

Comparative Evaluation of Analgesic, Antipyretic & Anti-Inflammatory Effects of Various Extracts of Dried Fruit of *Illicium verum* Hook.f (Star anise) in Rodents

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Abstract

The current investigation was designed to evaluate the analgesic, antipyretic, and anti-inflammatory effects of various extracts (methanol, ethanol, and aqueous) of dried fruit of *Illicium verum* hook.f, using 3 different doses (150, 250, and 350 mg/kg p.o) to verify the traditional uses of this spice. In the hot plate model of analgesia, ethanol extract showed a significant reduction in pain in a dose-dependent manner compared to the control group. The maximum effect was observed at 350 mg/kg dosage i.e., 16.90 ± 0.17 s compared to the control group i.e., 5.03 ± 0.05 s. The antipyretic activity was assessed in rats by Brewer's yeast induction. The methanol and ethanol extracts produced a significant reduction in rectal temperature compared to the control group throughout the three doses. The maximum effect was observed at 350 mg/kg dosage of ethanol extract i.e., $37.1 \pm 0.8^*$ compared to the control i.e., 39.1 ± 0.3 . In the paw edema model, methanol and ethanol extracts disclosed a significant reduction in paw edema at 350 mg/kg of dose. The maximum effect was observed at 350 mg/kg dosage of ethanol extract i.e., $0.25 \pm 0.23^*$ compared to the control i.e., 0.97 ± 0.4 . In a behavioral study, locomotor activity (rearing) and exploratory activity (grooming) in mice was reduced significantly at higher doses (350 mg/kg p.o) involving the three extracts. However, scratching was increased non-significantly at all doses compared to the control group. This study concluded that various extracts of *Illicium verum* hook.f showed significant analgesic, antipyretic, and anti-inflammatory effects at different doses in a dose-dependent manner with varying potencies. The ethanol extract was found to be more potent among all, followed by methanol and aqueous extracts, whereas maximum effects were observed at 350 mg/kg of dose.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, Behavioral, *Illicium verum* hook.f

Introduction

Herbs and spices are traditionally used in medicine from generation to generation; therapeutically, they occupy a unique position in every culture for the prevention and treatment of disease due to their strong pharmacological activities and less toxicity [1]. Approximately, 80 % of the population in African and Asian countries relies on traditional medicines for their primary health care [2]. Nonetheless, many of them require scientific testing and validation [3]. Numerous herbs and spices are comprised of several active chemical constituents and secondary metabolites, which contribute a significant role in medicine [4].

Illicium verum hook.f is one of the spices, that belongs to the family of *Illiciaceae*. It is an average-sized native perennial tree grown in the Northeast Vietnam and Southwest China, formerly located in the

tropic and subtropic areas of Asia and utilized as a traditional medicine in the East Asia. In 2002, the Chinese Health organization confirmed and validated that star anise is one of the items that used as “both food and medicine” [5].

Traditionally, the tea of star anise fruit is prepared by adding 0.5 - 1 g of star anise in 150 mL of water, used for various indications, such as cold, cough, laxative, increase metabolism, and for stomach aches at 0.06 - 0.6 mL/day. Nevertheless, various encapsulated products, such as ointments and even lozenges, indicated for respiratory tract illness, contain star anise oil at the dose of 0.050 - 0.200 mL [6].

Therapeutics areas covered by *Illicium verum* include diuretic, antibacterial, stimulant, carminative, odontalgic, and stomachic. It has been used in congestion, cough, asthma, and bronchitis due to its strong anti-bacterial and anti-fungal properties [6-8]. It is also a chief constituent of anti-tussive remedies and is used to refresh breath and facilitate digestion [9]. Star anise oil has beneficial effects in the treatment of rheumatism and lower back pain. It possesses anti-oxidant properties due to the presence of linalool [10]. The oil has tranquilizing and sedative actions on the nervous system [6,11].

The literature review suggested hypothermic effect produced by methanol extract of *Illicium verum* at the dose of 3 g/kg and analgesic effect at the dose of 500 mg/kg on oral administration [12]. Some other studies reported the analgesic effect of shikimic acid derived from star anise, analgesic, and anti-inflammatory effect of aqueous extract of star anise at 0.78 and 3.90 g/kg of doses [13,14]. Recently one of the authors has reported in-vitro anti-inflammatory activity of plant extract. It was effective in inhibiting heat-induced albumin denaturation at different concentrations. Maximum inhibition of 77.87 ± 1.55 was observed at 500 µg/mL [15].

