Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 2770-2783. **OPEN ACCESS**

Phytochemical, Anti-inflammatory and Analgesic Screening of an ethnomedicinal plant *Engelhardia colebrookiana* Lindl.

Muhammad Ajaib[1], Musfirah Anjum[1], Saiqa Ishtiaq[2], Faiza Shafi[1], Samia Abid[1], Khizar Hayat Bhatti[3] and Muhammad Fiaz Qamar[4]

[1]Department of Botany, Mirpur University of Science and Technology (MUST), Mirpur-10250 (AJK), **Pakistan**
[2]University College of Pharmacy, University of the Punjab, Lahore-54000, **Pakistan**
[3]Department of Botany, Hafiz Hayat Campus, University of Gujarat - 50700, **Pakistan**
[4]College of Veterinary & Animal Sciences, 12-Km Chiniot Road, Jhang. **Pakistan**

***Correspondence:** majaibchaudhry@yahoo.com **Received 20-07-2020, Revised: 01-11-2020, Accepted: 20-11-2020 e-Published: 22-11-2020**

Engelhardia colebrookiana Lindl. is a plant of the Juglandaceae family locally known as Samma. Local uses in folk medicine include root and leaves as antiseptic and massage on teeth. There is no scientific evidence justifying its effectiveness as an analgesic and anti-inflammatory agent. Therefore, the aim of this study was to investigate the phytochemicals of methanolic extract of selected plant's leaves and bark on analgesic and anti-inflammatory activities *in vivo* using mice and rats as animal model at the doses of 100 mg/kg, 200 mg/kg and 300 mg/kg body weight. The phytochemical analysis revealed that alkaloids, flavonoids, triterpenoids, cardiac glycosides, proteins, carbohydrates and sterols were present in MeOH extract of leaf and bark of *E. colebrookiana*. The analgesic activity was examined against tail immersion, hot plate and formalin tests in mice. The anti-inflammatory activity was investigated by using carrageenan induced hind paw edema method in rats. This plant reduced the nociceptive response of the animals as evaluated in the formalin and carrageenan test. Inflammatory studies showed that, leaves and bark extracts of chosen plant produced significant inhibition of carrageenin induced rat paw edema during 2-3 hours ($p<0.05$) as compared to the control. It showed 42.5% and 44.9% reduction in paw edema by leaves and bark respectively at 2 hr with 300 mg/kg. There is no significant analgesic effect was seen at 100 mg/kg dose extract. However, maximum latency time of 40.4±0.4 and 39.8±0.8 seconds were observed with 300 mg/kg dose extracts of leaves and bark respectively at 2 hr for the hot plate method. Tail immersion method also showed a similar pattern of results starting from minimum 100 mg/kg to maximum 300 mg/kg of methanolic extracts of leaves i.e., 30.2±0.02 and bark i.e., 29.2±0.1 of *E. colebrookiana*. Results of standard and different doses of methanolic extract *E.* colebrookiana were statistically significant ($p<0.05$) when compared with control. Aspirin 300 mg/kg showed significant ($p<0.05$) reduction in edema when it was compared with control. Similarly, different concentrations (200 mg/kg and 300 mg/kg) of the respective plant showed significant ($p<0.05$) dose dependent reduction in edema in comparison to control. However, a dose of 100 mg/kg did not produce a significant effect. The results showed that the plant exhibited significant activity as antinociceptive agent. So, this plant can be used as reducing pain and inflammatory disorders in the future

Keywords: Analgesic, Anti-inflammatory, *Engelhardia colebrookiana*, Hot plate method, Medicinal plants, Juglandaceae.

INTRODUCTION

Medicinal plants used by traditional people for the relief of many diseases. Many plants contain compounds that have analgesic properties (Verpoorte, 1999). Analgesics are the substances which reduce pain sensation by increasing the pain threshold to outside stimuli, i.e., chemical, thermal and physical pressure (Tripathi, 2003). Inflammation is a biological response of tissues to injury (Ajaib et al. 2018). It characterized by enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair (Palladino et al., 2003; Ferrero-Miliani et al., 2007). For many years, inflammation documented as a simple allergic reaction.

Phytochemicals from medicinal plants showing antimicrobial, antioxidant and anti-inflammatory activities have the potential of filling this need because of structures are different from those of the more studied and their those of the more action may too very likely differ (Ajaib et al. 2016; Fabricant et al. 2001). In this growing interest, many of the Phytochemical bioactive compounds from a medicinal plants have shown many pharmacological activities (Prachayasittikul et al., 2008; Chen et al., 2008). Screening of various bioactive compounds from plants has led to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases (Siddiqui et al., 2017; Mukherjee et al., 2007).

There are many medicinal plants that have a long history of traditional use of the healing properties against several diseases (Ajaib et al. 2020). For the treatment of these diseases synthetic medicines are used. The greatest disadvantage of these medicines is their toxicity (Ajaib et al. 2016; Kamran et al. 2016; Verpoorte, 1999; Amresha et al. 2007; Juneja et al. 2007). Therefore, researchers have recently paid attention to safer Phytomedicine and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Anjum and Hussain, 2015; Mazhar et al. 2015; Ajaib et al. 2013).

Ethnomedicinally the stem bark, root bark and leaves of *E. colebrookiana* is used treat many disorders such as inflammation on gums, reduce toothache and healing of wounds (Ajaib et al. 2014). This study, therefore seeks to examine *Engelhardia colebrookiana* Lindle for anti-inflammatory and analgesic activity since pain is one of the cardinal signs of inflammation.

MATERIALS AND METHODS

Plant Material

Engelhardia colebrookiana Lindle belongs to family Juglandaceae collected from Mansoh hills District Kotli, Azad Jammu & Kashmir. It is locally known as Samma. The plant specimen was collected, dried and mounted on herbarium sheet and then submitted in Department of Botany, Mirpur University of Science and Technology, Bhimber Campus voucher no. MUST.BOT.5351.

E. colebrookiana

Methanolic Extract of Plant

About 250 g of shade dried leaves and bark of selected plant, i.e. *E. colebrookiana* was soaked in 1000 ml of methanol in airtight container and kept for a week. After a week filtered material with cotton cloth. Then it was filtered through Whatman filter paper. The filtrate obtained was evaporated using a rotary evaporator. Completely dried extract of methanol is obtained.

