Terpene-rich fractions of *Ficus mucoso* (*Welw*) modulate lipopolysaccharide-induced inflammatory mediators and aberrant permeability of the inner mitochondrial membrane in murine animal model

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Abstract

*Ficus mucoso* is traditionally used to treat bronchial infections. This study compared the efficacy of terpene-rich fractions of *F. mucoso* root bark on lipopolysaccharide (LPS)-induced inflammation, liver mitochondrial permeability transition (mPT), an index of mitochondrial health, and associated pathological alterations. Terpene-Rich Fractions of Dichloromethane (TRDF) and Ethylacetate Fractions of *F. mucoso* (TREF) were obtained according to standard procedures. To induce systemic inflammation, a single intraperitoneal injection of 1mgLPS/kgbw was given to mice. Spectrophotometric techniques were used to evaluate the effects of the oral administration of TRDF and TREF (3 days) on levels of pro-inflammatory mediators (TNF-α, IL-1β, IL-6) using ELSA techniques as well as antioxidant indices in normal and LPS-treated mice. The mPT pore opening, mitochondrial ATPase activity and lipid peroxidation were monitored spectrophotometrically. Our results revealed that treatment with LPS caused significant elevation in serum cytokine levels while administration of 50 and 100 mg/kg TRDF and TREF significantly reduced elevated serum levels of cytokines (TNF-α, IL-1β, IL-6) in LPS-challenged mice. In addition, activities of superoxide dismutase, catalase and liver marker enzymes (ALT and AST) as well as levels of mitochondrial lipid peroxides were significantly reduced in mice treated with TRDF and TREF relative to LPS-fed mice. Furthermore, LPS caused induction of opening of the liver mPT pore which was significantly inhibited by TRDF at 100 and 200 mg/kg bw by 71% and 88%, respectively, but only at 100 mg/kg TREF. Furthermore, mitochondrial ATPase activity was inhibited largely by TRDF. UPLC–ESI–MS analysis revealed the presence of terpenoid derivatives and a few aromatic metabolites in TRDF. The terpene dominance of TRDF metabolites was further justified on the 1H NMR fingerprint. Overall, TRDF is more effective as a cocktail of anti-inflammatory compounds than TREF against LPS-induced acute systemic inflammation.

Keywords Mitochondrial swelling · Inflammation · Lipopolysaccharide · Pro-inflammatory cytokines

Introduction

The etiology of many diseases stems from inflammation. This constitutes a global health concern (Glass et al. 2010; Furman et al. 2019). Although, inflammation regulates defense against infection and promotes repair to prevent further tissue damage, persistent inflammatory process usually drives the onset of a number of disorders arising from various cellular alterations and metabolic interactions that eventually sustain and worsen tissue damage (Medzhitov 2008; Chen et al. 2018). Administration of the lipopolysaccharide endotoxin (LPS) to specific hosts has been shown to cause acute inflammation and toxicity to
various organs including the liver, brain and kidney (Sun et al. 2006; Abarca-Vargas and Petricevich 2019). Most of the toxicities associated with LPS-induced organ injury include production of pro-inflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), interleukins (IL-1, IL-6, IL-8), as well as reactive oxygen species (ROS) (Sun et al. 2006; Abarca-Vargas and Petricevich 2019).

Recent scientific findings reveal that mitochondria are involved in the regulation of innate immunity and are also drivers of inflammatory responses (Mohanty et al. 2019; Missiori et al. 2020). Mitochondrial ROS has been shown to play a significant role in this regard (Rimessi et al. 2016). The mitochondrial permeability Transition (mPT) pore opening has been shown to occur in many disease conditions including inflammation (Crompton 1999; Crouser et al. 2004; Oyebode et al. 2019). The opening of the mPT pore is activated by a number of factors including excessive matrix calcium concentration, inorganic phosphate and oxidative stress (Halestrap et al. 2004; Halestrap and Richardsson 2015) which result in matrix swelling, enhancement of ATPase activity and Mitochondrial Outer Membrane Permeabilization (MOMP) (Crompton 1999; Bauer and Murphy 2020). On the other hand, pharmacological inhibitors of the pore or mPT pore sealing agents (e.g., cyclosporine A, spermine) have been reported to preserve mitochondrial function in many diseases that stem from inappropriate opening of the pore (Najafi et al. 2014; Salimi et al. 2019; Oyebode et al. 2020). Interestingly, several medicinal plants have also exhibited modulatory effects on the mPT pore under different conditions (Oyebode et al. 2017; Oludele et al. 2018; Olowofolahan et al. 2020).

Prolonged use of anti-inflammatory drugs have damaging effects on critical organs as well as on isolated mitochondria (Battaglia et al. 2005; Pourahmad, et al. 2011; Pereira-Leite et al. 2017; Salimi et al. 2019). Hence, the search for safe/secure anti-inflammatory agents is on the rise. Terpenes account for anti-inflammatory effects of medicinal plants alongside additional pharmacological roles (Abarca-Vargas and Petricevich 2019). *Ficus mucoso* belongs to the family of Moraceae comprising numerous species of trees and shrubs extensively found in tropical and subtropical countries (Corner 1962; Rahman and Khanom 2013). The stem bark of *Ficus mucoso* is traditionally used to treat bronchial infections (Bouquet 1969). Although, our previous study also revealed that *F. mucoso* prevents iron-induced oxidative stress in Drosophila melanogaster (Oyebode et al. 2021), there is paucity of information on the mechanism of its anti-inflammatory action. Given that mitochondria play a pivotal role in the regulation of inflammatory responses and that terpenes have been proven to be found in *F. mucoso*, the mechanistic role of the anti-inflammatory effects of terpene-abundant fractions in addition to their mitochondrial-protective potentials in LPS-driven inflammation were evaluated.

## Materials and methods

### Collection and extraction of plant material

The roots of *F. mucoso* were freshly harvested and obtained from Oje market, Ibadan, Oyo State, Nigeria. Identification and authentication of samples were carried out at the Department of Botany, University of Ibadan and a specimen voucher number (UIH-22946) was deposited in the Herbarium. Fresh *F. mucoso* roots were washed in clean water after which the bark were peeled and air dried for a couple of weeks in the laboratory. Thereafter, the dried root bark was milled into a fine powder. Milled root bark of *F. mucoso* (2.3 kg) was submerged with enough methanol (100%) in glass jars at room temperature for 72 h. The flasks were stoppered and left to stand for 72 h. The extracts were then filtered using Whatman No 1 filter paper after which the filtrate (crude methanol extract of *F. mucoso*) was concentrated using a rotary evaporator at 40 °C. The resulting filtrate was lyophilized to obtain a powdery form that could be easily weighed.

