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Research Article

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Phenolic profile, antioxidant, anti-inflammatory and anticancer activities of *Telephium imperati* L.

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ABSTRACT

Telephium imperati L. is one of the most valuable spices used by Algerians for food and different medicinal purposes. Here, we report the main phenolic compounds of *T. imperati* that grows in arid regions and its antioxidant, anti-inflammatory, and anticancer activities. The phenolic profiles of methanolic and aqueous extracts of *T. imperati* roots was obtained using HPLC-DAD, whereas antioxidant activity was evaluated using 2.2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, and Hydrogen peroxide (H_2O_2) assays. Moreover, the anti-inflammatory activity was investigated by measuring the level of protection of the human red blood cell (HRBC) membrane against hypotonicity-induced lysis. The anticancer activity was evaluated using the MTT assay against three human cancer cell lines (HT-29, PC-3, A-549) and one non-tumorigenic (CCD18-Co) cells. Our results showed that the methanolic extract was rich in phenols (493.17±0.0 mg GAE/g), flavonoids (271.56±0.1 mg QE/g), and tannins (106.50±2.3 mg CE/g). The HPLC-DAD analysis revealed that caffeic acid (78.61 mg/g), ferulic acid (49.12 mg/g), rosmarinic acid (42.80 mg/g), coumarin (39.87 mg/g), rutin (37.68 mg/g), myricetin (25.62 mg/g), syringic acid (18.90 mg/g), and quercetin (18.65 mg/g) were mainly present in *T. imperati* extracts. Although the DPPH assay showed slight antioxidant activity for both extracts, the methanolic extract exhibited significant potent anti-inflammatory action in all *in vitro* tests, protecting HRBC damage and BSA denaturation at rates of 80.18 and 97.62%, respectively. Likewise, the methanolic extract exhibited an important anticancer activity against the HT-29 and A-549 cell lines, with IC_{50} values ranging from 1.85 to 2.00 $\log_{10}$ µg/mL. Interestingly, no toxic effects on CCD18-Co were observed. Therefore, the present study revealed that *T. imperati* root extracts are rich in phenolic compounds and could be a promising source of anti-inflammatory and anticancer molecules.

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INTRODUCTION

Since ancient times, plant-based drugs have been used worldwide in folk medicine to treat and prevent various disorders, and over time, the health benefits of many of these natural products have been well established (Rios & Recio, 2005). Consequently, alternative medicines are becoming more popular and used increasingly nowadays (Gautam *et al.*, 2007). Indeed, herbs appear to be the most promising materials for discovering novel pharmacologically active compounds to treat common as well as incurable diseases, especially cancers (Hoareau & DaSilva, 1999). Besides their therapeutic potential, some species are almost daily involved in our food (Belhouala & Benarba, 2021). These dietary plants contain various antioxidant chemical substances that boost immunity. A plant-based diet can protect against chronic diseases associated with oxidative stress, and it has been hypothesized that plant phenols may contribute to this beneficial effect (Asif, 2015). Diet can modify the pathophysiological processes of metabolic disorders and can be effective in several pathological processes, most of which are known to contribute to oxidative damage (Ullah & Khan, 2008).

Of the plant foods, spices such as cloves, mint, thyme, and cinnamon have been used for centuries as food flavorings, preservatives, and ingredients in traditional medicine mainly due to their antioxidant and antimicrobial activities (Cortés-

Rojas *et al.*, 2014). Currently, many reports have confirmed the antibacterial, antifungal, antiviral, and anticancer properties of several promising spice plants (Shan *et al.*, 2005). Although many spices are known only to the indigenous population for their healing properties, they have not been scientifically investigated. Among them, *Telephium imperati* L., named in Algeria as "tassarghit/sarghina", is an herbaceous plant belonging to the family *Caryophyllaceae* (Yang *et al.*, 2017). It grows wild on rocky terrains and boulders up to 3000 m high (Nejjari *et al.*, 2019). This rare spice is well known to be used for culinary purposes. Its parts are usually consumed as soup spice for postpartum women in Algeria (Sahara and Kabyle region) (Belhouala & Benarba, 2021). *T. imperati* is used for medicinal purposes by local healers in Algeria to treat mouth ulcers and anemia (Belhouala & Benarba, 2021; Nejjari *et al.*, 2019).

Recently, the anticancer, anti-inflammatory, and antioxidant potential of phenolic compounds has been well documented (Silva *et al.*, 2024). Indeed, phenolics are considered the most important sources of dietary antioxidants owing to their free radical-scavenging potential attributed to the presence of hydroxyl groups in their structure (Tutun & Yipel, 2024). It has been demonstrated that higher phenol contents in foods, spices, and plants enhance antioxidant activity (Yen *et al.*, 2018; Muhammad *et al.*, 2020; Demir *et al.*, 2022). This antioxidant potential has been related to the chemopreventive and chemotherapeutic effects exhibited by phenolic compounds against different cancers (Srivastava *et al.*, 2023). The cytotoxic effects of phenolic compounds have been well demonstrated (Kamto *et al.*, 2023; Tavallali *et al.*, 2024). The anticancer effects of phenolics are exerted by different mechanisms including, apoptosis induction, cell cycle arrest, enzyme modulation, receptor binding, gene alteration, and modulation of oxidative stress (Maheshwari & Sharma, 2023).