PhytoChemical Analysis (Qualitative tests)

The Bioactive compounds were analysed by the qualitative tests for the plant extracts following the methodology of Evens (2009) and Shah (2010). It was screened for triterpenoids, sterols, cardiac glycosides, flavonoids, alkaloids, proteins, carbohydrates, saponins, lipids and tannins by using standard procedures. The all tests analysed are given below.

Tests for Sterols

Liebermann Burchard test:

2ml of MeOH extract of sample plant mixed with 2 ml of chloroform, 2 ml of glacial acetic acid along the test tube walls and then added 2 ml of Conc. H_2SO_4. Appearance of green colour in chloroform layer showed the presence of sterols.

Sulphur test:

A pinch of sulphur added in 2 ml of test solution of plant extract. Sulphur sank into the solution indicated the presence of sterols.

Tests for Triterpenoids:

Salkowaski test

2 ml of methanolic extract of plant added in 1 ml of chloroform then added carefully 1ml Conc. H_2SO_4 along test tube wall. Yellow colour layer of chloroform indicated the presence of triterpenoids.

Liebermann Burchard test

2ml of MeOH solution of *E. colebrookiana* extract added in 1 ml of chloroform and 1 ml of glacial acetic acid along the test tube wall then add carefully 1 ml of Conc. Sulphuric acid. Appearance of deep red colour indicated the presence of triterpenoids.

Tests for Flavonoids

Zinc-hydrochloride acid-reduction test

1 ml of plant extract solution mixed with Zinc dust then added few drops of HCl. Presence of flavonoids is indicated by appearance of green colour precipitates.

Alkaline reagent test

Took 2 ml of MeOH extract of *E. colebrookiana* in test tube then added few drops of dilute Sodium hydroxide. Yellow colour develops, which become colourless on addition of dilute HCl indicated the presence of flavonoids.

Test for Alkaloids

Mayer's test

Took 2 ml of plant extracts solution in test tube and added 1 ml of Mayer's reagent, formation of cream color precipitate indicated the presence of alkaloids.

Hager's test

2 ml of Hager's reagent added to 1 ml test solution. Appearance of yellow color precipitates indicated the presence of alkaloids.

Tests for Proteins

Biuret test

1 ml of 40% NaOH added into 2 ml of test solution, then added Copper sulphate solution. Blue colour appeared due to presence of proteins.

Xanthoproteic test

2 ml MeOH extract of *E. colebrookiana* and added 1 ml of Conc. H_2SO_4. Yellow colour ppt formed, indicated presence of protein.

Tests for Cardiac glycosides

Keller-killani test

Took 1 ml of MeOH extract of *E. colebrookiana* in test tube and added 2-3 ml of glacial acetic acid, then added 2 ml of Conc. H_2SO_4 along walls of test tube. Two layers formed, in the lower layer reddish brown colour appeared and in upper layer bluish colour appeared, indicated the presence of cardiac glycosides.

Tests for Carbohydrates

Barfoed's test

1 ml of Barfoed's reagent added in 2 ml of MeOH extract of plant. On heating formation of brick red precipitates indicated the presence of carbohydrates.

Animals

In the present study, total 12 albino mice (15-35 g) and 5 adult albino rats (150-200 g) of either sex were obtained from Animal House of College of Pharmacy Punjab University, Lahore. These animals were acclimatized for about 7 days under standard environmental conditions and were maintained on regular pellet food and clean water. The study was conducted following approval by the institute.

Analgesic activity

Analgesic activity of *E. colebrookiana* was carried out by using 3 different methods:

Hot Plate Method

Analgesic activity was tested in mice using the hot plate method (Janseen and Jagneau, 1957). 25 mice of either sex were grouped in five (n=5 per group). Each group received one dose of the leaf or bark extract, i.e., 100, 200, 300 mg/kg, Aspirin (Standard drug) 300mg/kg and Control. After 30 min extract given, animals were lowered onto the surface of hot plate (50±2^o C) enclosed with large beaker and start stop watch. Time for the mice to raise or lick the hind limb was noted as reaction time (RT). Don't exceed the time above 90s to prevent mice from being burnt (Sharma et al. 1982).

Tail Immersion Method

The mice were screened for sensitivity by immersing 2-3 cm the tail of the mice gently in hot water maintained at 55^{o} C. The mice that lifted their tails from hot water within 5 sec were selected for the study. Overnight fasted mice were divided into five groups (n=5). Each group received on dose of leaf or bark extract, i.e., 100, 200, 300 mg/kg, Aspirin (300mg/kg) and one group served as control. 9-10 readings were measured after every 30 min (Gupta et al.2015).

Formalin induced paw licking in mice:

It is one day experiment, overnight fasted mice was treated with oral dose of extracts or drug before 30 min, 1.5 µl formalin injected into the dorsal lateral surface of the left hind paw. Counted the number of licking and biting by each animal at the interval of 10 min for 30-40 min (Coderre and Melzack, 1992). The response was bi-phasic, the initial nociceptive response (0-5 mins) after formalin injection indicated the early phase while (15-30 mins) indicated the late phase. This experiment was carried out in a large beaker.

Anti-inflammatory activity

Carrageenan induced paw edema in rats

Anti-inflammatory activity of methanolic extract of *E. colebrookiana* was investigated by using carrageenan induced rat paw edema method. Inflammation was induced in male albino rats by injecting 0.1 ml of 1% Carrageenan into sub-plantar surface of the right hind paw of rats half an hour after oral administration of plant extracts or drug (Winter et al., 1962). The hind paw volume was measured just before and after every 30 min after Carrageenan injection using digital vernier caliper. Diclofenac sodium (300 mg/kg) is used as standard in this method. Percentage inhibition of edema was computed using the formula:

$$\% \, \boldsymbol{inhibition} = \frac{(\boldsymbol{Ct} - \boldsymbol{Co}) \, \boldsymbol{Control} - (\boldsymbol{Ct} - \boldsymbol{Co}) \, \mathbf{test} \times \mathbf{100}}{(\boldsymbol{Ct} - \boldsymbol{Co}) \boldsymbol{Control}}$$

Where Co= paw size before Carrageenan injection; Ct=paw size at time t-hour after Carrageenan injection.

