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ORIGINAL ARTICLE

Inhibition of proinflammatory pathways by bioactive fraction of *Tinospora cordifolia*

Jenny Jacob[1] · Bashi M. Babu[1] · Mohind C. Mohan[1] · A. P. Abhimannue[1] · B. Prakash Kumar[1]

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Abstract *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thomson, a known immunomodulatory agent extensively used in ayurveda, has not been effectively validated for the mechanisms involved in immunomodulation and the identification of the active principles. The bioactive fraction of *T. cordifolia* (TBF) in methanol was used for nitric oxide (NO) radical scavenging activity, lipoxygenase (LOX) and cyclooxygenase (COX) dual inhibition and cytotoxicity studies. Production of the proinflammatory cytokines, tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in dendritic cell (DC) suspensions treated with lipopolysaccharide (LPS) was also studied. The bioactive principles involved were identified with ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometric (UPLC-Q-ToF MS/MS) system. The results indicate significantly higher potency of TBF as compared to positive standards for LOX/COX inhibition with moderate NO radical scavenging activity and the fraction was also found to be non-cytotoxic to monocyte cells. A significant inhibition was also observed in TNF-α and IL-1β production in LPS-treated DC suspensions as compared to standards, rolipram and dexamethasone, respectively. 11 compounds were identified from TBF by MS/MS system. The potent inhibition of LOX and COX enzymes with moderate NO scavenging was indicative of a free radical scavenging-independent mechanism of immunomodulation. Further investigations into the active principles identified would result in the development of lead candidates with potent therapeutic implications.

Keywords Cytokines · TNF-α · *Tinospora cordifolia* · Medicinal plants · Nitric oxide · Immunomodulation · LOX · COX · IL-1β

Introduction

Immunomodulation is the modification of the immune response brought about by therapeutic mechanisms and includes regulation of cytokine production and the primary cellular defence system. Proinflammatory cytokines function in the initiation of immune response and the activation of nociceptive sensory neurons (Zhang and An 2007) and includes majorly IL-1, IL-6 and particularly TNF (Dinarello 2000). Cellular therapies in immunomodulation include the manipulation of monocytes and dendritic cells to provide basic defence for the human system. Monocytes differentiate into macrophages in tissues with exposure to antigens and act as the first line of defence against any infection or inflammation of the human system. The dendritic cells are central to the immune system in determining the differentiation of T cells to produce a T helper cells (T_H1 or T_H2) immune response (Steinman 1991). Dendritic cells have been the focus of immunotherapy on the basis of two strategies where the cells are either directly targeted to dendritic cells or are reintroduced after they are preprimed (Caminschi et al. 2009).

Immunomodulatory drugs are particularly involved in the modulation of the immune response by regulating the type, duration or magnitude of the immune response.

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✉ B. Prakash Kumar
prakashkumar@mgu.ac.in

[1] School of Bioscience, Mahatma Gandhi University, Priyadarsini Hills, Kottayam, Kerala 686560, India

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Currently used immunomodulators include interferons in multiple sclerosis, monoclonal antibodies in rheumatoid arthritis and cytostatics like azathioprine. These drugs show partial effectiveness in disease control but have several adverse effects on prolonged usage including reactivation of latent tuberculosis, chronic liver diseases, pancreatitis, and general susceptibility to infections. Ayurveda has been the major force behind the use of medicinal plants as effective immunomodulators. The therapeutic strategy behind these herbal drugs is the enhancement of the person's natural defence towards the cause of infection rather than action on the infectious agent directly (Singh and Deepa 2015). These mechanisms of immunomodulation acquire significance with the use of micro and nanodoses of pharmaceutical preparations to modulate the immune system without creating a sudden and massive effect on the regulation of immune response. Such immunomodulatory herbs are more appropriately termed as rasayanas in ayurveda. *T. cordifolia* is one such immunomodulator which has been an integral part of ayurvedic drugs.

