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Preliminary Phytochemical Screening, Analgesic and Anti Inflammatory Activity of *Ficus glomerata* Fruit Extract

Roksana Rahman[1], Ummul Khayer Fatema[1] and Israt Jahan Bulbul[2*]

[1]Department of Pharmacy, North South University, Bashundhara, Dhaka, Bangladesh.
[2]Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author IJB designed the study. Author RR carried out the tests. Author UKF helped to carry out the study. Author IJB helped to coordinate the biological assay, draft the manuscript, checked the grammatical errors and corrected the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study was to investigate the preliminary phytochemicals, analgesic and anti-inflammatory potential of the ethanolic extract of *Ficus glomerata* Roxb. (Moraceae) fruits on Swiss albino mice.
Study Design: The freshly prepared crude extract was qualitatively tested for the identification of preliminary phytochemical constituents. 250 and 500 mg/kg body weight doses were assessed for analgesic and anti-inflammatory activities on animal models.
Place and Duration of Study: Department of Pharmacy, North South University, Dhaka, Bangladesh, from January to June 2015.
Methodology: The analgesic activity was evaluated using formalin induced paw licking test, Eddy's hot plate method. Carrageenan induced hind paw edema was performed to evaluate anti-inflammatory activity.

**Corresponding author: E-mail: israt_jahanb872@yahoo.com;*

Results: Preliminary phytochemical analysis of *F. glomerata* fruit indicated the presence of tannins, flavonoids, saponins, gum carbohydrates, alkaloids, reducing sugar and terpenoids in ethanolic extracts. In analgesic activity using formalin induced paw licking test, the test and standard drugs significantly (p<.001) reduced the number of licking and biting the hind paw. Analgesic activity studied by hot plate method showed all the test and standard drugs significantly (p<.001) reduced the pain as compare to the control group. Ethanolic extract of *F. glomerata* significantly (p<.001) inhibited carrageenan induced paw edema.
Conclusion: Our study reveals that *F. glomerata* fruit extract possess significant analgesic and anti-inflammatory activities and the dose 500 mg/kg body weight is more significant than 250 mg/kg body weight in all of the methods.

Keywords: F. glomerata; phytochemical screening; analgesic; anti-inflammatory.

1. INTRODUCTION

There are number of synthetic steroidal and non-steroidal anti-inflammatory drugs (NSAID's) are discovered to treat inflammation and pain. These drugs are potent in action but show wide range of adverse effects whereas, herbal drugs bear comparatively less side effects [1,2]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost.

Ficus glomerata commonly known as Gular fig, cluster fig, country fig and redwood fig [3] is found throughout greater part of India and also found in Burma, China, Indonesia, Malaysia, and Australia [4]. It is often cultivated round villages in India for its edible fruits [5]. All parts of this plant (leaves, fruits, bark, latex, and sap of the root) are medicinally important in the traditional system of medicine in India [6].

F. glomerata leaves are used in bilious infections [6], in dysmenorrhea [7], as a wash for wounds and ulcers, for boils blisters and measles [8]. The fruit is used as an astringent, stomachic, carminative. Both fruit and bark are given in menorrhea, hemoptysis [9], visceral obstruction, diarrhea and constipation [10,11], leprosy, diabetes [7]. The bark is highly efficacious in threatened abortions, recommended in uropathy, asthma and piles [6]. Paste of stem bark is used in burns, swelling, and leucorrhea [12]. The latex is aphrodisiac and is administered in hemorrhoids [13].

The crude extracts of *F. glomerata* has been reported as antidiarrhoeal [14], antihyperglycemic [15], anti-inflammatory [16], antibacterial [17], hepatoprotective [18], analgesic [19], antidiuretic [20], antitussive [21], wound healing [22], anthelmintic [23], antidiarrhoeal [24], antipyretic [25], anticholinesterase [17], hypolipidemic [26], larvicidal [27], antiulcer [28], antifilarial [29].