### Partitioning of crude methanol extract of *F. mucoso* (MEFM) using vacuum liquid chromatography (VLC)

MEFM (10 g) was adsorbed to Silica gel 60 (0.040–0.063 mm, MERCK) (20 g). A sintered funnel for Vacuum Liquid Chromatography (VLC) was packed with 20 g Silica gel (Hopkins and Williams, England). To de-fat the sample, n-hexane was first added while fractions were collected in the order of increasing polarity using dichloromethane, ethylacetate and methanol. The sequentially eluted fractions were concentrated using a rotary evaporator under reduced pressure to obtain concentrated solvent-free fractions, dichloromethane, ethylacetate, and methanol fractions of the plant. Given that terpenes are non-polar, dichloromethane and ethylacetate-free solvent fractions were exhaustively washed with petroleum ether according to the method described by Ferguson (1956) to obtain Terpene-Rich Dichloromethane fraction (TRDF) and Terpene-Rich Ethylacetate fractions of *F. mucoso* (TREF) root bark. These fractions were stored in glass sample bottles and kept in the refrigerator until use.

### Detection of terpenes by thin layer chromatography (TLC)

The crude fractions of dichloromethane (DFFM) and ethylacetate fractions (EFFM) as well as their terpene-rich fractions (TRDF and TREF) were tested for the presence of terpenes via thin layer chromatography. It was carried out using TLC aluminum sheets of pre-coated silica gel using...
the solvent system comprising hexane/ethyl acetate (1:1). Samples were prepared by diluting the fractions with respective solvent and then applied 1–10 µl to a TLC plate using capillary tubes. The spotted plate was placed in the TLC glass chamber (solvent saturated) for development of the chromatogram. The plates were then air dried and spots on TLC were visualized by spraying with 10% vanillin–sulfuric acid in methanol, followed by heating at 110 °C for 1–2 min.

**Metabolite profiling of terpene-rich fractions of *F. mucoso***

To obtain a profile of metabolites, the TRDF and TREF were characterized using a Waters UPLC coupled in sequence to Waters SYNAPT™ HDMS™ system (Waters Corporation, MA, USA). An optimized chromatographic separation was obtained on Waters UPLC utilizing a Waters HSS T3 C18 column (150 mm × 2.1 mm, 1.8 µm), temperature controlled at 60 °C and a binary solvent mixture of water (Eluent A) and acetonitrile (Eluent B) both containing 10 mM formic acid (pH of water adjusted to 2.4) was used. The initial conditions were 98% A at a flow rate of 0.4 mL/min, maintained for 1 min, followed by a linear gradient to 2% A at 16 min. First, the conditions were kept constant for 1 min and afterwards changed to the initial conditions. The runtime was 20 min while the injection volume was 2 µL. The samples were then maintained at 8 °C in the Waters Sample Manager during the analysis.

The SYNAPT G1 mass spectrometer used in V-optics and operated in electrospray mode allowed detection of all compounds compatible with electrospray ionization (ESI). Leucine enkephalin (50 pg/mL) was used as reference calibrant to obtain typical mass accuracies between 1 and 5 mDalton (mDa). Both ESI-positive and -negative modes were obtained from the mass spectrometer (capillary voltage of 2.5 kV, sampling cone at 30 V, extraction cone at 4.5 V). The scan time was 0.1 s covering the 50–1000 Dalton mass range with an interscan time of 0.02 s. The source temperature was 120 °C while desolvation temperature was set at 450 °C. Nitrogen gas was used as the nebulization gas at a flow rate of 550 L/h and cone gas was added at 50 L/h. The massLynx 4.1 (SCN 872) software was employed to control the hyphenated system and to process the data. Identification of compounds was further enhanced by analyzing all samples with both low and high collision energy settings of the collision cell. To reduce compound fragmentation, a low energy setting of 3 eV was used, while a collision energy ramp of 10–40 eV was used to enhance fragmentation of molecules. Argon was used as the collision gas employed in the collision cell was argon. Additional characterization of the TRDF was carried out by ¹H Nuclear Magnetic Resonance (NMR) fingerprinting. The choice of TRDF was based on its improved pharmacological effect/terpene dominance compared to TREF. The NMR spectrum was recorded using deuterated chloroform (CDCl₃) (Merck, Darmstadt, Germany) at room temperature on an Agilent ProPulse 500 MHz spectrophotometer (USA). The ¹H NMR data were processed using MestReNova software Version 14.2.0-26256 (Mestrelab Research S.L. (USA)).

**Chemicals and reagents**

Lipopolysaccharide (Escherichia coli serotype 0111:B4) was procured from AK Scientific, China. Calcium, trichloroacetic acid (TCA), Tris–HCl, bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), Folin–Ciocaltéau, spermine, mannitol, sucrose, sodium succinate and rotenone and all other chemicals were obtained from Sigma Chemical Co., (St. Louis, MO, USA). All solvents used are of high analytical grade.

**Animal grouping and experimental design**

**Experimental animals**

Male Swiss albino mice (15 g) were obtained from the Veterinary Medicine Animal House, University of Ibadan, Nigeria. The animals were acclimatized for a couple of weeks in the Animal House, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. They were given water and rat chow without restriction. They were kept under standard conditions of temperature and 12-h dark/light cycle.

**Experimental design**

**Determination of LPS dose induction** To determine the suitable dose of LPS in the strain of mice, two doses (0.5 mg/kg, 1.0 mg/kg) of LPS were intraperitoneally administered to mice for 3 days (72 h) in a pilot study after which they were sacrificed by cervical dislocation.

**Induction of inflammation/ treatment** To evaluate the protective effect of terpene-rich fractions of *F. mucoso* (TRDF and TREF) on LPS-induced damage, eighty mice were randomly distributed into ten (10) groups of eight (8) animals each. LPS was dissolved in normal saline which served as the vehicle and was also administered to control mice. The groups were as follows:

- **Group 1**: Normal control (normal saline); **Group 2**: LPS only (1 mg/kg).
- **Group 3**: TRDF (100 mg/kg); **Group 4**: TRDF (200 mg/kg).
- **Group 5**: LPS + TRDF (100 mg/kg); **Group 6**: LPS + TRDF (200 mg/kg).
Group 7: TREF (100 mg/kg); Group 8: TREF (200 mg/kg).

Group 9: LPS + TREF (100 mg/kg); Group 10: LPS + TREF (200 mg/kg).

Mice were initially intraperitoneally challenged with LPS for first 3 days in groups 5, 6, 9 and 10 after which they were orally treated with different doses of TRDF and TREF or fractions alone (without LPS) for the last 3 days before the termination of the experiment. LPS was dissolved in normal saline (0.9% NaCl solution).

**Estimation of pro-inflammatory cytokines**

Whole blood samples were drawn into plain tubes and allowed to clot for 2 h at room temperature after which they were centrifuged at 3000 rpm for 15 min at 4 °C. The clear supernatants were stored as serum. Serum samples exposed to LPS and/or treated with or without TRDF and TREM were used to evaluate TNF-α, IL-1β, and IL-6 levels. These cytokines were evaluated using Elabscience (CA, USA) standard ELISA kits according to the manufacturer’s instructions. The results were calculated based on the absorbance levels of complex cytokine antibodies, and the units of cytokines were described as pg/ml.

