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Antioxidant, anti-inflammatory activities and HPLC quantification of flavonoids in *Pteris tripartita* Sw. a critically endangered medicinal fern from India

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ABSTRACT

In this present study, we aimed to evaluate *in vitro* antioxidant and *in vivo* anti-inflammatory activities of a giant bracken fern, *Pteris tripartita* (PT) ethanol frond extract. Ethanol frond extract of PT was studied for their *in vitro* antioxidant activities using ABTS•+ and hydrogen peroxide radical scavenging activities and *in vivo* anti-inflammatory activity using carrageenan-induced rat paw edema volume method (200 mg/kg and 400 mg/kg of ethanol frond extract and 50 mg/kg of standard flavonoid, rutin). Significant ABTS•+ radical scavenging activity was observed in both ethanol and ethyl acetate extracts. Higher percentages of hydrogen peroxide radical scavenging activity were observed in both hexane extract and chloroform extract, respectively. Moreover, ethanol frond extract of PT exhibited potent anti-inflammatory activity after 3 h of treatment. We identified two flavonoids namely, rutin and quercetin in ethanol frond extract of PT. Our results revealed that *P.tripartita* fern could be used as a natural antioxidant and a potent therapeutic drug for ailments.

1. Introduction

Researchers screening medicinal plants to identify bioactive compounds for human ailments since several decades, (Sheeja and Kuttan, 2007; Mukherjee et al., 2007). Although pteridophytes have boundless medicinal properties, phytochemical validation has not yet been explored (Singh et al., 2001, 2008a, 2008b; Gogoi, 2002; Chen et al., 2005). Biochemically, pteridophytes are less versatile than angiosperms. Monoterpenes and sesquiterpenes are relatively less abundant in pteridophytes which produce a singular series of triterpenoids and phytoecdysones (Kumari et al., 2011). Pteridophytes have a long geological history as pioneer plants and colonized the planet millions of years ago. These plants also occupy a significant place in primary health care for cultural and economic reasons. Due to the economic crisis in developing countries, pteridophytes presently utilize for human ailments (Ho et al., 2011). Besides, ferns possess numerous bioactivities such as anti-microbial, anti-viral, anti-inflammatory, anti-tussive and anti-tumor (Chang et al., 2011). Furthermore, following medicinal ferns have been documented for their anti-inflammatory activity namely, *Pteris multifida* (Lee and Lin, 1988), *Cheilanthes farinosa* (Yonathan et al., 2006), *Blechnum occidentale* (Nonato et al., 2009), *Cyathea phalerata* (Appel Hort et al., 2008), *Cyathea gigantea* (Madhukiran and Ganga Rao, 2011), *Davallia mariesii* (Chang et al., 2007), *Microsorum scolopendria* (Bloomfield, 2002), *Phyllitis scolopendrium* (Bonet and Valles, 2007), and *Polypodium leucotomos* (Lucca, 1992). Ferns also reported for wound healing activities namely, *Davallia solida* (Whistler, 1992a), *Polystichum*

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pungens (Grierson and Afolayan, 1999), *Angiopteris evecta* (Cambie and Ash, 1994), *Nephrolepis cordifolia*, *Ophioglossum reticulatum, Thelypteris arida* (Upreti et al., 2009), *Microsorum scolopendria* (Whistler, 1992b), *Phyllitis scolopendrium* (Oniga et al., 2004), *Pityrogramma calomelanos* (De Feo, 2003), some *Polypodium* sp. (Liu et al., 1998) and *Phlebodium decumanum* (Punzon et al., 2003), respectively.

In our previous studies, spore germination percentage, gametophyte growth, apogamous sporophyte development, phytochemical analysis, biosynthesis of silver nanoparticles, antioxidants and antimicrobial of PT were reported (Baskaran and Jeyachandran, 2010, 2012; Baskaran et al., 2014, 2015a, 2015b). Biosynthesized silver nanoparticles using PT extract proved significant antioxidant, anti-inflammatory, and antimicrobial activities in our earlier study (Baskaran et al., 2016). But, there are no scientific reports on anti-inflammatory activity of this medicinal valuable fern's extract alone. Therefore, we assessed *Pteris tripartita* Sw. extracts for their antioxidant and anti-inflammatory activities and HPLC analysis for their bioactive compounds identification.

2. Materials and methods

2.1. Chemicals

Azinobis 3-ethylbenzothiazoline-6-sulfonate ($ABTS^{\bullet+}$), rutin, quercetin, carrageenan, indomethacin, potassium persulfate, eosin were purchased from Sigma-Aldrich chemical company (USA). All solvents were purchased from Merck (Germany). For the biochemical study of blood serum, standard analytical kits obtained from Diasys diagnostic systems (Germany).