Immunomodulatory effects of *T. cordifolia* were reported in its polysaccharide fractions having arabinogalactan content which contributed to the in vitro mitogenic stimulation of mice spleen lymphocytes (Verma et al. 2006). The exact mechanisms of significant immunomodulatory and adaptogenic activities of *T. cordifolia* have not been studied in detail. The focus of the present study is to find the effect of *T. cordifolia* on cytokine production in lipopolysaccharide (LPS)-stimulated monocytes and dendritic cells. The anti-inflammatory activity of *T. cordifolia* on the production of eicosanoids, lipid mediators of inflammation has also been evaluated. NO scavenging experiments are more relevant in chronic inflammatory conditions in biological systems because increased levels of nitric oxide can increase mortality (Broderick et al. 2006) through genotoxicity and deaminate DNA affecting DNA repair proteins. An attempt was also made into identifying the active constituents of the bioactive fraction (TBF) of *T. cordifolia* using hybrid mass spectrometry and fractionation of compounds.

Materials and methods

Materials and chemicals

The chemicals used in the study are analytical grade reagents from either HiMedia laboratories, India or from Merck Chemicals, India unless specified otherwise. The reagents for mass spectrometric studies were of LC/MS grade from Fischer Scientific, India. Consumables used for dendritic cell culture were procured from Thermo Fisher Scientific Inc., USA.

Preparation of bioactive fraction of *T. cordifolia* methanol extract

Fresh stems of *T. cordifolia* (Willd.) Miers ex Hook. f. & Thomson were collected from Kottayam, Kerala in March 2010 and identified by Dr. Jomy Augustine of St. Thomas College, Palai, Kottayam. A voucher specimen of the plant was deposited at the Kerala Forest Research Institute Herbarium with voucher no: JA 17151. The dried powder of the stems of *T. cordifolia* was initially defatted with hexane, extracted with methanol and fractionated as described previously (Jacob et al. 2014) and the fraction with highest activity against LOX enzymes was selected for further studies (TBF). The TBF sample was filtered for possible interference with endotoxins using the endotoxin removing columns (Thermo Fisher Scientific Inc., USA) and stored frozen immediately.

Scavenging of nitric oxide (NO) radical

NO radical scavenging was carried out as per the protocol described by Tsai et al. (2007). The reaction mixture of different concentrations of TBF and sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was incubated at 25 °C for 60 min and aliquots were treated with Greiss reagent containing sulphanilamide (1%), phosphoric acid (2%) and naphthylethylenediamine dihydrochloride (NEDD) (HiMedia Laboratories) (0.1%). The absorbance of the chromophore was read at 546 nm and referred to the positive standard ascorbic acid (Merck India). A control in the absence of plant extract and a blank of solvent was also treated similarly. The percentage of NO scavenging of the plant extracts was calculated as per the following equation:

$$\text{NO scavenging}(\%) = \frac{[\text{Absorbance of control} - \text{Absorbance of TBF}] \times 100}{\text{Absorbance of control}}.$$

LOX inhibition assay

LOX inhibition assay was carried out as described earlier (Jacob and Kumar 2013) for 5-LOX, 12-LOX and 15-LOX enzymes (Cayman Chemicals, USA) with sodium linoleate (HiMedia Laboratories, India) as substrate and Nordihydroguaiaretic acid (NDGA) (Cayman Chemicals, USA) as positive standard. A blank with methanol and a control without plant extract were also prepared and at least three independent experiments conducted. The reaction rate at 560 nm was determined using the chromogen extinction coefficient of 9.47 mM^{-1} adjusted for the path length. One

unit of enzyme utilises one μmol of linoleic acid per minute at 25 °C.

$$\text{LOX activity } (\mu\text{mol/min/ml}) = \frac{[\text{Absorbance}_{560}(\text{TBF}) - \text{Absorbance}_{560}(\text{blank})] \times \text{dilution}}{9.47\,\text{mM}^{-1} \times \text{Time of incubation}},$$

$$\text{Percent of inhibition}(\%) = \frac{(\text{EA of } C - \text{EA of } B) - (\text{EA of TBF} - \text{EA of } B)}{(\text{EA of } C - \text{EA of } B)} \times 100,$$

where EA stands for enzyme activity, C for control and B for blank.