**Quantification of catalase and superoxide antioxidant activity**

The post-mitochondrial fraction was kept for estimation of antioxidant activity. Catalase (CAT; EC 1.11.1.6) activity was determined by a procedure described by Aebi (1984). The reaction mixture containing 1.8 mL potassium phosphate buffer (pH 7.0), 180 mL of 300 mM H₂O₂, and 20 mL of sample (1: 50 dilution) was carried out by monitoring the clearance of H₂O₂ at 240 nm at 25 °C. The decrease in H₂O₂ was monitored for 2 min (10-s intervals), at 240 nm using a UV–visible spectrophotometer (Shimadzu) and expressed as mmol of H₂O₂ consumed/min/mg of protein. The method of Misra and Fridovich (1972) was employed in the determination of superoxide dismutase (SOD) activity. The ability of SOD to inhibit the autooxidation of epinephrine (pH 10.2) is the basis for the simple assay for SOD. The reaction mixture containing 50 μl of sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and 0.3 mL of epinephrine, mixed by inversion in a cuvette while change in absorbance was monitored every 30 s for 2.5 min at 480 nm.

**Evaluation of histopathology of liver and serum biomarkers**

The livers excised from mice were fixed in 10% formalin and were processed to paraffin blocks, sectioned (5 μm) and stained with hematoxylin–eosin (H&E) for histological analysis. These were counter stained in 10% aqueous eosin, incubated and mounted for photomicrography. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were measured by monitoring the concentration of pyruvate hydrazone formed according to the method described by Reitman and Frankel (1957). Levels of serum ALT and AST were determined using standard kits supplied by Fortress Diagnostic Limited (UK).

**Isolation of low-strength ionic rat liver mitochondria**

Rat liver mitochondria were isolated according to the method described by Johnson and Lardy (1967). The animals were killed by cervical dislocation, dissected and the tissues of interest were immediately excised. The liver was washed several times in isolation buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES and 1 mM EGTA at pH 7.4) to have a blood-free tissue. A 10% suspension was prepared by homogenizing the weighed and minced liver in a Teflon-glass cup homogenizer. The suspended tissue in isolation buffer was implored into a refrigerated Sigma (3e30 K, Germany) centrifuge, where the nuclear fraction and cell debris were sedimented by low speed centrifugation twice at 2300 rpm for 5 min. The supernatant was spun at 13,000 rpm for 10 min to pellet the mitochondria (the rate limiting step). The mitochondria (pellet) obtained after the supernatant was discarded was washed to remove impurities by re-suspending in washing buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES and 50% BSA at pH 7.4) and centrifuged at 12,000 rpm for 10 min. This washing stage was done twice. The mitochondria were immediately suspended in a solution of ice-cold MSH Buffer (Mannitol, Sucrose, HEPES–KOH, pH 7.4), then dispensed in Eppendorf tubes in aliquots and placed on ice for immediate use. To preserve the integrity of the mitochondria, temperature was maintained at 4 °C throughout the entire process.

**Measurement of mitochondrial swelling**

Mitochondrial swelling was determined according to the method described by Lapidus and Sokolove (1994). Uncoupled mitochondria (0.4 mg/ml) were incubated in the presence of 0.8 μM rotenone and MSH buffer for 3.5 min prior to the addition of 5 mM sodium succinate, the respiratory substrate. To assess Ca²⁺-induced swelling, mitochondria were pre-incubated in 0.8 μM rotenone and MSH buffer for 3 min. Ca²⁺ was then added to the reaction mixture while sodium succinate was added 30 s later in a total reaction volume of 2.5 ml. Spermine was used as the standard inhibitor of mitochondrial swelling, and was added prior to mitochondrial pre-incubation with rotenone. Change in absorbance was estimated at 540 nm at 30-s interval for 12 min in a T70 UV–visible spectrophotometer (PG Instrument Ltd). Mitochondrial swelling was measured as decrease in absorbance at 540 nm. Permeability transition in the mitochondria isolated
from the treated groups was carried out on the same mitochondrial protein content from the control group and the absorbance monitored accordingly. The mitochondrial protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

**Assay for mitochondrial FoF1 ATPase (mATPase) activity**

The mATPase activity was determined as described by Olorunsogo and Malomo (1985). The reaction mixture comprises 65 mM Tris–HCl buffer pH 7.4, 0.5 mM KCl, 1 mM ATP and 25 mM sucrose (pH 7.4) with a total volume of 2 ml. The reaction was initiated by the addition of a mitochondria (of known protein) and was allowed to proceed in a shaker water bath for 30 min at 27 °C. The reaction was stopped by the addition of 1 ml of a 10% of sodium dodecyl sulfate (SDS) and 1 ml of the resulting mixture was kept for phosphate determination. The zero time tube was prepared by addition of ATP to the reaction vessel with immediate addition of SDS, but for 30-s intervals for other reaction vessels.

**Estimation of inorganic phosphate** The concentration of inorganic phosphate released from ATP hydrolysis was measured according to the procedure described by Bassir (1963) and as modified by Olorunsogo et al. (1979).

**Determination of mitochondrial lipid peroxidation (in vivo)**

Mitochondrial lipid peroxides were estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the mitochondria, based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxides according to the method described by Varshney and Kale (1990). An aliquot of 0.4 ml of test sample (mitochondria) was mixed with 1.6 ml of Tris–KCl buffer to which 0.5 ml of 30% TCA was added. Then, 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 min at 80 °C. This was immediately cooled on ice to room temperature and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured against a reference blank of distilled water at 532 nm. The concentration of TBARS was calculated using extinction co-efficient of 0.156/μM/cm (Adám-Vizi and Seregi 1982).

Lipid peroxidation (nmole TBARS/ mg protein) = Absorbance × volume of mixture.

E532nm × volume of sample × mg protein/ml.

**Statistical analysis**

Using one-way ANOVA analysis of variance followed by Tukey’s test, multiple comparisons were made after analysis using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). All the values were expressed in mean ± standard deviation (SD) and differences were considered significant at p < 0.05.

**Results**

**Effects of varying doses of LPS administration on serum pro-inflammatory cytokines, antioxidant enzymes and aminotransferase activities in mice**

To determine the dose of LPS suitable for induction of inflammation in the mice strain, intraperitoneal administration of two doses (0.5, 1.0 mg/kg) of LPS was tested alongside a control group that received normal saline alone. The results revealed that the levels of serum pro-inflammatory cytokines—TNF-α, IL-1β and IL-6, in animals that received LPS increased significantly (p < 0.05; p < 0.01) when compared to control (Fig. 1a–c). Similarly, there were marked increase in levels of serum aspartate and alanine aminotransferases in LPS-administered animals with respect to control (Fig. 1f). However, LPS-treated mice had significant (p < 0.05) decreases in activities of the antioxidant enzymes (SOD and catalase) when compared to control (Fig. 1d, e).