To the best of our knowledge, there are no published reports on the phenolic composition or investigating the biological activities of *T. imperati*. Therefore, the current investigation was conducted on the extracts of the roots of *T. imperati* to evaluate its antioxidant, anticancer, and anti-inflammatory activities and establish their phenolic profile.

MATERIALS AND METHODS

Plant Material

Telephium imperati was collected from Biskra, Algeria, in June 2021. Botanical identification was conducted by Prof. Dr. Bachir Benarba from the department of Biology, University of Mascara, Algeria, and the voucher specimen (Voucher No. LRSBG/AB/20/158) was deposited in the LRSBG (Laboratory Research on Biological Systems and Geomatics) herbarium, a certified herbarium at the Department of Biology, University of Mascara, Algeria. The fresh roots were prewashed and dried for several days at ambient temperature (20-30 °C) in the Laboratory (Figure 1). After 10 days, the samples were crushed using an IKA M20 Laboratory universal grinder. The recovered powder was stored in a black container.

Figure 1: *T. imperati* roots

Chemicals

Chemicals such as 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), methanol, ethanol, hydrochloric acid, glacial acetic acid, ferric chloride, concentrated sulfuric acid, magnesium ribbon, Hager's reagent, sodium hydroxide solution, DPPH, phosphomolybdate reagent, Phosphate buffer, and 30% H_2O_2 solution were obtained from Sigma-Aldrich.

Preparation of Plant Extracts

The dried root powder (20 g) was mixed with 200 mL of distilled water, heated to 100 °C with continuous stirring for 20 min, cooled to room temperature, and then filtered. The residue was re-extracted twice following the same process. All filtrates were combined, concentrated, lyophilized, and stored at 4 °C until use (Yadav & Agarwala, 2011). The methanolic extract was prepared by macerating 40 g of dried roots with 400 mL of methanol for seven days (Sioud *et al.*, 2020). The mixture was continuously stirred daily at room temperature and then stored overnight at 4 °C. The obtained extracts were filtered, concentrated, and stored at 4 °C until further use.

Phytochemical Characterization

To establish the phytochemical profile of *T. imperati* root extracts, phytochemical content screening followed by HPLC-DAD analysis was performed.

Qualitative Tests

Phytochemical screening of the extracts was performed using standard methods adapted from previous studies. The presence of alkaloids, flavonoids, steroids, terpenoids, reducing sugars, saponins, tannins, cardiac glycosides, and anthraquinones was detected using Hager, Ammonia, Salkowski, Salkowski, Fehling, frothing, $FeCl_3$, Killer-Killani, and Chloroform layer tests, respectively (Sofowora, 1993; Trease & Evans, 2002; Marsoul *et al.*, 2020; Alqethami & Aldhebiani, 2021).

Quantitative Screening

Total phenolic content (TPC)

The total phenolic content of the extracts was determined using the Folin-Ciocalteu colorimetric method (Hosu *et al.*, 2014). Briefly, 1.5 mL of Folin-Ciocalteu reagent (0.2 mol/L) and 300 µL of each extract (0.1 g) were added to 1.2 mL of 0.7 mol/L Na_2CO_3, mixed, and incubated for 120 min in the dark at room temperature. The absorbance was then measured at 760 nm. A calibration curve was prepared using gallic acid at different concentrations under the same conditions. The phenolic content was calculated as gallic acid equivalent GAE/g of dry plant material. The values were determined in triplicate.

Total flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric-colorimetric method by mixing 400 µL of each extract and 120 µL of 5% $NaNO_2$ and resting for 5 min before the addition of 120 µL of 10% $AlCl_3$. 800 µL of 1 M NaOH solution were then added, and the resulting mixture was incubated for 30 min at room temperature. The absorbance was measured at 510 nm, and TFC was expressed as quercetin equivalent (QE) mg/g dry weight. Values were determined in triplicate (Marsoul *et al.*, 2020).

Determination of condensed tannin content

The condensed tannin content (TTC) was determined using the Vanillin-HCl method (Bikoro Bi Athomo *et al.*, 2018). An aliquot (0.5 mL) of the extract was mixed with 3 mL of 3% vanillin/methanol (w/v) and 1.5 mL HCl (37%). The resulting mixture was incubated at room temperature for 15 min, and absorbance was measured at 500 nm. TTC is expressed as catechin equivalent (CE) mg/g dry weight. Values were determined in triplicate.

HPLC-DAD analysis

The phenolic profiles of the *T. imperati* extracts were analyzed using HPLC-DAD as previously reported under standard conditions (Deveci *et al.*, 2019; Çayan *et al.*, 2020). The phenolic compounds were monitored at 280 nm with a photodiode array detector (PDA) and then identified in terms of retention time and UV data by comparison with commercial standards. The calibration curve was drawn via the injection of standard compounds at different concentrations, and the results are presented as mg/g extract dry weight (dw).

