Inhibition of the Chemiluminescence and Chemotactic Activity of Phagocytes for Selected *Ficus* Species Extract and Their Lipoxygenase and Xanthine Oxidase Enzyme Inhibitory

(Perencatan Aktiviti Kemiluminesens dan Kemotaktik Fagosit oleh Ekstrak Spesies *Ficus* Terpilih dan Kesan Perencatan terhadap Enzim Lipoksigenase dan Xantina Oksidase)

SHUKRANUL MAWA, IBRAHIM JANTAN, FADZUREENA JAMALUDDIN & KHAIＲANA HUＳAIN*

**ABSTRACT**

The effects of crude methanol from seven *Ficus* species on reactive oxygen species (ROS) production were evaluated using luminol based chemiluminescence assay and their inhibition of PMN chemotaxis was investigated using Boyden chamber technique. Crude methanol of *F. aurantiaca*, *F. parietalis*, and *F. annulata* showed strong activity against PMN chemotaxis with IC$_{50}$ values of 1.4, 0.3 and 2.2 µg/mL, respectively, while crude methanol from *F. aurantiaca* and *F. parietalis* on whole blood and ROS production of PMNs exhibited high inhibitory activity for luminol enhanced chemiluminescence with IC$_{50}$ values of 1.4, 0.9, and 1.0, 0.4 µg/mL, respectively. In xanthine oxidase inhibition assay, crude methanol from *F. aurantiaca* (stem), *F. parietalis* (stem), and *F. annulata* (leaf) exhibited greater than 92% inhibition at concentration of 100 µg/mL (with IC$_{50}$ values of 0.9, 1.0 and 8.9 µg/mL, respectively) while some extracts of other species showed more than 60% inhibitions at this concentration. In soybean lipoxygenase (SBL) assay, the methanolic extracts of these 3 species showed potent SBL inhibition with IC$_{50}$ values of 0.3, 0.7, and 6.3 µg/mL, respectively. The overall results suggest that *F. parietalis*, *F. annulata*, and *F. aurantiaca* might be a prospective source of anti-inflammatory mediators and as a source of new immunomodulatory agents.

**Keywords:** Chemiluminescence; chemotaxis; medicinal plants; soybean lipoxygenase enzyme; xanthine oxidase

**INTRODUCTION**

Phagocytes play an important role in one’s innate immune system defence mechanism. It serves as an important link between adaptive and innate immune mechanisms. Polymorphonuclear phagocytes (often called neutrophils or PMNs) are an important group of phagocytes. Neutrophils constitute a majority of blood leucocytes and develop from early precursors as monocytes and macrophages. During the phagocytosis, an increasing glucose and oxygen consumption is seen, which is referred to as the respiratory burst. The consequence of respiratory burst is a number of oxygen-containing compounds, which kill the phagocytosed bacteria during respiratory burst. Based on the types of superoxide produced, the destruction of microorganisms can be divided into two categories, namely destruction by reactive oxygen species and destruction by reactive nitrogen species (Hiyoshi et al. 2018).
Chemoattractants attract cells towards chemical signals emitted at the site of injury. The phagocytic cells are recruited to the site of infection or tissue damage by certain chemical stimulants that act as chemoattractants. They include the products of microorganisms, phospholipids released by injured mammalian cells, and the complement component C5a (Lee et al. 2019). Immunomodulators and anti-inflammatory drugs are widely used as the inhibitors of phagocyte reactive oxygen species (ROS) production in order to treat a variety of disorders along with inflammation. Therefore, the innovation of a novel plant with immune system-modulating activities has become a progressively important area of research, particularly in the search for new-generation vaccine adjuvant.

