Journal of Ayurveda and Integrative Medicine 11 (2020) 281–286

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article (Experimental)

# Bioassay guided fractionation of *Cyclea peltata* using *in vitro* RAW 264.7 cell culture, antioxidant assays and isolation of bioactive compound tetrandrine

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ARTICLE INFO

*Article history:*
Received 2 October 2017
Received in revised form
2 April 2018
Accepted 2 May 2018
Available online 23 October 2018

*Keywords:*
*Cyclea peltata*
Tetrandrine (TET)
iNOS
COX-2
TNF-α
HPTLC

ABSTRACT

*Background:* *Cyclea peltata* is one of the herbs mentioned in ancient scriptures of Ayurveda and is used in different types of Ayurvedic gritham preparations. Moreover, in traditional/tribal medicine *C. peltata* is used as digestive, anti-inflammatory, diuretic and to treat jaundice, digestive disorders, etc.
*Objective:* Activity guided fractionation of *C. peltata* and in correlation with the levels of bioactive compound tetrandrine.
*Materials and methods:* Preliminary phytochemical screening, estimation of total alkaloid content, preparation of different extracts of *C. peltata* (crude extract CP, hexane extract HCP, chloroform extract CCP, methanol extract MCP, alkaloid fraction ACP). *In vitro* anti-inflammatory studies using RAW 264.7 cells and *in vitro* antioxidant assays of the different extracts of *C. peltata*. HPTLC estimation of tetrandrine (TET) was carried out using solvent system toluene: ethyl acetate: diethylamine (7.2: 2: 0.8) and isolation of TET from ACP.
*Results:* Preliminary phytochemical studies of *C. peltata* showed the presence of alkaloid content in all extracts. Whereas, saponins, steroids and terpenoids were detected in CP and CCP. ACP and TET showed significant *in vitro* anti-inflammatory and antioxidant activity when compared to other extracts. ACP and TET (100 μg/ml) treatment significantly inhibited the mRNA expression of iNOS, COX-2, TNF-α in LPS treated RAW 264.7 cells. HPTLC estimation of bioactive compound tetrandrine was highest in ACP-228.4 μg/mg followed by CP-29.62 μg/mg, CCP-23.46 μg/mg, MCP-18.82 μg/mg and HCP-1.25 μg/mg. TET has been isolated from ACP.
*Conclusion:* The results of the present *in vitro* assays revealed that the alkaloid fraction (ACP) is the most active fraction when compared to other extracts and has a positive correlation with the levels of bioactive compound tetrandrine.


## 1. Introduction

*Cyclea peltata* (Poir.) Hook. f. & Thoms. is locally called 'Padathaali/Padakkilangu' which belongs to the family of Menispermaceae. The family contains mostly climbing plants found in tropical climate recognized for their important pharmacological activities [1]. *C. peltata,* a slender twining shrub with sparingly pilose stems and branches. Leaves simple, alternate, flowers small, greenish in axillary panicles. The tuberous root is cylindrical, irregular, curved with greyish brown surface and white starchy cortex.

*C. peltata* is one of the herbs mentioned in ancient scriptures of Ayurveda. It is used in different types of Ayurvedic gritham preparations like *Mahatikthaka gritham*, *Panchagavya gritham*, *Saraswatham gritham*, *Thikthaka gritham* and *Gulguluthikthaka gritham*. In Indian traditional medicine the roots are also used to treat jaundice and digestive disorders [2]. The Kurichiya tribe of Kerala used the tuberous roots of this plant along with a little salt to treat stomach pain [3]. The Garo tribe of Balphakram sanctuary in Meghalaya use the crushed root extract as a remedy against small

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Peer review under responsibility of Transdisciplinary University, Bangalore.

https://doi.org/10.1016/j.jaim.2018.05.009

pox [4]. Roots are used as digestive, anti-inflammatory, diuretic, vitiated conditions of kapha, cough, bronchitis, splenomegaly, ulcers, wounds, haemorrhoids, vomiting, hyperdipsia and cardiac disorders. The leaves are cooling, ophthalmic and are useful in dandruff, burning sensation of the eyes, fever and used as soap [5].

