JSML
Journal of Science and Mathematics Letters
ISSN 2462-2052 • eISSN 2600-8718

Research Article

Phenolic Composition and In Vitro Biological Activities of *Anacyclus valentinus* L.: Antioxidant, Anti-Inflammatory and Cytotoxic Potential of an Algerian Edible Spice

Khadidja Belhouala[1,2], Cansu Korkmaz[3], Meriem Benyelles[1], Adel Gouri[4], Meltem Tas-Kücükaydın[5], Selcuk Kücükaydın[6], Mehmet Emin Duru[5], Bachir Benarba[2,7]*

[1] Department of Agri-Food, Faculty of Science and Technology. University of Ain Temouchent, 46000 Ain Temouchent, Algeria
[2] Laboratory Research on Biological Systems and Geomatics, Mustapha Stambouli University, 29000 Mascara, Algeria
[3] Department of Biology, Faculty of Science, MuğlaSıtkıKoçman University, 48000 Muğla, Turkey
[4] Laboratory of Biochemistry, Faculty of Medicine, Badji Mokhtar University, 23000 Annaba, Algeria
[5] Department of Chemistry, Faculty of Science, MuğlaSıtkıKoçman University, 48000 Muğla, Turkey
[6] Department of Medical Services and Techniques, Köyceğiz Vocational School of Health Services, MuğlaSıtkıKoçman University, 48800 Köyceğiz-Muğla, Turkey
[7] Thematic Research Agency in Health and Life Sciences, 31000 Oran, Algeria
* Corresponding author: b.benarba@atrss.dz

ARTICLE HISTORY

Received
11 February 2026
Revised
8 May 2026
Accepted
15 May 2026
Published
22 May 2026

KEYWORDS

Anacyclus valentinus
Cytotoxicity
Antioxidant
Anti-inflammatory
Anticancer
HPLC-DAD

ABSTRACT

This study evaluated the phenolic composition, antioxidant, anti-inflammatory, and cytotoxic properties of aqueous and methanolic extracts from the aerial parts of *Anacyclus valentinus* L., an edible spice widely used in Algerian cuisine and traditional medicine. Phenolic constituents were profiled by HPLC-DAD, while antioxidant activity was assessed using phosphomolybdate, DPPH, FRAP, and H_2O_2 scavenging assays. Anti-inflammatory potential was determined through human red blood cell membrane stabilization, albumin denaturation, and proteinase inhibition assays, whereas cytotoxicity was evaluated using the MTT assay against HT-29, PC-3, A549, and non-tumor CCD18-Co cell lines. HPLC-DAD analysis revealed vanillin (45.93 mg/g), ferulic acid (20.31 mg/g), and quercetin (11.45 mg/g) as major phenolics in the methanolic extract. The aqueous extract exhibited higher total phenolic (280.83 mg GAE/g) and flavonoid contents (65.12 mg QE/g) than the methanolic extract. Both extracts demonstrated notable antioxidant activity, with the aqueous extract showing superior total antioxidant capacity (18.73 mg AAE/g) and H_2O_2 scavenging (72.44% at 500 µg/mL). DPPH activity followed the order: aqueous > methanolic > standard. Moderate anti-inflammatory effects were observed, with protein denaturation inhibition lower than the standard drug. Cytotoxic evaluation indicated weak antiproliferative activity (IC_{50} > 200 µg/mL) against all tested cancer cell lines, with no toxicity toward normal cells. These findings highlight *A. valentinus* as a promising natural source of antioxidant compounds with potential applications in functional foods and nutraceuticals.

https://doi.org/10.37134/jsml.vol14.2.13.2026

1. INTRODUCTION

Herbs and spices have long been utilized by local populations and are increasingly recognized as valuable sources of bioactive compounds with significant health-promoting effects, particularly in combating inflammation, oxidative stress, and cancer. These biological activities are largely attributed to diverse phytochemicals, including phenolics, tannins, alkaloids, and terpenoids, which are widely reported to exhibit antioxidant, anti-inflammatory, and cytotoxic properties (Kadir et al., 2022; Liu et al., 2020; Shakri et al., 2020; Salleh et al., 2015). Common culinary herbs such as basil, thyme, ginger, mustard, and garlic are extensively consumed and valued not only for their flavor but also for their therapeutic benefits, often with relatively low toxicity. Nevertheless, despite their widespread use and importance, many plant species remain inadequately explored, particularly in terms of their detailed phytochemical composition and pharmacological potential (Belhouala and Benarba, 2021; Azhar and Salleh, 2020).

Within this context, the genus *Anacyclus* (Asteraceae) comprises predominantly annual herbaceous species distributed across the Mediterranean basin, especially in North Africa and Southern Europe (Houicher et al., 2018). Members of this genus have a long history of use in traditional medicine for the treatment of various ailments, including inflammatory disorders, female sexual dysfunction, chest pain, and chronic cough (Hussain, 2024). Phytochemical investigations have demonstrated that *Anacyclus* species are rich in diverse secondary metabolites such as alkylamides, polyphenols, tannins, terpenoids, and essential oils. Correspondingly, these species have been reported to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and hypoglycemic effects, highlighting their pharmacological relevance (Ainseba et al., 2024; Tuersong et al., 2024; Sissi et al., 2022).

Anacyclus valentinus L. is one of the notable species within this genus and is widely recognized as a medicinal spice in Algeria, where it is locally known as “Guertoufa.” This herb is characterized by its yellow flowers and mild aromatic scent, and it is naturally distributed across the Mediterranean region, particularly in North Africa, where it grows spontaneously in the Hamada regions of southern Algeria (Julve, 2015). In addition to its use as a food condiment in certain regions, particularly in the Algerian Sahara, *A. valentinus* is extensively employed in traditional medicine for the treatment of bacterial and fungal infections, gastrointestinal and metabolic disorders such as diabetes, and for its potential role in reducing tumor development (Selles et al., 2013; Belhouala et al., 2024; Benarba et al., 2024). Despite these traditional applications, scientific validation of its bioactive potential remains limited.

The growing interest in medicinal plants is strongly linked to the role of oxidative stress and chronic inflammation in the pathogenesis of numerous diseases, including cancer, metabolic disorders, and cardiovascular conditions (Altanam et al., 2025). Natural products have been widely reported to possess significant antioxidant and anti-inflammatory properties, making them promising candidates for therapeutic development (Salihu et al., 2024; Azhar et al., 2020; Salleh et al., 2019). Mechanistically, many natural compounds function as antioxidants by scavenging free radicals and preventing oxidative damage to biological systems. In parallel, various phytochemicals can modulate key inflammatory signaling pathways, thereby exerting anti-inflammatory effects with fewer adverse effects compared to synthetic drugs (Bernstein et al., 2018). Furthermore, the cytotoxic and anticancer potential of plant-derived compounds is well established, often involving mechanisms such as apoptosis induction, cell cycle arrest, and inhibition of angiogenesis, which collectively support their potential application in cancer therapy.

To date, no study has simultaneously evaluated the phenolic profile alongside antioxidant, anti-inflammatory, and cytotoxic activities of both aqueous and methanolic extracts of this species. Therefore, the present study aimed to characterize the phenolic constituents and assess these biological activities in extracts obtained from the aerial parts of *A. valentinus*. The findings are expected to provide scientific validation for its traditional use and to highlight its potential as a natural source of bioactive compounds. To the best of our knowledge, this study represents the first comprehensive report addressing both the phenolic composition and in vitro biological activities of *A. valentinus* extracts.

2. METHODOLOGY

2.1. Plant Material

The plant material (Voucher No. 337.LRSBG/AB/23/12) was authenticated and deposited at the herbarium of the LRSBG, University of Mascara, Algeria. *A. valentinus* was collected on May 25, 2023, from Teniet-El-Makhzen, Ghardaïa, located in northern-central Algeria. The aerial parts were harvested fresh and subsequently processed to obtain the crude plant material for further analysis (Figure 1).

Figure 1. Aerial parts of *Anacyclus valentinus* collected from Ghardaïa (Algeria)

2.2. Preparation of Plant Extracts

Dried and powdered aerial parts of *A. valentinus* were extracted using both aqueous and methanolic methods. For the aqueous extract, 20 g of plant material was treated with 200 mL of distilled water preheated to 100°C, allowed to cool to room temperature, and filtered through Whatman No. 42 filter paper. The residue was re-extracted twice under identical conditions to ensure maximum recovery of water-soluble constituents. The combined filtrates were concentrated, freeze-dried (lyophilized), and stored at 4°C until further use. For the methanolic extract, 40 g of dried aerial parts was macerated in 400 mL of methanol at room temperature for 7 days with continuous stirring, followed by storage at 4°C overnight to enhance extraction efficiency. The mixture was then filtered, and the solvent was removed under reduced pressure to obtain the crude extract, which was subsequently stored at 4°C for further analyses.

