In vitro pro-inflammatory enzyme inhibition and anti-oxidant potential of selected Sri Lankan medicinal plants

Hettiarachchige Dona Sachindra Melshandi Perera¹, Jayanetti Koralalage Ramani Radhika Samarasekera¹*, Shiroma Mangalika Handunnetti², Ovitigala Vithanage Don Sisira Jagathpriya Weerasena², Hasitha Dhananjaya Weeratunga¹, Almas Jabeen³ and Muhammad Iqbal Choudhary⁴

Abstract

Background: The extracts of the ten selected Sri Lankan medicinal plants have been traditionally used in the treatment of inflammatory mediated diseases. The extracts were investigated for anti-inflammatory and anti-oxidant potential in vitro to identify bio-active extracts for further chemical characterization.

Methods: In vitro anti-inflammatory activities of total ethanol extracts were investigated measuring the inhibitory activities of four pro-inflammatory enzymes, arachidonate-5-lipoxygenase (A5-LOX), hyaluronidase (HYL), xanthine oxidase (XO) and inducible nitric oxide (iNO) synthase. Cytotoxicity of extracts were determined by MTT assay. Oxidative burst inhibition (OBI) on human whole blood (WB) and isolated polymorphonuclear neutrophils (PMNs) was carried out for a selected bio-active extract. Anti-oxidant activities of the extracts were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), ferrous ion chelation (FIC) and oxygen radical absorbance capacity (ORAC) assays. Total polyphenol and total Flavonoid contents of the extracts were also determined. The most active plant extract was analysed using Gas chromatography-Mass spectrometry (GC-MS) and High Performance Liquid Chromatography (HPLC).

Results: The ethanol bark extract of Flacourtia indica showed the highest A5-LOX (IC₅₀: 22.75 ± 1.94 μg/mL), XO (70.46 ± 0.18%; 250 μg/mL) and iNOs inhibitory activities on LPS-activated RAW 264.7 macrophage cells (38.07 ± 0.93%; 500 μg/mL) with promising OBI both on WB (IC₅₀: 47.64 ± 2.32 μg/mL) and PMNs (IC₅₀: 5.0 ± 20.38 μg/mL). The highest HYL inhibitory activity was showed by the leaf extracts of Barathranthus nodiflorus (42.31 ± 2.00%; 500 μg/mL) and Diospyros ebenum (41.60 ± 1.18%; 500 μg/mL). The bark and leaf extracts of Callophyllum innophyllum (IC₅₀: 6.99 ± 0.02 μg/mL) and Symlocos cochinichinesis (IC₅₀: 9.85 ± 0.28 μg/mL) showed promising DPPH free radical scavenging activities. The GC-MS analysis of ethanol bark extract of F. indica showed the presence of two major bio-active compounds linoleic acid ethyl ester and hexadecanoic acid, ethyl ester (> 2% peak area). The HPLC analysis showed the presence polyphenolic compounds.

Conclusion: The ethanol bark extract of F. indica can be identified as a potential candidate for the development of anti-inflammatory agents, which deserves further investigations. The bio-active plant extracts may be effectively used in the applications of cosmetic and health care industry.

Keywords: Anti-inflammatory, Enzyme inhibition, Anti-oxidant, Medicinal plants, F. indica, Gas chromatography-mass spectrometry, High performance liquid chromatography

* Correspondence: radhika@iti.lk
¹Industrial Technology Institute (ITI), 363, Baudhaloka Mawatha, Colombo 07, Sri Lanka
Full list of author information is available at the end of the article

© The Author(s). 2018 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
In Sri Lanka, medicinal plants have always been used and still remain a major source in the treatment of number of diseases including inflammatory and oxidative-stress associated chronic diseases. Free radicals can be either beneficial or deleterious to the body depending on the level. The excess levels of free radicals will cause damage to most cellular macromolecules such as proteins (enzymes), lipids and DNA leading to a condition called oxidative stress [1]. Oxidative stress has been recognized as a key factor in the pathogenesis of many diseases including inflammatory diseases [2]. The excess of reactive oxygen species (ROS) generated will lead to inflammation by stimulating cytokines and activation of pro-inflammatory enzymes such as lipooxygenase, hyaluronidase, inducible nitric oxide synthase and xanthine oxidase [3]. Lipooxygenases are capable of generating lipid mediators such as leukotrienes and prostaglandins, which can provoke several inflammatory diseases such as bronchial asthma, allergic rhinitis, cardiovascular diseases, rheumatoid arthritis and certain types of cancer [4]. Hyaluronidase will lead to degranulation of mast cells and release inflammatory mediators leading to several pathological conditions including rheumatoid arthritis [5]. Upon activation of inflammatory cells, inducible nitric oxide synthase (iNOs) will generate excessive amount of nitric oxide (NO), which can cause inflammation [6]. Xanthine oxidases also play a major role in the metabolic disease called gout, which is closely associated with inflammation and some other inflammatory mediated diseases due to the formation of free radicals during the catalytic function of the enzyme. It is evident that these pro-inflammatory enzymes play an important role in the pathogenesis of inflammation via different pathways. Hence, inhibition of these enzymes is considered as targets for the management of diseases associated with oxidative stress and inflammation [7].

Oxidative burst is characterized by the production and rapid release of reactive oxygen species (ROS) from immune cells, mainly by neutrophils. Though it is considered to play an important role as a defense mechanism in phagocytosis, the higher levels of ROS released during the oxidative burst has been identified to cause severe tissue injury and inflammation. Therefore inhibition of oxidative burst has been recognized as an interesting strategy in the research arena of anti-inflammatory drug research [8]. Anti-oxidants also play an important role in the management of inflammation. The efficacy of antioxidants and anti-inflammatory drugs derived from medicinal plants in the management of inflammatory diseases has been extensively documented. In this concern, medicinal plants are considered as valuable sources of potential therapeutic agents. A number of modern drugs have been isolated from medicinal plants based on the traditional use. There is an emerging interest in the use of natural products mainly those derived from medicinal plants in therapeutic applications [9]. In Sri Lanka, the practice of Ayurveda and traditional system of medicine has been implemented systematically for more than two thousand years to treat various diseases including inflammatory mediated diseases. Around 1414 of plant species including several endemic species have been used for the treatment and prevention of diseases. Among them, around 200 species are in general use and of them, nearly 50 species have been identified as heavily used plant species in Ayurveda and traditional system of medicine. With the estimated annual consumption of 2.2 million Kg, the potential for commercial exploitation of medicinal plants has risen high [10]. In the existing scenario of emerging global interest for natural products of high therapeutic potential, exploring bio-activities of Sri Lankan medicinal plants is of great importance and high demand to support traditional claims as well as to discover unexploited bio-active properties. Moreover, the bio-assay guided isolation of bio-active compounds from identified bio-active medicinal plant extracts may come up with more effective and safer therapeutic agents against various diseases including inflammatory diseases and other oxidative stress associated chronic diseases. Also the bioactive ingredients are of high commercial potential in health care and pharmaceutical industries.

Based on this rationale, we investigated A5-LOX, hyaluronidase, xanthine oxidase, nitric oxide production and oxidative burst inhibitory properties along with anti-oxidant capacities of ten selected Sri Lankan medicinal plants, which have been used in the traditional system of medicine in the management of diseases, associated with inflammation (Table 1).