Xanthine oxidase (XO) serves as a significant biological foundation of oxygen-derived free radicals, which yields oxidative break to living tissues implicated in many pathological processes such as inflammation, atherosclerosis, cancer, and ageing (Xinglong et al. 2018). It is the enzyme responsible for the formation of uric acid from the purines, namely hypoxanthine, and xanthine, and is responsible for a medical condition known as gout due to their overproduction. Since xanthine oxidase inhibitors (XOIs) have minor side effects in contrast with other anti-inflammatory mediators such as uricosuric, it is greatly efficient for inflammation. The clinically used XOIs is only allopurinol and causes various side effects, namely hypersensitivity syndrome, Stevens–Johnson syndrome, and renal toxicity. Although it is usually known to be safe, some contraindications are applicable for approximately 2% of the general population. This is a prominent fact, leading researchers to search for novel XOIs among natural products. Until now, phenolics and particular flavonoids have been found to be a large number of chemical skeletons with XO inhibitory properties, and their functions have been methodically evaluated (Liu et al. 2017; Yang et al. 2019). Therefore, the exploration of novel XOIs from natural plants may be helpful for the treatment of gout and a variety of inflammatory illnesses.

5-Lipoxygenase enzyme (5-LOX), 12-lipoxygenase enzyme (12-LOX), and 15-lipoxygenase enzyme (15-LOX) are a family of enzymes called lipoxygenase (LOX). The catalysis of 5-LOX synthesizes fatty acid signal molecules leukotrienes from arachidonic acid in the cell. The overproduction of leukotrienes causes a reduction in the soft muscle’s inside layer, which is the source of inflammation such as asthma and allergic diseases. Furthermore, hypersensitive inflammatory reactions like asthma, ulcerative colitis, rheumatoid arthritis, psoriasis, glomerular nephritis, and cancer are brought out by these arachidonic acid metabolites. The 5-LOX enzyme is a soybean lipoxygenase (SBL) similar to human LOX, which further catalyses the oxidation of linoleic acid, arachidonic acid, and other unsaturated fatty acids. Consequently, the SBL inhibition test may be applied to find new material that is actively functioning as the inhibitors of mammalian lipoxygenases; an instance is 5-LOX (Srivastava et al. 2016).

Ficus (Moraceae) comprises one of the largest genera of angiosperms with more than 800 species of trees, shrubs, hemi-epiphytes, and climbers in the tropics and subtropics worldwide. It is traditionally used for the treatment of wound healing, headache, toothache, and inflammation. A number of Ficus species are well-known in Asia as medicinal plants and widely used in folk medicine for the treatment of flu, malaria, tonsillitis, bronchitis, rheumatism, and various inflammatory diseases (Burkill 1966). One of the most biologically active species of Ficus is F. carica, which has many uses according to different ethnomedical reports (Mawa et al. 2013). In Malaysia, Ficus has been traditionally used for the treatment of inflammation, headache, wound, and toothache (Kamaruddin & Latif 2002). It is widespread in the lowland forests of Kelantan, Terengganu, Perak, Pahang, Selangor, Melaka, and Johor. Malaysia has a large number of medicinal plants and some plants have been used to treat gout; however, until now, no traditional usage of Ficus has been reported. In this study, seven species of Ficus from Malaysian therapeutic plants have been chosen to screen their XO inhibitory action based on the ethnomedical uses of the genus in various inflammatory disorders, such as rheumatism, swellings, and hyperglycaemia (Khajuria et al. 2017; Yang et al. 2018).

MATERIALS AND METHODS

PLANT SAMPLES

The stem (500.0 g) and leaf (4.5 kg) of F. aurantiaca Griff. (SM 2109), leaf (2.3 kg) of F. grossularioides Burm. F. (SM2110), leaf (5.1 kg) of F. obscura Blume. (SM2133), leaf (4.9 kg) of F. heteropodera Blume. (SM2130), leaf (3.6 kg) of F. annulata Blume. (SM2131), stem (300.2 g) and leaf (3.5 g) of F. parietalis Blume. (SM2132) and leaf of F. bengalensis L. (SM2133) were collected from Banting, Selangor, Malaysia. The voucher specimens were identified and deposited at the Herbarium of Universiti Kebangsaan Malaysia (UKM), Bangi.

