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Research Article

Isolation of Ferulic Acid - A Bioactive Phenolic Compound from Pineapple Peel and Evaluation of its *In-vitro* Anti-inflammatory Potential

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ABSTRACT

Fruit wastes and their byproducts may cause serious environmental problems, since it accumulates in agro-industrial yards without having any significant and commercial value. Pineapple(Family - Bromeliaceae) is one of the leading edible tropical fruit produced in the world. Pineapple peel is the massive waste produced by the pineapple processing industry leading to environmental pollution. In this regard, efforts have been made in order to tap the potential of this bio-waste as source of bioactive compounds by isolating, purifying and characterizing known as well as novel secondary metabolites from it and can be used for studying its application in various industries. A phenolic compound namely Ferulic acid is isolated from this bio-waste and its structure has been elucidated by Fourier Transform Infrared (FT-IR) spectroscopy, Liquid Chromatography-Mass Spectrometry (LC-MS), High Performance Liquid Chromatography (HPLC), ^{1}H NMR and ^{13}C Nuclear Magnetic Resonance (NMR) spectra. The anti-inflammatory potential of Ferulic acid was studied *in vitro* on Lipopolysaccharide (LPS) stimulated Ralph and Williams (RAW) and Tamm-Horsefall Protein-1 (THP-1) cell lines. The levels of pro-inflammatory enzymes such as cyclooxygenase (COX), lipooxygenase (LOX), and myeloperoxidase (MPOX) activities on THP-1 cell lines and the tested compound, exhibited a dose-dependent anti-inflammatory activity. Ferulic acid showed remarkable activity by COX (78.47%) and LOX (78.63%) inhibition in LPS stimulated THP-1 cells at 100 µg/mL. Different concentrations of Ferulic Acid (FER) effectively decreased the MPOX (92.76%) level in a dose dependent manner and the results were comparable to standard drug Indomethacin. The isolated phenolic compound, ferulic acid, effectively lowered inflammatory cytokines like Tumour Necrosis Factor Alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin-1 Beta (IL-1β) along with prostaglandin E2 and leukotriene C4 and cellular nitrate levels in LPS stimulated RAW cells.

Introduction

Plants have always been a source of a wide array of secondary metabolites with potential pharmacological properties. The use of secondary metabolites obtained from plant origin could be an advantage and best solution to narrow down unhealthy products available in the market.[1] Studies on plant secondary metabolites especially phenolic compounds have gained enormous popularity due to their potential health benefits. This opened new doors in pharmacology, as pure, isolated chemicals, instead of crude plant extracts, as the standard for treating diseases. Polyphenolic compounds ubiquitously of plant origin have many health benefits due to their potential antioxidant, anti-inflammatory and cancer-preventive activities.[2] Fruit peels are an important source of bioactive compounds including antioxidants, pectins, phenolics and proteins. The seed and rind of some fruits have higher vitamins, fibres, minerals and secondary metabolites than the pulp fractions. The potential uses of fruit peels as a rich source of natural antioxidants is mainly due to their phenolic constituent such as carotenoids, flavonoids, phenolic acids and anthocyanin's. Phenolic compounds in the fruit peels

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play an important role as a protector for inner material from insects and microorganisms.[3]

The Pineapple (*Ananas comosus* (L.) Merr.) is one of the world's most important edible tropical fruits. The increasing production of pineapple-based processed food products have resulted in massive waste generation. Pineapple fruit residues especially peel may cause serious environmental problems, such as water pollution, unpleasant odours, asphyxiation and greenhouse gas emission due to its rapid decay and eventually becoming a source of pest and pathogen multiplication. High level of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in peel waste creates further problems in their disposal. Since disposal of these wastes is expensive due to high costs of transportation and limited availability of landfills, they are unscrupulously disposed, causing serious environmental concerns. Hence, efficient, inexpensive and eco-friendly methods for the utilization of pineapple peel are becoming a need of the time.

Pineapple peel remains as the primary byproduct left over after processing the fruit and studies have shown that the peel is a potential source for various phytochemicals. Their isolation and characterization may help develop economically important high value products with application in chemical, agricultural, and pharmacological fields, thereby reducing their environmental impact if left unused or dumped. In this scenario, the present work has been carried out to explore the possibility of utilization of pineapple peel for isolating phenolic compounds and to determine its *in vitro* anti-inflammatory potential by evaluating the inhibitory levels of pro inflammatory enzymes such as cyclooxygenase, lipoxygenase and myeloperoxidase on THP-1 cell lines. The effect of the isolated compound on cellular nitrite concentration (NO) and pro-inflammatory cytokines namely TNF-α, IL-6, IL-1β along with prostaglandin E_2 and leukotriene C_4 in LPS induced RAW cells were also studied.

Materials and Methods

Plant Material

The material used for the study was the peel of Mauritius variety of pineapple fruit (the most popular cultivar grown in Kerala) collected from Pineapple Research Station, Vazhakulam, Muvattupuzha, which is the main cultivation region of pineapple in Kerala, India. The plant specimen collected was preserved as herbarium specimen in the CCK Herbarium having specimen number HCCK108 as reference material.

General Experimental Procedures

IR spectra were recorded with IR Affinity 1, Shimadzu, Japan. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-Avance III 400 MHz FT-NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The chemical shifts were expressed as δ ppm (parts per million) referring to internal standard, tetramethylsilane (Me_4Si). All the solvents, used in the study were purchased from Merck, Bengaluru, India and were of AR grade.

Methodology

Extraction and Isolation of Chemical Constituents

Dried pineapple peel powder (1 kg) was extracted continuously using soxhlet apparatus with petroleum ether followed by ethyl acetate, ethanol and water for 8 hours each. Removal of the solvent *in vacuo* using a Rotatory evaporator (Rotavapour) yielded 70 g of petroleum ether, 44 g of ethyl acetate, 80 g of ethanol and 68 g of aqueous extract. The ethyl acetate extract was subjected to silica gel (60–120 mesh and 100-200 mesh, Rankem) column chromatography (CC) for isolating the pure compounds. A gradient mixture of chloroform and ethyl acetate were used as mobile phase.

Analysis of Isolated Compound I

Purity of the isolated compound were tested by TLC, FT-IR, ^{1}H and ^{13}C NMR and LC-ESI-MS analysis data of isolated compound 1 were interpreted as described in results and discussion section.

Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) was performed using a commercially prepared TLC plate (Aluminium pre-coated TLC plate; $60F_{254}$ 20 × 20 cm, 0.2 mm thick; Merck, Germany). The mobile phase employed for TLC was ethyl acetate and chloroform in the ratio 9:1.

High-Performance Liquid Chromatography (HPLC) Analysis

The purity of the polyphenol compound extracted from ethyl acetate extracts of pineapple peel were analysed using a Prominence HPLC system. The isolated compound (1 mg/mL) is filtered through 0.45 µm PTFE filter; 20 µL was injected into the HPLC system. The analysis was performed on a prominence HPLC system containing LC-20AD system controller, phenomenex Gemini C18 column (250 × 4.6 mm, 5 µm), a column oven (CTO-20A), a Rheodyne injector (USA) with a loop of 20µl volume and a diode array detector (SPD-M20A). The mobile phase used was, solvent A; methanol- acetic acid – water (10:2:88, v/v) and solvent B; methanol – acetic acid – water (90:2:8, v/v) with gradient program 0–15 minutes 15% B, 16-20 minutes 50% B, 21-35min 70% B, 36–50 minutes 100% B and finally the column was regenerated in 10min. The injection volume was 20 µL, and the flow rate was kept at 1 mL/min. Along with the isolated compound, std. ferulic acid at a 1 mg/mL concentration was also subjected to HPLC analysisin the above condition. The column was maintained at room temperature and eluted fractions were monitored at 280 nm. Sample peaks were identified by comparing with retention times of standard peaks. LC Lab solution software was used for data acquisition and analysis.

LC (Liquid Chromatography) Conditions

The LC analysis was carried out with chromatographic separation achieved at 25°C on an AcquityUPLC BEH C18 column (50 mm×2.1 mm id, 1.7 m) using gradient elution of 0.1% formic acid in water (A) and acetonitrile (B) as mobile phase. The gradient elution was programmed as follows:0–0.70 min, 5–15% B; 0.7–2.5 min, 15–23% B; 2.5–2.8 min, 23– 33%B, 2.8–4.0 min, 33–40% B; 4.0–4.8 min, 40–95% B; 4.8–6.8 min,95– 95% B; 6.8–7.5 min, 95–5% B and finally, the initial conditions was held for 1.5 min for re-equilibration. The flow rate was kept at 0.3 mL/min throughout the analysis. The sample injection volume was 4 µL.

MS (Mass Spectrometry) Conditions

The detection of analytes was carried out in negative electrospray ionization (ESI) by precursor ion scanning (Q1 scan) in the range of m/z 100–1000. Simultaneous quantitative determination of analytes was performed using MRM acquisition mode at unit resolution. The compound dependent MRM parameters of precursor to product ion transition specific for each analyte including declustering potential (DP), entrance potential (EP), collision energy (CE) and cell exit potential (CXP) were optimized. The dwell time for analytes and the internal standard was set at 200 ms. The optimized ion source parameters were as follows: ion spray voltage, −4200 V; curtain (CUR) gas, 20 psi; nebulizer gas (GS1), 20 psi; heater gas (GS2), 20 psi; ion source temperature, 550°C; collision activated dissociation (CAD) gas, medium and the interface heater was on.

In-vitro Anti-inflammatory Activity Using the Isolated Phenolic Compound

Cell Culture

RAW 264.7 murine macrophage cells (ATCC, Rockville, MD, USA) were cultured in DMEM medium supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin sulfate (100 µg/mL) at 37°C.

Measurement of NO, PGE2, TNF-α, IL-1β and IL-6

1×10^6 cells/well density of macrophage cells were plated in a 12-well plate and incubated for 24 hours. The cells were treated with different doses of isolated phenolic acid for 1 hour, and then triggered with 1 µg/m LLPS for another 24 hours. Then, the cells were centrifuged at 2000 g for 10 minutes and stored at -80°C until used for analysis.

Estimation of Cellular Nitrite Levels

The level of nitrite level was estimated by the method of Lepoivre *et al*.,1990.[4] To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

Levels of Prostaglandin E_2 (PGE_2), TNF-α, IL-1β, IL-6 and Leukotriene C_4 (LTC_4) in cultured media were quantitated by ELISA, as per the manufacturer's protocols. Experimental sample was treated with three different concentrations of isolated compound. Indomethacin was used as reference compound.

Cultured THP1 Cell lines

THP1 (Human monocytic)cell lines cultured in RPMI 1640 [HIMEDIA] media supplemented with 10% heat-inactivated FBS, antibiotics (Penicillin and Streptomycin) and1.5% sodium bicarbonate. The media was filtered using 0.2 µm pore-sized cellulose acetate filter (Sartorius) in completely aseptic conditions. The cells were then grown till 60% confluency followed by activation with 1 µL LPS (1 µ/mL). LPS stimulated THP 1 cells were exposed with different concentrations of samples such as 25 µg/mL, 50 µg/mL, 75 µg/mL and 100 µg/mL.

The anti-inflammatory effects of samples were determined by assessing the inhibition of COX, LOX and Myeloperoxidase (MPOX) levels spectrophotometrically. The isolation was done by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200 µL of cell lysis buffer (1MTris HCl, 0.25M EDTA, 2 M NaCl, 0.5% Triton) was added. The incubation done for 30 minutes at 4°C and enzymes assay was done in pellet suspended in a small amount of supernatant.

Cyclooxygenase (COX) Activity

The COX activity was assayed by the method of Walker & Gierse, 2010.[5] The cell lysate was incubated in Tris-HCl buffer (pH 8), Glutathione 5 mM/L, and Hemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by adding Arachidonic acid 200 mM/L and terminated after 20 minutes incubation at 37°C, by adding 10% Trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 632 nm.

