

## Chemical composition and anti-inflammatory effect of *Phellodendron amurense* Rupr. stem bark extract

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### Abstract

In many diseases inflammation and oxidative stress coexist and are therapeutic targets. *Phellodendron amurense* Rupr. has anti-inflammatory and antioxidant effects, but the mechanisms are not completely elucidated. Therefore, first *P. amurense* stem bark extract was analysed regarding chemical compounds such as total polyphenol content (TPC), polyphenols (gallic acid, 4-hydroxybenzoic acid, caffeic acid and ferulic acid) and alkaloids (berbamine, jatrorrhizine, palmatine, and berberine). Quantitative determination of alkaloids revealed that berberine had the highest concentration of  $2.44 \pm 0.22$  mg/g. *In vitro* antioxidant activity was significant. Then, the *in vivo* anti-inflammatory and antioxidative effects of *P. amurense* extract were studied before and after acute experimental rat inflammation induction in order to find some mechanisms of these effects. The *P. amurense* stem bark extract had a good anti-inflammatory activity by reducing nitric oxide, 3-nitrotyrosine and NF- $\kappa$ B, and an antioxidant activity by lowering oxidants and increasing antioxidants. This study provides scientific knowledge and could contribute to the development of novel drugs based on *P. amurense* stem bark extract for the treatment of inflammatory diseases and prevention of oxidative stress.

**Keywords:** alkaloids; Amur cork tree; anti-inflammatory; antioxidant; polyphenols

Received: 23 Jul 2023. Received in revised form: 05 Sep 2023. Accepted: 06 Sep 2023. Published online: 11 Sep 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

## Introduction

Inflammation is a complex adaptive response to cell injury triggered by exogenous and endogenous stimuli. As long as inflammatory response continues it is a risk for the development of chronic inflammation (Allegra, 2019). An excessive or chronic inflammatory response is involved in the pathophysiology of many chronic diseases and cancer. In such conditions, inflammation and oxidative stress are tightly linked processes because phagocytic and nonphagocytic cells produce excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS), induce local oxidative stress and cause tissue injury (Balázová *et al.*, 2022). Moreover, it has been demonstrated that bilateral connections exist between oxidative stress and the inflammatory response. Alterations of the inflammatory response may trigger oxidative stress, and oxidative stress may cause dysfunction of inflammatory cells (Allegra, 2019). Therefore, treatment should target simultaneously both inflammation and oxidative stress starting from the early stages of the inflammatory response.

The available conventional synthetic anti-inflammatory and antioxidants drugs have important side effects. In an attempt to develop new anti-inflammatory and antioxidant drugs that are as potent as current synthetic drugs, safe plant products with anti-inflammatory and antioxidant activity are attractive (Allegra, 2019). Plants contain phytochemicals responsible for the pharmacological effects, and many of them significantly modulate the inflammatory response and the oxidative stress. The mechanisms can be direct antioxidant activity, radical-scavenging activity, or by acting on the ROS/RNS cell signalling pathways (Allegra, 2019).

*Phellodendron amurense* Rupr., commonly called the Amur cork tree, belongs to the Rutaceae family, and it is mainly distributed in northern forests of Japan, China, and Korea (Xian *et al.*, 2014). In Asia it is widely used in traditional medicine for the treatment of infectious diseases, like pneumonia, tuberculosis and dysentery, to improve blood circulation (Jung *et al.*, 2017), as well to treat some digestive problems, like diarrhea, dyspepsia, hepatitis and gastroenteritis (Balázová *et al.*, 2022). A study suggested that in HIV patients with neurological diseases *P. amurense* bark extract use represents a lower risks of all-cause mortality (Chiou *et al.*, 2023). Besides these, in ethnopharmacology the dried trunk bark of *P. amurense* was used as an anti-inflammatory treatment in chronic inflammatory diseases as rheumatoid arthritis (Lipsky and Tao, 1997) and lupus erythematosus (Qin *et al.*, 1981). Furthermore, some studies proved the anti-inflammatory *P. amurense* bark extract properties on animal models of acute inflammation, such as the croton oil-induced ear edema and an acetic acid-induced capillary permeability, and of chronic inflammation, like the cotton pellet test and a delayed-type hypersensitivity test (Cuéllar *et al.*, 2001; Park *et al.*, 2007). *P. amurense* bark extract also protects cartilage destruction and osteoarthritis progression (Kim *et al.*, 2011). Berberine, an isoquinoline alkaloid component of *P. amurense*, and *P. amurense* have Cox-2 inhibition activities and other anti-inflammatory effects (James *et al.*, 2011; Li *et al.*, 2019). Moreover, Phellodendri cortex stimulated bone growth by upregulating insulin-like growth factor (IGF)-1 and bone morphogenetic protein (BMP)-2 in the growth plate (Lee *et al.*, 2018), exerts a beneficial gastroprotective effect through a neurohumoral mechanism regulation (Wang *et al.*, 2017).

The tumour microenvironment includes malignant cells and non-malignant cells such as macrophages, neutrophils, endothelial cells, fibroblasts, and others. Initially, the non-malignant cells have tumour suppression activity, but later tumour cells trigger complex inflammatory and immune responses, and then non-tumour cells become tumour promoter (Santos *et al.*, 2018). Because in cancer inflammation and oxidative stress are closely correlated pathophysiological processes, one recent therapeutic strategy is to look for compounds that target both inflammation and oxidative stress (Biswas, 2016a). In traditional Chinese medicine the cortex of *P. amurense* has been used to treat patients who suffer from cancer because it is considered to have antitumor, immunostimulatory and anti-inflammatory effects (Park *et al.*, 1999; Chen *et al.*, 2010). The *P. amurense* bark extract and berberine were studied for the antitumor activity. It has been

found that oral administration of berberine in its pure form or as a part of *P. amurense* extract inhibits some types of lung (James *et al.*, 2011), prostate (James *et al.*, 2011), pancreatic, breast (Mi *et al.*, 2023) and skin cancers (Balážová *et al.*, 2022). On the market there are some preparations of *P. amurense* bark extract used for the anti-inflammatory, analgesic, and anticancer effects (e.g., Nexrutine®, Relora®) (Balážová *et al.*, 2022).

Recent studies have shown that *P. amurense* extracts possess antioxidant functions too (Jung *et al.*, 2017). Little is known about the mechanisms involved in the antioxidant activities of *P. amurense* stem bark extract. Therefore, this study primary aim was not to report a novel biological activity of the *P. amurense* stem bark extract, then to provide experimental evidence for some antioxidant mechanisms that were not thoroughly investigated. Because previous research analysed just the therapeutic treatment effects, the second aim was to compare the effects of the prophylactic and therapeutic treatments with *P. amurense* stem bark extract.

## Materials and Methods

### *Plant extract preparation*

*P. amurense* Rupr. was collected from the “Alexandru Borza” Botanical Garden of Cluj-Napoca (46°45'36"N and 23°35'13"E) in June 2020 and was identified by Dr. M. Parvu, Babes-Bolyai University of Cluj-Napoca. The plants were taxonomically identified, authenticated and voucher specimens (CL 669022) were deposited in the Herbarium of “Alexandru Borza” Botanical Garden, “Babeş-Bolyai” University, Cluj-Napoca, Romania.

Fresh *P. amurense* stem bark (fragments of 0.5-1 cm) was extracted with 70% ethanol (Merck, Bucuresti, Romania) by cold reperloration method, at room temperature, for 3 days (Pârvu *et al.*, 2019). The *P. amurense* extract, containing 1 g plant material in 1 mL of 30% ethanol (w/v), was obtained by filtration (Pârvu *et al.*, 2019).