The leaves, barks and fruits are reported to contain sterols, triterpenoids, flavonoids, glycosides, tannins, carbohydrates [18,25,30-32], Fruits contain β-sitosterol, gluanol acetate, hentriacontane, tiglic acid of taraxasterol, lupeol acetate [33-36], gallic acid, ellagic acid [37] and α-amyrin acetate [38]. Bark contains leucocyanidin-3-*O*-β-D-glucopyrancoside, leuco-pelargonidin-3-*O*-β-D-glucopyranoside, gluanol acetate, leucopelargonidin-3-*O*-α-L-rhamnopy-ranoside, ceryl behenate, α-amyrin acetate [3], lupeol [33,34,39], friedelin, behanate [40], stigmasterol, β-sitosterol-D-glucoside [33-35,39], gluanol acetate [27], and quercetin [41], bergenin, racemosic acid [42,43], friedelin [39], β-amyrin, and lupeol acetate [44].

Since the plant contains flavonoids and alkaloids in ample quantity and there is no previous report on the analgesic, anti-inflammatory activity of *F. glomerata* the present study was carried out to assess analgesic and anti-inflammatory activity using validated methods.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

All the chemicals used in this study were of analytical grade. Carrageenan was purchased from Sigma-Aldrich, USA.

2.2 Plant Material Collection and Authentication

The plant sample of *F. glomerata* fruit was collected on the month of October 2012. The plant was identified by Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB Accession no. 38465).

The freshly separated different Fruit parts were cut into small pieces, and sun dried as require to grind these into coarse powder. The dried powder was preserved in airtight container against the re-absorption of moisture, oxidation, excessive heat or humidity, growth of moulds and bacteria and infestation by insects and rodents. The plant powder is properly stored to protect the drugs from all the above deteriorating factors and agents and maintain a high degree of quality in them.

2.3 Preparation of Extract

About 500 gm of powdered fruit was taken in a flat bottom glass container and soaked in ethanol. The container with its content was sealed with aluminum foil and kept at room temperature for a period of 7 days accompanying occasional shaking and stirring. The extract was filtered through fresh cotton plug followed by Whatman No.1 filter paper (Bibby RE-200, Sterilin Ltd., UK). The filtrate (ethanol extract) obtained was evaporated by Rotary evaporator (R-210, Buchi, Switzerland) at 5 to 6 rpm and at 68℃ temperature. It rendered a gummy concentrate of dark brown color. This gummy concentrate was designated as ethanol extract of *F. glomerata* (EEFG). Then the crude extract was dried by freeze drier and preserved at 4℃.

2.4 Experimental Animal

Swiss Albino mice of either sex, aged 4-5 weeks of average wt. 25-30 gm, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiment. They were kept in standard environmental conditions (relative humidity 55-65%, r.t. 23.0±2.0℃ and 12 h light: dark cycle) and fed ICDDR; B formulated rodent food and water according to the institutional animal ethics, Department of Pharmacy, North South University.

2.5 Acute Oral Toxicity Test

Acute oral toxicity test was carried out in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals, 420 [45] Swiss albino mice maintained under standard laboratory conditions were used for acute toxicity study. A total of three mice from each group received a single oral dose (500, 1000, and 2000 mg/kg b.w. of the extract. Animals were kept over-night fasting prior to administration. After administration of the extracts, food was withheld for further 3 to 4 h. The animals were then individually observed (with special attention during the first 4 h) for possible behavioral changes, allergic reactions (skin rash, itching), eyes and mucous membrane, and mortality for the next 72 h.

2.6 Preliminary Phytochemical Screening

The freshly prepared crude extract was qualitatively tested for the identification of preliminary phytochemical constituents by following the methods of Adegoke et al. [46] for alkaloids and saponins, Shahid-Ud-Duaula and Anwarul [47] for flavonoids, Molisch test for gums, Boxi et al. [48] for tannins and reducing sugars, Obianime and Uche [49] for terpenoids.

2.7 Analgesic Activity Test

2.7.1 Hot plate

Eddy's hot plate method was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at 55 ± 0.5℃. The reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test groups received ethanolic extracts of *F. glomerata* (EEFG) at 250 and 500 mg/kg dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Pentazocine (10 mg/kg, i.p.) and control group received only 1 ml of 2% Tween 80 solution. The reaction times were determined before and after 30 minutes, 1 hour, 2 hours and 3 hours period with reference to the control group receiving only vehicle.

