Current Trends in Biotechnology and Pharmacy
Vol. 18(4) 2024-2035, October 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2024.4.48

GC-MS Analysis and Quantification of Some Secondary Metabolites of the Algerian *Phragmites australis* Leaf Extract and Their Biological Activities

Ahlem Frahtia[1,2], Samir Derouiche [1,2*], and JanettaNiemann[3]

[1] Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El-Oued, El-Oued 39000, Algeria
[2] Laboratory of Biodiversity and Application of Biotechnology in the Agricultural Field, Faculty of Natural Sciences and Life, University of El-Oued, El-Oued 39000, Algeria
[3] Department of Genetics and Plant Breeding, Poznań University of Life Sciences, Dojazd11, 60-632 Poznań, Poland

*Corresponding author: dersamebio@gmail.com

Abstract

This study aims to assess the biological impacts and phytochemical compounds present in the leaves of *Phragmites australis*. Standard protocols were used for qualitative and quantitative chemical screening, where DPPH and FRAP assays were used to estimate the antioxidant activity. Regarding the anti-inflammatory potential, it was tested through the red blood cells' protection against hemolysis and the protection of protein from denaturation. Using GC-MS, volatile compounds can be identified. Results show that more than 200 volatile compounds in this plant were identified by the GC-MS analysis including 1-Dodecanol (48.25%), Pyrazine, tetramethyl (1.28%), Heptadecane, 2,6,10,15-tetramethyl (0.91%), Cyclopentasiloxane, decamethyl (0.93%) and Cyclotetrasiloxane, octamethyl (0.56%). Qualitative phytochemical tests show the richness of the aqueous extract of different biocompounds such as polyphenols, flavonoids, terpenoids, tannins, and alkaloids. Total phenolic content and total flavonoids exhibit paramount concentration (70,74±1,94 mg GA eq/ g dry extract and 3,64±0,98 mg Q eq/ g dry extract). Results reveal a moderate antioxidant activity compared to the anti-inflammatory one whose values are prominent. We conclude that *P. australis* is a promising source of bioactive compounds that could be exploited in the pharmaceutical field.

Keywords: *P. australis;* GC-MS; Polyphenols; Antioxidant; Anti-inflammatory; IC_{50}.

Introduction

Plants continue to be a major source of new physiologically active chemicals. Many medicinal plant species are being evaluated for biological activity due to the growing interest in phytomedicine (1). The phytochemical components of medicinal plants are responsible for the wide spectrum of pharmacological effects (2). *Phragmites australis*, or common reed, is a wetland species known for its abundance of bioactive components in aqueous plant extracts such as tannins, phenolic compounds, flavonoids, terpenoids, and glycosides (3). The genus Phragmites contains more than ten species in the world (4). It is used in traditional medicine to cure a variety of human and livestock ailments (5). Aquatic extracts of *P. australis* rhizomes were shown to have antioxidant and hepatopro-

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tective effects. Furthermore, leaf extract has anti-melanogenesis and antioxidant properties (6, 7). Another study reported the antiviral activity of the plant aqueous extract (8). The intention of this study is to identify the phytochemical profile of *Phragmites australis'* leaves using GC-MS analysis and to figure out the medicinal effectiveness of the Algerian *P. australis* regarding inflammation and oxidative stress disorders.

Materials and methods

Plant material and extraction method

Phragmites australis specimens (figure 1) were gathered in October 2022 from Touggourt state in Algeria. Leaves were separated from specimens and cleaned with water to eliminate dust and debris and left to dry out in the shade before being ground to fine powder. Leaves' aqueous extract was prepared as follows: 5 g of *Phragmites australis* leaves' powder was added to 50 ml of distilled water. The solution was exposed to a temperature of 50° C while stirring it for two hours. The preparation was then left to be macerated for 24 hours at room temperature. After filtring with a muslin cloth and filter paper, the filtrate was dried in a laboratory oven and saved in the freezer for further use (9).