Lipoxygenase (LOX) Activity

The determination of LOX activity was carried out as per the method of Axelrod *et al*., 1981.[6] Briefly, the reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate and Sodium linoleate (200 µL). The LOX activity was monitored as an increase of absorbance at 234 nm, which reflects the formation of 5-hydroxyeicosatetraenoic acid. Percentage inhibition of the enzyme was calculated using the formula:

$$\%\ \text{inhibition} = \text{Absorbance of control} - \left(\frac{\text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100$$

Myleoperoxidase (MPO) Activity

Cell lysate was homogenized in a solution containing 50 mM Potassium Phosphate buffer and 0.57% Hexadecyl Trimethyl Ammonium Bromide (HTAB). Homogenized mixture was frozen in liquid nitrogen and thawed. After freeze thawing 3 times, the samples were centrifuged at 2000 g for 30 minutes at 4°C, and the supernatant was assayed for MPO activity. MPO in the sample was activated by adding 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL Guaiacol and 0.0005% H_2O_2. The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. One unit of MPO activity was defined as that degrading 1 µM of peroxide per minute at 25°C.[7]

Statistical Analysis

The values obtained are expressed as mean ± SEM. The data was analyzed by one-way variance (ANOVA) analysis, followed by Dunnett's post-test using Prism 5.0 (GraphPad Software, Inc., USA). P value less than 0.05 (P\0.05) was considered to be significant.

Results and Discussion

Isolation and Purification of a Polyphenol Compound from Pineapple Peel

For the isolation and purification of thecompounds from pineapple peel the ethyl acetate extract was purified via repeated column chromatography. 10 g of the extract was subjected to silica gel (300g, 100-200 mesh, Rankem) column chromatography for isolating the pure compounds. A gradient mixture of chloroform and ethyl acetate (EtOAc) were used as mobile phase for eluting the fractions. Initially 8 fractions were obtained on elution with 10% EtOAc. Based on TLC profile of the fractions they were clubbed as F1. From fraction 9 to 120, the mobile phase was gradually changed from 15% EtOAc to 80% EtOAc and the fractions were combined on the basis of their TLC profile. From the fraction 121 at 85% EtOAc compound 1 started to elute and it was monitored by TLC profile of the fractions collected. The mobile phase employed for TLC was a gradient mixture of chloroform and ethyl acetate (1:9). The isolated compound 1 showed an intense band at a R_f of 0.48 in the above solvent system along with few other bands. The fractions 121 to 180 (1.2 g) were combined to together and it was re-chromatographed over silica gel (30 g, 200-400 mesh,Rankem) for further purification using a gradient mixture of chloroform and methanol as solvent system. The compound 1 was obtained in a purified form as a yellow coloured liquid, from fractions from 11 to 48. It showed a single spot on TLC in (Chloroform, 9:1, EtOAc) solvent system with a R_f value 0.48 The compound 1 was tentatively confirmed as ferulic with by doing a Co-TLC with std ferulic acid and extract in the solvent system and comparing the measured R_f value with that of the previously reported standard values.[8]The residual weight of the compound 1 (40 mg) was also noted.

Spectral Analysis of Compound 1

In order to characterize and elucidate the structure of the compound 1 various spectral analysis such as FT-IR, ^1H and ^{13}C NMR, LC-MS and HPLC analysis were carried out.

FT-IR (Fourier Transform Infrared) Spectroscopy

FT-IR spectroscopy was used for the characterization of functional groups in the phenolic compound. The FT-IR analysis of the purified compound 1 from pineapple peel showed two absorption bands at 3605 and 3584 cm^{-1} (Fig. 1). The sharp IR spectral band at 3605 and 3584 cm^{-1} refers to stretching vibrations of OH groups such as phenols. The FT-IR spectrum of compound 1 confirmed the presence of polyphenol namely ferulic acid.[9] Some researches have reported that FT-IR spectrum of ferulic acid in *Parthenium hysterophorus* L. FT-IR spectroscopy was used to characterize the ferulic acid isolated from the brewery spent grains.[10]Infrared spectrum analysis reflecting the panorama of chemical constituents in a complex system is the most credible method to validate and identify the compounds used in traditional and herbal medicine.[11] FT-IR analysis in *Spilanthus acmella*, an acutely threatened medicinal plant revealed the presence of phenols and other organic constituents.[12] Research in food technology, pharmaceutical and medicinal studies utilizing FT-IR for the analysis of bioactive compounds.

NMR Analysis of Compound 1

Further the structure elucidation by ^1H NMR spectrum of compound 1 displayed peaks at δ12.13 ppm revealing a COOH group in the compound. The OH groupis found to resonate at δ 9.55 ppm. The signal corresponding to the aromatic protons resonated in the range of δ 6.35-7.52 ppm. A singlet at δ 3.82 ppm confirms the presence of a methoxy (OCH_3) group. Doublet peaks δ 6.37 ppm and δ 7.08 ppm shows the presence of an alkene side chain (CH=CH) (Fig. 2).

^1H NMR of compound 1: ^1H NMR (400 MHz, DMSO-D6) δ = 12.13 (brs, 1H, COOH), 9.55 (s, 1H, OH), 7.49 (d, J = 15.89 Hz, 1H), 7.29 (s, 1H), 7.09 (d, J = 8.18 Hz, 1H), 6.79 (d, J = 8.11 Hz, 1H), 6.37 (d, J = 15.91 Hz, 1H), 3.82 (s, 3H, O-CH_3,) ppm.