Methods
Chemicals and equipment
A5-LOX (soybean), linoleic acid, baicalein, hyaluronic acid potassium salt (human umbilical cord), hyaluronidase (bovine testes), calcium chloride, sodium hydroxide, p-Dimethylaminobenzaldehyde (PDMAB), sodium borate, tannic acid, xanthine oxidase (bovine milk), xanthine, allo-p-Dimethylamino benzaldehyde (PDMAB), sodium borate, tannic acid, xanthine oxidase (bovine milk), xanthine, allo-purinol, Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum (FCS), bacterial lipopolysaccharide (LPS), trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), NG-Monomethyl-L-arginine-dimethylamino benzaldehyde (NMMA), HBSS+ (Hanks Balanced Salt Solution, containing calcium chloride and magnesium chloride) [Sigma, St. Louis, USA], serum opsonized zymosan (SOZ) [Fluka, Buchs, Switzerland], HBSS− (Hanks Balanced Salt Solution without calcium chloride and magnesium chloride), folin-ciocalteu reagent, gallic acid, quercetin, ethylenediaminetetraacetic acid disodium salt (EDTA-Na2), dimethylsulfoxide (DMSO),
Table 1 Traditional uses of ten Sri Lankan medicinal plants

| Plant name/(FAMILY) | Local name/English name | Part used in the study/ Voucher specimen No. | Traditional uses |
|---------------------|-------------------------|---------------------------------------------|------------------|
| Sphaeranthus indicus (Asteraceae) | Musumahana/ East India Globe Thistle | Leaf/SEL/15/11 | Swelling in the neck, acute laryngitis and bronchitis, piles [42]. |
| Acronychiopedunculata L. (Rutaceae) | Ankenda/Claw flowered laurel | Leaf/APL/15/15 | Skin diseases, rheumatism, ulcers asthma [43]. |
| Calophyllum inophyllum Linn. (Clusiaceae) | Dombay/Alexandrian laurel | Bark/CIB/15/21 | Skin diseases, piles, sore eyes, migraine [44]. |
| Symlocos cochinchinesis (Lour.) S. Moore. (Symplcaceae) | Sewalambomu/Lodh tree | Bark/SCB/15/27 | Leptosy, tumors, menorrhagia, inflammation and urination problems [45]. |
| Tinospora cordifolia (Willd.) (Menisperms) | Rasakinda/heartleaf moonseed | Bark/TCB/15/32 | Skin diseases, Jaundice, Diabetes, rheumatic pain, syphilis [46]. |
| Flacourtia indica (Burm.f. Merr.) (Flacourica) | Uguress/Governor’s Plum | Bark/FIB/15/34 | Rheumatoid arthritis, gout, intermittent fever [40, 47]. |
| Leucus zeylanica L. (Lamiaceae) | Gata thumba/Ceylon slitwort | Leaf/LZL/15/41 | Jaundice, scorpion, snake bite [48]. |
| Barathranthus nodiflorus Thw. (Loranthaceae) | Pilila | Leaf/BNL/15/44 | Bone fractures [49]. |
| Diospyros ebenum J.Koenig ex Retz (Ebenaceae) | Kaluwaraya/Ceylon ebony | Leaf/DEL/15/47 | Snake bite, diarrhoea, ulcers, biliousness [32]. |
| Argyreia populifolia Choisy (Convolvulaceae) | Elephant creeper | Leaf/APL/15/48 | Swellings [50]. |

2,2-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (trolox), potassium persulphate, 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein, 2,4,6-tripyridyl-s-triazine (TPTZ) and 4,4'-disulfonic acid sodium salt (ferrozine) were purchased from Sigma-Aldrich (USA). All chemicals and reagents used in the experiment were of analytical grade. The bio-assays were performed using high throughput micro-plate readers (Spectra Max Plus384, Molecular Devices, USA) and were conducted in well-ventilated conditions (Relative humidity: 65–75%), at room temperature (25 ± 2 °C) for 72 h and ground to make coarse powder using a mechanical grinder [11, 12]. Powdered plant materials (100 g) were soaked in ethanol (500 mL) overnight and stirred for 1 h using a mechanical stirrer at room temperature (25 ± 2 °C) followed by suction filtration through a celite bed, packed in a sintered funnel. The filtrates were concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain the ethanol extracts [11]. The solvent free extracts were stored in air-tight glass containers at –20 °C until used [13].

Enzyme inhibitory activity
Arachidonate S-lipoxygenase (A5-LOX) inhibitory assay
A5-LOX inhibitory activity of plant extracts was determined by a modified spectrometric method [14]. Plant extracts were assayed within the concentration range of 10–1000 μg/mL. Briefly, sodium phosphate buffer (110 μL, 100 mM, pH 8.0), plant extracts dissolved in methanol (10 μL), and A5-LOX solution (55 μL) were incubated for 10 min at 25 °C followed by the addition of linoleic acid solution (25 μL, 0.08 mM). Absorbance was measured at λ = 234 nm for 10 min at 25 °C.
Percentage inhibition of A5-LOX was determined by comparison of reaction rates of extracts relative to control using the formula $(E - S)/E \times 100$, where $E$ and $S$ are activities of the enzyme with and without extracts, respectively. IC$_{50}$ values were determined. Baicalein was used as the reference standard.

**Hyaluronidase inhibitory assay**

Hyaluronidase inhibitory activity of plant extracts was evaluated by a spectrometric method with modifications [15]. Extracts were assayed at the concentrations of 100 and 500 μg/mL. Extracts (50 μL) were incubated with hyaluronidase enzyme solution (10 μL) at 37 °C for 10 min followed by the addition of calcium chloride (12.5 mM, 20 μL) and re-incubation at 37 °C for 10 min. Sodium hyaluronate (50 μL) was added to the reaction mixture and incubated at 37 °C for 40 min followed by the addition of Sodium hydroxide (0.9 M, 10 μL) and Sodium borate (0.2 M, 20 μL) and incubation at 100 °C for 3 min. p-Dimethylaminobenzaldehyde (PDMAB), (50 μL, 67 mM) was added to the reaction mixture and incubated at 37 °C for 10 min. Absorbance was measured at $\lambda = 585$ nm. Percent enzyme inhibition was calculated as given below, compared to the control. Tannic acid was used as the reference standard.

$$\text{Inhibition (\%)} = \left[ \frac{(\text{Abs. control } - \text{Abs. sample})}{\text{Abs. control}} \right] \times 100$$

**Xanthine oxidase inhibitory activity**

Xanthine oxidase inhibitory activity of plant extracts was determined by a kinetic method [16] with slight modifications. Extracts were tested at the assay concentration of 250 μg/mL. Briefly, sodium phosphate buffer (150 μL, 50 mM, pH 7.4), extracts (10 μL) and xanthine oxidase solution (10 μL) were incubated at 25 °C for 10 min. The reaction was then initiated with the addition of xanthine solution (0.1 mM). Absorbance was monitored with the change of absorbance at $\lambda = 295$ nm for 15 min at 25 °C. Percentage inhibition of xanthine oxidase was calculated using the formula $(E - S)/E \times 100$, where $E$ is the activity of enzyme without extracts and $S$ is the activity of enzyme with extracts. Allopurinol was used as the reference standard.

**Nitric oxide production inhibitory activity and viability of LPS-activated RAW 264.7 macrophages**

**Cell culture**

Murine macrophage (RAW 264.7) cell lines were purchased from ATCC, VA, USA. The RAW 264.7 cells were cultured and maintained in DMEM, supplemented with streptomycin sulfate (100 μg/mL), penicillin G sodium (100 units/mL), amphotericin B (0.25 μg/mL) and 10% fetal bovine serum (FBS) (Humidified atmosphere, 5% CO$_2$, 37 °C). Cells were split twice a week.