2.7.2 Formalin test

To investigate the analgesic effect of plant extracts, the formalin test [50] was used. For this

method, animals were kept into four groups and were treated in the following manner: Group I received vehicle, group II received indomethacin as standard drug at the dose of 10 mg/kg body weight and group III-IV received different doses (250 and 500 mg/kg) of the extract. Thus, 30 min after injection of different doses of the extract or the carrier, 20 µl of 5% formalin was injected into the right back paw of mice using a Hamilton syringe and the animal was immediately placed in the formalin test container. The number of paw licking was measured in each mouse from 0-5 min and 20-30 min.

2.8 Anti-inflammatory Activity

2.8.1 Carrageenan-induced rat paw edema

This method was previously described by Yin [51] and was used with some modifications. Mice were divided into 4 groups (5 animals/ group). The vehicle control group received distilled water. The positive control group received Indomethacin at 20 mg/kg p.o and two treatment groups received ethanolic extracts of *F. glomerata* at 250 mg/kg, and 500 mg/kg. The mice were injected subcutaneously with 0.1 ml of 1% carrageenan solution in normal saline (0.9% w/v NaCl) into the sub-plantar region of the left hind paw. Paw volume was measured before and 1, 2, 3 and 6 hrs after injection of carrageenan using a plethysmometer. All the test substances and reference drugs were administered 60 min before injection of carrageenan. The percent increase in paw volume was calculated and compared with the vehicle control.

2.9 Statistical Analysis

Statistical analysis was carried out using one-way ANOVA followes by Dunnet's multiple comparisons for analgesic screening tests. The results obtained were compared with the vehicle control group. P values <.05, <.01 and <.001 were considered to be statistically significant.

3. RESULTS

3.1 Acute Toxicity Test

In the acute toxicity assay no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with Toxicity, such as convulsion, ataxy, diarrhoea or increased Diuresis.

3.2 Preliminary Phytochemical Screening

The crude ethanolic extract of *F. glomerata* fruit contained Tannins, saponins, Steriods, Alkaloids, Flavonoids and Terpiniods (Table 1).

Table 1. Phytochemical analysis of the crude ethanolic extract of *F. glomerata* fruit

Name of the phytochemical constituents	Result
Alkaloids	+++
Flavonoids	++
Saponins	+
Gums carbohydrates	+++
Tannins	+++
Terpenoids	+++
Reducing sugar	+

Symbol (+++) indicates presence in high concentration, Symbol (++) indicates presence in moderate concentration, Symbol (+) indicates presence in trace concentration, and (-) indicates absence of the respective phytochemical

3.3 Analgesic Activity

3.3.1 Hot plate

In the hot plate method of analgesic activity the extract exhibited potent activity. The response time observed was significantly increased when compared to normal control. However, the standard pentazocine was found to be better active than the extract during 240 min response. The maximum percentage protection after 240 min for pentazocine is 69% where 64.83% and 67% were for 250 mg/kg and 500 mg/kg dose of the ethanolic extract of *F. glomerata* respectively (Fig. 1) and found to be significant at the level of P<.001 (Table 2).

3.3.2 Formalin induced paw licking test

The effects of the ethanol extract of *F. glomerata* fruit on formalin induced paw licking in mice are shown in Table 3. In first phase of formalin induced pain model, ethanol extract at 500 and 250 mg/kg body weight produced 69.41% and 65.84% inhibition of pain response whilst at second phase *F. glomerata* fruit extract produced 70.45% and 67.93% inhibition of pain response respectively (Fig. 2). We found that *F. glomerata* fruit extract showed analgesic effect both in the early and late phase of formalin test which indicates that the extract exerts its analgesic effect through the peripheral and central mechanism.