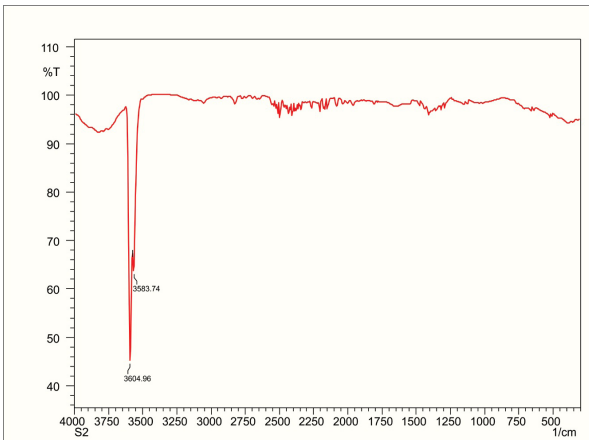

Fig. 1: IR spectrum of Compound 1

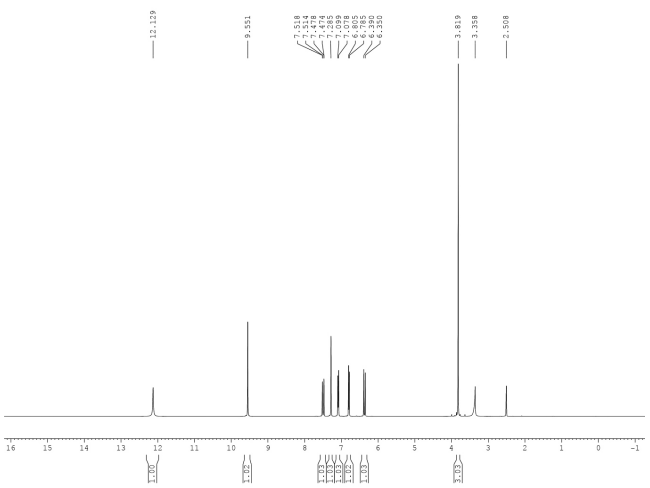

Fig. 2: ^{1}H-NMR spectrum of Compound 1

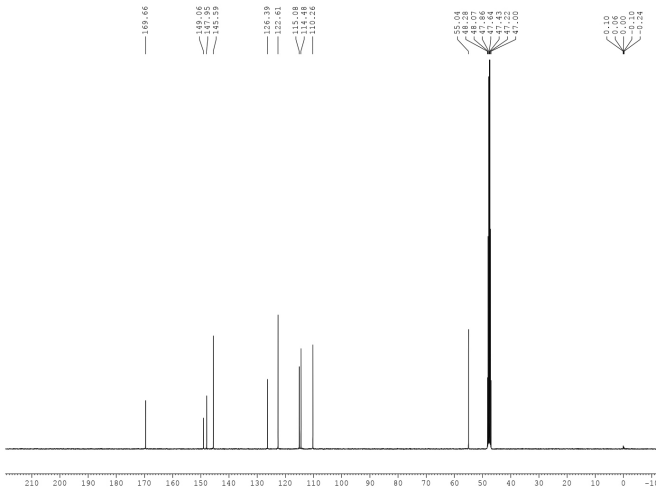

Fig. 3: ^{13}C-NMR spectrum of compound 1

The ^{1}H NMR data is in agreement with that ofthe polyphenol namely ferulic acid. NMR analysis of extracts prepared from freeze-dried fruits led to the identification of more than 40 compounds and this formed the basis for a quantitative analysis of the unintended metabolic consequences of the transformation.[13] In addition to ^{1}H NMR, carbon-13 NMR spectrum of compound 1 showed carbonyl peak corresponding to -COOH group at δ 169.6 ppm. A peak at δ 55.0 ppm confirmed the presence of OCH_3 group at 4' position. Further two peaks at δ 145.6 and 115.1 ppm were observed corresponding to acetylenic carbons at 1' and 2' positions. A total of 10 signals (6 aromatic carbon and 4 aliphatic chain) were observed in the ^{13}C-NMR spectrum (Fig. 3).

The spectral values were in agreement with the reported values corresponding to Ferulic acid (Fig. 4). Hence, the structure of compound 1 is confirmed as Ferulic acid by comparison of their NMR spectral data with those reported in the literature.

Ferulic acidisolated from *P. hysterophorus* L. and its structural characterization using ^{1}H NMR and ^{13}C-NMR were carried out.[14,15] isolated ferulic acid from aerial parts of *Kelussia odoratissima* and its structure elucidation done using ^{13}CNMR.

HPLC (High Performance Liquid Chromatography) Analysis

The polyphenol compound isolated from ethyl acetate extract of pineapple peel was analyzed using high-performance liquid chromatography. The HPLC analysis of compound 1 (Ferulic acid) showed only one peak corresponding to the retention time 28.653 min. On comparison with HPLC profile of 13 std. phenolic acids the isolated compound 1 was found to match with the profile of std. ferulic acid (Fig. 5). The results of HPLC analysis completely matched with the spectral analysis of the compound 1 and the compound 1 is confirmed as ferulic acid. Further HPLC analysis of pineapple peel extract

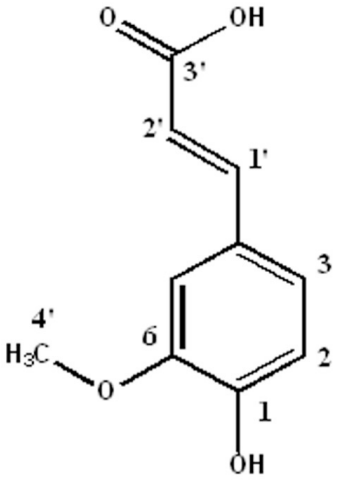

Fig. 4: Structure of Ferulic acid (Compound 1)

sample compared with isolated ferulic acid confirmed that ferulic acid is distributed in all the sample of pineapple peel extracts.

Ferulic acid is well-known for its physiological functions such as anti-microbial, anti-inflammatory and anti-cancer activities. It lowers cholesterol level in serum and increases sperm viability. It also acts as a natural protector against ultra-violet radiation known to cause skin disorders such as cancer.[16] The HPLC analysis of the algae *Amphiroa anceps* resulted in polyphenolic compounds found to be an effective source of antioxidants that may have potential applications in the food industries.[17] HPLC currently represents the most popular and reliable technique for the analysis of phenolic acids. Ferulic acid isolated from cereal extracts using reverse-phase HPLC with a C-18 stationary-phase column coupled with diode array detector.[18,19] Further, we extracted the highest amount of ferulic acid from maize bran by HPLC method.