Monolayer cells were plated in 96-well micro-plates at a density of 1 × 10$^5$ cells/well followed by the incubation (humidified atmosphere, 5% CO$_2$, 37 °C) for 24 h. The plated cells were treated with extracts (500 μg/mL) and incubated for 30 min (humidified atmosphere, 5% CO$_2$, 37 °C), followed by the incubation with bacterial lipopolysaccharide (LPS, 1 μg/mL) for 24 h [17].

**Nitric oxide production inhibition**

The inhibition of nitric oxide production was determined using the Griess assay [6]. After 24 h incubation with LPS, cell culture supernatants (100 μL) were reacted with Griess reagent (100 μL) and incubated for 10 min at room temperature and absorbance was measured at $\lambda = 540$ nm. The nitrite concentration was determined using a standard curve of sodium nitrite ($y = 0.012x + 0.036, R^2 = 0.999$). Percentage inhibition of nitric oxide formation by extracts was calculated [18].

**Cell viability**

The cytotoxicity of the extracts on RAW 264.7 cells was determined by MTT assay [19]. Cells were initially incubated (humidified atmosphere, 5% CO$_2$, 37 °C) for 6 h and with plant extracts (500 μg/mL) for 30 min. The cells were treated with LPS (1 μg/mL) and incubated for 24 h. MTT solution (20 μL, 5 mg/mL in PBS) and FBS free DMEM (180 μL) were added to the cells and incubated (humidified atmosphere with 5% CO$_2$ at 37 °C) for 4 h. DMSO (100 μL) was added to dissolve the formed formazan salt and absorbance was measured at $\lambda = 570$ nm. Percentage cell viability was determined [18].

**Oxidative burst inhibition**

Oxidative burst inhibition assay was conducted at Dr. Panjwani Center for Molecular Medicine and Drug Research, International Centre for Chemical and Biological Sciences, University of Karachi, Pakistan. The institute has obtained the ethical clearance for studies on human blood from independent ethics committee, ICCBS, UoK. No: ICCBS/IEC-008-BC-2015/Protocol/1.0.

**Isolation of human polymorphonuclear neutrophils (PMNs)**

Venous blood was collected from a healthy adult male volunteer (25–30 years age) to a heparinized tube and density gradient centrifugation was carried out to isolate neutrophils [20]. Briefly, whole blood (10 mL), HBSS$^-$ (10 mL) and lympho separation medium (LSM, 10 mL) were mixed and kept at room temperature for 45 min for serum separation. The separated serum was centrifuged at 400 g for 20 min and sedimented cells were re-centrifuged with an equal volume of LSM at 300 g,
4 °C for 10 min. The cells were re-suspended in HBSS− and cell count was adjusted to 1 x 10⁶ cells/mL.

Chemiluminescence assay Luminol-enhanced chemiluminescence assay was performed according to a kinetic method [21] with modifications. Briefly, 25 μL of diluted whole blood in HBSS++ was incubated with the plant extract (25 μL) at 37 °C for 15 min and 25 μL of serum opsonized zymosan (SOZ) and 25 μL of luminal were added into each well, except blank wells. The level of the ROS was recorded and inhibition of ROS production (%) was calculated. IC₅₀ values were determined. Ibuprofen was used as the reference standard.

Antioxidant activity
2,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity
The DPPH free radical scavenging activity of plant extracts was determined using a spectrophotometric method with modifications [22]. Extracts were assayed within the concentration range of 10–500 μg/mL. Extracts (100 μL) were incubated with DPPH solution (40 μg/mL, 200 μL) at room temperature (25 ± 2 °C) in dark for 10 min and absorbance was measured at 517 nm. The DPPH free radical scavenging activity was calculated using the following equation and IC₅₀ values were determined. Trolox was used as the reference standard.

Scavenging activity (%) = [(Abs. control – Abs. sample)/ Abs. control] × 100.

Ferric reducing antioxidant power (FRAP)
The assay was performed according to a spectrophotometric method [23] with slight modifications. Extracts were tested within the assay concentration range of 25–500 μg/mL. Extracts (50 μL) were incubated with freshly prepared FRAP reagent (Acetate buffer of 300 mM and pH 3.6, TPTZ in 10 mM in 40 mM HCl, and 20 mM ferric chloride hexahydrate solution mixed at 10:1:1, v/v/v) (150 μL) at room temperature (25 ± 2 °C) for 8 min. Absorbance was recorded at λ = 593 nm. FRAP of extracts was expressed as mg trolox equivalents (TE)/g of extract using a standard curve of trolox (y = 0.008x + 0.046, R² = 0.996).

Ferrous ion chelating (FIC) activity
Ferrous ion chelating activity was determined according to a spectrophotometric method [24] with modifications. Plant extracts were assayed in the assay concentration range of 100–5000 μg/mL. Extracts (140 μL) were incubated with ferrous sulfate solution (1 mM, 20 μL) at room temperature (25 ± 2 °C) for 10 min. After the incubation, ferrozine (40 μL) was added to the reaction mixture and re-incubated at room temperature (25 ± 2 °C) for 10 min. Absorbance was measured at 562 nm. Percentage chelating effect was calculated with compared to control based on the following equation and IC₅₀ values were determined. EDTA-Na₂ was used as the reference standard.

Chelating activity (%) = [(Abs. control – Abs. sample)/ Abs. control] × 100.

Oxygen radical absorbance capacity (ORAC)
The oxygen radical absorbance capacity (ORAC) assay was conducted using a kinetic method [25] with modifications. Plant extracts were assayed in the assay concentration range of 1–100 μg/mL. Extracts (10 μL) were pre-incubated with phosphate buffer (40 μL) and fluorescein solution (4.8 μM, 100 μL) at 37 °C for 10 min and freshly prepared AAPH solution (40 μg/mL, 50 μL) was added. The decay of fluorescein was monitored at 1 min intervals for 35 min at the wavelengths of 494 nm (excitation) and λ = 535 nm (emission). Trolox was used as the reference standard. The net area under the curve of decay of fluorescein was determined using the calibration curve of trolox (y = 0.035x + 0.08, R² = 0.999) and expressed as mg trolox equivalents (TE)/g of extract.

Determination of Total polyphenolic content
The total polyphenolic content (TPC) of plant extracts was quantified by the modified Folin-Ciocalteu method [26]. Extracts were assayed within the assay concentration range of 50–500 μg/mL. Plant extracts (110 μL) were incubated with folin-ciocalteu reagent and sodium carbonate solution (10% w/v, 70 μL) for 30 min at room temperature (25 ± 2 °C). Absorbance was recorded at 765 nm. TPC was calculated using the calibration curve of Gallic acid standard curve (y = 0.053x + 0.105, R² = 0.993) and expressed as mg gallic acid equivalents (GAE)/g of extract.

Determination of Total flavonoid content
The total flavonoid content (TFC) of plant extracts was quantified by the aluminium chloride method [27]. Extracts were tested within the assay concentration range of 50–500 μg/mL. Extracts (100 μL) were incubated with AlCl₃-methanol solution (2%, 100 μL) for 10 min at room temperature (25 ± 2 °C) and absorbance was recorded at 415 nm. TFC was calculated using a calibration curve of quercetin (y = 0.033x–0.002, R² = 0.999) and expressed in terms of mg quercetin equivalents (QE)/g of extract.

