Applied Biological Research 25(1): 36-43; (2023)
DOI: 10.5958/0974-4517.2023.00004.6

IN VIVO ANTI-INFLAMMATORY POTENTIAL, MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION OF FERULIC ACID, ISOLATED FROM PINEAPPLE WASTE

Lubaina Abdulhadeef Shereefa[1]*, Renjith Raveendran[1], Ragesh R Nair[2] and Praveen Kumar[3]

[1]PG and Research Department of Botany, Christian College, Kattakada, Thiruvananthapuram - 695 572, Kerala (India)
[2]PG Department of Botany, Iqbal College, Peringammala, Thiruvananthapuram - 695 563, Kerala (India)
[3] PG and Research Department of Zoology, Government College for Women, Thiruvananthapuram – 695 014, Kerala (India)
*****e-mail: *lubainanizam@gmail.com*

(Received 2 August, 2022; accepted 3 January, 2023)

ABSTRACT

Compounds isolated from organic waste are used as a source for the discovery of new entities against inflammation. Pineapple peel, a by-product of pineapple processing industry, is a thrown away waste. The current work aimed to study the *in-vivo* anti-inflammatory potential and determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ferulic acid - a phenolic acid isolated from the pineapple peel. *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), *Bacillus cereus* (ATCC 10876) and *Staphylococcus aureus* (ATCC 33591) were used for MIC and MBC study. Acute toxicity screened after giving graded doses of ferulic acid and analysed it's *in vivo* anti-inflammatory potential using carrageenin induced paw oedema and xylene induced ear oedema in Wistar rats. NaCl and Indomethacin were used as both negative and positive controls, respectively. The isolated phenolic compounds significantly reduced inflammation at all the dose tested. From the study it may be concluded that the phenolic compound isolated from pineapple peel exhibit significant anti-inflammatory activities and display strong bactericidal potentiality against *Staphylococcus aureus* and *Pseudomonas aeruginosa* indicating its anti-inflammatory and bactericidal potential.

Keywords: Ferulic acid, *in-vivo* anti-inflammatory potential, pineapple waste, *Pseudomonas aeruginosa, Staphylococcus aureus*

INTRODUCTION

The mechanisms linked with inflammation is a major challenge in human health care. Inflammation causes depressing symptoms such as fever, redness, swelling and pain that necessitates immediate pharmaceutical therapy (Sharma *et al*., 2020). Rheumatoid arthritis, pulmonary fibrosis, atherosclerosis and chronic hepatitis are all inflammatory illnesses caused by excessive production of mediators such as leukotrienes, prostaglandins, cytokines like interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL-10), nitric oxide, serotonin, histamine, tumour necrosis factor-alpha (TNF-α), and platelet-activating factor (PAF) (Gupta *et al*., 2014). Reactive oxygen species (ROS) produced by free radicals combine with molecular oxygen, generating inflammation and an imbalance between the antioxidant mechanisms of the body and oxidising chemicals, resulting in oxidative stress. This

oxidative stress triggers inflammatory pathways, causing biological components to be damaged (Amri *et al*., 2018). Blocking these inflammation-causing factors from natural sources is being studied as a promising possibility for anti-inflammatory medicines.

Because of the various negative impacts of medications, it's critical to produce safer drugs derived from plant material that have a higher therapeutic index and lesser negative consequences. As per the World Health Organization (WHO), nonconventional medicines derived from plant sources are used as the primary health care system by 70–80 % of the world's population for treating a variety of maladies (Roy *et al*., 2011). Because of the increased antibiotic resistance shown by infectious pathogens, compounds isolated from plants are being tested for possible antimicrobial action (Burt, 2004). Secondary metabolites from plants found to be active constituents give therapeutic value.

Ethnopharmacologically, fruits wastes are used to treat oedema and inflammation. The anti-inflammatory potentialities of phenolic compounds isolated from fruit wastes helps to prevent oxidative stress and thereby inflammation-related diseases. Pineapple peel - a waste product from the pineapple industry, account for 40-50% of total pineapple weight usually thrown away as waste. With sustainable utilization of pineapple peel using novel scientific and technological methods, valuable products could be obtained. The present research was aimed to determine the *in vivo* anti-inflammatory potential, minimum inhibitory concentration and minimum bactericidal concentration of ferulic acid - a potential phenolic compound isolated from pineapple peel.

