

ANTI-INFLAMMATORY, ANTI-NOCICEPTIVE AND ANTIPYRETIC POTENTIAL OF *TERMINALIA CITRINA* FRUIT EXTRACTS

Ammara Saleem<sup>1,2\*</sup>, Aqeel Javeed<sup>2\*</sup>, Muhammad Ashraf<sup>2</sup>, Muhammad Furqan Akhtar<sup>1,2\*</sup>, Bushra Akhtar<sup>4</sup>, Ali Sharif<sup>2,3</sup>, Kanwal Akhtar<sup>5</sup>, Moosa Raza<sup>3</sup>, Irfan Hamid<sup>6</sup>, Sohaib Peerzada<sup>3</sup>, Shoaib Ahmad<sup>3</sup>, Sania Atta<sup>3</sup>

<sup>1</sup>Faculty of Pharmaceutical Sciences, GC University, Faisalabad, Pakistan. <sup>2</sup>Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore, Pakistan. <sup>3</sup>Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan. <sup>4</sup>Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. <sup>5</sup>Centre of Excellence in Solid State Physics, University of the Punjab, Pakistan. <sup>6</sup>Mirpur University of Science and Technology, AJK, Pakistan.

\*Corresponding Author E-mail: [Amarafurqan786@hotmail.com](mailto:Amarafurqan786@hotmail.com), [furqan.pharmacist@gmail.com](mailto:furqan.pharmacist@gmail.com), [aqeel.javeed@uvas.edu.pk](mailto:aqeel.javeed@uvas.edu.pk)

**Article History**

Received: 29, May, 2016

Revised Received: 13, Aug, 2016

Accepted: 15, Aug, 2016

Published Online: 01, Oct, 2017

**Abstract**

**Background:** Plants and herbs have long been used as remedies without scientific evidences. The objective of the present study was to explore the anti-inflammatory, anti-nociceptive and antipyretic potential of ethanolic and aqueous extracts of *Terminalia citrina* fruits in mice.

**Materials and Methods:** Extracts of *Terminalia citrina* fruits were evaluated at doses of 200mg/kg, 400mg/kg and 600mg/kg in albino mice for preventive effect in inflammatory edema, peripheral pain sensation and pyrexia. Carrageenan induced paw edema method was utilized to evaluate anti-inflammatory activity. Analgesic appraisal of extracts was demonstrated using acetic acid induced writhing model of pain. Antipyretic potential was determined by brewer's yeast induced pyrexia model. Statistical analysis was conducted by ANOVA following post hoc test.

**Results:** Both extracts exhibited significant and dose-dependent anti-inflammatory, analgesic and antipyretic activities. The ethanolic extract was more effective in reducing inflammatory edema, pyrexia and pain sensation than aqueous extracts in all tested doses.

**Conclusion:** It can be concluded that fruit extracts of *Terminalia citrina* may be effective in reducing inflammation, pyrexia and pain sensation in animals.

**Key words:** Ethnopharmacology; anti-inflammatory; writhing; pyrexia; pain; Brewer's yeast

**Introduction**

Nature provides us food and remedies for most of the diseases the mankind suffers from, in the form of plants, herbs and microbes with fewer side effects (D Mogosanu, 2015; El Gharras, 2009; Reegan, 2015). Although progress in dosage form development provides more effective alternatives, however, various communities in South Asia use medicinal plants for curing their diseases (Akhtar et al., 2011; Bhaskar & Balakrishnan, 2009; Szabo, 2010). The traditional system of medicine well established by the name of Hikmat in South Asia. This system utilizes approximately 600-1000 medicinal plants which are used on folklore basis for the treatment of various pathological conditions (Saeed, 2011).

Inflammation occurs in response to an injurious stimulus such as infection, physical injuries and antibodies. Noxious stimulus results in inflammatory process culminating in loss of tissue function (Vikrant & Arya, 2011). Leukocytes, prostaglandins and other inflammatory mediators surge at the site of inflammation. Although protective in nature, survival in an environment rich in pathogens and injuries is dependent on the body's ability to cope with the process of inflammation.

