

# Hydromethanolic Extract of *Artemisia campestris* Targets Acetylcholinesterase and Butyryl Esterase for Sustainable Insect Control

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## Abstract

*Artemisia campestris* is a medicinal plant species endemic to Algeria, particularly abundant in the southern regions and central Sahara. Its long-standing use in traditional medicine has recently gained scientific attention, prompting further investigation into its bioactive potential. This study focuses on the phytochemical composition and biological activity of its hydromethanolic extract, with particular emphasis on its ability to inhibit neural enzymes associated with insect physiology. Preliminary screening revealed a diverse array of secondary metabolites, including tannins (catechic and gallic), flavonoids, quinones, glucosides, terpenoids, saponins, coumarins, and alkaloids; anthocyanins were not detected. Quantitative analysis confirmed high concentrations of total phenolics ( $80.91 \pm 1.58$  mg GAE/g), flavonoids ( $60.45 \pm 2.02$  mg RE/g), phenolic acids ( $4.24 \pm 0.38$  mg CAE/g), and condensed tannins ( $2.26 \pm 0.29$  mg CE/g). Enzyme inhibition assays were performed using Ellman's method, and  $IC_{50}$  values were calculated by nonlinear regression analysis based on dose-response curves. The extract demonstrated significant activity against acetylcholinesterase (AChE) and butyryl esterase (BuE), with  $IC_{50}$  values of  $13.79 \pm 0.79$   $\mu$ g/ml and  $8.34 \pm 0.58$   $\mu$ g/ml, respectively, based on five independent replicates ( $n = 5$ ). These results were benchmarked against galantamine, a reference inhibitor, which exhibited  $IC_{50}$  values of  $1.50 \pm 0.12$   $\mu$ g/ml under the same conditions. Although galantamine showed superior potency, the relatively low  $IC_{50}$  values of the *A. campestris* extract highlight its promising inhibitory potential and support its relevance as a natural alternative. The findings suggest that *A. campestris* may offer a natural and environmentally friendly approach to pest control due to its ability to disrupt insect neural function. Further research is needed to validate its efficacy and safety in real-world applications.

## Introduction

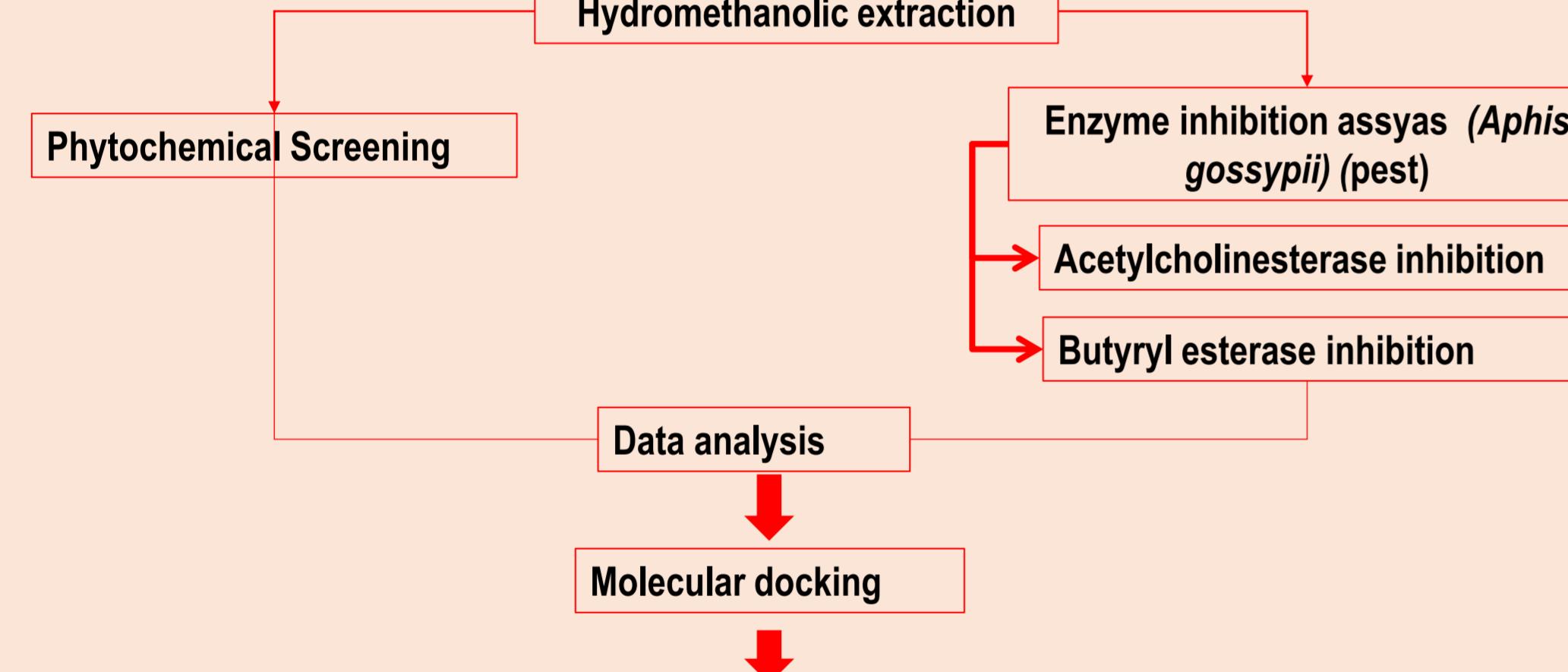
*Artemisia campestris*, known locally as "Tgouffot," is a small shrub widely used in Algerian traditional medicine to treat obesity, diabetes, skin conditions, and hypertension (Taibi et al., 2020, 2021b; Djahafi et al., 2021). In recent years, however, growing concerns about the safety of synthetic chemicals in medicine and food including antibiotic resistance and the toxicity of synthetic antioxidants have intensified interest in natural alternatives (Mothana & Lindequist, 2005; Djeridane et al., 2006). Phytochemical profiling of *Artemisia* species has revealed essential oils, flavonoids, tannins, polyphenols, and saponins (Ghlissi et al., 2016; Younsi et al., 2017; Golubkina et al., 2022). These metabolites are known to interfere with insect neural function, leading to overstimulation and paralysis (Smith & Brown, 2020; Zhang et al., 2022). Given its rich ethnopharmacological heritage and diverse bioactivities, *A. campestris* represents a promising candidate for modern scientific validation. This study evaluates the phytochemical composition and inhibitory activity of its hydromethanolic extract against acetylcholinesterase (AChE) and butyryl esterase (BuE), highlighting its insecticidal potential and relevance for eco-friendly pest control.