Antioxidant Activity

Total antioxidant capacity assay

Total antioxidant capacity (TAC) was determined using the phosphomolybdate assay, as previously described (Ahmed *et al.*, 2014). 3 mL of phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to 300 µL of the extract, and the resulting mixture was incubated in a water bath at 95 °C for 90 min. After cooling the samples to room temperature, the absorbance was measured at 765 nm against a blank (300 µL of the extract's vehicle instead of plant sample). A calibration curve was generated using different concentrations (25, 50, 100, 250, 500 µg/mL) of ascorbic acid. TAC was expressed as mg/g of ascorbic acid equivalents (mg/g AAE).

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated as described by Brand-Williams *et al.* (1995). Briefly, 1 mL of the extract or ascorbic acid (as positive control) at varying concentrations (25-500 µg/mL) was mixed with 0.1 mM methanolic DPPH solution (4 mg/100 mL) freshly prepared. The absorbance of the reaction mixture was measured at 517 nm against the DPPH methanolic solution as a control and the extraction solvent as a blank after incubating in the dark at room temperature for 16 min.

The DPPH free radical scavenging activity was expressed using the following formula:

$$\%\ \text{Scavenging activity} = \frac{A1\text{-}A2}{A1} \times 100$$

A_1: absorbance of the control (DPPH solution) without extract.
A_2: absorbance after the addition of the extract.
IC_{50} (half-maximal inhibitory concentration) value was calculated by linear regression analysis.

Reducing power assay

The reducing power was measured using the potassium ferricyanide assay (Oyaizu, 1986). Briefly, 2.5 mL of the plant extract (or standard) was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide solution (1%). After incubation at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, and centrifuged at 650 rpm for 10 min. The supernatant (5 mL) was mixed with 5 ml distilled water and 1 mL of ferric chloride solution (0.1%). The absorbance was measured at 700 nm against a blank (2.5 mL methanol instead plant sample), and the results are expressed in mg AAE/g extract.

H_2O_2 assay

The ability of the extracts to scavenge H_2O_2 was evaluated using the method of Ruch *et al.* (1989). In brief, 100 µL of the extracts or ascorbic acid (positive control) (25-500 mg/mL) were added to 3 mL of H_2O_2 (2 mM). The mixture was vortexed, and after 10 min of reaction time, the absorbance was measured at 230 nm. The ability to scavenge H_2O_2 was calculated using the following formula:

$$\%H_2O_2\ \text{Scavenging} = \frac{A1\text{-}A2}{A1} \times 100$$

A_1: absorbance of the control
A_2: absorbance of the sample

Anti-inflammatory Activity

HRBC membrane stabilizing assay

HRBC membrane stabilization activity of the extracts was determined using heat- and hypotonic solution-induced hemolysis as previously described (Sunmathi *et al.*, 2016). HRBC suspensions were prepared by mixing equal volumes of blood (O^+) obtained from healthy volunteers who had not been taking any $NSAID_S$ for the past two weeks with ELSIVER solutions (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl), followed by centrifugation at 3000 rpm for 10 min. The pellet was washed three times with an equal volume of PBS (pH=6.3), and red cells were re-suspended in PBS (10% v/v) (Azeem *et al.*, 2010). The reaction mixture (1mL of various concentrations of the extracts (25, 50, 100, 250, 500 µg/mL), 0.5 ml HRBC suspension, 1 mL phosphate buffer, and 2 mL hyposaline (0.25% w/v NaCl) was incubated at 37 °C for 30 min and then centrifuged at 300 rpm for 20 min. The control was prepared by omitting the plant extract, and the reference standard drug was sodium diclofenac (s-DCF).

The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm against the blank (Sakat *et al.*, 2010).

$$\text{Membrane stabilization}(\%) = 100 - \left(\frac{A1 - A2}{A1}\right) \times 100$$

A_1: Absorbance of the control (hypotonic-buffered saline solution alone)
A_2: Absorbance of the sample

Heat-induced hemolysis assay

1 mL of *T. imperati* extract or s-DCF (standard drug) was mixed with 1 mL of 10% HRBC suspension, and the resulting mixture was incubated in a water bath at 56 °C for 30 min, cooled under running tap water, and then centrifuged at 3000 rpm for 5 min. The supernatant absorbance was measured at 560 nm, and the percentage inhibition of hemolysis was calculated as follows (Sangeetha & Vidhya, 2016; Shinde *et al.*, 1999):

$$\% \text{ inhibition of haemolysis} = \frac{\text{Absorbance control - Absorbance test}}{\text{Absorbance control}} \times 100$$

Albumin denaturation assay

Protein denaturation is correlated with inflammatory disorders through the formation of antigens. Thus, a substance or extract that inhibits protein denaturation could exert important anti-inflammatory effects. Three protein denaturation assays were performed: bovine serum albumin and egg albumin denaturation assays and protease inhibition (Rahman *et al.*, 2015).