Figure 1: leaves of *Phragmites australis*

Phytochemical analyses

P. australis leaf extract was analyzed for the presence of several compounds such as phenols, tannins, alkaloids, steroids, saponins, flavonoids, and terpenoids using conventional protocols for phytochemical screening (10-12).

Determination of total phenol content

The total phenolic contents (TPC) of *P. australis* leaf extract was determined by the Folin Ciocalteu method (13).100 µl of the extract is mixed with 500 µl of the FC reagent and 400 µl of Na_2CO_3 at 7.5% (w / v). After 10 minutes of being stirred and incubated in the dark at room temperature, the absorbance of the solution is measured at 760 nm using a UV spectrophotometer. The results are expressed in mg gallic acid equivalent/ g of dry extract with reference to the calibration curve of gallic acid.

Determination of total flavonoid content

The total flavonoid contents (TFC) of *P. australis* leaf extract was determined as described by (Dehpour A et al., 2009) (14). 500 µl of the extract, 100 µl $AlCl_3$, 100µl of 1 M sodium acetate, and 2.8 ml of distilled water. The mixture is incubated in the dark and at room temperature for 30 minutes after being well stirred. The blank is made by replacing the extract with 95% methanol and the absorbance is measured at 415nm using a UV spectrophotometer. The results are expressed in mg equivalent quercetin / g of dry extract material with reference to the quercetin calibration curve.

Gas chromatography and mass spectroscopy

Plant extract of leaves was prepared in universal solvent methanol for which 1µl plant extract was employed to quantify the volatile compounds by GCMS analysis. For extraction of volatiles headspace solid-phase micro-extraction (SPME) with DVB/CAR/PDMS fiber was used. Firstly, the fiber was conditioned in the GC injection port at 270°C for 4 h. Then the fiber was put into the vial with the sample using

an adapter for 15 min at room temperature. After that, the fiber was put into the injection port of a gas chromatograph for desorption. Desorption time was 10 min at 260°C in the splitless mode. For analysis was used a 7890A GC system (Agilent Technologies, Santa Clara, United States) coupled with a 5975C VL Triple-Axis mass detector (Agilent Technologies, Santa Clara, United States). Separation was run on a DB-5MS capillary column (25 m × 0.2 mm; 0.33 µm film thickness; J&W, Folsom, California) with helium as a carrier gas at a flow rate of 0.6 mL/min. The temperature of the injector and transfer line were 260°C and 280°C, respectively. The oven program of temperature was: the initial temperature at 40°C was held for 3 min, then increased at 4°C/min to 160°C and further increased at 10°C/min to 280°C, with the final temperature held for 3 min. The masses were scanned from 33 to 333 Da. The ionization energy value was set to 70 eV. The result interpretation of GCMS data was evaluated using the National Institute of Standards and Technology (NIST) database. The comparative assessment assisted in identifying unknown chemicals when compared to the stored NIST library in order to investigate the available plant extract data. The molecular chemical data was derived by determining the name, formula, weight, and structure of the sample's volatile components.

Data presentation and analysis

The results were expressed as mean ± standard deviation (SD), calculated from duplicate determinations and the linear relationship was visually determined.

In vitro biological activities

The anti-oxidant activity is measured using two assays: DPPH free-radical scavenging activity as described by Nwidu et *al.* (15) and the ferric reducing anti-oxidant power assay (FRAP) as described by Oyaizu (16); while the anti-inflammatory effect of *Phragmites australis* and biosynthesized copper NPs was investigated *in vitro* using two methods. The first one is a measure of protein denaturation inhibition as described by Vennila, et *al*. (17), and the second one is a measure of red blood cells protection against hemolysis as stated by Vinjamuri et al. (18).

Results and Discussion

Phytochemical screening

The phytochemical analyses show that leaf extract is rich in different active components (Table 1). A (+) indicates the presence of phytochemicals, whereas a (-) indicates its absence.