Gas chromatography - mass spectroscopy (GC-MS) analysis
The total ethanol extract of bark of F. indica was analysed by GC-MS using Thermoscientific Trace 1300 GC system, coupled with ISQ QD mass detector (EI mode,
mass range of m/z 40–450). The GC system is equipped with a programmable temperature vaporization (PTV) inlet and a Supelcowax capillary column (30 m × 0.25 mm × 0.25 μm), fused with silica and polyethylene glycol as the stationary phase.

Sample was dissolved in ethanol (0.60 g/mL) and 500 μL of head space gas was introduced to the PTV inlet. The injector temperature was set at 250 °C with an initial oven temperature of 60 °C, which was set to increase at a rate of 5 °C min⁻¹ to reach up to 220 °C. He- lium was used as the carrier gas (Flow rate: 1 mL min⁻¹). The compounds in the extract were matched and identified using mass spectral database of NIST 11, USA.

Analysis of phenolic compounds using high Performance liquid chromatography (HPLC)
The ethanol extract of *F. indica* was dissolved in methanol (5 mg/mL) and filtered through a membrane filter (0.25 μm) for HPLC analysis. The HPLC system was equipped with an Agilent 1260 Infinity II system, consisting of a quaternary pump (G7111A), vial sampler, column heater and Diode array detector (WR G7115A). Separation was achieved on a reversed phase column C₁₈ (250 mm × 4.6 mm × 5 μm). The eluates were detected at 254, 280 and 320 nm. Two solvent mixtures were used as the mobile phase in a gradient system. Water/formic acid (1000/0.005 v/v) was used as solvent A and methanol was used as solvent B. The total flow rate was 0.5 mL/min. The gradient profile of the mobile phase was from 10% B linearly to 70% B in 60 min followed by an isocratic flow for 10 min and back to 10% B at 90 min followed by isocratic flow for 10 min to re-equilibrate [28].

Statistical analysis
All analysis was carried out in triplicate and experimental results were expressed as mean ± standard error (SE), analysed with one-way ANOVA. Turkey’s multiple range tests was applied for mean separation, when ANOVA was significant (p < 0.05). IC₅₀ values were calculated using linear regression analysis. Pearson’s correlation coefficient was used for the correlation analysis (p < 0.05) (IBM SPSS Statistics 22.0).

Results
Enzyme inhibitory activities of selected medicinal plants
**Arachidonate 5-lipoxygenase inhibitory activity**
Based on the percent inhibition in the screening, the results revealed that, the ethanol bark extract of *F. indica* had the highest A5-LOX inhibitory activity followed by the extracts of *S. cochinchinesis* and *C. innophyllum*, while the extract of *T. cordifolia* had the lowest activity (Table 2). Apart from the extracts of *A. pedunculata*, *L. zeylanica* and *B. nodiflorus*, the other extracts showed high to moderate A5-LOX inhibitory activities. Based on the IC₅₀ values, the extract of *F. indica* (Fig. 1) showed the highest A5-LOX inhibitory activity followed by *S. cochinchinesis* and *C. innophyllum* in a dose dependent manner confirming the results of the initial screening (Table 2). The activities of the extracts were found to be significantly different from the positive control baicalein, which showed a strong dose dependent activity against A5-LOX enzyme activity.

**Table 2 Anti-A5-LOX activity of ethanol extracts of medicinal plants**

| Plant name                      | A5-LOX Inhibition (%) | Anti-A5-LOX activity IC₅₀ (μg mL⁻¹) |
|---------------------------------|-----------------------|-------------------------------------|
| *Sphaeranthus indicus*          | 32.45 ± 0.45[^a]      | 136.69 ± 1.79[^a]                   |
| *Acronychiapedunculata*         | 17.45 ± 0.94[^b]      | 294.68 ± 2.23[^b]                   |
| *Calophyllum innophyllum*       | 67.14 ± 0.51[^c]      | 74.82 ± 1.35[^c]                    |
| *Symlocos cochinchinesis*       | 70.12 ± 0.36[^d]      | 39.01 ± 0.91[^d]                    |
| *Tinospora cordifolia*          | 12.71 ± 0.71[^e]      | 393.63 ± 1.74[^e]                   |
| *Filocourcia indica*            | 89.35 ± 1.24[^f]      | 22.75 ± 1.94[^f]                    |
| *Leucus zeylanica*              | 19.37 ± 0.11[^g]      | 258.03 ± 1.91[^g]                   |
| *Barathranthus nodiflorus*      | 9.38 ± 0.32[^h]       | 213.27 ± 1.55[^h]                   |
| *Diospyros ebenum*              | 34.91 ± 0.84[^i]      | 143.76 ± 1.03[^i]                   |
| *Argyreia populifolia*          | 32.89 ± 0.17[^j]      | 152.41 ± 1.00[^j]                   |
| Baicalein                       | 97.64 ± 0.65[^k]      | 1.76 ± 0.15[^k]                     |

Data represented as mean ± SE (N = 3). *A5-LOX Inhibition at 100 μg/mL. Mean within each column followed by the same letter are not significantly different at p < 0.05

![Fig. 1](image-url)  
**Fig. 1** A5-LOX inhibitory activities of ethanol extract of bark of *Flacourtia indica*. Results are presented as mean ± SE (N = 3). Means followed by the same letter are not significantly different at p < 0.05.
dependent inhibitions within the concentration range of inhibitory activity showed significant (p < 0.05), dose dependent inhibitions within the concentration range of 31.25–500 μg/mL with a IC_{50} value of 176.62 ± 0.7 μg/mL (Fig. 2; Allopurinol: IC_{50}: 47.64 ± 2.32 μg/mL). The highest DPPH free radical scavenging activities were showed by the extracts of C. innophyllum and S. cochinchinesis. The ethanol bark extract of F. indica showed significant oxidative burst inhibitory effect when tested on isolated PMNs (IC_{50}:5.02 ± 0.38 μg/mL) (Fig. 3b), which is comparable with the reference drug Ibuprofen (IC_{50}: 5.12 ± 0.45 μg/mL).

**Anti-oxidant activity**

Anti-oxidant capacities of the extracts were evaluated using four different methods including the DPPH free radical scavenging, FIC, FRAP and ORAC assays. The extracts showed high to low DPPH free radical scavenging, FIC, FRAP and ORAC activities, having the IC_{50} values within the range of 6.26 ± 0.93 to 70.74 ± 0.95%. The results revealed that, the ethanol bark extract of F. indica had the highest inhibitory activity followed by the extracts of S. cochinchinesis and C. innophyllum (Table 3). The extracts of A. pedunculata and A. populifolia showed significantly low inhibitory activities against xanthine oxidase enzyme with respect to the reference standard allopurinol (Table 3). The extract of F. indica, which showed the highest xanthine oxidase inhibitory activity showed significant (p < 0.05), dose dependent inhibitions within the concentration range of 31.25–500 μg/mL with a IC_{50} value of 176.62 ± 0.7 μg/mL (Fig. 2; Allopurinol: IC_{50}: 2.33 ± 0.51 μg/mL).

**Nitric oxide production inhibitory activity and viability of LPS-activated RAW 264.7 macrophages**

The inhibitory activities of extracts were moderate to low in comparison to the reference standard L-NMMA and ranged from 3.63 ± 0.69% to 38.07 ± 0.93%. Of the tested plant extracts, the extracts of F. indica, S. cochinchinesis and C. innophyllum showed significantly high activities, while that of S. indica, T. cordifolia, L. zeylanica and B. nodiflorus showed significantly low activities (Table 4). The extracts showed no cytotoxicity (Cell viability: > 80%) at the tested concentration (Table 4).