Bovine serum albumin (BSA) denaturation assay

0.45 mL of BSA (0.5% w/v aqueous solution) was mixed with 0.05 mL of various concentrations (50, 100, 250, 500 µg/mL) of the extract or s-DCF as the standard drug. The resulting mixtures were incubated at 37 °C for 20 min, and the temperature was increased to maintain the samples at 57 °C for 3 min. After cooling, 2.5 mL of phosphate buffer (pH=6.6) was added, and absorbance was measured at 255 nm against a blank. The control solution consisted of 0.45 mL 0.5% BSA and 50 µL distilled water (Rahman *et al.*, 2015). The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition of protein denaturation} = 100 - \left(\frac{A1 - A2}{A1}\right) \times 100$$

A_1: Absorbance of the sample
A_2: Absorbance of the control

Egg albumin denaturation assay

The prevention of egg albumin denaturation was carried out according to the method described by Sunmathi *et al.* (2016). 2 mL of varying concentrations of the extract (50, 100, 250, 500 µg/mL) or s-DCF (positive control) were mixed with 0.2 mL of egg albumin (0.5% w/v aqueous solution) and 2.8 mL of PBS (pH=6.4). After incubation at 37 °C for 15 min, the mixture was heated at 70 °C for 5 min, and absorbance was then measured at 660 nm against the solvent as blank and distilled water instead of the extract as a control. Inhibition of protein denaturation (%) was calculated using the following formula:

$$\% \text{ inhibition of protein denaturation} = \frac{A1 - A2}{A1} \times 100$$

A_1: Absorbance of the sample
A_2: Absorbance of the control

Proteinase inhibition

The test was performed according to the method of Oyedapo and Femurewa (1995), modified by Sakat *et al.* (2010). The reaction mixture (2 mL) containing 0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH 7.4), and 1 mL test samples of different concentrations was incubated at 37 °C for 5 min. Then, 1 mL of 0.8% (w/v) casein was added, and the mixture was incubated for 20 min. 2 mL of 70% perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against the buffer as blank. The experiment was performed in triplicate. The percentage of proteinase inhibitory activity was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance control - Absorbance test}}{\text{Absorbance control}} \times 100$$

Anticancer Activity

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium reduction assay

The cytotoxic effects of *T. imperati* extracts on the viability of three human cancer cell lines (HT-29, PC-3, A 549) and normal human (CCD18-Co) cells were determined using the colorimetric MTT assay. In brief, cells were seeded in 96-well plates at a density of $1x10^4$ cells/well and incubated for 24 h in a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C. Cells were then treated with increasing concentrations (from 0 to 200 µg/mL) of *T. imperati* extract and incubated for 72 h. At the end of treatment, 10 µL of MTT (0.5 mg/mL) were added. After 4 h of incubation, the medium was gently removed and 100 µL of pure DMSO was added to dissolve the formazan blue crystals. The plate was incubated for 10 min under obscurity, and absorbance was recorded at 540 nm using a microplate reader. All experiments were performed in triplicate (Wangchuk *et al.*, 2011).

Statistical Analysis

The data were expressed as the mean±standard deviation (SD) of triplicate independent experiments and analyzed via two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison using EXCEL PRO 2019-QI Macros and GraphPad Prism version 8 Software. $p<0.05$, <0.01, <0.001 and <0.0001 were considered statistically significant.

RESULTS AND DISCUSSION

Extraction Yield, Quantitative and Qualitative Phytochemical Screening

The aqueous and methanol extracts of the powdered roots of *T. imperati* gave yields of 1.6% and 1.4%, respectively. Regarding quantitative phytochemical screening, our results showed significantly higher values for the alcohol extract compared with water extract ($p<0.05$), except for TTC, the difference was not statistically significant (Table 1).

Indeed, TPC, TFC, and TTC in the methanolic extract were found to be 493.17±0.0 mg GAE/g, 271.56±0.1 mg QE/g and 106.50±2.3 mg CE/g, whereas those of the aqueous extracts were 275.83±0.2 mg GAE/g, 191.79±0.5 mg QE/g and 64.83±0.9 mg CE/g, respectively. This suggests that the use of MeOH resulted in a higher amount of TPC with almost double the quantity of phenols, flavonoids, and tannins. In this study, methanol was found to be more efficient than other solvents for extracting the phenolic compounds of *T. imperati*. This was expected since previous studies consistently found higher concentrations of phenols in alcohol extracts. In fact, alcohols, which are less polar solvents, can break down plant cell walls more efficiently and thus extract the targeted components (Lapornik *et al.*, 2005; Spigno *et al.*, 2007; Adedapo *et al.*, 2011). Our study reports phytochemical content of *T. imperati* prepared using two extraction methods. The water extraction method used in this study was consistent with the traditional preparations used by healers in Sahara and Kabylie regions of Algeria (Belhouala & Benarba, 2021). Methanol extracts are preferentially involved in most pharmacological investigations studying the activity of medicinal herbs (Cieniak *et al.*, 2015).