Statistical analysis

The results of the experiments were expressed as mean ± S.E.M. Statistical analysis was performed to find out variance by two-way ANOVA in MS word 2007 (analysis of variance).

P value < 0.05 was considered as statistically significant (Lee et al., 2003).

RESULTS AND DISCUSSION

The phytochemical screening of leaf and bark extracts of *Engelhardia colebrookiana* Lindl. (Table 1) was carried out employing standard protocols to characterize active principles responsible for its ethno-medicinal properties. Hot plate method's results are shown in Table 2. The *E. colebrookiana* extracts at the all doses showed significant results Fig 1, 2 and 3. The maximum effect was observed with 300 mg/kg at the 2nd hour of study, leaf extract showed 40.4±0.4 latency time and bark with 39.8±0.8, which was comparable to the standard drug Aspirin i.e., 27.6±0.58. Respective plant extracts at 300 mg/kg revealed a progressive increase in latency time compared to control. The overall results were found to be statistically significant ($p < 0.05$ and $p < 0.01$).

The results of the tail immersion test in mice are shown in table 3. The results showed that the leaf and bark extracts at the dose of 300 mg/kg and drug Aspirin significantly increases the pain reaction time (PRT) when compared to control group. At the dose of 100 and 200 mg/kg, there was a marginal increase in the mean of PRT. The results from 8.6±0.56 shown by leaves extract of 100 mg/kg at 0 hr increased to 30.2±0.02 of 300 mg/kg dose at 2 hr. Bark extracts also exhibited significant results shown in Fig 4, 5 and 6.

Table 4 showed the results obtained from Formalin Pain induced method. All the oral extracts doses reduced the number of paw shakes, licking and biting the formalin-injected paw in both phases. The leaves and bark of plant extracts caused a significant inhibitory effect on both phases of formalin-induced pain as compared to control. With increase of doses of 100 mg/kg, 200 mg/kg and 300 mg/kg, the plant showed significant results. The total paw licking and biting event decreasing with increases of extract concentration.

Leaf with 100 mg/kg, 200 mg/kg and 300 mg/kg showed 8.4±0.37, 4.8±0.43 and 8.6±0.68 respectively at 0 min. The values become decrease with time, after 30 min 0.2±0.1, 0.2±0.37 and 0.0±0.0 shown by respected doses, 0.1±0.01 recorded from Aspirin Fig 7, 8 and 9.

Results of extracts, standard and control groups of anti-inflammatory activity is summarized in table 5. Leaf, bark and standard drug showed significant reduction in paw thickness when compared with control. 100 mg/kg dose did not produce significant results, i.e, 1- 13.5 % inhibition shown from 0hr – 4hr by leaf extract and 12.2-11.6% of bark extract.

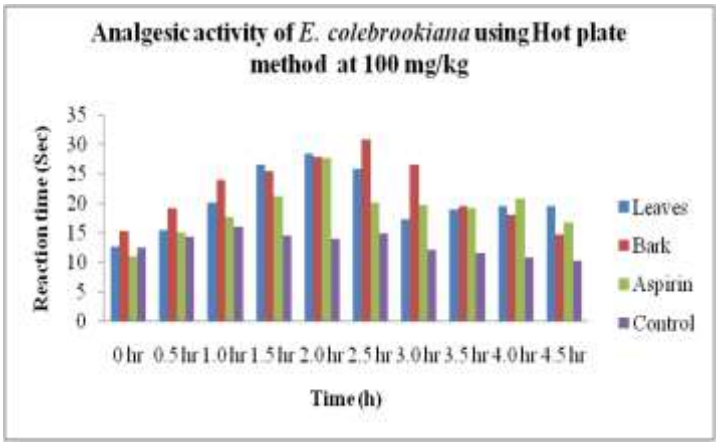

Figure 1: Graphical representation of analgesic effect of 100 mg/kg extracts of *E. colebrookiana* using Hot Plate method

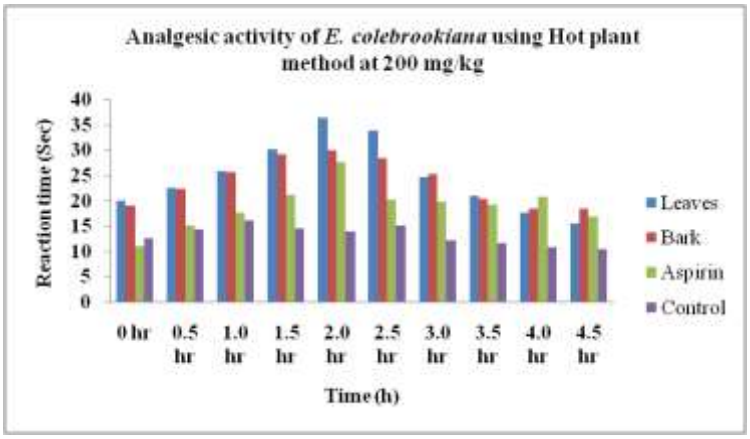

Figure 2: Graphical representation of Analgesic effect of 200 mg/kg extracts of *E. colebrookiana* using Hot Plate method

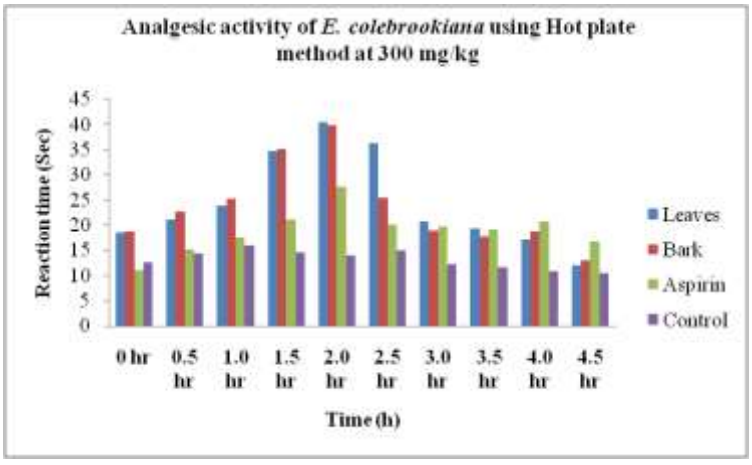

Figure 3: Graphical representation of Analgesic effect of 300 mg/kg extracts of *E. colebrookiana* using Hot Plate method

Table 1: Phytochemical analysis of the leaf and bark macerates of *Engelhardia colebrookiana* Lindl.