2.3. Phytochemical Characterization

Preliminary phytochemical screening of *A. valentinus* extracts was carried out using standard qualitative procedures. The extracts were subjected to a series of specific chemical tests to identify the presence of major phytochemical classes. These included the ammonia test for flavonoids, Hager's test for alkaloids, Salkowski test for steroids and terpenoids, frothing test for saponins, Fehling's test for reducing sugars, ferric chloride ($FeCl_3$) test for tannins, Keller-Killiani test for cardiac glycosides, and the chloroform layer test for anthraquinones. Quantitative estimations of selected phytochemical groups, including total phenolics, flavonoids, and condensed tannins, were performed using established spectrophotometric methods as described above.

2.3.1. Total Phenolic Content (TPC)

The TPC was determined using the Folin-Ciocalteu colorimetric method, following the procedure described by Hosu et al. (2014) with slight modifications. Briefly, 300 µL of the extract solution (corresponding to 0.1 g of dry plant material) was mixed with 1.5 mL of Folin-Ciocalteu reagent (0.2 mol/L). After initial mixing, 1.2 mL of sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was incubated at room temperature for 120 min in the dark. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer. A calibration curve was constructed using standard solutions of gallic acid at different concentrations. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry plant material (mg GAE/g). All measurements were carried out in triplicate, and the results were reported as mean ± standard deviation.

2.3.2 Total Flavonoids Content (TFC)

The TFC was determined using the aluminium chloride colorimetric assay, following the method of Sioud et al. (2020) with slight modifications. Briefly, 400 µL of the extract was mixed with 120 µL of 5% sodium nitrite ($NaNO_2$). After 5 min, 120 µL of 10% aluminium chloride ($AlCl_3$) was added, followed by the addition of 800 µL of 1 M sodium hydroxide (NaOH). The reaction mixture was then incubated at room temperature for 30 min. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer. A calibration curve was prepared using standard solutions of quercetin at different

concentrations. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

2.3.3. Condensed Tannin Content (CTC)

The CTC was determined using the vanillin-HCl colorimetric method, following the procedure described by Bikoro et al. (2018) with slight modifications. Briefly, 0.5 mL of the extract was mixed with 3 mL of 3% vanillin in methanol (w/v), followed by the addition of 1.5 mL of concentrated hydrochloric acid (HCl, 37%). The reaction mixture was incubated at room temperature for 30 min under the same conditions as previously described. The absorbance was measured at 500 nm using a UV-Vis spectrophotometer. A calibration curve was constructed using catechin as the standard. The condensed tannin content was expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

2.4. HPLC-DAD Analysis

The phenolic profile of *A. valentinus* extracts was determined using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD), following the method described by Çayan et al. (2020) with slight modifications. The extracts were dissolved in a water-methanol mixture (80:20, v/v) and filtered prior to analysis. Chromatographic separation was carried out on an Inertsil ODS-3 reverse-phase C18 column (5 µm, 250 mm × 4.6 mm i.d.). The injection volume was 20 µL, and the flow rate was maintained at 1.0 mL/min. The column temperature was set at 40°C. Detection of phenolic compounds was performed at 280 nm using a photodiode array (PDA) detector. The mobile phase consisted of solvent A (0.5% acetic acid in water) and solvent B (0.5% acetic acid in methanol), applied under appropriate elution conditions. Identification of phenolic compounds was achieved by comparing retention times with those of authentic reference standards. Quantification was performed using external calibration curves constructed from standard solutions at concentrations ranging from 0-1 ppm. The results were expressed as milligrams per gram of extract dry weight (mg/g dw).

2.5. Antioxidant Activity

2.5.1 Total Antioxidant Capacity (TAC) Assay

The TAC was evaluated using the phosphomolybdate method, as described by Ahmed et al. with slight modifications. Briefly, 300 µL of the extract was mixed with 3 mL of phosphomolybdate reagent, consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was incubated in a water bath at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 765 nm against a blank containing 300 µL of the extraction solvent instead of the sample. A calibration curve was constructed using ascorbic acid at concentrations ranging from 25 to 500 µg/mL. The total antioxidant capacity was expressed as mg of ascorbic acid equivalents per gram of extract (mg AAE/g). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

2.5.2. DPPH Radical Scavenging Assay

The free radical scavenging activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following a previously reported method with slight modifications (Salleh and Ahmad, 2016). A freshly prepared 0.1 mM DPPH solution in methanol (4 mg/100 mL) was used for the analysis. Briefly, 1 mL of DPPH solution was mixed with 1 mL of the extract or ascorbic acid (positive control) at various concentrations (25-500 µg/mL). The reaction mixture was incubated in the dark at room temperature for 16 min, after which the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The radical scavenging activity was determined based on the decrease in absorbance of the DPPH solution. The percentage of inhibition was calculated using the following equation:

$$I\% = [\,(\text{Absorbance}_{DPPH} - \text{absorbance}_{extract}) / \text{absorbance}_{DPPH}\,] \times 100$$

The IC_{50} value, defined as the concentration of extract required to scavenge 50% of the DPPH radicals, was determined by linear regression analysis. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.5.3. Reducing Power Assay

The reducing power was evaluated using the potassium ferricyanide method. Briefly, 2.5 mL of the extract or standard solution was mixed with 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min, followed by centrifugation at 650 rpm for 10 min. Subsequently, 2.5 mL of 10% trichloroacetic acid (TCA) was added to terminate the reaction. A volume of 5.0 mL of the resulting supernatant was then mixed with 5.0 mL of distilled water and 1.0 mL of 0.1% ferric chloride ($FeCl_3$). The formation of Perl's Prussian blue complex was monitored by measuring the absorbance at 700 nm using a UV-Vis spectrophotometer. A calibration curve was constructed using ascorbic acid as the reference standard, and the results were expressed as milligrams of ascorbic acid equivalents per gram of extract (mg AAE/g). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

2.5.4. Hydrogen Peroxide (H_2O_2) Assay

The hydrogen peroxide scavenging activity was determined using a previously reported method with slight modifications (Benarba et al., 2025). Briefly, 100 µL of the extract or ascorbic acid (positive control) at different concentrations (25-500 µg/mL) was added to 3 mL of hydrogen peroxide solution (2 mM) prepared in phosphate buffer. After incubation for 10 min at room temperature, the reaction mixture was vortexed, and the absorbance was measured at 230 nm using a UV-Vis spectrophotometer against a suitable blank. The ability of the extracts to scavenge hydrogen peroxide was calculated using the following equation:

$$H_2O_2 \text{ scavenging inhibition (\%)} = [\,(\text{Absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}) / \text{absorbance}_{\text{control}}\,] \times 100$$

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6. Anti-inflammatory Activity

2.6.1. HRBC Membrane Stabilizing Assay

The anti-inflammatory activity was evaluated using the human red blood cell (HRBC) membrane stabilization method, based on heat- and hypotonic solution-induced hemolysis, as described by Sunmathi et al. (2016) with slight modifications. Fresh human blood (O^+) was obtained from healthy volunteers who had not taken non-steroidal anti-inflammatory drugs (NSAIDs) for at least two weeks, following informed consent and institutional ethical guidelines. The collected blood was mixed with Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.42% NaCl, and 0.5% citric acid) and centrifuged at 3000 rpm for 10 min. The packed cells were washed three times with phosphate-buffered saline (PBS, pH 6.3) and reconstituted as a 10% (v/v) HRBC suspension. The reaction mixture consisted of 1.0 mL of extract at various concentrations (25-500 µg/mL), 0.5 mL of HRBC suspension, 1.0 mL of phosphate buffer, and 2.0 mL of hypotonic saline (0.25% w/v NaCl). The mixture was incubated at 37°C for 30 min and then centrifuged at 3000 rpm for 20 min. The control contained all reagents except the extract, while sodium diclofenac was used as the reference standard. The absorbance of the supernatant was measured at 560 nm using a UV-Vis spectrophotometer to determine hemoglobin release. The percentage of membrane stabilization was calculated using the following equation:

$$\text{Membrane stabilization (I\%)} = 100 - [\,(\text{Absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}) / \text{absorbance}_{\text{control}}\,] \times 100$$

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6.2. Heat Induced Hemolysis Assay

The anti-inflammatory activity was further evaluated using the heat-induced hemolysis method. Briefly, 1.0 mL of the extract at different concentrations or sodium diclofenac (standard drug) was mixed with 1.0 mL of 10% (v/v) HRBC suspension. The reaction mixture was incubated in a water bath at 56°C for 30 min, followed by cooling under running tap water. The samples were then centrifuged at 3000 rpm for 5 min, and the absorbance of the supernatant was measured at 560 nm using a UV–Vis spectrophotometer. The percentage inhibition of hemolysis was calculated using the following equation:

$$\text{Haemolysis inhibition (I\%)} = [\,(\text{Absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}) / \text{absorbance}_{\text{control}}\,] \times 100$$

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6.3. Albumin Denaturation Assay

In this study, the anti-inflammatory potential was evaluated using protein denaturation-based assays, following the method described by Rahman et al. (2015) with slight modifications. Three complementary approaches were employed, including protease inhibition, bovine serum albumin (BSA) denaturation, and egg albumin denaturation assays. These methods collectively assess the ability of the extracts to stabilize proteins under stress conditions and prevent denaturation. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation.