### *Phytochemical analysis*

#### Total polyphenols content

The total polyphenol content (TPC) of the *P. amurense* stem bark extract was measured using the Folin–Ciocalteu method with some modifications. The absorbance was measured at 760 nm with a JASCO UV-VIS spectrophotometer, and results were expressed as gallic acid equivalents (mg GAE/g d.w. plant material). The assay was performed in triplicate (Pfingstgraf *et al.*, 2021).

#### Identification and quantification of polyphenolic compounds by HPLC-DAD

The identification and determination of the most important analytes in the studied samples was achieved using a HPLC-DAD approach. Analysis was performed on an Agilent 1200 HPLC system (Waldbronn, Germany) equipped with an on-line vacuum degasser and a quaternary pump. The samples to be analysed were placed in a temperature-controlled sample tray and the injection was performed using an automatic injector. The chromatographic column was placed in a thermostat compartment and the detection was done using a DAD detector. The chromatographic separations were run on an Eclipse XBD-C18 column (150 mm x 4.6 mm, 5 µm particle size) from Agilent (Waldbronn, Germany). The injection volume was 10 µL (10 times diluted extract using methanol followed by 0.2 µm filtering), the column temperature was set to 30°C and the flow rate was 1.0 mL/min. Preliminary trails were employed for method optimization by varying the gradient steps. The optimum method consisted of a gradient elution using solvent A, 0.1% TFA and solvent B as acetonitrile. The gradient was as follows: 0-2 min isocratic at 5% B, 2-20 min from 5 to 25% B, 20-29 min isocratic at 25% B, 29-30 min from 25 to 30%, 30-35 min from 30% to 100% B, 35-37 min isocratic at 100% B and 37-37.5 min back to 5% B where was kept until 40 min. As standards there were gallic acid, 4-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, berbamine, jatrorrhizine, palmatine and

berberine, of analytical grade purity for different commercially available sources. Calibration curve was constructed for each compound at 11, 22, 44, 88, 175, 340  $\mu\text{g}/\text{mL}$  using the area of the peak by integration employed by the Agilent soft. The limit of detection (LOD) and limit of quantification (LOQ) were for the same chromatographic system was previously determined. The UV-VIS detection has been done using the DAD detector that measured the entire spectrum in 190-550 nm region, every 1 s and the chromatograms were monitored at 235, 280, 345 and 430 nm. The identification of the compounds was performed using both chromatographic retention time and spectral similarities which were done by the built-in soft. The chromatograms were digitally exported and the graphs were performed in Excel.

#### *Antioxidant activity evaluation*

##### Chemicals

Methanol, ferrous ammonium sulfate, vanadium chloride (III) ( $\text{VCl}_3$ ), sulfanilamide (SULF), N- (1-Naphthyl) ethylenediaminedihydrochloric acid (NEDD), xylenol orange [ocresosulfonphthalein-3,3-bis (sodium methyliminodiacetate)], hydrochloric acid, ortho dianisidine dihydrochloric acid (3-3'-dimethoxybenzidine), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), sulfuric acid, thiobarbituric acid, o-phthalaldehydewere purchased from Merck (Darmstadt, Germany); Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Alfa-Aesar (Karlsruhe, Germany). The 3-nitrotyrosine ELISA kit was purchased from ABNOVA (KA0445-ABNOVA EMBLEM, Heidelberg, Germany), and the nuclear factor- $\kappa\text{B}$  (NF $\kappa\text{B}$ ) ELISA kit from Biothec (EU2560- Fine Biothec, Wuhan, China).

#### *In vitro antioxidant activity*

##### DPPH radical scavenging activity

Analysis DPPH radical scavenging activity of *P. amurense* stem bark extract was measured as previously described (Biswas, 2016b). Briefly, 3 mL of *P. amurense* stem bark extract, 1 mL DPPH, and 0.1 mM methanol solution were added. After 30 min at room temperature and in the dark, absorbance was measured at 517 nm against a blank, and the percentage of radical scavenging activity (AA%) was calculated by the formula:  $\text{AA}\% = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100$ . A Trolox standard solutions calibration curve (0.5-5  $\mu\text{g}/\text{mL}$ ) was used, and AA% was converted to  $\mu\text{g}$  Trolox equiv./g d.w. plant material (Balea *et al.*, 2020).

##### Ferric reducing antioxidant power

The reduction capacity of the *P. amurense* stem bark extract was measured with the ferric reducing antioxidant power (FRAP) assay as previously described (Fernandes *et al.*, 2016). Briefly, 3.4  $\mu\text{L}$  FRAP reagent and 100  $\mu\text{L}$  *P. amurense* stem bark extract sample were added and mixed thoroughly. After 30 min, the absorbance was taken at 593 nm using a JASCO UV-VIS spectrophotometer, and the results were expressed as IC<sub>50</sub> in ( $\mu\text{g}$  Trolox equiv./g d.w. plant material).

##### Hydrogen peroxide scavenging activity

The ability of the *P. amurense* stem bark extract to scavenge hydrogen peroxide was determined as previously described (Bhatti *et al.*, 2015). Briefly, *P. amurense* stem bark extract in distilled water was added to  $\text{H}_2\text{O}_2$  solution and after 10 min absorbance was taken at 230 nm against a blank solution of phosphate buffer by using a JASCO UV-VIS spectrophotometer. The percentage of  $\text{H}_2\text{O}_2$  scavenging was calculated as follows:  $\% \text{ scavenged } \text{H}_2\text{O}_2 = (\text{A control} - \text{A sample}) / \text{A control} \times 100$  (Chera *et al.*, 2022). The results were expressed as IC<sub>50</sub> in mg Trolox equiv./g d.w. plant material.

##### Nitric oxide radical scavenging

The Nitric Oxide Radical Scavenging Assay was performed as previously described (Taukoorah and Mahomoodally, 2016). Nitric oxide (NO) was generated from sodium nitroprusside, and it was measured by

the Griess reagent. Briefly, *P. amurense* stem bark extract (0.5 mL) was added to an SNP solution (2 mL SNP and 0.5 mL PBS, pH 7.4), and then incubated for 2.5 h at 25 °C; 0.5 mL of this mixture was added to 1 mL sulphanic acid; after 5 min 1 mL Naphthylethylene-diamine-dihydrochloride was added to the mixture; the final mixture was vortexed and incubated for 30 min. The absorbance was read at 546 nm, using a JASCO UV-VIS spectrophotometer and the percentage of inhibition was calculated as follows: % inhibition = (A blank – A sample/A blank) × 100. Results were expressed as IC<sub>50</sub> in mg Trolox equiv./g d.w. plant material. All antioxidant assays were performed in triplicate.

#### *In vivo anti-inflammatory and antioxidant activity*

##### Animal subjects

For the evaluation of *P. amurense* stem bark extract effect on the acute experimental inflammation Wistar albino male rats (strain Ctrl:WI) with a mean weight of 250 (± 15) g, obtained from the Animal Centre (Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca) were used. During the study animals were housed in polypropylene cages, with free access to standard pellet food and water, and a proper laboratory environment (12 h light/dark cycles, at an ambient temperature of 21 °C). All *in vivo* experiments were conducted in triplicate.