COX inhibition: tetra methyl-*p*-phenylenediamine (TMPD) assay

The COX-TMPD assay (Copeland et al. 1994) measures the peroxidative function of COX enzymes, COX-1 and COX-2 (Cayman Chemicals, USA) and was compared with the positive standard, Indomethacin (HiMedia Laboratories, India). The assay mixture contained Tris–HCl buffer (100 mM, pH 8.0), Hemin (15 μM), EDTA (3 μM), enzyme and TBF preincubated at 25 °C for 15 min and initiated by the addition of arachidonic acid (Cayman Chemicals, USA) (100 μM) substrate and TMPD in total volume of 1 mL. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25 s of the reaction, following the increase in absorbance at 603 nm. The COX activity was calculated from the reaction rate at 603 nm using the TMPD extinction coefficient of 0.00826 μM^{-1} adjusted to the path length. One unit of enzyme activity was defined as the amount of enzyme that causes the oxidation of 1.0 nmol of TMPD per minute at 25 °C. Prostaglandin G_2 (PGG_2) reduction to prostaglandin H_2 (PGH_2) requires the oxidation of two molecules of TMPD.

$$\text{COX activity (nmol/min/ml)} = \frac{\text{Absorbance}_{603}(\text{TBF}) - \text{Absorbance}_{603}(\text{blank}) \times \text{Dilution}}{0.0086\,\mu\text{M}^{-1} \times \text{Volume of COX} \times \text{Time of incubation}} \div 2.$$

Generation of dendritic cells

Fresh blood purchased from the blood bank, medical college, Kottayam was used for isolation of monocyte cells with density gradient lymphocyte separation medium, (HiSep LSM 1077) (HiMedia Laboratories, India). Dendritic cells (DC) were generated from the isolated monocyte cells using the DC generation medium (DCGM, PromoCell GmbH, Germany) as per the manufacturer's instructions. The DC cells were then harvested from the supernatant and resuspended. The cell suspension was checked for viability.

Cytotoxicity assay

The basal cytotoxicity of TBF was measured in isolated monocytes seeded at a density of 1×10^6 cells/mL in 96-well flat bottom culture plates and incubated for 1 h. The cells were then treated with TBF, diluted in 0.1% dimethyl sulphoxide (DMSO) and incubated for 2 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (HiMedia Laboratories, India) was then performed as per the manufacturers' instructions. The results were expressed as the survival rate index in percentage.

Production of proinflammatory cytokines (TNF-α and IL-1β) in LPS-stimulated DC

The dendritic cell suspension was seeded at a density of 5×10^5 cells/mL in 24-well plates with different concentrations of TBF and incubated for 2 h. The cells were then stimulated with 20 ng/mL LPS (*Escherichia coli)* (Sigma-Aldrich, India) concentrations for 16 h. Untreated cells were considered as control and medium was considered as blank. Supernatants were collected and assayed for TNF-α (BioLegend, San Diego, USA) and IL-1β (BioLegend, San Diego, USA) according to the manufacturers' instructions. Rolipram (Sigma-Aldrich, India) and Dexamethasone (Sigma-Aldrich, India) were used as positive standards for TNF-α and IL-1β, respectively. The data were plotted as absorbance versus log concentration using a 4-parameter logistic curve fit. The concentration of each sample was determined from standard curve with appropriate dilutions and used to calculate the percent inhibition as per the formula given below:

$$\text{Percent inhibition TNF}\alpha/\text{IL1}\beta\ (\%) = \frac{(\text{Activity of control} - \text{Activity of TBF})}{\text{Activity of control}} \times 100$$