PREPARATION OF PLANT EXTRACT

Each plant material was air-dried at room temperature and ground. The ground powders of the stem (100.0 g) and leaf (132.1 g) of F. aurantiaca, leaf (57.5 g) of F. grossularioides Burm. F, leaf (150 g) of F. obscura Blume, leaf (140.0 g) of F. heteropodera Blume, stem (50.0 g) and leaf (100.0 g) of F. parietalis Blume, leaf (100.0 g) of F. annulata Blume, and leaf (100.0 g) of F. bengalensis L. were macerated with methanol (1:3 v/v) every three days and filtration was repeated three times.
times. The extract was evaporated to remove the solvent by using a rotary evaporator in order to obtain a crude extract.

CHEMICALS, REAGENTS AND EQUIPMENTS
Luminol, serum opsonised zymosan A, phosphate buffer saline (PBS), Hanks balanced salt solutions (HBSS)**, Ficoll, dextran, dimethylsulfoxide (DMSO), N-formylmethionyl-leucyl-phenylalanine (NMLP), hematoxyline, oil immersion, trypan blue, aspirin and ibuprofen were purchased from Sigma (St Louis, Missouri, USA). Chemicals for xanthine oxidase and soybean lipoxygenase enzyme inhibition assay: Xanthine oxidase (XO) enzyme from cow’s milk (20 units/mL) was obtained from Roche Diagnostics Pt Ltd. (Cat No. 10110434001, St. Louis, MI, USA). Lipoxygenase enzyme, type 1B, soybean (80 units/well) was obtained from Sigma (St Louis, Missouri, USA). Chemicals for xanthine oxidase and soybean lipoxygenase enzyme inhibition assay: Xanthine oxidase (XO) enzyme from cow’s milk (20 units/mL) was obtained from Roche Diagnostics Pt Ltd. (Cat No. 10110434001, St. Louis, MI, USA). Lipoxygenase enzyme, type 1B, soybean (80 units/well) was obtained from Sigma (Cat No. L-73951, St. Louis, MI, USA) while xanthine substrate (0.15 mM), allopurinol and phosphate buffer (KH₂PO₄, 50 mM, pH 7.5), substrate linoleic acid (0.3 mM, Sigma cat no. L-8134, FW 302.4), DMSO, phosphate buffer (NaH₂PO₄, 100 mM, pH 8.0) and phenidone were obtained from Sigma (St. Louis, MI, USA). All other chemicals have been used in this research were got hold of commercially and were of analytical grade. The working solution of substrate xanthine in buffer (pH 7.5), the xanthine oxidase enzyme solution (0.2 unit/ well) in buffer (pH 7.5) and the working solution of substrate linoleic acid in phosphate buffer (pH 8.0), the soybean lipoxygenase (SBL) enzyme solution (80 unit/well) in buffer (pH 8.0) were prepared immediately before use.

While chemiluminescence measurements were carried out on a Luminoscan Ascent luminometer (Thermo Scientific, UK). A Boyden chamber (48-well) with 2 µm polycarbonate membrane filter separating the upper and lower compartments was purchased from Neuro probe, Cabin Jhon, MD, USA), carbon dioxide incubator (Thermo scientific, UK), Haemocytometer (Hauser Scientific, USA and low-power microscope (Olympus, Germany) were also used in this assay.

ISOLATION OF POLYMORPH NUCLEAR LEUCOCYTES (PMNS)
Human blood was collected from a healthy volunteer who had fasted for at least 8 h. PMNs were isolated by Ficoll gradient separation method as described (Yuandani et al. 2013). The use of human blood in this study was approved by the human ethics committee of Universiti Kebangsaan Malaysia Medical Centre (HUKM), Cheras, with a recorded permission number of FF-220-2008. Cell counts were performed using a haemocytometer.

CELL VIABILITY
Cell viability test was carried out using trypan blue dye exclusion method with additional procedure modification as described by Koko et al. (2008). The neutrophils (1 × 10⁶/mL) were incubated with 6.25 µg/mL and 100 µg/mL of plants extracts in triplicate at 37 °C for 1 to 2 h. In the chemotaxis assay, the lowest concentration of 6.25 µg/mL and the highest concentration of 100 µg/mL were utilised. Cell death was indicated by the blue dye uptake and percentage of cell viability was considered from the total cell counts.