## Materials and Methods

The aerial part of *A. campestris* was harvested during spring 2023 in Oued N'ssa area (N  $32.633235^\circ$ , E  $4.281273^\circ$ ), Ghardaïa province.



Figure 1. *Artemisia campestris*.



## 3. Phytochemical content

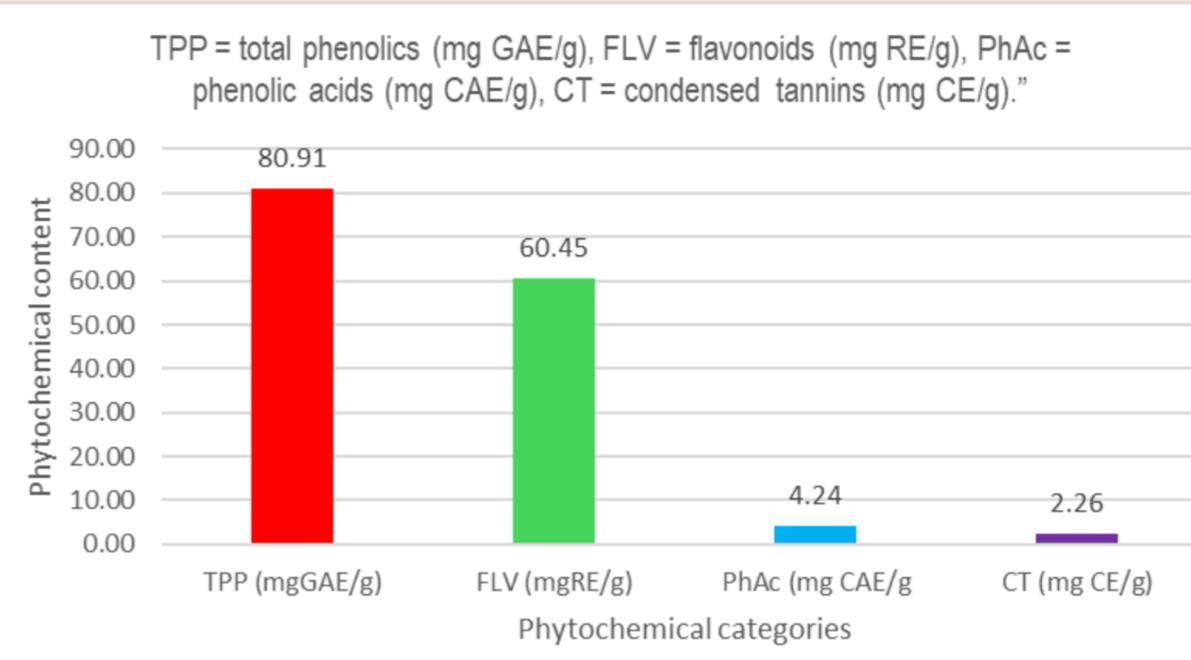


Figure 2. Phytochemical content of *A. campestris* extract.