##### Experimental protocol

For the evaluation of the prophylactic and therapeutic anti-inflammatory and antioxidant activities of the *P. amurense* stem bark extract acute inflammation was induced by a single turpentine oil (6 mL/kg BW, i.m.) administration, respectively in the prophylaxis groups in day 8 and in the treatment groups in the first day. Nine groups (n = 5) were used: (1) negative control (Control), with no inflammation and no treatment; (2) inflammation (Inflam), with inflammation and no treatment, (3) seven days of treatment with *P. amurense* stem bark extract 100% (1 mL/200 g BW) followed by inflammation in day 8 (Phel100/Inflam); (4) seven days of treatment with *P. amurense* stem bark extract 50% (1 mL/200g BW) followed by inflammation in day 8 (Phel50/Inflam); (5) seven days of treatment with *P. amurense* stem bark extract 25% (1 mL/200 g BW) followed by inflammation in day 8 (Phel25/Inflam); (6) inflammation followed by seven days of treatment with *P. amurense* stem bark extract 100% (1 mL/200 g BW) (Inflam/Phel100); (7) inflammation followed by seven days of treatment with *P. amurense* stem bark extract 50% (1 mL/200 g BW) (Inflam/Phel50); (8) inflammation followed by seven days of treatment with *P. amurense* stem bark extract 25% (1 mL/200 g BW) (Inflam/Phel25); (9) inflammation followed by seven days of treatment with diclofenac (20 mg/kg BW) (Inflam/Diclo). All prophylactic and therapeutic treatments were performed by gavage (1 mL/day p.o.). On day 9, animals were anesthetized (20 mg/kg BW xylazine, 50 mg/kg BW ketamine), blood samples were collected by retro-orbital puncture and the obtained serum was kept at –80 °C until analysis (Balea *et al.*, 2020). Afterward, the animals were sacrificed by cervical dislocation.

#### *In vivo anti-inflammatory and oxidative stress markers*

The anti-inflammatory activity was assessed by measuring serum nuclear factor-kB (NF-kB), 3-nitrotyrosine (3NT), and total nitrates and nitrites (NO<sub>x</sub>). NF-kB and 3NT were measured according to the ELISA kits instructions. The Griess reaction was used to indirectly determine NO<sub>x</sub> and the results were expressed as nitrite μmol/L (Miranda *et al.*, 2001; Ghasemi *et al.*, 2007).

The serum oxidative stress was evaluated with general markers, as total antioxidant capacity (TAC) (Erel, 2004), total oxidant status (TOS) (Erel, 2005), and oxidative stress index (OSI) (Yıldız *et al.*, 2008), plus some specific markers, like total thiols (SH) (Erel and Neselioglu, 2014), and malondialdehyde (MDA) (Tomşa *et al.*, 2021) by using colorimetric assays. TAR results were expressed as mmol Trolox equiv/L, and TOS results were expressed in μmol H<sub>2</sub>O<sub>2</sub>equiv/L. OSI was calculated: OSI (arbitrary unit) = TOS (μmol H<sub>2</sub>O<sub>2</sub>equiv/L)/TAR (mmol Trolox equiv/L). Malondialdehyde (MDA), a lipid peroxidation marker, was

measured using thiobarbituric acid and the results were expressed as nM/mL of serum. Total thiols (SH) were estimated using Ellman's reagent and the results were expressed as mM GSH/mL. All serum spectrophotometric measurements were performed using a Jasco V-530 UV-VIS spectrophotometer (Jasco International Co. Ltd., Tokyo, Japan).

#### Statistical analysis

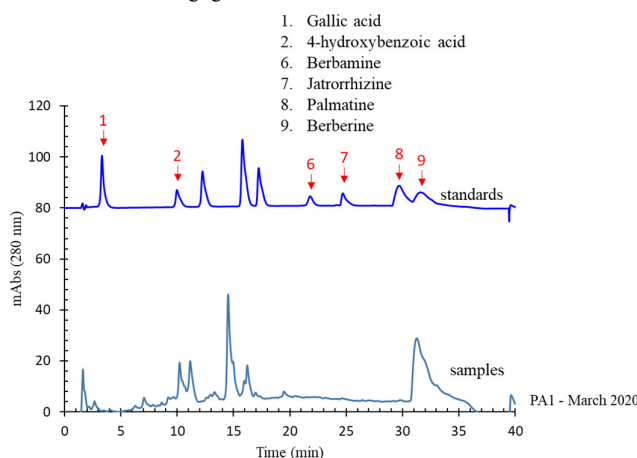
All results were expressed as mean  $\pm$  standard deviation (SD) whenever data were normally distributed. Experimental groups were compared by using the one-way ANOVA test and the post hoc Bonferroni–Holm test. The correlations analysis between each group parameter was performed with the Pearson test. Principal Component Analysis (PCA) was also performed. A  $p < 0.05$  was considered statistically significant. The statistical analysis was performed by using IBM SPSS Statistics, version 20 (SPSS Inc. Chicago, IL, USA).

## Results

#### Phytochemical analysis

TPC was 13,125 mg GAE/g d.w. HPLC-DAD of the *P. amurense* stem bark extract which was calibrated for five polyphenols (gallic acid, 4-hydroxybenzoic acid, caffeic acid and ferulic acid) and four alkaloids (berbamine, jatrorrhizine, palmatine, and berberine) (Table 1; Figure 1) obtained from *P. amurense* stem ethanolic extracts. We were able to show that the concentration of gallic acid for PA1 is of  $0.10 \pm 0.01$  mg/g and the concentration of 4-hydroxybenzoic acid for PA1 is  $0.38 \pm 0.03$  mg/g. Ferulic acid, p-coumaric acid and caffeic acid were not detected in the analysed sample.

Analysing four alkaloids in the *P. amurense* stem bark ethanol extract, it was found that berberine had the highest concentration of  $2.44 \pm 0.22$  mg/g.



**Figure 1.** HPLC-DAD chromatogram of *Phellodendron amurense* alcoholic extract

**Table 1.** Quantitative determination of chemical compounds in *Phellodendron amurense* stem bark extract by HPLC-DAD

Nr.	Compounds	Elution time (min)	<i>Phellodendron amurense</i> (mg/g)
1	gallic acid	3.32	0.10±0.01
2	4-hydroxybenzoic acid	9.98	0.38±0.03
3	caffeic acid	12.25	<LOD
4	p-coumaric acid	15.79	<LOD
5	ferulic acid	17.24	<LOD
6	berbamine	21.80	<LOD
7	jatrorrhizine	24.70	<LOD
8	palmatine	29.70	<LOD
9	berberine	31.59	2.44±0.22