Table 1**:** Phytochemical screening of *P. australis* leaves aqueous extract.

Phytochemical compound	***P. australis* leaves aqueous extract**
Polyphenols	+
Flavoniods	+
Alkaloids	+
Tanins	+
Saponins	+
Terpenoids	+
Reducing compounds	+

Total phenolic content is expressed in terms of gallic acid equivalents (mg GA eq/ g of dry plant extract), whereas total flavoniods content is expressed in terms of quercetin equivalents (mg Q eq/ g of dry plant extract), using the following equation based on the calibration curve: Y= 0,007x+0,009 (R2=0,985) for phenolic compound and Y=0,006x+0,031 for flavonoids compounds (R2=0,993). The results are represented in Table 2.

Table 2: Total phenol and flavonoid contents in *P. australis* leaves aqueous extract.

Compounds	**Total phenol content mg GA eq/ g dry extract**	**Total flavonoid content mg Q eq/ g dry extract**
P. australis leaves aqueous extract	70.74±1.94	3.64±0.98

GC-MS analysis

The screening and the identification of volatile compounds of *P. australis* leaf extract were conducted through GC-MS chromatogram (Fig. 2). Using the GC-MS technique, more than 200 components were identified (Table 3). The chemical constituents of *P. australis* were dominated by 1-Dodecanol (48.25%), Pyrazine, tetramethyl (1.28%), Heptadecane, 2,6,10,15-tetramethyl (0.91%), Cyclopentasiloxane, decamethyl (0.93%) and Cyclotetrasiloxane, octamethyl (0.56%).

Table 3: Quantification of volatile compounds by GC-MS of *P. australis* leaf extract.

Peak #	Name	Formula	R.T. (s)	Area %
	Pyridine, 2,4,6-trimethyl-	$C_8H_{11}N$	519.375	0.185
	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	525.98	0.566
	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	560.425	0.198
	Cyclohexene, 4-ethenyl-1,4-dimethyl-	$C_{10}H_{16}$	563.796	0.179
	Pyrazine, tetramethyl-	$C_8H_{12}N_2$	623.786	1.287
	Linalyl acetate	$C_{12}H_{20}O_2$	637.587	0.071
	Nonanal	$C_9H_{18}O$	641.938	0.088
	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	691.491	0.939
	Undecane	$C_{11}H_{24}$	738.646	0.049
	Cyclodecanol	$C_{10}H_{20}O$	744.805	0.054
	1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	$C_{14}H_{24}O_2$	792.808	0.057
	m-Ethylacetophenone	$C_{10}H_{12}O$	804.602	0.007
	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	816.363	0.029
	(1R,3R,4S,5S)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-3-yl acetate-rel	$C_{12}H_{20}O_2$	829.467	0.031
	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	833.846	0.081
	Undecanal	$C_{11}H_{22}O$	841.331	0.019
	Trimethylsilylcatecholpyruvatetris(trimethylsilyl) ether	$C_{21}H_{40}O_5Si_4$	856.708	0.49
	Triacetin	$C_9H_{14}O_6$	875.414	0.201
	Tridecane, 5-methyl-	$C_{14}H_{30}$	882.295	0.02
	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	$C_{12}H_{24}O_3$	887.91	0.305
	Dodecane, 3-methyl-	$C_{13}H_{28}$	890.952	0.092
	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	$C_{12}H_{24}O_3$	905.368	0.477
	Octadecane, 6-methyl-	$C_{19}H_{40}$	922.984	0.015
	Dodecanal	$C_{12}H_{24}O$	931.787	0.063
	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	$C_{15}H_{24}$	953.746	0.04
	1-Dodecanol	$C_{12}H_{26}O$	986.66	48.251
	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	988.172	0.083
	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)-	$C_{10}H_{14}O$	999.155	0.031