*A. campestris* revealed a clear dominance of total phenolics ( $80.91 \pm 1.58$  mg GAE/g) and flavonoids ( $60.45 \pm 2.02$  mg RE/g), while phenolic acids ( $4.24 \pm 0.38$  mg CAE/g) and condensed tannins ( $2.26 \pm 0.29$  mg CE/g) were present at much lower levels ( $<10$  mg/g). This distribution confirms the plant's polyphenolic richness, which underpins its strong antioxidant and enzyme-inhibitory potential. Our findings are consistent with previous reports on Algerian *A. campestris* (Brahmi et al., 2024).

## 4. Enzyme Inhibition

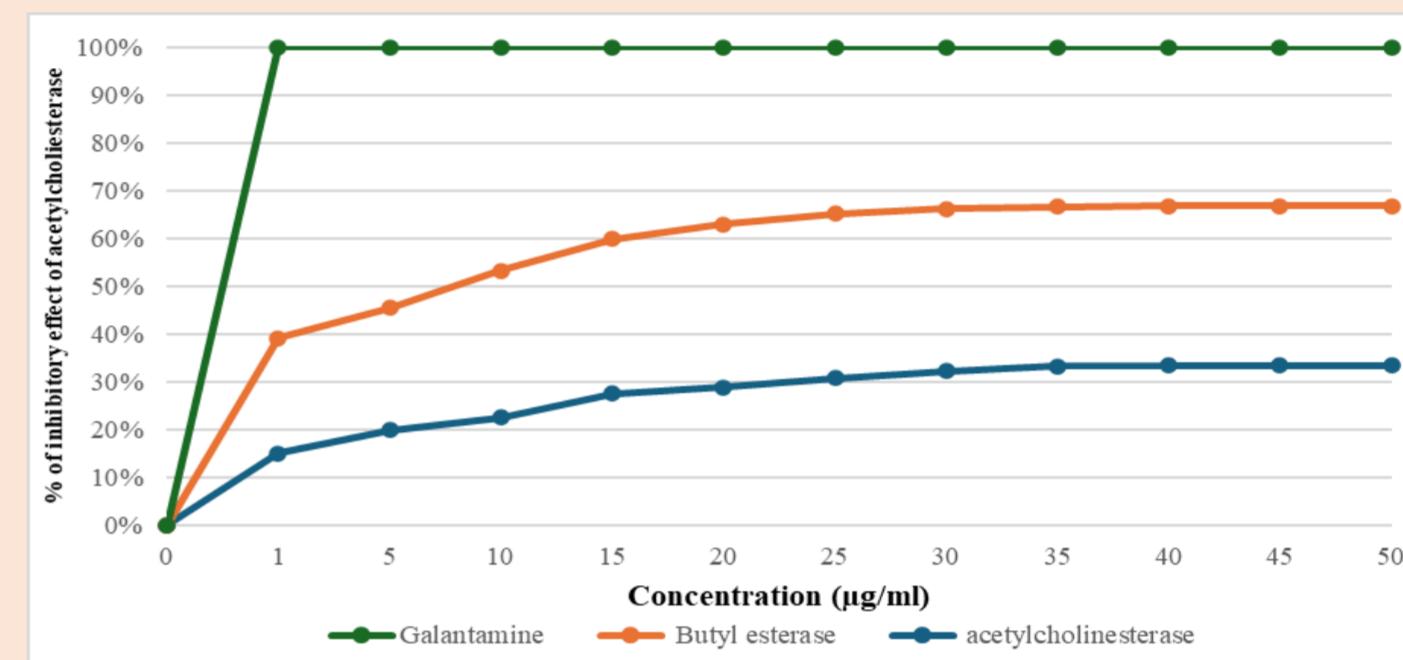


Figure 3. Dose-Response Curves for Enzyme Inhibition by *Artemisia campestris* Extract.