2.6.4. Bovine Serum Albumin (BSA) Denaturation Assay

The inhibition of protein denaturation was evaluated using the bovine serum albumin (BSA) assay. Briefly, 0.05 mL of the extract at various concentrations (50–500 µg/mL) or sodium diclofenac (standard drug) was mixed with 0.45 mL of 0.5% (w/v) aqueous BSA solution. The mixture was incubated at 37°C for 20 min, followed by heating at 57°C for 3 min to induce denaturation. After cooling to room temperature, 2.5 mL of phosphate buffer (pH 6.6) was added, and the absorbance was measured at 255 nm using a UV-Vis spectrophotometer against a blank. The control consisted of 0.05 mL of distilled water and 0.45 mL of 0.5% BSA solution treated under the same conditions. The percentage inhibition of protein denaturation was calculated using the following equation:

$$\text{Protein denaturation inhibition (I\%)} = 100 - [(\text{Absorbance}_{\text{sample}} - \text{absorbance}_{\text{control}}) / \text{absorbance}_{\text{sample}}] \times 100$$

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6.5. Egg albumin Denaturation Assay

The inhibition of egg albumin denaturation was evaluated according to the method described by Sunmathi et al. (2016) with slight modifications. Briefly, 2.0 mL of the extract at various concentrations (50-500 µg/mL) or sodium diclofenac (standard drug) was mixed with 0.2 mL of 0.5% (w/v) aqueous egg albumin and 2.8 mL of phosphate-buffered saline (PBS, pH 6.4). The reaction mixture was incubated at 37°C for 15 min, followed by heating at 70°C for 5 min to induce protein denaturation. After cooling to room temperature, the absorbance was measured at 660 nm using a UV-Vis spectrophotometer. The solvent served as the blank, while distilled water was used as the control. The percentage inhibition of protein denaturation was calculated using the following equation:

$$\text{Protein denaturation inhibition (I\%)} = [(\text{Absorbance}_{\text{sample}} - \text{absorbance}_{\text{control}}) / \text{absorbance}_{\text{sample}}] \times 100$$

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6.6. Proteinase Inhibition Assay

The proteinase inhibitory activity was evaluated using a modified method based on trypsin-casein digestion. Briefly, the reaction mixture consisted of 2 mL of extract solution at different concentrations, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 0.06 mg of trypsin. The mixture was incubated at 37°C for 5 min. Subsequently, 1.0 mL of 0.8% (w/v) casein solution was added, and the reaction was allowed to proceed for 20 min at 37°C. The reaction was terminated by adding 2 mL of 70% perchloric acid. The resulting mixture was centrifuged to remove precipitated proteins, and the absorbance of the supernatant was measured at 210 nm using a UV-Vis spectrophotometer against a buffer blank. The percentage inhibition of proteinase activity was calculated using the following equation:

$$\text{Protein denaturation inhibition (I\%)} = [(\text{Absorbance}_{\text{sample}} - \text{absorbance}_{\text{control}}) / \text{absorbance}_{\text{sample}}] \times 100$$

All experiments were performed in duplicate, and the results were expressed as mean ± standard deviation.

2.7. Cytotoxicity

The cytotoxic activity was evaluated against one normal human cell line (CCD-18Co) and three human cancer cell lines, namely HT-29 (colon carcinoma), PC-3 (prostate cancer), and A549 (lung carcinoma), using the MTT assay. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air to allow cell attachment. Subsequently, the cells were treated with various concentrations of the extracts (0-200 µg/mL) and further incubated for 72 h. After treatment, 10 µL of MTT solution (0.5 mg/mL) was added to each well, and the plates were incubated for an additional 4 h to allow the formation of formazan crystals. The culture medium was then carefully removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plates were kept in the dark for 10 min

to ensure complete dissolution. The absorbance was measured at 540 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells. All experiments were performed in triplicate, and the results were reported as mean ± standard deviation.

2.8. Statistical Analysis

Statistical analysis was performed using two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to evaluate significant differences between groups. The analyses were conducted using Microsoft Excel (with QI Macros) and GraphPad Prism. Differences were considered statistically significant at $p < 0.05$, while highly significant differences were defined at $p < 0.0001$.

3. RESULTS AND DISCUSSION

3.1. Extraction Yields

The extraction yields of *A. valentinus* obtained using aqueous and methanolic solvents were comparable, both reaching approximately 11%. This similarity suggests that both polar solvent systems are equally effective in extracting bioactive constituents from the aerial parts of the plant. These findings are consistent with previous reports indicating that water and methanol can yield comparable quantities of extractable compounds, particularly phenolics (Chatepa et al., 2023). However, earlier investigations have reported significant differences in extraction yields depending on the solvent system used (Benarba et al., 2025; Belhouala et al., 2025). Such variations are likely attributed to differences in solvent polarity and extraction efficiency. It has been demonstrated that aqueous extraction preferentially recovers highly hydrophilic phenolic compounds, whereas methanolic or hydro-methanolic systems are more efficient for extracting moderately polar constituents, including flavonoids and condensed tannins (Turay et al., 2025). These observations emphasize the critical role of solvent selection in influencing both extraction yield and phytochemical composition.

3.2. Phytochemical Screening

Qualitative phytochemical analysis of *A. valentinus* extracts revealed the presence of various secondary metabolites, including phenols, flavonoids, terpenoids, quinones, saponins, steroids, phytosteroids, tannins, anthocyanins, and carbohydrates (Table 1). Both aqueous and methanolic extracts tested negative for alkaloids and glycosides. Notably, coumarins were detected exclusively in the methanolic extract. These findings are partially consistent with previous reports on Algerian species, where crude aqueous extracts were shown to contain flavonoids, saponins, tannins, cardiac glycosides, coumarins, mucilage, and amino acids. However, in contrast to the present study, alkaloids were also detected in that report (Benarba et al., 2024).

Table 1. Qualitative phytochemical screening of *A. valentinus* aqueous and methanolic extracts.

Phytochemicals	Aqueous extract	Methanolic extract	Phytochemicals	Aqueous extract	Methanolic extract
Anthocyanins	++	++	Flavonoids	+	+
Iridoids	-	-	Terpenoids	++	++
Quinones	+++	+++	Phenols	++	++
Coumarins	-	++	Proteins and amino acids	-	+
Anthraquinones	+	-	Reducing sugars	+++	+
Phlobatannins	-	-	Alkaloids	-	-
Glycosides	-	-	Cardiac glycosides	+	+
Saponins	+	+	Catechic tannins	+	+
Steroids	+	++	Gallic tannins	-	-
Phytosteroids	+	++			

(+++) very abundant; (++) abundant; (+) slightly abundant; (−) not detected

Quantitative analysis of total phenolics, flavonoids, and condensed tannins is presented in Figure 2. The aqueous extract exhibited higher total phenolic content (280.83 ± 0.001 mg GAE/g) and flavonoid content (65.12 ± 0.013 mg QE/g) compared to the methanolic extract (247.50 ± 0.001 mg GAE/g and 56.44 ± 0.009 mg QE/g, respectively). In contrast, the methanolic extract showed a higher condensed tannin content (64.83 ± 0.111 mg CE/g), whereas the aqueous extract contained a lower amount (27.61 ± 0.089 mg CE/g). These variations reflect the influence of solvent polarity on the selective extraction of different classes of phytochemicals (Benarba et al., 2025).

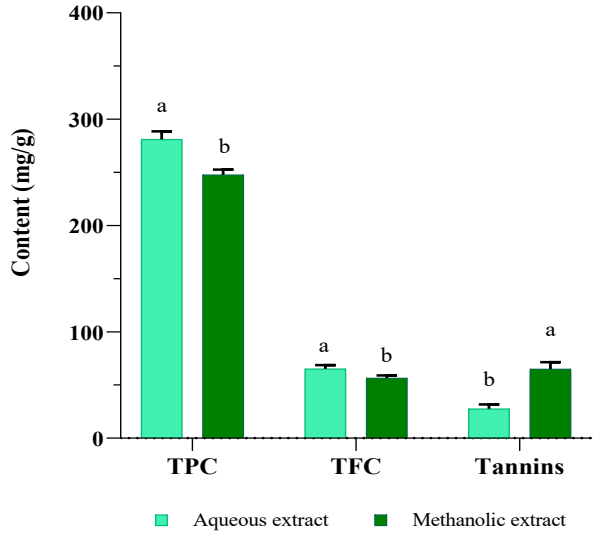

Figure 2. Total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content of *A. valentinus* extracts. Values are expressed as mean ± SD (n = 3). Different letters (**a**,**b**) indicate statistically significant differences between extracts at $p < 0.05$.