Activity guided fractionation is an effective and fruitful strategy to identify the bioactive compounds from the herbs. Both *in vitro* and *in vivo* methods can be utilized to identify the pharmacological activities of most potent extract or fraction or molecule. Knowledge about structure, characteristics, stability aspects of phytoconstituents are essential to provide medicinal effects with respect to their bio-absorption, bioavailability, tolerance, toxicity and intensity of providing therapeutic effects. Hence different modern phytochemical methods should be used to ensure the presence of potent phytoconstituents in the medicinal plants. Further quantitative and qualitative estimation of these phytocostituents carried out using modern technique such as chromatographic and spectroscopic methods. Chromatographic techniques are the most useful and popular method, among which high performance thin layer chromatography is effective and powerful tool for linking the chemical constituent profiling and estimation of the plant extracts [6].

*C. peltata* roots are reported to contain alkaloids like fangchinoline, tetrandrine, D-isochondrodendrine, cycleapeltine, cycleadrine, cycleacurine, cycleanorine, etc. [7,8]. Indian samples of *C. peltata* reported to have tetrandrine (TET) as the major alkaloid [9]. TET is well known to possess activities including antioxidant, plasma glucose lowering [10], anti-inflammatory, immunosuppressive [11], anti-fibrotic, anticancer, hypertension and silicosis [12,13].

Estimation of the bioactive compound tetrandrine in different extracts of *C. peltata* has great significance due to its therapeutic role. In the present study, we have assessed the *in vitro* antioxidant and anti-inflammatory activities of different extracts of *C. peltata*. Further the bioactive compound tetrandrine was estimated in different extracts using HPTLC.

## 2. Materials and methods

### 2.1. Plant material

*C. peltata* roots were collected from Thiruvananthapuram district of Kerala, India and authenticated by Dr. Mathew Dan, plant taxonomist of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI). A voucher specimen had been deposited at the JNTBGRI Herbarium (TBGT 13814).

### 2.2. Chemicals and standards

Tetrandrine (TET) and lipopolysaccharide (LPS) were purchased from Sigma Chemicals Co., USA. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Hi-media, India. Hexane, chloroform, methanol, toluene, diethylamine, ether, sulphuric acid ($H_2SO_4$), hydrochloric acid (HCl) and ammonia solution were purchased from SD Fine Chem Ltd., Mumbai, India. Precoated TLC plates of silica gel 60 $F_{254}$ was purchased from Merck, India.

### 2.3. Preparation of extracts

#### 2.3.1. Preparation of various extracts of C. peltata

100 g plant powder was sequentially extracted with hexane, chloroform and methanol using a Soxhlet apparatus. Concentrated the extracts under reduced pressure, which yielded 8 g hexane extract (HCP), 10 g chloroform extract (CCP), 6 g methanol extract (MCP). Separately another 100 g of powdered plant material was extracted with ethanol for 48 h at 27 °C and dried under reduced pressure to yield 15 g crude extract (CP).

#### 2.3.2. Preparation of C. peltata alkaloid fraction

100 g of plant material was extracted with methanol for 48 h using Soxhlet apparatus and dried under reduced pressure using rotory evaporator (Buchi R-210) to yield 20 g methanol crude extract (CP1). Total alkaloid fraction was isolated in accordance with the standard method [14]. 20 g of the crude extract (CP1) was dissolved in dil. $H_2SO_4$, filtered and pH was adjusted to 9.5. Free alkaloid was extracted with chloroform. The chloroform layer was filtered and concentrated under reduced pressure to yield 9 g alkaloid fraction (ACP).

### 2.4. Phytochemical studies

#### 2.4.1. Phytochemical screening

The different extracts of *C. peltata* (HCP, CCP, MCP, CP) were analysed for the presence of secondary metabolites as per the standard methods [15].

#### 2.4.2. Isolation of tetrandrine from the alkaloid fraction (ACP) using column chromatography

ACP (5 gm) was subjected to column chromatography over neutral Alumina (50 gm) using toluene: ethyl acetate: diethylamine (6.9: 3: 0.1) isocratic elution. 10 ml fractions were collected and fractions 6–8 gave pure compound (9.4 mg). The UV spectrum of the isolated compound has been carried out at 209 nm. TLC of the isolated compound using standard tetrandrine has been carried out using solvent system toluene: ethyl acetate: diethylamine (7.2: 2: 0.8). This isolated TET has been used for the *in vitro* studies.