LOD –limit of detection

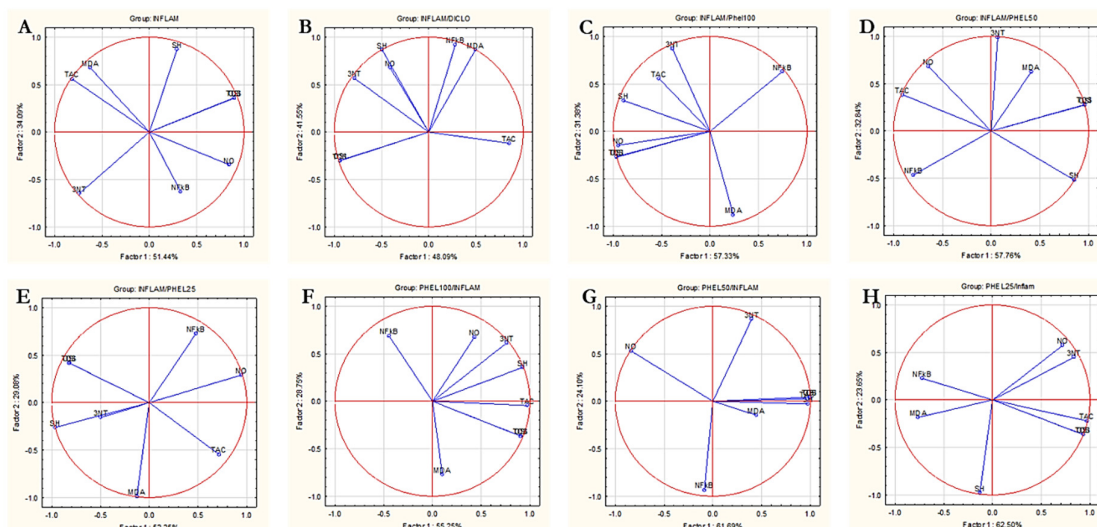
*In vitro antioxidant activity*

The *P. amurense* stem bark extract DPPH scavenging effect with IC<sub>50</sub> 155 µg Trolox equiv./mL was significantly higher than that of Trolox with IC<sub>50</sub> 11.20 µg /mL. The FRAP scavenging activity of *P. amurense* bark ethanol extract was 27,42 mmol Trolox equiv./ g d.w. Hydrogen peroxide scavenging activity IC<sub>50</sub> 21,53 µg Trolox equiv./g d.w. was higher than that of Trolox (IC<sub>50</sub> 10,77 µg /mL) too. No scavenging activity evaluated as IC<sub>50</sub> 115,68 µg Trolox equiv./g d.w. was not better than that of Trolox (IC<sub>50</sub> 86,82 µg /mL).

*In vivo anti-inflammatory and antioxidative effect*

Effects of *P. amurense* stem bark extract was evaluated for anti-inflammatory activity in an animal model of turpentine oil induced acute inflammation. The anti-inflammatory activity of *P. amurense* stem bark extract was evaluated by measuring serum levels of NO<sub>x</sub>, 3NT, and Nf-kB after prophylactic and therapeutic treatment plans. Compared with the control group inflammation increased NO<sub>x</sub>, 3NT and Nf-kB significantly ( $p < 0.001$ ), and the changes were correlated (Figure 2A). Diclofenac caused an important correlated reduction of the same parameters (Table 1 (Figure 2B)). Only therapeutic treatment lowered NO<sub>x</sub> concentration in a dose dependent way and correlated with 3NT (Figure 2 C, D, E), the Inflam/Phel100 having the best inhibitory effect ( $p < 0.001$ ). Furthermore, the therapeutic treatment plan efficiency on NO<sub>x</sub> was comparable to that of diclofenac. In the prophylactic treatment only Phel100/Inflam had a small inhibitory activity ( $p < 0.05$ ) on NO<sub>x</sub> production, and it was correlated with 3NT and NFkB (Figure 2 F). The 3NT was significantly reduced by both treatment plans ( $p < 0.001$ ), prophylactic treatment being a little more efficient. As compared to diclofenac, *P. amurense* stem bark extract inhibitory activity on 3NT production was better.

The therapeutic treatments with *P. amurense* stem bark extract caused a very significant reduction of NF-kB ( $p < 0.001$ ) in a dose dependent way, and the prophylactic treatment had a smaller inhibitory activity ( $p < 0.01$ ). The effect of diclofenac on NF-kB was significantly smaller than that of the therapeutic treatments with *P. amurense* stem bark extract ( $p < 0.01$ ), but better than the prophylactic treatment ( $p < 0.05$ ) (Table 2).



**Figure 2.** Anti-inflammatory and oxidative stress tests PCA correlation monoplots of rats with turpentine oil-induced acute inflammation and *P. amurense* bark extract treatments. (A) Inflam - inflammation group; B) Diclo - inflammation group treated with diclofenac; C) Inflam/Phel100 - inflammation group with *P. amurense* extract 100% treatment; D) Inflam/Phel50 - inflammation group with *P. amurense* extract 50% treatment; E) Inflam/Phel25 - inflammation group with *P. amurense* extract 25% treatment; F) group with Phel100/Inflam - *P. amurense* extract extract 100% prophylaxy followed by inflammation; G) group with Phel50/Inflam - *P. amurense* extract extract 50% prophylaxy followed by inflammation; H) group with Phel25/Inflam - *P. amurense* extract extract 25% prophylaxy followed by inflammation

**Table 2.** Serum inflammatory markers of the rats with turpentine oil induces inflammation treated with *P. amurense* stem bark extract

Groups	NOx (ng/mL)	3NT (ng/mL)	NF-kB(ng/mL)
Control	27.73±1.75	28.43±3.94	202.33±25.06
Inflam	35.35±4.56 <sup>###</sup>	76.13±8.61 <sup>###</sup>	358.75±37.31 <sup>##</sup>
Diclo	26.52±2.47 <sup>**</sup>	40.77±8.60 <sup>***</sup>	194.67±10.36 <sup>***</sup>
Inflam/Phel100	24.75±2.61 <sup>***</sup>	35.40±6.42 <sup>***</sup>	99.67±7.31 <sup>***</sup>
Inflam/Phel50	25.74±3.07 <sup>**</sup>	39.55±7.77 <sup>***</sup>	103.00±18.46 <sup>***</sup>
Inflam/Phel25	29.92±5.78 <sup>*</sup>	36.65±8.50 <sup>***</sup>	163.00±13.57 <sup>***</sup>
Phel100/Inflam	31.16±1.19 <sup>*</sup>	29.97±9.31 <sup>***</sup>	225.50±14.50 <sup>**</sup>
Phel50/Inflam	33.30±4.62	28.73±6.00 <sup>***</sup>	268.83±31.55 <sup>**</sup>
Phel25/Inflam	36.78±4.32	34.04±6.53 <sup>***</sup>	248.00±28.61 <sup>**</sup>

Data represent mean ±SD of five animals. Animals with inflammation and no treatment serve as positive control and are compared to untreated negative control group animals. Animals with inflammation and *P. amurense* stem bark extract treatment are compared to positive control group animals.

Abbreviations: NOx—nitrites and nitrates; 3NT—3-nitrothiosine; NFkB—nuclear factor kB; Control—negative control; Inflam—inflammation; Diclo—inflammation treated with diclofenac; Inflam/Phel100— *P. amurense* extract 100% treatment; Inflam/Phel50— *P. amurense* extract 500% treatment; Inflam/Phel25— *P. amurense* extract 25% treatment; Phel100/Inflam— *P. amurense* extract 100% prophylaxy; Phel50/Inflam— *P. amurense* extract 50% prophylaxy; Phel25/Inflam— *P. amurense* extract 25% prophylaxy.

<sup>#</sup> Statistical significance vs Control: <sup>##</sup>p < 0.01; <sup>###</sup>p < 0.001; <sup>\*</sup> statistical significance vs Inflam; <sup>\*</sup> p < 0.05; <sup>\*\*</sup> p < 0.01; <sup>\*\*\*</sup> p < 0.001.

Oxidative stress markers of the Inflam animals were significantly changed as compared to the Control, respectively TOS, OSI and MDA were increased, TAC and SH were decreased (Table 3). OSI was positively correlated with TOS and NO, and negatively with TAC (Figure 2 A). The *P. amurense* stem bark extract

prophylactic treatments only increased TAC ( $p < 0.01$ ), the therapeutic treatment, and diclofenac having no significant effect.

