Segueni K *et al*. (2023)
Notulae Scientia Biologicae
Volume 15, Issue 4, Article number 11772
DOI:10.15835/nsb15411772
Research Article

Phytochemical profile, antioxidant and anti-inflammatory activities of crude latex (*Pergularia tomentosa* L.) in Algerian Saharan

Khaoula SEGUENI[1,2]*, Atef CHOUIKH[1,3], Mohammed Laid TLILI[1,2]

[1]*El Oued University, Laboratory of Biology, Environment and Health (LBEH), Algeria; khaoula-segueni@univ-eloued.dz (*corresponding author); chouikhateff@gmail.com; laidtlili2@gmail.com*
[2]*El Oued University, Faculty of Natural Science and Life, Department of Cellular and Molecular Biology, Algeria*
[3]*El Oued University, Faculty of Natural Science and Life, Department of Biology, B.P. 789, 39000, El Oued, Algeria*

Abstract

This study aimed to evaluate the dry crude latex extract from *P. tomentosa*, endemic to the Sahara, by determining the total content of polyphenols and flavonoids, and detecting the plant compounds by HPLC chromatography. Also, Antioxidant activity was measured through three tests: (BCB) beta-carotene/linoleic acid bleaching assay, (DPPH) radical scavenging and (FRAP) ferric reducing ability assay. In addition, anti-inflammatory activity. The results showed that the dry crude latex extract of *P. tomentosa* showed moderate content of polyphenols and flavonoids in the dry crude latex extract of *P. tomentosa*. Besides, qualitative HPLC analysis led to the detection of a group of phenolic compounds of this extract that have therapeutic properties. Also, the studied extract had strong activity in the beta-carotene/linoleic acid bleaching test and the values obtained were very close to the reference gallic acid. The results also indicated significant antioxidant activities in the DPPH and FRAP assay compared to ascorbic acid. In addition, the strong effect of dry crude latex extract from *P. tomentosa* in inhibiting protein denaturation induced by albumin hyperthermia, compared to *ASPEGIC*®, which is used as a reference drug in infections. In conclusion, the results indicate that the dry crude latex extract of *P. tomentosa* has antioxidant and anti-inflammatory activity, which confirms the use of this extract in folk medicine, which could be a first step in introducing it into therapeutic applications.

Keywords: antioxidant activity; anti-inflammatory activity; crude latex; HPLC chromatography; *Pergularia tomentosa* L.

Introduction

Since ancient times, human have been interested in including natural products in the use of traditional medicine (Haidan *et al*., 2016). It has been used more 35.000 species medicinal purposes (Hafsia *et al*., 2020), improving health; Plants remain promising sources of bioactive compounds (Abdel-Aty *et al*., 2019) they protect cells from the damage of oxidative stress and various diseases caused by free radicals (ROS) (Yıldırım *et al*., 2019; Hafsia *et al*., 2020).

Received: 25 Oct 2023. Received in revised form: 15 Nov 2023. Accepted: 21 Nov 2023. Published online: 23 Nov 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae 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.

Therefore, the researchers focused their attention on natural antioxidants, especially those derived from plants that are able to clean and repair damage caused by free radicals that have antioxidant and anti-inflammatory activities (Ernst Kerche-Silva *et al*., 2017; Yıldırım *et al*., 2019).

P. tomentosa belongs to the Asclepiadaceae family (Lahmar *et al*., 2022). It is a wild perennial plant that grows in the Mediterranean regions, and in arid and semi-arid regions and it is 50 cm high (Al-Hinai *et al*., 2018; Lahmar *et al*., 2018; Lahmar *et al*., 2022). It is classified as a poisonous plant with a distinctive odor (Al-Hinai *et al*., 2018; Ads *et al*., 2021). It contains a sap called latex, which is used in folk medicine to accelerate wound healing and treat skin infections (Lahmar *et al*., 2022). Also, it has been shown to possess antioxidant and antifungal activity (Lahmar *et al*., 2018).

Generally, latex is a viscous polymer exuded from various plant parts (Abdel-Aty *et al*., 2019; Salomé Abarca *et al*., 2019). It is produced and stored in specialized cells called laticifers (Ramos *et al*., 2019; Castelblanque *et al*., 2020). Due to the chemical diversity that distinguishes latex from other plant secretions, it has different biological and pharmacological activities. Popularly, latex has been used in the treatment of many ailments (Konno, 2011; Salomé Abarca *et al*., 2019; Castelblanque *et al*., 2020). As an anti-inflammatory, anti-diarrheal (Roy *et al*., 2005; Hua *et al*., 2015). In addition, it is used as an antifungal and antiviral (Yıldırım *et al*., 2019). In addition to these biological effects, it has powerful: antioxidant, antimicrobial and anticancer pharmacological properties (Abdel-Aty *et al*., 2019).