2.2. Plant material collection

Mature frond of *P. tripartita* Sw. (Pteridaceae) without spores was collected during March 2010 from Alagar hills reserve forest of Eastern Ghats with 650 m elevation (10°0′–10°30′ N and 75°55′–78°20′ E) in Madurai, Tamil Nadu (India). Voucher specimen compared with previous reference specimen available in St. Xavier's College Herbarium (XCH), Palayamkottai, Tamil Nadu (India) and numbered (XCH 25403). For extraction, collected fern material was washed in running tap water and shade dried at room temperature.

2.3. Plant extraction

Shade air-dried plant material (fern frond) of *Pteris tripartita* pulverized and extracted separately with hexane, acetone, ethyl acetate, chloroform, ethanol, methanol, and water. Ground powder (10 g) of PT placed inside a thimble and loaded into Soxhlet extractor. Total extraction time was 48 h and the total amount of solvent was 60 mL to reflux over the sample, continuously. The solvent extraction was performed at solvent boiling temperatures. After extraction, the solvent removed from the solute mixture using rotary evaporator and stored at 4 °C.

2.4. HPLC analysis

HPLC analysis was performed in liquid chromatography (Shimadzu LC-8A, Japan) pumps equipped with SPD-20 A UV/Vis detector. HPLC profile of ethanol frond extract of PT was recorded with a modified method of Govindarajan et al. (2007). The elution was carried out at a flow rate of 0.8 mL/min with water:phosphoric acid (99.7:0.3 v/v) as solvent A and acetonitrile:water: phosphoric acid (79.7:20:0.3 v/v) as solvent B using gradient elution in 0–5 min with 88–85% A, 5–6 min with 85–82% A, 6–9.5 min with 82–75% A, 9.5–10.5 min with 75–74% A, 10.5–12 min with 74–73% A, and 12–20 min with 73–70% A. Detection was carried out at 264 nm. Standard solutions of both standards, rutin, and quercetin were analyzed. Quantification of flavonoid compounds achieved by absorbance recorded using chromatogram relative external standards with the following equation: C(c)=(A(c))/(A(st)) × C (st). In which, C (c) is the concentration of compound in the sample, A (c) is the peak area of compound in sample chromatograms, C (st) is the concentration of standard in the reference solution and A (st) is the area of peak for the standards in reference chromatograms (Vinholes et al., 2011).

2.5. In vitro antioxidant activities

2.5.1. Azinobis 3-ethylbenzothiazoline-6-sulfonate ($ABTS^{\bullet+}$) assay

The ABTS radical cation decolorization assay was measured to estimate the total antioxidant activity of PT frond extracts according to Re et al. (1999). $ABTS^{\bullet+}$ produced by reacting 7 mM $ABTS^{\bullet+}$ aqueous solution with 2.4 mM Potassium persulfate in dark for 12–16 h at room temperature. Reagent solution diluted in ethanol (about 1:89 v/v) and equilibrated at 30 °C to give an absorbance at 734 nm of 0.7 ± 0.02. After adding 1 mL of diluted $ABTS^{\bullet+}$ solution to different concentrations of a sample or Trolox standards (final concentration 0–15 μM) in ethanol, absorbance measured at 734 nm after 30 min. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity and expressed as μmol/g sample.

2.5.2. Hydrogen peroxide (H_2O_2) scavenging activity

The ability of PT frond extracts to scavenge hydrogen peroxide was determined according to Ruch et al. (1989). A solution of hydrogen peroxide (2 μmol/L) was prepared in phosphate buffer (0.2 M, pH 7.4) and its concentration was determined at 230 nm using spectrophotometer from absorption with molar absorptivity 81 M^{-1}/cm. The PT frond extracts (10 μL) suspended solution was added to 3.4 mL of phosphate buffer together with a hydrogen peroxide solution (0.6 mL). The identical reaction mixture without sample was taken as a negative control. The absorbance of hydrogen peroxide was determined at 230 nm after 10 min against blank solution (Phosphate buffer). The scavenging activity in percentage was calculated as follows: Scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where, A_0 is the absorbance of the control (reaction mixture without sample) and A_1 is the absorbance of the sample/standard.

2.6. In vivo anti-inflammatory activity

2.6.1. Animals

Female Wistar albino rats (120–150 g) were kept in polypropylene cages with sterile husk materials. Experimental animals were maintained under controlled environmental conditions at 23 ± 2 °C and relative humidity (55 ± 10%) for 12 h dark/light cycle. All the experimental rats were allowed to acclimatize for one week along with standard pellet diet and water. Experimental protocols used on the animals were approved by the Institutional Animal Care and Use Committee of Sri Krishnadevaraya University at Anantapur, Andhra Pradesh, India (Reg. No. 25/1/99/AWD).

2.6.2. Acute toxicity

According to the Organization for Economic Co-operation and Department (OECD), the acute oral toxicity studies were carried out by random sampling technique (Ecobicon, 1997). Six Wistar albino adult female rats were selected and fasted for 12 h with free access of water only. Ethanol frond extract suspended solution was orally administered at a dose of 5 mg/kg and their mortality observed for three days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as a toxic dose. Moreover, if the mortality was observed in only one rat out of six animals, then the same dose was repeated with higher doses such as 50, 300, 500, 1000 and 2000 mg/kg. The general behaviors such as motor activity, tremors, convulsions, Straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin color were observed 1 h and 24 h later.