3.3. Chemical Constituents of A. valentinus using HPLC-DAD

In this study, comparison with 26 authentic standards by HPLC-DAD enabled the identification of several phenolic compounds in both methanolic and aqueous extracts (Table 2). The major constituents detected were vanillin (45.93 and 28.90 mg/g), ferulic acid (20.31 and 11.28 mg/g), quercetin (11.43 and 7.20 mg/g), coumarin (8.15 and 2.05 mg/g), myricetin (7.60 and 4.20 mg/g), chrysin (4.10 and 2.41 mg/g), and chlorogenic acid (3.10 and 2.87 mg/g), respectively (Figure 3). In addition, *p*-coumaric acid was detected in minor amounts (1.08 and 0.75 mg/g). Luteolin (1.08 mg/g) and kaempferol (0.75 mg/g) were exclusively identified in the methanolic extract. These findings are in agreement with previous studies on species of the *Anacyclus* genus, which reported high levels of chlorogenic acid, followed by quercetin, *p*-coumaric acid, and ferulic acid. However, rutin was identified as the predominant compound in ethyl acetate extracts of *A. maroccanus* and *A. radiatus* (27.23 ± 0.35 and 33.91 ± 0.55 mg/g, respectively), whereas vanillin was the dominant compound in the present study (Sissi et al., 2026).

The predominance of vanillin, ferulic acid, and quercetin is notable, as these compounds are well known for their antioxidant and anti-inflammatory activities. Vanillin exhibits radical scavenging and cytoprotective effects, while ferulic acid contributes to free radical stabilization and inhibition of lipid peroxidation. Flavonols such as quercetin and myricetin further enhance antioxidant capacity through hydrogen donation and metal-chelating mechanisms. The variation in phenolic composition between methanolic and aqueous extracts reflects the influence of solvent polarity, with methanol favoring flavonoid aglycones (e.g., luteolin and kaempferol) and water promoting the extraction of more polar phenolic acids (Babbar et al., 2014).

Table 2. Phenolic composition of aqueous and methanolic extracts of *A. valentinus* determined by HPLC–DAD (mg/g extract).

RT (min)	Phenolic compounds	Methanolic extract	Aqueous extract	RT (min)	Phenolic compounds	Methanolic extract	Aqueous extract
5.14	Chlorogenic acid	3.10	2.87	27.35	Myricetin	7.60	4.20
19.00	Vanillin	45.93	28.90	30.43	Quercetin	11.43	7.20
25.61	p-Coumaric acid	1.08	0.75	31.70	Luteolin	4.84	N/A
25.89	Ferulic acid	20.31	11.28	33.21	Kaempferol	2.29	N/A
26.49	Coumarin	8.15	2.05	38.40	Chrysin	4.10	2.41

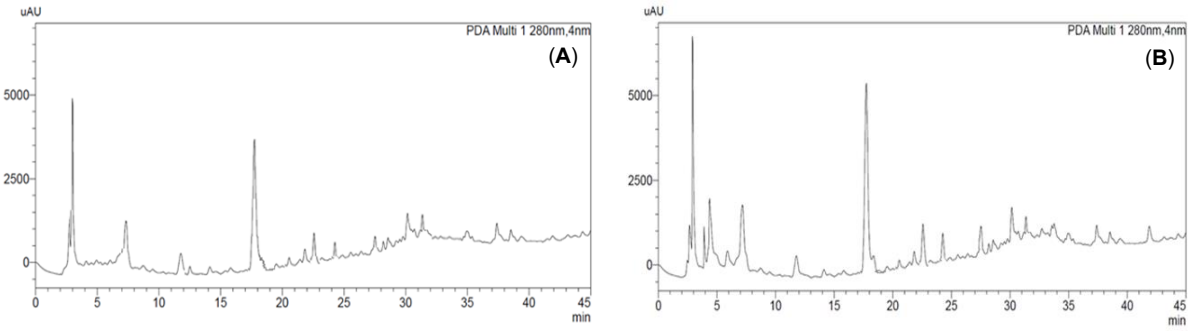

Figure 3. HPLC-DAD chromatograms of *A. valentinus* (**A**: aqueous, **B**: methanolic) extracts.

3.4. Antioxidant Activity

The total antioxidant capacity (TAC) of the extracts, expressed as ascorbic acid equivalents (AAE), showed that both extracts possessed strong antioxidant activity, with the aqueous extract (18.73 ± 0.03 mg AAE/g) slightly higher than the methanolic extract (17.78 ± 0.04 mg AAE/g), and the difference was statistically significant (Figure 4). This higher activity in the aqueous extract is likely due to its greater phenolic and flavonoid content, which are known to act as effective electron donors and reduce oxidized species. The strong reducing ability indicates that *A. valentinus* extracts contain potent antioxidants capable of neutralizing reactive species. Interestingly, ascorbic acid showed a much lower TAC value (1.05 ± 0.01 mg AAE/g), which may be explained by the nature of the phosphomolybdate assay that reflects the combined and synergistic effects of multiple compounds in plant extracts rather than the activity of a single pure compound (Shahidi and Samarasinghe, 2025).

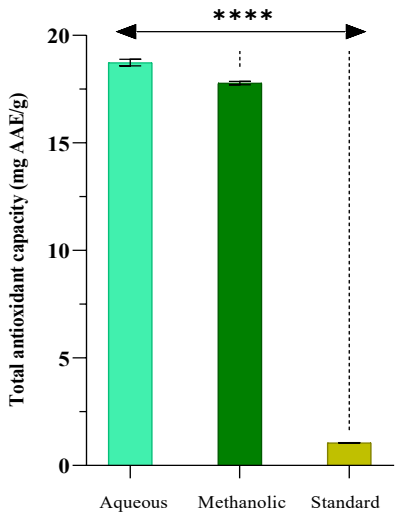

Figure 4. Total antioxidant capacity of aqueous and methanolic extracts of *A. valentinus* compared with ascorbic acid. Results are expressed as mean ± SD (n = 3), in mg ascorbic acid equivalents (AAE) per g of extract.

The DPPH assay is a simple and reliable method to evaluate the free radical scavenging activity of plant extracts. In this study, both aqueous and methanolic extracts of *A. valentinus* showed strong, concentration-dependent activity. At 500 µg/mL, the aqueous extract exhibited slightly higher inhibition (57.58 ± 0.00%) than the methanolic extract (56.82 ± 0.00%), although the difference was not significant ($p = 0.91$) (Figure 5). The IC_{50} values supported this trend, with the aqueous extract (6.61 ± 0.03 µg/mL) being slightly more active than the methanolic extract (7.29 ± 0.04 µg/mL). In comparison, ascorbic acid showed lower maximum inhibition (49.86 ± 0.00%) but a lower IC_{50} (4.43 ± 0.00 µg/mL), indicating higher potency at lower concentrations. This difference highlights that the extracts are more effective at higher doses, while the standard is more efficient at lower concentrations. The strong activity of *A. valentinus* extracts is likely due to their high phenolic and flavonoid content, including compounds such as quercetin, ferulic acid, and vanillin, known for their hydrogen-donating properties (Cai et al., 2004).

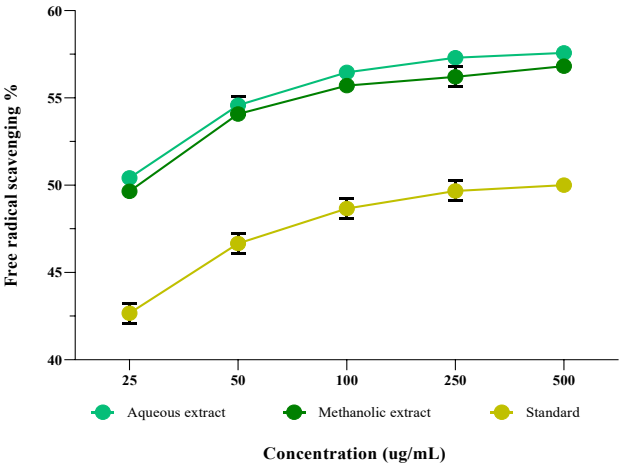

Figure 5. DPPH radical scavenging activity of aqueous and methanolic extracts of *A. valentinus* at different concentrations (25-500 µg/mL), compared with ascorbic acid. Results are expressed as mean ± SD (n = 3).

The reducing power of *A. valentinus* extracts, expressed as IC_{50} values, is presented in Table 3. The methanolic extract exhibited a significantly stronger reducing capacity, with a lower IC_{50} value (194.48 ± 0.001 µg/mL) compared to the aqueous extract (324.13 ± 0.001 µg/mL). These results indicate that the methanolic extract possesses a higher electron-donating ability, reflecting its superior reducing potential. The enhanced reducing activity of the methanolic extract may be attributed to its higher content of moderately polar compounds such as flavonoid aglycones and condensed tannins, which are known to act as effective reductants by donating electrons to stabilize reactive intermediates. In contrast, the aqueous extract, although rich in total phenolics, may contain a higher proportion of highly polar compounds with comparatively lower reducing efficiency in this assay system. These findings highlight the influence of solvent polarity on the extraction of compounds with distinct redox properties. The significant difference observed between the two extracts suggests that methanol is more effective in extracting compounds contributing to ferric ion (Fe^{3+}) reduction (Benarba et al., 2025).