#### 2.4.3. Estimation of tetrandrine using high performance thin layer chromatography (HPTLC)

HCP, CCP, MCP, CP and ACP were analysed by HPTLC following the modified method [16]. HCP, CCP, MCP, CP and ACP were prepared (5 mg/ml concentration each) and applied as 40 µl HCP, 10 µl CCP, 10 µl MCP, 2.5 µl ACP and 10 µl CP respectively on precoated TLC silica gel 60 $F_{254}$ plate using Linomat V. Standard tetrandrine was prepared in mg/ml concentration and applied 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 µg of tetrandrine on precoated TLC silica gel 60 $F_{254}$ plate using Linomat V. The plate was then developed using the solvent system (Toluene: Ethyl acetate: Diethyamine; 7.2: 2: 0.8) and scanned densitometrically at 209 nm using CAMAG TLC Scanner 3.

### 2.5. In vitro free radical scavenging effects of plant extracts, fraction and TET

#### 2.5.1. Assessment of hydroxyl radical scavenging

Hydroxyl radicals generated from $Fe^{3+}$-ascorbate-EDTA-$H_2O_2$ were estimated by the degradation of deoxyribose that resulted in thiobarbituric acid reacting substances, (TBARS) formation. Curcumin was used as reference compound [17].

#### 2.5.2. Assessment of superoxide scavenging

Superoxide radical scavenging activity of plant extracts (CP, HCP, CCP, MCP, ACP) and TET were determined by nitroblue tetrazolium (NBT) reduction [18]. The percentage inhibition of superoxide production was evaluated by comparing the absorbance of control and experimental tubes. Curcumin was used as reference compound.

**Table 1**
Primer sequence used for the present study.

| Sl. No | Target gene | Forward sequence | Reverse sequence |
|---|---|---|---|
| 1 | TNF-α | 5′-TTAACCTA-CGCGCTGAGTTG-3′ | 5′-CCTGTAGCCCACGR-CGRAGC-3′ |
| 2 | COX-2 | 5′-AAGACTTGCCAGGCTGAACT-3′ | 5′-CTTCTGCAGTCCAGGTTCAA-3′ |
| 3 | iNOS | 5′-CCT CCT CCACCC TAC CAA GT-3′ | 5′-CACCCAAAGTGCTTCAGTCA-3′ |
| 4 | β-Actin | 5′-ATGCTCCTGCTTGAGTATGT-3′ | 5′-GGAGGAAGAG-GATGCGGCAGT-3′ |

*2.5.3. Assessment of DPPH radical scavenging*

DPPH radical scavenging activity was measured by the spectrophotometric method [19]. Curcumin was used as reference compound. Curcumin was used as reference compound.

*2.5.4. Assessment of anti − lipid peroxidation*

The anti-lipid peroxidant effect of plant extracts (CP, HCP, CCP, MCP, ACP) and TET were assessed by the modified method [20].

*2.6. In vitro anti-inflammatory studies*

*2.6.1. In vitro RAW 264.7 cell culture studies of plant extracts, fraction, TET and diclofenac sodium*

Raw 264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37 °C in 5% $CO_2$. The murine macrophage cell line RAW 264.7 cells were grown to 60% confluence followed by activation with 1 μl lipopolysaccharide (LPS) (1 μg/ml). LPS stimulated RAW cells were exposed with different concentrations (25, 50, 100 μg/mL in 0.1% DMSO) of plant extract/fractions (CP, HCP, CCP, MCP, ACP), TET and diclofenac sodium, (a standard anti-inflammatory drug) incubated for 24 h. LPS stimulated RAW 264.7 cells treated with 0.1% DMSO was treated as LPS control. RAW 264.7 cells treated with 0.1% DMSO without LPS was served as normal control. After incubation of 24 h at 37° C in a 5% $CO_2$ humidified incubator, the anti-inflammatory assays were performed using the cell lysate [21], COX activity [22], LOX activity [23], myeloperoxidase (MPO) activity [24], Cellular nitrite level [25]. Percentage inhibition of COX and LOX activity was calculated using the formula given below.

Percentage inhibition = Absorbance of LPS control − Absorbance of test/Absorbance of LPS control × 100.