**Table 3.** Serum oxidative stress markers of the rats with turpentine oil induces inflammation treated with *P. amurense* stem bark extract

Group	TAC (mM Trolox equiv./L)	TOS (mM Trolox equiv./L)	OSI	MDA (nM/L)	SH (mM/L)
Control	1.0935±0.0016	3.52±0.16	3.23±0.15	3.53±0.24	452.00±47.09
Inflam	1.0889±0.0005 <sup>#</sup>	9.03±1.07 <sup>###</sup>	8.29±0.98 <sup>###</sup>	5.03±0.87 <sup>#</sup>	268.80±29.65 <sup>###</sup>
Diclo	1.0907±0.0015	6.31±1.41 <sup>**</sup>	5.79±1.29 <sup>**</sup>	4.63±0.31 <sup>**</sup>	370.00±38.21 <sup>**</sup>
Inflam/Phel100	1.0907±0.0006	8.88±1.00	8.15±0.91	4.27±0.28 <sup>**</sup>	409.00±75.43 <sup>**</sup>
Inflam/Phel50	1.0885±0.0007	7.33±1.30	6.74±1.20	4.55±0.33 <sup>**</sup>	345.67±65.99 <sup>*</sup>
Inflam/Phel25	1.0890±0.0010	6.33±0.55 <sup>**</sup>	5.98±0.51 <sup>**</sup>	4.33±0.40 <sup>**</sup>	250.50±29.14
Phel100/Inflam	1.0909±0.0011 <sup>**</sup>	4.62±1.31 <sup>###</sup>	4.23±1.20 <sup>###</sup>	4.32±0.48 <sup>**</sup>	304.00±69.13
Phel50/Inflam	1.0923±0.0003 <sup>**</sup>	3.79±0.57 <sup>###</sup>	3.47±0.52 <sup>###</sup>	4.36±0.46 <sup>**</sup>	316.67±50.05
Phel25/Inflam	1.0927±0.0006 <sup>**</sup>	4.12±0.53 <sup>###</sup>	3.77±0.48 <sup>###</sup>	3.63±1.01 <sup>**</sup>	317.00±65.92

Data represent mean ±SD of five animals. Animals with inflammation and no treatment serve as positive control and are compared to untreated negative control group animals. Animals with inflammation and *P. amurense* stem bark extract treatment are compared to positive control group animals.

Abbreviations: TAC—total antioxidant capacity; TOS—total oxidative status; OSI—oxidative stress index; MDA—malondialdehyde; SH—total thiols; Control—negative control; Inflam—inflammation; Diclo—inflammation treated with diclofenac; Inflam/Phel100—*P. amurense* extract 100% treatment; Inflam/Phel50—*P. amurense* extract 50% treatment; Inflam/Phel25—*P. amurense* extract 25% treatment; Phel100/Inflam—*P. amurense* extract 100% prophylaxy; Phel50/Inflam—*P. amurense* extract 50% prophylaxy; Phel25/Inflam—*P. amurense* extract 25% prophylaxy.

<sup>#</sup> statistical significance vs Control: <sup>#</sup> $p < 0.01$ ; <sup>###</sup> $p < 0.001$ ; <sup>\*</sup> statistical significance vs Inflam: <sup>\*</sup> $p < 0.05$ ; <sup>\*\*</sup> $p < 0.01$ ; <sup>\*\*\*</sup> $p < 0.001$ .

TOS and OSI were significantly reduced by *P. amurense* stem bark extract in prophylactic administration ( $p < 0.001$ ), and from the therapeutic treatments only PHEL25 had an inhibitory effect ( $p < 0.01$ ). On TOS and OSI diclofenac had a lower effect as compared to *P. amurense* stem bark extract administrated as a prophylactic treatment, a comparable effect with therapeutic treatment with Phel25, and a better effect than therapeutic administration of PHEL100 and PHEL50. TOS and OSI changes after *P. amurense* stem bark extract treatments were negatively correlated to NFkB (Figure 2 C-H).

Acute inflammation induction increased MDA ( $p < 0.01$ ). Diclofenac and all *P. amurense* stem bark extract treatments caused MDA reduction ( $p < 0.01$ ). These changes were correlated with TOS and OSI only in the prophylactic treatments (Figure 2 F, G, H).

SH was significantly reduced by the inflammation ( $p < 0.001$ ). Diclofenac ( $p < 0.01$ ) and therapeutic administration of Inflam/Phel100 ( $p < 0.01$ ) and Inflam/Phel50 ( $p < 0.05$ ) increased SH, but Phel25 and prophylactic treatments had no significant effect ( $p > 0.05$ ) (Table 3). These changes were not correlated with TAC (Figure 2 C, D).

In order to evaluate the relationship between the parameter correlation analysis with Pearson test followed by a PCA was performed (Figure 2).

## Discussion

This study investigated the anti-inflammatory and antioxidant effects of the *P. amurense* cortex in order to develop a natural anti-inflammatory herbal medicine with no side effects like those of the nonsteroidal anti-

inflammatory drugs. The extract effectively counteracted turpentine oil-induced injury through an anti-inflammatory and an antioxidant mechanism with an efficacy comparable to that of diclofenac and Trolox.

The composition of a plant extract can vary considerably according to the geographical area, season, parts and organs of plants, plant's lifecycle and growth phase, the conservation procedure (Allegra, 2019). Moreover, the concentration of total polyphenols and alkaloids differ according to the type of extraction procedure. The concentration of TPC in *P. amurense* bark extracts obtained as decoctum, infusum, and tincture measured using Folin–Ciocalteu reagent and expressed as hyperoside g/L, had the highest value in the tincture (Balážová *et al.*, 2022). When comparing TPC in aqueous and ethanol extracts of *P. amurense* bark it was about 2 fold higher in ethanol extract ( $23.45 \pm 4.52$  mg GAE/g) than in the aqueous extract ( $13.92 \pm 3.37$  mg GAE/g) (Wang *et al.*, 2009). Therefore, only alcohol extracts were used in this study. Another study of *P. amurense* bark extract included into a soybean protein isolate at a concentration of 22.5% w/w, found a TPC of 14.87 mg GAE/g, soybean protein isolate having a TPC of 4.17 mg GAE/g (Liang and Wang, 2018). Our ethanol extract of *P. amurense* bark 1:1 w:v had a lower TPC. Perhaps the geographical area, shorter warm season and extraction method influenced that.

In HPLC-DAD analysis of the *P. amurense* stem bark extract, from the five measured polyphenols, gallic acid, 4-hydroxybenzoic acid, caffeic acid and ferulic acid, only gallic acid, 4-hydroxybenzoic acid were detected, and their concentration was small. The polyphenols content does not correlate with isoquinoline alkaloids concentration in different types of extracts of the same plant product. A study that analysed *P. amurense* bark isoquinoline alkaloids in ethanolic tincture found berberine in the concentration of 32.96 mg/kg (Balážová *et al.*, 2022). Another study that performed a LC–ESI-MS/MS analysis of the alkaloids from cortex *P. amurense* detected important quantities of phellodendrine (42.4–424.0  $\mu\text{g/mL}$ ), magnoflorine (0.459–45.5–455.0  $\mu\text{g/mL}$ ), columbamine (0.88–8.8  $\mu\text{g/mL}$ ), jatrorrhizine (30.5–305.0  $\mu\text{g/mL}$ ), berberine (109.0–1090.0  $\mu\text{g/mL}$ ), and palmatine (51.0–510.0  $\mu\text{g/mL}$ ) (X. Xian *et al.*, 2014). When contents of jatrorrhizine, palmatine, and berberine from the extracts of *Phellodendron chinense* Schneid and of *P. amurense* bark were compared, significant differences were found, *P. chinense* having a higher content of these alkaloids (Xian *et al.*, 2011; Kamimura *et al.*, 2019).