CHEMILUMINESCENCE ASSAY
Luminol enhanced chemiluminescence assay was carried out using a modified version of the described method (Mawa et al. 2016; Yuandani et al. 2013). In brief, 25 µL of human whole blood or 25 µL of isolated PMN cells were suspended in HBSS** into each well of 96-well microplate. The plate was incubated with 25 µL of tested samples and aspirin at five different concentrations (12.5, 6.25, 3.18, 1.56, and 0.78 µg/mL) of each sample for 50 min at 37 °C in luminoscan, while 25 µL of HBSS** was used as for the negative control. The cells were induced with 25 µL of Serum Opsonised Zymosan, followed by 25 µL of luminol into each well. Then, the HBSS** solution was added into each well to ensure the final volume of 200 µL. Aspirin was used as the positive control while the negative control contained zymosan, luminol, DMSO (0.5%), HBSS**, and cells. The percentage of inhibition was calculated by the measurement of RLU (reading luminometer unit) of peak and total integral values, with repeated scans conducted.

CHEMOTAXIS ASSAY
The assay was carried out using the modified 48-well Boyden chamber method (Yuandani et al. 2016). The Boyden chamber contains 48 wells with a diameter of 8 µm and is divided into two compartments by a filter separation. In brief, 25 µL of chemoattractant, fMLP (10⁻⁸ M, diluted with chemoattractant buffer solution) was added to the lower compartment of the Boyden chamber. Then, 45 µL of PMN cell suspension with 5 µL of test samples and ibuprofen at five concentrations (6.25, 12.5, 25, 50, and 100 µg/mL) were added to the upper compartment of the chamber, whereas the negative control contained 45 µL of PMN cell suspension and 5 µL of chemoattractant buffer. Ibuprofen was used as a positive control. The final concentrations of test samples and ibuprofen in the wells were 0.625, 1.25, 2.5, 5, and 10 µg/mL. The chamber was incubated in 5% carbon dioxide incubator for 1 h at 37 °C. After incubation, the polycarbonate membrane (where the migrated cells remained) was stained with PBS, methanol (99.5%), haematoxylin, distilled water, ethanol (70%) and 95.8%) and xylene. The distance of cell migration was measured using a low power microscope with 40x magnifications.

In vitro XANTHINE OXIDASE INHIBITION ASSAY
All extracts were investigated for in vitro XO inhibitory activity and the assay was run spectrophotometrically in an aerobic environment (Mamat et al. 2014). The
combination for the assay was prepared with 1 mL of test solutions (6.25, 12.5, 25, 50, and 100 µg/mL), 2.9 mL of phosphate buffer (pH 7.5), and 0.1 mL of enzyme solution (0.01 units/mL in phosphate buffer, pH 7.5). They were made instantly before use. After pre-incubation for 15 min at 25 °C, the reaction was started by adding 2 mL of substrate solution (i.e. 0.15 mM xanthine in phosphate buffer). The mixture was incubated for 30 min at 25 °C and the reaction was then blocked by adding 1 mL of 1N hydrochloric acid, whereby its absorbance was calculated at 295 nm with a UV spectrophotometer. In the same way, a blank was arranged but the enzyme solution was replaced with phosphate buffer. Another reaction mixture was prepared (control) and had 100 µL of DMSO instead of test samples in order to generate the maximum uric acid formation. The reading was taken with three parallel measurements and the assay was done in triplicate. The total amount of enzyme necessary to make 1 mmol of uric acid per min at 25 °C was thus defined as one unit of xanthine oxidase (XO). Xanthine oxidase inhibitory activity was calculated to evaluate the degree of inhibitory activity using a reported equation (Xinglong et al. 2018). Allopurinol as a recognised inhibitor of XO has been used as a positive control, while the IC_{50} values have been considered from the mean values of data.

Percentage of inhibition (%) = \( \frac{(1 - \beta/\alpha) \times 100}{\beta} \)

where \( \alpha \) is the optical density of control or \( \alpha \) is the activity of XO without plant extract, and \( \beta \) is the optical density of test samples or \( \beta \) is the activity of XO with plant extract.