2.6.3. Anti-inflammatory study

In vivo anti-inflammatory activity of ethanol frond extract of PT was evaluated in Wistar albino adult female rats using standard Carrageenan-induced paw edema method (Winter et al., 1962). The animals were divided into six different groups and each group contains six rats allowed for fasting overnight only with water. Group I rats (negative control) were received water alone while group II (positive control) rats were treated with Carrageenan (10 mg/kg) to induce inflammation. The group III animals were treated with indomethacin (10 mg/kg) as a standard drug. Group IV and V rats were treated individually with 200 and 400 mg/kg of ethanol frond extract of PT, respectively. Group VI rats were treated with standard flavonoid compound, rutin (50 mg/kg). The drugs, rutin, indomethacin and ethanol frond extract of PT were orally administered 30 min before the administration of Carrageenan (Hi-Media, Mumbai) in right hind paws of experimental rats. Finally, paw volume was measured using Pliethysmometer (Tokyo, Japan) at 0, 1, 2, 3 and 24 h. Percentage of inhibition of paw edema was calculated by the following formula: Percentage of inhibition = (Vc-Vt)/Vc × 100. Where V_t is the increase in paw volume of rats treated with the sample drug; V_c is the increase in paw volume of the positive control group.

2.6.4. Biochemical assay

All experimental animals were sacrificed after 24 h of administration of Carrageenan. Both blood serum and liver samples of the sacrificed animals were collected and analyzed for biochemical and histopathological studies. The non-heparinized blood samples were allowed to coagulate before centrifugation (4000 rpm for 20 min) and their serums were separated to estimate glucose, cholesterol, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) contents using standard analytical kits from Diasys diagnostic systems (Germany) by auto chemistry analyzer (Mindray BS 300, China).

2.6.5. Histopathological examinations

A portion of the median lobe of liver samples was dissected and fixed at 10% of neutral formalin buffer solution for 24 h. Tissues were dehydrated in descending grades of isopropyl alcohol and cleared in xylene. Then, tissues were embedded in molten paraffin wax for microtomic sections about 5 µm thickness which deparaffinized and rehydrated. Tissue sections were stained using hematoxylin and eosin to study the histopathological changes of a liver sample under a light microscope (Damodara Reddy et al., 2010).

2.7. Statistical analysis

All the data represented as mean ± standard error (SE). Differences between groups were analyzed using SPSS 17.0 software (Chicago, USA) with an analysis of variance (ANOVA) followed by DMRT test and their relationships were considered to be statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Plant extracts yield (%)

Various solvent extracts of *Pteris tripartita* Sw. was quantified and represented in Table 1. Of them, the yield of plant extract was significantly higher in both water (22.71%) and methanol extracts (21.59%), respectively.

3.2. HPLC quantification of rutin and quercetin

Retention times and percentage area of flavonoids such as rutin, and quercetin were given in Fig. 1. Retention times of flavonoids namely, rutin, and quercetin were of 3.427 and 9.799 min, respectively.

Table 1
Percentage of extracts yield and their *in vitro* antioxidant activities of *Pteris tripartita* Sw.

Plant extracts	Extract yields (%)	$ABTS^{\bullet+}$ assay (µmol Trolox/g)	H_2O_2 Assay (%)
Hexane	1.82	456.29 ± 36.2	47.20 ± 0.4
Chloroform	4.93	603.44 ± 56.6	45.26 ± 0.2
Acetone	6.18	828.22 ± 42.9	14.50 ± 2.5
Ethyl acetate	4.93	1494.44 ± 61.4	31.70 ± 1.0
Ethanol	17.76	937.56 ± 4.2	3.20 ± 0.5
Methanol	21.59	702.67 ± 95.8	4.96 ± 0.1
Water	22.71	920.69 ± 11.7	26.18 ± 0.6

All values are expressed as Mean±SE of triplicate. All data were analyzed using SPSS 17.0 software with one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) and relationships were considered to be statistically significant when $p<0.05$.