Pyrexia caused by some infectious disease, malignancy, or any other etiology is in fact the inherent ability of the body, creating a hostile environment for damaged cells and microbes so as to prevent their survival. (Chattopadhyay et al., 2005). Pain is a debilitating condition and may be of peripheral or central in origin. Analgesia renders the body to be insensitive to pain even when conscious (Pradhan, 2009). For pyrexia and pain management, various therapeutic agents have been employed such as NSAIDs and opioids, however, many of these drugs, on chronic uses, produce tolerance and dependence while others exhibit hepatic, renal, gastrointestinal and cardiovascular side effects (Sharif et al., 2011). It prompted using modified release formulations or common traditional medicines for disease cure owing to their cheaper availability and fewer side effects. The plant *Terminalia citrina* belongs to family Combrataceae. *Terminalia citrina* (Gaertn.) Roxb. ex Fleming is commonly known as Yellow Myrobalan. It is widely distributed in India and Pakistan. Its fruits are used in various crude drug preparations (Ingle & Dhabe, 2011). The present study was designed to elucidate pharmacological potential of ethanolic and aqueous extracts of *Terminalia citrina* fruits against inflammatory edema, fever and peripheral pain sensation.

## Materials and Methods

### Acquisition of Materials

Paracetamol (Tianjin Bofa Pharmaceuticals), Carrageenan (Sigma Lambda, USA), Indomethacin, Acetic acid and Brewer's yeast (Merck, Germany) were acquired for experimental purpose and were of analytical grade.

### Experimental Animals

Male albino mice (20-30g) were purchased from Veterinary Research Institute (VRI), Lahore, Pakistan. They were kept in polyacrylic cages with free access to dry pellet diet and water ad libitum in a 24±2<sup>0</sup>C with light/dark cycle. Animals were kept for 7 days to acclimatize to laboratory conditions. Prior to each experiment, animals were being fasted overnight.

The study was conducted according to the guidelines and approval from Animal Ethics Committee, University of veterinary and animal sciences Lahore and Principles of Laboratory Animal Care were strictly adhered.

### Preparation of extracts

Dried fruits of the plant were purchased from local markets and its fruits were subjected to identification from Government College University, Lahore. The voucher sample was also preserved there with its allotted voucher number "GC. Herb. Bot. 2205". Ethanolic and aqueous extracts were prepared by triple maceration of ground dried fruits of the plant. Extracts were dried by rotary evaporator after filtration.

### Carrageenan-induced paw edema method

For evaluating anti-inflammatory activity, complete randomized block design (CRBD) was applied. The albino mice were divided into eight groups of five mice in each group. Group A received 1ml normal saline (0.9% w/v NaCl) and served as negative control and group B received indomethacin (10mg/kg) as positive control. Group C, group D and group E received 200 mg/kg, 400 mg/kg and 600 mg/kg of aqueous extract, respectively, while group F, group G and group H received 200 mg/kg, 400 mg/kg and 600 mg/kg of ethanolic extract respectively by oral route.

After one hour, 0.1ml of 1% freshly prepared carrageenan solution was injected subcutaneously (S/C) in sub-plantar surface of right hind paw of every mice (Karan et al., 2011; Nwafor, 2013). For the measurement of inflammation, the diameter of hind paw was measured using digital vernier caliper at 0 hr, 1 hr, 2 hr and 3 hrs after carrageenan injection according to the previously described method (Shojaii, 2015).