Constituents	Phytochemical tests	MeOH extracts of *E. colebrookiana*	
		Leaf	Bark
Triterpenoids	Salkowaski test Liebermann's test	+ +	+ +
Sterols	Liebermann's test Sulphur test	+ +	-- +
Cardiac glycosides	Keller-killani test	+	+
Flavonoids	Zinc-hydrochloride acid-reduction test Alkaline reagent test	+ +	-- +
Alkaloids	Mayer's test Hager's test	+ +	-- +
Proteins	Biuret test Xanthoproteic test	+ --	+ --
Carbohydrates	Barfoed's test	+	+

*Phytochemical detection key: − = Absent, + = Present

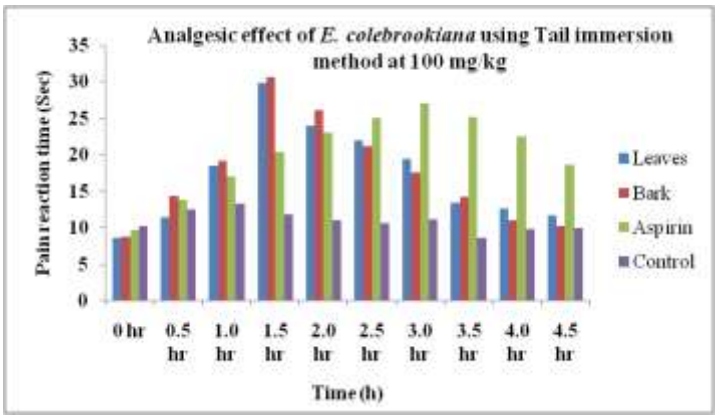

Figure 4: Graphical representation of Analgesic effect of 100 mg/kg extracts of *E. colebrookiana* using Tail immersion method

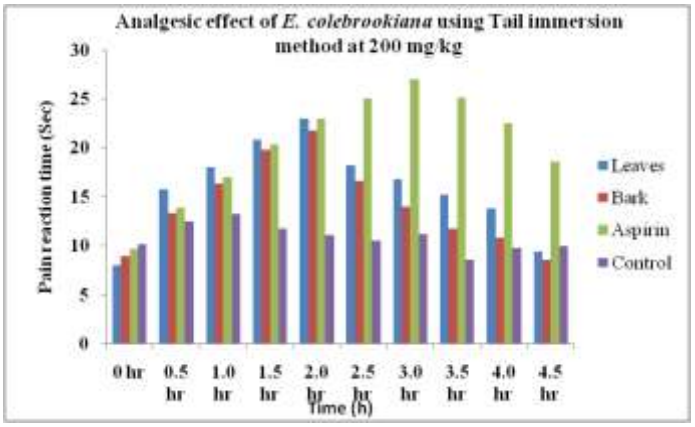

Figure 5: Graphical representation of Analgesic effect of 200 mg/kg extracts of *E. colebrookiana* using Tail immersion method

Table 2: Analgesic effect of MeOH extracts with different concentrations of *E. colebrookiana* using Hot Plate method

	Leaf			Bark			Aspirin	Control
	100 mg/kg	200 mg/kg	300 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	300 mg/kg	
0 hr	12.8±0.86	20.0±0.45	18.6±0.24	15.2±0.20	19.0±0.32	18.8±0.37	11.0±0.04	12.6±0.58
0.5 hr	15.5±0.20	22.6±0.24	21.2±0.37	19.2±0.58	22.4±0.75	22.6±0.24	15.1±0.06	14.4±0.37
1.0 hr	20.1±0.80	25.8±0.37	23.8±0.73	24.0±0.60	25.6±0.24	25.2±0.37	17.6±0.24	16.0±0.54
1.5 hr	26.6±0.73	30.2±0.37	34.8±0.37	25.4±0.40	29.2±0.37	35.2±0.58	21.1±0.10	14.6±0.81
2.0 hr	28.4±1.47	36.4±0.24	40.4±0.4	27.8±0.73	30.0±0.45	39.8±0.8	27.6±0.58	14.0±1.64
2.5 hr	25.8±1.53	34.0±0.89	36.4±0.37	22.8±0.49	28.4±0.40	25.4±0.6	20.1±0.06	15.0±0.89
3.0 hr	17.4±0.68	24.8±1.5	20.8±0.37	20.6±0.50	25.2±0.60	19±0.37	19.8±0.36	12.2±0.37
3.5 hr	19.0±0.45	21.0±0.45	19.4±0.24	19.6±0.40	20.4±0.68	17.8±0.37	19.2±0.58	11.6±0.40
4.0 hr	19.6±0.51	17.6±0.68	17.2±0.37	18.0±0.31	18.4±0.67	18.8±0.37	20.8±0.78	10.8±0.37
4.5 hr	19.6±0.24	15.4±1.03	12.0±0.77	14.8±0.37	18.4±0.51	13±0.37	16.8±0.78	10.4±0.24

Results were expressed as Mean± SEM (standard error mean), n=5. P < 0.05 were considered statistically significant

Table 3: Analgesic effect of MeOH extracts with different concentrations of *E. colebrookiana* using Tail immersion method
Results were expressed as Mean± SEM (standard error mean), n=5. P < 0.05 were considered statistically significant