The extract inhibited acetylcholinesterase (AChE) with  $IC_{50} \approx 13.8 \pm 0.4$   $\mu$ g/ml and butyrylcholinesterase (BuE) with  $IC_{50} \approx 8.3 \pm 0.3$   $\mu$ g/ml. In comparison, the standard drug galantamine was more potent ( $IC_{50} \approx 1.5 \pm 0.1$   $\mu$ g/ml). Although less active than galantamine, these values fall within the range reported for natural inhibitors from *Artemisia* species, supporting the insecticidal potential of the extract and validating its ethnopharmacological use (Politeo et al., 2023).

## 5. In silico validation: molecular docking

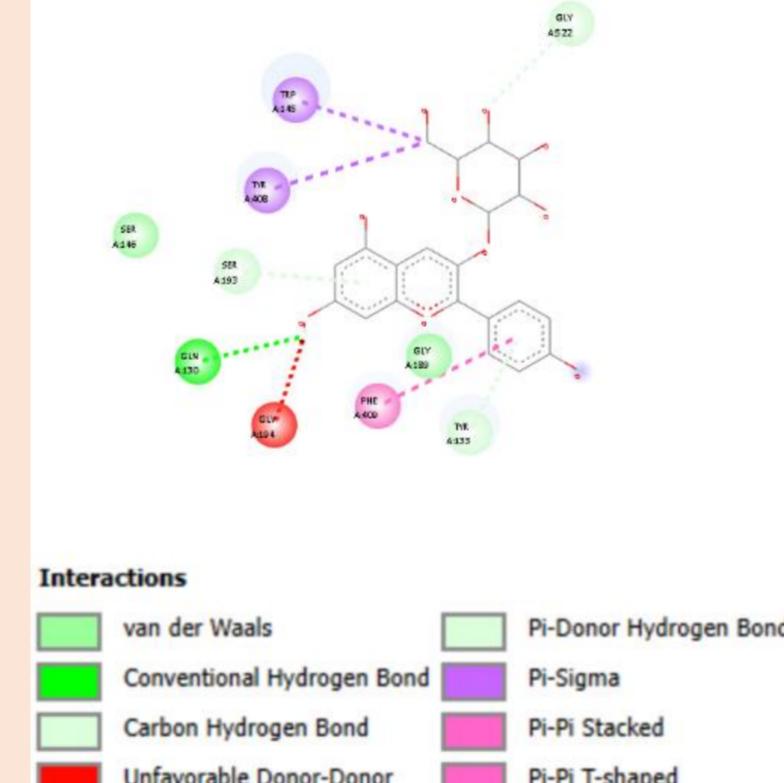


Figure 4. 2D Docking Interaction Map of *A. campestris* Metabolite in AChE Active Site

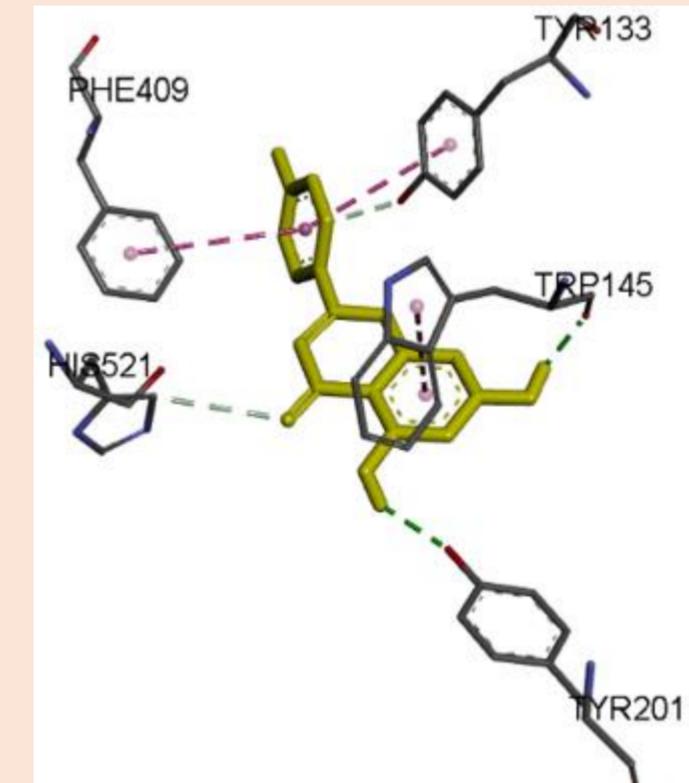


Figure 5. Ligand-Residue Interactions of *A. campestris* Metabolite in AChE Active Site



Figure 6. Ribbon diagram: Modeled Structure of *Aphis gossypii* Acetylcholinesterase