MATERIALS AND METHODS

Plant material

Fruit peel of Mauritius variety of pineapple (the most common cultivar in Kerala) collected from the Pineapple Research Station, Vazhakulam, Muvattupuzha, Kerala, India was used for the analyses. The plant material was deposited as a herbarium specimen at the Christian College, Kattakada Herbarium, with voucher number HCCK 108 as reference material.

Drug and chemicals

The chemicals carrageenan, xylene, and other compounds were bought from commercial sources of analytical grade. Before usage, all medication solutions utilised in the study were freshly prepared.

Test microorganisms

The reference bacterial species; *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 33591) and *Bacillus cereus* (ATCC 10876), were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India**.** All microbiological works were carried out according to Clinical & Laboratory Standards Institute (CLSI) guidelines.

In vivo anti-inflammatory studies

Acute toxicity Acute toxicity tests were carried out in accordance with the recommendations of the Organization for Economic Cooperation and Development (Nath and Yadav, 2015). Healthy albino Wistar rats of 8-12 week's age groups of six were used (n = 6). Animals were kept overnight fasting prior to oral drug administration only with unrestricted access to water and after administration of drug sample, food was withheld for a further 3-4 h. The isolated phenol was given orally to four groups of rats at varied dosages of 75, 150, 300, 600 mg kg^{-1} and studied after 14 days for mortality, physical, and behavioural abnormalities. Ferulic acid was adequately suspended in 1% carboxymethyl cellulose and given to the animals orally. Control (group I) was given distilled water only (p.o. for 8 days). Treatment with 0.9% sodium chloride (NaCl) was considered as vehicle group (group II). The Indomethacin treated group received 100 mg kg^{-1} day^{-1} by the intraperitoneal (i.p.) route (Group III). Group IV to VII received 75, 150, 300, 600 mg kg^{-1} body weight of purified phenol. Individual animals

were monitored at least once during the first 30 min after treatment, on a regular basis for the next 24 h (with specific focus during the first 4 h), and daily for the next 14 days.

Anti-inflammatory analysis in carrageenan-induced paw oedema in rats

The anti-inflammatory effect of ferulic acid was analysed in rat paw oedema induced by carrageenan. Sub-planar tissues of the right-hand paw of experimental rats were injected with carrageenan (1%) i.e., 0.1 mL in 0.9 mL NaCl) for 30 min prior to i.p administration of isolated phenolic acid (25-100 mg kg^{-1}). The positive control group received 10 mg kg^{-1} Indomethacin and the negative control group received saline only (0.9% NaCl).

The thickness of the paw was noted from ventral to the dorsal surface using a plethysmometer (Classic Scientific, Maharashtra, India) prior to injection of carrageenan and followed by measurement at 2h intervals for 5 h. Data were expressed as a volume of increase in thickness compared with pre-injection values. The difference in paw thickness at 0 h and at the various experimental periods was measured. The measurements were taken after the 1st, 3rd, and 5th h after the carrageenan injection. The swelling inhibition effect was determined using the formula below.

$$\text{Oedema (\%) inhibition} = \frac{1 - D}{C}\ x\ 100$$

Where, D indicates the percentage variation in increased paw volume after the rats were given test drugs. C denotes the percentage difference in increased volume between the control and experimental groups.

Xylene-induced ear oedema in rats

The anti-inflammatory effect of ferulic acid against xylene-induced ear oedema in rat was carried out as per the protocol of Ma *et al*. (2013). Five classes of rats with six animals in each group were used in this study. The negative control rats were given distilled saline water (0.9% NaCl). Indomethacin (10 mg kg^{-1}) was given to the 2nd group of animals as a positive control. Ferulic acid (25, 50, and 100 mg kg^{-1}) was administered to the experimental groups. After 60 min, 0.03 mL of xylene was applied to the right anterior and posterior ear surfaces to induce ear oedema. The left ear (untreated) was taken as the control. After 60 min, the rats were sacrificed using ether anaesthesia and 6 mm punches were carried out using a borer in the right and left ears of each mouse. The weight of each ear punch was carried and the differences in weight between the two ear punches were tabulated. The anti-inflammatory potential was recorded as the % of inhibition of oedema in the treated mice in comparison with the control rats.