Any change in paw diameter was obtained by subtracting the initial paw diameter from the paw diameter at different time interval. The average value of edema was calculated by taking the average of each group at different hours in millimeters (mm). Percentage inhibition of paw inflammation was calculated for each group with respect to its control group as

$$\text{Percentage Inhibition of Inflammation} = \frac{A - B}{A} \times 100$$

Where A = Mean paw diameter in control group; and B = Mean paw diameter in treated group

### Acetic acid-induced writhing test

For evaluating peripheral analgesia, complete randomized block design (CRBD) was used. The albino mice were divided into eight groups of five mice in each group. Group A received 1ml normal saline (0.9% w/v NaCl) and served as

negative control and group B received indomethacin (10mg/kg) as positive control. group C, group D and group E received 200 mg/kg, 400 mg/kg and 600 mg/kg of aqueous extract, respectively while group F, group G and group H received 200 mg/kg, 400 mg/kg and 600 mg/kg of ethanolic extract respectively by oral route.

After one hour, 0.1ml of 1% acetic acid was injected intraperitoneally (i.p) to each mouse. After injecting acetic acid, mice were placed in cages and writhes were counted for each animal. The number of writhes (abdominal muscle contractions), trunk twisting, stretching of hind limb was observed and counted for 20 minutes after acetic acid injection (Dhara, Suba, Sen, Pal, & Chaudhuri, 2000; Du et al., 2007; Franzotti et al., 2000). Percent inhibition was calculated for each group of mice by following formula (Sharma, Sharma, Singh, Sutar, & Singh, 2010).

$$\text{Percentage Inhibition of writhings} = \frac{N - N_t}{N} \times 100$$

N = number of writhing of control group; and N<sub>t</sub> = the number of writhing of treated group.

### Brewer's yeast-induced pyrexia

For evaluating anti-pyretic activity, complete randomized design (CRD) was used. Fever was induced by injecting 20mg/kg of 20% brewer's yeast suspension in normal saline subcutaneously below the nape of the neck. Initial temperature of rectum of each mouse was recorded. After 18hour, mice that exhibited a rise of 0.5-0.9°F in temperature of rectum were selected for further studies. The albino mice were divided into eight groups of five mice in each group. Group A received 1ml normal saline and served as negative control whereas group B received acetaminophen (150 mg/kg) and served as positive control. Group C, Group D and Group E received 200 mg/kg, 400 mg/kg and 600 mg/kg of aqueous extract respectively while Group F, Group G and Group H received 200 mg/kg, 400 mg/kg and 600 mg/kg of ethanolic extract respectively by oral route. The rectal temperature (°F) was measured with clinical thermometer at 0, 1, 2 and 3 hours post treatment (Pradhan, et al., 2009; Santos & Rao, 1998). Percentage reduction in fever was calculated for each group of mice (Chomchuen, Singharachai, Ruangrunsi, & Towiwat, 2010) by following formula.

$$\text{Percentage reduction in fever} = \frac{\text{Yeast induced pyrexia} - \text{Pretreatment temperature}}{\text{Yeast induced pyrexia}} \times 100$$

### Statistical analysis

The data was statistically analyzed by SPSS (Statistical package for social sciences). The results of anti-inflammatory, analgesic and antipyretic activities were expressed as means ± SD (Standard Deviation). Data of analgesic activity was statistically analyzed by one way ANOVA followed by post hoc test for multiple comparisons. Data of anti-inflammatory activity and antipyretic activity was statistically analyzed by two way ANOVA followed by post hoc test for multiple comparison.

## Results

### Percentage yield of plant extracts

Extraction of dried *Terminalia citrina* was carried out by triple maceration with two solvents i.e. ethanol and water. The percentage yield of aqueous and ethanolic extracts was found to be 32.8 and 39.6% respectively (Table 1).