Nowadays, phytotherapy, based on science, has become an important alternative trend in the world to find effective alternative natural medicines without unwanted effects. According to previous literature survey We found a limited number of studies of latex extract of *P. tomentosa* and due to the common use of latex without any studied background.

Therefore, in this study, we sought to evaluate the phytochemical properties of latex extract of *P. tomentosa* by estimating the total phenolic and flavonoid content, performing qualitative analysis by HPLC chromatography, and determining anti-inflammatory activity. In addition, the antioxidant activity of three assays were measured: they included a ß-carotene/linoleic acid bleaching assay, DPPH radical scavenging and (FRAP) Ferric reducing power assay.

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu Reagent (99%), Chloroform (99.8%) from Biochem Chenopharma Co, France, Aluminum Trichloride ($AlCl_3$), Ferric Chloride $FeCl_3$ (99.99%), Sodium Carbonate Na_2CO_3 (99.5%), Trichloroacetic Acid (99%) from Prolabo (USA). Ascorbic Acid (99%), Gallic Acid (99%), DPPH (2,2-Diphenyl-1-picrylHydrazyl radical) (99%), Potassium Ferricyanide ($K_3[Fe(CN)_6]$(99%), Quercetin (95%), Methanol (99.7%), Acetonitrile (99.9%) and the phenolics standards all (99%) and all other reagents of analytical Chemicals were received from Sigma Aldrich co, St. Louis, MO, USA).

Collection and preparation of plant material

Latex was collected from the aerial parts of *P. tomentosa* in December 2022 in the Meguibra 34°15'20.7"N 6°00'50.4"E" (El oued region). After identification of the plant material by Pr. Chouikh Atef, Head of the Laboratory of Biology, Environment and Health (LBEH), University of El oued, Algeria. A voucher specimen (LOST. St 17/010) has been deposited in the herbarium of Faculty of Natural Science and Life, University of El oued, Algeria. Sample were dried at room temperature away from light and moisture. Then the sample was ground and stored at 4 °C until future use.

Quantification of phytochemical compounds of crude latex from P. tomentosa

Estimation of the total phenolic content (TPC)

Total phenolic content of using the Folin-Ciocalteu method by Guha *et al*. (2010), with some modifications. 0.2 mL was taken from of dry crude latex extract from *P. tomentosa* was taken and 1 mL of Folin-Ciocalteu reagent (10%) was added to it after 5 min of shaking. Add to the mixture 0.8 mL of diluted sodium carbonate (7.5%). After 40 min incubation at room temperature. The absorbance was measured at 765 nm. We reported the total the results for phenolic content in milligrams of Gallic acid equivalent per gram of dry crude latex extract.

Estimation of total flavonoids content (TFC)

The flavonoid content of crude latex extract was calculated using the Aluminum trichloride method, according to Chouikh (2020). We blended 0.5 mL of crude latex extract from *P. tomentosa* plant with 0.5 mL of $AlCl_3$ (2%) solution after 15 min.

The absorbance was measured at 430 nm of flavonoids content of dry crude latex in milligrams of quercetin equivalent (QE) per gram of dry crude latex.

Qualitative analysis of by high-performance liquid chromatography (HPLC)

In this study, in order to detect phenolic compounds, we use high-performance liquid chromatography (HPLC), which is the most widely used technique for the separation of phenolic compounds, a high-performance liquid chromatography (HPLC) system, a Shimadzu LC 20 AL equipped with a universal injector (Hamilton 25 µL) The analytical column used was a Shimadzu VP-ODS C18 package (4.6 mm × 250 mm, 5 µm), type (Shimadzu). A UV- VIS detector SPD 20A (Shimadzu) was used. 20 µL of a dry crude latex extract solution was injected into the mobile phase consisted in (acetonitrile /acetic acid 0.1% v/v) flow. Also, separated compounds were determined using the column for 40–50 min, Quantification of the separated peaks was performed by titration with standards with detection at $\lambda = 268$ nm (Chouikh, 2020).