Furthermore, quantities of rutin and quercetin flavonoids were of 4.634 µg/mg and 11.507 µg/mg respectively in ethanol frond extract of *P. tripartita*. In earlier studies, both flavonoids, rutin and quercetin were quantified in *Pteris multifida*, *P. vittata* and *P. semipinnata*, in which larger quantity of rutin than quercetin was present and other flavonoids like hyperin; isoquercitrin and kaempferol were also analyzed (Wang et al., 2010). In our present study, a higher percentage of quercetin was present than rutin in ethanol frond extract of *P. tripartita*. According to John de Britto et al. (2012), *Pteris biaurita* crude extracts revealed the presence of steroids, triterpenoids, reducing sugars, alkaloids, phenolics, flavonoids, saponins, and tannins. Flavonoids act as antioxidant agents by direct, free radical scavenging, transition metal chelation, and maintenance of endogenous antioxidants such as glutathione and superoxide dismutase systems (Tourino et al., 2005). Several earlier studies proved the antioxidant activities of flavonoids namely, quercetin and rutin (Conforti et al., 2007; Sagrantini et al., 2008; Filippini et al., 2010). Moreover, flavonoid quercetin was also reported in *Pteris vittata*, *P. plumula* and *P. deflexa* (Salatino and Prado, 1998). Apart from *Pteris* genus, some ferns like *Adiantum tetraphyllum*, *Blechnum regnellianum*, *Cheilanthes goyazensis, Doryopteris concolor*, *D. ornithopus*, *Pellaea gleichenioides, Pteris cymbiformis, P. pinnata* and *P. riedelii* were also reported for the presence of quercetin (Salatino and Prado, 1998; Melos et al., 2007). Several flavonoids are widely distributed in fern species. The 3, 8-Di-C-arabinosyl luteolin, 3-O-(2″, 3″-di-O-*p*-coumaroyl)-glucosides, 7-O-rhamnoside and 7-O-*p*-hydroxybenzoate, and three di-C-glycosyl flavones have been separated from *P. vittata* (Imperato, 2002, 2003; 2004, 2006). The main chemical ingredients in *Pteris multifida* are tannins and flavonoids such as rutin, luteolin and apigenin (Lu et al., 1999). Besides, a new flavonoid is known as, kaempferol 3-O-α-L-rhamnopyranoside-7-O-[α-D-apiofuranosyl-(1–2)-β-D-glucopy ranoside] has been isolated and determined from *Pteris ensiformis* (Chen et al., 2007). Flavonoid components of *Pteris multifida* were extracted as active ingredients to treat hepatitis (Wang and Zhang, 2008). Giannasi and Mickel (1979) were obtained the glycosides of kaempferol and quercetin from *Hemionitis* sp. leaf tissues. The Pteridaceae family ferns contain quercetin, kaempferol, apigenin and luteolin in an earlier study (Salatino and Prado, 1998).

3.3. $ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ assay is an excellent tool to determine hydrogen-donating antioxidants (scavenging aqueous phase radicals) and chain-breaking antioxidants (scavenging lipid peroxyl radicals). The antioxidant activity of *P. tripartita* ranged from 456.29 to 1494.44 µmol Trolox/g extract (Table 1). Lowest antioxidant activities were obtained in both hexane and chloroform extracts (456.29 and 603.44 µmol Trolox/g extract). In a previous study, low polar solvent extracts like petroleum ether and chloroform extracts of *Ficus talboti* fruit showed less activity due to a small number of bioactive compounds with minimal ranges (Arunachalam and Parimelazhagan, 2014). Significant Trolox equivalent