**Table1:** Percentage yield of *Terminalia citrina* after triple maceration

Extracts	Ground fruit used For extraction (Grams)	Dried extracts obtained (Grams)	Percentage yield % (w/w)
Aqueous	250	82	32.8±0.85
Ethanol	250	98	39.6±0.74

Result expressed as mean ±S.D. (n=3)

## Carrageenan-induced paw edema method

Percent inhibition of inflammation using ethanol and aqueous extracts was more with higher doses than at lower doses after standard drug. While in negative control, the inflammation increased with the passage of time. Results showed that all the doses reduced inflammation in mice when compared to normal saline (Table 2). Ethanolic extract of *Terminalia citrina* exhibited more percentage reduction in inflammation than the aqueous extract of the same plant and both extracts reduced inflammation in a dose dependent manner.

**Table2:** Anti-inflammatory activity of aqueous and ethanolic extracts of *Terminalia citrina*, at different time intervals

Group	Time	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H
Diameter (mm) of left hind paw	0hr	2.4±0.04 3	2.39±0.04 5	2.37±0.0 33	2.35±0.04 08	2.34±0.04 26	2.45±0.03 1	2.44±0.02 3	2.39±0.0 51
	1hr	3.94±0.0 39	2.95±0.01 1* , (25.07%)	2.99±0.0 32* , (24.11%)	2.98±0.01 41* , (24.36%)	2.976±0.0 13* , (24.46%)	2.984±0.0 55* , (23.84%)	2.974±0.0 54* , (24.13%)	2.96±0.0 15* , (24.38%)
	2hr	3.96±0.0 58	2.45±0.02 7* , (37.76%)	2.83±0.0 29* , (28.12%)	2.79±0.05 8* , (28.98%)	2.76±0.02 12* , (29.94%)	2.78±0.00 5* , (29.12%)	2.77±0.02 5* , (30.14%)	2.55±0.0 19* , (35.68%)
	3hr	4.00±0.0 2	2.408±0.0 57* , (39.83%)	2.71±0.0 35* , (32.23%)	2.67±0.00 5* , (33.13%)	2.62±0.02 58* , (34.33%)	2.65±0.02 9* , (33.48%)	2.62±0.02 3* , (34.43%)	2.44±0.0 23* , (38.88%)

Result expressed as mean ±S.D. (n=5). Statistically result was significant at  $P \leq 0.05$  as compared to the negative control group. \*= Significant.

Figures in parentheses indicated the % inhibition of inflammation.

## Acetic acid-induced writhing test

Results of analgesic activity showed that percentage inhibition of writhing was reduced more with standard drug indomethacin. 600mg/kg dose reduced writhing more than 400mg/kg and 200mg/kg. Results of the writhing test indicated that more inhibition of pain was observed with ethanolic extracts than with aqueous extract of the plant (Table 3).

**Table 3:** Anti-nociceptive activity of aqueous and ethanolic extracts of *Terminalia citrina*

Group	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H
Mean of writhing± S.D.	62.2 ± 1.095	15.6 ± 0.894*	30.6 ± 1.341*	28 ± 1.414*	23.6 ± 2.073*	39.8 ± 1.483*	33 ± 2*	28.6 ± 0.894*
% age Inhibition of Writhing	--	74.91	53.98	54.98	62.05	35.598	46.601	53.721

Result expressed as mean ±S.D. n=5. Statistically result was significant at  $P \leq 0.05$  as compared to the negative control group.

## Brewer's yeast induced pyrexia

It was found that the fever was reduced more with acetaminophen than with 600mg/kg dose of both extracts whereas 400mg/kg and 200mg/kg doses reduced fever less effectively than 600mg/kg. Results indicated that more fever was reduced with ethanol than with aqueous extract of *Terminalia citrina* fruit (Table4). Percentage

reduction in fever by the ethanolic and aqueous extract of the plant indicated that the ethanolic extract was more effective in reducing fever than the aqueous extract of *Terminalia citrina* fruit.