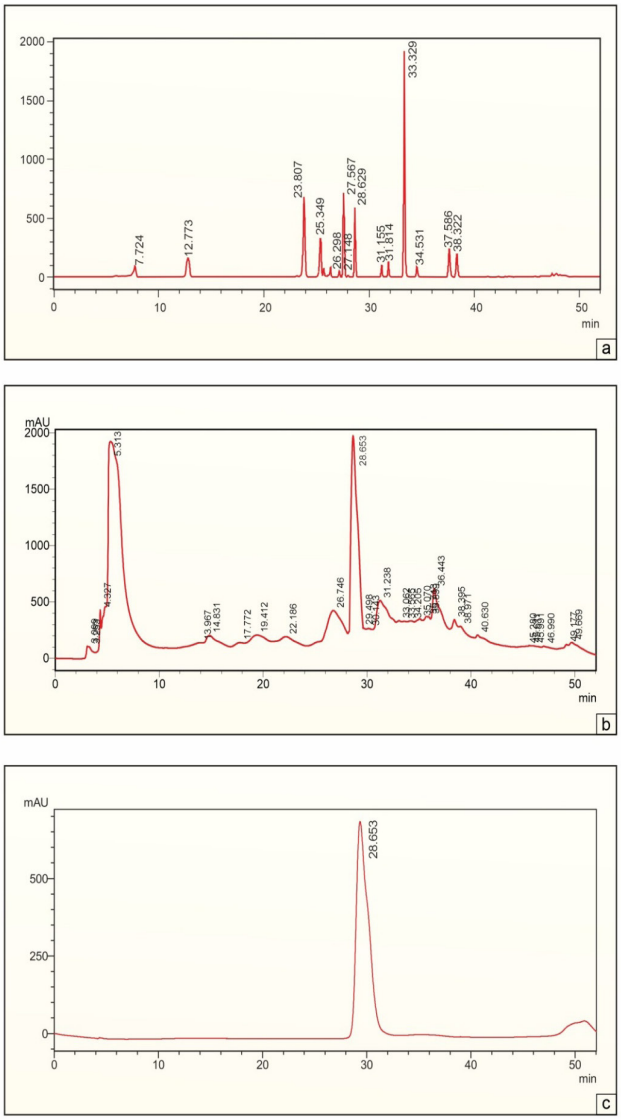

Fig. 5: (a) HPLC chromatogram of 13 std. phenolic acids; Ferulic acid at 28.629 min, (b) HPLC chromatogram of ethyl acetate extract of pineapple peel; Ferulic acid at 28.653 min (c) HPLC chromatogram of isolated Ferulic acid

LC-MS Identification of Compound 1

The combination of LCMS (Liquid chromatography mass spectrometry) facilitates accurate fractionation and identification of phenolic compounds.Mass spectrometry provides abundant information for elucidating the structure of the compounds. Electrospray ionization (ESI) is a preferred source due to its high ionization efficiency for phenolic compounds. The identity of the compound 1 was determined as Ferulic acid with their retention time and MS spectra of the reference standards. The structure of compound 1 obtained by NMR spectrum was supported by the ESI-MS data which revealed a parent peak having m/z 193.2 (M-Hpeak) corresponds to the compound ferulic acid (molecular formula $C_{10}H_{10}O_4$) after comparing with MS library (Fig. 6).

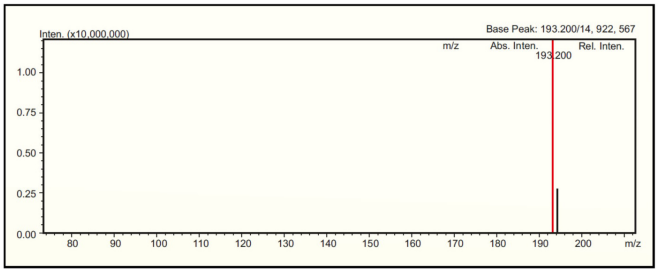

Fig. 6: ESI-MS mass spectrum of compound 1

Using mass spectrometry, relative molecular mass (molecular weight) can be determined with high accuracy and an exact molecular formula can be also known.[20]

RAW Cell Line

Cytokines like TNFα, IL-6, IL-1β along with PGE_2 and TLC_4 play important roles in the regulation of inflammatory responses by controlling proliferation, differentiation, and effective function of immune cells. Nitric Oxide is a gas with little half-life, also a crucial mediator leading to inflammatory response and generated *via* the oxidation of the terminal guanidine nitrogen atom of L-arginine by Nitric Oxide Synthase(NOS). RAW cell lines induced with LPS showed increased production of these cytokines and the treatment with Ferulic acid effectively lowered the levels of inflammatory cytokines which is evident from the ELISA analysis of cell exudates. LPS challenge significantly increased NOS activity as evident from the high level of NO in the toxin group (43.56 ± 3.84 µM/mL) and administration of FER (100 µg/mL) reduced it to 12.08 ± 1.87 µM/mL which is almost comparable to the values obtained for the standard Indomethacin group as shown in (Fig. 7). The expression of cytokines like TNF*α*, IL-1*β*& IL-6 in LPS induced toxin group was significantly increased to 27.12 ± 2.34 ng/mL, 84.73 ± 4.28 ng/mL and 112.89 ± 5.38 pg/mL, when compared with the normal control. The administration of higher dose of FER at 100 µg/mL reduced the level of these cytokines to 10.27 ± 1.63 ng/mL, 35.41 ± 2.03 ng/mL and 31.62 ± 2.24 pg/mL which is comparable to the standard Indomethacin treated group as shown in the (Fig. 8) PGE_2 & TLC_4 levels in toxin group were elevated to 275.67 ± 12.35 pg/mL and 80.92 ± 3.26 pg/mL when compared to the normal control group. Treatment with various doses of FER reduced the levels of PGE_2& TLC_4 in a dose dependent manner. FER (100 µg/mL) was found to be the most effective does, which reduced the levels of these inflammatory factors to 60.25 ± 5.19 pg/mL and 23.62 ± 1.18 pg/mL, respectively. The results obtained for the higher dose of FER at 100 µg/mL in all the parameter studied were almost comparable to the reference standard Indomethacin.

Phenolic compounds are plant secondary metabolites known for their anti-oxidative and anti-inflammatory properties.[21,22] It was then, reported that the phenolic compounds extracted from *Nymphaea mexicana* showed

an anti-inflammatory property towards induced RAW 264.7 macrophage cells. It has been suggested that the anti-inflammatory property of the phenolic compounds was due to their scavenging activities on the reactive oxygen species or reactive nitrogen species.[23,24] Some researchers have studied the role of quercetin to exhibit anti-inflammatory effects through regulation of nitric oxide and TNFα production in LPS-stimulated macrophages. The effect of ellagic acid on inflammation also has been studied using *in vitro* and *in vivo* models.[25] Pro-inflammatory cytokines such as TNFα, IL-6, IL-1β and also prostaglandin E2 and leukotriene C_4 are produced and play critical roles in the inflammation processes. Among anti-inflammatory cytokines, TNFα has been highlighted as a main mediator in the inflammatory diseases mechanism.[26] High levels of pro-inflammatory cytokines, including TNFα have been detected in psoriatic skin lesions and joints of patients with the inflammatory disease.[27] TNFα may have a significant role in pathogenesis of several inflammatory diseases.