Recently, it is reported that essential oil of *Illicium verum* showed against *Acinetobacter baumannii*, a major human pathogen causing hospital-acquired infections [16]. According to some *in-vitro* studies this plant also possesses following activities: antioxidant, antidiabetic [17] antihelminth [18], and antifungal [19].

Based on the conventional consumptions of this spice in traditional Chinese medicine and its reported activities in the literature, we, therefore, designed this study to compare and evaluate the analgesic, anti-inflammatory, and anti-pyretic effects of 3 different extracts, i.e., methanol, ethanol, and aqueous extracts of dried fruit of *Illicium verum* hook.f.

Materials and methods

Plant material

Dried fruits of star anise (*Illicium verum* hook.f) were purchased from the Imtiaz Super Market, Gulshan branch, Karachi, recognized and authenticated by Prof. Dr. Iqbal Azher, Dean, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan. The voucher specimen number (IV-01-17) was issued and the sample was deposited in the Department of Pharmacognosy, University of Karachi, Pakistan.

Preparation of plant extracts

Methanol/ethanol extract preparation

The dried fruits of Star anise (*Illicium verum* hook.f) in the form of raw material were air-dried then crushed after washing. The extract was prepared by addition of 500 mL methanol in approximately 100 g of star anise dried sample (in the coarse powder form). It was soaked for 24 h at room temperature with occasional shaking. The saturated material was then purified with the help of filter paper and the filtrate was collected separately. Afterward, methanolic extract was evaporated under reduced pressure in a rotary evaporator at 40 °C, followed by freeze-drying at -30 °C in a freeze dryer. The final extract in the dried form was kept at -20 °C until used. The same procedure is repeated for ethanol extract in which we used ethanol as a solvent. The percentage yield of methanol and ethanol extracts was 31.7 and 34.5 %, respectively.

Aqueous extract preparation

500 g of dried fruit of *Illicium verum* hook.f was washed, air-dried, and crushed. It was taken in the round bottom flask and 500 mL distilled water was added to cover the material fully. Later, it was boiled in the water bath for 1 h at 90 - 95 °C. The supernatants were removed and repeated. The supernatant materials were purified with the help of filter paper (Whatman no.1). Afterward, lyophilization was performed to concentrate the filtrate and the residue was termed as an aqueous extract. The aqueous extract was stored in an amber glass bottle at -20 °C until further use. The percentage yield of aqueous extract was found to be 24.4 %.

Phytochemical studies

Different extracts of dried fruit of *Illicium verum* (Star anise) were subjected to preliminary phytochemical screening by applying qualitative tests for confirming the presence of phytoconstituents [21,22]. The presence of phytoconstituents was confirmed by TLC and the results are presented in **Table 1**.

Table 1 Qualitative analysis of various extracts of dried fruit of *Illicium verum* hook.f.

Phytochemical	Methanol Extract of <i>I.Verum</i> (MEIV)	Ethanol Extract of <i>I.Verum</i> (EEIV)	Aqueous Extract of <i>I.Verum</i> (AEIV)
Alkaloids	+	++	+
Flavonoids	+	++	+
Glycosides	+	+	-
Phytosterols	+	+	+
Phenolic compound	+	++	-
Steroids	+	+	+
Saponins	+	++	-
Terpenoids	+	++	+
Triterpenoids	+	+	-
Tannins	+	++	+

Absence of phytochemicals (-), Presence of phytochemicals (+), Presence of phytochemicals at high concentration (++)

Qualitative tests

Test for alkaloids (Wagner's test)

Two mL of each extract was dissolved in 1 % dilute hydrochloric acid and filtered. Filtrate treated with Wagner's reagent (Iodine in potassium Iodide). Reddish-brown precipitate indicates the presence of alkaloids.

Test for flavonoids

One mL of each extract was taken added with 1 mL of 10 % lead acetate solution. The formation of a yellow precipitate indicates the presence of flavonoids.

Test for glycosides

Each extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. The formation of a red precipitate indicates the presence of glycosides.

Phytosterols

One mL of each extract was added into 2 mL of acetic anhydride and 2 mL of concentrated H₂SO₄. Change in color from violet to blue indicates the presence of sterol.

Test for phenolic compounds

One drop of FeCl₃ was added into each extract and showed an intense violet color, indicating the presence of phenolic compounds.