$$\%\ \text{Oedema degree} = M_{right} - M_{left}$$

$$\%\ \text{Inhibition} = \frac{\text{Oedema degree}_{control} - \text{Oedema degree}_{treated}}{\text{Oedema degree}_{control}}\ x\ 100$$

Where M_{right} is the weight of the right earpiece and M_{left} is the weight of the left earpiece

Minimum inhibitory concentration and minimum bactericidal concentration of plant extract

The MIC of plant extract was determined by the Microtiter broth dilution method as per the CLSI guidelines (Dubin, 1998). The stock solution of plant extract was prepared in DMSO (dimethyl sulfoxide) to a final concentration of 100 mg mL^{-1}. The overnight bacterial suspension was adjusted to 0.5 McFarland standard and then diluted to 1×10^6 CFU mL^{-1}. From this suspension, 100 µL was inoculated into each well. The 100 µL plant extract was prepared in Muller Hinton broth (Murray and Zeitinger, 1983) and added to the well in the first row and 2-fold serial dilution was obtained vertically (from row A to H), so as to obtain 5×10^5 CFU mL^{-1} in each test well. The positive control was 5×10^5 CFU mL^{-1}, and the negative control was 100 µL of medium alone. The microtiter plates were incubated overnight at 37°C. MIC values were determined visually and interpreted as the lowest concentration of plant extract displayed no visible growth (clear well). The experiment was repeated in six times. The lowest extract concentration that kills 99.9% of bacterial growth is known as the minimum bactericidal concentration (MBC). Pipetted out 10 µL each from the well exhibiting MIC value and the subsequently two wells showing above the MIC value, and cultured onto Mueller–Hinton agar plates overnight at 37°C. The concentrations showing < MBC value was set at 10 colonies.

Statistical analysis

The statistical analysis of *in vivo* anti-inflammatory activity and measurement of MIC and MBC of ferulic acid was done using ANOVA and Dunnett's test, and the values were reported as mean ± SD. $P \leq 0.05$ was used to determine if the differences in treatment mean and control groups were significant.

RESULTS AND DISCUSSION

Acute toxicity

During 14 days of monitoring, no fatalities or dangerous symptoms were seen in mice following an acute oral therapy with Indomethacin (10 mg kg^{-1}) and different doses of ferulic acid such as 75, 150, 300 and 600 mg kg^{-1}. Ferulic acid administration up to 600 mg kg^{-1} body weight did not produce any morbidity or mortality in drug-treated animals. Therefore, the LD_{50} value was > 600 mg kg^{-1}. As compared to the zero day data, all of the animals in the experimental group did not demonstrate any noticeable drop in food, bodyweight or water intake during 14 days. Administration of the isolated compound revealed no significant behavioural changes in animals under study. Table 1 showed the general cage-side observations for all of the parameters tested.

Table 1: **Cage-side observations of animals administered with ferulic acid (75-600 mg kg^{-1})**

Parameters studied	Observations
Condition of the fur	Normal
Skin	Normal
Subcutaneous swellings	Nil
Abdominal distension	Nil
Eyes – dullness	Nil
Eyes – opacities	Nil
Pupil diameter	Normal
Ptosis	Nil
Colour and consistency of faeces	Normal
Wetness or soiling of perineum	Nil
Condition of teeth	Normal
Breathing abnormalities	Nil
Gait	Normal

Anti-inflammatory analysis in carrageenan-induced paw oedema

The high dose of ferulic acid (100 mg kg^{-1}) considerably decreased the carrageenan induced rat paw oedema in comparison to the control group by 35.41% at 1 h, 53.23% at 3 h and 68.08 % at 5 h after administration (Table 2). The medium dose of ferulic acid (50 g kg^{-1}) might have a huge impact on oedema by 52.18% at 5 h after the administration. The positive control indomethacin (10 mg kg^{-1}) markedly lowered the carrageenan-induced rat paw oedema by 72.11% at 5 h after administration (Fig. 1).