	Leaf			Bark			Aspirin	Control
	100 mg/kg	200 mg/kg	300 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	300 mg/kg	
0 hr	8.6±0.56	8±0.43	11.2±0.37	8.8±0.68	9.0±0.83	10.1±0.63	11.6±0.04	10.2±0.37
0.5 hr	11.4±0.4	15.8±0.71	17.6±0.4	14.4±0.4	13.4±0.75	18.3±0.2	14.1±0.06	12.5±0.03
1.0 hr	18.5±0.5	18±0.63	22.4±0.05	19.2±0.73	16.4±0.42	25.4±0.51	18.8±0.24	13.3±0.03
1.5 hr	29.8±0.8	20.8±0.37	26.4±0.24	30.6±0.6	19.8±0.1	26.5±0.02	19.4±0.10	11.8±0.62
2.0 hr	24±0.02	23±0.45	30.2±0.02	26.1±0.02	21.8±0.17	29.2±0.1	25.3±0.58	11.1±0.44
2.5 hr	22±0.04	18.2±0.72	27.3±0.55	21.1±0.04	16.6±0.45	28.6±0.36	19.0±0.06	10.6±0.45
3.0 hr	19.4±0.4	16.8±0.04	24.3±0.51	17.6±0.2	14±0.8	25.6±0.61	20.6±0.36	11.2±0.82
3.5 hr	13.5±0.75	15.2±0.2	20.6±0.1	14.2±0.02	11.8±0.3	19±0.24	20.0±0.58	8.6±0.04
4.0 hr	12.6±0.6	13.8±0.58	17.8±0.37	11±0.33	10.8±0.55	16.7±0.53	20.8±0.78	9.8±0.2
4.5 hr	11.7±0.01	9.4±0.5	14.8±0.01	10.2±0.4	8.6±0.28	15.9±0.7	14.7±0.6	10±0.43

Table 4: Analgesic effect of MeOH extracts with different concentrations of *E. colebrookiana* using Formalin pain induced

	Leaf			Bark			Aspirin	Control
	100 mg/kg	200 mg/kg	300 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	300 mg/kg	
0 min	8.4±0.37	4.8±0.43	8.6±0.68	7.0±0.15	6.8±0.78	7.6±0.29	5.8±0.37	7.9±0.2
10 min	1.2±0.1	1.6±0.71	0.4±0.37	1.8±0.01	2.2±0.15	1.0±0.1	1.9±0.04	3.2±0.05
20 min	1.0±0.02	0.4±0.63	0.2±0.03	1.2±0.1	0.6±0.01	0.6±0.02	0.5±0.1	4±0.02
30 min	0.2±0.1	0.2±0.37	0.0±0.0	1.0±0.37	0.2±0.2	0.0±0.0	0.1±0.01	2±0.1

Results were expressed as Mean± SEM (standard error mean), n=5. P < 0.05 were considered statistically significant

Table 5: Anti-inflammatory evaluation and % inhibition of MeOH extracts with different concentrations of *E. colebrookiana* using Carrageenan Induced Paw Edema method

	Leaf			Bark			Standard	Control
	100 mg/kg	200 mg/kg	300 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	Diclofenac sodium	
0 hr	2.98±0.17 1%	2.60±0.16 13.6%	2.45±0.13 18.6%	2.64±0.2 12.2%	3.2±0.35 6.3%	2.22±0.25 26.2%	2.7±0.22 10.2%	3.01±0.24
1 hr	3.97±0.11 6.36%	3.34±0.24 21.2%	3.35±0.28 20.9%	4.03±0.28 4.9%	3.59±0.27 15.3%	3.4±0.30 19.8%	3.32±0.27 21.6%	4.24±0.1
2 hr	3.3±0.01 19.3%	2.58±0.01 36.9%	2.35±0.1 42.5%	3.45±0.02 14.6%	2.45±0.02 40%	2.25±0.6 44.9%	2.96±0.1 27.6%	4.09±0.14
3 hr	3.05±0.33 14.3%	2.39±0.2 32.8%	2.14±0.32 39.8%	3.23±0.19 9.2%	2.29±0.17 35.6%	2.18±0.01 38.7%	2.08±0.01 41.5%	3.56±0.37
4 hr	2.67±0.02 13.5%	2.22±0.37 28.1%	2.07±0.02 33%	2.73±0.1 11.6%	2.3±0.1 25.5%	2.11±0.03 31.7%	2.00±0.2 35.2%	3.09±0.01

Results were expressed as Mean± SEM (standard error mean), n=5. P < 0.05 were considered statistically significant

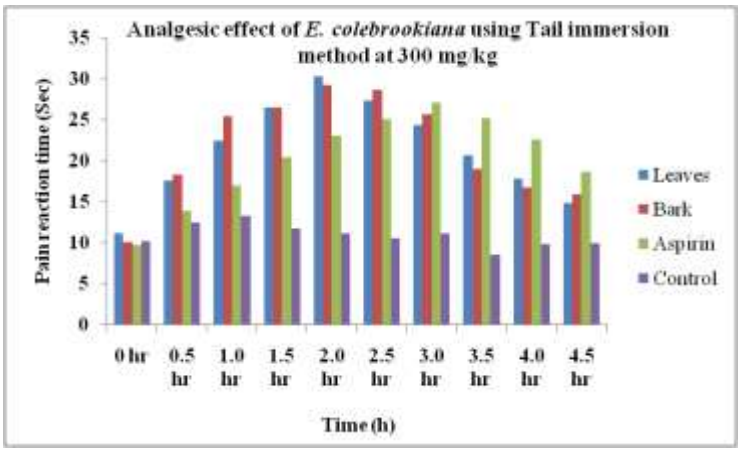

Figure 6: Graphical representation of Analgesic effect of 300 mg/kg extracts of *E. colebrookiana* using Tail immersion method

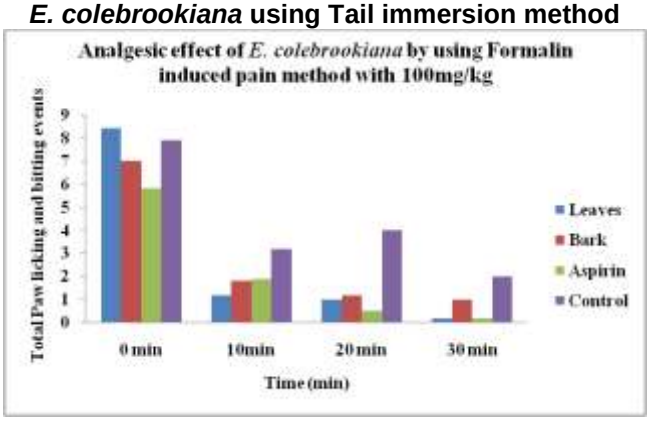

Fig 7: Graphical representation of analgesic effect of *E. colebrookiana* with 100 mg/kg