	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{17}H_{50}O_7Si_7$	1005.56	0.363
	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	1007.09	0.186
	Oxirane, dodecyl-	$C_{14}H_{28}O$	1017.19	0.026
	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	1026.9	0.123
	Ethyl dodecyl ether	$C_{14}H_{30}O$	1032.92	0.086
	Lilial	$C_{14}H_{20}O$	1036.4	0.039
	Hexadecane	$C_{16}H_{34}$	1041.66	0.028
	Decane, 5-propyl-	$C_{13}H_{28}$	1044.73	0.015
	Benzene, (1-propylheptyl)-	$C_{16}H_{26}$	1048.45	0.016
	Dodecane, 5-methyl-	$C_{13}H_{28}$	1049.38	0.019
	Hexane, 3,3-dimethyl-	C_8H_{18}	1060.39	0.011
	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	1064.15	0.03
	2-Methyl-1-undecanol	$C_{12}H_{26}O$	1080.98	0.19
	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	1086.69	0.185
	Diethyl Phthalate	$C_{12}H_{14}O_4$	1087.61	0.164
	Diphenyl sulfide	$C_{12}H_{10}S$	1088.24	0.025
	trans-2-Dodecen-1-ol	$C_{12}H_{24}O$	1097.83	0.01
	Benzene, (1-butylheptyl)-	$C_{17}H_{28}$	1117.73	0.036
	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	$C_{13}H_{22}O_3$	1132.68	0.243
	Octane, 1,1'-oxybis-	$C_{16}H_{34}O$	1135.74	0.056
	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	1138.43	0.355
	Tetradecane, 2,2-dimethyl-	$C_{16}H_{34}$	1140.89	0.018
	Benzene, (1-ethylnonyl)-	$C_{17}H_{28}$	1141.76	0.021
	n-Hexyl salicylate	$C_{13}H_{18}O_3$	1152.93	0.025
	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	1162.12	0.159
	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	1166.71	0.11
	Hydrazinecarboxamide	CH_5N_3O	1186.1	0.012
	Undecane, 4,6-dimethyl-	$C_{13}H_{28}$	1194.08	0.051
	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1203.45	0.041
	Hydrazinecarboxamide	CH_5N_3O	1225.78	0.006
	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	1233.77	0.109
	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	1240.95	0.079
	Benzene, (1-methylundecyl)-	$C_{18}H_{30}$	1243.14	0.017
	Benzoic acid, 2-hydroxy-, 2-methylbutyl ester	$C_{12}H_{16}O_3$	1245.87	0.013
	13-Methyltetradecanal	$C_{15}H_{30}O$	1246.8	0.026
	Isoamyllaurate	$C_{17}H_{34}O_2$	1265.1	0.411