*2.6.1.1. mRNA expression of iNOS, COX-2 and TNF-α using qRT-PCR analysis of fraction, TET and diclofenac sodium.* Total RNA of RAW cells, RAW cell + LPS, and RAW cells exposed LPS along with ACP, TET, diclofenac sodium (100 μg/ml) groups were extracted using (Trizol, Ambion Life technologies, USA) from RAW cells. The purity and the concentration of total RNA was determined using Qubit 3.0 (Life technologies, USA) flurimetric analysis. Template complementary DNA was synthesized using the cDNA preparation kit (High Capacity cDNA synthesis Kit, Thermoscientific USA). Real-Time qRT-PCR analysis was carried out using SYBR Green Master Mix (Applied Biosystem, Life technologies). All reactions were performed in triplicates and data were analysed according to $\Delta\Delta C_t$ method using Light cycler analysis software (Light cycler 96 SW 1.1). The primer sequences used were summarized in Table 1.

*2.7. Statistical analysis*

The results were expressed as mean ± standard deviation of mean (SD). ANOVA was done to compare and analyse the data followed by Duncan's multiple range test. Effects were considered significant at $p \leq 0.01$.

## 3. Results

*3.1. Preliminary phytochemical screening*

The result showed the presence of alkaloids in CP, CCP, MCP and HCP. The presence of saponins, steroids and terpenoid were detected in CCP and CP (Table 2).

*3.2. Estimation of tetrandrine (TET) using high performance thin layer chromatography (HPTLC)*

The amount of tetrandrine in CP, HCP, CCP, MCP and ACP were estimated by HPTLC-densitometry and was expressed as μg/mg extract. The analysis showed that Tetrandrine content was found to be high in ACP 228.4 μg/mg compared to that detected in CP 29.62 μg/mg. The tetrandrine content was also estimated in HCP, CCP and MCP extracts and found to be 23.46 μg/mg (CCP), 18.82 μg/mg (MCP) and 1.25 μg/mg (HCP) (Fig. 1 and 2).

*3.3. Effect of plant extracts, fraction, TET and curcumin on in vitro free radical scavenging*

*3.3.1. Effect of plant extracts, fraction, TET and curcumin on in vitro hydroxyl radical scavenging*

Hydroxyl radical generated by $Fe^{3+}$/ascorbate/EDTA/$H_2O_2$ system initiated the degradation of deoxyribose which was inhibited significantly by the TET, ACP and plant extracts (CP, CCP, MCP and HCP). The $IC_{50}$ value for TET − 20 μg/ml, ACP was 27.21 μg/ml and for CP, CCP, MCP, HCP were 52.25 μg/ml, 58.32 μg/ml, 61.25 μg/ml and 117.26 μg/ml respectively. The standard, curcumin exhibited $IC_{50}$ value of 2.89 μg/ml (Fig. 3).

**Table 2**
Preliminary phytochemical analysis of *Cyclea peltat* extracts (HCP, CCP, MCP, CP).

| Extracts | Glycosides | Alkaloids | Saponins | Steroids | Terpenoids | Tannins | Flavanoids | Coumarins |
|---|---|---|---|---|---|---|---|---|
| HCP | − | + | − | − | − | − | − | − |
| CCP | − | ++ | + | + | + | − | − | − |
| MCP | − | ++ | − | − | − | − | − | − |
| CP | − | ++ | + | + | + | − | − | − |

+++ highly present, ++ moderately present, + Low, − absent.