On the other hand, the qualitative phytochemical screening of *T. imperati* revealed the presence of cardiac glycosides, flavonoids, phenols, phytosteroids, tannins, and quinones. These classes were detected in both extracts, whereas the methanolic extract exclusively contained alkaloids, terpenoids, and saponins. Moreover, the aqueous extract revealed the presence of anthraquinones.

Phenolic Profile (HPLC-DAD Analysis)

On the other hand, HPLC-DAD analysis revealed remarkable qualitative and quantitative phenolic compounds in *T. imperati*. As shown in Table 2, 19 compounds were detected in this plant, including caffeic acid (78.61, 33.77 mg/g), ferulic acid (49.12 mg/g), rosmarinic acid (42.80, 23.35 mg/g), coumarin (39.87 mg/g), rutin (37.68 mg/g), myricetin (25.62, 18.03 mg/g), syringic acid (18.90 mg/g), quercetin (18.65, 12.37 mg/g), and p-hydroxy benzoic acid (17.63 mg/g) in methanolic and aqueous extracts, respectively. It is obvious that the methanolic extract is richer in these compounds than aqueous extract, indicating the important presence of caffeic acid, ferulic acid, and rosmarinic acid. However, the aqueous extract contained higher amounts of kaempferol, apigenin, and chrysin than the methanolic extract. Moreover, trans-cinnamic acid was only detected in the alcoholic extract.

Due to the lack of phytochemical studies on this species, no information could confirm these results. However, Nejjari *et al.* (2019) reported that the ethanolic extract of *T. imperati* leaves contained saponins and flavonoids, which corroborate our results showing the detection of saponins exclusively in the methanolic extract. Moreover, quercetin was found to be one of the components of the chloroform and diethyl ether extracts of plant leaves.

Overall, the results revealed *T. imperati* contains a variety of phytchemicals, and the methanolic root extract of this plant

Table 1: Extraction yield, total phenolic, flavonoid and tannin contents of *T. imperati* extracts

Extract	Yield %	Phenols (mg AGE/g)	Flavonoids (mg QE/g)	Tannins (mg CE/g)
Aqueous	1.6	275.83±0.2****	191.79±0.5**	64.83±0.9
Methanol	1.4	493.17±0.0****	271.56±0.1**	106.50±2.3

****<0.0001, **=0.0047

showed the presence of most of the tested phenolic compounds, such as caffeic acid, rutin, rosmarinic acid, quercetin, luteolin, and kaempferol. Although a solvent effect appears clearly in the variety of phytochemical contents of *T. imperati*, this effect was not noted for some compounds (gallic acid). Hence, these phytochemicals may have important biological activities, as previously mentioned (Wangchuk *et al.*, 2011).

Table 2: Phenolic composition of the extracts (mg/g extract)

S. No.	Phenolic compounds	RT (min)	TEL-Met	TEL-Aqu
1.	Gallic acid	5.70	5.18	7.90
2.	Protocatechuic acid	8.75	8.66	6.49
3.	Chlorogenic acid	12.35	8.56	6.07
4.	p-hydroxybenzoic acid	12.77	17.63	15.82
5.	6.7-Dihydroxy coumarin	14.10	2.41	1.04
6.	Caffeic acid	15.09	78.61	33.77
7.	3-hydroxybenzoic acid	15.98	1.55	0.78
8.	Syringic acid	16.56	18.90	7.24
9.	Ferulic acid	22.14	49.12	29.50
10.	Coumarin	24.49	39.87	12.47
11.	Rutin	25.30	37.68	19,43
12.	Rosmarinic acid	26.77	42.80	23.35
13.	Myricetin	27.35	25.62	18.03
14.	Quercetin	30.43	18.65	12.37
15.	trans-cinnamic acid	31.33	3.37	-
16.	Luteolin	31.70	10.58	5.88
17.	Kaempferol	33.21	3.96	5,12
18.	Apigenin	33.77	11.31	15.26
19.	Chrysin	38.40	4.58	9.39

Antioxidant Activities

It has been found that disturbances of the oxidative status may contribute to the emergence and development of a number of diseases (Rahman *et al.*, 2012). Therefore, the antioxidant contents of medicinal plants have been hypothesized to account for the ability of these plants to improve many pathological conditions (Hegde *et al.*, 2008).

Data from the antioxidant assays shown in Figure 2. The total antioxidant capacity of the extracts ranged from 0.18 to 0.42 mg AAE/g, which was less than that of the standard (1.05 mg AAE/g) (Figure 2a). Regarding the DPPH scavenging activity, the same results were obtained for both extracts, which exhibited a dose-dependent scavenging activity lower than that of ascorbic acid. Actually, both methanolic and aqueous extracts of *T. imperati* dried roots had maximum values of 7.74 and 7.30 % at the highest concentration (500 µg mL^{-1}) (Figure 2b).

Likewise, our results showed that the methanolic extract exhibited considerable reducing activity (0.97 mg AAE g^{-1}). The value of the latter was lower than that exhibited by the standard (1.52 mg AAE g^{-1}) (Figure 2c). Furthermore, a slight hydrogen peroxide scavenging activity for both extract (IC_{50}>500 µg mL^{-1}) was observed, which was not comparable to that of ascorbic acid (Figure 2d).