**Effects of LPS on mice liver mitochondrial Permeability Transition pore**

Figure 2 shows the representative profile of changes in absorbance over a period of 12 mins of mitochondria isolated from mice previously intraperitoneally administered doses (0.5, 1.0 mg/kg) of LPS for 3 days. First, swelling of mitochondria isolated from control mice was determined. The data presented indicate that over a period of 12 mins, there was no significant change in the absorbance of mitochondria isolated from the control mice in the presence of succinate. However, on the addition of calcium, there was a large amplitude swelling and the mPT pore was opened by 5.1-fold compared to control (no calcium). Interestingly, the calcium-induced swelling of the pore was almost completely reversed (90%) by spermine (a standard inhibitor). Furthermore, the data clearly show that the mPT pore of mitochondria isolated from LPS-treated mice was significantly opened. In this regard, induction of pore opening of 4.0- and 4.9-folds were observed in livers of mice intraperitoneally administered 0.5 and 1.0 mg/kg LPS. The higher dose of 1 mg/kg LPS was selected for further study because it gave a higher inductive effect in vivo.
TLC profiling of the terpene-rich fractions of *F. mucoso* (TRDF and TREF)

Figure 3 shows the TLC plate of terpene-rich fractions (TRDF and TREF) of *F. mucoso*. Different chromogenic reagents usually give distinct colors for the specific phytochemicals. A purple color against a white background when sprayed with vanillin–sulfuric acid (10%) indicates the presence of terpenes. Figure 3, therefore, indicates that

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Fig. 1 Effects of administration of doses of lipopolysaccharide (LPS) on liver antioxidant parameters, aminotransferases and pro-inflammatory cytokines in mice. Charts are represented as: tumour-necrosis factor (TNF-α) (A), Levels of IL-1 β (B), Levels of IL-6 (C) Superoxide dismutase activity (D), Catalase activity (E), levels of aminotransferases (F). Values are expressed as Mean ± Standard Error of Mean with 3 replicates per treatment group. Significant differences from the control are indicated by "*"($p < 0.05$).
Terpene-rich fractions of *Ficus mucoso* (Welw) modulate lipopolysaccharide-induced inflammation, alteration in antioxidant status and elevation in hepatic enzyme activities

As shown in Fig. 4, LPS-induced mice exhibited higher levels of serum TNF-α, IL-1β and IL-6 when compared with normal mice, while administration of TRDF and TREF (100 mg/kg and 200 mg/kg per day) without LPS treatment maintained basal levels of the pro-inflammatory cytokines. Interestingly, co-administration of TRDF and TREF in LPS-treated mice significantly \((p < 0.05)\) reduced serum levels of TNF-α, IL-1β, and IL-6. However, the terpene-rich dichloromethane fraction (TRDF) produced a greater effect in a dose-dependent manner in reducing elevated levels of pro-inflammatory cytokines in LPS-induced mice.

Similarly, mice treated with doses of terpene-rich fractions (TRDF and TREF) in the absence of LPS administration preserved activities of antioxidant enzymes-CAT and SOD to varying extent with respect to the control while LPS-induced reduction in CAT and SOD activities were restored by doses of TRDF and TREF in a dose-independent manner.

Again, elevated levels of serum AST and ALT in LPS-treated mice were significantly reduced by administration of TRDF (100 mg/kg and 200 mg/kg per day) while administration of TREF alone had no significant effect on the activities of the enzymes when compared with control. Contrarily, administration of TREF alone brought about a significant elevation in the levels of the liver marker enzymes relative to control. This effect was more pronounced at the higher dose (200 mg/kg bw). Furthermore, when TREF (100 mg/kg and 200 mg/kg per day) were co-administered with LPS-induced mice, the lower dose (100 mg/kg bw) exhibited a reduction in serum levels of ALT and AST when compared with LPS-induced mice while the higher dose (200 mg/kg bw) had no significant effect whatsoever.

Histological evaluation of the liver in TRDF- and TREF-treated rats with LPS-induced inflammation

Fig. 4 Effects of administration of terpene-rich fractions of *F. mucoso* on liver antioxidant parameters, aminotransferases and pro-inflammatory cytokines in Lipopolysaccharide-treated mice. Charts are represented as: Tumour-necrosis factor TNF-α (A), Levels of IL-1β (B), Levels of IL-6 (C), Superoxide dismutase activity (D), Catalase activity (E), levels of aminotransferases (F). TRDF- Terpene-Rich Dichloromethane Fraction of *F. mucoso*. TREF- Terpene-Rich Ethylacetate Fraction of *F. mucoso*. Values are expressed as Mean ± Standard Error of Mean with 3 replicates per treatment group. Significant differences from the control are indicated by \(^a(p < 0.05)\). Significant differences from the LPS-treated group indicated by \(^b(p < 0.05)\)
macro vesicular steatosis and marked periportal infiltration by inflammatory cells. Interestingly, exposure of LPS pre-treated mice to TRDF (100 mg/kg and 200 mg/kg per day) significantly recovered damaged hepatocytes in contrast to TREF. However, the lower dose of TREF (100 mg/kg bw) had a mild protective effect on LPS-induced liver inflammation while the higher dose (200 mg/kg) TREF had no significant effect on LPS-induced liver injury.

**Effects of terpene-rich fractions of *F. mucoso* on liver mitochondrial permeability transition pore in LPS-induced inflammation**

Figure 6 is a representative profile of changes in absorbance of mitochondria isolated from normal, LPS-induced mice and those treated with TRDF and TREF after being challenged with LPS. The data obtained reveal that control mice had intact inner mitochondrial membrane as shown by an insignificant change in absorbance at 540 nm. Similarly, administration of doses of TRDF (in the absence of LPS) had no effect whatsoever on the mPT pore. However, administration of TREF alone at 100 mg/kg and 200 mg/kg activated opening of the mPT pore by 1.1- and 2.4-fold, respectively. LPS administration significantly induced opening of the pore by 3.3-fold at 1 mg/kg LPS and the rate of mitochondrial swelling demonstrated by LPS was comparable to that of calcium (3.6-fold), the standard triggering agent of the mPT pore. Interestingly, mitochondrial swelling induced by LPS was significantly inhibited in the presence of TRDF (100 mg/kg; 200 mg/kg) and low dose of TREF (100 mg/kg) by 71, 88 and 67%, respectively; while spermine, the standard pore inhibitor, has an inhibitory effect of 64%. In contrast, the high dose of TREF induced opening of the pore by 5.2-fold.
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Effect of terpenes from *F. mucoso* on liver mitochondrial ATPase activity and lipid peroxidation in LPS-induced mice

To further monitor mitochondrial health with respect to its ATP generating function, the investigation of the mitochondrial ATPase (mATPase) activity and degree of lipid peroxidation of livers of mice previously exposed to TRDF and TREF in LPS-treated mice were carried out (Fig. 7). There results showed that there was significant enhancement of mitochondrial ATPase activity (increased levels of inorganic phosphate) at physiological pH in...