	Dodecanoic acid, 1,1-dimethylpropyl ester	$C_{17}H_{34}O_2$	1267.63	0.081
	Caffeine	$C_8H_{10}N_4O_2$	1275.72	0.017
	Hydrazinecarboxamide	CH_5N_3O	1281.58	0.002
	(1R,2R,4S)-2-(6-Chloropyridin-3-yl)-7-methyl-7-azabicyclo[2.2.1]heptane	$C_{12}H_{15}ClN_2$	1282.25	0.008
	Pentadecane	$C_{15}H_{32}$	1302.02	0.05
	Benzenemethanol, α-[1-(ethylmethylamino)ethyl]-, [R-(R*,S*)]-	$C_{12}H_{19}NO$	1312.82	0.105
	Benzene, (1-methylnonadecyl)-	$C_{26}H_{46}$	1313.44	0.017
	Hydrazinecarboxamide	CH_5N_3O	1326	0.024
	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1340.11	0.075
	Dibutyl phthalate	$C_{16}H_{22}O_4$	1348.07	0.146
	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	1355.79	0.173
	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	1367.08	0.022
	Semicarbazide	CH_5N_3O	1395.08	0.006
	Hydrazinecarboxamide	CH_5N_3O	1419.5	0.001
	Nickel tetracarbonyl	C_4NiO_4	1440.13	0.001
	Hydrazinecarboxamide	CH_5N_3O	1443.12	0.008
	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{17}H_{50}O_7Si_7$	1449.59	0.121
	dl-Alanyl-l-alanine	$C_6H_{12}N_2O_3$	1451.96	0.003
	Semicarbazide	CH_5N_3O	1458.17	0.001
	Hydrazinecarboxamide	CH_5N_3O	1466.48	0.001
	Benzenemethanol, α-(1-aminoethyl)-	$C_9H_{13}NO$	1468.36	0.002
	Semicarbazide	CH_5N_3O	1512.72	0.002
	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	1534.62	0.108
	Hydrazinecarboxamide	CH_5N_3O	1548.59	0.007
	dl-Alanyl-l-alanine	$C_6H_{12}N_2O_3$	1558.04	0.001
	Hydrazinecarboxamide	CH_5N_3O	1564.48	0.009
	dl-Alanyl-l-alanine	$C_6H_{12}N_2O_3$	1566.33	0.001
	Hydrazinecarboxamide	CH_5N_3O	1581.87	0.006
	Semicarbazide	CH_5N_3O	1602.56	0.002
	Benzoic acid, octadecyl ester	$C_{25}H_{42}O_2$	1610.92	0.025
	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	1613	0.111
	Semicarbazide	CH_5N_3O	1615.36	0.001
	1,2-Propanediamine	$C_3H_{10}N_2$	1625.08	0.001
	Carbonic acid, bis(2-ethylhexyl) ester	$C_{17}H_{34}O_3$	1656.5	0.01
	Benzoic acid, hexyl ester	$C_{13}H_{18}O_2$	1665.64	0.011

	Semicarbazide	CH_5N_3O	1676.88	0.002
	Nickel tetracarbonyl	C_4NiO_4	1679.45	0.001
	2-(2,2-Dimethyl-propionyl)-1-(hydroxy-phenyl-methyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid	$C_{22}H_{25}NO_4$	1680.59	0.017
	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	1712.86	0.043
	Hexanoic acid, 2-ethyl-, anhydride	$C_{16}H_{30}O_3$	1717.27	0.028
	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	1732.3	0.912
	Nickel tetracarbonyl	C_4NiO_4	1754.93	0.002
	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	1757.19	0.114
	N-(4-Nitrophenyl)-2-pyrrolidinecarboxamide, N'-acetyl	$C_{13}H_{15}N_3O_4$	1758.46	0.008
	Semicarbazide	CH_5N_3O	1776.52	0.002
	L-Prolinamide	$C_5H_{10}N_2O$	1786.44	0.004
	Semicarbazide	CH_5N_3O	1796.76	0.001
	Nickel tetracarbonyl	C_4NiO_4	1800.03	0
	3-Methylhexan-2-amine	$C_7H_{17}N$	1800.99	0.001
	Hydrazinecarboxamide	CH_5N_3O	1809.92	0.001
	Semicarbazide	CH_5N_3O	1829.39	0.002
	Squalene	$C_{30}H_{50}$	1835.7	0.13
	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	1839.53	0.104
	Hydrazinecarboxamide	CH_5N_3O	1843.67	0.057
	Hydrazinecarboxamide	CH_5N_3O	1896.43	0.005
	1,2-Propanediamine	$C_3H_{10}N_2$	1910.64	0.002
	dl-Alanyl-l-alanine	$C_6H_{12}N_2O_3$	1915.17	0.001
	Hydrazinecarboxamide	CH_5N_3O	1936.29	0.004
	Semicarbazide	CH_5N_3O	1958.34	0.002

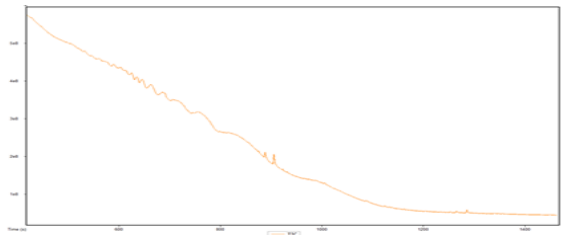

Figure 2: Spectrum of GCMS analysis and their volatile compounds representation of *P. australis* leaf extract.