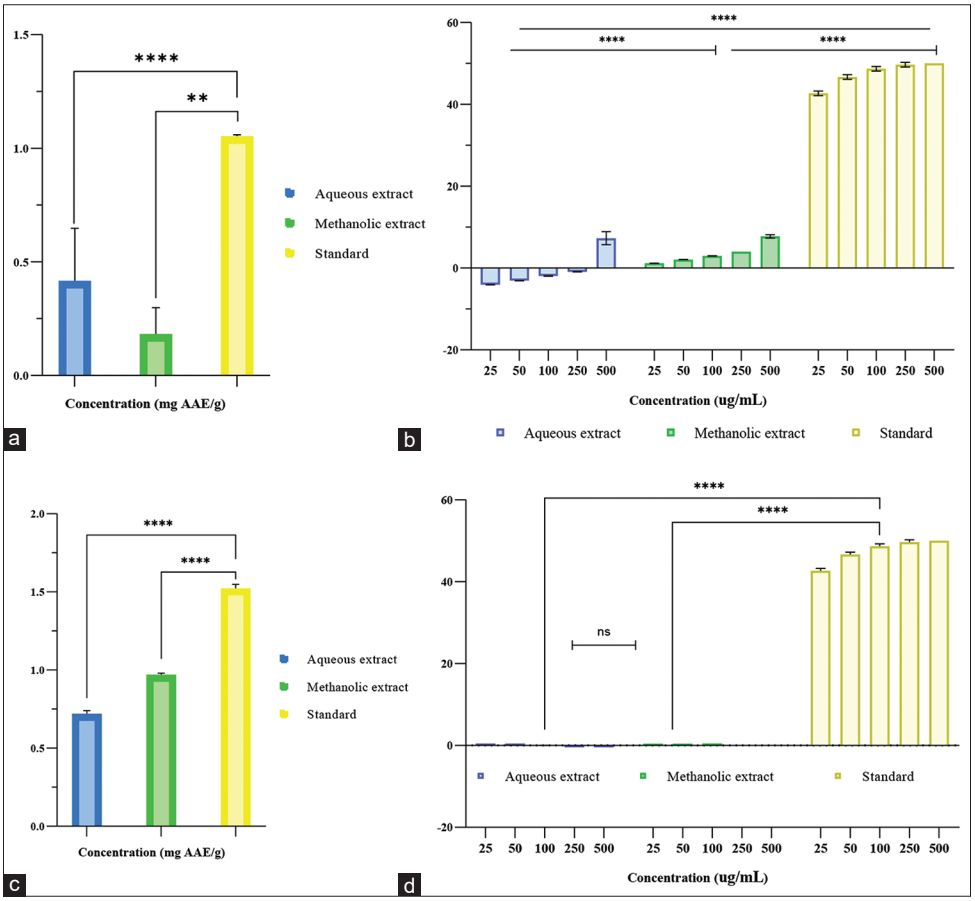

Figure 2: Antioxidant activity of *T. imperati*. a) Total antioxidant capacity, b) DPPH, c) Reducing power, and d) H_2O_2

Although high-quality phenolic compounds were detected in *T. imperati*, no significant antioxidant activity was observed. These results seem to suggest that there is no relationship between the phenolic content and the antioxidant activity of the *T. imperati* root extracts tested, confusingly suggesting that the phenolic compounds of this plant would not contribute to the radical scavenging activity of the species or that there are other compounds in which their combination suppresses the activity. A previous study by Liu *et al.* (2022) reported that the formation of caffeic acid and tartaric acid ester bonds could inhibit the antioxidant capacity of caffeic acid. In contrast to our findings, several studies have evaluated the relationship between the antioxidant activity of plant extracts and their phenolic content. It has been reported that phenolic compounds were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity (Wojdyło *et al.*, 2007).

Anti-inflammation Activity

In our study, we focused on the anti-inflammatory effect of *T. imperati* due to its contribution to the treatment of many inflammation-related diseases. The absorbance of hemoglobin was determined using an HRBC membrane stabilization method in which hemoglobin is released as a result of lysates of the RBC membrane due to less stabilization of the membrane (Sangeetha & Vidhya, 2016).

The plant extracts exhibited membrane stabilization via hypotonic and heat-induced lysation of the erythrocyte membrane, as shown in Figure 3. The results showed that the extracts (at concentration 500 µg/mL) were significantly potent in human erythrocytes, adequately protecting them against hypotonic solution and heat-induced lyses, compared with the standard drug (s-DCF) ($p<0.0001$). In a dose-dependent manner, the highest stabilization effect was obtained by the methanolic extract, with an increased stabilization percentage of 80.18% at the highest dose, while the aqueous extract reached a lower activity of 66.72%, followed by that of the standard (19.73%). Regarding heat-induced HRBC hemolysis, plant extracts significantly showed an interesting protective effect, reducing the percentage of lysis to no lysis action by the methanolic extract and almost zero by the aqueous extract compared with s-DCF (5.91%).