**Fig. 7** Mitochondrial ATPase (mATPase) activity and Degree of lipid peroxidation of mitochondria of livers of mice previously exposed to terpenes from *F. mucoso* in lipopolysaccharide (LPS)-treated mice (B). TRDF- Terpene-Rich Dichloromethane Fraction of *F. mucoso*. TREF- Terpene-Rich Ethylacetate Fraction of *F. mucoso*. DNP-2,4-dinitrophenol. Values are expressed as Mean ± Standard Error of Mean with 3 replicates per treatment group. Significant differences from the control are indicated by * (p < 0.05). Significant differences from the LPS-treated group indicated by † (p < 0.05)
Profiles matching the chromatographic peaks in TRDF and TREF of *F. mucoso* (Figs. 8 and 9, respectively) were determined by full-scan MS and MS/MS analysis using both positive- and negative-ion modes based on their accurate mass, and fragment ions in comparison with those available on databases like MassBank and SciFinder® including data reported in literature. Twelve compounds each were identified in the TRDF and TREF of the *F. mucoso* extract with detailed reports of the compounds presented in Tables 1 and 2, respectively. These compounds comprise derivatives of mono-, sesqui-, di-, sester- and triterpenes. Some aromatic compounds, much of which occurred in TREF were also identified. Compounds common to both fractions are loliolide and azelaic acid. Two nitrogen-containing compounds (nonanamide and nopalnic acid) were found only in TRDF.

The full and regions-expanded $^1$H NMR spectra of TRDF is presented in Fig. 10 with chemical shifts of compounds ranging from 0.54 to 9.77 ppm. The full spectrum revealed a dense upfield region and a less dense to very low intensity downfield region.

**Discussion**

A causal connection between mitochondrial disorders and organ damage is fast gaining acceptance as a new concept for the etiology of inflammation-induced organ dysfunction (Brealey et al. 2002; Supinski et al. 2020). The intraperitoneal administration of lipopolysaccharide (LPS) is an experimental model for inducing systemic and hepatic inflammation in rodents (Hamesch et al. 2015). Oxidative stress and release of pro-inflammatory cytokines have been shown to be a well-known mechanism of LPS-induced hepatic injury (Hamesch et al. 2015; Liu et al. 2017).

Superoxide dismutase (SOD), an important antioxidant enzyme, catalyzes the dismutation of the superoxide anion ($O_2^-$) into hydrogen peroxide and molecular oxygen (Magnani et al. 2000). Given that hydrogen peroxide is highly deleterious to the cell, the decomposition of hydrogen peroxide ($H_2O_2$) to water and oxygen is brought about by another antioxidant enzyme, catalase. This study compared the therapeutic effects of terpene-rich fractions of *F. mucoso* on LPS-induced alteration in hepatic mitochondrial permeability transition, oxidative stress and inflammatory mediators in mice. First, the suitable dose of LPS for hepatic inflammation in the mice strain was determined in a pilot study. The observation that administration of doses of LPS brought about a rise in levels of key pro-inflammatory mediators (TNF-α, IL-1β and IL-6) which was consistent with increased activities of the serum liver marker enzymes (AST and ALT) and decreased antioxidant enzyme activities (SOD and CAT) in the post-mitochondrial fraction indicates that hepatic inflammation has been induced in the challenged mice. Furthermore, findings that doses of LPS promoted large amplitude matrix swelling in a dose-dependent manner is an attestation to the earlier reported role of mitochondria in inflammation (Missiroli et al. 2020). In addition, the inductive effect of LPS on liver mPT pore is in tandem with the reports of Crouser et al. (2004) that mitochondrial dysfunction results from alteration in permeability of liver mitochondrial membranes during acute endotoxemia. Taken together, these data suggest that induction of hepatic inflammation occurred via production of cytokines, overwhelming antioxidant capacity possibly through generation of oxidant species and activation of mPT pore opening. Given the pronounced effects observed at the higher dose, 1 mg/kg LPS was adopted for the main study.

The presence of terpenes in *F. mucoso* as revealed by the TLC profile of terpene-rich fractions (TRDF and TREF) is in consonance with the findings of Djemgou et al. (2009) who reported the presence of terpenes in *F. mucoso*. However, the observation that terpene-rich dichloromethane fraction (TRDF) had a higher content of terpenes when compared with terpene-rich ethylacetate fraction (TREF) is in order since terpenes are non-polar, they are, therefore, expected to be present in greater amount in a non-polar solvent (dichloromethane) relative to a more polar solvent (ethylacetate).

Plant terpenes have been shown to exhibit potential anti-inflammatory and antioxidant activity (González-Burgos et al. 2012; Vega et al. 2018; Li et al. 2019). In this study, the observation that TRDF and TREF ameliorated LPS-induced production of inflammatory cytokines and restored antioxidant enzyme activity may be attributed to the anti-inflammatory and antioxidant activities of the terpenes.
present in the fractions. Furthermore, the observation that the terpene-rich fractions alone maintained basal levels of pro-inflammatory cytokines while preserving antioxidant activity is a pointer to the anti-inflammatory and antioxidant potential of the plant (Bouquet 1969; Essien et al. 2016; Oguntoye et al. 2016;). These data also give credence to our recently published study showing prevention of iron-induced oxidative stress in *Drosophila melanogaster* (Oyebode et al. 2021). It emphasizes the fact that iron plays a role in inflammation (Cornelissen et al. 2019), hence *F. mucoso* modulates both processes.

In addition, the finding that TRDF is more potent than TREF in reducing levels of pro-inflammatory cytokines may be due to higher content of terpenes present in TRDF than TREF.
Increase in levels of serum/plasma AST and ALT usually serve as specific indicators of liver injury clinically (Senior 2012). The results obtained in this study agree with those of Chen et al. (2019), who reported that LPS induced inflammatory cell infiltration and caused significant increases in levels of AST and ALT, as well as accompanying hepatocyte necrosis. The findings that a combination of LPS with TRDF significantly reduced LPS-induced elevation of AST and ALT is a pointer to the hepatoprotective potential of this fraction (TRDF). In addition, the histological analysis confirmed/underscored the hepatic pathological change in livers of LPS-induced mice (Jiang et al. 2018) as well as the dose-dependent protective effect of TRDF with or without LPS challenge, while the histological evaluation of control
livers showed the expected normal architecture with no pathological signs.

In this study, the administration of TREF alone brought about a significant elevation in the levels of the liver marker enzymes relative to control while a mild reduction in enzyme activities was observed when the low dose (100 mg/kg) of TREF was co-administered with LPS while the high dose (200 mg/kg) had no effect on liver AST and ALT. These are indications that although TREF possesses antioxidant and anti-inflammatory activities, these pharmacological potentials could not protect the liver from injury as further emphasized by histological examination. This prompted further investigation into the mechanism of protective effects offered largely by TRDF, but not TREF.