**In vitro SOYBEAN LIPOXYGENASE ENZYME INHIBITION ASSAY**

Soybean lipoxygenase (SBL) inhibition was evaluated spectrophotometrically with the measurement of change in the absorbance at 234 nm following the modified published method (Zhang et al. 2018). The structure of hydroperoxylinoleic acid was then converted to linoleic acid. The blank, control and samples were made with the following compositions:

- **Blank:** 700 µL of sodium phosphate buffer (100 mM, pH 8.0) was put into 50 µL of DMSO solution and 250 µL of linoleic acid (250 µM solution in phosphate buffer) was added to formulate the 1 mL volume of reaction.
- **Control:** Sodium phosphate buffer (200 µL, 100 mM) was added to DMSO (50 µL) solution, enzyme (500 µL, 400 units/mL in phosphate buffer), and linoleic acid (250 µL, 250 µM) to make the 1 mL volume of reaction.
- **Sample:** Sodium phosphate buffer (200 µL, pH 8.0) was added to 50 µL of extracts at various concentrations (6.25-100 µg/mL in DMSO), enzyme (500 µL, 400 unit/mL in phosphate buffer), and linoleic acid (250 µL) to make the volume of reaction 1 mL.

Phenidone was used as a standard SBL inhibitor. Each experiment was done in triplicates. Percentage of inhibition was considered using the following formula:

\[ \% \text{ of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \]

**STATISTICAL ANALYSIS**

Data were analyzed using One-way ANOVA, post hoc, Turkey. P < 0.05 was considered to be statistically significant using SPSS 17.0 statistical software. All values were characterized as mean ± S.E.M. Probit programme was used to determine the IC_{50} values for active samples.

**RESULTS AND DISCUSSION**

**REACTIVE OXYGEN SPECIES (ROS) INHIBITORY ACTIVITY OF CRUDE EXTRACTS ON HUMAN WHOLE BLOOD AND PMNs**

This study used different parts of varying species. Different plant parts may contain different chemical constituents, thus each part will show different activities for the same assay. Screening the extracts from seven *Ficus* species on whole blood showed that the crude methanol of *F. aurantiaca* (stem), *F. parietalis* (stem), and *F. annulata* (leaf) exhibited significant inhibition for luminol-enhanced chemiluminescence with IC_{50} values of 1.4, 0.9 and 6.1 µg/mL, respectively (Table 1). In contrast, the first two were lower than that of the positive control, namely aspirin (2.1 µg/mL). The highest percentage of inhibition on whole blood was shown by the crude extract of *F. aurantiaca* stem. The crude extracts were further evaluated for their effects on the oxidative burst of PMNs. Among all extracts, crude methanol of five species showed inhibition on PMNs, whereas crude methanol of *F. aurantiaca* (stem), *F. parietalis* (stem), and *F. annulata* (leaf) showed the most potent activity against PMNs with the IC_{50} values of 1.0, 0.4 and 4.1 µg/mL, respectively. The IC_{50} values were lower than that of the positive control aspirin (1.7 µg/mL) (Table 1). The activation of phagocytosis by serum opsonised zymosan marks the simultaneous production of ROS and the release of hydrolytic enzymes. The process was initiated by the reaction of ROS on the surface of phagocytic cells. The released ROS were then measured by luminol-enhanced chemiluminescence assay. Luminol increases in detecting the specific ROS affected by the extracts because luminol can enter the cells due to its small molecular weight, and then react with intracellular and extracellular ROS (Hiyoshi 2018). The dose-dependent effects of active extracts are shown in Figure 1(a) and 1(b).
Cells were viable (>95%) at the concentration of 6.25 and 100 µg/mL of the extracts after incubation of 2 h. Of all the extracts of seven species, *F. aurantiaca* (stem), *F. parietalis* (stem), and *F. annulata* (leaf) showed strong inhibitory activity with dose-dependent effects on the migration of PMNs towards the chemotactant (fMLP) with IC$_{50}$ values of 1.4 and 0.3 µg/mL, respectively. *F. bengalensis* also inhibited the migration of PMNs. The IC$_{50}$ values of *F. aurantiaca* (1.4 µg/mL) and *F. parietalis* (0.3 µg/mL) were lower than that of positive control ibuprofen (1.5 µg/mL). The cell viability test was performed using trypan blue to determine the toxicity of concentrations for all plant extracts. The elevated cell viability indicated that the extracts were nontoxic to immune cells and could highly adapt the cellular immune response in the reaction mixtures. Chemotactant buffer (DMSO and HBSS, 1:1 ratio) was used as a control and ibuprofen, which was found to be the most effective drug in a study to determine the effect of selected NSAIDs in blocking the migration of PMNs (Lee et al. 2019). The percentage of inhibitions (%) and the IC$_{50}$ values of active extracts with dose-dependent effects are shown in Figure 2 and Table 1, respectively.
TABLE 1. IC$_{50}$ values of active crude methanol and positive control for chemiluminescence and chemotaxis activity. Data represents mean ± SEM (n = 3) in triplicate measurement