Test for steroids (Liebermann Burchard reaction)

Two hundred mg of each extract was added with 10 mL of chloroform. Two mL of this filtrate reacted with 2 mL of acetic anhydrides and concentrated H₂SO₄. The formation of a blue-green ring indicates steroids' presence.

Test for saponins

One mL of each extract was diluted with 20 mL of distilled water in the test tube. It was shaken with a hand for 15 min. The appearance of the foamy lather layer indicates the presence of saponins.

Test for terpenoids (Salkowski test)

Five mL of each extract was mixed with 2 mL of chloroform with the addition of 3 mL conc.H₂SO₄. The formation of reddish-brown color indicates the presence of terpenoids.

Test for triterpenoids

One mL of each extract was mixed with chloroform (2 mL) and then acetic anhydride (1 mL) and concentrated sulphuric acid (1 mL) was added to the solution. The formation of reddish-violet color indicates the presence of triterpenoids.

Test for tannins

About 0.5 g of each extract was boiled in 10 mL of water in the test tube and then filtered. The brownish-green color appeared after the addition of 0.1 % FeCl₂ which indicates the presence of tannins.

Chemicals and reagents

All chemicals and drugs utilized were of analytical grade; Indomethacin (Indogesic, Wilson's pharmaceuticals, Pakistan), Aspirin (Disprin, Reckitt Benckiser Pakistan LTD), Acetaminophen (Paracetamol, GlaxoSmithKline), Carrageenan (Sigma Aldrich), and Brewer's yeast (GNC) was used as a standard.

Animals selection

The study was performed on healthy Wister albino rats of either sex, weighing between (150 - 250 g), and albino mice 25 - 30 g of (6 - 8 weeks). Animals were purchased from the animal house of Dow University of Health Sciences, Karachi. Animals were housed in separate cages under standard conditions, humidity (55 - 60 %), and temperature (23±2 °C). The circadian cycle of 12 h light and 12 h darkness was maintained. Animals were fed with the standard diet and water *ad libitum*. The apparent physical condition of these animals was monitored during this particular period. The inspection was done in the laboratory environment for a week before the administration of drugs. The entire experimental procedures, including the handling of animals, were operated by the National Institutes of Health guidelines and approved by the International Centre for Chemical and Biological Sciences (ICCBS) Animal Care and Use Committee.

Mice are preferably used for behavioral studies due to their similar anatomical, biochemical, and cellular features, similar brain functions such as memory, anxiety, aggression, and other emotional responses and genetic resemblance with humans. However, a rat is preferred over a mouse as a model of human disease and for cognition studies because of its large size and similar physiology. The proportional

size of substructures facilitates the assessment of how much of the organ is involved in the experimental lesion and the distant effects of drug administration to the specific anatomical areas [22,23].

Acute toxicity testing

The acute toxicity was evaluated according to the OECD 423 guidelines [24]. Different doses of all extracts (100, 200, 500, 700, 1,000, 1,200, 1,500, 1,800, 2,000 mg/kg p.o) were administered to the 3 groups of mice. However, the control group received only a vehicle. Groups were observed for 48 h period for any toxic signs and symptoms and mortality. Results of acute toxicity showed that this drug is safe up to the dose of 2,000 mg/kg.

Behavioral study

The behavior of animals such as rearing, grooming, and scratching were examined after 20 min (oral administration) of *Illicium verum* extracts (methanol, ethanol, and aqueous) at 3 different doses (150, 250, and 350 mg/kg), each having ($n = 6$) animals. **Table 2** depicts the results of behavior pattern changes, which were recorded by the comparison between the extracts and control group that received 0.5 mL normal saline correspondingly.

Table 2 Observation of behavioural activities (Rearing, Grooming, and Scratching) of methanol, ethanol, and aqueous extracts of *Illicium verum* hook.f.

Treatment	Dose (mg/kg)	Rearing	Grooming	Scratching
Control	0.5 mL	12.6±1.3	19.2±2.0	8.9±1.7
Methanol Extract	150	6.9±2.3*	8.9±1.2*	9.3±1.5
(MEIV)	250	4.6±1.4*	6.7±1.8*	9.5±2.4
	350	3.8±2.5*	3.3±0.7*	10.3±1.8
Ethanol Extract	150	8.4±2.7	7.4±1.4*	9.4±3.3
(EEIV)	250	7.2±3.1	3.6±0.6*	10.1±2.1
	350	3.6±2.3*	2.9±0.8*	10.7±1.7
Aqueous Extract	150	9.1±1.2	7.1±1.5*	8.9±2.3
(AEIV)	250	6.6±2.2	3.1±0.5*	9.4±2.2
	350	3.7±3.2*	2.3±0.2*	9.9±3.6
Morphine	5	3.5±1.3*	3.4±0.7*	11.3±0.9

N = 6

MEIV = Methanol extract of *Illicium verum*

EEIV = Ethanol extract of *Illicium verum*

AEIV = Aqueous extract of *Illicium verum*

All values represent mean±S.E.M

* $p < 0.05$ as compared to controls.