Evaluation of antioxidant activity

$DPPH^{\bullet}$ free radical scavenging activity

Scavenging activities were measured using 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH), as per Ao *et al*. (2008), with some modifications. The reaction mixture consisted of 0.2 mL of different concentrations of dry crude latex extract from *P. tomentosa* with 1 mL of DPPH (0.1×10^{-3}mol) solution prepared in methanol, repeated in triplicate. After the mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. Methanol used as a control, ascorbic acid as a positive standard, Inhibition of DPPH radical was calculated using the equation:

$$I\,(\%) = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Where Abs control is the absorbance of the control and Abs sample is the absorbance of the tested sample or positive standard. The IC_{50} value which represented the concentration of the samples that caused 50% inhibition was determined for all test samples according to Asamenew *et al*. (2011). it was expressed in mg/mL.

ß-Carotene / Linoleic Acid Bleaching assay (BCB)

The lipid peroxidation counteracting activity of latex from *P. tomentosa* using ß-Carotene/Linoleic Acid were determined, according to Nickavar and Esbati (2012), with some modification. A solution was prepared consisting of 2 mg ß-Carotene, 10 mL chloroform, 45 µL linoleic acid, and 400 mg Tween 40. The chloroform was evaporated, then distilled water saturated with oxygen was added until it reached 200 mL. 0.5 mL was taken for each sample prepared with methanol from several concentrations and 4.5 mL of the prepared ß-carotene solution was added to it, after which the tubes were incubated in a water bath at 50 °C for 2h.

Inhibition percentage of bleaching calculated using the following equation:

$I_{bleaching}$ (%) = (Abs after 2h of assay/ Initial Abs) × 100

The absorbance was measured immediately at initial absorbance and after absorbance after 2h of assay at 470 nm. Gallic acid was used as a positive control.

Reducing power assay (FRAP)

The ability to reduction of the ferric cyanide (Fe^{3+}) to the ferrous cyanide (Fe^{2+}) was measured according to the protocol described by Amarowicz *et al*. (2004). With a little modification. Briefly, 0.5 mL of latex extract were mixed with 1 mL of phosphate buffer (M:0.2, pH 6.6), 1 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min and then 1 mL of trichloroacetic acid (10%) was added in order to stop the reaction. The tubes were then centrifuged at 3000 rpm for 10 min. Then 1 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.2 mL of $FeCl_3$ (0.1%).

The absorbance was determined at 700 nm. Ascorbic acid was used as a positive standard. The effective concentration of dry crude latex extract from *P. tomentosa*, Ascorbic acid EC_{50} was calculated at absorbance 0.5.

Anti-inflammatory activity of dry crude latex of P. tomentosa

The egg albumen protein denaturation method was adopted as an anti-inflammatory test. According to Boudebia *et al*. (2022): We mixed 0.05 mL of egg albumen (fresh chicken eggs) with 0.7 mL of phosphate-buffered saline (M: 0.1, pH: 6.4) and 0.5mL of Different concentrations of dry crude latex extract. The mixture was incubated at 37 °C for 15 min and then immediately placed in a water bath at 70 °C for 5 min. After cooling, it was centrifuged at 3000 rpm for 10 min for latex extract or *ASPEGIC®*. Absorbance was measured at 660 nm. The adoption of *ASPEGIC®* as a positive control (reference drug). The percentage inhibition of protein denaturation was calculated using the following formula:

Inhibition (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$

Where in: Abs control is the absorbance in the absence of extract; Abs sample is the absorbance in the presence of the latex or the standard.

Statistical analysis

All the analyses of the tests were triplicated. Data were analysed and presented as an average and calculate the IC_{50} and EC_{50} values using Microsoft Excel (version. 2013), which was also used to plot the different curves.

Results

Analyse qualitative by HPLC of dry crude latex extracted from P. tomentosa

This work is the first study to investigate the phenolic compounds of latex. HPLC analysis is the best method for the chemical characterization of a plant extract (Afsar *et al*., 2016). We used HPLC analysis to identify and quantify the concentration of phenolic compounds. HPLC analysis of our sample showed a high variation in phenolic acids as well as some flavonoids).

The results are shown (Table 1) and (Figure 1). Where 03 phenolic compounds were detected: Quercetin (1235.33 µg/g dry crude Latex). followed by Chlorogenic Acid (41.99 µg/g dry crude latex) and p-Coumaric Acid (14.84 µg/g dry crude Latex). Most of the phenolic compounds identified in the latex have various therapeutic properties besides their antioxidant, antitumor and anti-carcinogenic properties (Abdel-Aty *et al*., 2019).