| Plant species       | Parts   | IC$_{50}$ value ± SEM (µg/mL) | Chemiluminescence | Chemotaxis |
|---------------------|---------|--------------------------------|--------------------|------------|
|                     |         |                                | PMNs               | WB         |
| $F$. anulata        | leaf    | 4.1±1.5                        | 6.1±1.1            | 2.2±0.3    |
| $F$. aurantiaca     | leaf    | 17.1±1.1                       | -                  | -          |
| $F$. aurantiaca     | stem    | 1.0±0.3                        | 1.4±0.7            | 1.4±0.4    |
| $F$. parietalis     | stem    | 0.4±0.07                       | 0.9±0.7            | 0.3±0.1    |
| $F$. heteropleura   | leaf    | 15.3±1.2                       | 20.8±3.4           | -          |
| $F$. grossularioidae| leaf    | -                              | -                  | -          |
| $F$. bengalgensis   | leaf    | -                              | -                  | -          |
| $F$. obscura        | leaf    | -                              | -                  | -          |
| Aspirin             |         |                                |                    |            |
| (positive control)  |         | -                              | 1.7±0.3            | 2.1±0.9    |
| Iboprunfen          |         |                                |                    | 1.5±0.3    |

FIGURE 2. Dose dependent percentage of inhibition on PMNs chemotaxis
Data are mean ± SEM (n = 3). *P<0.05 is significant difference compared to the negative control.
**In vitro XANTHINE OXIDASE INHIBITORY ACTIVITY**

Of the 11 crude extracts of different parts from seven species screened in this study, five extracts demonstrated XO inhibition at the concentration of 100 µg/mL, whereas three extracts showed more than 92% inhibition at this concentration. Four crude extracts showed more than 50% inhibitory activity at the lowest concentration of 6.25 µg/mL. Among the extracts screened for XO inhibition, the methanolic extracts from the stem of *F. aurantiaca* and *F. parietalis* and leaf of *F. annulata* showed the highest inhibitory activity with IC$_{50}$ values of 0.9 and 1.0 and 8.9 µg/mL, respectively. Any crude extracts of *F. grossolarioidae*, *F. heteropleura*, and *F. obscura* did not exhibit any important activity up to 100 µg/mL concentrations while *F. bengalgensis* showed more than 60% inhibition only at a concentration of 100 µg/mL. Finally, the methanolic extracts of *F. parietalis* (stem), *F. aurantiaca* (stem), and *F. annulata* (leaf) exhibited potent xanthine oxidase inhibition that is comparable to that of positive control allopurinol. Allopurinol was used as a standard drug to compare the studied results, which showed a 92.5% inhibitory activity at a concentration of 100 µg/mL at the IC$_{50}$ value of 0.96 µg/mL. The methanolic extracts of *F. aurantiaca* (stem), and *F. parietalis* (stem) exhibited greater than 92% inhibitory activity, which was higher than that of allopurinol. Many published literature have shown different anti-inflammatory and antioxidant properties of various species of *Ficus* (Yang et al. 2018) due to the existence of flavonoids, triterpenoids, lignans, alkaloid, diterpene, phenolics, and polyphenols (Cheng et al. 2019; Mawa et al. 2016). Accordingly, XO inhibitory activity of the extracts from the studied species of *Ficus* might be credited to the existence of active chemical constituents against inflammation. The percentage of inhibitions (%) and IC$_{50}$ values of active extracts with dose-dependent effects are shown in Figure 3 and Table 2, respectively.