In our study HPLC-DAD analysis of the *P. amurense* stem bark extract collected from the “Alexandru Borza” Botanical Garden of Cluj-Napoca looked for four alkaloids, berbamine, jatrorrhizine, palmatine and berberine. Like in other studies, berberine had the more significant concentration. The literature considers berberine as an important compound that can be found not only in *P. amurense*, but also in other plants, such as *Berberis vulgaris* (Neag *et al.*, 2019) or *Mahonia aquifolium* (Andreicut *et al.*, 2018). Moreover, all the active compounds detected in the *P. amurense* stem bark extract possess many different pharmacologic effects, and they exhibit low toxicity to normal cells and tissues (Balážová *et al.*, 2022). Even berberine that had the higher concentration in our extract, is considered more likely to correlate *P. amurense* stem bark extract effect to the content of total alkaloids rather than only to the content of berberine (Chen *et al.*, 2010).

It was demonstrated that for *P. amurense* bark ethanol extract FRAP and DPPH scavenging activities were better than those of the aqueous extract, and that higher content of phenolics and flavonoids exhibited a stronger antioxidant activity (Wang *et al.*, 2009). In another study, DPPH radical-scavenging activity of *P. amurense* stem bark extract was significant and dose dependent (Liang and Wang, 2018).

The preliminary study demonstrated that *P. amurense* stem bark extract has strong *in vitro* antioxidant effect, because DPPH, FRAP, NO, and H<sub>2</sub>O<sub>2</sub> scavenging activities were significantly higher than those of Trolox. Trolox protects against oxidative stress by decreasing ROS formation, as a direct ROS scavenger, and by stimulating the activity of antioxidant enzymes (Do *et al.*, 2017). Thus, we used Trolox as a positive control to confirm the antioxidant effects of *P. amurense* stem bark extract.

Considering that oxidative stress is an important pathological mechanism in inflammation, we further tested *in vivo* the anti-inflammatory and antioxidant activities of the *P. amurense* stem bark extract. Moreover,

because many anti-inflammatory drugs currently in use have side effects, we aimed to test *P. amurense* stem bark extract for the development of a new drug without side effects. The *in vivo* anti-inflammatory effect of *P. amurense* stem bark extract was evaluated in animal models of turpentine oil induced acute inflammation.

*P. amurense* stem bark extract from our sample and other's analysis contain a number of alkaloids, such as berberine, palmatine, jatrorrhizine, and obacunone, which are thought to have anti-inflammatory effect by inhibiting the activity of NF- $\kappa$ B, a key transcription factor involved in inflammation (Cicero and Baggioni, 2016; Jung *et al.*, 2017; Balážová *et al.*, 2022). Our extract reduced significantly NF- $\kappa$ B concentration, mostly in the therapeutic treatment plan, in a dose dependent way.

In inflammation nitric oxide (NO) is a pro-inflammatory mediator that plays a pivotal role in many diseases. It is synthesized by inducible nitric oxide synthase (iNOS). The expression of the iNOS gene is induced by NF- $\kappa$ B and pro-inflammatory cytokines, like IL-1 $\beta$ . Excessive cytokine production and excessive iNOS could trigger NO overproduction with tissue injury. Therefore, NO reduction is a sensitive marker for the evaluation of the anti-inflammatory effects of the plant extracts (Fujii *et al.*, 2017; Sun *et al.*, 2019). A different composition of the *P. amurense* stem bark extract may modulate the anti-inflammatory effects differently (Fujii *et al.*, 2017). According to the previous studies, *P. amurense* stem bark extract has anti-inflammatory properties due to inhibition of NF- $\kappa$ B and NO synthesis (Kim *et al.*, 2021). The *in vitro* and *in vivo* anti-inflammatory activity of berberine was associated with the reduction of proinflammatory cytokines (Balážová *et al.*, 2022) and iNOS (Sun *et al.*, 2019). Because in our extract berberine was the major active compound (Choi *et al.*, 2021), we presumed that berberine had an important contribution in NO reduction. In the same time it has to take into consideration that the nonalkaloid *P. amurense* stem bark extract can lower NO production and iNOS gene expression via a NF- $\kappa$ B-mediated pathway too (Sun *et al.*, 2019).

NO is a small molecule that can diffuse through cell membranes. In inflammation when it is synthesized in excess, NO reacts with superoxide producing peroxynitrite, an oxidizing and nitrating agent that has the capacity to damage proteins, lipids, and DNA (Biswas, 2016b). Proteins nitration cause structural and functional alterations and have important pathological consequences. Through the nitration of proteins tyrosine residues 3NT is generated and it can be used as a biomarker for inflammation. *P. amurense* stem bark extract lowered significantly 3NT in both treatment plans, but in the therapeutic plan the effects were stronger than in the prophylactic therapy.

Taking together, these results confirm that *in vivo* *P. amurense* stem bark extract has anti-inflammatory effects by interfering with NF- $\kappa$ B, NO and 3NT biosynthesis pathways (Choi *et al.*, 2014).

It has been demonstrated that complex interconnections exist between the inflammatory response and oxidative stress (Allegra, 2019), because inflammatory cells produce ROS leading to oxidative stress, and ROS can increase proinflammatory mediators (Biswas, 2016b). Oxidative stress is the consequence of a disrupted balance between oxidants and antioxidants, and it leads to oxidative damage of lipids, proteins, and DNA macromolecules, damage of cells, subsequently triggering cells death (Tan *et al.*, 2018). Lipid peroxidation lead to membrane damages with receptors, enzymes, and ion channels inactivation (Biswas, 2016b). Major specific biomarkers of oxidative stress include TOS and OSI, lipid oxidation products, like MDA, protein carbonyls, advanced glycation end products, DNA/RNA oxidation products (Picardo and Dell'Anna, 2010). Like in other studies (Do *et al.*, 2017), the acute experimental inflammation increased TOS, OSI and MDA. *P. amurense* extracts used by the traditional medicine in Korea and China was found to reduce the intracellular ROS levels. Furthermore, by lowering lipid peroxidation and increasing antioxidant enzymes activities *P. amurense* extracts prevented skin oxidative stress induced by ultraviolet radiation (Do *et al.*, 2017). In our experiments *P. amurense* stem bark extract treatments reduced oxidative stress markers TOS, OSI and MDA. The results also confirmed that the antioxidant activity of *P. amurense* stem bark extract is dose dependent (Sun *et al.*, 2019). Moreover, *P. amurense* stem bark extract had a better inhibitory effect than diclofenac.

Naturally, the organism has several endogenous antioxidant mechanisms: antioxidant enzymes, like catalase, glutathioneperoxidase, and superoxide dismutase; non-enzymatic ROS scavengers, like  $\beta$ -carotene, vitamin C, vitamin E, and uric acid. Some exogenous antioxidants, natural or synthetic compounds can reduce oxidative stress too ( Chatterjee, 2016; Tan *et al.*, 2018). Polyphenols are plants secondary metabolites with antioxidative activities, such as ROS scavenging, inhibition of ROS generation, and antioxidant mechanisms upregulation. The molecular mechanisms of the antioxidant activity of berberine, the main compound from *P. amurense* stem bark extract, seem to be up regulation of SOD, and downregulation of NADPH oxidase expression (Tan *et al.*, 2018). Major specific biomarkers of the antioxidants are TAC, glutathione, protein thiols, and others (Picardo and Dell'Anna, 2010). In our experimental inflammation TAC and SH were reduced, and only *P. amurense* stem bark extract prophylactic treatments increased TAC, suggesting that other antioxidants then SH were involved. Diclofenac and therapeutic administration of *P. amurense* stem bark extract increased SH, without influencing TAC, indicating that in this treatment plan not all antioxidant mechanisms were up regulated.