Anti-oxidant activity

Table 04 shows the IC_{50} values of the anti-oxidant activity of leaves aqueous extract in comparison with ascorbic acid for DPPH (2,2-Diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays respectively.

Table 4: IC_{50} values of *P. australis* leaf extract and ascorbic acid according to DPPH and FRAP assays.

Test	Leaves aqueous extract	Ascorbic acid
	IC_{50} µg/ml	IC_{50} µg/ml
DPPH	1.339	0.033
FRAP	0.201	0.035

Anti-inflammatory activity

Figure 3 shows the IC_{50} levels of protein denaturation assay using BSA and leaves aqueous extract where diclofenac is used as a standard.

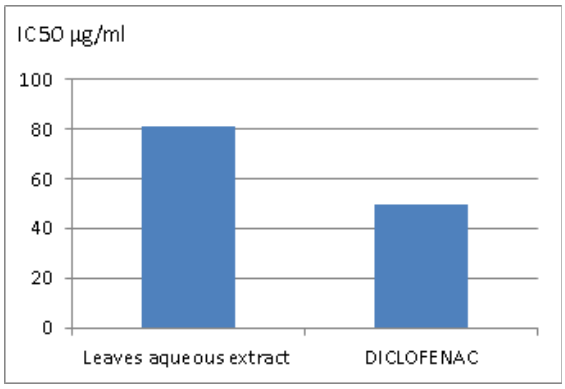

Figure 3: IC_{50} levels of protein denaturation assay using *P. australis* leaves aqueous extract in comparison with diclofenac

Hemolysis test

Figure 4 shows the results of red blood cell hemolysis in the presence of *P. australis* leaves aqueous extract, diclofenac is used as a standard.

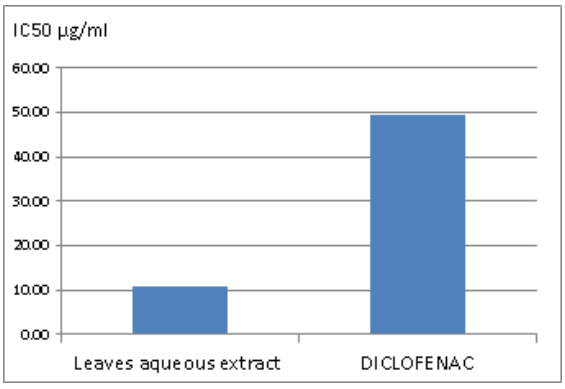

Figure 4: IC_{50} levels of red blood cells hemolysis assay using *P. australis* leaves aqueous extract in comparison with diclofenac