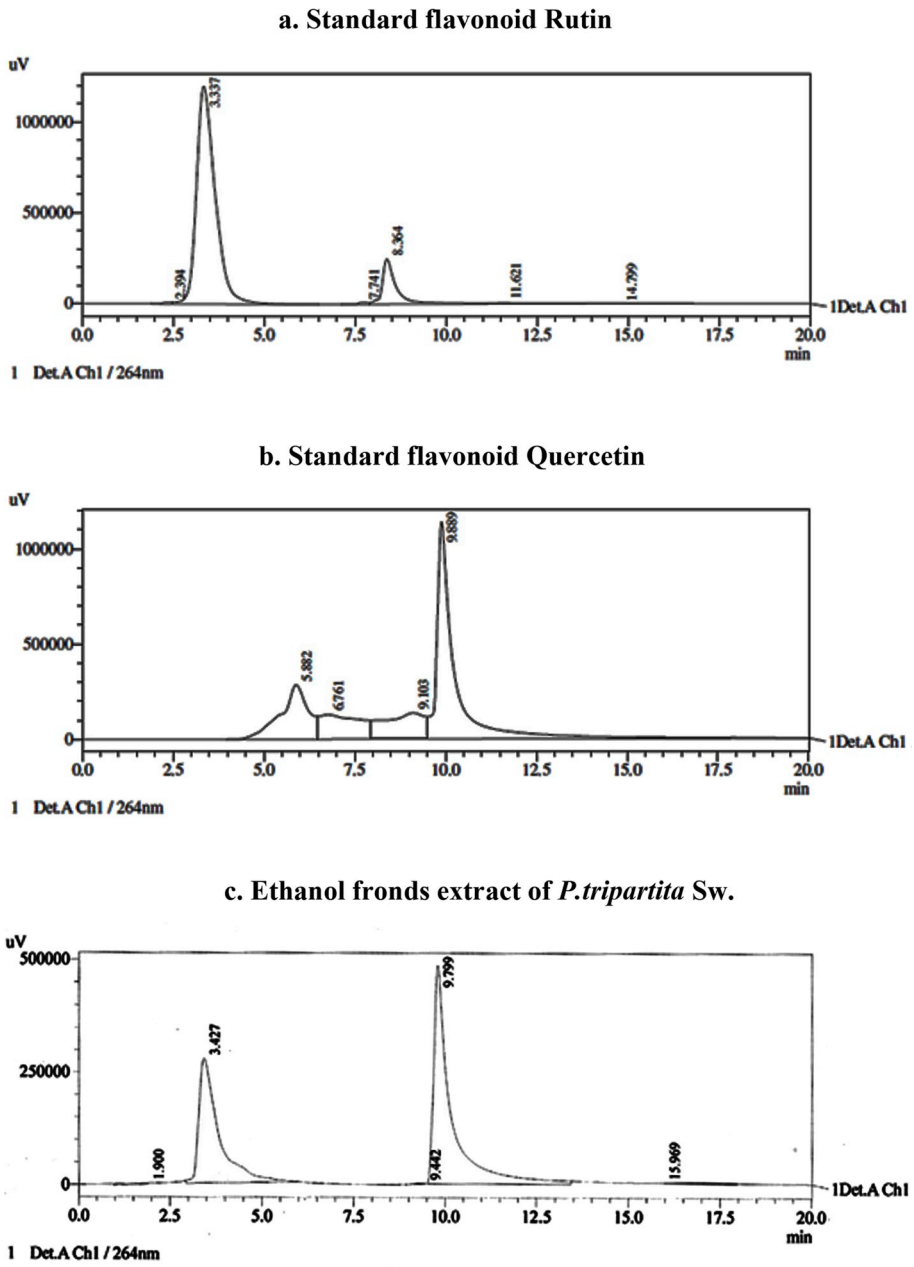

Fig. 1. HPLC analysis of (a) standard rutin, (b) quercetin and (c) ethanol frond extract of *P. tripartita* Sw.

antioxidant capacity (TEAC) was observed in ethanol (937.56) and ethyl acetate (1494.44 µmol Trolox/g extract) extracts of PT while hexane (456.29 µmol Trolox/g extract), chloroform (603.44 µmol Trolox/g extract), acetone (828.22 µmol Trolox/g extract), methanol (702.67 µmol Trolox/g extract) and water extracts (920.69 µmol Trolox/g extract) showed moderate activities in our present study (Fig. 2). Hagerman et al. (1998) reported that high molecular weight phenolics (tannins) exhibit better ability to quench free radicals ($ABTS^{\bullet+}$) and its effectiveness depends on molecular weight, number of aromatic rings and nature of hydroxyl group substitution than the specific functional groups. Due to the high content of phenolic compounds, antioxidant capacity can be observed in plant extracts. The activity of putative antioxidants has been attributed to various mechanisms, binding of transition metal ion catalysts and reductive capacity (Hayouni et al., 2007). Hydrolyzable tannins showed antioxidant activity in medicinal plants (Boulekbache-Makhlouf et al., 2013). Also, a significant amount of total phenols, flavonoids, tannins and vitamins were quantified in frond

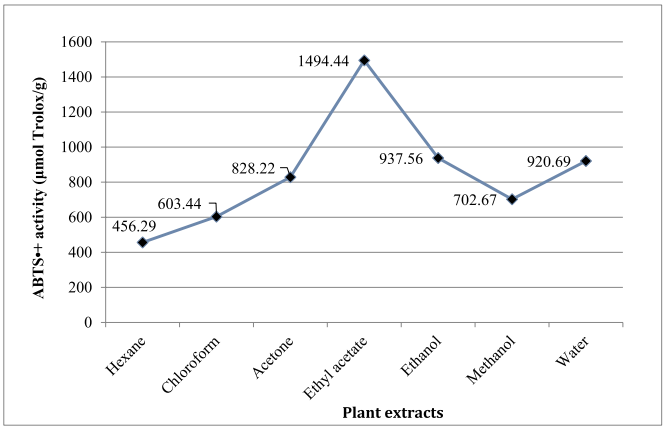

Fig. 2. ABTS•+ assay of *P. tripartita* Sw. frond extracts.

extracts of *P. tripartita* in our earlier study (Baskaran and Jeyachandran, 2010). Some phenolic antioxidants have been identified in Sword brake fern, *Pteris ensiformis* Burm. (Chen et al., 2007).

3.4. Hydrogen peroxide scavenging capacity

Both ethanol and methanol extracts of *P. tripartita* showed least scavenging activities (3.20% and 4.96%) whereas, hexane (47.20%) and chloroform extracts (45.26%) revealed maximum percentages of antioxidant capacity in hydrogen peroxide radical scavenging assay. The inhibitory activity of ethyl acetate and water extracts was found to be 31.70% and 26.18% while acetone extract exhibited 14.50% (Table 1). In previous report, major phenolic components namely, 4-hydroxycinnamic acid (ρ-coumaric), 3 methoxy-4-hydroxycinnamic acid (ferulic), 3,4-dihydroxycinnamic acid (caffeic), 3-methoxy-4-hydroxybenzoic acid and 3-caffeoilquinic acid (chlorogenic) were found in *Polypodium leucotomos* fern extract and proved to be strong antioxidant properties using H_2O_2 assay (Gombau et al., 2006).