Biopsies of paws were done 5 h after the initial injection of carrageenan for histological analysis. All tissue samples fixed in 10% neutral buffered formalin and embedded in paraffin wax were cut into 5 m thick slices, and stained with eosin and haematoxylin. Histopathological analysis of rat's paw after treatment with higher doses of ferulic acid (100 mg kg^{-1}) showed reduced necrosis, oedema and proliferation of collagenous tissue with normal cellular architecture (Fig. 2). The impact of different concentrations of the isolated phenolic compound on carrageenan-induced paw oedema was comparable to that of the positive control in

Table 2: **Effect of ferulic acid on paw oedema in carrageenan induced and normal rats**

Treatments	Dose	1st h after treatment	3rd h after treatment	5th h after treatment
Control (normal diet)		0.46 ± 0.05	0.61 ± 0.06	0.76 ± 0.09
Indomethacin	10 mg kg^{-1}	0.31 ± 0.04	0.26 ± 0.06	0.20 ± 0.06*
Ferulic acid	25 mg kg^{-1}	0.43 ± 0.07	0.50 ± 0.09	0.57 ± 0.05
Ferulic acid	50 mg kg^{-1}	0.38 ± 0.05	0.42 ± 0.07	0.34 ± 0.08**
Ferulic acid	100 mg kg^{-1}	0.32 ± 0.05	0.29 ± 0.07	0.23 ± 0.08**

Values stated as mean ± SD, n = 6. In comparison to the control; *p <0.05 and **p <0.01

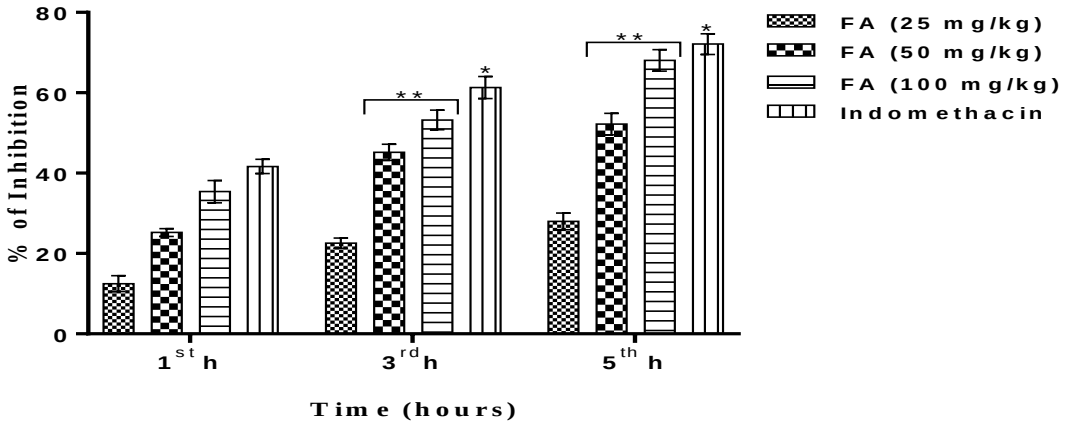

Fig. 1: Impact of ferulic acid on carrageenan-induced paw oedema in rats. The values are mean ± SD, n = 6, one-way ANOVA followed by Dunnett's multiple comparison test; *p < 0.05, **p < 0.01 show a substantial difference as compared to the negative control.