Analgesic activity

Hot plate method

Mice and rat paws are extremely sensitive to heat and temperatures. As a result, the reaction of animals is licking and withdrawal of paws with jumping [25]. A hot plate comprises of the electrically heated surface, generally made up of copper or heated glass surface. Fifty-five to fifty-six °C temperature is controlled on an electric hot plate. The animal was placed on the hot plate and the time was recorded by stopwatch until the occurrence of liking or jumping. Evaluation of analgesic activity was checked on Wister albino rats of either sex weighing between 150 - 250 g. Animals were distributed in 5 groups and each comprised of 6 were marked individually. The solution of Aspirin (300 mg/kg/10 mL) was prepared in normal saline water that was used as a standard drug.

Three extracts of (*Illicium verum* hook.f) dried fruit at different doses such as (150, 250 & 350 mg/kg/10 mL p.o), were given to 3 different groups of animals. Food was withdrawn 12 h prior to the administration of drugs and extracts until the completion of the experiment. The animals were placed on a hot plate after 60 min and observation was noted after 0, 0.5, 1, 2, and 3 h time intervals. The results of this experiment are tabulated in **Table 3**.

Table 3 Analgesic effects of methanol, ethanol, and aqueous extract of *Illicium verum* hook by using hot-plate thermal induce pain responses in rats.

Treatment	Dose		Hot plate Reaction Time (s)			
	(mg/kg)	0 h	0.5 h	1 h	2 h	3 h
Control	0.5 mL	5.41±0.03	5.73±0.02	5.12±0.02	5.44±0.23	5.03±0.05
Methanol Extract (MEIV)	150	4.36±0.18	4.35±0.27	5.46±0.30	5.68±0.3	6.81±0.41
	250	4.78±0.23	5.34±0.34	5.46±0.50	5.49±1.3	6.31±0.36
	350	4.79±0.78	5.21±0.56	5.53±0.16	5.91±1.2	6.45±0.38
Ethanol Extract (EEIV)	150	4.29±0.03	6.19±0.30	7.98±0.37	8.51±0.31	11.46±0.30*
	250	4.28±0.04	6.51±0.17	5.42±0.52	8.93±0.12	12.81±0.12*
	350	4.29±0.12	7.64±0.25	8.24±0.35	9.89±0.56	16.90±0.17*
Aqueous Extract (AEIV)	150	4.98±1.21	5.23±0.43	5.81±0.34	6.11±0.12	7.22±1.21
	250	4.87±0.89	5.51±0.45	5.93±0.21	6.42±0.34	7.73±1.03
	350	4.67±0.78	5.81±0.95	6.32±0.45	6.65±0.67	7.94±0.98
Acetylsalicylic acid (ASA)	300	4.7±1.2	6.6±4.5	9.90±0.7*	13.9±0.6*	19.34±1.3*

N = 6

MEIV = Methanol extract of *Illicium verum*

EEIV = Ethanol extract of *Illicium verum*

AEIV = Aqueous extract of *Illicium verum*

All values are presented as mean±S.E.M.

*P < 0.05 as compared to controls.

Antipyretic activity

The antipyretic activity was evaluated on Wister albino rats distributed into 5 groups, each contains 6 animals. A solution of 15 % yeast (10 mL/kg/bodyweight) was used to induce pyrexia in rats by subcutaneous injection [26]. The rectal temperature was recorded before and after 24 h injection of yeast solution. However, the test group was given 10 mL orally of different doses i.e. 150, 250, and 350 mg/kg/body weight of methanol, ethanol, and aqueous extract respectively. Normal saline (1 mL) was given to the control group and the standard reference drug group was treated with 1 mL Paracetamol (100 mg) in an aqueous solution. The post-treatment temperature of each animal was recorded before drug 18 h and after 0,1, 2, 3, and 4 h drug treatment. The results of antipyretic effects are presented in **Table 4**.