Taking together, the results of this study provide scientific knowledge on the anti-inflammatory and antioxidant activities of *P. amurense* stem bark extract in experimental acute inflammation. These effects are dose dependent and are influenced by the treatment type, prophylactic or therapeutic.

## Conclusions

In summary, it appears from the results of this study that *P. amurense* stem bark extract has a good anti-inflammatory activity by reducing NF-kB, NOx and 3NT. These effects were better in the therapeutic treatment plan than in the prophylactic one. In the same time *P. amurense* stem bark extract has antioxidant activity by lowering oxidants and increasing antioxidants. Related to the oxidative stress reduction, the prophylactic treatment plan seemed to be more efficient than the therapeutic one. All these activities were dose dependent, the higher doses being more efficient.

In conclusion, these results could contribute to the development of novel drugs based on *P. amurense* stem bark extract for the treatment of inflammatory diseases and prevention of oxidative stress.

## Authors' Contributions

Conceptualization SEE, MP, AEP; Data curation SEE, AC, ACM; Formal analysis SEE, AAD, AJVM; Funding acquisition MP, ACM; Investigation SEE, AAD, AJVM, AC, ACM; Methodology AEP, MP, ACM; Project administration SEE, MP; Resources SEE, MP, AEP, ACM; Software AEP; Supervision MP, ACP; Validation AEP, MP, ACM; Visualization SEE, AEP, MP, ACM; Writing - original draft SEE, AC, ACM; Writing - review and editing AEP, MP. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

The study was conducted according to the guidelines of the Declaration of Helsinki, and the experiments were approved by the Ethics Committee of the "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca (302/04.04.2022).

## Acknowledgements

This research was funded by Babes-Bolyai University, under the doctoral grant of Sabina-Emanuela Erhan. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Allegra M (2019). Antioxidant and anti-inflammatory properties of plants extract. *Antioxidants* 8(11):549. <https://doi.org/10.3390/antiox8110549>
- Andreicut AD, Pârvu AE, Mot AC., Pârvu M, Fodor EF, Cătoi AF, ... Irimie A (2018). Phytochemical analysis of anti-inflammatory and antioxidant effects of *Mahonia aquifolium* flower and fruit extracts. *Oxidative Medicine and Cellular Longevity* 66(1):49-58. <https://doi.org/10.1155/2018/2879793>
- Balážová L, Kurhajec S, Kello M, Bedlovičová Z, Zigová M, Petrovová E, ... Eftimová J (2022). Antiproliferative effect of *Phellodendron amurense* Rupr. based on angiogenesis. *Life* 12(5). <https://doi.org/10.3390/life12050767>
- Balea ȘS, Pârvu AE, Pârvu M, Vlase L, Dehelean CA, Pop TI (2020). Antioxidant, anti-inflammatory and antiproliferative effects of the *Vitis vinifera* L. var. Fetească Neagră and Pinot Noir pomace extracts. *Frontiers in Pharmacology* 11:1-11. <https://doi.org/10.3389/fphar.2020.00990>
- Bhatti MZ, Ali A, Ahmad A, Saeed A, Malik SA (2015). Antioxidant and phytochemical analysis of *Ranunculus arvensis* L. extracts. *BMC Research Notes* 8(1):1-8. <https://doi.org/10.1186/s13104-015-1228-3>
- Biswas SK (2016). Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative Medicine and Cellular Longevity* 2016:17-19. <https://doi.org/10.1155/2016/5698931>
- Chatterjee, S. (2016). Oxidative stress, inflammation, and disease. In *Oxidative Stress and Biomaterials*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-803269-5.00002-4>
- Chen ML, Xian YF, Ip SP, Tsai SH, Yang JY, Che CT (2010). Chemical and biological differentiation of *Cortex phellodendri chinensis* and *Cortex phellodendri amurenensis*. *Planta Medica* 76(14):1530-1535. <https://doi.org/10.1055/s-0030-1249774>
- Chera EI, Pop RM, Pârvu M, Sorițău O, Uifălean A, Cătoi FA, ... Pârvu AE (2022). Flaxseed ethanol extracts' antitumor, antioxidant, and anti-inflammatory potential. *Antioxidants* 11(5):892. <https://doi.org/10.3390/antiox11050892>
- Chiou J-S, Chou C-H, Ho M-W, Tien N, Liang W-M, Chiu M-L, ... Lin Y-J (2023). Effect of Chinese herbal medicine therapy on risks of all-cause mortality, infections, parasites, and circulatory-related mortality in HIV/AIDS patients with neurological diseases. *Frontiers in Pharmacology* 14:1-13. <https://doi.org/10.3389/fphar.2023.1097862>
- Choi J, Moon MY, Han GY, Chang MS, Yang D, Cha J (2021). *Phellodendron amurense* extract protects human keratinocytes from pm2.5-induced inflammation via par-2 signalling. *Biomolecules* 11(1):1-13. <https://doi.org/10.3390/biom11010023>
- Choi YY, Kim MH, Han JM, Hong J, Lee TH, Kim SH, Yang WM (2014). The anti-inflammatory potential of *Cortex phellodendron* *in vivo* and *in vitro*: Down-regulation of NO and iNOS through suppression of NF-kB and MAPK activation. *International Immunopharmacology* 19(2):214-220. <https://doi.org/10.1016/j.intimp.2014.01.020>
- Cicero AFG, Baggioni A (2016). Berberine and its role in chronic disease. *Advances in Experimental Medicine and Biology* 928:27-45. [https://doi.org/10.1007/978-3-319-41334-1\\_2](https://doi.org/10.1007/978-3-319-41334-1_2)