**Oxidative burst inhibition**

The ethanol bark extract of F. indica, which was identified as a highly anti-inflammatory extract was assessed for the effect on oxidative burst response on human whole blood and isolated PMNs. The extract showed a significant inhibition of ROS production on human whole blood (IC_{50}: 47.64 ± 2.32 μg/mL), which was found to be dose-dependent (12.5–200 μg/mL) (Fig. 3a). Interestingly, the extract showed promising, significant oxidative burst inhibitory effect when tested on isolated PMNs (IC_{50}:5.02 ± 0.38 μg/mL) (Fig. 3b), which is comparable with the reference drug Ibuprofen (IC_{50}: 5.12 ± 0.45 μg/mL).

**Table 3 Xanthine oxidase and hyaluronidase enzyme inhibitory activities of total ethanol extracts of medicinal plants**

| Plant Name          | Xanthine oxidase inhibition | Hyaluronidase inhibition |
|---------------------|-----------------------------|--------------------------|
| Sphaeranthus indicus| 30.35 ± 0.32a, f             | NI                       |
| Acronychia pedunculata| 7.86 ± 0.14b, d             | 36.60 ± 1.02a           |
| Calophyllum inophyllum| 38.95 ± 1.28c              | NI                       |
| Symlocos cochinchinesis| 44.86 ± 1.43c              | 27.49 ± 1.09b           |
| Tinospora cordifolia| 17.92 ± 1.73d               | NI                       |
| Flacourtia indica   | 70.46 ± 0.18a               | 36.67 ± 2.23a           |
| Leucus zeylanica    | 13.26 ± 0.25a               | 24.38 ± 2.09b           |
| Barathranthus nodiflorus| 6.26 ± 0.93b               | 42.31 ± 2.00d           |
| Diospyros ebenum     | 24.76 ± 2.16a               | 41.60 ± 1.18d           |
| Argyreia populifolia| 32.79 ± 2.16f               | 16.27 ± 1.00d           |
| Allopurinol         | 99.26 ± 0.18f               | NA                       |
| Tannic acid         | NA                          | 90.69 ± 0.50f           |

Inhibition (%) of xanthine oxidase and hyaluronidase is recorded at 250 and 500 μg/mL assay concentrations respectively. Data represented as mean ± SE (N = 3). Means followed by the same letter are not significantly different at p < 0.05. NA Not applicable.
| Plant name                | Nitric oxide production (μM) | % NO inhibition | % Cell viability |
|--------------------------|------------------------------|-----------------|-----------------|
| Sphaeranthus indicus     | 31.11 ± 0.17                 | 9.46 ± 0.21     | 87.21 ± 0.50    |
| Acronychia pedunculata   | 30.39 ± 0.07                 | 11.56 ± 0.21    | 89.76 ± 0.35    |
| Calophyllum innophyllum  | 23.67 ± 0.27                 | 31.12 ± 0.78    | 88.89 ± 0.82    |
| Symplacos cochinichinesis| 22.50 ± 0.28                 | 34.52 ± 0.78    | 82.15 ± 0.31    |
| Tinospora cordifolia     | 32.89 ± 0.24                 | 2.40 ± 0.69     | 94.56 ± 2.57    |
| Flacourtia indica        | 21.28 ± 0.32                 | 38.07 ± 0.93    | 87.31 ± 0.50    |
| Leucus zeylanica         | 33.11 ± 0.24                 | 3.63 ± 0.69     | 93.02 ± 0.67    |
| Barathranthus nodiflorus | 31.22 ± 0.25                 | 9.13 ± 0.72     | 87.11 ± 1.16    |
| Diospyros ebenum         | 29.08 ± 0.29                 | 15.36 ± 0.85    | 85.43 ± 1.22    |
| Argyrea populifolia      | 27.64 ± 0.15                 | 19.56 ± 0.43    | 86.10 ± 0.61    |
| L-NMMA                   | 0.97 ± 0.12                  | 97.10 ± 0.56    | 97.54 ± 0.47    |

Data represented as mean ± SE (N = 3). Mean within each column followed by the same letter are not significantly different at $p < 0.05$. *Inhibition (%) at the assay concentration of 500 μg/mL.

**Fig. 3** Oxidative burst inhibitory activities of ethanol extract of bark of Flacourtia indica on human whole blood (a) and polymorphonuclears (b). Results are presented as mean ± SE(N = 3). Means followed by the same letter are not significantly different at $p < 0.05$. 
**Table 5** Antioxidant activities of ethanol extracts of medicinal plants

| Plant Name/Standard          | DPPH IC_{50} (μg/mL⁻¹) | IC_{50} (μg/mL⁻¹)/Inhibition (%) | FRAP values (mg TE/g) | ORAC values (mg TE/g) |
|-----------------------------|-------------------------|----------------------------------|-----------------------|-----------------------|
| *Sphaeranthus indicus*      | 109.33 ± 1.19           | 19.21 ± 0.56*                    | 326.15 ± 3.53         | 1018.71 ± 9.96        |
| *Acronychiapedunculata*     | 743.49 ± 1.94           | 974.56 ± 2.31                    | 741.64 ± 1.75         | 322.67 ± 1.94         |
| *Calophyllum innophyllum*   | 6.99 ± 0.02             | 19.50 ± 0.71*                    | 2613.00 ± 7.23        | 2110.0 ± 6.35         |
| *Symposium cochinchinesis*  | 9.85 ± 0.28             | 1093.53 ± 4.04                   | 2181.61 ± 2.16        | 2910.7 ± 12.9         |
| *Tinospora cordifolia*      | 389.20 ± 0.75           | NI                               | 586.66 ± 3.29         | 121.29 ± 2.12         |
| *Flacourtia indica*         | 26.37 ± 0.49            | 8.29 ± 0.26*                     | 375.20 ± 2.79         | 1480.20 ± 11.5        |
| *Leucus zeylanica*          | 352.65 ± 2.12           | 33.46 ± 0.66*                    | 157.69 ± 1.85         | 63.69 ± 1.16          |
| *Barathranthus nodiflorus*  | 282.22 ± 1.78           | 33.37 ± 0.40*                    | 427.29 ± 2.07         | 18.07 ± 0.42          |
| *Diospyros ebenum*          | 177.32 ± 1.03           | NI                               | 369.18 ± 0.61         | 95.24 ± 0.00          |
| *Argyreia populifolia*      | 288.81 ± 1.45           | NI                               | 268.01 ± 1.53         | 479.55 ± 1.80         |
| Trolox                       | 5.29 ± 0.09             | NA                               | NA                    | NA                    |
| EDTA-Na₂                    | NA                      | 13.07 ± 0.64                     | NA                    | NA                    |
| GreenTea                    | NA                      | NA                               | NA                    | 1662.82 ± 0.22        |

Data represented as mean ± SE (N = 3). Mean within each column followed by the same letter are not significantly different at p < 0.05. *Inhibition (%) at the assay concentration of 1000 μg/mL. NA Not applicable

**cochinchinesis**. For the remaining extracts, which showed chelating activity, dose response studies were not carried out due to the interference of turbidity of the reaction mixture at higher concentrations (> 1000 μg/mL) (Table 5).

In FRAP assay, the extracts showed low to high reducing power within the range of 157.69 ± 1.85–2613.00 ± 7.23 mg TE/g. The extract of *C. innophyllum* showed the highest FRAP value followed by the extracts of *S. cochinchinesis* and *A. pedunculata*. The extract of *L. zeylanica* showed the lowest FRAP (Table 5).