Table 4 Antipyretic effects of methanol, ethanol, and aqueous Extract of *Illicium verum* hook.f on brewer's yeast induced pyrexia in rats.

Treatment	Dose mg/kg	Rectal Temperature °C					
		Before Drug		After Drug			
		-18 h	0 h	1 h	2 h	3 h	4 h
Control	0.5 mL	36.7±0.3 5	37.4±1.3	39.8±0.5	39.4±0.6	39.4±0.4	39.1±0.3
Methanol	150	36.7±0.4	38.1 ±0.4	39.0±0.7	38.5±0.1	38.3±0.2	37.2±0.12*
Extract	250	37.2±0.6	38.0±0.1	39.1±0.5	38.6±0.3	37.9±0.1	37.5±0.41*
(MEIV)	350	37.8±0.3	38.1±0.5	39.0±0.2	38.4±0.4	37.6±0.2*	37.3±0.61*
Ethanol	150	38.2±0.1	38.8±0.8	38.3±0.6	38.0±0.2	37.8±0.3	37.2±0.7*
Extract	250	37.4±0.5	38.0±0.1	38.0±0.3	37.9±0.1	37.4±0.1*	37.2±0.3*
(EEIV)	350	37.8±0.3	38.7±0.5	38.1±0.9	37.8±0.3	37.5±0.2	37.1±0.8*
Aqueous	150	37.7±0.6	38.9±0.4	38.8±0.3	38.5±0.9	38.3±0.5	37.9±0.1
Extract	250	37.5±0.3	38.7±0.7	38.4±0.2	38.4±0.3	38.2±0.4	37.8±0.3
(AEIV)	350	37.6±0.1	38.4±0.5	38.7±0.2	38.3±0.5	38.1±0.6	37.9±0.4
Paracetamol	100	37.5±0.4	38.9±0.06	38.7±0.7	37.5±0.01*	37.6±0.3*	37.3±0.2*

N = 6

MEIV = Methanol extract of *Illicium verum*

EEIV = Ethanol extract of *Illicium verum*

AEIV = Aqueous extract of *Illicium verum*

All values are presented as mean±S.E.M

*P < 0.05 as compared to control.

Anti-inflammatory activity

Model of carrageenan-induced paw edema

Carrageenan-induced paw edema test was performed as described by Winter *et al.* [27]. Animals (rats) were divided into 5 groups and each group contained 6 animals. After 30 min, control group animals were orally treated with distilled water (10 mL/kg). The standard group of animals was given indomethacin (10 mg/kg) and test drug methanol, ethanol, and aqueous extracts of *Illicium verum* hook.f (150, 250, and 350 mg/kg) were given to their respective groups. Edema was induced by injection of carrageenan (1 % w/v) in normal saline (0.1 mL/kg) into the right hind paw subplantar of each animal. The edema of paw thickness was measured after 1, 2, 3, 4, and 5 h by using a plethysmometer presented in **Table 5**.

Table 5 Anti-inflammatory effects of methanol, ethanol, and aqueous extract of *Illicium verum* hook on carrageenan induced paw edema model in rats.

Treatment	Dose (mg/kg)	Time (h)					Average edema formation (mm)
		0	1	2	3	4	
Control	0.5 mL	-	0.51±0.06	0.79±.06	0.89±0.7	0.97±0.4	0.78±0.47
Methanol Extract (MEIV)	150	-	0.63±0.3	0.83±0.45	0.73±0.34	0.53±0.91	0.68±0.15
	250	-	0.61±0.12	0.57±0.36	0.54±0.44	0.51±0.34	0.55±0.31
	350	-	0.31±0.24	0.29±0.20	0.26±0.16	0.26±0.26*	0.29±0.21*
Ethanol Extract (EEIV)	150	-	0.51±0.33	0.49±0.31	0.42±0.07	0.39±0.21	0.45±0.22
	250	-	0.47±0.12	0.41±0.34	0.34±0.06	0.28±0.33	0.37±0.20*
	350	-	0.23±0.37	0.27±0.4	0.39±0.18	0.25±0.23*	0.28±0.45*
Aqueous Extract (AEIV)	150	-	0.63±1.2	0.66±0.8	0.78±0.18	0.73±0.23	0.71±0.60
	250	-	0.53±0.6	0.67±1.2	0.69±0.45	0.56±0.33	0.61±0.64
	350	-	0.36±0.87	0.45±1.7	0.55±1.6	0.59±0.48	0.48±1.16
Indomethacin	10	-	0.39±0.7	0.47±0.5	0.43±0.3	0.33±0.45*	0.25±0.48*

N = 6

MEIV = Methanol extract of *Illicium verum*

EEIV = Ethanol extract of *Illicium verum*

AEIV = Aqueous extract of *Illicium verum*

All values are presented mean±S.E.M

*P < 0.05 compared to control.