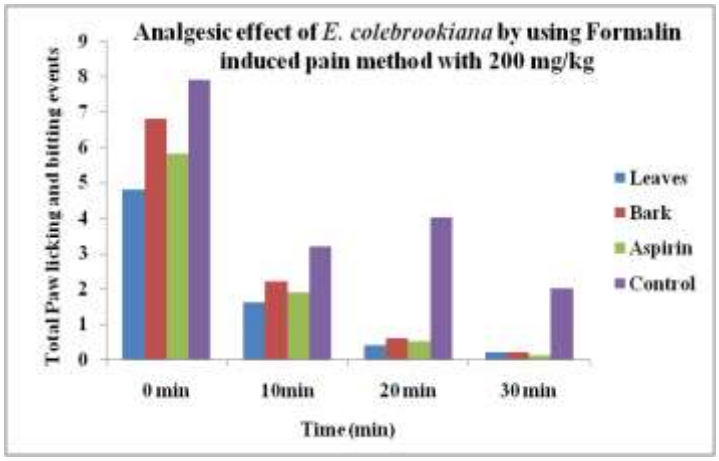

Figure 8: Graphical representation of analgesic effect of *E. colebrookiana* with 200 mg/kg

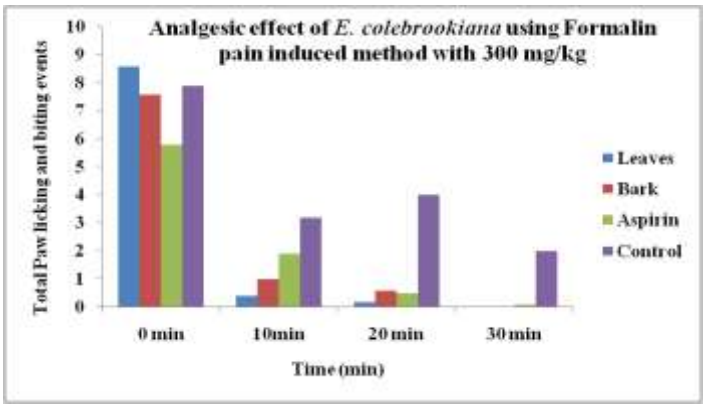

Figure 9: Graphical representation of analgesic effect of *E. colebrookiana* with 300 mg/kg

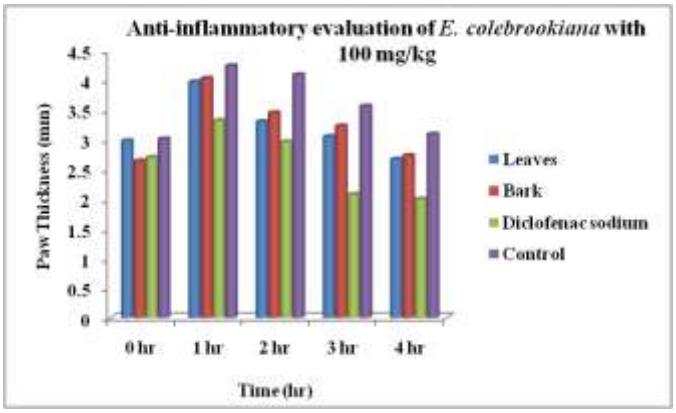

Figure 10: Anti-inflammatory effect of *E. colebrookiana* with 100 mg/kg

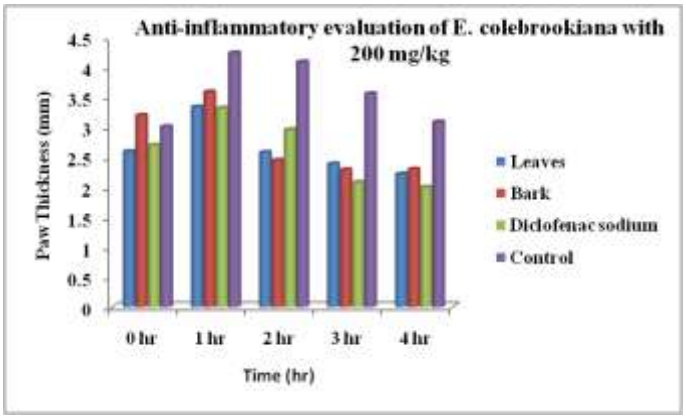

Figure 11: Anti-inflammatory effect of *E. colebrookiana* with 200 mg/kg

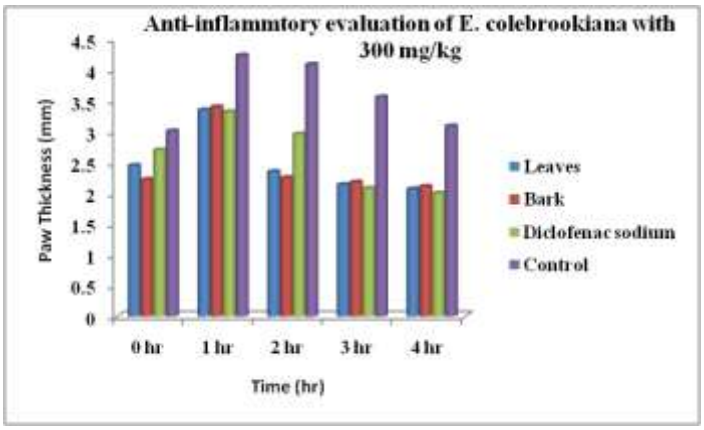

Figure 12: Anti-inflammatory effect of *E. colebrookiana* with 300 mg/kg

Maximum % inhibition shown by bark extract with 300 mg/kg i.e., 44.9% (Table 4).

The result of the phytochemical investigation of the methanolic extract of *E. colebrookiana* indicates the presence of alkaloids, flavonoids, sterols, carbohydrates, cardiac glycosides, proteins and triterpenoids (Asif, 2013). The presence of alkaloids represents the possibility of some biological activity of the extracts of *E. colebrookiana*; such as anti-microbial, analgesic and anti-tumor. The presence of flavonoids responsible for anti-allergic and anti-inflammatory therapies (Wu et al. 2008).

In this study the animal models of analgesia and inflammation showed that the MeOH extract of *Engelhardia colebrookiana* possesses potent analgesic and anti-inflammatory property, especially at 300 mg/kg. The results of the present study described that the MeOH extract of selected plant contains analgesic activity evident in the three analgesic models and inflammatory pain model, which is suggestive of the presence of both peripheral and centrally mediated mechanisms.