Table 3. Antioxidant activity of *A. valentinus* extracts, expressed as IC_{50} (µg/mL) for DPPH and H_2O_2 scavenging assays, and as ferric reducing antioxidant power (FRAP).

Samples	DPPH (µg/mL)	FRAP (µg/mL)	H_2O_2 (µg/mL)
Aqueous extract	>500	324.13 ± 0.001*	>500
Methanolic extract	>500	194.48 ± 0.001*	>500
Ascorbic acid	>500	N/A	>500

*Significant difference compared to ascorbic acid ($p < 0.05$)

The hydrogen peroxide (H_2O_2) scavenging activity of *A. valentinus* extracts is presented in Figure 6. Both aqueous and methanolic extracts demonstrated a concentration-dependent scavenging effect. At the highest tested concentration (500 µg/mL), the aqueous extract exhibited the strongest activity (72.44%), followed by the methanolic extract (67.76%), while the reference standard, ascorbic acid, showed a lower activity (52.84%). Despite these relatively high inhibition percentages at elevated concentrations, the IC_{50} values for all samples were greater than 500 µg/mL, indicating comparatively low scavenging efficiency at lower concentrations. This suggests that higher doses are required for effective neutralization of H_2O_2. The superior performance of the aqueous extract may be attributed to its higher content of hydrophilic phenolic compounds, which are known to act as effective hydrogen peroxide scavengers by donating electrons and decomposing H_2O_2 into water and oxygen. The moderate activity observed for the methanolic extract further supports the contribution of phenolic constituents, although differences in compound composition and polarity may influence their reactivity (Benarba et al., 2025).

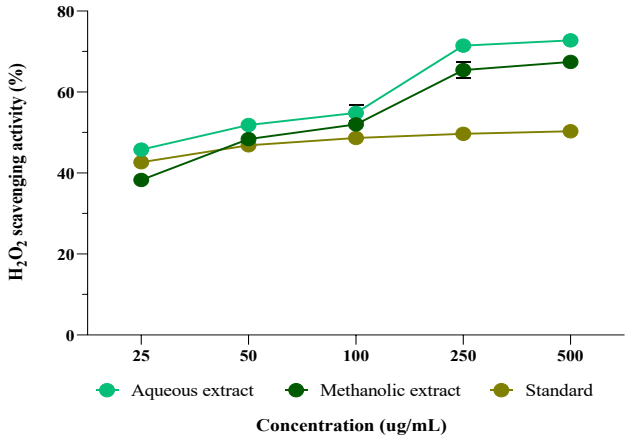

Figure 6. Hydrogen peroxide (H_2O_2) scavenging activity of aqueous and methanolic extracts of *A. valentinus* at different concentrations (25-500 µg/mL), compared with ascorbic acid. Results are expressed as mean ± SD (n = 3).

The present findings consistently demonstrate that both aqueous and methanolic extracts of *A. valentinus* exhibit significant antioxidant activity across multiple assays (TAC, DPPH, FRAP, and H_2O_2 scavenging). The use of complementary methods strengthens the reliability of these results, as each assay reflects a different antioxidant mechanism. The extracts showed notable efficiency in radical scavenging and ferric ion reduction, indicating strong electron- and hydrogen-donating capacities. This activity is primarily governed by the structural features of phytochemicals, particularly the number and position of hydroxyl groups in phenolic compounds, which facilitate the reduction of Fe^{3+} to Fe^{2+} and stabilization of reactive species. These observations are consistent with the high levels of total phenolics and flavonoids identified in the extracts. The results are in agreement with previous studies, including Ainseba et al. (2023), which reported significant antioxidant activity of *A. valentinus* essential oil, particularly in oxygenated fractions evaluated by DPPH and β-carotene bleaching assays. Similarly,

phenolic compounds are well recognized as effective DPPH radical scavengers (Sultana et al., 2009; Lekmine et al., 2025). The strong antioxidant activity observed in the present study may be attributed to the predominance of key phenolics such as vanillin, ferulic acid, and quercetin, which have been widely reported to exhibit potent antioxidant effects (Wang et al., 2024). Interestingly, in certain assays, *A. valentinus* extracts showed higher activity than ascorbic acid, which may be explained by synergistic interactions among multiple phytochemicals, resulting in a greater cumulative effect compared to a single standard compound. However, this observation should be interpreted with caution, as antioxidant performance is highly dependent on assay conditions and underlying reaction mechanisms (Tai et al., 2011).

3.5. Anti-inflammatory Activity

In the hypotonicity-induced haemolysis assay, both aqueous and methanolic extracts of *A. valentinus* exhibited weak membrane-stabilizing effects across the tested concentration range (25-500 µg/mL). The percentage stabilization remained low and nearly identical for both extracts (9.85 ± 0.00%), indicating minimal protective capacity against osmotic stress (Figure 7). In contrast, the reference drug diclofenac sodium demonstrated a markedly higher stabilization effect (19.73 ± 0.00%), approximately twofold greater than that of the plant extracts. These findings clearly indicate the comparatively lower efficacy of *A. valentinus* extracts in preserving erythrocyte membrane integrity under hypotonic conditions. Similarly, in the heat-induced haemolysis assay, both extracts exhibited moderate inhibition of haemolysis but without a clear dose-dependent trend. The aqueous and methanolic extracts showed inhibition values of 28.31 ± 0.14% and 27.23 ± 0.14%, respectively, which remained nearly constant across all tested concentrations (25-500 µg/mL). This absence of a dose-response relationship suggests limited potency or suboptimal concentration of active constituents responsible for membrane stabilization. In comparison, Diclofenac sodium showed a lower haemolysis value (5.91 ± 0.09%), indicating stronger membrane protection and superior anti-inflammatory activity. The results demonstrate that *A. valentinus* extracts possess relatively weak anti-inflammatory activity in the HRBC model. The limited membrane-stabilizing effect may be attributed to the low abundance or reduced bioactivity of phenolic compounds present in the extracts (Mounnissamy et al., 2007).

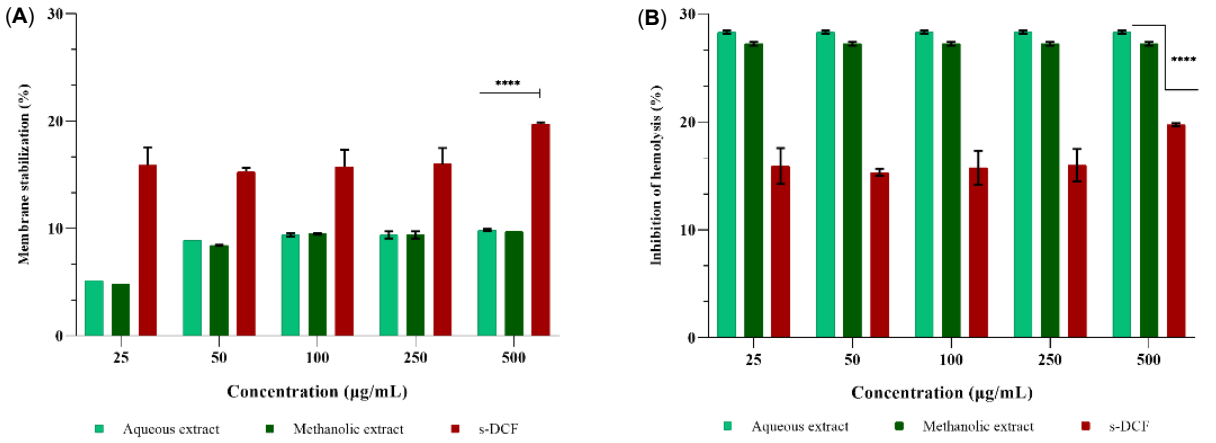

Figure 7. Anti-inflammatory activity of aqueous and methanolic extracts of *Anacyclus valentinus* at different concentrations (25–500 µg/mL). (**A**) Membrane stabilization activity assessed by hypotonic solution-induced haemolysis; (**B**) Inhibition of heat-induced haemolysis. Results are expressed as mean ± SD (n = 3).