Discussion

Numerous bioactive components, including phenolics, terpenoids, tanins, alkaloides, carbohydrates, and flavonoids, were identified in *P. australis* leaves aqueous extract after phytochemical screening. The phenolic compounds are known to be antioxidants that neutralize free radicals, which are triggers of oxidative damage (9, 19, 20). Those secondary metabolites have played a major role in preventing and treating a variety of diseases including cancer, inflammation-related illnesses, diabetes, osteoporosis, cardiovascular disease, and neuro-degenerative diseases (21). Terpenoids have anticancer, anti-inflammatory, antibacterial, antiviral, and antimalarial properties, as well as the ability to enhance transdermal absorption, prevent and cure cardiovascular disease, and have hypoglycemic properties. Furthermore, earlier research has discovered that terpenoids have a wide range of potential uses, including insect resistance, immunoregulation, antioxidation, antiaging, and neuroprotection (22). As for the total phenolic content and the total flavonoids of *P. australis* leaves aqueous extract, it was 70,74±1,94 mg GA eq/ g dry extract and 3,64±0,98 mg Q eq/ g dry extract respectively (Table 02). The presence of hydroxyl groups (-OH) in the structure of phenolic compounds and flavonoids is responsible for free radical scavenging and functioning as antioxidants (19). The richness of *P. australis* leaves aqueous extract of different bioactive compounds and their variable application justify its wide use in traditional medicine. GC-MS analysis indicated that more than 200 components were identified in *P. australis* leaf extract which is dominated by 1-Dodecanol, Pyrazine, tetramethyl, Heptadecane, 2,6,10,15-tetramethyl, Cyclopentasiloxane, decamethyl, and Cyclotetrasiloxane, octamethyl.1-Dodecanol also known as lauryl alcohol is an organic fatty alcohol (23). Studies have shown that 1-dodecanol has antibacterial activity against S. aureus with a MIC of 6.25 µg/ml (24). It is also shown to be an antimycobacterial agent (25). Pyrazine, tetramethyl also called ligustrazine, is an organic volatile compound that many studies have been conducted to investigate its medical usefulness, including the decrease of platelet

aggregation, renal ischemia/reperfusion damage, and the prevention of atherosclerosis (26). Another study revealed its antioxidant activity (27). Tetramethylpyrazine's effects on improving postoperative adhesion of tissues have been reported in pharmacological investigations and therapeutic applications (28). Heptadecane, 2,6,10,15-tetramethyl is a compound that has demonstrated antituberculous activity (29) and anti-inflammatory activity (30). As for cyclopentasiloxane, decamethyl and cyclotetrasiloxane, octamethyl, they are well-known antimicrobial substances derived from many plant species (31, 32).

Regarding the antioxidant activity done by DPPH assay, it is increased when DPPH interacts with an antioxidant in the tested sample that might supply hydrogen. The color goes from deep violet to pale yellow (5). The potential of scavening free radicals is expressed by IC_{50} values which is the extract concentration capable of blocking 50% of DPPH free radicals (33), the lower is IC_{50} value, the higher the antioxidant power and vice versa (19). FRAP test is about reducing power which is related to a compound's capacity to transfer electrons, it can be used to anticipate its future antioxidant activity. This is due to the extract's polyphenols' predisposition to transfer electrons (34). Talking about anti-inflammatory activity, inflammation is a complicated pathological process that is a protective reaction of live tissue with the vascular system to inflammatory factor harm (35). The most significant drawback of the currently available powerful synthetic anti-inflammatory medications is their toxicity and recurrence of symptoms after withdrawal. As a result, individuals are going back to natural goods in the hope of finding safety and security (36). The aim of using plant extract is to increase the chances of having a synergic effect on all its components, otherwise, it might disappear when using each component on its own. Several pharmaceutical studies, including those for anti-inflammatory efficacy, revealed this (37). Results of the anti-inflammatory tests presented in Figure 02 and Figure 03 expressed by the IC_{50} levels reveal a distinguished anti-inflammatory effect, *P. australis* leaves aqueous extract has protected red blood cells from hemolysis effectively (IC_{50} = 10,73 µg/ml). Our results are consistent with Zhu *et al.* who deduced that *P. australis* the crude extract has strongly repressed macrophages responsible for inflammation (8).

Conclusion

Phytochemical screening results, GC-MS analysis results, and *in vitro* biological activities study validate the abundance of bioactive compounds in *P. australis* leaves aqueous extract that places it in a position to be a potential source of natural antioxidant and anti-inflammation bio-molecules.

Acknowledgement

The authors thank all the laboratory staff in the College of Natural and Life Sciences and outside the country who helped us in completing all the biological analyzes.

Conflict of interests: None declared.

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