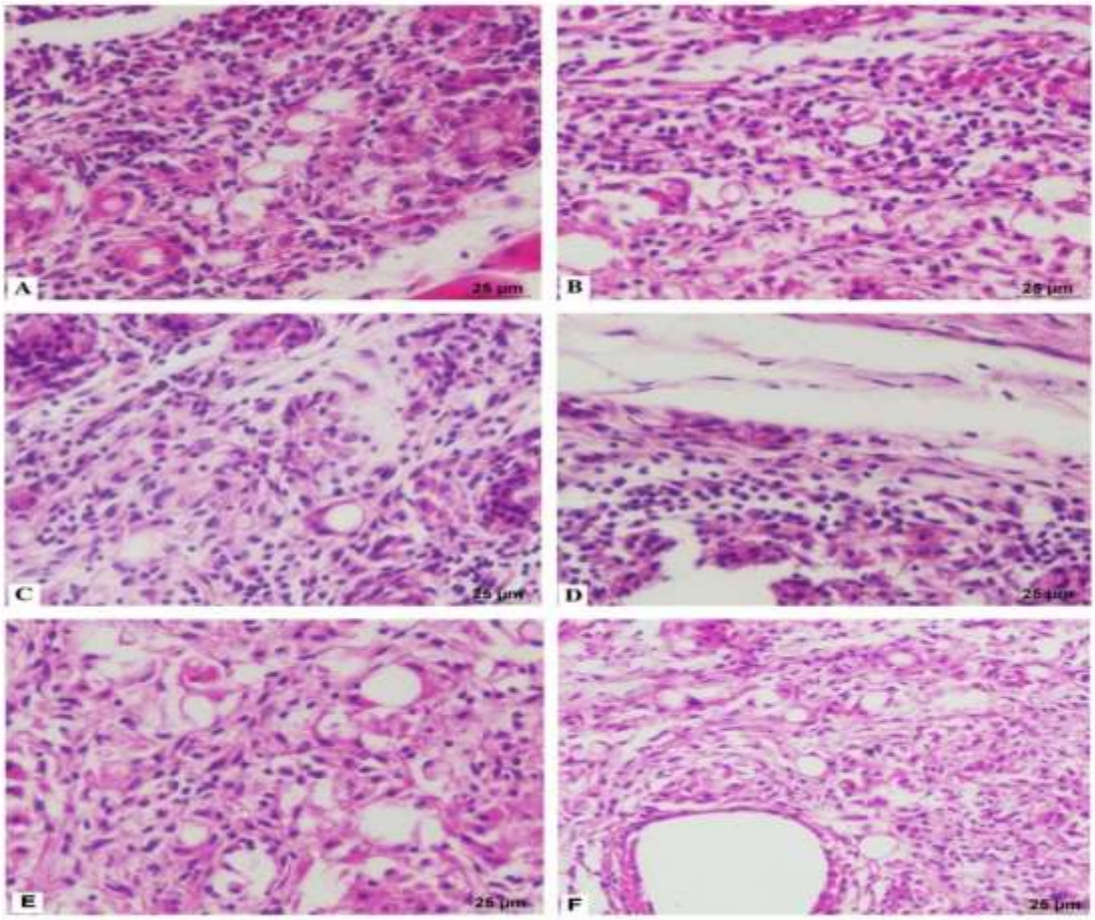

Fig. 2: Effect of ferulic acid on histopathology of carrageenan induced anti-inflammatory study. A) Normal control rat paw with normal histopathology; B) Toxin treated with tissue necrosis, proliferation of collagenous tissue, paw tissue swelling, oedema and leukocyte infiltration; C) Standard Indomethacin treated group with reduced infiltration, oedema and cellular necrosis; and D-F) Ferulic acid-treated groups (25, 50 and 100 mg kg^{-1}) of which the higher dose treated rats paw histopathology showed reduced necrosis, oedema and proliferation of collagenous tissue with normal cellular architecture.

anti-inflammatory activity based on percentage inhibition of paw oedema volume.

Carrageenin-induced paw oedema has been used to assess the anti-oedematous effect of natural products. In rat, the carrageenin-induced inflammatory process is separated into three phases, each of which involves the sequential release of several mediators. During initial phase (1.5 h) of inflammation, histamine and serotonin are released (Lin *et al*., 1995), The second phase occurs from 1.5 to 2.5 h after carrageenin injection, is handled by bradykinin, and the third phase, regulated by prostaglandins, takes place between 2.5 and 6 h following carrageenin injection (Suba *et al*., 2005). In inflammatory reactions, the third step looked to be the most intriguing that prostaglandins play a significant role in the inflammatory process due to their action as modulators of inflammatory responses. The decrease of carrageenin-induced paw edoema after the 3rd h was found significantly correlated with therapeutic dosages of the most promising anti-inflammatory drugs (Panthong *et al*., 2007). Methanolic extract of *Trichosanthes dioica* seeds showed significant dose-dependent *in vivo* anti-inflammatory activity in carrageenan-induced paw oedema in rats (Arora and Gill, 2021). Ferulic acid extracted from pineapple peel greatly decreased the carrageenan-induced paw oedema in rats, in turn revealed its anti-inflammatory potential.