3.5. In vivo anti-inflammatory activity

Carrageenan is a noxious agent and widely used to induce experimental inflammation to screen anti-inflammatory activity. This phlogistic agent injects in rat hind paw which produces a severe inflammatory reaction within 30 min (Ojewole, 2006; Gurib-Fakim, 2006). Numerous flavonoid components from medicinal ferns were reported namely, apigenin, luteolin, naringenin and kaempferol which showed strong antioxidant capacity and biomedical activities such as, reducing blood lipid levels, liver protection, resisting inflammation, relaxing coronary arteries and antibacterial activities (Milovanovic et al., 2007; Ho et al., 2011). Besides, polyphenolic compounds were recognized as anti-inflammatory agents which inhibit hydrolytic and oxidative enzymes (Frankel, 1995).

Table 2 summarized the anti-inflammatory potential of flavonoid rutin and ethanol frond extract of *Pteris tripartita* Sw. using carrageenan-induced paw volume test. Both rutin flavonoid and ethanol extract of *P. tripartita* Sw. showed significant anti-inflammatory activity at independent doses of extract. There was an increased paw volume in carrageenan-treated group rats. However, ethanol extract (200 mg/kg) of *P. tripartita* showed better paw edema inhibition (52.09%) after 3 h whereas standard drug indomethacin exhibited 60.30% of inhibition. The inhibition value slightly reduced in ethanol frond extract of *P. tripartita* at 400 mg/kg. Of them, 51.52% of inhibition was observed in 400 mg/kg, while standard flavonoid rutin showed 50.19% of inhibition after 3 h (Fig. 3). However, ethanol frond extract of *P. tripartita* at 200, 400 mg/kg and standard rutin (50 mg/kg) were displayed significant anti-inflammatory activities and their edema inhibition was of 62.63%, 48.64% and 59.08% after 24 h, respectively. In the previous study, alcoholic extract of *Equisetum arvense* showed anti-inflammatory and analgesic activities (Do Monte et al., 2004). The fern, *Cyathea phalerata* extracts showed hypoglycemic and anti-inflammatory activities (Pizzolatti et al., 2007). Methanol crude extracts of *Blechnum occidentale* showed antinociceptive and anti-inflammatory activities (Nonato et al., 2009). Several authors reported that plant ethanol extracts, rutin, and quercetin were proved various bioactivities (Afanaseva et al., 2001; Guardia et al., 2001; Selloum et al., 2003; Rotelli et al., 2009; Rogerio et al., 2010; Rajamanickam et al., 2010; Gurudeeban et al., 2010, 2013; 2014; Lee et al., 2013; Selvaraj et al., 2014; Azevedo et al., 2013; Satyavani et al., 2015, 2018; Vijayakumar et al., 2018; Divya et al., 2018; Bharathi et al., 2018; Sathasivam et al., 2019).

In our study, ethanol frond extract of *Pteris tripartita* showed scavenging activities of free radicals. According to Lee and Lin (1988), spider brake fern has multiple flavonoids and possesses dampness-eliminating, heat-clearing, antipyretic, detoxify, antibiotic, anti-inflammation and antimutagenic activities. Various mono- and sesquiterpenes of essential oils proved their anti-inflammatory effect by several authors (Peana et al., 2002; Sayyah et al., 2007; Fernandes et al., 2007; Ko et al., 2008; Dung et al., 2009). In our previous study, terpenoid was also reported in ethanol frond extract of *P. tripartita* (Baskaran and Jeyachandran, 2010). Moreover, sesquiterpenes are an important group of secondary metabolites which proved various biological activities such as, anticancer, anti-inflammatory, cytotoxic, antimicrobial properties and plant growth regulation (Baruah et al., 1994). Anti-inflammatory activities of following fatty acids namely, myristic, palmitic, stearic, oleic–linoleic and eicosatrienoic compounds are widely known (Bremner and Heinrich, 2002). Stearic acid exhibited both anti-inflammatory and hepatoprotective effects (Pan et al., 2010). Moreover, plant secondary metabolites such as, phenolic compounds (Sakat et al., 2010; Roy et al., 2010; Garg et al., 2010; Delgado-Adamez et al., 2012), flavonoids (Amaral et al., 2009), terpenoids (Neukirch et al., 2005) and tannins (Fawole et al., 2010) proved their anti-inflammatory activities.