Upon treatment with LPS, RAW 264.7 cells produce induced NO and with prostaglandin E_2 as well as other inflammatory cytokines.[28] NO is an important inflammatory product produced by NOS under physiological and pathophysiological conditions primarily involved in promoting inflammatory responses. NO production by immune cells has been used as an indicator of the presence and extent of inflammation. The isolated ferulic acid (FA) from pineapple waste reduces NO accumulation and also pro-inflammatory cytokines namely TNFα, IL-6, IL-1β along with prostaglandin E_2 and leukotriene C_4in the culture medium of LPS induced macrophage cells. Decrease in the pro cytokinin, prostaglandin E_2 and leukotriene C_4levels by FER suggests that it may be useful in a variety of inflammatory conditions.

THP-1 Cell Line

When THP-1 cells are stimulated with LPS, the pro inflammatory mediators such as COX, LOX and MPO are markedly up-regulated. The present study clearly

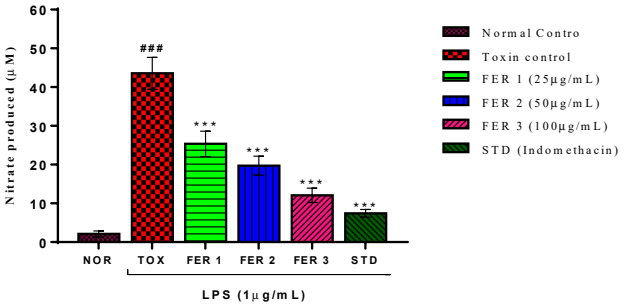

Fig. 7: The effect of Ferulic acid on NO production in LPS stimulated RAW 264.7 macrophage cells. The values are expressed as mean ± SEM, n=4, as one way ANOVA followed by Dunnett's multiple comparison test, ###p < 0.001, the LPS-treated group versus the control group; ***p < 0.001, the Ferulic acid treated group significantly different from LPS-treated group.

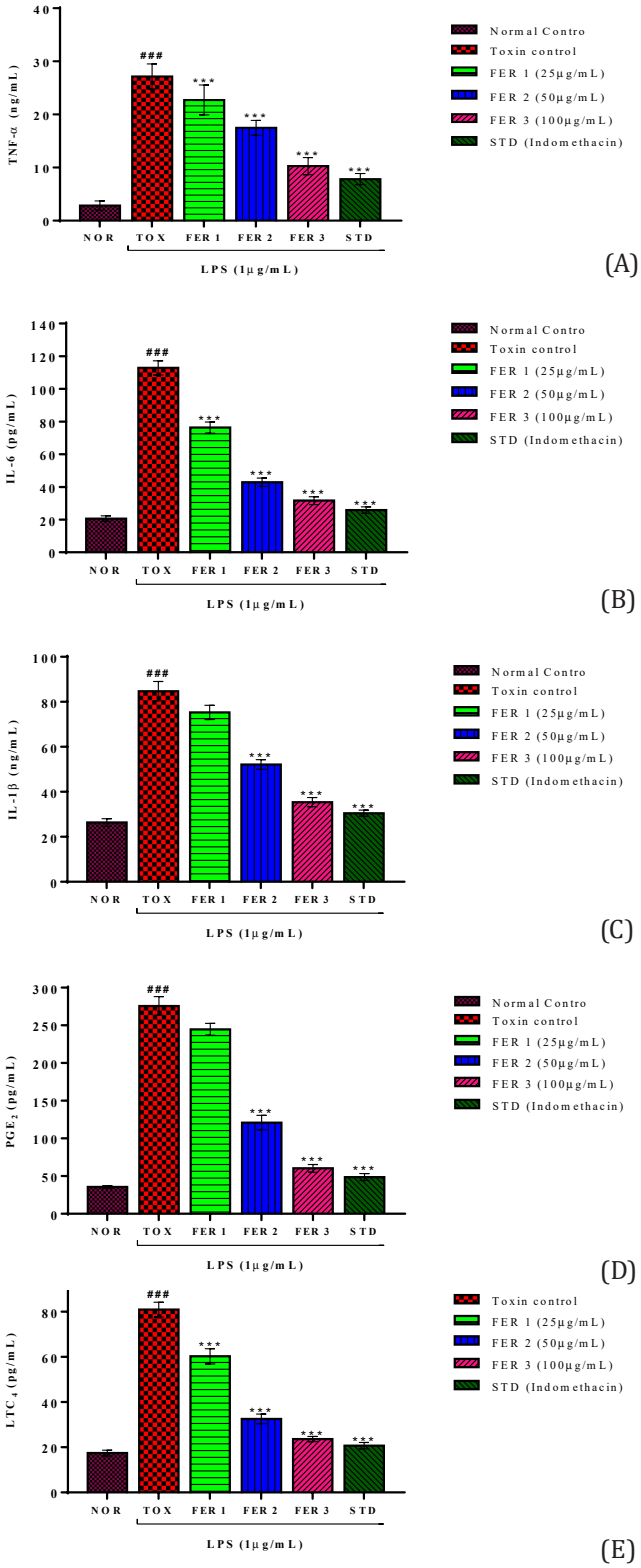

Fig.8: The effect of Ferulic acid on (a). TNF-α, (b). IL-6, (c). IL-1β, (d). PGE_2& (e). TLC_4 production in LPS stimulated RAW 264.7 macrophage cells. The values are expressed as mean ± SEM, n=4, one way ANOVA followed by Dunnett's multiple comparison test, ###p < 0.001, the LPS-treated group versus the control group; ***p < 0.001, the Ferulic acid treated group significantly different from LPS-treated group.

demonstrated that Ferulic acid significantly reduced the production of inflammatory enzymes like COX, LOX and MPO in LPS induced THP-1 cell lines without cytotoxicity at all tested concentrations. The isolated compound-Ferulic acid (FER) showed 78.47% of COX inhibition at (100 µg/mL) and the standard Indomethacin showed 87.91% of inhibition at the highest concentration used. The IC_{50} obtained was 44.92 µg/mL for FER and 12.52 µg/mL for the reference standard as shown in the (Fig. 9) The LOX activity in LPS stimulated THP-1 cells was inhibited to 78.63% by FER and 90.42% by Standard drug at 100 µg/mL. The IC_{50} obtained against LOX inhibition was 46.62 µg/mL and 8.69 µg/mL for FER and Indomethacin respectively. Different concentration of FER effectively decreased the MPO levels in a dose dependent manner. FER at 100 µg/mL showed maximum activity by reducing MPO production to 0.0012 U/mL and the value obtained for the standard drug was 0.000412U/mL which is almost comparable.