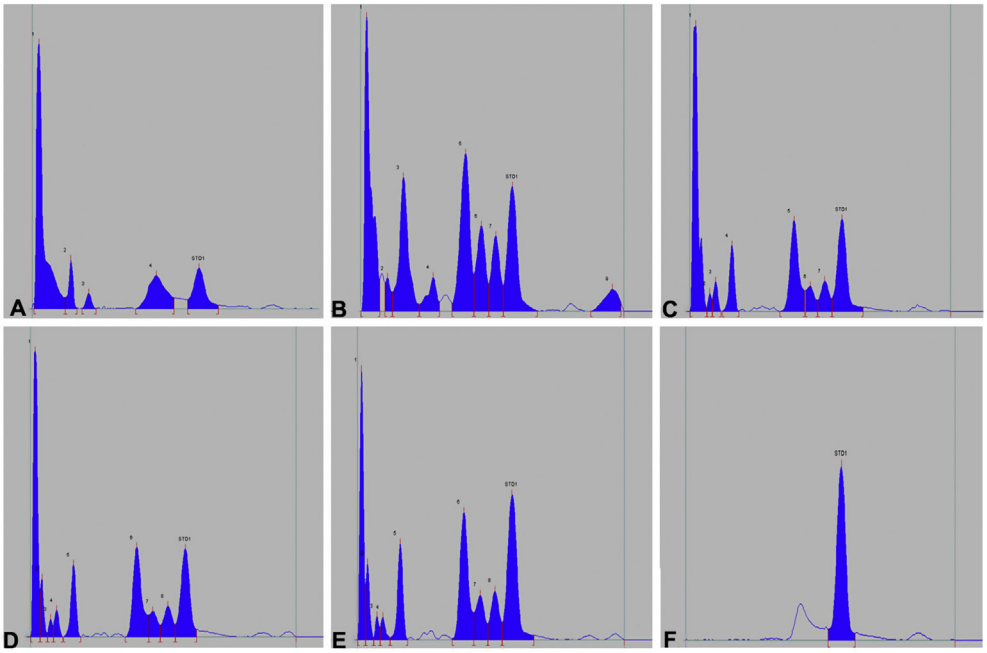


**Fig. 1. A–F.** HPTLC chromatogram of different extracts/fraction of *C. peltata* **(A)** HPTLC chromatogram of hexane extract of *C. peltata* (HCP). **(B)** HPTLC chromatogram of chloroform extract of *C. peltata* (CCP). **(C)** HPTLC chromatogram of methanol extract of *C. peltata* (MCP). **(D)** HPTLC chromatogram of crude ethanol extract of *C. peltata* (CP). **(E)** HPTLC chromatogram of alkaloid fraction of *C. peltata* (ACP). **(F)** HPTLC chromatogram of standard tetrandrine (TET).

### *3.3.2. Effect of plant extracts, fraction, TET and curcumin on in vitro superoxide scavenging*

Percentage inhibition of superoxide radical generation by TET, ACP, CP, CCP and MCP was found to be increasing in a dose dependent manner. ACP showed better protection than other groups. The $IC_{50}$ value for TET – 25 μg/ml, ACP was found to be 35.21 μg/ml and CP, CCP, MCP and HCP 62.31 μg/ml, 66.43 μg/ml, 70.25 μg/ml and 125.52 μg/ml respectively when compared to the standard curcumin 5.25 μg/ml (Fig. 3).

### *3.3.3. Effect of plant extracts, fraction, TET and curcumin on in vitro DPPH radical scavenging*

The DPPH Scavenging assay of the plant extracts showed significant antioxidant activity with ACP than other treatments. The $IC_{50}$ value for TET – 15 μg/ml, ACP was 31 μg/ml and CP, CCP, MCP and HCP as 48 μg/ml, 48 μg/ml, 56 μg/ml and 120 μg/ml respectively. Whereas, standard curcumin having $IC_{50}$ value of 2.26 μg/ml (Fig. 3).

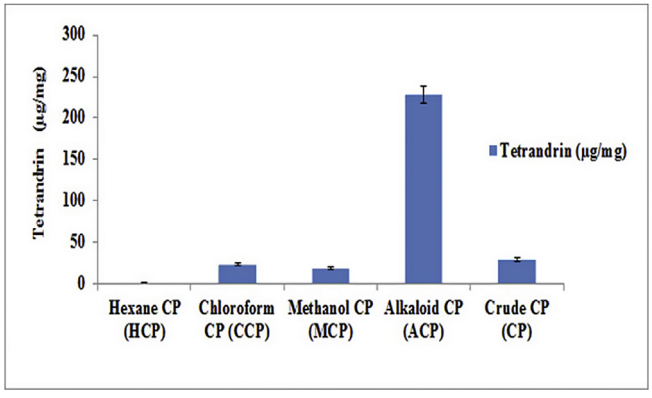


**Fig. 2.** HPTLC estimation of Tetrandrine (TET) in different extracts/fraction of *C. peltata* hexane (HCP), chloroform (CCP), methanol (MCP), crude ethanol (CP) and alkaloid fraction (ACP).