Statistical analysis

SPSS version 23 was utilized for statistics. Obtained results are presented as mean ± S.E.M. One-way ANOVA followed by post hoc Tukey's Honest Significant Difference (HSD) test was carried out for comparison with control. P-value < 0.05 was considered as significant.

Results and discussion

Behavioral activities were altered by the administration of 3 different extracts (methanol, ethanol and aqueous) of *Illicium verum* at the doses of (150, 250 & 350 mg/kg). **Table 2** shows that the locomotor activity (rearing) and exploratory activity (grooming) in mice was reduced significantly ($p < 0.05$) at higher doses (350 mg/kg p.o) of all 3 extracts used. However, scratching was increased in animals at all doses as compared to the control group.

Analgesic activities of 3 extracts (methanol, ethanol, and aqueous) of *Illicium verum*, dried fruit were assessed at the doses of (150, 250, and 350 mg/kg respectively) by the hot plate method. All the three doses of the ethanol extracts showed a significant reduction in pain response as shown in **Table 3**. Enhanced significant result ($p < 0.05$) was observed after 3 h at the dose of 350 mg/kg p.o of ethanol extract i.e., 16.90±0.17 s as compared to the control group i.e., 5.03±0.05 s. However, the methanol and aqueous extracts showed non-significant results as compared to the control group. On the other hand, standard drug acetylsalicylic acid (Aspirin) at the dose of 300 mg/kg showed a more significant result i.e., 19.34±1.3 s as compared to the control group i.e., 5.03±0.05 s.

Values of antipyretic activity after 15 % yeast solution administration in rats of all 3 extracts (methanol, ethanol, and aqueous) are tabulated in **Table 4**. The results revealed that methanol and ethanol extract at a dose of 250 and 350 mg/kg p.o produced more significant results ($p < 0.05$) to reduce the rectal temperature of rats after 3 and 4 h, as compared to the control group. However, the aqueous extract of *Illicium verum* showed non-significant effects at all doses as compared to the control group. On the other hand, standard drug paracetamol at 100 mg/kg p.o produced more significant effects after 2, 3, and 4 h of pyrexia induction by 15 % yeast solution in rats. The dose-dependent antipyretic effects of 3 extracts showed variation in their potency. The potency of antipyretic effects of all used 3 extracts were found as follows, EEIV > MEIV > AEIV. Results revealed that ethanol extract of *Illicium verum* is more potent and effective than methanol and aqueous extract of *Illicium verum* in reducing the rectal temperature in rats as shown in **Table 4**.

The results of the decrease of inflammation in paw edema of rats, which was induced by carrageenan 1 % w/v solution are presented in **Table 5**. The inflammation of paw volume in rats was significantly reduced ($P < 0.05$) in methanol and ethanol test groups of *Illicium verum* as compared to the control group. The ethanol extract exhibited significant effects in edema reduction (0.25 ± 0.23 mm) after 4 h as compared to the control group 0.97 ± 0.4 mm at 350 mg/kg of dosage. The average value of edema formation of ethanol extract was 0.27 ± 0.21 mm; however, the control group showed a 0.78 ± 0.47 mm value. However, the effective reduction of paw edema by 3 extracts followed this order EEIV > MEIV > AEIV.

Discussion

Previous researchers reported various pharmacological activities of *Illicium verum* hook.f (Star anise), encompassing carminative, stomachic, anti-rheumatic, chemopreventive, and anti-flu drug [28,29]. Phytochemical studies have revealed numerous valuable biomedical active constituents present in this spice, including anethole, anisaldehyde, *trans*-anethole, limonene, shikimic acid, sesquiterpenes, caryophyllene, α -pinene, squalene, eucalyptol, and several others from different part of star anise [30,31]. Anethole is the major constituent, more than 90 % present in star anise responsible for several medicinal properties [32]. The current study investigated and identified the potency and effectiveness of the active constituents present in *Illicium verum* of three different extracts of previously reported analgesic, antipyretic, and anti-inflammatory activities. The three different extracts (methanol, ethanol and aqueous) of the *Illicium verum* showed dose-dependent variation in pharmacological activities.