Mitochondrial swelling (matrix expansion) is an important parameter to assess inner membrane permeability transition and this provides information, in part, as to the health of the organelle. The mPT pore is a potential drug target for the treatment of a myriad of diseases (Kalani et al. 2018; Bhosale and Duchen et al. 2019). In this regard, inducers of pore opening trigger cell death in diseases characterized by cell death insufficiency, while mPT pore inhibitors are useful in preventing pathological changes associated with excessive cell death. As observed earlier in the preliminary study, LPS significantly stimulated liver mitochondrial swelling indicating that the status of the mitochondria could be manipulated by bacteria during infection. The observation that mitochondria isolated from mice treated with TRDF (without LPS) has intact inner mitochondrial membrane while those isolated from mice that received a combination of LPS and TRDF significantly inhibited LPS-induced pore opening is an attestation to the protective effect of TRDF. It is also noteworthy that doses of TRDF inhibited mitochondrial swelling better than spermine, a standard mPT blocker. Furthermore, the inductive effect of TREF alone on mPT pore may account for the pathological effects seen in the

| S/N | tR (min) | Compound identified | Formula | DBE | Found mass (m/z) | Adduct |
|-----|---------|---------------------|---------|-----|----------------|--------|
| 1   | 5.68    | Esculetin           | C₉H₅O₄ | 7   | 177.0189       | [M−H]⁻ |
| 2   | 5.80    | Loliolide           | C₁₁H₁₇O₃ | 4 | 197.1177       | [M+H]⁺ |
| 3   | 6.13    | Azelaic acid        | C₉H₁₃O₄ | 2 | 187.0970       | [M−H]⁻ |
| 4   | 9.24    | Nonanamide          | C₉H₁₈NO  | 1 | 158.1529       | [M+H]⁺ |
| 5   | 9.33    | Nopalanic acid      | C₁₀H₁₉N₂O₆ | 3 | 263.1255       | [M+H]⁺ |
| 6   | 12.64   | Galvaquinone A      | C₅H₁₇O₅ | 12 | 353.1376       | [M+H]⁺ |
| 7   | 13.26   | Octadecanoic acid   | C₁₂H₂₃O₄ | 2 | 313.2406       | [M−H]⁻ |
| 8   | 14.26   | Osajin               | C₂₀H₂₅O₅ | 14 | 403.1536       | [M−H]⁻ |
| 9   | 14.67   | (3β,5α,6β)-Androstane-3,5,6-triol | C₁₅H₂₃O₆ | 3 | 309.2444 | [M+H]⁺ |
| 10  | 14.93   | Ursolic acid        | C₉H₁₇O₃ | 7 | 455.3515       | [M−H]⁻ |
| 11  | 15.92   | Glycol stearate     | C₂₀H₄₁O₃ | 1 | 327.2919       | [M−H]⁻ |
| 12  | 17.64   | α-D-Glucopyranoside, β-D-fructofuranosyl, 6-tricosanoate | C₁₈H₃₁O₁₂ | 3 | 679.4569       | [M+H]⁺ |

Table 2 UPLC-ESI–MS report of Terpene-Rich Ethylacetate Fraction (TREF)
histological sections of the liver as well as observed elevated liver-specific enzymes. Interestingly, the finding that TREF inhibited opening of the mPT pore at the low dose (100 mg/kg), but had a higher magnitude of mitochondrial swelling (5.2-fold) at high dose (200 mg/kg) greater than LPS alone (3.3-fold) suggests that TREF may have beneficial or protective effects only at low doses.

The finding that TRDF and TREF inhibited LPS-induced increase in mitochondrial lipid peroxidation at all the doses used in this study to varying extents confirm that the modulation of pore opening effects exerted by these fractions was not due to any disruption of the mitochondrial membrane bilayer. These data are in consonance with the studies of Singh et al. (2012) which revealed that natural terpenes prevented oxidative mitochondrial dysfunction during toxicant-induced hepatic damage.

Although, it is well established that disruption or opening of the mPT pore results in bioenergetics collapse or decrease in ATP levels which is usually seen in enhancement of mATPase activity, it is still a matter of debate as to whether the ATP synthase is a strong candidate of the pore (Bernadi 2018). An assessment of mATPase in mice that received TRDF with LPS revealed that TRDF significantly inhibited LPS-enhanced mATPase activity. This suggests that TRDF preserved the bioenergetics status of the mitochondria, while TREF with (except at the low dose) / without LPS compromised the energy status of the organelle as shown by high levels of inorganic phosphate released relative to the control.

In our attempt to further understand the underlying bioactive metabolites that are possibly responsible for the observed pharmacological activities in this study, terpene-rich solvent fractions of F. mucoso was analyzed with UPLC hyphenated with a high definition ESI–MS. The identified constituents of both TRDF and TREF revealed some similarities in the compound classes as observed with the fatty acid derivatives, esters, lactone bearing compounds and isoprenylated flavonoids. However, variation in the pharmacophoric combinations across the compounds which impose either positive or negative synergy is likely responsible for the bioactivity differences between TRDF and TREF. The functional groups that constitute each pharmacophore interact with the target receptors in varying degrees, positively or negatively (Efferth and Koch 2011).

The monoterpene lactone, loliolide, which was identified in both TRDF and TREF was recently described as a potent anti-inflammatory and neuroprotective metabolite (Silva et al. 2021). Similarly, aesculetin and several other coumarins derivatives are well known anti-inflammatory agents (Grover and Jachak 2015). A common pharmacophore that may be responsible for activities observed in loliolide and

Fig. 10. $^1$H NMR fingerprint of terpene-rich dichloromethane fraction
A terpene-dominated \(^1\)H NMR fingerprint was obtained for TRDF as evident in the highly overlapping upfield chemical shifts with strong intensities. The strong singlets between \(\delta_H 0.79\) and 1.29 are characteristic of angular methyl protons of the terpenoids. The olefinic proton(s) triterpenes and some other terpenoids often find resonances between \(\delta_H 5.13\) and 5.36 (for example, \(\delta_H 5.21\) in ursolic acid), although terminal methylene protons resonate around \(\delta_H 4.50–4.69\). Oxygenated methylene and methine signals which are major components of (\(3\beta,5\alpha,6\beta\))-androstane-3,5,6-triol, ursolic acid, glycol stearate, the glycolipid and other terpenoids present in TRDF must have given rise to the signals observed around \(\delta_H 3.23\) and 4.58 which may be further deshielded to about \(\delta_H 5.42\) for some anomic protons. Presence of few aromatic metabolites was registered in TRDF which were revealed by low intensity peaks between \(\delta_H 6.11\) and 7.72. Amongst the identified aromatic compounds are aesculetin, galvaquinone A and osajin. The \(^1\)H NMR fingerprint, therefore, provides qualitative credence to the identity of the metabolites identified in TRDF. Although these phenolics with other non-terpene constituents, including fatty acids and their derivatives may be present in reduced quantities, it is possible that their additive effect on the fractions contributed to the observed anti-inflammatory activities.

## Conclusion

Put together, we have shown for the first time the anti-inflammatory potential of *Ficus mucoso*. The TRDF is more potent than TREF with respect to beneficial hepatoprotective effects against LPS-induced inflammation. Thus, augmenting the antioxidant defense system and inhibition of mPT opening is important especially during infections or periods of chronic oxidative insult. Therefore, isolation and characterization of terpenes in *F. mucoso* as well as their molecular targets is highly desirable in finding novel specific anti-inflammatory drugs.