Table 1. Retention time and concentration of phenolic compounds identified from latex of *P. tomentosa*

Phenolic compound	Retention time (min)	Equation	Concentration (µg/g dry crude Latex)
Quercetin	45.10	Y= 45378X	1235.33
Chlorogenic acid	13.39	Y=21665X	41.99
p-Coumaric acid	23.63	Y= 49495X	14.84

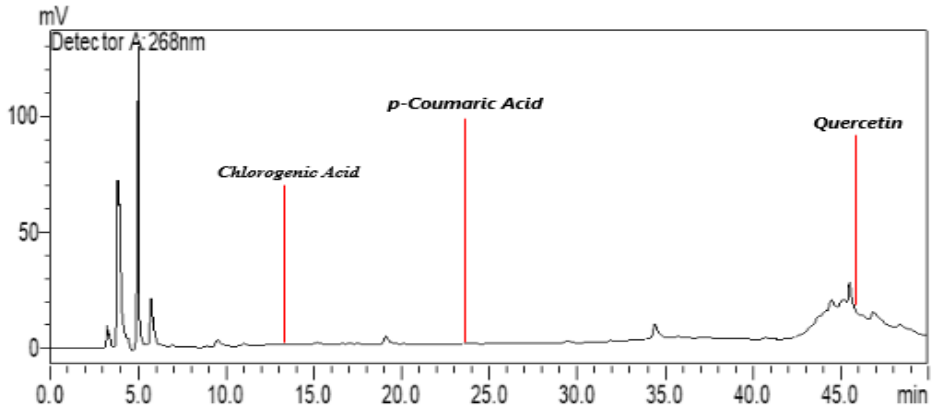

Figure 1. HPLC chromatographic profile of dry crude latex extracted from *P. tomentosa*

Evaluation quantification of polyphenols and flavonoids content of dry crude latex of P. tomentosa

The results are shown in (Figure 2) after using the equations of calibration curve (y= 13.99x +0.007, R^2= 0.99) for total polyphenols and (y= 34.071x -0.028, R^2= 0.98) for total flavonoid. The total polyphenols and flavonoids content of dry crude latex of *P. tomentosa* were (32.86 ± 0.001 mg GAE/g dry crude latex), (4.49 ±0.000 mg QE/g dry crude latex), respectively.

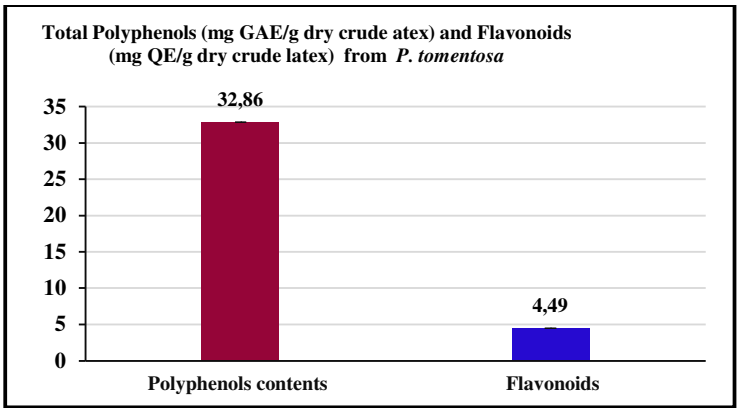

Figure 2. Polyphenols and flavonoids content of dry crude latex of *P. tomentosa*

As a result of surveying previous literature on this topic, there are a very limited number of studies on the latex extract of *P. tomentosa*. Shows the total phenolic and flavonoid content and their chemical composition. Based on previous studies, results obtained in this study differed from a study conducted by Lahmar *et al*. (2022), the total phenolic content TPC and total flavonoid content TFC of latex extract of *P. tomentosa* were reported as (62.3 mg GAE/g and 24.8 mg QE/g), respectively.

In another study conducted by Abdel-Aty *et al*. (2019), on three different plant species on latex extracts of *Ficus carica, Ficus sycomorus* and *Euphorbia tirucalli* the total content phenolic and flavonoid was 50.2, 88.0, 10.5 mg GAE/g latex, respectively and 12.5, 34.0 and 4.3 mg CE/g latex, respectively.

Plant secondary metabolites are bioactive compounds with diverse pharmacological properties (Afsar *et al*., 2016). Among them are phenolic compounds and flavonoids, which play an important role as antioxidants and may be useful in protecting against many chronic diseases (Nickavar and Esbati, 2012), and anti-inflammatories (Afsar *et al*., 2016).

The different plant species of latex extract highlight this difference in plant chemistry. Therefore, it is believed that different environmental conditions, in addition to the difference in the entire collection period, may affect in one way or another the amount of synthesis of phytochemical metabolites, including polyphenol content (Sihoglu Tepe, 2021).

Evaluation of antioxidant activity of dry latex of P. tomentosa

The results indicate in (Table 2) and (Figure 3) the antioxidant Activity estimated by IC_{50} and EC_{50} values of dry crude latex extract of *P. tomentosa*.