### *3.3.4. Effect of plant extracts, fraction, TET and curcumin on in vitro anti – lipid peroxidation*

The estimation of malondialdehyde (MDA) levels in $FeCl_2$-AA treated rat liver homogenate is direct value of the level of lipid peroxidation. The TET and plant extracts/fraction showed significant reduction in the MDA levels dose dependently *in vitro*. The concentration needed for the 50% reduction of lipid peroxidation by the TET/plant extracts/fraction were 64.24 μg/ml 74.45 μg/ml, 86.48 μg/ml, 119.15 μg/ml and 182.86 μg/m respectively for ACP, CP, CCP, MCP, HCP and the TET exhibited $IC_{50}$ value of 46.5 μg/ml (Table 3).

### *3.4. Effect of plant extracts, fraction, TET and diclofenac sodium on in vitro anti-inflammatory studies*

### *3.4.1. Effect of plant extracts, fraction, TET and diclofenac sodium on LPS induced inflammation in RAW 264.7 cell culture*

RAW 264.7 cells treated with 1 μl LPS (1 μg/ml) caused an increase in the activity of COX, LOX, myeloperoxidase and increased

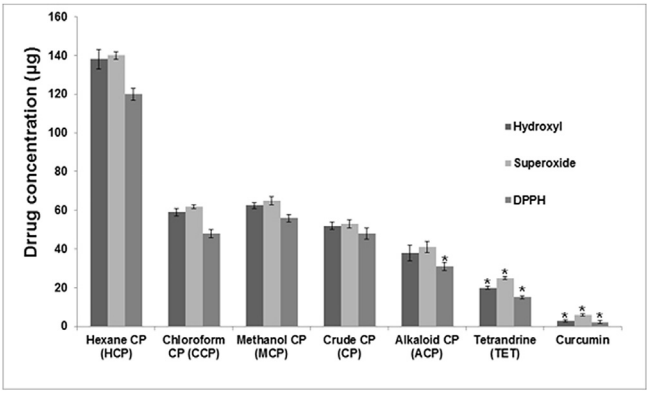


**Fig. 3.** Effect of plant extracts, fraction, tetrandrine (TET) and curcumin on *in vitro* hydroxyl radical, superoxide radical, DPPH radical scavenging studies. Values are mean ± SD, $n = 3$, ANOVA followed by Duncan's multiple range test. $^{*}p \leq 0.01$ vs control.

**Table 3**
Inhibitory effect of different extracts, fraction of *C. peltata* (HCP, CCP, MCP, CP, ACP), tetrandrine (TET) and curcumin on $FeCl_2$-ascorbic acid-induced lipid peroxidation in rat liver homogenate *in vitro*.

| Treatments | Extract concentration (μg/ml) | MDA inhibition (%) |
|---|---|---|
| CP | 25 | 8.6[a] |
| | 50 | 12.24[a,b] |
| | 75 | 60[e] |
| | 100 | 68.65[e] |
| HCP | 25 | 2[a] |
| | 50 | 8.4[a] |
| | 75 | 18.6[b] |
| | 100 | 22[b,c] |
| CCP | 25 | 6.21[a] |
| | 50 | 10[a] |
| | 75 | 40[d] |
| | 100 | 56[e] |
| MCP | 25 | 4[a] |
| | 50 | 8[a] |
| | 75 | 29[c] |
| | 100 | 35[c,d] |
| ACP | 25 | 12[a,b] |
| | 50 | 31[c] |
| | 75 | 70[f] |
| | 100 | 78[f,g] |
| TET | 25 | 40[d] |
| | 50 | 65[e] |
| | 75 | 80[g] |
| | 100 | 92[h] |

Values are the mean ± SD, n = 6, Analysis of variance (ANOVA) followed by Duncan's multiple range test, means bearing different superscripts differ significantly. ($p \leq 0.01$). Parentheses indicate the percentage of MDA Inhibition *in vitro*.

cellular nitrite levels *in vitro*. Treatment with ACP, CP, CCP, MCP, HCP and the standard drug-diclofenac sodium could reduce the COX, LOX, myeloperoxidase and cellular nitrite levels in a dose dependent manner. ACP (100 μg/ml) showed significant *in vitro* protection than the other extracts and comparable to that of standard drug-diclofenac sodium (Fig. 4A-D).