Table 2. Effect of ethanolic extracts of *F. glomerata* by hot plate method in mice

Treatment	Reaction time (s)				
	Initial	Time after drug administration			
	0 min	60 min	120 min	180 min	240 min
Control	8.3000±0.63	7.5000±0.39	7.5000±0.45	7.1888±0.33	6.7800±0.32
Standard	7.4200±0.2887	10.5800±0.3813**	11.9800±0.4789***	13.3800±0.2709***	12.5400±0.2502***
EEFG 250 mg/kg	7.82±0.34	10.64±0.49*	11.89±0.20**	13.65±0.12***	12.89±0.12***
EEFG 500 mg/kg	8.00±0.29	11.08±0.35*	12.76±0.32**	13.78±0.26***	13.36±0.16***

*Values are expressed as mean ± S.E.M. (n=5); significance at *p<.05, **p<.01, ***p<.001 as compared to control. Ethanolic extracts of F. glomerata (EEFG)*

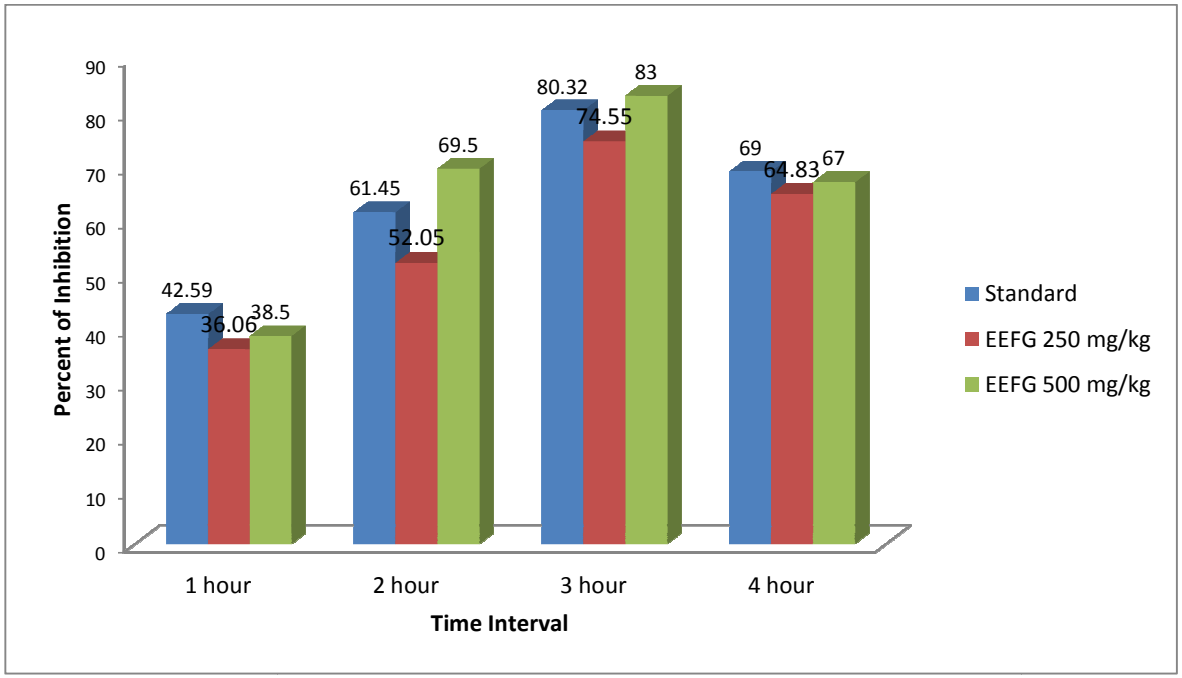

Fig. 1. Percentage of inhibition of *F. glomerata* fruit extract by hot plate method