The ORAC of extracts ranged from 18.07 ± 0.42–2910.7 ± 12.9 mg TE/g. The extract of *S. cochinchinesis* showed the highest among the extracts and even significantly higher ORAC in comparison to the standard green tea extract. The extracts of *C. innophyllum*, *F. indica* and *S. indicus* also showed high ORAC values and that of *L. zeylanica* showed the lowest ORAC, compared to other extracts (Table 5).

**Total polyphenol and flavonoid contents**

The total polyphenol contents of the extracts ranged from 10.63 ± 0.22–661.42 ± 2.67 mg GAE/g. The extract of *S. cochinchinesis* showed the significantly highest polyphenol content followed by that of *C. innophyllum* and *A. pedunculata*. The extracts of *L. zeylanicus* and *D. ebenum* showed the lowest polyphenolic contents (Fig. 4).

The flavonoid contents of the extracts ranged from 3.69 ± 0.15–72.74 ± 0.76 mg QE/g. The extract of *S. cochinchinesis* showed the highest flavonoid content and that of *S. indicus* and *F. indica* showed the lowest flavonoid contents. The extracts of *B. nodiflorus* (32.94 ± 0.88 mg QE/g) and *D. ebenum* (30.68 ± 0.30 mg QE/g) also showed significant flavonoid contents (Fig. 4).

**Correlation between assays**

The correlation analysis is important to get an understanding of statistical relationships between different assays. The p values resulted from the correlation analysis among ten assays are given in Table 6.

**Gas chromatography - mass spectroscopy (GC-MS) analysis of ethanol extract of bark of Flacoutia indica**

The GC-MS analysis of ethanol extract of bark of *F. indica* revealed the presence of six phytoconstituents including acid esters and fatty acid derivatives (Table 7). Propan-2-yl tetradecanoate, [1,1'-Bicyclopentyl]-2-octanoic acid, 2'-hexyl-, methyl ester, Linoleic acid ethyl ester, Hexadecanoic acid, ethyl ester and Benzoic acid, ethyl ester were identified as major compounds (>2% peak area) (Fig. 5).

**Analysis of phenolic compounds using high performance liquid chromatography (HPLC)**

The HPLC analysis produced a well resolved chromatogram representing peaks corresponding to retention time of phenolic compounds. The HPLC chromatogram of ethanol bark extract of *F. indica* at 254, 280 and 320 nm is given in Fig. 6.

**Discussion**

Enzyme inhibitory activities of selected medicinal plants

**Arachidonate 5-lipoxygenase inhibitory activity**

In this assay, all the extracts showed significant (p < 0.05) A5-LOX inhibitory activities at the tested concentrations.
Fig. 4 Total polyphenolic contents (a) and total flavonoid Contents (b) of plant extracts. Results are presented as mean ± SE (N = 3). Means followed by the same letter are not significantly different at p < 0.05. Si: Sphaeranthus indicus, Ap: Acronychia pedunculata, Ci: Calophyllum inophyllum, Sc: Symlocos cochinchinesis, Tc: Tinospora cordifolia, Fi: Flacourtia indica, Lz: Leucus zeylanica, Bn: Barathranthus nodiflorus, De: Diospyros ebenum, Ar.p: Argyria populiflora.

Table 6 Pearson’s correlation coefficients of in-vitro anti-inflammatory activities, antioxidant activities, total phenolic and total flavonoids content of extracts

|        | LOX  | HYL  | XO   | NO   | DPPH | FRAP | FIC  | ORAC | TPC  | TFC  |
|--------|------|------|------|------|------|------|------|------|------|------|
| AS-LOX | 1    | 0.327* | 0.930** | 0.950** | −0.736** | .545** | .150** | .844** | .540** | 0.228** |
| HYL    | 1    | 0.326* | 0.371* | −0.022* | −0.297* | .115* | .043* | .081* | −0.059* |       |
| XO     | 1    | 0.864** | −0.728** | .287** | −0.041* | .696** | .326** | 0.069* |       |       |
| NO     | 1    | −0.656* | 0.592* | 0.243* | 0.829* | 0.587* | 0.368* |       |       |       |
| DPPH   | 1    | −0.417* | 0.270* | −0.675** | −0.416* | −0.324** |       |       |       |       |
| FRAP   | 1    | −0.429* | 0.811** | 0.607** |       |       |       |       |       |       |
| FIC    | 1    | 0.436* | 0.707** | 0.520* |       |       |       |       |       |       |
| ORAC   | 1    | 0.857** | 0.523* |       |       |       |       |       |       |       |
| TPC    | 1    | 0.821** |       |       |       |       |       |       |       |       |
| TFC    | 1    |       |       |       |       |       |       |       |       |       |

The statistical significance is represented with **p < 0.01 and *p < 0.05. ns non-significant.
The ethanol bark extract of *F. indica* showed the highest A5-LOX inhibitory activity followed by that of *S. cochinchinesis* over the other extracts. When compared with the reported A5-LOX inhibitory activity of Caffeic acid, (IC$_{50}$: 57.0 μg/mL), [29] which is a known lipoxygenase inhibitor, the IC$_{50}$ values of bark extracts of *F. indica* and *S. cochinchinesis* are about 2.5 fold and 1.5 fold lower than that of caffeic acid, respectively so that could be considered as even more potent than caffeic acid as A5-LOX inhibitors. To best of our knowledge, no previous studies have been conducted on A5-LOX inhibitory potential of extracts of *F. indica* and *S. cochinchinesis*.

**Hyaluronidase inhibitory activity**

In the hyaluronidase inhibitory assay, the extracts showed moderate to low anti-hyaluronidase activities at the tested concentration in comparison to the reference standard tannic acid. The extracts of *B. nodiflorus* and *D. ebenum* exhibited the highest anti-hyaluronidase activities compared to the other extracts. *B. nodiflorus* and *D. ebenum* are two medicinal plants endemic to Sri Lanka, that have been less exploited in the field of scientific research. The hyaluronidase inhibitory properties of these two plant extracts are recorded for the first time to upgrade the medicinal value of the species.

**Xanthine oxidase inhibitory activity**

All the extracts studied showed significant xanthine oxidase inhibitory activity (p < 0.05). The extract of *F. indica* showed the highest, xanthine oxidase inhibitory activity compared to the other extracts tested. This promising anti-xanthine oxidase potential of the extract of *F. indica* may be further supported by the traditional use of extracts of *F. indica* in the treatment of gouty arthritis. Specifically bark extracts have been used in the treatment of gout in the Unani system of medicine, where xanthine oxidase enzyme plays a major role in pathogenesis by imparting inflammation and catalyzing the formation of uric acid crystals leading to arthritic conditions [30].

**Nitric oxide production inhibitory activity and viability of LPS-activated RAW 264.7 macrophages**

Of the studied extracts, *F. indica* bark showed the highest NO production inhibitory activity. The NO production inhibitory activity could be either because of the direct inhibition of iNOs enzyme catalytic activity or expression of nitric oxide synthase. The high cell viability observed in the MTT cytotoxicity assay is evident for the non-toxic nature of the tested extracts confirming, that the observed NO production inhibitions are not due to any cytotoxic effect of extracts.
Oxidative burst inhibitory activity
The bark extract of *F. indica*, showed a pronounced oxidative burst inhibition in comparison to ibuprofen, when tested on PMNs. This observed activity may be due to the inhibition of NADPH oxidize enzyme, which catalyzes the generation of ROS or the direct scavenging of ROS upon the stimulation of opsonized zymosan [31].

