolic and aqueous seed extract of *Trachyspermum ammi* (Ajwain)

Sr. No	Constituents	Alcoholic Extract	Aqueous Extract
1	**Carbohydrates**		
	i. Molisch test	-	++
	ii. Barfoed's test	++	+
	iii. Fehling (reducing sugar test)	+	+
	iv. Fehling (combined reducing sugar test)	+++	++
2	**Alkaloid**		
	Dragendroff's test	+	++
3	**Flavonoids**		
	FeCl3 test	+	+

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4	**Saponins** Frothing test	 +	 +
5	**Terpenes and steroids** Libarman-Burchard's test	 +	 +
6	**Tannins** i. FeCl3 test ii. Lead acetate test	 + ++	 +++ ++
7	**Anthraquinones test** Borntragen's test	 -	 -

- = Neagtive (absent); + = Positive(slightly present); ++ = Positive (moderately present)

In-vivo study (Anti-inflammatory model):

Paw Edema Group:

Sr. No.	**No. of Groups**	**No. of Rat**
1	Control	6
2	Standard (Ibuprofen) (50 mg/kg)	6
3	Aqueous Extract 1 (200 mg/kg)	6
4	Alcoholic Extract 1 (200 mg/kg)	6
5	Aqueous Extract 2 (200 mg/kg)	6
6	Alcoholic Extract 2 (200 mg/kg)	6
7	Aqueous Extract 1 + Aqueous Extract 2 (200mg/kg)	6
8	Alcoholic Extract 1 + Alcoholic Extract 2 (200mg/kg)	6
Total		48

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Group (n=6)	**Drug**	**Dose (mg, ml/kg PO)**	**Hind paw volume**					
			BDA	**ADA**				
				½ h	**1 h**	**3 h**	**5 h**	**6 h**
I	DW	10	2.25±0.05	3.56±0.13	4.42±0.11	5.88±0.06	3.45±0.17	3.33±0.16
II	IB	50	2.16±0.08	3.52±0.12	4.45±0.16	5.88±0.04	3.45±0.16	3.33±0.16
III	Aq. Extract 1 CS	200	2.30±0.09	3.55±0.16	4.43±0.11	5.87±0.05	3.44±0.15	3.32±0.15
IV	Al. Extract 1 CS	200	2.33±0.08	3.51±0.14	4.42±0.12	5.85±0.07	3.45±0.17	3.33±0.16
V	Aq. Extract 2 TA	200	2.29±0.07	3.53±0.13	4.46±0.13	5.86±0.06	3.43±0.16	3.30±0.14
VI	Al. Extract 2 TA	200	2.25±0.08	3.52±0.15	4.44±0.14	5.84±0.05	3.45±0.16	3.32±0.16
VII	Aq. Extract 1 CS + Aq. Extract 2 TA	200	2.26±0.09	3.49±0.11	4.41±0.11	5.79±0.06	3.39±0.17	3.29±0.17
VIII	Al. Extract 1 CS + Al. Extract 2 TA	200	2.24±0.08	3.47±0.12	4.39±0.12	5.78±0.05	3.38±0.16	3.26±0.17

(n, no. of animals; DW, distilled water; PO, per orally; BDA, before drug administration; ADA, after drug administration; CS**,** *Camellia sinensis*; TA, *Trachyspermum ammi;* IB, Ibuprofen; One-way ANOVA followed by Dunnett's test)

*P<0.05; **P<0.01;$^{+}$P<0.001 in comparison to control (Group I)

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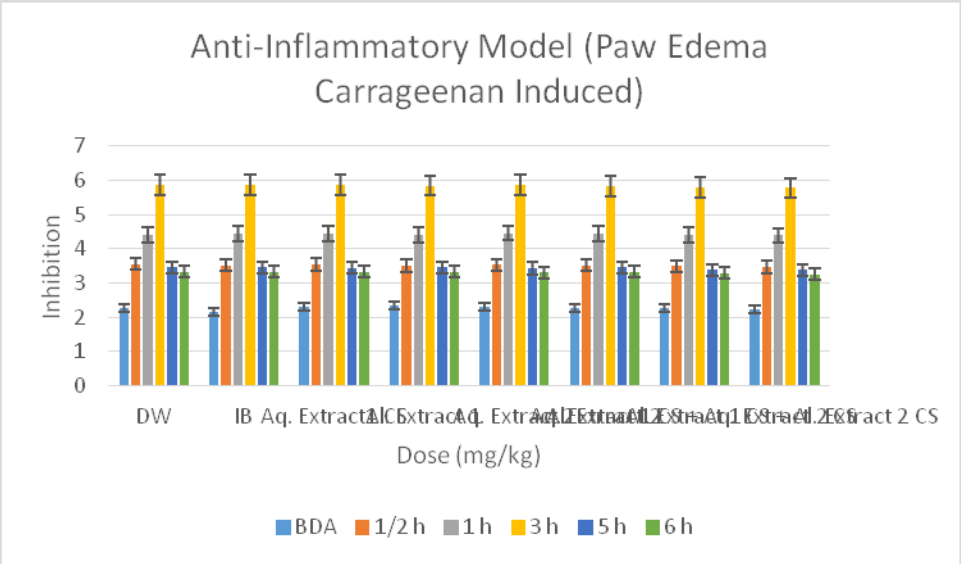

Figure: Effect of Extracts of the *Camellia sinensis* and *Trachyspermum ammi* on carrageenan induced rat paw edema

Discussion:

Both the *Camellia sinensis* and *Trachyspermum ammi* extracts demonstrated statistically significant (P<0.001) anti-inflammatory effect, which peaked at 3 hours. In comparison to the control, the anti-inflammatory effects began to show at 1 hours and persisted for 5 hours. The combined effects of the alcoholic and aqueous extract on the anti-inflammatory activity are considerable (P< 0.01).

According to our study's results, *Camellia sinensis* and *Trachyspermum ammi* may be beneficial as adjuvants in addition to conventional anti-inflammatory drugs since they potentiated the anti-inflammatory action of ibuprofen in the relevant inflammatory models. It may reduce both the dosage required and the side effects of conventional anti-inflammatory medications.

In conclusion, extracts from *Trachyspermum ammi* and *Camellia sinensis* can be used orally as an adjuvant treatment in addition to conventional anti-inflammatory drugs.

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