Identification of bioactive compounds with UPLC-Q-ToF mass spectrometer

TBF was analysed for its bioactive constituents with the Waters Xevo G2 Q-ToF mass spectrometer with a Waters Acquity H class Ultra-Performance Liquid Chromatography (UPLC) system and an ACQUITY UPLC™ BEH C18 column (50 mm × 2.1 mm × 1.7 μm; Waters, Milford, MA, USA) at 30 °C. The mixture of (A) 0.1% formic acid in water and (B) acetonitrile was chosen as the mobile phase using a gradient program: 0–6 min: 10–95% B, 6–6.5 min: 95% B and 6.5–7.5 min: 95–10% B. The back pressure was set at 1500 psi, nebulizer gas, nitrogen at 900 L/h and temperature at 350 °C under positive ion

electrospray ionisation (ESI) modes. The cone gas (nitrogen) set at a flow rate of 50 L/h, and source temperature at 135 °C with capillary voltage at 3.5 kV and cone voltage at 35 V. The ToF data were collected between *m/z* 50 and *m/z* 1000 and triplicate MS/MS experiments were performed using a range of collision energies for fragment ion information through the MS^E centroid technology. The flow rate was 0.2 mL/min and the injection volume was 10 µL. The extract was analysed using the Lock Spray reference (leucine-enkephalin, $[M + H]^+ m/z$ 556.2771). The accurate mass and composition for the precursor ions and for the fragment ions were calculated using the Mass Lynx V4.1 software.

Statistics

The results were expressed as mean ± standard deviation (SD) for n determinations where $n = 3$ unless otherwise stated. The statistical analyses were performed using one-way analysis (ANOVA) followed by the post hoc Dunnett's test. These calculations were performed using the SigmaPlot software V 12.5. $p < 0.05$ and $p < 0.001$ were considered to be statistically significant as per the data analysed.

Results

Inhibition of nitric oxide radicals

The NO scavenging assay protocol measures the ability of the antioxidant in inhibiting the production of nitric oxide and subsequent measurement of nitrite produced from NO. The effective concentration, EC_{50} value, was as given in Fig. 1 and TBF shows inhibition of NO scavenging but was not as effective as that of ascorbic acid.

TBF inhibition of 5-LOX, 12-LOX and 15-LOX enzymes

The inhibition of 5-LOX, 12-LOX and 15-LOX enzymes was carried out using the FOX assay and the oxidation of the ferrous ion was measured. The IC_{50} values were calculated as shown in Fig. 2. The TBF extract shows a potent inhibition of the 5-LOX and 12-LOX enzymes which are better than NDGA standard. The inhibition for 15-LOX enzyme was effective but not as that of NDGA standard.

TBF inhibition of COX-1 and COX-2 activity

The inhibition of COX-1and COX-2 enzymes was carried out using the TMPD-COX assay. The IC_{50} values were calculated as shown in Fig. 3. The plant extracts did not exhibit a significant inhibition of COX isoenzymes as compared to positive control, Indomethacin. However, the low concentration of the IC_{50} values of COX 2 is indicative of possible presence of molecules which have the potential for development as lead molecules for inhibition of inflammation-induced COX-2.

Basal cytotoxicity of TBF for monocyte cells

The TBF fraction of *T. cordifolia* was screened for cytotoxic activity against monocytes by the MTT assay as described earlier. The results as shown in Fig. 4 indicate that the bioactive fraction was found to be non-cytotoxic to monocytes. Monocytes cells were taken as a reference standard for cell-based studies with TBF.

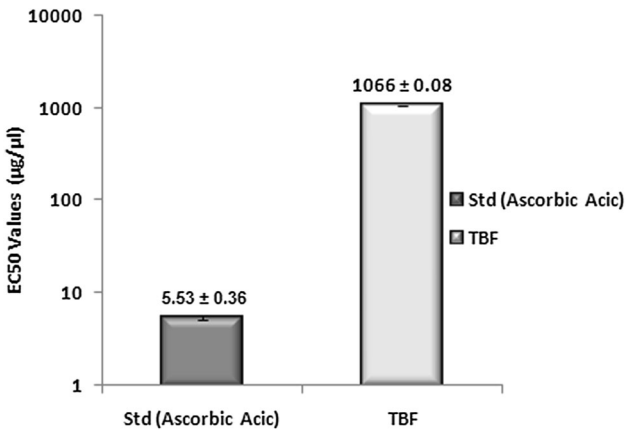

Fig. 1 Effect of bioactive fraction of *T. cordifolia* on NO scavenging. The EC_{50} values of TBF fraction on nitric oxide scavenging activity were compared to the ascorbic acid standard. Results expressed as mean ± SD. $p < 0.001$ versus standard