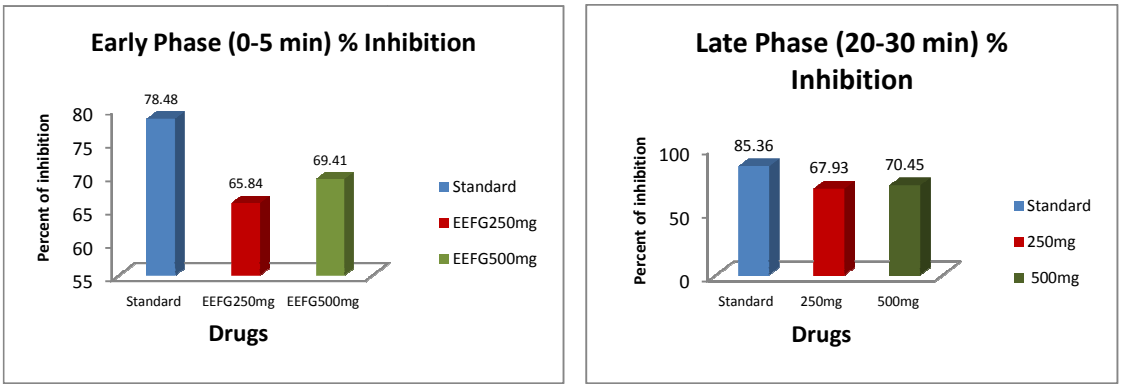

Fig. 2. Percentage of inhibition of *F. glomerata* fruit extract by formalin test

Table 3. Effects of the ethanolic extracts of *F. glomerata* fruit on formalin induced paw licking test

Group	Early phase (0-5 min)	Late phase (20-30 min)
	No. of paw licking	No. of paw licking
Control	32.20±4.63	22.20±4.18
Standard	6.93±0.58***	3.25±0.66***
EEFG 250 mg	11.00±2.44**	7.12±2.91**
EEFG 500 mg	9.85±2.73***	6.56±2.79***

*Values are expressed as mean ± S.E.M. (n=5); significance at *p<.05 **p<.01, ***p<.001 as compared to control. Ethanolic extracts of F. glomerata (EEFG)*

3.4 Anti-inflammatory Activity

3.4.1 Carrageenan-induced rat paw edema

Anti-inflammatory effect of ethanolic extract was observed and found to be significant at the level of P<.001 when compared with the vehicle distilled water (control group) and indomethacin (Standard). The percent inhibition (Fig. 3) in paw edema after 3 hrs were recorded 41.00% in case of indomethacin and 37.07% and 39.33% in 250 mg/kg and 500 mg/kg dose of the ethanolic extract of *F. glomerata* respectively. The observations are given in Table 4.

4. DISCUSSION

In the present study analgesic activity of *F. glomerata* was evaluated by hot plate and formalin induced paw licking methods. These tests allow analyzing peripheral and centrally mediated antinociceptive responses. Hot plate test have selectivity for opioid derived centrally mediated analgesics [52]. The Hot plate test useful in the elucidating centrally mediated antinociceptive responses that focused mainly on changes above the spinal cord level. Animals treated with the extract of *F. glomerata* showed significantly longer latency than the control group indicating that the extract cause analgesia by their actions at CNS. Formalin causes an increase in peritoneal fluids of PGE2 and PGF2α, serotonin and histamine involved in part, which is a model commonly used for screening peripheral analgesics [53]. In formalin induced paw licking test the early phase (immediately after injection) seems to be caused by C-fiber activation due to the peripheral stimulus. The late phase (20 min after formalin injection) appears to depend on the combination of an inflammatory reaction, activation of NMDA and non-NMDA receptors and NO cascade [54] in the peripheral tissue and the functional changes in the dorsal horn of the spinal cord. The extract significantly inhibited the formalin induced paw licking at both the doses (250 mg/Kg and 500 mg/Kg) indicating their potent activity by both peripheral and central antinociceptive action.

In the present investigation anti-inflammatory activity of ethanolic exracts of *F. glomerata* was studied by using carrageenan induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. Carrageenan-induced inflammation is a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents [55]. Edema formation in the rat paw is a biphasic histamine, serotonin, and kinins, whereas the second phase is due to the release of prostaglandin and slow reacting substance [56]. The ethanolic extract of *F. glomerata* significantly reduced the paw edema. In this experiment the suppression of inflammation may

Table 4. Anti inflammatory effects using Carrageenan-induced rat paw edema of the ethanolic extracts of *F. glomerata* fruit