Xylene induced ear oedema

The effects of ferulic acid on xylene-induced acute inflammation in rats is presented in Table 3. In comparison to the control group, the higher dose of ferulic acid (100 mg kg^{-1}) inhibited ear oedema to 62.63% ($p < 0.01$), which was found an effective dose and comparable to the standard treated group. The medium dose of ferulic acid (50 mg kg^{-1}) inhibited oedema to 46.2% ($p \leq 0.01$) while lower dose showed 23.36% ($p \leq 0.05$) inhibition. The standard indomethacin-treatment of 10 mg kg^{-1}) inhibited ear oedema to 66.46 % ($p \leq 0.01$).

Table 3: Effect of ferulic acid on ear weight of xylene induced and normal rats

Treatment	Dose	Oedema degree (mg)	% inhibition
Control (normal diet)		14.25 ± 2.84	-
Indomethacin	10 mg kg^{-1}	4.78 ± 1.95	66.46 %**
Ferulic acid	25 mg kg^{-1}	10.92 ± 1.04	23.37 %
Ferulic acid	50 mg kg^{-1}	7.67 ± 1.53	46.18 %*
Ferulic acid	100 mg kg^{-1}	5.34 ± 2.06	62.63 %**

The results were mean ± SD for six rats in each group. In comparison to the control, *p <0.05 and **p <0.01 were found.

The production of inflammatory mediators that promote vasodilation and increase vascular permeability may contribute to ear oedema. The anti-inflammatory characteristics of natural products have long been assessed using xylene-induced ear oedema, and showed a good predictive value in anti-inflammatory medication (Parveen *et al*., 2007; Zhang *et al*., 2008). The extracted phenolic acid from pineapple peel has been shown to significantly reduce xylene-induced ear oedema in rats, thus it may have an anti-inflammatory effect. During the acute phase of inflammation, ferulic acid may have anti-inflammatory actions by inhibiting inflammatory mediators.

Determination of MBC and MIC

The MBC/MIC ratio of the ferulic acid is shown in Table 4. The MBC/MIC ratio for bacterial isolates was 2, and the isolated compound possessed bactericidal activity against all the test bacterial isolates. The results demonstrated that the isolated phenolic acid was most active against Gram-positive bacterial strain *Staphylococcus aureus* and Gram -ve bacterial strain *Pseudomonas aeruginosa.* A MBC/MIC ratio of < 4 the antimicrobial action is considered bactericidal (Mogana *et al*., 2020).

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ferulic acid against the test bacteria

Bacterial species	MIC (μg mL^{-1})	MBC (μg mL^{1})	MBC/MIC ratio
Klebsiella pneumonia	2048	4096	2
Pseudomonas aeruginosa	512	1024	2
Straphylococcus aureus	512	1024	2
Bacillus cereus	1024	2048	2

Disc diffusion technique was used to examine the bacteriostatic and bactericidal capabilities of the isolated

phenolic acid. The MBC validated by the lack of bacterial growth of the tested strains, which were streaked from the inhibitory zone corresponding to their lowest MICs. The variance in MIC of plant extracts appears due to the changes in their chemical makeup and the volatile nature of components (Mostafa *et al*., 2018). The antimicrobial agents in plants such as terpenoids, alkaloids and phenolic chemicals interact with enzymes and proteins of the microbial cell membrane, disrupting it and producing a flow of protons towards the cell exterior, causing cell death or blocking amino acid biosynthesis (Gill and Holley, 2006).

Conclusion: The study provides the scientific validation of a thrown away waste, thereby established a sustainable strategy for waste management. The isolated natural product can be used as an alternative source for the discovery of new entities in pharmaceutical industry. The anti-inflammatory effects of ferulic acid investigated in albino rats using carrageenan-induced paw oedema and xylene-induced ear oedema showed that the isolated compound had significant anti-inflammatory action within 10 min of administration. The MIC and MBC values of the isolated compound revealed its antibacterial action. More studies are needed to standardize this waste for the development of a novel multi therapeutic herbal drug used for various ailments with least side effects.

Conflict of interest: The authors declare that they have no conflicts of interest.

Acknowledgements: The authors are grateful to Kerala State Council for Science, Technology, and Environment, Thiruvananthapuram, Kerala (Order No. 320/2020/KSCSTE dated 07-06-2020) for the financial support.