Molecular docking demonstrated that *Artemisia campestris* metabolites, particularly cyanidin-3-O-glucoside (-8.5 kcal/mol), malvidin-3-O-glucoside (-8.4 kcal/mol), and apigenin (-8.2 kcal/mol), exhibited the strongest affinities for *Aphis gossypii* acetylcholinesterase, interacting with catalytic residues such as Trp145, Tyr133, Gly189, and Tyr408. Recent docking experiments on *Artemisia* show that flavonoids and anthocyanins act as important binders in the enzyme's active site gorge, stabilizing it by hydrogen bonding and  $\pi$ -stacking (Politeo et al., 2023). The overlap between our findings and published papers demonstrates that phytochemicals from *A. campestris* can efficiently inhibit cholinesterase activity, supporting the plant's environmentally friendly insecticidal potential as well as the *in vitro* inhibitory data.

## Conclusion

Collectively, our *in vitro* and *in silico* results demonstrate that *Artemisia campestris* is a viable, promising eco-friendly candidate for sustainable insect control.

Protein modeling	Validation	Pocket detection	Ligand selection	Docking	Visualization
Protein modeled with AlphaFold	Validated ProSA-web, ERRAT, Verify3D, Ramachandran	Identified (FPocketWeb)	PubChem	AutoDock Vina	Discovery Studio

Table 1. Molecular Docking Workflow

## Results & Discussion

### 1. Phytochemical screening

Compounds	<i>Artemisia campestris</i>
Tannins	+++
Catechic tannins	+
Gallic tannins	+++
Flavonoids	+++
Anthocyanins	-
Free quinones	+++
Glucosids	+++
Terpenoids	+++
Saponins	++
Coumarines	++
Alkaloids	wagner assay+/mayer assay-

Table 2. Qualitative phytochemical profile of *Artemisia campestris* extract

According to the findings in Table 1, *A. campestris*'s aerial portion (leaves and stems) contains various secondary metabolites. Catechic and gallic tannins, flavonoids, free quinones, glucosides, and terpenoids were all found to be strongly present. Coumarins and saponins were found in moderate amounts. Although Wagner's assay did not confirm alkaloids, Mayer's assay did. Our findings are consistent with recent reports on *Artemisia campestris* phytochemistry (Bendifallah & Merah, 2023)

### 2. Yield of Hydromethanolic Extract

Extraction yields across five replicates of *Artemisia campestris* ranged from 11.78% to 13.01%, with an overall mean of 12.48  $\pm$  0.46%. An excellent yield from the extraction confirmed that it is a suitable source of bioactive chemicals. Comparable yields are reported in similar investigations conducted on Algerian communities, confirming its traditional usage (Zahnit et al., 2022).

### 3. Secondary metabolites of *Artemisia campestris* interaction with modeled acetylcholinesterase of *Aphis gossypii*

Molecule	Pubchem CID	Binding energy	Binding amino acids
15-O- $\beta$ -D-glucopyranosyl-11 $\beta$ , 13-dihydro urospermal A	5280633	-6.5	Trp145, Ser146, Gly189, Ser193, Glu275, Ser276, Tyr408
5-O-caffeoquinic acid	443648	-7.3	Tyr133, Trp145, Ser146, Gly189, Ser193, Trp145, Tyr145, Ser193, Gly194, Tyr194, Phe409, Gly522
Cyanidin-3-O-glucoside	441667	-8.5	Trp145, Gly188, Gly189, Gly194, Tyr194, Phe409, Gly522
Malvidin-3-O-glucoside	443652	-8.4	Trp145, Gly188, Gly189, Gly194, Tyr194, Phe409, Gly522
Esculetin-6-O-glucoside	521417	-7.6	Trp145, Gly188, Gly189, Gly194, Glu275, Ser296, Tyr201, Phe409
3-hydroxyphloretin - 6'-O-glucoside	1020685873	-6.5	Trp145, Gly188, Gly189, Gly194, Tyr194, Phe409, Gly522
Apigenin	5280443	-8.2	Trp145, Gly188, Gly189, Gly194, Tyr194, Phe409, His521
Acacetin	5280442	-7.7	Trp145, Gly188, Gly189, Gly194, Tyr194, Phe409, His521
Chlorpyrifos	2730	-6.0	Trp145, Gly188, Gly189, Gly194, Tyr194, Phe409, His521, Met525

Table 3. Secondary metabolites of *Artemisia campestris* interaction with modeled acetylcholinesterase of *Aphis gossypii*

## References

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