**FIGURE 3.** Xanthine oxidase inhibitory effects of active crude methanol

Data are mean ± SEM (n = 3). *P<0.05 is significant difference compared to the negative control

**In vitro SOYBEAN LIPOXYGENASE (SBL) INHIBITORY ACTIVITY**

Among all extracts of the seven *Ficus* species, the crude methanol from the stem of *F. aurantiaca*, and *F. parietalis*, leaf of *F. annulata*, and *F. obscura* showed SBL inhibition with the IC$_{50}$ values of 0.3, 0.7, 6.3, and 53.2 µg/mL, respectively. Meanwhile, the IC$_{50}$ values of crude methanol from the stem of *F. parietalis*, and *F. aurantiaca* showed remarkable activities compared to standard drug phenidone with an IC$_{50}$ value of 0.35 µg/mL. The phytochemical analysis of different species of *Ficus* showed the presence of flavonoids, coumarins, alkaloids, steroids, pentacyclic triterpenes, simple phenols, and salicylic acids (Cheng et al. 2019; Mawa et al. 2016). Meanwhile, the inhibition of lipoxygenase was mostly due to the attachment of the keto group. The percentage of inhibitions (%) and the IC$_{50}$ values of active extracts with dose-dependent effects are shown in Figure 4 and Table 2, respectively.
TABLE 2. IC_{50} values of active crude methanol and positive control for soybean lipoxygenase and xanthine oxidase activity.

Data represent mean ± SEM (n = 3) in triplicate measurement.

| Plant species | Parts       | Soybean lipoxygenase IC_{50} ± SEM (µg/mL) | Xanthine oxidase IC_{50} ± SEM (µg/mL) |
|---------------|-------------|------------------------------------------|---------------------------------------|
| *F. annulata* | leaf        | 6.36±0.3                                  | 8.90±0.4                              |
| *F. parietalis* | leaf      | 34.97±2.3                                 | 22.08±0.2                             |
| *F. parietalis* | stem      | 0.71±0.1                                  | 1.03±0.4                              |
| *F. aurantiaca* | stem      | 0.30±2.1                                  | 0.90±0.8                              |
| *F. bengalgensis* | leaf      | -                                        | 30.32±5.8                             |
| *F. obscura* | leaf        | 53.20±3.0                                  | -                                     |
| *F. grossularioidae* | leaf   | -                                        | -                                     |
| *F. heteropleura* | leaf       | -                                        | -                                     |
| Allopurinol (positive control) | - | -                                        | 0.96±0.3                              |
| Phenidone (positive control) | - | 0.35±0.1                                  | -                                     |

Data are mean ± SEM (n = 3). *P < 0.05 is significant difference compared to the negative control.
CONCLUSION
This report described biological screening for PMNs chemotaxis, ROS inhibitory activity of crude methanol extracts on human whole blood and PMNs, soybean lipoxygenase and xanthine oxidase enzyme inhibitory activities of specific species of Ficus. A total of seven Ficus species were extracted from different parts. Among all extracts, the crude methanol from the stem of F. aurantiaca and F. parietalis, and leaf of F. annulata showed strong inhibition in all assays. Crude methanol extracts from F. parietalis, F. aurantiaca, F. annulata, and F. obscura thus supported the phytochemical investigation and their ethnopharmacological properties for wound healing and inflammation. Therefore, F. aurantiaca (stem), F. parietalis (stem), and F. annulata (leaf) may be prospective source of new leads in the development of new immunomodulatory agents, as well as being functional as remedies in hyperuricaemia and gout. Additionally, this study can establish the source for exploring further the appropriate phytochemical investigation and drug improvement on other mechanisms of immunomodulatory responses.

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