Membrane stabilization is closely associated with the ability of bioactive compounds to prevent the leakage of intracellular constituents and the subsequent release of inflammatory mediators, which are critical events in the progression of inflammation. In the present study, *A. valentinus* extracts exhibited weak stabilization of the erythrocyte membrane, suggesting a limited capacity to inhibit the release of lytic enzymes and the activation of inflammatory pathways. This observation is consistent with the relatively low percentages of membrane protection recorded in both hypotonicity- and heat-induced haemolysis assays. In contrast, previous reports have demonstrated markedly stronger anti-inflammatory activities within the *Anacyclus* genus. For instance, Jawhari et al. (2020) reported that *A. pyrethrum* extracts from different plant parts exhibited inhibition percentages of 98%, 94%, and 100%, highlighting potent anti-inflammatory, analgesic, and wound healing properties. Similarly, Bouriche et al. (2016) suggested that *A. clavatus* could serve as a promising source of anti-inflammatory agents. The discrepancy between these findings and the present results should be interpreted with caution, as earlier

studies primarily focused on essential oils, which are rich in lipophilic constituents with higher affinity for biological membranes, thereby enhancing their stabilizing effects. Furthermore, variations in chemical composition among *Anacyclus* species may arise from differences in geographical origin, environmental conditions, harvesting stage, and extraction techniques. Notably, the pronounced anti-inflammatory activity of *A. pyrethrum* has been attributed to its high content of bioactive alkamides (Jawhari et al., 2020), compounds that were not detected in the extracts of *A. valentinus* in the present study.

Protein denaturation is a well-recognized mechanism in inflammatory processes, leading to structural alterations and loss of protein function, which subsequently triggers inflammatory responses. In the present study, the anti-denaturation potential of *A. valentinus* extracts was evaluated using bovine serum albumin (BSA), egg albumin, and proteinase inhibition assays. As shown in Figure 8, both aqueous and methanolic extracts exhibited their highest inhibition of protein denaturation in the BSA assay at 500 µg/mL. However, the inhibitory effect remained relatively low, with values of 39.48 ± 0.04% and 38.21 ± 0.24%, respectively. In contrast, Diclofenac sodium demonstrated a markedly higher inhibition (96.09 ± 0.33%) at the same concentration, indicating a substantially stronger protective effect against protein denaturation. Similarly, in the egg albumin denaturation and proteinase (trypsin) inhibition assays, both extracts showed limited activity, with no significant enhancement across the tested concentrations. Meanwhile, Diclofenac sodium exhibited pronounced inhibitory effects, with values of 75.60 ± 0.27% and 51.00 ± 0.13% in egg albumin and trypsin assays, respectively. These findings further confirm the comparatively weak anti-inflammatory potential of *A. valentinus* extracts in preventing protein denaturation and proteolytic activity.

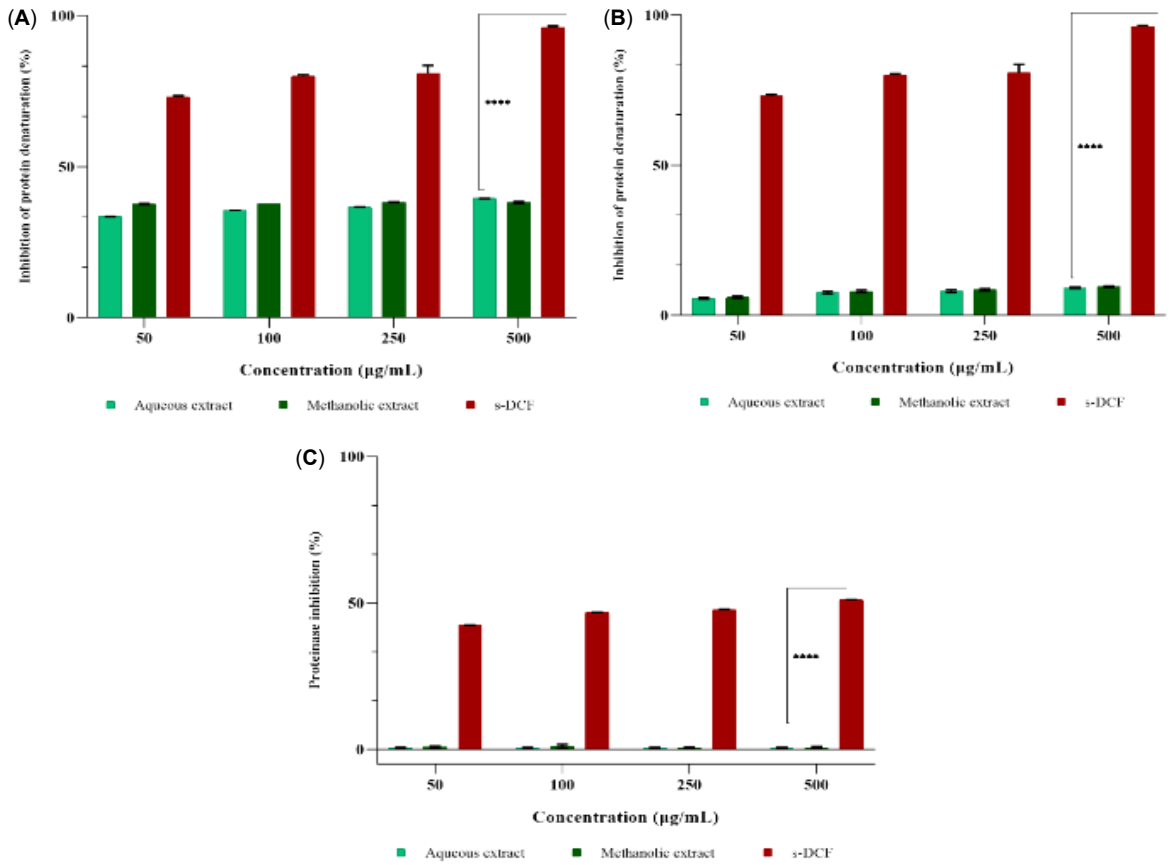

Figure 8. Anti-inflammatory activity of *A. valentinus* extracts showing (**A**) BSA denaturation inhibition, (**B**) egg albumin denaturation inhibition, and (**C**) proteinase inhibition.

Despite the inhibition of BSA denaturation, both aqueous and methanolic extracts of *A. valentinus* exerted limited egg albumin denaturation and proteinase activity. This indicates that the extracts possess only a limited capacity to prevent protein denaturation and proteolytic processes associated with inflammation. Therefore, the anti-inflammatory potential inferred from these assays should be considered weak under the tested conditions.Nonetheless, previous studies have reported stronger anti-inflammatory activities of Anacyclus species. For instance, Ainseba et al. (2023) found that essential oil of *A. valentinus* exhibited significant inhibition of egg albumin denaturation (92.7%), which exceeded that of diclofenac (90.3%) at concentration 1000 g/L. This discrepancy may be attributed to the richness

of essential oils in lipophilic bioactive compounds that may be responsible of enhanced anti-inflammatory activity through stronger interactions with different protein structures than those exerted by polar molecules present in aqueous and methanolic extracts. The modest activity exhibited by A. valentinus extracts may be attributed to the present of dominant phenolic compounds, such as quercetin or vanillin (Kumar et al., 2023; Bouriche et al., 2016). Furthermore, it was previously stated that the phenolic compounds inhibited *in vivo* acute and chronic inflammation by reduction the oxidative stress (Wu et al., 2007).

3.6. Cytotoxicity

The cytotoxic effects of aqueous and methanolic extracts of *A. valentinus* were evaluated against three human cancer cell lines, HT-29 (colon), PC-3 (prostate), and A549 (lung), as well as one non-tumorigenic cell line (CCD18-Co), using the MTT assay. As illustrated in Figure 9, both extracts exhibited weak antiproliferative effects within the tested concentration range (100-200 µg/mL). The aqueous and methanolic extracts slightly reduced the viability of HT-29 cells to 87.42% and 86.98%, respectively, while for PC-3 cells, viability decreased to 94.50% and 87.09%, respectively. A similar trend was observed for HT-29 cells treated with the aqueous extract, which showed modest reductions in cell viability (89.84% and 88.05%) at the same concentrations. In contrast, no significant cytotoxic effect was observed against A549 cells, indicating resistance of this cell line to the tested extracts. Importantly, both extracts showed no cytotoxicity toward the non-tumorigenic CCD18-Co cells, highlighting a favorable safety profile and suggesting selectivity toward cancer cells, albeit weak. The IC_{50} values for all tested cancer cell lines were greater than 200 µg/mL (Table 4), confirming the low cytotoxic potency of *A. valentinus* extracts under the studied conditions.

Table 4. Cytotoxic activity of *A. valentinus* extracts against cancer (HT-29, PC-3, A549) and normal (CCD-18Co) cell lines, expressed as IC_{50} values (µg/mL).