Hot plate method has been used to find out central anti-nociceptive effect (Akkol et al. 2009; Lavich et al. 2005). According to Pini et al. the hot plate test involved in central anti-nociceptive mechanism of extracts/drugs because only centrally acting drugs were able to affecting this test (Hosseinzadeh and Younessi, 2002). Centrally acting compounds/agents activated the release of endogenous peptide by PGA (Periaqueductal Gray Matter), which inhibits the pain muscle transmission within the dorsal horn in the spinal cord (Katzung, 2005). Inhibition of pain perception by *E. colebrookiana* indicates its central action. The effect observed was dose dependent and statistically significant.

The tail immersion method is the confirmatory test towards the centrally acting component of the pain mechanism (Santos et al. 1994), believed to be spinally mediated reflex. The central action of *E. colebrookiana* and Aspirin was proved as its effect was abolished by blocking action of naloxone, an antagonist to the μ-receptor (Clark, 1988; Wibool, 2008).

Analgesic activity of *E. colebrookiana* has been confirmed by formalin-induced paw licking and biting method. The formalin test in mice has been proposed as a chronic pain model which is sensitive to centrally active analgesic agents by Dubuisson and Dennis, 1977. Selection of formalin test was due to several advantages, i.e., sensitivity to mild analgesics, the ability to mimic human clinical pain conditions, production of tonic stimulus and sensitivity to non-steroidal anti-inflammatory drugs (Prado et al. 1990; Tjolsen et al. 1992; Santos et al. 1997; Hunskaar and Hole, 1987). According to this method, drugs acting through a central mechanism inhibit the early response called neurogenic phase where as those acting peripherally are good effective in the late phase known as inflammatory phase. The extract inhibited early and late phases of the formalin induced pain indicate that *E. colebrookiana* is a drug which acts both centrally and peripherally.

Carrageenan has been widely used to induce experimental inflammation used for the screening the selected plant possessing anti-inflammatory activity. It induces an inflammatory reaction in two different phases. The initial phase has been attributed to the release of serotonin, histamine and bradykinin on vascular permeability (Vinegar, 1969) and the later phase has been due to over production of prostaglandin in tissues (Di-Rosa, 1974). *E. colebrookiana* and Diclofenac sodium

produced a marked inhibition of Carrageenan-induced rat paw inflammation by inhibiting the mediators of acute inflammation inducing its anti-inflammatory activity.

CONCLUSION

The present work of analgesic and anti-inflammatory activity of leaf and bark extracts of *Engelhardia colebrookiana* was found to be potent ability. It has been observed that the selected plant has marked beneficial effects against peripheral, central and inflammatory pain models. This action may be due presence of secondary metabolites i.e., flavonoids, sterol etc. We would like to conclude that it is worthwhile to think, to use *E. colebrookiana* as drugs and further studies should be initiated to establish exact mechanism of action and elaborative phytochemical investigations to find out which active compound is responsible for analgesic and anti-inflammatory activity. These reports may serve as a foot step in the research of potent analgesic and anti-inflammatory drug.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

MA designed and MA performleed the experiments and both also wrote the manuscript. SI, FS, SA, KHB and MFQ help in experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Ajaib M, Ishtiaq S and Siddiqui MF, 2018. Comparative Analgesic Evaluation of Different Parts of *Himalrandia tetrasperma* and *Wendlandia exserta* of family Rubiaceae After Induction of Pain in Mice. Pak. J. Pharm. Sci. 31(6): 2509-2514.

Ajaib M, M Ishtiaq, Shafi F, Bhatti KH and Zahid MT, 2020. Antimicrobial and antioxidant analysis of *Strobilanthes glutinous*: an unexplored medicinal plant. Bioscience Research 17(2): 1521-1534.

Ajaib M and Khan Z, 2014. Ethnobotanical Studies of Useful Trees of District Kotli, Azad Jammu and Kashmir. Biologia (Pakistan) 60: 63-71.

Ajaib M, Zikrea A, Khan KM, Perveen S, Shah S and Karim A, 2013. *Rivina humilis* L.: A Potential Antimicrobial and Antioxidant Source. J. Chem. Soc. Pak. 35: 1384-1398.

Ajaib M, Latif M, Kamran SH, Khan KM, Perveen S, Shah S and Kareen A, 2016. Comparative Anti-Diabetic Evaluation of Different Parts of *Himalrandia tetrasperma* in Alloxan Induced Diabetic in Mice. J. Chem. Soc. Pak. 38:313-317.

Ajaib M, Hussain T, Farooq S and Ashiq M, 2016. Analysis of Antimicrobial and Antioxidant Activities of *Chenopodium ambrosioides*: An Ethnomedicinal Plant. Journal of Chemistry 2016: 1-11.

Akkol EK, Güvenç A and Yesilada E, 2009. A comparative study on the antinociceptive and anti-inflammatory activities of five *Juniperus* taxa. J. Ethnopharmacol. 125: 330-336.

Amresha G, Reddya GD, Raoa CV and Singh PN, 2007. Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. J. Ethnopharmacol. 110: 526–531.

Anjum M and Hussain MA, 2015. Antibacterial Screening of Different Parts *Datura alba* Nees. *Pharm.* Pharma. Int. J. 2(5): 1-8.

Asif M, 2013 A review on spermicidal activities of *Azadirachta indica.* J. Pharmacog. Phytochemistry 1: 61-79.

Chen IN, Chang CC, Wang CY, Shyu YT, Chang TL. 2008. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods Human Nutrition 63:15-20.

Clark JS, Follenfant LR and Smith WT, 1988. Evaluation of opioidinduced antinociceptive effects in anaesthetized and conscious animals. Br. J. Pharmacol. 95: 275-283.

Coderre TJ and Melzack R, 1992. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin induced tissue injury. J. Neuroscience 12: 3665-3670.

Di Rosa M, 1974. Effects of non-steroidal Anti-inflammatory drugs on Leukocyte Migration. In: Velo GP, Willoughby PA, editors. Future Trends in Inflammation. Piccin Medical

Books: Padova. pp. 829–39.

Dubuisson D and Dennis SG, 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain bark stimulation in rats and cats. Pain, 4: 161–174.

Eveans WC, 2009. Trease and Evans Pharmacognosy (16th ed) Sounders Elsevier. London, UK, PP. 193-223.