- Cuéllar MJ, Giner RM, Recio MC, Máez S, Ríos JL (2001). Topical anti-inflammatory activity of some Asian medicinal plants used in dermatological disorders. *Fitoterapia* 72(3):221-229. [https://doi.org/10.1016/S0367-326X\(00\)00305-1](https://doi.org/10.1016/S0367-326X(00)00305-1)
- Do GY, Kim JW, Park HJ, Yoon SB, Park JY, Yang SG, ... Koo DB (2017). Native plants (*Phellodendron amurense* and *Humulus japonicus*) extracts act as antioxidants to support developmental competence of bovine blastocysts. *Asian-Australasian Journal of Animal Sciences*, 30(9):1245-1252. <https://doi.org/10.5713/ajas.16.0985>
- Erel O (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry* 37(4):277-285. <https://doi.org/10.1016/j.clinbiochem.2003.11.015>
- Erel O (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry* 38(12):1103-1111. <https://doi.org/10.1016/j.clinbiochem.2005.08.008>
- Erel O, Neselioglu S (2014). A novel and automated assay for thiol/disulphide homeostasis. *Clinical Biochemistry* 47(18):326-332. <https://doi.org/10.1016/j.clinbiochem.2014.09.026>
- Fernandes RPP, Trindade MA, Tonin FG, Lima CG, Pugine SMP, Munekata PES, ... de Melo MP (2016). Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. *Journal of Food Science and Technology* 53(1):451-460. <https://doi.org/10.1007/s13197-015-1994-x>
- Fujii A, Okuyama T, Wakame K, Okumura T, Ikeya Y, Nishizawa M (2017). Identification of anti-inflammatory constituents in *Phellodendri cortex* and *Coptidis rhizoma* by monitoring the suppression of nitric oxide production. *Journal of Natural Medicines* 71(4):745-756. <https://doi.org/10.1007/s11418-017-1107-4>
- Ghasemi A, Hedayati M, Biabani H (2007). Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess Assay. *Journal of Medical and Scientific Research* 2:29-32. <http://jmsr.org/index.php/journal/article/viewFile/27/71>
- James MA, Fu H, Liu Y, Chen D-R, You M (2011). Dietary administration of berberine or *Phellodendron amurense* extract inhibits cell cycle progression and lung tumorigenesis. *Molecular Carcinogenesis* 50(1):1-7. <https://doi.org/10.1053/j.gastro.2016.08.014.CagY>
- Jung HW, Kim KH, Park YK (2017). Inhibitory effect of the extract of *Phellodendron amurense* Ruprecht root on collagen-induced arthritis in mice. *Chinese Journal of Integrative Medicine* 23(10):755-762. <https://doi.org/10.1007/s11655-016-2094-5>
- Kamimura S, Nishihara M, Osumi Y, Shiota H (2019). Simultaneous quantitative analysis of berberine and other alkaloids in powdered *Phellodendron* Bark. In *YAKUGAKU ZASSHI* 139(11):1471-1478. <https://doi.org/10.1248/yakushi.19-00097>
- Kim HJ, Kim YJ, Park W (2021). Berberine modulates hyper-inflammation in mouse macrophages stimulated with polyinosinic-polycytidylic acid via calcium-CHOP/STAT pathway. *Scientific Reports* 11(1):1-10. <https://doi.org/10.1038/s41598-021-90752-z>
- Kim JH, Huh JE, Baek YH, Lee JD, Choi DY, Park DS (2011). Effect of *Phellodendron amurense* in protecting human osteoarthritic cartilage and chondrocytes. *Journal of Ethnopharmacology* 134(2):234-242. <https://doi.org/10.1016/j.jep.2010.12.005>
- Lee SH, Lee HJ, Lee SH, Kim YS, Lee D, Chun J, ... Chang GT (2018). Effects of Huang Bai (*Phellodendri Cortex*) on bone growth and pubertal development in adolescent female rats. *Chinese Medicine (United Kingdom)* 13(1):1-9. <https://doi.org/10.1186/s13020-017-0156-7>
- Li S, Zhang Y, Shi D, Hou W, Xia J, Liu C (2019). Screening and isolation of cyclooxygenase-2 inhibitors from the stem bark of *Phellodendron amurense* Ruprecht by ultrafiltration with liquid chromatography and tandem mass spectrometry, and complex chromatography. *Journal of Separation Science* 42(10):1905-1914. <https://doi.org/10.1002/jssc.201801262>
- Liang S, Wang L (2018). A natural antibacterial-antioxidant film from soy protein isolate incorporated with cortex *Phellodendron* extract. *Polymers* 10(1). <https://doi.org/10.3390/polym10010071>
- Mi S, Liu X, Zhang L, Wang Y, Sun L, Yuan S, ... Liu Y (2023). Chinese medicine formula 'Baipuhuang Keli' inhibits triple-negative breast cancer by hindering DNA damage repair via MAPK/ERK pathway. *Journal of Ethnopharmacology* 304:116077. <https://doi.org/10.1016/j.jep.2022.116077>

- Miranda KM, Espey MG, Wink DA (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5(1):62-71. <https://doi.org/10.1006/niox.2000.0319>
- Neag MA, Bocsan IC, Catinean A, Vesa SC, Balan GG, Parvu M, ... Buzoianu AD (2019). Effects of berberis vulgaris extract on lipid profile, kidney and liver function in experimental dyslipidemia. *Revista de Chimie* 70(2):614-618. <https://doi.org/10.37358/rc.19.2.6968>
- Park E-K, Rhee HI, Jung H-S, Ju SM, Lee Y-A, Lee SH, ... Kim KS (2007). Antiinflammatory effects of a combined herbal preparation (RAH13) of *Phellodendron amurense* and *Coptis chinensis* in animal models of inflammation. *Phytotherapy Research* 21:746-750. <https://doi.org/10.1002/ptr>
- Pârvu M, Moț CA, Pârvu AE, Mircea C, Stoeber L, Roșca-Casian O, Țigu AB (2019). *Allium sativum* extract chemical composition, antioxidant activity and antifungal effect against *Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa* causing onychomycosis. *Molecules* 24(21):1-16. <https://doi.org/10.3390/molecules24213958>
- Pfingstgraf IO, Taulescu M, Pop RM, Orăsan R, Vlase L, Uifălean A, ... Pârvu AE (2021). Protective effects of taraxacum officinale L. (dandelion) root extract in experimental acute on chronic liver failure. *Antioxidants* 10(4):160-165. <https://doi.org/10.3390/antiox10040504>
- Picardo M, Dell'Anna ML (2010). Oxidative stress. *Vitiligo* 231-237. [https://doi.org/10.1007/978-3-540-69361-1\\_27](https://doi.org/10.1007/978-3-540-69361-1_27)
- Santos J, Brito M, Ferreira R, Moura AP, Sousa T, Batista T, ... Sobral M (2018). Th1-biased immunomodulation and in vivo antitumor effect of a novel piperine analogue. *International Journal of Molecular Sciences* 19(9):1-22. <https://doi.org/10.3390/ijms19092594>
- Sun Y, Lenon GB, Yang AWH, Amado JRR (2019). Phellodendri Cortex: a phytochemical, pharmacological, and pharmacokinetic review. *Evidence-Based Complementary and Alternative Medicine* 2019. <https://doi.org/10.1155/2019/7621929>
- Tan BL, Norhaizan ME, Liew WPP, Rahman HS (2018). Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Frontiers in Pharmacology* 9:1-28. <https://doi.org/10.3389/fphar.2018.01162>
- Taukoorah U, Mahomoodally MF (2016). Crude aloe vera gel shows antioxidant propensities and inhibits pancreatic lipase and glucose movement *in vitro*. *Advances in Pharmacological Sciences* 2016. <https://doi.org/10.1155/2016/3720850>
- Tomșa A, Răchișan A, Pandrea S, Benea A, Uifălean A, Parvu A, Junie L (2021). Accelerated lipid peroxidation in a rat model of gentamicin nephrotoxicity. *Experimental and Therapeutic Medicine* 22(5):1-8. <https://doi.org/10.3892/etm.2021.10652>
- Wang L, Wang X, Zhu XM, Liu YQ, Du WJ, Ji J, ... Yuan CS (2017). Gastroprotective effect of alkaloids from *Cortex phellodendri* on gastric ulcers in rats through neurohumoral regulation. *Planta Medica* 83(3-4):277-284. <https://doi.org/10.1055/s-0042-114044>
- Wang W, Zu Y, Fu Y, Reichling J, Suschke U, Nokemper S, Zhang Y (2009). In vitro antioxidant, antimicrobial and anti-herpes simplex virus type 1 activity of *Phellodendron amurense* Rupr. from China. *American Journal of Chinese Medicine* 37(1):195-203. <https://doi.org/10.1142/S0192415X09006655>
- Xian X, Sun B, Ye X, Zhang G, Hou P, Gao H (2014). Identification and analysis of alkaloids in cortex *Phellodendron amurense* by high-performance liquid chromatography with electrospray ionization mass spectrometry coupled with photodiode array detection. *Journal of Separation Science* 37(13):1533-1545. <https://doi.org/10.1002/jssc.201400012>
- Xian YF, Mao QQ, Ip SP, Lin ZX, Che CT (2011). Comparison on the anti-inflammatory effect of *Cortex phellodendri chinensis* and *Cortex phellodendri amurenensis* in 12-O-tetradecanoyl-phorbol-13-acetate- induced ear edema in mice. *Journal of Ethnopharmacology* 137(3):1425-1430. <https://doi.org/10.1016/j.jep.2011.08.014>



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