Table 2. The antioxidant activities of latex extract of *P. tomentosa*

Extract /standard	Antioxidant activities		
	EC_{50} mg/mL	IC_{50} mg/mL	
Dry Crude latex of *P. tomentosa*	Ferric reducing power assay	Free radical scavenging activity	ß-Carotene / Linoleic Acid Bleaching assay
	2.42 ± 0.065	0.69 ± 0.066	0.04 ± 0.014
Ascorbic acid	0.03 ± 0.001	0.03 ± 0.001	/
Gallic acid	/	/	0.01 ± 0.000

IC_{50} value is the concentration of dry crude latex extract of *P. tomentosa*. Required to scavenge 50% of either free radical scavenging activity DPPH or ß-Carotene /Linoleic Acid Bleaching assay. EC_{50} is the effective concentration of extract at which the absorbance was 0.5. Values are presented as means ± S.E. (n = 3).

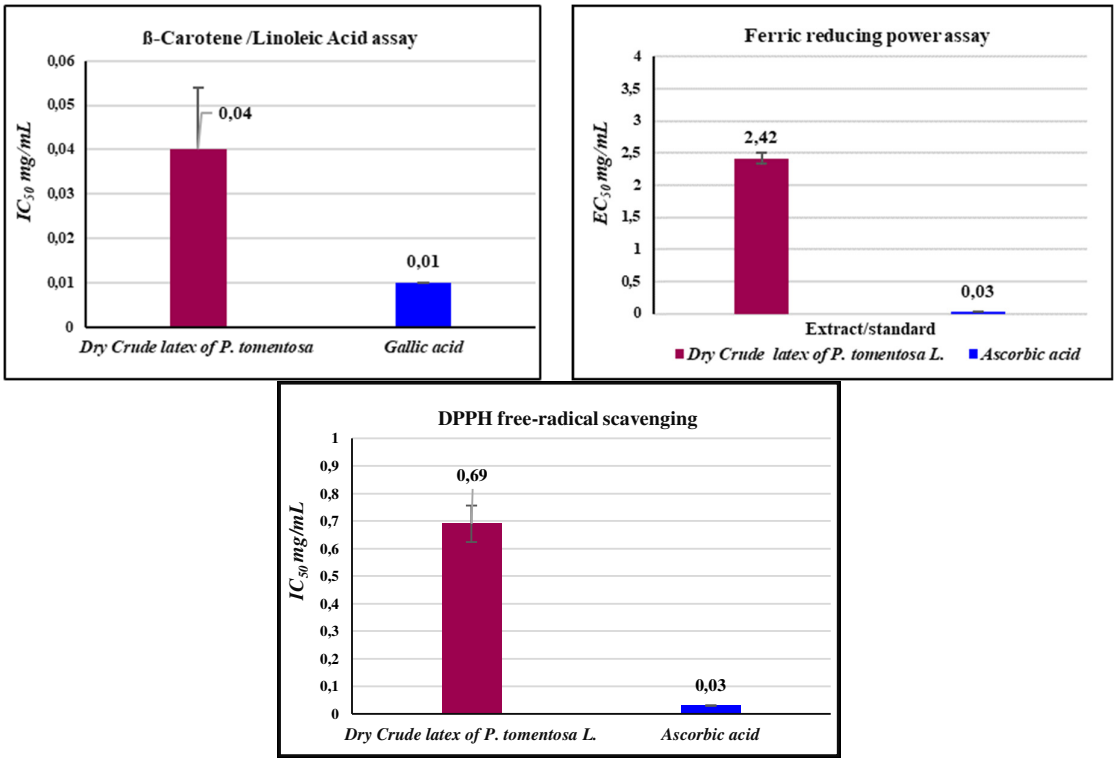

Figure 3. Antioxidant activities of dry crude latex of *P tomentosa* extract

The results of the ß-carotene/linoleic acid bleaching test resulted in a strong antioxidant activity for the latex extract of *P. tomentosa*, the values obtained were very close to the standard reference IC_{50}: 0.04 ± 0.014 mg/mL, compared to the gallic acid IC_{50}: 0.01 ± 0.000 mg/mL. We can explain this to the presence of antioxidants that block the whitening of ß-Carotene by balancing linoleic acid and other free radicals formed in the process. Accordingly, the color of the reaction solution has been preserved for a long time in the presence of antioxidants (Afsar *et al*., 2016).