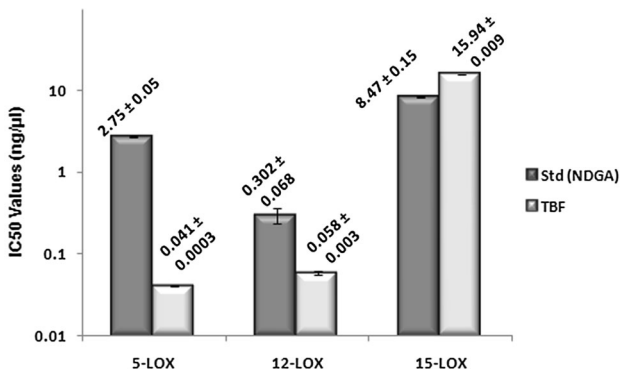

Fig. 2 Effect of bioactive fraction of *T. cordifolia* on LOX isoenzymes. The IC_{50} values of TBF fraction on LOX activity were compared to the NDGA standard. Results expressed as mean ± SD. $p < 0.001$ versus standard

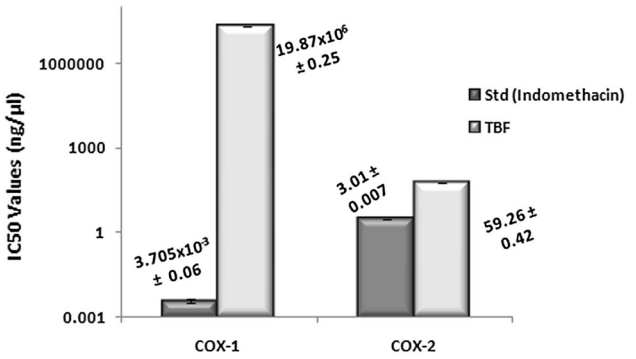

Fig. 3 Effect of bioactive fraction of *T. cordifolia* on COX isoenzymes. The IC_{50} values of TBF fraction on COX-1 and COX-2 activity were compared to the indomethacin standard. Results expressed as mean ± SD. $p < 0.001$ versus standard

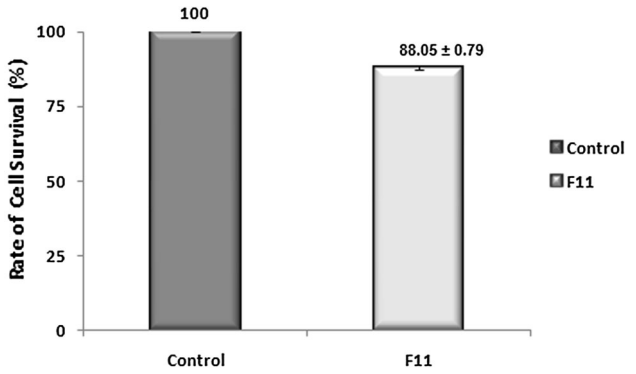

Fig. 4 Cytotoxicity study in monocytes of the bioactive fraction of *T. cordifolia*. Cytotoxic effect of TBF on monocytes in culture. Culture wells treated with MTT and formation of formazan crystals were measured at 570 nm. Data expressed as mean ± SD. Control were the cells without extract treatment. $p < 0.05$ versus standard

Effect of TBF on the production of TNF-α and IL-1β in LPS-stimulated DC

Dendritic cells have a crucial role in the innate and adaptive immune responses mediated through cytokine regulation. The study on the effect of TBF on release of cytokines by the dendritic cells was conducted after LPS-induced stimulation for 16 h. The IC_{50} value of TBF as given in Fig. 5 indicates a better inhibition of TNF-α production than standard, Rolipram while the inhibition of IL-1β production was lower than the Dexamethasone standard.