## References

Abourashed EA, Abraha A, Khan SI et al (2015) Potential of Horse Apple Isoflavones in targeting inflammation and Tau Protein Fibrilization. Nat Prod Commun 10:1577–1580

Abarca-Vargas R, Petricevich VL (2019) Extract from bougainvillea xbutitana (Variety Orange) inhibits production of LPS-induced inflammatory mediators in macrophages and exerts a protective effect in vivo. BioMedRes Int 2034247. https://doi.org/10.1155/2019/2034247

Adám-Vizi V, Seregi A (1982) Receptor independent stimulatory effect of noradrenaline on Na, K-ATPase in rat brain homogenate. Role of Lipid Peroxidation. Biochem Pharmacol 31:2231–2236. https://doi.org/10.1016/0006-2952(82)90106-x

Aebi H (1984) [13] Catalase in Vitro. Methods Enzymol 105:121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

An R-B, Sohn D-H, Kim Y-C (2006) Hepatoprotective compounds of the roots of Cudrania tricuspidata on tacrine-induced cytotoxicity in Hep G2 cells. Biol Pharm Bull 29:838–840. https://doi.org/10.1016/S0076-6879(84)05016-3

Bassir O (1963) Handbook of practical biochemistry. Ibadan University Press, Ibadan, p 13

Battaglia V, Salvi M, Toninello A (2005) Oxidative stress is responsible for mitochondrial permeability transition induction by salicylate in liver mitochondria. J Biol Chem 280:33864–33872. https://doi.org/10.1074/jbc.M502391200

Bauer TM, Murphy E (2020) Role of mitochondrial calcium and the permeability transition pore in regulating cell death. Circ Res 126:280–293. https://doi.org/10.1161/CIRCRESAHA.119.316306

Bernardi P (2018) Why F-ATP synthase remains a strong candidate as the Mitochondrial Permeability Transition Pore. Front Physiol 9:1543. https://doi.org/10.3389/fphys.2018.01543
Bhosale G, Duchen MR (2019) Investigating the Mitochondrial Permeability Transition Pore in Disease Phenotypes and Drug Screening. Curr Protoc Pharmacol 85:e59. https://doi.org/10.1002/cpph.59

Bouquet A (1969) Féticheurs et médecine traditionnelles du Congo (Brazzaville). Mémoires O.R.S.T.O.M., 36: 282

Brealey D, Brand M, Hargreaves I et al (2002) Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 360:219–223. https://doi.org/10.1016/S0140-6736(02)09459-X

Chen L, Deng H, Cui H et al (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9:7204–7218. https://doi.org/10.18632/oncotarget.23208

Chen, Z, Yang, Y, Mi S et al (2019) Hepatoprotective effect of chlorogenic acid against chronic liver injury in inflammatory rats. J Funct Foods 62:103540. https://doi.org/10.1016/j.jff.2019.08.014

Corner EJH (1962) The Classification of Moraceae. The Gardens’ bulletin, Singapore 19:187–252

Crompton M (1999) The mitochondrial permeability transition pore and its role in cell death. Biochem J 341(1 Pt 2):233–249

Crouser ED, Julian MW, Huff JE et al (2004) Abnormal permeability of inner and outer mitochondrial membranes contributes independently to mitochondrial dysfunction in the liver during acute endotoxemia. Crit Care Med 32:478–488. https://doi.org/10.1097/01.CCM.0000109449.99160.81

Cruzer FC, Ngandeu F, Hegazy ME et al (2009) GC-MS analysis of terpenes from ficus mucuso. Pharmacogn Res 1:197–201

Ellerth T, Koch E (2011) Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. Curr Drug Targets 12:122–132. https://doi.org/10.2174/138945011793591626

Essien E, Newby J, Walker T et al (2016) Essential oil constituents, anticancer and antimicrobial activity of Ficus mucoso and Casuaria equisetifolia leaves. Am J Essent Oils Nat Prod 4:1–6

Ferguson NM (1956) A textbook of pharmacognosy. California Medi-

Furman D, Campisi J, Verdin E et al (2019) Chronic inflammation in the mitochondria. Methods Enzymol 10:94–96. https://doi.org/10.1016/0076-6879(67)10018-9

Glass CK, Saijo K, Winner B et al (2010) Mechanisms underlying inflammation-associated diseases. Curr Protoc Pharmacol 85:e59. https://doi.org/10.1002/cpph.59

Halestrap AP, Richardson AP (2015) The mitochondrial permeability transition: a current perspective on its identity and role in ischaemia/reperfusion injury. J Mol Cell Cardiol 78:129–141. https://doi.org/10.1016/j.yjmcc.2014.08.018

Halestrap AP, Clarke SJ, Javadov SA (2004) Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. Cardiovasc Res 61:372–385. https://doi.org/10.1016/S0308-6636(03)00533-9

Hamesh K, Borkham-Kamphorst E, Strnad P, Weiskirchen R (2015) Lipopolysaccharide-induced inflammatory liver injury in mice. Lab Anim 49:37–46. https://doi.org/10.1177/0023677215570087

Hu X, Wu J-W, Zhang X-D et al (2011) Isoprenylated flavonoids and adipogenesis-promoting constituents from Morus murgas. J Nat Prod 74:816–824. https://doi.org/10.1021/nl101009d

Jiang Z, Meng Y, Bo L et al (2018) Sophocarpine Attenuates LPS-Induced Liver Injury and Improves Survival of Mice through Suppressing Oxidative Stress, Inflammation, and Apoptosis. Mediators Inflamm 2018:1–12. https://doi.org/10.1155/2018/5871431

Johnson D, Lardy H (1967) [15] Isolation of liver or kidney mitochondria. Methods Enzymol 10:94–96. https://doi.org/10.1016/0076-6879(67)10018-9

Kalani K, Yan SF, Yan SS (2018) Mitochondrial permeability transition pore: a potential drug target for neurodegeneration. Drug Discovery Today 23:1983–1989. https://doi.org/10.1016/j.drudis.2018.08.001

Lapidus RG, Sokolove PM (1994) The mitochondrial permeability transition. Interactions of sphingomyelin, ADP, and inorganic phosphate. J Biol Chem 269:18931–18936

Li F, Zhang J, Lin M et al (2019) Anti-inflammatory terpenes from Schefflera rubriflora C. J. Tseng & G. Hoo with their TNF-α and IL-6 inhibitory activities. Phytochemistry 163:23–32. https://doi.org/10.1016/j.phytochem.2019.03.021

Lin KW, Liu CH, Tu HY et al (2009) Antioxidant prenylflavonoids from Artocarpus communis and Artocarpus elaeis. Food Chem 115:558–562. https://doi.org/10.1016/j.foodchem.2008.12.059

Liu Y, Li F, Zhang L et al (2017) Taurine alleviates lipopolysaccharide-induced liver injury by anti-inflammation and antioxidants in rats. Mol Med Rep 16:6512–6517. https://doi.org/10.3892/mmr.2017.7414

Lowry Oh, Nj R, Al F, Rj R (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275. https://doi.org/10.1016/0922-338X(96)89160-4