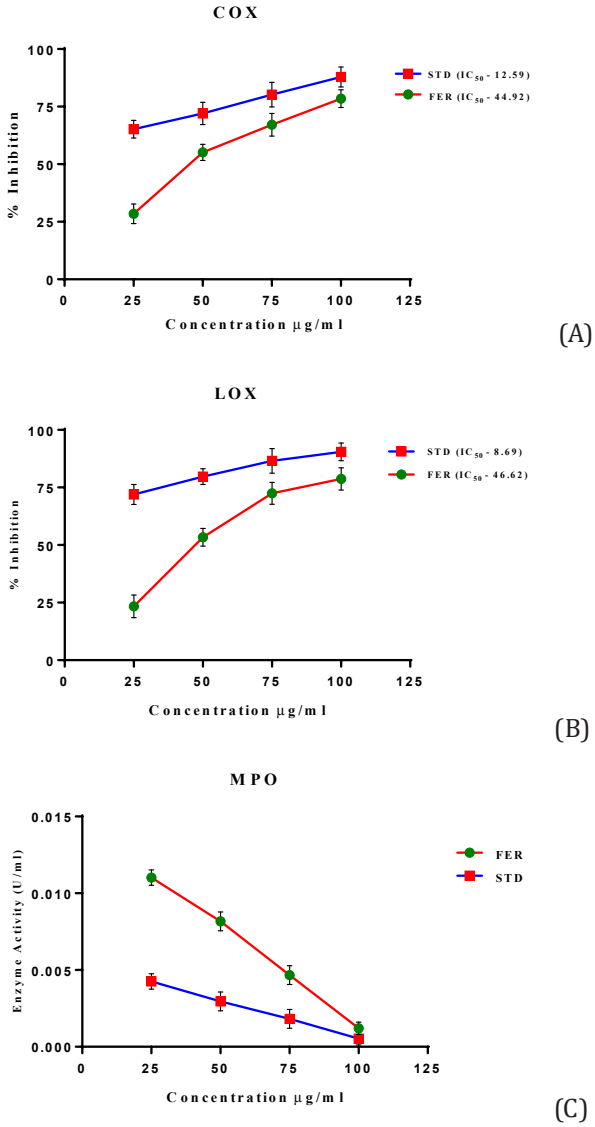

Fig. 9: The effect of Ferulic acid on (a). COX, (b). LOX and (c). MPO production in LPS stimulated THP-1 cell lines. The values are expressed as mean ± SEM, n=3

LOX is the first indexed enzyme involved in the arachidonic acid pathway produce leukotrienes and its expression connected with inflammation. LOX inhibitors are prompted as promising molecules for the treatment of inflammatory disorders on the basis of their role in LOX pathways.[29] proved that *Premna integrifolia* extracts act as dual inhibitors of COX-2/5-LOX and thereby effective against inflammation and also as immune modulators. The present results showed that FER inhibited COX and LOX enzyme activity effectively in a concentration dependent manner. In the inflammatory reactions, oxidative stress is triggered by phagocytes containing MPOX, and these uplift ROS synthesis and down regulates oxidative defense.[30] revealed that in *Syzygium aromaticum* aqueous extract inhibits MPOX and protects mice from LPS-induced lung inflammation. As an important indicator of neutrophil leaching, MPOX activity should be regulated during treatments with secondary metabolites. Here the protective effect of the isolated phenolic compound, FER balancing oxidative stress via inhibiting the MPOX synthesis.

Conclusion

The present study revealed that pineapple peel is a potential source of the bioactive phenolic compound Ferulic acid. It was isolated by column chromatography of ethyl acetate extract of pineapple peel. The structure of the compound was confirmed through FT-IR, LC-MS, HPLC, ^{1}H-NMR and ^{13}C NMR spectra followed by the evaluation of *in vitro* anti-inflammatory potential of the isolated compound. Anti-inflammatory potential of the ferulic acid was evaluated by estimation of pro-inflammatory enzymes such as cyclooxygenase (COX), lipooxygenase (LOX), and myleoperoxidase (MPOX) on THP-1 cells and pro-inflammatory cytokines namely TNFα, IL-6, IL-1β along with prostaglandin E_2 and leukotriene C_4on Macrophage RAW 264.7 cell lines stimulated with LPS. FER exhibited significant inhibition activity of COX (78.47%) and LOX (78.63%) in LPS stimulated THP-1 cells at 100 µg/mL. Varied concentration of FER effectively reduced the MPOX (92.76%) level in a dose dependent manner. The isolated ferulic acid was also able to inhibit the production of NO, PGE2, TLC4 and pro-inflammatory cytokines such as TNFα, IL-6, IL-1β whichin turn revealed that Ferulic acid could be considered as a potential anti-inflammatory agent which in turn pointing out the therapeutic potential of this non-edible under explored material.

Further studies are warranted to analyze the *in vivo* anti-inflammatory and antimicrobial potentialities of the isolated polyphenol to study its functional role(s). Clinical study is also warranted to support traditional use of this formulation for various ailments.