The results revealed the radical scavenging potential of DPPH of latex extract of *P. tomentosa* values for were IC_{50}: 0.69± 0.066 mg/mL. Compared to the reference standard of ascorbic acid 0.03 ±0.001 mg/mL, and a result of surveying previous literature,

In another study in this regard conducted on the latex extract of *P. tomentosa* by Lahmar *et al*. (2022) indicates that it has an antioxidant activity for a high DPPH and its value at IC_{50}: 12 µg/mL.

Also, another study on latex extract showed three plant species: *Ficus sycomorus* and *Euphorbia tirucalli* to evaluate its antioxidant activity by assay free radicals scavenging DPPH, at values of IC_{50}: 13.6, 7.0 and 6.0 µg GAE/mL, respectively (Abdel-Aty *et al*., 2019).

Contrary to the results obtained in this study, the reason for this difference is due to the different environmental conditions between the two countries, in addition to the difference in the collection time (Sihoglu Tepe, 2021).

In the FRAP test the dry crude latex showed *P. tomentosa*. less reduction capacity of values of EC_{50}: 2.42 ±0.065 mg/mL compared to ascorbic acid, which was estimated at 0.03 ±0.001 mg/mL. Therefore, the efficiency of the latex extract of *P. tomentosa* in antioxidant assays can be attributed to the formation of hydrogen atoms of polyphenols contained in the latex extract under study with free radicals (Afsar *et al*., 2016).

The qualitative analysis by HPLC chromatography revealed the presence of quercetin, chlorogenic acid and p-Coumaric Acid from the dry crude latex extract of *P. tomentosa*. Which could be enough for this plant

to be a natural source of antioxidants prevent oxidative damage that causes many different diseases (Dimitrios, 2006), enhance health by preventing the formation of free radicals and converting them into less toxic compounds, in addition to restoring damaged molecules (Ali Amin *et al*., 2022) and for use in the food industry as a natural additive due to their natural antioxidant content (Kaska, 2018).

Finally, these results conclude that the differences in antioxidant activity tests between the studied latex extract and these species may be attributed to the difference in geographical environments, in addition to the difference in the mechanism of action of these assays and the interaction between them on the one hand, and the variation in phenolic content between them on the other hand. As a result, these species had different antioxidant activity than what we obtained in this study (Hafsia *et al*., 2020).

Anti-inflammatory activity of dry crude latex of P. tomentosa

In this study, we used fresh egg albumen as a protein to test: the results are shown in (Table 3) and (Figure 4), the effectiveness of dry crude latex extract of *P. tomentosa* in protecting egg whites from denaturation (%) using a temperature increase of 70 °C, which was estimated at IC_{50}: 0.19 ± 0.004 mg/mL compared to the reference drug *ASPEGIC*® with: 0.05 ±0.004 mg/mL.

Table 3. Anti-Inflammatory activity of dry crude latex of *P. tomentosa*

Extract /standard	Anti-Inflammatory Activity Inhibition (%)
	IC_{50} mg/mL
Dry crude latex of *P. tomentosa*	0.19 ±0.004
***ASPEGIC*®**	0.05 ±0.004

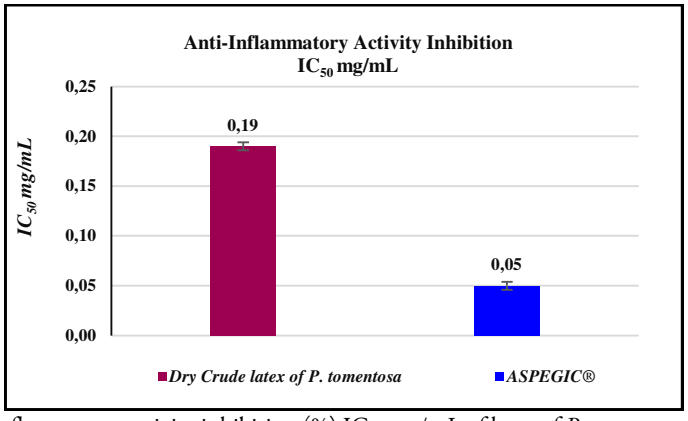

Figure 4. Anti-inflammatory activity inhibition (%) IC_{50} mg/mL of latex of *P. tomentosa* extract

Accordingly, we conclude that the latex extract under study is effective and this is due to its phytochemical properties such as quercetin, chlorogenic acid and p-coumaric acid, which have effective Antioxidant and anti-inflammatory activities (Zhen *et al*., 2016; Yıldırım *et al*., 2019).

Numerous studies have documented the causes of inflammatory diseases and arthritis, and one such factor can be attributed to tissue protein denaturation *in vivo*. Therefore, controlling before this occurs may be a viable alternative in finding and developing new anti-inflammatory drugs (Chandra *et al*., 2012).