Bioactive constituents in TBF

The UPLC-MS and MS/MS data were detected and noise reduced such that only true analytical peaks were considered by the Waters Mass Lynx software. The Lock Spray is an electrospray of reference compounds directly into the ion source simultaneously with the sample providing

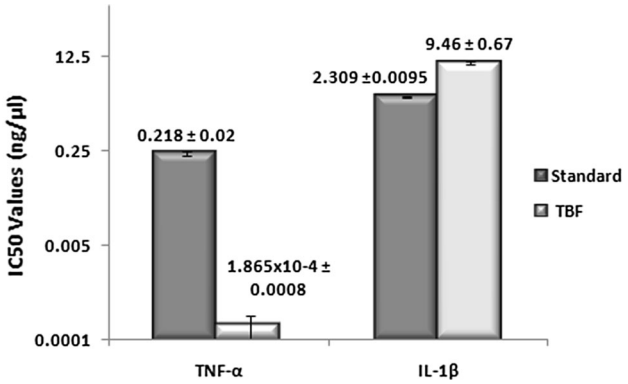

Fig. 5 Effect of bioactive fraction of *T. cordifolia* on proinflammatory cytokines. Culture supernatants from LPS-induced TBF treated and untreated dendritic cells were immunoassayed for TNF-α and IL-1β production by ELISA and compared with standards, rolipram and dexamethasone, respectively. $p < 0.001$ versus standard

corrected exact mass in MS and MS/MS modes to within 5 ppm root mean square (RMS) mass with high specificity. The Mass Lynx software generates the calculated masses, mass accuracy (ppm) and FIT confidence % values (the confidence of the i-FIT value which is based on the percentage of identity between the elemental composition and the isotopic pattern of the peaks obtained). The molecular formula generated by the software was used to find positive matches in structural databases. The compound of interest from the database was further inserted into the MassLynx software to create theoretical mass spectrum. The major parameters of the MS/MS data along with the retention time of compounds have been summarised in Table 1 and the 11 bioactive constituents identified in Fig. 6.

Discussion

The study of the inhibition of LOX enzymes can be justified by the implication of LOX metabolites in arthritis (Gheorghe et al. 2009), general inflammation (Fiorucci et al. 2001) and asthma (De Caterina and Zampolli 2004). IC_{50} values for inhibition of LOX enzymes indicate specificity for the 5-LOX and 12-LOX enzymes than the 15-LOX enzyme. The dual role of 15-LOX in inflammation reported by Hsi et al. (2002) with opposing stimulatory and inhibitory effects on cancer cell proliferation has proven the low inhibition of the 15-LOX enzyme to be beneficial. Analysis of the inhibition of COX isoenzymes shows that TBF is a potent inhibitor with IC_{50} values in nanogram quantities for COX-2 and a minimal inhibition for COX-1. This result becomes significant when considering the fact that COX-1 enzyme has a constitutive expression while COX-2 enzyme is induced under conditions of inflammation leading to the need for selectivity towards COX-2 enzyme (Parente 2001).

Table 1 The details of parent ions identified in bioactive fraction of *T. cordifolia*

Name of compound	t_R (min)	Elemental composition	$[M + H]^+$		
			Measured mass	Theoretical mass	Mass error (ppm)
TC1	3.01	$C_{20}H_{19}NO_4$	338.1377	338.1386	−2.66
TC2	4.13	$C_{14}H_{20}O_5$	269.1390	269.1383	2.16
TC3	4.19	$C_{20}H_{43}NO_3$	346.3316	346.3315	0.28
TC4	4.65	$C_{27}H_{38}O_{10}$	523.2524	523.2537	−2.48
TC5	4.66	$C_{24}H_{51}NO_4$	418.3908	418.3890	4.3
TC6	4.7	$C_{24}H_{36}O_9$	469.2436	469.2432	0.85
TC7	6.2	$C_{18}H_{30}O_7$	359.2038	359.2064	−7.23
TC8	6.2	$C_{31}H_{52}O_8$	551.3572	551.3578	1.08