3.6. Biochemical parameter and histology of liver

In biochemical assays, blood-glucose levels were reduced in both 200 and 400 mg/kg of ethanol frond extract of *P. tripartita* while compared to the control group (Normal). Furthermore, lysosomal enzymes such as alanine transaminase, aspartate transaminase, alkaline phosphatase and acid phosphatase were elevated in the sacrificed animal blood serums. Of them, lower amount of AST (282.33 U/l), ALT (39.16 U/l), ALP (229.66 U/l) and ACP (0.36 U/l) were obtained in 200 mg/kg, while 400 mg/kg extract increased AST (355.83 U/l), ALT (64.83 U/l), ALP (270.83 U/l) and ACP (0.51 U/l) (Table 3). Standard flavonoid rutin reduced the amount of ACP (0.41 U/l). Glucose (137 mg/dl), cholesterol (49.33 mg/dl), AST (214.33 U/l), ALT (42.16 U/l) and ALP (139.50 U/l) than normal group, accordingly. In previous studies, alanine amino transferase, aspartate amino transferase, alkaline phosphatase were elevated in plant extracts treated animals than control groups (Adeyemi and Akanji, 2010; Kamisan et al., 2014; Iqbal et al., 2018).

The normal group liver tissue showed normal architecture of hepatocyte, portal hepatic vein and lack of pyknosis (Fig. 4a). Ethanol frond extract of PT (200 mg/kg) showed mild perivascular inflammation, slight portal vein damage and lack of sinusoidal damage, fatty changes or any other changes (Fig. 4b). Ethanol frond extract of PT (400 mg/kg) showed mild disarray, cholestasis, more inflammation, cytoplasmic granularity, absence of fatty change, normal architecture of hepatocytes

Table 2
In vivo anti-inflammatory activities of an ethanol frond extract of *P. tripartita* Sw.

Animal Groups	Drugs (mg/kg)	0 h	1 h	2 h	3 h	24 h
I	Normal (Negative)	2.38 ± 0.08	2.72 ± 0.09	2.53 ± 0.14	2.58 ± 0.07	2.49 ± 0.04
II	Carrageenan (Positive)	4.33 ± 0.38	4.75 ± 0.46	4.94 ± 0.44	5.24 ± 0.31	4.79 ± 0.38
III	Indomethacin 10	2.55 ± 0.13 (41.10%)	2.30 ± 0.09 (51.57%)	2.53 ± 0.13 (48.78%)	2.08 ± 0.03 (60.30%)	1.48 ± 0.11 (69.10%)
IV	EPT 200	3.29 ± 0.11 (24.01%)	3.35 ± 0.19 (29.47%)	3.08 ± 0.17 (37.65%)	2.51 ± 0.14 (52.09%)	1.79 ± 0.07 (62.63%)
V	EPT 400	3.55 ± 0.32 (18.01%)	3.13 ± 0.31 (34.10%)	2.58 ± 0.17 (47.77%)	2.54 ± 0.10 (51.52%)	2.46 ± 0.16 (48.64%)
VI	Rutin 50	3.21 ± 0.18 (25.86%)	2.70 ± 0.26 (43.15%)	2.71 ± 0.31 (45.14%)	2.61 ± 0.28 (50.19%)	1.96 ± 0.08 (59.08%)

EPT= Ethanol frond extract of *Pteris tripartita* Sw. All values are expressed as Mean±SE of six rats. All data were analyzed using SPSS 17.0 software with one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) and relationships were considered to be statistically significant when $p<0.05$.

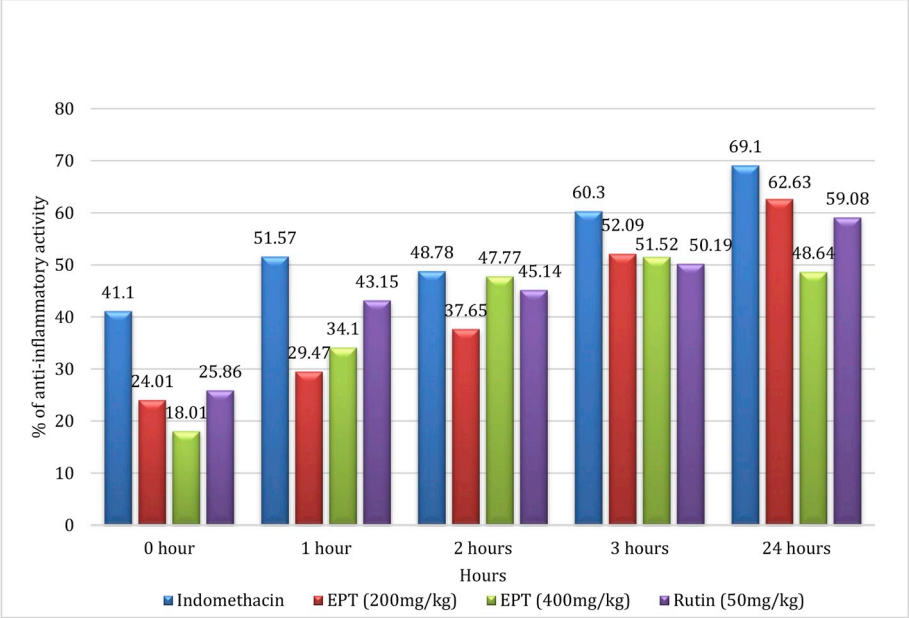

Fig. 3. Effects of frond ethanol extract of *P. tripartita* Sw. (EPT) and rutin on carrageenan induced Swiss albino rats.