On the other hand, chronic inflammation can lead to many serious problems, such as arthritis (Jayaprakasam & Ravi, 2012). This inflammation is caused by protein denaturation, which is taregetd by anti-inflammatory drugs (steroids) to decrease inflammation in tissues and reducing the immune activity responsible for inflammation (Kanimozhi *et al.*, 2022).

The anti-inflammatory activity of *T. imperati* was also determined by egg albumin and BSA denaturation at various concentrations of 50, 100, 250 and 500 µg/mL. The results of the anti-denaturation activity of the extracts from *T. imperati* root are given in Figure 4. The methanolic extract showed a marked inhibition of protein denaturation, with significant values of 97.62 and 85.84% for both assays (BSA and egg albumin), respectively, exceeding those of the aqueous extract (96.92 and 84.91%). Interestingly, the protective effect of the plant extracts against egg albumin denaturation was significantly greater than that of the standard drug. Furthermore, the methanolic extract showed total inhibition of protease action, reaching a maximum percentage of 99.48%, whereas the aqueous extract resulted in 97.11% inhibition (Figure 4c). Interestingly, both extracts exhibited significantly higher anti-protease activity than s-DCF. On the basis of these results, it is demonstrated that extracts of *T. imperati* roots have significant anti-inflammatory potential comparable with that of s-DCF (standard drug).

Our findings corroborate those reported by Nejjari *et al.* (2019) demonstrating the wound-healing potential activity of the hydroalcoholic extract of *T. imperati* on Wistar rat skin. In this study, treatment with *T. imperati* promoted cell proliferation and formation of a thick granulation tissue in an accelerated manner, with a decrease in inflammatory cells, suggesting an anti-inflammatory activity related to burn wound healing.

Obviously, the phenolic compounds found in *T. imperati* could contribute to the anti-inflammatory activity of this plant, such as caffeic acid, which was found to be the major phenolic compound found in the methanolic (78.61 mg/g) and the aqueous extract (33.77 mg/g). Indeed, caffeic acid has been shown to directly inhibit IRAK1, IRAK4, and JNK, along with strong suppression of the nuclear translocation of AP-1 family proteins acting as an anti-inflammatory drug (Yang *et al.*, 2013). Furthermore, this compound can simultaneously suppress

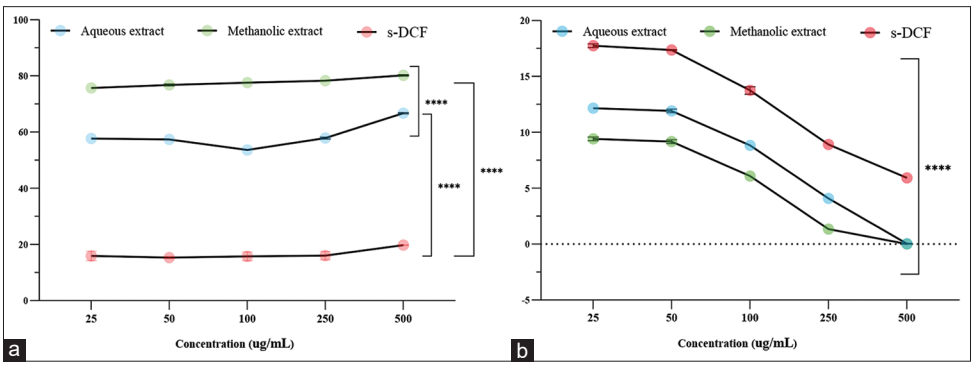

Figure 3: HRBC membrane stabilization and hemolysis inhibition (percentage) of *T. imperati* extracts compared with diclofenac sodium control. a) HRBC membrane stabilization and b) hemolysis inhibition.