Fabricant DS, Fansworth NR, 2001. The value of plants used in traditional medicine for drug discovery. Environment Health Perspective 109:69-75.

Ferrero-Miliani L, Nielsen OH, Anderson PS and Girardin SE, 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin- 1 beta generation. Clin. Exp. Immunol. 147 (2): 227-235.

Gupta AK, Parasar DA, Sagar V, Choudhary BS, Chopra R and Khatri N, 2015. Analgesic and anti-inflammatory properties of gelsolin in acetic acid induced writhing, tail immersion and carrageenan induced paw edema in mice. PloS One 10(8), 1-16.

Hosseinzadeh H and Younessi HM, 2002. Antinociceptive and anti-inflammatory effects of. *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacology 2, 7.

Hunskaar S and Hole K, 1987. The Formalin Test in Mice. Dissociation between Inflammatory Pain. Pain 30: 103-114.

Janssen PAJ, Jageneau AH, 1957. A new series of potent analgesics: Dextro 2,2-diphenyl-3-methyl-H-morholino-butryprolidine and related amides. Part 1. Chemical structure and pharmacological activity. J. Pharm. Pharmacol. 9:38.

Juneja D, Shrivastava PN, Guha MK and Saxena RC, 2007. Preliminary phytochemical screening of some folklore medicinal plants for their antiinflammatory activity. Pharmacognosy Magazine 11: 201-203.

Kamran SH, Ahmad M, Shahwar D and Ajaib M, 2016. Anti-diabetic and anti-oxidant status of *Loranthus pulverulentus* obtained from two different hosts. Bangl. J. Pharmacol. 11: 181-189.

Katzung BG, 2005. *Basic and Clinical Pharmacology*, 6th ed. Appleton and Lange, Connecticut, pp. 297–302.

Lavich TR, Cordeiro RSB, Silva PMR and Martins MA, 2005 A novel hotplate test sensitive to hyperalgesic stimuli and non-opioid analgesics. Braz. J. Med. Biol. Res. 38: 445-451.

Lee SE, Hwang HJ, Ha JS, Jeong HS and Kim JH, 2003. Screening of medicinal plant extracts for antioxidant activity. Life Sci. 73(2): 167-179.

Mazhar F, Khanum R, Ajaib M and Jahangir M, 2015, Potent AChE enzyme inhibition activity of *Zizyphus oxyphylla*: A new source of antioxidant compounds. Pak. J. Pharm. Sci. 28:2053-2059.

Mukherjee PK, Kumar V, Houghton PJ, 2007. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytotherapy Research 21:1142-1145.

Palladino MA, Bahjat FR, Theodorakis EA and Moldawer LL, 2003. Anti-TNF- α therapies: the next generation. *Nat. Rev.* Drug Discovery 2: 736-746.

Pini LA, Vitale G, Ottani A and Sandrini M, 1997. Naloxone- reversible antinociception by paracetamol in the rat. J. Pharmacol. Exp. Ther. 280: 934–940.

Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, IsarankuraNa-Ayudhya C, Ruchirawat S, Prachayasittikul V, 2008. Antimicrobial and antioxidant activity of bioreactive constituents from Hydnophytum formicarum Jack. Molecules 13:904-921.

Prado WA, Tonussi CR, Rego EM and Corrado AP, 1990. Antinociception induced by intraperitoneal injection of gentamicin in rats and mice. Pain 41: 365–371.

Sajeli B, Bhagawati S, Madhur G, Rakesh R, Vijaya BJ, Rao V, Sairam K and Mahendra S, 2010. Study of anti-inflammatory, analgesic and antipyretic activities of seeds of *Hyoscyamus niger* and isolation of a new coumarinolignan. Fitoterapia 81: 178-184.

Santos ARS, Filho VC, Niero R, Viana AM, Morenof N, Campos MM, Yunes RA and Calixto JB, 1994. Analgesic effects of callus culture extracts from selected species of Phyllantus in mice. J. Pharmacy and Pharmacol. 46: 755–759.

Santos FA, Rao VSN and Silveira RA, 1997. Anti-inflammatory and analgesic activities of the essential oil of *Psidium guianense*. Fitoterapia 68: 65-68

Shah B and Seth A, (2010). Textbook of Pharmacognosy and Phytochmistry (1st ed). Elsevier Health Sciences, New Delhi, India, PP. 161, 189, 233, 234, 364.

Sharma KK, Khanna T, Sen P, 1982. Current status of centrally acting peptides. In: (Dhawan, BN, ed) *Advances in Biosciences*

Vol 38, Oxford: Pergamon Press, p. 147.

Shulan S, Tuanjie W, Jino D, Wei Z, Yong QH, Tanga YP, Yua LI and Da WQ, 2011. Anti-inflammatory and analgesic activity of different extracts of *Commiphora myrrha*. J. Ethnopharmacol. 134: 251-258.

Siddiqui SZ, Saleem H, Abbasi MA, Aziz-ur-Rehman and Ajaib M, 2017. *Lonicera quinqueloculari*s: A rich source of antioxidant for protection against chronic diseases. Pak. J. Pharm. Sci. 30(2): 347-353.

Tjolsen A, Berge OG, Hunskaar S, Rosland JH and Hole K, 1992. The formalin test: An evaluation of the method. Pain 51: 5-17.

Tripathi KD, 2003. *Essential of Medical Pharmacology*. New Delhi: Jayapee Brother Medical Publisher. 219.

Verpoorte R, 1999. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discovery Today 3: 232-238.

Vinegar R, Schreiber W, Hugo R, 1969. Biphasic development of Carrageenan edema in rats. J. Pharmacol. Exp. Ther. 166: 96–103.

Wibool R, Chutha S, Wantana R and Malinee W, 2008. Antinociceptive activity of the methanolic extract of *Kaempferia galangal* Linn. in experimental animals. J. Ethnopharmacol. 118: 225–230.

Winter CA, Risley EA and Nuss CW, 1962. Carrageenan-induced edema in the hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol Med. 111: 544-547.

Wu JH, Tung YT, Chien SC, Wang SY, Kuo YH, 2008. . Effect of phytocompounds from the heartwood of Acacia confusa on inflammatory mediator production. J. Agric. Food Chem. 56: 1567-1573.