The table represents the retention time of each parent ion identified and the molecular weight determined for each mass. The mass error is indicative of the accuracy of the determined mass

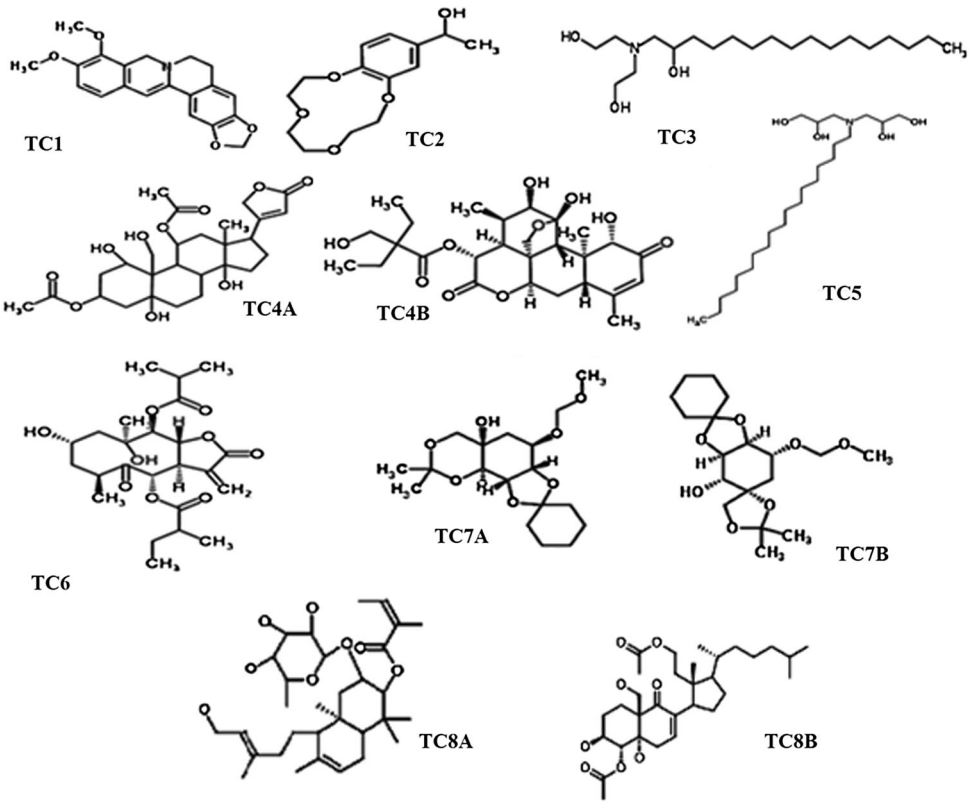

Fig. 6 Compounds identified in bioactive fraction of *T. cordifolia* by UPLC/Q-ToF/MS/MS. TC1: dihydroberberine, TC2: 1-(2,3,5,6,8,9-hexahydro1,4,7,10–benzotetraoxacyclododecin-12-yl) ethanol, TC3: *N*-(2-hydroxyhexadecyl) diethanolamine, TC4A: 3Beta,11Alpha-Di-O-acetylouabagenin, TC4B: (1β, 11β, 12α, 15β)-1, 11, 12-trihydroxy-2, 16-dioxo-11, 20-epoxypicras-3-en15-yl 2-ethyl 2-hydroxymethyl butanoate, TC5: 3,3′-(octadecylimino) bispropane-1,2-diol,TC6: cardivin B, TC7A: (3a′S,4′R,5a′R,9a′R,9b′S)-4′-(methoxymethoxy)-8′,8′-dimethyltetrahydro-3a′Hspiro[cyclohexane-1,2′-[1,3]dioxolo[4,5 h][1,3]benzodioxin]-5a′ (6′H)-ol, TC7B: (3a′R,4′R,5′R,7′R,7a′S)7′ (methoxymethoxy) -2″,2″dimethyltetrahydro'H dispiro[cyclohexane-1,2′[1,3benzodioxole-5′,4″[1,3]dioxolan]-4′-ol, TC8A: (2R,3S,4aR,5 S, 8aS)-3[6-deoxy-α-mannopyranosyl)oxyl]5[(3E)-5-hydroxy-3-methyl-3-penten-1-yl]-1,1,4a, 6-tetramethyl-1,2,3,4,4a,5,8,8a-octahydro-2-naphthalenyl(2Z)-2-methyl-2-butenoate, TC8B: blancasterol