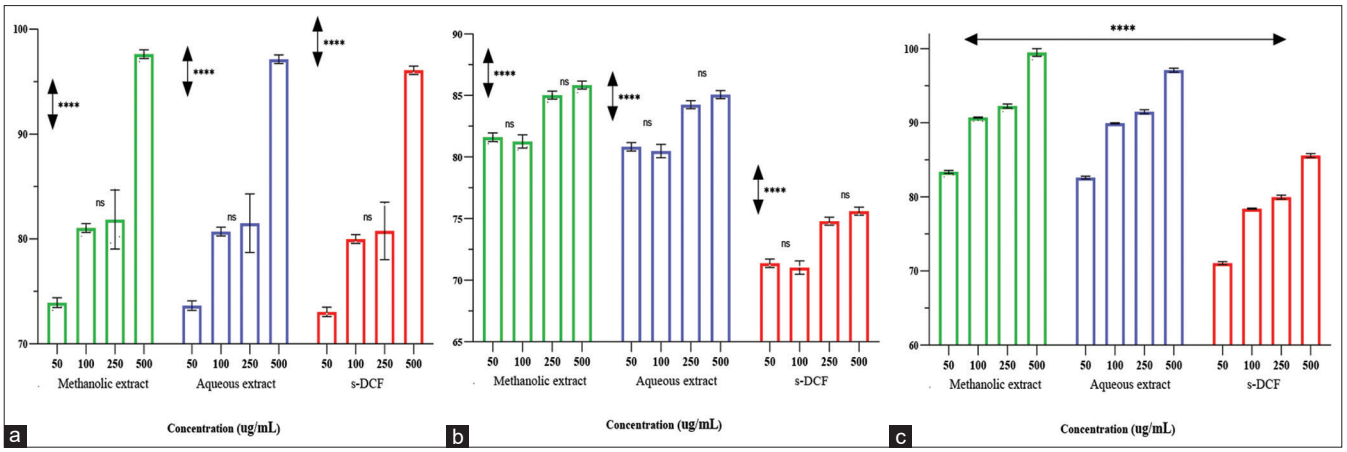

Figure 4: Inhibition of albumin denaturation by *T. imperati* aqueous and methanolic extracts compared with the diclofenac sodium control. a) BSA, b) Egg and c) Protease.

Table 3: Inhibition concentrations (IC_{50} in μg/mL) of *T. imperati* toward cancerous and nontumorigenic cell lines as determined using the MTT assay

T. imperati	Canser Cell Lines			Human Cell Line
	HT-29 IC_{50} (log10 μg/mL)	PC-3 IC_{50} (μg/mL)	A 549 IC_{50} (log10 μg/mL)	Nontumorigenic (CCD18-Co) IC_{50} (μg/mL)
Methanolic	1.85	>200	2	>200
Aqueous	1.71	>200	>200	>200

Values are presented as mean±SD (n=3)

the activation of various transcription factors involved in inflammation, such as NFAT, NF-κB, and AP-1 (Feng *et al.*, 2005; Lee *et al.*, 2012). Moreover, Nile *et al.* (2016) found that gallic, ferulic, and caffeic acid, abundant compounds in *T. imperati*, are promising anti-inflammatory agents that inhibit xanthine oxidase and cyclooxygenase-2. Additionally, another major compound, rosmarinic acid (methanolic: 42.80; aqueous: 23.35 mg/g) has been revealed to have an *in vitro* and *in vivo* anti-inflammatory effect against various inflammatory diseases like arthritis, colitis, and atopic dermatitis (Luo *et al.*, 2020). Although, our results were observed using experimental data, the mechanism of anti-inflammatory activity of *T. imperati* is unknown.

Anticancer Activity

Our results showed that the methanolic extract exerted significant antiproliferative effects against two human cancer cell lines from two different tissues, HT-29 and A549. Maximum growth inhibition (92%) was observed against HT-29 with low IC_{50}=1.85 $\log_{10}{}^{\mu}g$/mL, followed by 76% cytotoxicity against A-549 (IC_{50}=2 $\log_{10}{}^{\mu}g$/mL), and 23% against PC-3 with high IC_{50}>200 µg/mL (Table 3). The aqueous extract of *T. imperati* suppressed only the proliferation of HT-29 with IC_{50}=1.71 $\log_{10}{}^{\mu}g$/mL. Interestingly, both extracts from *T. imperati* exhibited no *in vitro* cytotoxic activity against non-tumorigenic cells (CCD18-Co) (>200 µg/mL). Thus, we demonstrated here that the methanolic extract of *T. imperati* displayed significant anticancer efficacy against carcinoma cells without affecting healthy cells. Most drugs used in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic, and teratogenic effects in non-tumor cells. Therefore, there is a need for alternative natural drugs that are less toxic and have fewer side effects. The anticancer activity exhibited by *T. imperati* could be attributed to the phenolic compounds. We found that the major phenolic compounds found in both *T. imperati* extracts were caffeic acid, ferulic acid, rosmarinic acid, coumarin, rutin, and myricetin. Caffeic acid was demonstrated to possess multiple biological activities, including anticancer potential (Min *et al.*, 2018), by targeting MMP-9 (Singh *et al.*, 2018). Moreover, ferulic acid has been shown to be a potent anticancer agent despite its low bioavailability (Sweed *et al.*, 2024). The promising anticancer effects of rosmarinic acid, coumarin, rutin, myricetin, and other phenolic compounds have been documented (Benarba *et al.*, 2019; Bouyahya *et al.*, 2022; Konstantinou *et al.*, 2023; Belhouala *et al.*, 2024).

CONCLUSION

To the best of our knowledge, this is the first study on this plant including, a phenolic analysis and biological investigation. We found that *T. imperati* methanolic and aqueous extracts contained various phenolic compounds with the dominance of caffeic acid, ferulic acid, rosmarinic acid, coumarin, rutin, and myricetin. Moreover, we demonstrated that *T. imperati* anti-inflammatory effect was higher than that of the drug reference, suggesting that phenolic compounds are probably responsible for this impressive effect. Interestingly, both extracts exhibited anticancer activity with no cytotoxic effect against non-tumorigenic cells. Taken together, our results demonstrate that *T. imperati* could be a promising source of anti-inflammatory

and anticancer agents. Further studies are needed to establish the complete phenolic profile of the plant and clarify the mechanisms responsible for these activities.

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