Anti-oxidant activity
The bark extract of *C. innophyllum* showed the highest DPPH free radical scavenging activity and FRAP over
other extracts, which may be attributed to the presence of major chemical compounds such as xanthones and coumarins abundantly present in the extracts [23]. C. innophyllum is known as a medicinal plant with number of curative properties and it has been extensively studied worldwide [23]. The present study ascertains the significant free radical scavenging activity of the Sri Lankan variety of C. innophyllum free radical scavenging activity with the means of marked DPPH free radical scavenging and FRAP. Lower chelating properties of the extracts may be due to the low contents of effective metal chelating compounds in the extracts.

ORAC evaluates a hydrogen atom transfer mechanism of anti-oxidants [25]. Significant ORAC of extracts indicates the peroxy radical absorbance capacities of the extracts at different degrees. The extract of S. cochinchinesis showed the highest ORAC over the other extracts as well as the standard green tea extract. The presence of triterpenoids and flavonoid glycosides may be attributed to the anti-oxidant potential of the extracts of S. cochinchinesis. It has been extensively studied for its anti-diabetic properties [32].

**Total polyphenol and flavonoid contents**

The moderate to weak and non-significant correlations of antioxidant and enzyme inhibitory activities with TPC and TFC (Table 6), respectively suggested that the polyphenolic and flavonoid compounds of the extracts may not be solely responsible for the enzyme inhibitory and antioxidant activities. It is further confirmed by the fact, that the ethanol extract of bark of F. indica has exhibited the highest bio-activities irrespective of possessing moderate to low contents of polyphenols and flavonoids (Fig. 4) Among the identified phytoconstituents, hexadecenoic acid, ethyl ester is known to possess anti-oxidant properties as well as other bio-activities such as hypcholesterolemic, nematicide, pesticide, anti-androgenic, hemolytic and 5-alpha reductase inhibitory properties. Also the compound has flavor properties [33] which may be attributed to the strong aroma of the ethanol bark extract of F. indica. Linoleic acid ethylester is another bio-active compound detected in the extract of bark of F. indica which is known to possess anti-inflammatory properties and many more of bio-activities including anti-arthritic, anti-histaminic, anti-eczemic and anti-acne properties [33].

**Correlation between assays**

The A5-LOX, XO and NO inhibitory activities of plant extracts showed high, negative and significant correlations with DPPH free radical scavenging activity (IC50 values) \( (r = -0.736, -0.728 \text{ and } -0.656 \text{ respectively at } p < 0.01) \) and high, positive and significant correlations with ORAC assay \( (r = 0.844, 0.796 \text{ and } 0.696 \text{ respectively at } p < 0.01) \). This observation may be indicative of the dual function of bio-actives of plant extracts as free radical, peroxy radical scavenging and A5-LOX, XO and NO production inhibitory compounds. Moreover, the A5-LOX, XO and NO inhibitory activities of plant extracts have showed high, positive and significant correlations with each other supporting the fact, that some common group of bio-actives of the extracts could be attributed for the enzyme inhibitory activities of the extracts (Table 6). FRAP of the extracts has showed a high, positive and significant correlation with TPC \( (r = 0.810, p < 0.01) \) so that the FRAP of the extracts may be due to the presence of polyphenols, which are well known to participate in redox reactions.

**Gas chromatography - mass spectroscopy (GC-MS) analysis of ethanol extract of bark of Flacourtia indica**

In previous studies the GC-MS analysis of methanol extract of root of F. indica has showed the presence of 4-Benzoyl-3-methoxyisocoumarin as the major compound [34], which was not detected in the bark extract in this study. No other GC-MS analysis has been previously carried out on any species of the genus Flacourtia except for root of F. indica and this is the first report of the GC-MS analysis of Bark of F. indica.

**Analysis of phenolic compounds using high performance liquid chromatography (HPLC)**

According to a reported study, liquid chromatography-mass spectroscopy (LC-MSn) analysis of aqueous methanolic fruit extract of F. indica has enabled identification of 35 phenolic compounds including rutin, feruloylquinic acid, esculin, gentisic acid glycoside, salicylic acid glycoside and derivatives of caffeoylquinic acids, quercetin and kaempferol [28]. The HPLC peak pattern of the ethanol bark extract of F. indica showed similarities with the reported HPLC peak pattern of aqueous methanolic fruit extract of F. indica based on the retention times of the peaks under similar experimental conditions. Therefore, the reported compounds of the fruit extract may also be present in the bark extract of F. indica. Further, the HPLC chromatogram may be used for standardization of ethanol bark extract of F. indica. However, further chemical Characterisation is needed for the identification of the compounds and activity guided fractionation is in progress.

In addition, presence of several other bio-active phyto-constituents including phenolic glycosides [35] coumarins (scoparon) [36, 37] and different types of polyphenolic compounds having radical scavenging properties such as coumaroylglycospyranose, tannins and butyrolactones [38] in the extracts of F. indica have been reported. Phenolic glycosides and coumarins have long been recognized as potent A5-LOX and xanthine oxidase inhibitors as well as
antioxidants [39]. The bark of *F. indica* has been traditionally used to treat rheumatoid arthritis, [40] which is mediated by inflammation. According to previous studies, The extracts of *F. indica* are known to possess a broad range of pharmacological activities including anti-inflammatory properties [34–36, 41] yet the pharmacological profile needs to be further investigated using more in vivo, in vitro and clinical studies [40]. This is the first report investigating the bio-activities of extract from bark of *F. indica* except for one study in which, the methanol extract of bark of *F. indica* has shown good DPPH free radical scavenging activity (IC50: 17.5 ± 1.0 μg/ml) [38].

**Conclusion**

The extracts showed significant anti-inflammatory activities in vitro in terms of A5-LOX, xanthine oxidase, hyaluronidase and nitric oxide inhibitory activities along with promising anti-oxidant activities. Among the ten extracts, the ethanol extract of bark of *F. indica* showed the highest anti-inflammatory activity with good radical scavenging activities. Therefore, the ethanol extract of bark of *F. indica* is identified as a source of anti-inflammatory agents, which will be further studied to isolate and characterize bio-active constituents.

To the best of our knowledge, through this study, the pro-inflammatory enzyme inhibitory and anti-oxidant potential of ethanol bark extract of *F. indica* was identified and analysed by GC-MS and HPLC for the first time and selected for further bio-logical and chemical characterization.

**Compounds studied**

Benzoic acid, ethyl ester, Propan-2-yl tetradecanoate, Estra-1,3,5(10)-trien-17α-ol, Hexadecanoic acid, ethyl ester, Linoleic acid ethyl ester, [1,1’-Bicyclopropyl]-2-oc- tanoic acid, 2’-hexyl-2-methyl ester.

**Abbreviations**

A5-LOX : Arachidonate-5-lipoxygenase; AAPH: Azobis (2-amidinopropane) dihydrochloride; DMEM: Dulbecco’s modified Eagle’s medium; DMSO : Dimethyl sulfoxide; DPPH: 2,2-diphenyl-2-picryl-hydrazyl; EDTA-Na2: Ethylenediamine tetra acetic acid disodium salt; FCS: Fetal calf serum; FIC: Ferrous ion chelating; FRAP: Ferric reducing antioxidant power; GC-MS: Gas chromatography-Mass spectrometry; HBSS: Hanks Balanced Salt Solution; HPLC: High performance liquid chromatography; LPS: Bacterial lipopolysaccharide; L-NMMA: NG-Monomethyl-L-arginine; LPS: Lipo polysaccharide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NO: Nitric oxide; ORAC: Oxygen radical absorbance capacity; PDMAB: p-Dimethylaminobenzaldehyde; SOZ: Serum opsonized zymosan; XO: Xanthine oxidase

**Acknowledgements**

The technical staff at Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi is highly acknowledged for their support.