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References

1. Russell W, Duthie G. Plant secondary metabolites and gut health: The case for phenolic acids. Proceedings of the Nutrition Society. 2011; 70:389-396.
2. Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. Resources and biological activities of natural polyphenols. Nutrients. 2014; 6:6020-6047.
3. Tropea A, Wilson D, La Torre LG, Curto RB, Saugman P, Troy-Davies P, Dugo G, Waldron KW. Bioethanol production from pineapple wastes. Journal of Food Research. 2014; 3:60-70.
4. Lepoivre M, Chenais B, Yapo A, Lemaire G, Thelander L, Tenu JP. Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. Journal of Biological Chemistry. 1990; 265:14143-14149.
5. Walker MC, Gierse JK. In vitro assays for cyclooxygenase activity and inhibitor characterization. In: Ayoub SS, Flower RJ, Seed MP (Eds). Cyclooxygenases-Methods and Protocols. United States: Humana Press; 2010. p. [131-144].
6. Axelrod B, Cheesbrough TM, Laakso S. [53] Lipoxygenase from soybeans: EC 1.13. 11.12 Linoleate: oxygen oxidoreductase. In: Lowenstein JM (Ed). Methods in enzymology. United States: Academic Press; 1981 p. [441-451].
7. Pulli B, Ali M, Forghani R, Schob S, Hsieh KL, Wojtkiewicz G, Linnoila JJ, Chen JW. Measuring myeloperoxidase activity in biological samples. PloS one. 2013; 8:e67976.
8. Touchstone JC. Practice of thin layer chromatography. 3rd Ed. New York. John Wiley & Sons; 1992.
9. Kumar N, Pruthi V. Potential applications of ferulic acid from natural sources. Biotechnology Reports. 2014; 4:86-93.
10. Anuradha V, Srinivas PV, Rao RR, Manjulatha K, Purohit MG, Rao JM. Isolation and synthesis of analgesic and anti-inflammatory compounds from *Ochna squarrosa* L. Bioorganic & Medicinal Chemistry. 2006;14:6820-6826.
11. Hirun N, Dokmaisrijan S, Tantishaiyakul V. Experimental FTIR and theoretical studies of gallic acid-acetonitrile clusters. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012; 86:93-100.
12. Preetha TS, Mohan SN, Deepthi SR. Chemical Fingerprinting of *Spilanthes acmella* L. (Murr.)-An Acutely Threatened Medicinal Plant of Pharmaceutical Importance by HPLC, FTIR and UV-VIS Spectroscopic Tools. World Journal of Pharmacy and Pharmaceutical Science. 2014; 3:1275-1287.
13. Le Gall G, Colquhoun IJ, Davis AL, Collins GJ, Verhoeyen ME. Metabolite profiling of tomato (*Lycopersicon esculentum*) using ^{1}H NMR spectroscopy as a tool to detect potential unintended effects following a genetic modification. Journal of Agricultural and Food Chemistry. 2003; 51:2447-2456.
14. Kumar N, Pruthi V. Structural elucidation and molecular docking of ferulic acid from *Parthenium hysterophorus* possessing COX-2 inhibition activity. 3 Biotech. 2015; 5:541-551.
15. Sajjadi SE, Shokoohinia Y, Moayedi NS. Isolation and Identification of Ferulic Acid from aerial parts of *Kelussia odoratissima* Mozaff. Jundishapur Journal of Natural Pharmaceutical Products. 2012; 7:159-162.
16. Asif M, Khodadadi E. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. Archives of Advances in Biosciences. 2013; 4:119-138.
17. Nagai T, Yukimoto T. Preparation and functional properties of beverages made from sea algae. Food chemistry. 2003; 81:327-332.
18. Benoit I, Navarro D, Marnet N, Rakotomanomana N, Lesage-Meessen L, Sigoillot JC, Asther M, Asther M. Feruloyl esterases as a tool for the release of phenolic compounds from agro-industrial by-products. Carbohydrate Research. 2006; 341:1820-1827.
19. Tilay A, Bule M, Kishenkumar J, Annapure U. Preparation of ferulic acid from agricultural wastes: Its improved extraction and purification. Journal of Agricultural and Food Chemistry. 2008; 56: 7644-7648.
20. Christophoridou S, Dais P, Tseng LH, Spraul M. Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with post column solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). Journal of Agricultural and Food Chemistry. 2005; 53:4667-4679.
21. Dussossoy E, Brat P, Bony E, Boudard F, Poucheret P, Mertz C, Giaimis J, Michel A. Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican Noni juice (*Morinda citrifolia* L.). Journal of ethnopharmacology. 2011; 133:108-115.
22. Hsu CL, Fang SC, Yen GC. Anti-inflammatory effects of phenolic compounds isolated from the flowers of *Nymphaea mexicana* Zucc. Food and function. 2013; 4:1216-1222.
23. Sergent T, Piront N, Meurice J, Toussaint O, Schneider YJ. Anti-inflammatory effects of dietary phenolic compounds in an *in vitro* model of inflamed human intestinal epithelium. Chemico-Biological Interactions. 2010; 188:659-667.
24. Nakamura M, Omura S. Quercetin regulates the inhibitory effect of monoclonal non-specific suppressor factor β on tumor necrosis factor-α production in LPS-stimulated macrophages. Bioscience, Biotechnology and Biochemistry. 2008; 72:1915-1920.
25. Anitha P, Priyadarsini RV, Kavitha K, Thiyagarajan P, Nagini S. Ellagic acid coordinately attenuates Wnt/β-catenin and NF-κB signaling pathways to induce intrinsic apoptosis in an animal model of oral oncogenesis. European Journal of Nutrition. 2013; 52: 75-84.
26. Maxia A, Sanna C, Frau MA, Piras A, Karchuli MS, Kasture V. Anti-inflammatory Activity of *Pistacia lentiscus* essential oil: Involvement of IL-6 and TNF-α. Natural Product Communications. 2011; 6:1543-1544.
27. Mease PJ. Tumour necrosis factor (TNF) in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors. Annals of the Rheumatic Diseases. 2002; 61:298-304.
28. Zhao BT, Hung ND, Lee JH, Min BS, Woo MH. Lignan derivatives from *Selaginella tamariscina* and their nitric oxide inhibitory effects in LPS-stimulated RAW 264.7 cells. Bioorganic and Medicinal Chemistry letters. 2017; 27:524-529.
29. Deepa J, Aleykutty NA, Jyoti H. Comparative evaluation of *in vitro* anti-inflammatory activity of *Psidium guajava* and *Syzygium cumini* leaves. International Journal of Ayurveda and Pharma Research. 2017; 5:33-41.
30. Tian R, Ding Y, Peng YY, Lu N. Inhibition of myeloperoxidase-and neutrophil-mediated hypochlorous acid formation in vitro and endothelial cell injury by (–)-epigallocatechin gallate. Journal of Agricultural and Food chemistry. 2017; 65:3198-3203

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