*3.4.1.1. Effect of fraction, TET and diclofenac sodium on mRNA expression of iNOS, COX-2 and TNF-α on in vitro RAW 264.7 cell.* LPS treated RAW cells showed increase levels of mRNA expressions of TNF-α, COX-2 and iNOS. ACP (100 μg/ml) treated LPS + RAW cells showed significant decline in the levels of mRNA expression of TNF-α, COX-2 and iNOS (Fig. 5A-C).

## 4. Discussion

Different extracts and fraction of *C. peltata* were screened for the *in vitro* antioxidant, anti-inflammatory activities. The studies revealed alkaloid fraction (ACP) was identified as the most active among other treatment groups. Thus, ACP could significantly scavenge free radicals generated *in vitro*. Moreover, ACP showed significant anti-inflammatory effect via inhibition of mRNA expression of iNOS, COX-2, TNF-α in LPS treated RAW cells. *C. peltata* was reported to have bisbenzyl isoquinoline alkaloids, tetrandrine (TET., $C_{38}H_{42}O_8N_2$; MW 622.30), fangchinoline (FAN., $C_{37}H_{40}N_2O_6$; MW 608.288) and coclaurine (CoCl., $C_{17}H_{19}NO_3$; MW 285.33) [7,8] which we have previously confirmed using DART-MS [26]. Indian samples of *C. peltata* were reported to contain tetrandrine as the major compound; hence in the present study we have assessed the *in vitro* activity of different extracts of *C. peltata* and correlated the observed activity in relation to the TET content in *C. peltata*. Our, HPTLC estimation of bioactive compound tetrandrine (TET) showed higher level in ACP followed by CP, CCP and MCP. When compared to ACP other extracts CP, CCP and MCP showed moderate radical scavenging and anti-inflammatory activity *in vitro*. Phytochemical and *in vitro* pharmacological studies could positively correlate the TET content and pharmacological activity. Hence, in the present study we have isolated TET from ACP and evaluated *in vitro* antioxidant and anti-

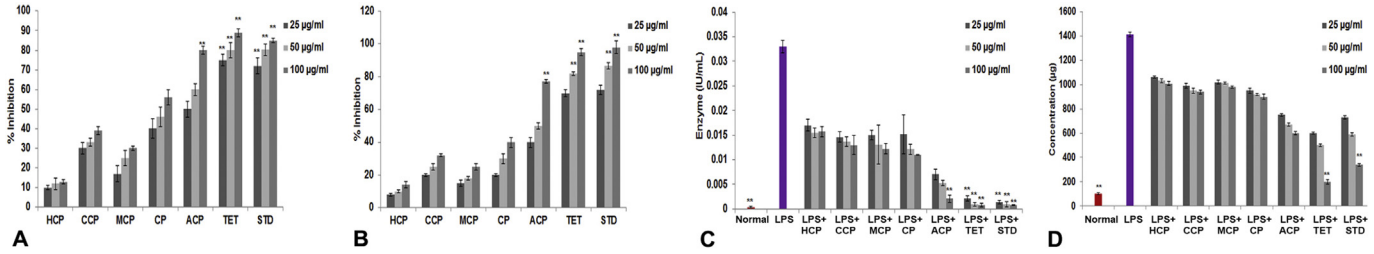


**Fig. 4. A–D** Effect of different extracts/fraction of *C. peltata*, tetrandrine (TET) and standard drug diclofenac sodium (STD) in different concentrations (25, 50, 100 μg/ml) on COX, LOX, myeloperoxidase activity and cellular nitrite levels in LPS induced RAW 264.7 cells (**Fig. 4A**). COX activity (**Fig. 4B**). LOX activity (**Fig. 4C**) Myloperoxidase activity (**Fig. 4D**) Cellular nitrite levels. Values are mean ± SD, $n = 3$, ANOVA followed by Duncan's multiple range test. $^{**}p \leq 0.01$ vs LPS control.