Conclusions

In conclusion, the results presented in this study provide a significant insight into the potential of dry crude latex of *P. tomentosa* and its therapeutic effects especially antioxidants and anti-inflammatory activities.

The studied latex extract is characterized by its richness of active bio compounds, which largely indicates its high abilities to inhibit the oxidation of linoleic acid, which can be included within the food industry as a promising additive due to its natural antioxidant content.

In addition, it contains powerful therapeutic anti-inflammatory properties, which can be attributed to the phenolic compounds detected by high-performance chromatography (HPLC), such as: quercetin, chlorogenic acid, and p-Coumaric acid.

Finally, we recommend further phytochemical analysis of the extract under study to identify and evaluate *in vivo* the specific compounds responsible for these powerful biological and pharmacological effects that may qualify for use as natural antibiotics against several diseases caused by oxidative stress and Which may conclude with the manufacture of a natural drug for some of these diseases and their prevention.

Authors' Contributions

SK: Plant collection, Laboratory analysis, Investigation and Writing. CA: Investigation, Methodology and Supervision and TLILI Mohammed Laid: revision and Supervision assistant. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The author, Khaoula SEGUENI, would like to thank Dr. Ouafa BOUDEBIA for her encouragement the completion of this work.

This study falls within PRFU project D01N01UN390120220003.

Conflict of Interests

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

References

Abdel-Aty A, Hamed M, Salama W, Ali M, Fahmy A, Mohamed S (2019). *Ficus carica*, *Ficus sycomorus* and *Euphorbia tirucalli* latex extracts: phytochemical screening, antioxidant and cytotoxic properties. Biocatalysis and Agricultural Biotechnology 20(101199):1878-8181. *https://doi.org/10.1016/j.bcab.2019.101199*

Ads E, Abouzied A, Alshammari M (2021). Evaluation of cytotoxic effects of methanolic extract of *Pergularia tomentosa* L growing wild in KSA. Asian Pacific Journal of Cancer Prevention 22:67-72. *https://doi.org/10.31557/apjcp.2021.22.s1.67*

Afsar T, Razak S, Khan M, Mawash S, Almajwal A, Shabir M, Ulhaq I (2016). Evaluation of antioxidant, anti-hemolytic and anticancer activity of various solvent extracts of *Acacia hydaspica* R. Parker Aerial Parts. BMC Complementary and Alternative Medicine 16(1):258. *https://doi.org/10.1186/s12906-016-1240-8*

Al Hinai H, Al-Subhi W, Al-Rubaiai F, Hassan S, Sherwani N, Fatope M (2018). Lupane and ursane-type triterpenoids from *Pergularia tomentosa*. Chemistry of Natural Compounds 54(4):790-792. *https://doi.org/10.1007/s10600-018-2477-x*

Ali Amin A, Ekin S, Bakir A, Yildiz D (2022). Antioxidant properties of *Lycianthes rantonnetii* and contents of vitamin and element. International Journal of Secondary Metabolite 9(2):194-207. *https://doi.org/10.21448/ijsm.1030207*

Amarowicz R, Pegg R, Rahimi-Moghaddam P, Barl B, Weil J (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry 84:551-562. *https://doi.org/10.1016/S0308-8146(03)00278-4*

Ao C, Li A, Elzaawely A, Xuan T, Tawata S (2008). Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. Extract. Food Control 19(10):940-948. *https://doi.org/10.1016/j.foodcont.2007.09.007*

Asamenew G, Bisrat D, Mazumder A, Asres K (2011). *In vitro* antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlana* reynolds. Phytotherapy Research 25:1756-1760. *https://doi.org/10.1002/ptr.3482*

Boudebia O, Medila I, Toumi I (2022). Evaluation of biological activities of *Chamaeleo chamaeleon*: a reptile used in traditional folk medicine in Algeria. Journal of Biochemical Technology 13(4):15-19. *https://doi.org/10.51847/ed9gjaf2j7*

Castelblanque L, Andrade J, Arias C, Rodríguez J, Escaray F, Fenollosa E, Jaques J, Vera P (2020). Opposing roles of plant laticifer cells in the resistance to insect herbivores and fungal pathogens. Plant Communications 1:100112. *https://doi.org/10.1016/j.xplc.2020.100112*

Chandra S, Chatterjee P, Dey P, Bhattacharya S (2012). Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine 2(1):178-180. *https://doi.org/10.1016/s2221-1691(12)60154-3*

Chouikh A (2020). Phytochemical profile, antioxidant, analgesic and hypolipidaemic effects of *Ephedra alata* decne. female cones extract. Farmacia 68(6):1011-1020. *https://doi.org/10.31925/farmacia.2020.6.7*