Samples	Cancer cell lines			Normal cells
	HT-29	PC-3	A 549	CCD18-Co
Methanolic	>200	>200	>200	N/A
Aqueous	>200	>200	>200	N/A

Values are shown as mean ± SD (n = 3)

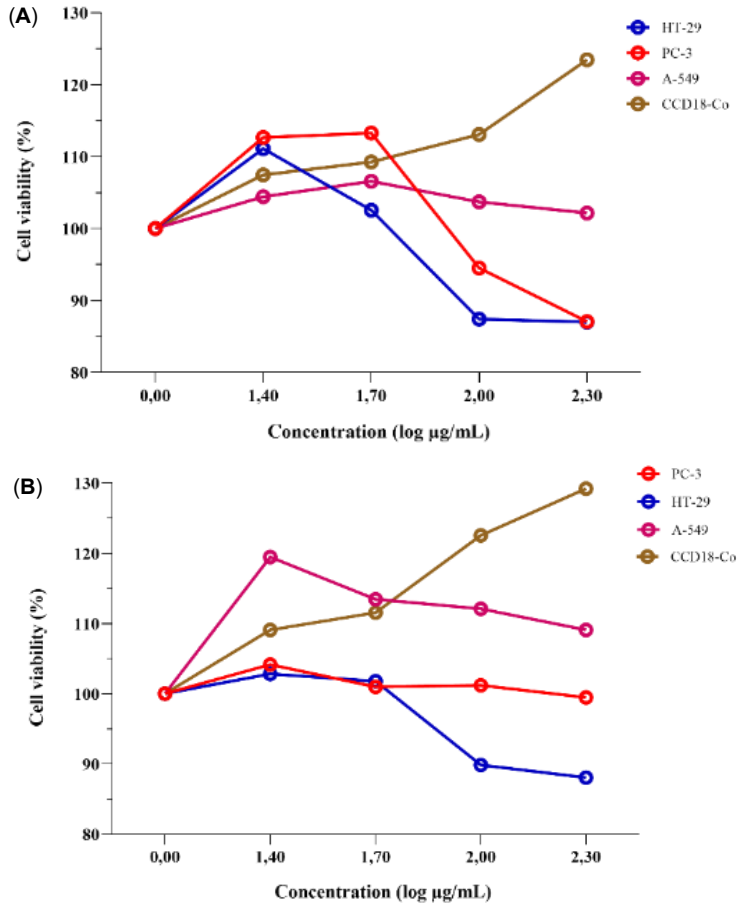

Figure 9. Cytotoxic activity of *A. valentinus* extracts against HT-29, PC-3, A-549, and CCD-18Co cell lines after 72 h of treatment, showing (**A**) methanolic extract and (**B**) aqueous extract; cell viability (%) was assessed at concentrations of 0-200 µg/mL and expressed as mean ± SD (n = 3), where values above 100% indicate a potential proliferative effect

4. CONCLUSION

This study presents the first comprehensive evaluation of the phytochemical profile and biological activities of aqueous and methanolic extracts from the aerial parts of *A. valentinus*. HPLC-DAD analysis confirmed the presence of key phenolic constituents, with vanillin, ferulic acid, and quercetin identified as major compounds, which likely contribute to the observed bioactivities. Both extracts demonstrated notable antioxidant capacity across multiple assays, highlighting their potential as natural sources of antioxidant agents. In contrast, the anti-inflammatory activity assessed through membrane stabilization and protein denaturation assays was moderate to low when benchmarked against Diclofenac sodium, indicating limited efficacy in mitigating inflammatory processes under the tested conditions. Similarly, cytotoxic evaluation revealed weak antiproliferative effects against HT-29, PC-3, and A549 cancer cell lines, with IC_{50} values exceeding 200 µg/mL, while no toxicity was observed toward the non-tumorigenic CCD18-Co cells, suggesting a favorable safety profile. Overall, *A. valentinus* extracts exhibit promising antioxidant potential but limited anti-inflammatory and cytotoxic activities. These findings provide a valuable baseline for future investigations. Further studies focusing on bioassay-guided fractionation, isolation of active constituents, and mechanistic evaluations are necessary to fully elucidate the therapeutic potential of this species and to identify compounds with enhanced biological efficacy.

ACKNOWLEDGEMENT
The authors are grateful to Mr. Djaafri M from the technical services of the laboratories of the Faculty of Natural and Life Sciences of the University of Mascara for his help.

CONFLICT OF INTEREST
The authors declare no conflicts of interest

AUTHOR CONTRIBUTION
Bachir Benarba: Conceptualization, methodology, supervision, project administration, and funding acquisition; Khadidja Belhouala: Software, formal analysis, investigation, data curation, writing original draft preparation, writing, review and editing, visualization, validation, and resources; Mehmet Emin Duru: Validation, resources, and funding acquisition; Cansu Korkmaz: Formal analysis and investigation; Meltem Taş Küçükaydın, Selçuk Küçükaydın, Adel Gouri: Investigation. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY
All data generated or analyzed during this study are included in this published article.

DECLARATION OF GENERATIVE AI
Not applicable.

ETHICS
Not applicable.

REFERENCES

Ahmed D, Fatima K, Saeed R. (2014). Analysis of phenolic and flavonoid contents, and the antioxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. *Antioxidants*, 3(4), 671-683. doi:10.3390/antiox3040671

Ainseba N, Soulimane A, Dib MEA, Djabou N, Muselli A. (2023). Comparative study of the antioxidant, antimicrobial and anti-inflammatory activity between essential oil and hydrosol extract of the aerial parts of *Inula viscosa* L. *Journal of Applied Biotechnology Reports*, 10(4), 1169-1175. doi:10.30491/jabr.2023.409230.1654

Ainseba N, Soulimane A, Mami IR, Dib M, Muselli A. (2024). Evaluation of the antioxidant and anti-inflammatory activity of the Anacyclus *Valentinus* L. Essential Oil and its oxygenated fraction. *Combinatorial Chemistry & High Throughput Screening*, 27(5), 765-772. doi:10.2174/1386207326666230418093319

Altanam SY, Darwish N, Bakillah A. (2025). Exploring the interplay of antioxidants, inflammation, and oxidative stress: mechanisms, therapeutic potential, and clinical implications. *Diseases*, 13(9), 309. doi:10.3390/diseases13090309

Azhar MAM, Salleh WMNHW, Khamis S. (2020). Essential oil composition of three *Cryptocarya* species from Malaysia. *Zeitschrift für Naturforschung C Journal of Biosciences*, 75, 297-301. doi:10.1515/znc-2020-0079

Azhar MAM, Salleh WMNHW. (2020). Chemical composition and biological activities of essential oils of the genus *Litsea* (Lauraceae) - a review. *Agriculturae Conspectus Scientificus*, 85(2), 97-103.

Babbar N, Oberoi HS, Sandhu SK, Bhargav VK. (2014). Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. *Journal of Food Science and Technology*, 51(10), 2568-2575. doi:10.1007/s13197-012-0754-4

Belhouala K, Benarba B. (2021). Medicinal plants used by traditional healers in Algeria: A multiregional ethnobotanical study. *Frontiers in Pharmacology*, 12, 760492. doi:10.3389/fphar.2021.760492

Belhouala K, Korkmaz C, Taş Küçükaydın M, Küçükaydın S, Duru ME, Benarba B. (2024). Eco-friendly species *Evernia prunastri* (L.) Ach.: Phenolic profile, antioxidant, anti-inflammatory, and anticancer properties. *ACS Omega*, 9(46), 45719-45732. doi:10.1021/acsomega.3c10407

Belhouala K, Korkmaz C, Taş-Küçükaydin M, Küçükaydin S, Duru ME, Benarba B (2025). HPLC-DAD phenolic profile, antioxidant, anti-inflammatory, and anticancer activities of *Bryonia cretica* subsp. dioica (Jacq.) Tutin.(Cucurbitaceae). *Chemical Papers*, 79(9), 6011-6024. doi:10.1007/s11696-025-04169-3

Benarba B, Belhouala K, Korkmaz C, Küçükaydın MT, Küçükaydın S, Duru ME. (2025). Phytochemical composition and biological activities of *Ephedra alata* Decne.: Antioxidant, anti-inflammatory and anticancer potentials. *Journal of Science and Mathematics Letters*, 13(2), 33-47. doi:10.37134/jsml.vol13.2.3.2025

Benarba B, Belhouala K, Korkmaz C, Taş Küçükaydın M, Küçükaydın S, Duru ME. (2024). Phenolic profile, antioxidant, anti-inflammatory and anticancer activities of *Telephium imperati* L. *Journal of Phytology*, 16, 216-225. doi:10.25081/jp.2024.v16.9334

Bernstein N, Akram M, Daniyal M, Koltai H, Fridlender M, Gorelick J (2018). Antiinflammatory potential of medicinal plants: A source for therapeutic secondary metabolites. *Advances in Agronomy*, 150, 131-183. doi:10.1016/bs.agron.2018.02.003

Bikoro Bi Athomo B, Engozogho Anris SP, Safou-Tchiama R, Santiago-Medina FJ, Cabaret T, Pizzi A, Charrier B. (2018). Chemical composition of African mahogany (*Khaya ivorensis* A.Chev.) extractives and tannin structures of the bark by MALDI-TOF. *Industrial Crops and Products*, 113, 167-178. doi:10.1016/j.indcrop.2018.01.013

Bouriche H, Kherbache A, Kada S, Senator A, Demirtas I. (2016). Phenolic content, anti-inflammatory and antioxidant activities of *Anacyclus clavatus* extracts. *Environmental and Experimental Biology*, 14, 127-135.