Ethical statement: Ethical approval was given by the ethical committee of the Cape Biolab & Research Centre, Marthandam with approval number CBLRC/IAEC/10/01-2020 on 15/07/2020.

REFERENCES

Amri, O., Zekhnini, A., Bouhaimi, A., Tahrouch, S. and Hatimi, H. 2018. Anti-inflammatory activity of methanolic extract from *Pistacia atlantica* Desf. leaves. *Pharmacognosy Journal*, **10**: 71-76.

Arora, R. and Gill, N.S. 2021. Evaluation of anti-inflammatory activity of *Trichosanthes dioica* Roxb. seeds. *International Journal of Pharmaceutical Sciences* and *Drug Research*, **13**: 263-267.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential application in foods - A review. *International Journal of Food Microbiology*, **94**: 223-253.

Dubin, S.B. 1998. Assessment of fetal lung maturity: Practice parameter. *American Journal of Clinical Pathology*, **110**: 723-732.

Gill, A.O. and Holley, R.A. 2006. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *International Journal of Food Microbiology*, **108**: 1-9.

Gupta, D., Verma, N., Das, H.R. and Gupta, R.K. 2014. Evaluation of anti-inflammatory activity of *Dracaena cinnabari* Balf. f. resin. *Indian Journal of Natural Products and Resources*, **5**: 215-222.

Lin, C., Lin, W., Chang, C. and Namba, T. 1995. Anti-inflammatory and hepatoprotective effects of *Ventilago leiocarpa*. *Phytotherapy Research*, **9**: 11-15.

Ma, Y., Li, Y., Li, X. and Wu, Y. 2013. Anti-inflammatory effects of 4-methyl cyclopentadecanone on edema models in mice. *International Journal of Molecular Sciences*, **14**: 23980-23992.

Mogana, R., Adhikari, A., Tzar, M.N., Ramliza, R. and Wiart, C. 2020. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. *BMC Complementary Medicine and Therapies*, **20**: 55-62.

Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N. and Bakri, M.M. 2018. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, **25**: 361-366.

Murray, P.R. and Zeitinger Y.R. 1983. Evaluation of Mueller-Hinton agar for disk diffusion susceptibility tests. *Journal of Clinical Microbiology*, **18:** 1269-1271.

Nath, P. and Yadav, A.K. 2015. Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of *Hibiscus rosa-sinensis* L. in mice. *Journal of Intercultural Ethnopharmacology*, **4**: 1-4.

Panthong, A., Supraditaporn, W., Kanjanapothi, D., Taesotikul, T. and Reutrakul, V. 2007. Analgesic, anti-inflammatory and venotonic effects of *Cissus quadrangularis* Linn. *Journal of Ethnopharmacology*, **110**: 264-270.

Parveen, Z., Deng, Y., Saeed, M.K., Dai, R., Ahamad, W. and Yu, Y.H. 2007. Antiiflammatory and analgesic activities of *Thesium chinense* Turcz extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. *Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan*, **127**: 1275-1279.

Roy, P., Amdekar, S., Kumar, A. and Singh, V. 2011. Preliminary study of the antioxidant properties of flowers and roots of *Pyrostegia venusta* (Ker Gawl) Miers. *BMC Complementary Medicine and Therapies*, **11**: 69-73.

Sharma, V.C., Kaushik, A., Dey, Y.N., Srivastava, B., Wanjari, M. and Jaiswal, B. 2020. Analgesic, anti-inflammatory and antipyretic activities of ethanolic extract of stem bark of *Anogeissus latifolia* Roxb. *Clinical Phytoscience*, **6**: 22-28.

Suba, V., Murugesan, T., Kumaravelrajan, R., Mandal, S.C. and Saha, B.P. 2005. Anti-inflammatory, analgesic and antiperoxidative efficacy of *Barleria lupulina* Lindl. extract. *Phytotherapy Research*, **19**: 695-699.

Zhang, Z., Luo, P., Li, J., Yi, T., Wang, J., An, J. and Zhang, H. 2008. Comparison of the anti-inflammatory activities of three medicinal plants known as “Meiduoluomi” in Tibetan folk medicine. *Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan*, **128**: 805-810.