**Table 4:** The antipyretic activity of aqueous and ethanolic extract of *Terminalia citrina* in mice by Brewer's yeast induced pyrexia

Name of Groups		Group A (Negative Control)	Group B (Positive Control)	Group C (200mg Aqueous)	Group D (400mg Aqueous)	Group E (600mg Aqueous)	Group F (200mg Alcoholic)	Group G (400mg Alcoholic)	Group H (600mg Alcoholic)
Rectal temperature in °F at different hours	Before inducing fever	96.24±0.32	96.6±0.07	96.86±0.343	96.24±0.320	96.86±0.345	96.24±0.32	96.3±0.469	96.86±0.3435
	After 18hrs	99.22±0.228	99.74±0.512	99.86±0.055	99.7±0.187	98.12±0.045	99.78±0.16	99.32±0.465	99.62±0.438
Rectal temperature after treatment	1hr	99.96±0.25	96.24±0.763*, (3.72%)	98.68±0.164*, (1.06%)	97.5±0.342*, (1.42%)	97.36±0.288*, (2.38%)	99.4±0.514*, (0.56%)	98.46±0.482*, (1.50%)	97.84±0.676*, (2.32%)
	2hr	100.08±0.286	95.9±0.412*, (4.17%)	98.24±0.37*, (1.70%)	97.88±0.22*, (2.86%)	96.84±0.29*, (3.10%)	98.06±0.87*, (2.01%)	97.58±0.13*, (2.89%)	96.52±0.342*, (3.55%)
	3hr	100.14±0.054	95±0.685*, (5.13%)	97.96±0.34*, (2.13%)	96.54±0.449*, (3.55%)	96.28±0.204*, (3.81%)	97.79±0.19*, (2.41%)	96.18±0.46*, (3.45%)	95.7±0.424*, (4.43%)

Result expressed as mean ±S.D. (n=5); Statistically result was significant at  $P \leq 0.05$  as compared to the negative control group. \*= Significant.  
The figures in parentheses indicated the % reduction in fever.

## Discussion

A lot of researches have documented that extracts from medicinal plants contain such type of phyto-constituents that might be responsible for treating diseases (Abad, 2000). In the present research work, anti-inflammatory, analgesic and antipyretic activities of ethanolic and aqueous extracts of *Terminalia citrina* fruit evaluated using carrageenan induced paw edema method, acetic acid induced writhing test and brewer's yeast induced pyrexia model respectively.

Carrageenan induced edema is known to be the acute anti-inflammatory model sensitive to the cyclooxygenase inhibitors and is used to find the effect of nonsteroidal anti-inflammatory agents which inhibit cyclooxygenase involved in prostaglandin synthesis. Thus inhibitory effect of plant extract on carrageenan induce inflammation mean it inhibit cyclooxygenase enzyme and inturn PG synthesis (Karan, et al., 2011). Results of a percent reduction of inflammation indicated that ethanolic and aqueous extracts of *Terminalia citrina* reduced hind paw edema which may be due to effects on mediators of inflammation and also on prostaglandin synthesis. This blocked of PG synthesis may be due to flavonoids and tannins, saponins and polyphenolic compounds present in *Terminalia citrina* (Palasuwan, 2005).

In acetic acid induced writhing test, acetic acid is injected intraperitoneal and pain produced by increase in amount of PGE2 and PGF2 $\alpha$  in the peritoneal fluid (Bentley, 1983). If plant extracts have significant reduction of acetic acid induced writhing then underlying mechanism of plant extract can be linked partly to cyclooxygenase and/or lipooxygenase inhibition (Franzotti, et al., 2000). Thus, in our study, *Terminalia citrina* exerted analgesic activity in acetic acid induced writhing test might be by inhibiting cyclooxygenase and lipooxygenase pathway in mice.

Mostly antipyretic drugs act by blocking COX-2 expression for reducing elevated temperature of body by blocking PGE2 biosynthesis (Luo & BOHLIN, 2005) thus in our study, *Terminalia citrina* plant aqueous and ethanolic extracts might possessed antipyretic activity due to blocking of COX-2 expression and PGE2 biosynthesis. It was suggested that flavonoids and tannins are potent inhibitors of PGs (Manthey, 2000). It can be concluded that flavonoids present in *Terminalia citrina* might block PG synthesis in hypothalamus of mice and exerted antipyretic activity.