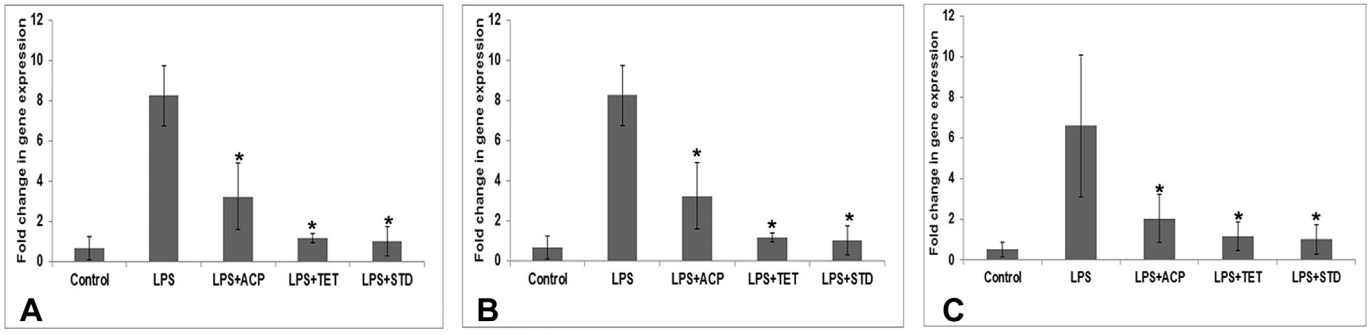


**Fig. 5. A–C.** Effect of alkaloid fraction of *C. peltata* (ACP-100 μg/ml), tetrandrine (TET-100 μg/ml) and standard drug diclofenac sodium (STD-100 μg/ml) on mRNA expression levels of TNF-α, COX-2 and iNOS on LPS induced RAW 264.7 cells (**Fig. 5A**). TNF-α mRNA levels were increased in LPS induced RAW 264.7 and were decreased in ACP and STD treatment (**Fig. 5B**). COX-2 mRNA levels were increased in LPS induced RAW 264.7 and were decreased in ACP and STD treatment (**Fig. 5C**). iNOS mRNA levels were increased in LPS induced RAW 264.7 and were decreased in ACP and STD treatment. * represents a significant difference from LPS control.

inflammatory property. These studies revealed TET was most promising when compared to STD.

Inflammation is a crucial pathway for many metabolic disorders, including atherosclerosis, multiple sclerosis, rheumatoid arthritis, obesity, liver fibrosis and type 2 diabetes [27,28]. LPS activates nuclear transcription factor kappa-B (NF-κB) nuclear translocation in macrophages through toll-like receptor 4 (TLR4) and potentially promote the target genes of pro-inflammatory cytokines such as iNOS, COX-2, TNF-α [29,30]. TET/ACP treatment could significantly inhibit the expression of TNF-α, COX-2 and iNOS mRNA in LPS induced RAW cells. This anti-inflammatory potential of TET/ACP may be due to the inhibition of NF-κB nuclear translocation and further inhibition of TNF-α, COX-2 and iNOS.

Non-steroidal anti-inflammatory drugs (NSAIDs) are main medication for inflammatory conditions like arthritis, pain etc. The NSAIDs are known to have adverse effects such as peptic ulcer, kidney damage, risk of serious thromboembolic events etc. [31,32]. Herbal medicines are becoming increasingly popular because of their relatively few side effects. Our previous studies showed *C. peltata* could significantly inhibit peptic ulcer and liver damage [16,26]. Thus from our present study *C. peltata* could be a promising herbal drug against inflammatory conditions. The observed anti-inflammatory activity of ACP may be due to the synergistic activity of major bioactive molecules such as tetrandrine present in ACP.

## 5. Conclusion

The observed results could correlate the *in vitro* antioxidant and anti-inflammatory activity of *C. peltata* with TET content in the extracts. These findings showed that TET may be one of the major bioactive compounds in *C. peltata*. Further detailed phytochemical studies coupled with *in vitro* and *in vivo* pharmacological studies are warranted to identify more bioactive compounds from *C. peltata*.

## Sources of funding

Indian Council of Medical Research (ICMR), New Delhi as Research Fellowship (45/9/2007/BMS/TRM) and Science and Engineering Research Board, Department of Science and Technology (SERB-DST), Government of India (SB/YS/LS-241/2013).

## Conflicts of interest

None.

## Acknowledgements

The authors thankfully acknowledge The Director, JNTBGRI for the facilities provided. Dr. B. Sabulal, Dr. J. Anil John, Mrs. Rajani Kurup Phytochemistry & Phytopharmacology Division, JNTBGRI for HPTLC analysis.

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