Cai Y, Luo Q, Sun M, Corke H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, 74(17), 2157-2184. doi:10.1016/j.lfs.2003.09.047

Çayan F, Deveci E, Tel-Çayan G, Duru ME. (2020). Identification and quantification of phenolic acid compounds of twenty-six mushrooms by HPLC-DAD. *Journal of Food Measurement and Characterization*, 14(3), 1690-1698. doi:10.1007/s11694-020-00417-0

Chatepa LEC, Masamba KG, Sanudi T, Ngwira A, Tanganyika J, Chamera F. (2023). Effects of aqueous and methanolic solvent systems on phytochemical and antioxidant extraction from two varieties of Roselle (*Hibiscus sabdariffa* L.) var. *sabdariffa* plant from Central Malawi. *Food and Humanity*, 1, 1172-1179. doi:10.1016/foohum.2023.09.006

Hosu VM, Cristea C, Cimpoiu C. (2014). Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: Prediction of antioxidant activities and classification of wines using artificial neural networks. *Food Chemistry*, 150, 113-118. doi:10.1016/j.foodchem.2013.10.153

Houicher A, Hamdi M, Hechachna H, Özogul F. (2018). Chemical composition and antifungal activity of *Anacyclus valentinus* essential oil from Algeria. *Food Bioscience*, 25, 28-31. doi:10.1016/j.fbio.2018.07.005

Hussain A. (2024). Potential medicinal uses of plants from the Asteraceae (Compositae) family in Pakistan: A literature review based meta-analysis. *Journal of Herbal Medicine*, 45, 100871. doi:10.1016/j.hermed.2024.100871

Jawhari FZ, El Moussaoui A, Bourhia M, Imtara H, Mechchate H, Es-Safi I. (2020). Anacyclus pyrethrum (L.): chemical composition, analgesic, anti-inflammatory and wound healing properties. *Molecules*, 25(22), 5469. doi:10.3390/molecules25225469

Kadir NHA, Salleh WMNHW. (2022). A systematic review on essential oils and biological activities of the genus *Syzygium* (Myrtaceae). *Rivista Italiana Delle Sostanze Grasse*, 99(2), 165-178.

Kumar RS, Naveena S, Praveen S, Yogadharshini N. (2023). Therapeutic aspects of biologically potent vanillin derivatives: A critical review. *Journal of Drug Delivery and Therapeutics*, 13(7), 177-189. doi:10.22270/jddt.v13i7.6159

Lekmine S, Benslama O, Bensalah B, Touzout N, Moussa H, Tahraoui H, Amrane A. (2025). Bioactive phenolics of *Hyoscyamus muticus* L. subsp. falezlez: a molecular and biochemical approach to antioxidant and urease inhibitory activities. *International Journal of Molecular Sciences*, 26(1), 370. doi:10.3390/ijms26010370

Lekmine S, Benslama O, Ola MS., Touzout N, Moussa H, Tahraoui H, Amrane A. (2025). Preliminary data on *Silybum marianum* metabolites: comprehensive characterization, antioxidant, antidiabetic and antimicrobial activities, LC-MS/MS profiling and predicted ADMET analysis. *Metabolites*, 15(1), 13. doi:10.3390/metabo15010013

Liu J, Bi J, McClements DJ, Liu X, Yi J, Lyu J. (2020). Impacts of thermal and non-thermal processing on structure and functionality of pectin in fruit- and vegetable-based products: A review. *Carbohydrate Polymers*, 250, 116890. doi:10.1016/j.carbpol.2020.116890

Mounnissamy VM, Kavimani S, Balu V, Quine SD. (2007). Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Cansjera rheedii* J. Gmelin. *Iranian Journal of Pharmacology and Therapeutics*, 6(2), 235-240.

Rahman H, Eswaraiah MC, Dutta AM. (2015). In vitro anti-inflammatory and anti-arthritic activity of *Oryza sativa* var. joha rice. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 15(1), 115-121. doi:10.5829/idosi.aejaes.2015.115.121

Salihu AS, Salleh WMNHW, Ogunwa TH. (2024). Chemical composition, acetylcholinesterase inhibition and molecular docking studies of essential oil from *Knema hookeriana* Warb. (Myristicaceae). *Natural Product Research*, 38(14), 2516-2521. doi:10.1080/14786419.2023.2184359

Salleh WMNHW, Ahmad F, Khong HY. (2015). Chemical constituents from *Piper caninum* and antibacterial activity. *Journal of Applied Pharmaceutical Sciences*, 5(6), 20-25. doi:10.7324/JAPS.2015.50604

Salleh WMNHW, Ahmad F. (2016). Antioxidant and anticholinesterase activities of essential oil of *Alseodaphne peduncularis* Meisn. (Lauraceae). *Turkish Journal of Pharmaceutical Science*, 13(3), 347-350. doi:10.4274/tjps.2016.11

Salleh WMNHW, Hashim NA, Khamis S. (2019). Chemical constituents and lipoxygenase inhibitory activity of *Piper stylosum* MiQ. *Bulletin of the Chemical Society of Ethiopia*, 33(3), 587-592. doi:10.4314/bcse.v33i3.19

Selles C, Dib MEA., Djabou N, Beddou F, Muselli A, Tabti B. (2013). Antimicrobial activity and evolution of the composition of essential oil from Algerian *Anacyclus pyrethrum* L. through the vegetative cycle. *Natural Product Research*, 27(23), 2231-2234. doi:10.1080/14786419.2012.739693

Shahidi F, Samarasinghe A. (2025). How to assess antioxidant activity? Advances, limitations, and applications of in vitro, in vivo, and ex vivo approaches. *Food Production, Processing and Nutrition*, 7(1), 50. doi:10.1186/s43014-025-00326-z

Shakri NM, Salleh WMNHW, Khamis S, Ali NAM, Shaharudin SM. (2020). Chemical composition of the essential oils of four *Polyalthia* species from Malaysia. *Zeitschrift für Naturforschung C Journal of Biosciences*, 75, 473-478. doi:10.1515/znc-2020-0097

Sioud F, Ben Toumia I, Lahmer A, Khlifi R, Dhaouefi Z, Maatouk M, Ghedira K, Chekir-Ghedira L. (2020). Methanolic extract of *Ephedra alata* ameliorates cisplatin-induced nephrotoxicity and hepatotoxicity through reducing oxidative stress and genotoxicity. *Environmental Science and Pollution Research*, 27(11), 12792-12801. doi:10.1007/s11356-020-07904-3

Sissi S, Di Giacomo S, Ferrante C, Angelini P, Macone A, Giusti AM. (2022). Characterization of the phytochemical composition and bioactivities of *Anacyclus maroccanus* Ball. and *Anacyclus radiatus* Loisel aerial parts. *Molecules*, 27(3), 692. doi:10.3390/molecules27030692

Sultana B, Anwar F, Ashraf M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14, 2167-2180. doi:10.3390/molecules14062167

Sunmathi D, Sivakumar R, Ravikumar K. (2016). In vitro anti-inflammatory and antiarthritic activity of ethanolic leaf extract of *Alternanthera sessilis* (L.) R.Br. ex DC and *Alternanthera philoxeroides* (Mart.) Griseb. *International Journal of Advanced Pharmaceutical and Biological Chemistry*, 5, 109-115.

Tai A, Sawano T, Yazama F, Ito H. (2011). Evaluation of antioxidant activity of vanillin using multiple antioxidant assays. *Biochimica et Biophysica Acta - General Subjects*, 1810(2), 170-177. doi:10.1016/j.bbagen.2010.11.004

Tuersong T, Yimingniyazi F, Maimaitimin B, Tureniyazi R, Xue B, Li Z,, Chen Y, Ma L, Yang X. (2024). Traditional use, chemical composition, and pharmacological activity of *Anacyclus pyrethrum* (L.) DC. *Clinical Traditional Medicine and Pharmacology*, 5(4), 200179. doi:10.1016/j.ctmp.2024.200179

Turay A, Kargbo HA, Conteh EB, Kamara IA. (2025). Comparative analysis of methanolic and aqueous extracts of four medicinal plants in Sierra Leone. *Journal of Pharmacognosy and Phytochemistry*, 14(3), 149-151. doi:10.22271/phyto.2025.v14.i3b.15360

Wang J, An W, Wang Z, Zhao Y, Han B, Tao H, Wang J, Wang X. (2024). Vanillin has potent antibacterial, antioxidant, and anti-inflammatory activities in vitro and in mouse colitis induced by multidrug-resistant *Escherichia coli*. *Antioxidants*, 13(12), 1544. doi:10.3390/antiox13121544

Wu YH, Zhou CX, Song LY, Li XP, Shi SY, Mo JX. (2007). Effect of total phenolics from *Laggera alata* on acute and chronic inflammation models. *Journal of Ethnopharmacology*, 108, 243-250. doi:10.1016/j.jep.2006.05.017