Group	0 hour	1 hour	2 hour	3 hour	6 hour
Control	0.73±0.038	1.31±0.193	1.58±0.017	1.78±0.032	1.69±0.020
Standard	0.692±.0348	0.962±0.05	1.34±0.049*	1.04±0.045**	0.85±0.021***
EEFG 250 mg/kg	0.79±0.0387	1.11±0.045	1.40±0.0599	1.42±0.028*	1.02±0.0306***
EEFG 500 mg/kg	0.83±0.02177	1.07±0.0403	1.37±0.0523*	1.26±0.043**	0.92±0.0193***

*Values are expressed as mean ± S.E.M. (n=5); significance at *p<.05, **p<.01, ***p<.001 as compared to control. Ethanolic extracts of F. glomerata (EEFG)*

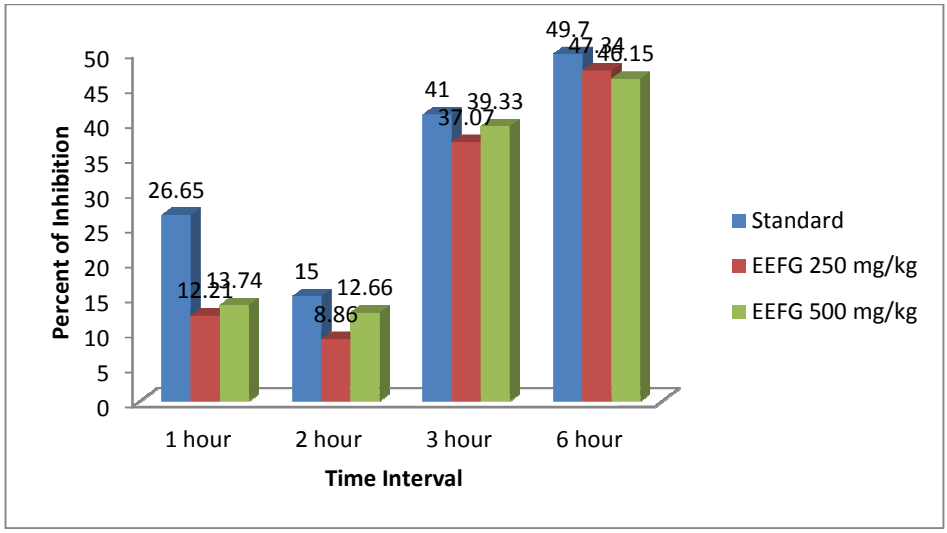

Fig. 3. Percentage of inhibition of *F. glomerata* fruit extract by carrageenan-induced rat paw edema test

be due to PG and kinin synthesis/ release inhibition and antihistamine activities. The maximum inflammation is seen approximately six hours post the carrageenan injection, after which it beings to decline.

The present study for preliminary phytochemicals showed the presence of tannins, saponins, steriods, alkaloids, flavonoids and terpiniods in *F. glomerata* fruit. Many of these natural compounds work by inhibiting the inflammatory pathways in a similar manner as NSAIDs. In addition to the COX pathway, they act to inhibit nuclear factor-kB (NF-kB) inflammatory pathways. Flavonoids/Phenolics are COX-2 inhibitors inhibit inflammatory-mediating cytokines. Polyphenolic compounds such as catechins, and epigallocatechin-3 galate inhibit IL-1–induced proteoglycan release and type 2 collagen degradation in cartilage explants. It also suppresses IL-1b and attenuates activation of the transcription factor NF-kB [57]. So, it can be interpreted that both central and peripheral analgesic and anti-inflammatory properties of *F. glomerata* are due to inhibition of proinflammatory factors by phytoconstituents. Further studies on understanding its precise mechanism of action are required.

5. CONCLUSION

Based on the results of this study, it may be concluded that ethanolic extract of *F. glomerata* of has an analgesic effect which may be related to the phytochemicals identified in the extract. Further studies are needed to elucidate the effect of the extract on inflammation and analgesic activity mediators COX, NO, PGE and IL-6. In addition, further toxicological studies are needed to determine the safety profile of *F. glomerata*.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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