Table 3
Effects of an ethanol frond extract of *Pteris tripartita* on glucose, total cholesterol, creatinine and serum enzymes in Carrageenan induced Wistar Albino female rats.

Parameters	Animal groups			
	Normal	EPT 200 (mg/kg)	EPT 400 (mg/kg)	Rutin 50 (mg/kg)
Glucose (mg/dl)	143.00 ± 6.28	141.16 ± 2.93	141.00 ± 3.56	137.00 ± 2.42
Creatinine (mg/dl)	0.29 ± 0.00	0.45 ± 0.02	0.29 ± 0.02	0.49 ± 0.01
Cholesterol (mg/dl)	64.66 ± 1.49	63.50 ± 3.97	50.50 ± 2.44	49.33 ± 0.95
AST (U/I)	283.66 ± 4.91	282.33 ± 19.6	355.83 ± 37.82	214.33 ± 23.24
ALT (U/I)	86.66 ± 3.47	39.16 ± 0.90	64.83 ± 4.81	42.16 ± 3.81
ALP (IU/I)	139.83 ± 18.49	229.66 ± 15.91	270.83 ± 14.91	139.50 ± 6.56
ACP (IU/I)	0.23 ± 0.03	0.36 ± 0.04	0.51 ± 0.09	0.41 ± 0.07

Each value represents the mean±SE of six rats. All data were analyzed using SPSS 17.0 software with one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at $P<0.05$. EPT: Ethanol frond extract of *Pteris tripartita* Sw., AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, ACP: Acid phosphatase.

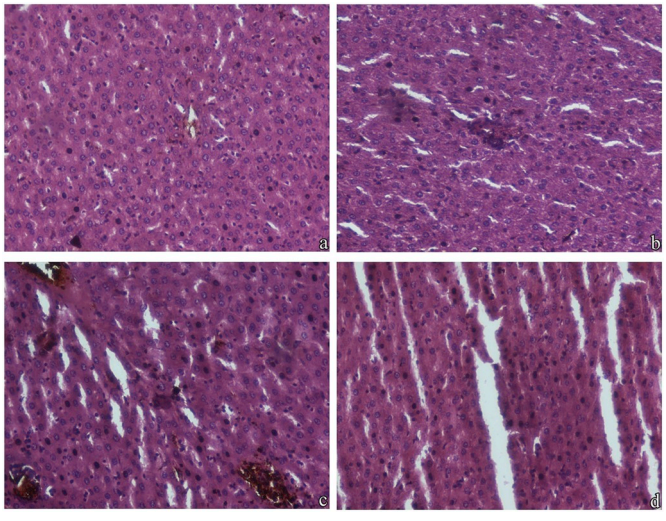

Fig. 4. Histology of liver in experimental rats (40x). a) Normal; b) *P. tripartita* Sw. ethanol frond extract (200 mg/kg b.w.); c) *P. tripartita* Sw. ethanol frond extract (400 mg/kg b.w.); d) Standard flavonoid Rutin (50 mg/kg b.w.).

without any cell damage and central portal vein damage (Fig. 4c). Liver tissue of standard flavonoid rutin showed mild inflammation, cholestasis, no fatty change or shrinkage, central hepatic veins without any damage and absence of necrosis or water infiltration (Fig. 4d).

On the other hand, *Pteris vittata* possess flavonoid rutin in aqueous methanol extract which showed antimicrobial activity (Singh et al., 2008b). Some other *Pteris* ferns such as *P. multifidi*, *P. biaurita*, *P. ensiformis* have been reported already for their anticancer, antioxidant, antibacterial activities due to phenolic acids, flavonoids and terpenoids (Jiangsu New Medical College, 1985; Chen et al., 2007; Dalli et al., 2007; Hao-bin et al., 2008; Hao-bin et al., 2009). However, standard flavonoid rutin and ethanol frond extract of *Pteris tripartita* Sw. possess significant antioxidant and *in vivo* anti-inflammatory activities.

4. Conclusion

Our present study reveals that the ethanol extract of *P. tripartita* exhibited significant antioxidant and anti-inflammatory activities. Due to bioactive principles, ethanol extract of *P.tripartita* Sw. was efficient to scavenge free radicals. The ethyl acetate, water, and ethanol extracts showed higher ABTS radical scavenging activity. Ethanol frond extract of *P. tripartita* did not possess any side effects, malnutrition, abnormal behavioral changes, and toxicity. A higher percentage of anti-inflammatory activity was exhibited by ethanol extract than flavonoid rutin due to several secondary metabolites in our plant extract. Hereby, further studies are needed to determine other bioactive compounds and

their mode of action of *P. tripartita* Sw.

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Conflicts of interest

There is none to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101304.

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