Dimitrios B (2006). Sources of natural phenolic antioxidants. Trends in Food Science & Technology 17(9):505-512. *https://doi.org/10.1016/j.tifs.2006.04.004*

Ernst Kerche-Silva L, Gomes Silva M, Silva Danna C, Gomes Silva A, Moratto Carrara I, Lourenço C, Yoshihara E, Eloizo J (2017). Free-radical scavenging properties and cytotoxic activity evaluation of latex c-serum from *Hevea brasiliensis* rrim 600. Free Radicals and Antioxidants 7(1):107-114. *https://doi.org/10.5530/fra.2017.1.16*

Guha G, Rajkumar V, Kumar R, Mathew L (2010). Aqueous extract of *Phyllanthus amarus* inhibits chromium (VI)-induced toxicity in mda-mb-435s cells. Food and Chemical Toxicology 48(1):396-401. *https://doi.org/10.1016/j.fct.2009.10.028*

Hafsia B, Sabah D, Noura S, Abdelfattah E, Najla H (2020). Phytochemistry and antioxidant activities of the methanolic extract obtained from the leaves of *Citrus limon* (L.) osbeck. International Journal of Secondary Metabolite 7(1):47-53. *https://doi.org/10.21448/ijsm.590522*

Haidan Y, Qianqian M, Li Y, Guangchun P (2016). The Traditional medicine and modern medicine from natural products. Molecule 21(559):12-18. *https://doi.org/10.3390/molecules21050559*

Hua J, Liu Y, Jing S, Luo S, Li S (2015). Macrocyclic diterpenoids from the latex of *Euphorbia helioscopia*. Natural Product Communications 10(12):2037-2039. *https://pubmed.ncbi.nlm.nih.gov/26882659*

Kaska A (2018). The phenolic content, antioxidant and cytotoxic activities of *Origanum sipyleum* from Turkey. International Journal of Secondary Metabolite 5(4):343-352. *https://doi.org/10.21448/ijsm.468418*

Konno K (2011). Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. Phytochemistry 72(13):1510-1530. *https://doi.org/10.1016/j.phytochem.2011.02.016*

Lahmar I, Ben Nasri-Ayachi M, Belghith K (2022). Laticifer identification, rubber characterization, phenolic content, and antioxidant activity of *Pergularia tomentosa* latex extract. Hindawi BioMed Research International 1-8. *https://doi.org/10.1155/2022/7158905*

Lahmar I, Radeva G, Marinkova D, Velitchkova M, Belghith H, Ben Abdallah F, Yotova L, Belghith K (2018). Immobilization and topochemical mechanism of a new β-amylase extracted from *Pergularia tomentosa*. Process Biochemistry 64:143-151. *https://doi.org/10.1016/j.procbio.2017.09.007*

Nickavar B, Esbati N (2012). Evaluation of the antioxidant capacity and phenolic content of three *Thymus* species. Journal of Acupuncture and Meridian Studies 5(3):119-125. *https://doi.org/10.1016/j.jams.2012.03.003*

Ramos M, Demarco D, DaCosta Souza I, Teixeira De Freitas C, (2019). Laticifers, latex, and their role in plant defense. Trends in Plant Science 24(6):553-567. *https://doi.org/10.1016/j.tplants.2019.03.006*

Roy S, Sehgal R, Padhy B, Kumar V (2005). Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. Journal of Ethnopharmacology 102(3):470-473. *https://doi.org/10.1016/j.jep.2005.06.026*

Salomé Abarca L, Klinkhamer P, Choi Y (2019). Plant latex, from ecological interests to bioactive chemical resources. Planta Medica 85:856-868. *https://doi.org/10.1055/a-0923-8215*

Sihoglu Tepe A (2021). Chemical compositions and antioxidant activities of four different *mushroom* species collected from turkey. International Journal of Secondary Metabolite 8(3):214-226. *https://doi.org/10.21448/ijsm.953923*

Yıldırım A, Sen A, Doğan A, Bitis L (2019). Antioxidant and anti-inflammatory activity of capitula, leaf and stem extracts of *Tanacetum cilicicum* (Boiss.) grierson. International Journal of Secondary Metabolite 6(2):211-222. *https://dx.doi.org/10.21448/ijsm.510316*

Zhen J, Villani T, Guo Y, Qi Y, Chin K, Pan M, Ho C, Simon J, Wu Q (2016). Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. Food Chemistry 190:673-680. *https://doi.org/10.1016/j.foodchem.2015.06.006*