The effect of NO scavenging is more relevant to the human biological system under chronic inflammatory conditions as compared to other antioxidative assays. In the present study, low NO radical scavenging as compared to ascorbic acid standard, but potent inhibition of LOX and COX enzymes, points to the fact that inhibition of LOX/COX enzymes is by a mechanism which is independent of Nitric oxide scavenging. The preferential flux of

arachidonic acid was towards the LOX pathway rather than the COX pathway (Yang et al. 2007) and highlights the importance of dual inhibition of these enzymes.

The presence of dendritic cells has been reported in atherosclerosis (Choi et al. 2009) and chronic eosinophilic airway inflammation in asthma (Lambrecht et al. 1998) and the typical proinflammatory cytokines produced by these cells on exposure to inflammatory stimuli are TNF-α, IL-1α, IL-1β, IL-6, and IL-12. Rolipram, a selective phosphodiesterase inhibitor, has been reported in several studies with significant inhibition of TNF-α production in LPS-induced monocytes and macrophages (Souness et al. 1996; Wang et al. 1997; Korhonen et al. 2013). Dexamethasone, a general anti-inflammatory agent, was found to mediate the specific inhibition of IL-1β production in several studies (Kern et al. 1988; Chang et al. 1990; Palmer et al. 2002) in human monocytes and articular chondrocytes. Rolipram was used as the positive standard for TNF-α inhibition and dexamethasone for IL-1β inhibition. The IC_{50} values for TNF-α inhibition by TBF indicate a potent inhibition comparable to Rolipram while the IC_{50} values for IL-1β inhibition, though potent, were lower than the standard, dexamethasone. Plasma levels of TNF-α and IL-1β were found elevated in undialyzed chronic renal failure indicating increased production in inflammation (Pereira et al. 1994) while use of anti-TNF-α drugs suppresses abnormal B cell activity (rheumatoid arthritis) leading to the associated side effects and anti-IL-1β drugs were still under clinical trials. These factors underline the feasibility of the use of natural compounds as potential drugs at low doses.

TC1 to TC9 were identified from TBF with the use of mass spectrometric fraction of the compounds at specific collision energies. TC1 was identified as a protoberberine alkaloid with reported activities of antioxidant action and anti-inflammatory properties (Li et al. 2014). TC2 is a macrocyclic polyether derived from catechol and is specifically called crown ethers. Though not reported in plants, specific studies on cytotoxicity have been reported (Marjanovic et al. 2007). TC3 and TC5 were identified as amino alcohols with no reported activities in plants. TC4A was identified to be a cardiac glycoside derivative of ouabain and TC4B as a diterpene quassinoid. TC6 could be identified as a derivative of podophyllotoxin type lignan. These plant lignans have potent anti-inflammatory activity (Gordaliza et al. 2004). TC7A was identified as a sesquiterpene compound while TC8B was a secosteroid similar to vitamin D in humans with reported antitumour activities (Pinto et al. 2010). The observed activities in the compounds identified in TBF were indicative of the anti-inflammatory potentials of these bioactive compounds.

Conclusion

The bioactive fraction of the methanol extract of *T. cordifolia* was investigated for its immunomodulatory activity and found to be a potent inhibitor of LOX enzymes. The extract also inhibits the production of TNF-α in LPS-stimulated dendritic cells and the exact mechanism of inhibition of TNF-α production needs to be further evaluated. These observed activities of the bioactive fraction could be due to the presence of the eleven compounds identified by mass spectroscopy and provide a source of lead candidates with anti-inflammatory potentials in *T. cordifolia*. Further investigations as to the exact nature of the bioactive compound(s) and the interaction of the compound(s) to produce its biological effects need to be studied.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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