Magnani L, Gaydou E, Hubaud J (2000) Spectrophotometric measurement of antioxidant properties of flavones and flavonols against superoxide anion. Anal Chim Acta 411:209–216. https://doi.org/10.1016/S0003-2670(00)00717-0

Medzhivot R (2008) Origin and physiological roles of inflammation. Nature 454:428–435. https://doi.org/10.1038/nature07201

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biochem 247:3170–3173

Missiroli S, Genovese I, Perrone M et al (2020) The Role of Mitochondrial Injury in Inflammation: From Cancer to Neurodegenerative Disorders. J Clin Med. https://doi.org/10.3390/jcm90303740

Mohanty A, Tiwari-Pandey R, Pandey NR (2019) Mitochondria: the indispensable players in innate immunity and guardians of the inflammatory response. J Cell Commun Signal 13:303–318. https://doi.org/10.1007/s12079-019-00507-9

Najafi M, Farajnia S, Mohammadi M et al (2014) Inhibition of mitochondrial permeability transition pore restores the cardioprotection by postconditioning in diabetic hearts. J Diabetes Metab Disord 13:106. https://doi.org/10.1186/s40200-014-0106-1

Oguntoso SO, Hamid AA, Ajibade SO et al (2016) GC-MS analysis, antioxidant and antimicrobial activities of extracts from Ficus mucoso leaves. Niger J Chem Res 21:51–65. https://doi.org/10.4314/njcrr.v21i1

Olorunsogo OO, Malomo SO (1985) Sensitivity of oligomycin-inhibited respiration of isolated rat liver mitochondria to perfluorodone, a fluorinated arylalkylsulfonamide. Toxicology 35:231–240. https://doi.org/10.1016/0300-483X(85)90018-6

Olorunsogo OO, Bababunni EA, Bassi O (1979) Effect of glycolosate on rat liver mitochondria in vivo. Bull Environ Contam Toxicol 22:357–364. https://doi.org/10.1007/BF02026955

Olowofolahan A, Oludele O, Olorunsogo O (2020) GCMS analysis of partially purified chloroform sub fractions of methanol extract of...
drymaria cordata (Linn) and their effects on mitochondrial membrane permeability transition pore. 8:3–11

Oludele O, Idris B, Benard O et al (2018) Mondia whitei, an African Spice Inhibits Mitochondrial Permeability Transition in Rat Liver. Prevent Nutr Food Sci 23:206–213. https://doi.org/10.3746/pnfs.2018.23.3.206

Oyebode TO, Adebusuyi OT, Akintimehin SE, Oluronsojo OO (2017) Modulation of Cytochrome C Release and Opening of Mitochondrial Permeability Transition Pore by Calliandra portoricensis (Benth) Root Bark Methanol Extract. Eur J Med Plants 20:1–14. https://doi.org/10.9734/EJMP/2017/35211

Oyebode OT, Ogunbiyi FO, Oluronsojo OO (2019) Opening of liver mitochondrial permeability transition pore in streptozotocin-induced diabetic rats and its inhibition by methanol fraction of Ficus mucoso (Welw) root bark. J Integr Med 17:446–454. https://doi.org/10.1016/j.joim.2019.10.001

Oyebode OT, Abolaji AO, Oluwadare JO et al (2020) Apigenin ameliorates D-galactose-induced lifespan shortening effects via antioxidative activity and inhibition of mitochondrial-dependent apoptosis in Drosophila melanogaster. J Funct Foods. https://doi.org/10.1016/J.JFF.2020.103957

Oyebode OT, Abolaji AO, Faleke H, Olorunsogo O.O. (2021). Methanol fraction of Ficus mucoso (Welw) Prevents Iron-induced Oxidative Damage and Alters Mitochondrial Dysfunction in Drosophila melanogaster. Drug and Chemical Toxicology (in press)

Pereira-Leite C, Nunes C, Jamal SK et al (2017) Nonsteroidal anti-inflammatory therapy: a journey toward safety. Med Res Rev 37:802–859. https://doi.org/10.1002/med.21424

Pourahmad J, Mortada Y, Eskandari MR, Shahraki J (2011) Involvement of Lysosomal Labilisation and Lysosomal/mitochondrial Cross-Talk in Diclofenac Induced Hepatotoxicity. IJPR 10:877–887

Rahman AHMM, Khanom A (2013) A Taxonomic and Ethno-Medicinal Study of Species from Moraceae (Mulberry) Family in Bangladesh Flora. Res Plant Sci 1:53–57. https://doi.org/10.12691/planet-1-3-1

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63. https://doi.org/10.1093/ajcp/28.1.56

Rimessi A, Previati M, Nigro F et al (2016) Mitochondrial reactive oxygen species and inflammation: Molecular mechanisms, diseases and promising therapies. Int J Biochem Cell Biol 81:281–293. https://doi.org/10.1016/j.biocel.2016.06.015

Salimi A, Neshat MR, Nasrzaadeh P, Pourrahmad J (2019) Mitochondrial Permeability Transition Pore Sealing Agents and Antioxidants Protect Oxidative Stress and Mitochondrial Dysfunction Induced by Naproxen, Diclofenac and Celecoxib. Drug Research 69:598–605. https://doi.org/10.1055/a-0866-9356

Senior JR (2012) Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury-past, present, and future. Clin Pharmacol Ther 92:332–339. https://doi.org/10.1038/clpt.2012.108

Silva J, Alves C, Martins A et al (2021) Loliolide, a New Therapeutic Option for Neurological Diseases? In Vitro Neuroprotective and Anti-Inflammatory Activities of a Monoterpenoid Lactone Isolated from Codium tomentosum. Int J Mol Sci. https://doi.org/10.3390/ijms22041888

Singh BK, Tripathi M, Chaudhari BP et al (2012) Natural terpenes prevent mitochondrial dysfunction, oxidative stress and release of apoptotic proteins during nimesulide-hepatotoxicity in rats. PLoS ONE 7:e34200. https://doi.org/10.1371/journal.pone.0034200

Sun S, Zhang H, Xue B, Wu Y, Wang J, Yin Z, Luo L (2006) Protective effect of glutathione against lipopolysaccharide-induced inflammation and mortality in rats. Inflamm Res. 55(11):504–510

Supinski GS, Schroder EA, Callahan LA (2020) Mitochondria and critical illness. Chest 157:310–322. https://doi.org/10.1016/j.chest.2019.08.2182

Varshney R, Kale RK (1990) Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol 58:733–743. https://doi.org/10.1080/09553009014552121

Vega RJS, Xolalpa NC, Castro AJA et al (2018) Terpenes from Natural Products with Potential Anti-Inflammatory Activity. Terpenoids. https://doi.org/10.5772/INTECHOPEN.71215

Wei B-L, Weng J-R, Chiu P-H et al (2005) Antiinflammatory flavonoids from Artocarpus heterophyllus and Artocarpus communis. J Agric Food Chem 53:3867–3871. https://doi.org/10.1021/jf047873n

Yu J, Ji X, Yu B, Chen D (2016) Isoflavones: anti-inflammatory benefit and possible caveats. Nutrients. https://doi.org/10.3390/nu8060361

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