Statistical analysis for evaluating anti-inflammatory, analgesic and antipyretic activities of *Terminalia citrina* indicated that results of experiment were significant at  $P \leq 0.05$ . According to post hoc test results of aqueous and ethanolic extracts of *Terminalia citrina* about anti-inflammatory, analgesic and antipyretic activities exhibited that difference of Group B, Group C, Group D and Group E significant as compared to Group A at  $P \leq 0.05$ . The results supported the use of fruit extracts of *Terminalia citrina* in treating fever, inflammatory conditions and painful conditions.

## Conclusion

The results of this study indicated that *Terminalia citrina* fruit extracts possessed significant anti-inflammatory, analgesic and antipyretic activity at 200mg/kg, 400mg/kg and 600mg/kg as compared to the negative control group. Effect of inhibition of inflammation and writhing and protection against fever was more at higher doses than at lower doses. Ethanolic extract of *Terminalia citrina* was more effective than the aqueous extract of the *Terminalia citrina*.

**Conflict of Interest:** Authors declare that there is no conflict of interest in carrying out this study.

## References

1. Abad, M., Guerra, J., Bermejo, P., Irurzun, A., & Carrasco, L. (2000). Search for antiviral activity in higher plant extracts. *Phytotherapy research*, 14(8), 604-607.
2. Akhtar, M., Rabbani, M., Sharif, A., Akhtar, B., Saleem, A., & Murtaza, G. (2011). Formulation and Characterization of Modified Release Tablets Containing Isoniazid Using Swellable Polymers. *African Journal of Traditional, Complementary, and Alternative Medicines*, 8(3), 250-259.
3. Bentley, G., Newton, S., & Starr, J. (1983). Studies on the antinociceptive action of  $\alpha$ -agonist drugs and their interactions with opioid mechanisms. *British journal of pharmacology*, 79(1), 125-134.
4. Bhaskar, V., & Balakrishnan, N. (2009). Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia* and *Carissa carandas*. *DARU Journal of Pharmaceutical Sciences*, 17(3), 168-174.
5. Chattopadhyay, D., Arunachalam, G., Ghosh, L., Rajendran, K., Mandal, A., & Bhattacharya, S. (2005). Antipyretic activity of *Alstonia macrophylla* Wall ex A. DC: an ethnomedicine of Andaman Islands. *Journal of*