**Funding**

The National Research Council of Sri Lanka (Grant No: 12–100).

**Availability of data and materials**

The data presented in this manuscript belong to the PhD work of Ms. H. D. Sachindra Melshandi Perera and has not been deposited in any repository yet. However, the materials are available to the researchers upon request.

**Authors’ contributions**

Conception and design of in vitro experiments: HDSPM, JKRRS, SMH, AJ, OVDSJW, MIC. Conducted in vitro experiments: HDSPM. Conception and design of chemical characterisation: HDSPM, JKRRS, HDW. Conducted chemical characterisation: HDSPM, HDW. Data analysis and interpretation: HDSPM, JKRRS, SMH, OVDSJW, AJ. Manuscript drafting: HDSPM. Final approval and critical revision: JKRRS, SMH, OVDSJW, AJ, MIC. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable for this submission.

**Competing interest**

Not applicable for this submission.

**Consent for publication**

Not applicable for this submission.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Author details**

1. Industrial Technology Institute (ITI), 363, Bauddhaloka Mawatha, Colombo 07, Sri Lanka. 2.Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, 90, Cumaratumunga Muniadasa Mawatha, Colombo 03, Sri Lanka. 3.Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan. 4.H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.

**Received**: 5 October 2017 **Accepted**: 24 September 2018 **Published online**: 03 October 2018

**References**

1. Sen S, Chakraborty R, Sridhar C, Pradesh A. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res. 2010;03:091–100.

2. Pala FS, Gürkan H. The role of free radicals in ethnopathogenesis of disease. Advances in Molecular Biology. 2008;1:1–9.

3. Dobrian AD, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL. Functional and pathological roles of the12- and 15-lipoxygenases. Progress in Lipids Research. 2011;50:115–31.

4. Schneider I, Bucar F. Lipoxygenase inhibitors from natural plant sources. Part 1: medicinal plants with inhibitory activity on Arachidonate 5-lipoxygenase and 5-lipoxygenase/cyclooxygenase. Phytother Res. 2005;19:81–102.

5. González-Peña D, Colina-Coca C, Char CD, Cano MP, Ancos B, Sánchez-Moreno C. Hyaluronidase inhibiting activity and radical scavenging potential of Flavonols in processed onion. J Agric Food Chem. 2013;61:4862–72.

6. Cheenpracha S, et al. Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells by the norsesterterpene peroxide, epimuqubilin a. Mar Drugs. 2010;8(3):429–37.

7. Yumitha A, Suganda AG, Sukandar EY. Xanthine oxidase inhibitory activity of some Indonesian medicinal plants and active fraction of selected plants. Int J Pharmacy Pharmac Sci. 2013;5:293–6.

8. Boukemara H, Hurtado-Nedelec, M, Marzaoli V., Bendjeddou, D, El Bennia, J, & Marie, JC, Anvillea garcinni extract inhibits the oxidative burst of primay human neutrophils. BMC Complement Altern Med, 2016;16(1):433.

9. Rates SMK. Plants as source of drugs. Toxicon. 2001;39:603–13.
10. Arambevelle LSR, Wimalasena S, Gunawardene N. Herbal medicine, phytopharmaceuticals and other natural products: trends and advances. 1st ed. Colombo: NAM S&T Centre, New Delhi and Institute of Chemistry Ceylon. 2006.

11. Perera HDSM, Samarasekara R, Handunnetti S, Weerasena OVDS. In vitro anti-inflammatory and antioxidant activities of ten medicinal plants, traditionally used to treat inflammatory diseases in Sri Lanka. Ind Crop Prod. 2016;94:610–20.

12. Samaradivakara SP, Samarasekara R, Handunnetti SM, Weerasena OVDS. Cholinesterase, protease inhibitory and antioxidant capacities of Sri Lankan medicinal plants. Ind Crop Prod. 2016;83:227–34.

13. Wu MJ, Wang L, Weng CY, Yen JH. Antioxidant activity of methanol extract of the N. nucifera leaf (Nelumbo nucifera gertn.). Chin Med J. 2003;31(5):987–98.

14. Tappei AL. Methods in enzymology. Academic press, New York. USA. 1962: 539.

15. Sahasrabudhe A, Dhedhar M. Anti-hyaluronidase, anti-elastase activity of Guaracinandia. Int J Bot. 2010;6(3):299–303.

16. Lee SK, Mbtambo ZH, Chung H, Luengi L, Gamez EJ, Mehta RG, Pezzuto JM. Evaluation of the antioxidant potential of natural products. Comb Chem High Throughput Screen. 1998;1(1):35–46.

17. Min HY, Kim MS, Jang DS, Park EJ, Seo BK, Lee SK. Suppression of lipopolysaccharide-stimulated inducible nitric oxide synthesize (iNOS) expression by a novel humulene derivative in macrophage cells. Int Immunopharmacol. 2009;9(7):844–9.

18. Yang EJ, Yim EY, Song G, Kim GO, Hyun CG. Inhibition of nitric oxide production by lipopolysaccharide-activated RAW 264.7 macrophages by jeju plant extracts. IntercellularToxicol. 2009;2(4):245–9.

19. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods. 1989;119(2):203–10.

20. Masaik MA, Ul-Haq Z, Radha K, Jorny J, Hisham A. Diuretic activity of the roots of Flacourtia indica. Hygeia J D Med. 2013;5:79–83.

21. Kundu J, Roy M, Bachar SC, Chum KS, Kundu JK. Analgesic, anti-inflammatory, and diuretic activity of methanol extract of Flacourtia indica. Arch Basic Appl Med. 2013;1:39–44.

22. Jayaweera D, Senaratna LK. Medicinal plants (indigenous and exotic) used in Ceylon. Part II. National Sci Found. 2006;69.

23. Patro SK, Behera PC, Wang ML, Liu MJ, Damu AG, Wu TS. Acetophenone derivatives from Acronychia pedunculata. J Nat Prod. 2003;66:990–3.

24. Prabakaran K, Britto J. Biology, agroforestry and medicinal value of Calophyllum inophyllum (Clusiaceae): a review. J Asian Nat Prod Res. 2012;4:23–33.

25. Vadiu R, Lakshmi KS. In vitro and in vivo anti-inflammatory activity of leaves of Symplocos cochinchinensis (Loui) Moore sip Laurina. Bangladesh J Pharmacol. 2008;12:1–4.

26. Jayaweera D, plants M. (indigenous and exotic) used in Ceylon. Part IV. National Sci Counc Sri Lanka. 1982:81.

27. Patro SK, Behera PC, Kumar PM, Sasdal D. Pharmacological review of Flacourtia sepiaria. SAJP. 2012;8:69–93.

28. Jan D, Baheti AM, Jain SR, Khandelwal KR. Use of medicinal plants among tribes in Satpura region of Dhubel and Jhalgon district of Maharasthra-an ethnobotanical survey. Indian J Tradit Know. 2010;9:152–7.

29. Samarayake GVP, Pushpakumara AAJ. A literary review on traditional medical systems for bone fractures in Sri Lanka. IJTRD. 2016;3:89.