The results of behavioral models of animal studies revealed that these three extracts (methanol, ethanol, and aqueous) decreased the grooming (exploratory) and rearing (locomotor) activities which might be due to the depression of the central nervous system. The excitatory monoamines neurotransmitter released from CNS is responsible for performing motor activities and exploratory behaviors [33]. Anethole is the main constituent of star anise, proven for psychoactive effects [34]. Therefore, the general depressant effects might be due to a decline in the release of neurotransmitters by these extracts as morphine also reduced the behavioral activities [35]. However, an increase in the body scratching activity in animals was observed when compared to the control group which received simple normal saline. It is also reported that active constituent anethole and *trans*-anethole present in star anise are responsible to produce strong anxiolytic and CNS effects [36].

Previous studies on some fraction of *Illicium verum* signified that this spice has anxiolytic-like effects and increase immobility in mice is due to the presence of *trans*-anethole. The results of the current investigations disclosed that ethanol and methanol extracts produced more significant effects on grooming and rearing. This supported and validated the prior findings of anxiolytic and sedative properties of this spice as reported earlier [35].

The evaluation of analgesic activity in the hot plate method by the three extracts (methanol, ethanol, and aqueous) showed that ethanol extract produced more significant results than methanol and aqueous extract of *Illicium verum*. Similarly, the potency of ethanol extracts was found more in reducing pyrexia in rats as compared to methanol and aqueous extract. Thus, the active constituents, e.g., alkaloids, flavonoids, and saponins in the ethanol, produce more pronounced effects. Squalene is one of the major

constituents widely present in star anise, has been proved as an immune-modulating agent to strengthen the body and resist fatigue [37].

The yeast contains protein which is responsible to produce fever by an inflammatory reaction, in addition to other pro-inflammatory cytokines in the brain, which is responsible for increasing body temperature including interleukin (IL-1 β and IL-6), interferon (IFN- α), tumor necrosis factor (TNF- α), prostaglandins (PGE2 and PGI2) [38]. For the management of pyrexia, standard drug paracetamol is used to reduce the level of prostaglandins by inhibiting cyclooxygenase enzymes, which release anti-inflammatory signals from the brain at the site of injury. *Illicium verum* showed similar antipyretic action to reduce body temperature as seen with standard drug paracetamol. The previous studies validated that the presence of active constituents 3,4 Oxoisopropylidene-shikimic acids (ISA) in *Illicium verum* produced anti-inflammatory and peripheral analgesic effects by the inhibition of chemical mediators such as cytokines and prostaglandin E2 production [39-41]. All the extracts of the *Illicium verum* showed favorable results and reduced inflammation. Although, the anti-inflammatory action of ethanol extract was found more significant than methanol and aqueous extracts in reducing paw edema of rats.

Numerous investigations of medicinal plants revealed the presence of secondary metabolites such as flavonoids and alkaloids, which are found to possess analgesic, antipyretic, and other therapeutic properties. The research reported that eucalyptol present in star anise is responsible for reducing pain and inflammation by the destruction of leukemia cells *in vitro* [41]. Flavonoids are the major component to impede biosynthesis of the eicosanoids pathway as well as inhibiting the release of arachidonic acid by reducing neutrophils degranulation. As a result of these, end products inflammatory mediators such as prostaglandin and lipoxigenase suppresses which is the leading cause of inflammation, fever and pain [42]. Recently, one of the study reported that anti-inflammatory activity of *Illicium verum* is partly due to the reduction of some inflammatory mediators by suppression of NF-kB pathway [43]. The preliminary screening of the *Illicium verum* confirmed the presence of the above-mentioned constituents. Therefore, it is assumed that antipyretic, anti-inflammatory, and analgesic activities of *Illicium verum* are due to the presence of phytochemical constituents within it.

Conclusions

This study concluded that various extracts of *Illicium verum* hook.f showed significant analgesic, antipyretic and anti-inflammatory effects at different doses in a dose-dependent manner with varying potencies. The ethanol extract was found to be more potent among all followed by methanol and aqueous extracts, whereas maximum effects were observed at 350 mg/kg of dose. Hence, maximum health benefits will be attained by regular consumption of probiotics of this spice. Further investigation is required to reveal the actual mechanism of their constituents.

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