- pharmacy & pharmaceutical sciences: a publication of the Canadian Society for Pharmaceutical Sciences, Société canadienne des sciences pharmaceutiques*, 8(3), 558.
6. Chomchuen, S., Singharachai, C., Ruangrunsi, N., & Towiwat, P. (2010). Antipyretic effect of the ethanolic extract of *Ficus racemosa* root in rats. *J Health Res*, 24(1), 23-28.
  7. D Mogosanu, G., M Grumezescu, A., Huang, K.-S., E Bejenaru, L., & Bejenaru, C. (2015). Prevention of Microbial Communities: Novel Approaches Based Natural Products. *Current pharmaceutical biotechnology*, 16(2), 94-111.
  8. Dhara, A., Suba, V., Sen, T., Pal, S., & Chaudhuri, A. N. (2000). Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of *Tragia involucrata* Linn. *J Ethnopharmacol*, 72(1), 265-268.
  9. Du, J., Yu, Y., Ke, Y., Wang, C., Zhu, L., & Qian, Z. M. (2007). Ligustilide attenuates pain behavior induced by acetic acid or formalin. *J Ethnopharmacol*, 112(1), 211-214.
  10. El Gharras, H. (2009). Polyphenols: food sources, properties and applications – a review. *International Journal of Food Science & Technology*, 44(12), 2512-2518.
  11. Franzotti, E., Santos, C., Rodrigues, H., Mourao, R., Andrade, M., & Antoniolli, A. (2000). Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L.(Malva-branca). *J Ethnopharmacol*, 72(1), 273-277.
  12. Ingle, P., & Dhabe, A. (2011). Pharmacognostic Studies on *Terminalia Citrina* (Gaertn) Roxb. Ex Fleming. *Pharmacognosy Journal*, 3(20), 63-65.
  13. Karan, T. K., Bhattacharya, S., Ghosh, A. K., Haldar, P. K., Biswas, M., & Biswas, K. (2011). Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. *Journal of Phytology*, 3(1).
  14. Luo, C., & BOHLIN, L. (2005). Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial. *Acta Pharmacologica Sinica*, 26(8), 926-933.
  15. Manthey, J. A. (2000). Biological properties of flavonoids pertaining to inflammation. *Microcirculation*, 7(S1), S29-S34.
  16. Nwafor, P. A., Nwajiobi, N., Uko, I. E., & Obot, J. S. (2013). Analgesic and anti-Inflammatory activities of an ethanol extract of *Smilax krausiana* leaf in mice. *African Journal of Biomedical Research*, 13(2), 141-148.
  17. Palasuwan, A., Soogarun, S., Lertlum, T., Pradniwat, P., & Wiwanitkit, V. (2005). Inhibition of heinz body induction in an invitro model and total antioxidant activity of medicinal Thai plants. *Asian Pacific Journal of Cancer Prevention*, 6(4), 458.
  18. Pradhan, D. K., Jana, G. K., Panda, S., Choudhury, N. K., & Patro, V. J. (2009). Analgesic, antipyretic and anti-inflammatory effect of the whole plant extract of *Desmostachya bipinnata* Stapf (Poaceae) in albino rats. *Drug Invention Today*, 1(2).
  19. Reegan, A. D., Kinsalin, A. V., Paulraj, M. G., & Ignacimuthu, S. (2015). Larvicidal, ovicidal and repellent activities of marine sponge *Cliona celata* (Grant) extracts against *Anopheles stephensi* Liston (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, 8(1), 29-34.
  20. Saeed, M., Muhammad, N., & Khan, H. (2011). Assessment of heavy metal content of branded Pakistani herbal products. *Tropical Journal of Pharmaceutical Research*, 10(4), 499-506.
  21. Santos, F., & Rao, V. (1998). A Study of the Anti-pyretic Effect of Quinine, an Alkaloid Effective Against Cerebral Malaria, on Fever Induced by Bacterial Endotoxin and Yeast in Rats. *Journal of pharmacy and pharmacology*, 50(2), 225-229.
  22. Sharif, A., Rabbani, M., Akhtar, M. F., Akhtar, B., Saleem, M., Farzana, K., et al. (2011). Design and evaluation of modified release bilayer tablets of Flurbiprofen. *Adv Clin Exp*, 20(3), 343-349.
  23. Sharma, U. S., Sharma, U. K., Singh, A., Sutar, N., & Singh, P. J. (2010). Screening of *Terminalia bellirica* fruits extracts for its analgesic and antipyretic activities. *JJBS*, 3, 121-124.
  24. Shojaii, A., Motaghinejad, M., Norouzi, S., & Motevalian, M. (2015). Evaluation of Anti-inflammatory and Analgesic Activity of the Extract and Fractions of *Astragalus hamosus* in Animal Models. *Iran J Pharm Res*, 14(1), 263-269.
  25. Szabo, M., Radu, D., Gavrilas, S., Chambre, D., & Idoiuiu, C. (2010). Antioxidant and antimicrobial properties of selected spice extracts. *International Journal of Food Properties*, 13(3), 535-545.
  26. Vikrant, A., & Arya, M. (2011). A review on anti-inflammatory plant barks. *International Journal of PharmTech Research*, 3(2), 899-908.