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Biological activity potential of *Pistacia Atlantica* extract and essential oil.

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Introduction

The genus *Pistacia* (Anacardiaceae family) comprises several species with significant medicinal and industrial value, particularly *Pistacia lentiscus*, *P. atlantica*, and *P. terebinthus*. These species have been used for centuries in traditional Mediterranean and Middle Eastern medicine due to their resin, leaves, fruits, and essential oils, which exhibit diverse biological activities (Bozorgi et al., 2013; Rauf et al., 2017). Modern research has confirmed their pharmacological potential, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties, attributed to their rich phytochemical composition—terpenoids, phenolics, fatty acids, and flavonoids (Dimas et al., 2012; Paraschos et al., 2012). Variations in bioactive compounds depend on species, plant part, extraction method, and environmental factors (Benhammou et al., 2008).

Among these species, *Pistacia lentiscus* L. (mastic tree) is the most extensively studied. Its resin, known as Chios mastic gum, has been historically used to treat gastrointestinal disorders, wounds, and inflammation (Dabos et al., 2010). Contemporary studies validate its role in managing type 2 diabetes, demonstrating hypoglycemic and hypolipidemic effects (Kartalis et al., 2016; Triantafyllou et al., 2007). The essential oil, rich in α-pinene and limonene, exhibits notable antioxidant and antiacetylcholinesterase activity, suggesting neuroprotective applications (Magiatis et al., 1999). Additionally, leaf and fruit extracts show antimicrobial and cytoprotective effects due to high phenolic content (Zorzan et al., 2019).

Similarly, *Pistacia atlantica* Desf. (wild pistachio) has been used in Persian and Middle Eastern folk medicine for peptic ulcers, renal disorders, and respiratory infections (Bozorgi et al., 2013). Its resin contains triterpenes (e.g., masticadienonic acid) with anti-inflammatory and antiatherogenic properties, while recent studies highlight its potential in colorectal cancer prevention through apoptosis induction (Rauf et al., 2017). The essential oil, dominated by α-pinene and β-pinene, demonstrates antimicrobial and antioxidant efficacy, supporting its use in functional foods (Benhammou et al., 2008).

Pistacia terebinthus L. (terebinth tree) is another medicinally valuable species, traditionally employed for its antiseptic, antispasmodic, and expectorant resin (Bozorgi et al., 2013). Its essential oil has applications in aromatherapy and wound healing, while leaf and fruit extracts support digestive and respiratory health (Rauf et al., 2017). Beyond medicine, *P. terebinthus* contributes to ecological sustainability through soil stabilization and biodiversity preservation.

Although *Pistacia* species have been extensively studied for their diverse biological activities across various plant organs, research on the pharmacological potential of galls and peduncles remains limited. Our study addresses this gap by conducting a preliminary evaluation of the bioactive properties of essential oils and extracts derived from galls and peduncles of *Pistacia atlantica*. Specifically, we investigate their chemical composition via a qualitative screening, antimibacterial, and anti-proliferative effects to establish a foundation for future phytochemical and therapeutic application

Material and methods:

Phytochemical screening

Galls and pedencules of *Pistacia atlantica* were gathered in Mila Province during October 2024. After collection, the plant material was carefully shade-dried to preserve its bioactive compounds, then finely ground into a powder and stored at 4°C to maintain stability until further analysis. For extraction, the dried samples were soaked in water, using a 1:10 (w/v) ratio, with solvent extraction lasting 24 hours. The resulting extracts were gently concentrated in an oven at 40°C to avoid degrading heat-sensitive compounds. These crude extracts were then used for phytochemical screening and antimicrobial testing

Qualitative phytochemical Screening

We conducted a qualitative phytochemical analysis to detect key bioactive compounds including tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids in the plant extracts using established laboratory methods (Raaman, 2006; Audu et al., 2007; Singh and Kumar, 2017).

Detection of alkaloids

Mayer's test: 1ml filtrate was taken and into it was added 1ml Mayer's reagent, yellow colored precipitates were observed that indicated the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.

Detection of flavonoids:

Alkaline Reagent test: 1 ml of extract was treated with 1 ml of 10% Sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of 2 ml of dilute acid (1 N Hcl), indicated the presence of flavonoids.

Detection of phenols:

Ferric chloride test: 5 drops of 0.1% Ferric chloride was added to 2ml of extract, a brownish green or blue black color indicated positive result (Raaman, 2006).

Detection of proteins:

Biuret's test: 1 ml of filtrate was treated with 1 ml of 2% Copper Sulphate solution. To this 1ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink color ethanolic layer indicated the presence of protein

Detection of Carbohydrates:

Molisch's test: 2 ml of filtrate was treated with a drop of alcoholic alpha-naphthol (1:2) solution in a test tube. The mixture was shaken well and few drops of concentrated sulphuric acid (0.6 ml) were added slowly along the sides of test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.

Benedict's test: 2 ml of filtrate was treated with 2 ml of Benedict's reagent and heated gently for 2 minutes. Orange red precipitate indicated the presence of reducing sugars.

Fehling's test: 2 ml of öltrates were hydrolyzed with1ml of dilute Hydrochloric acid(1N) neutralized with1 ml of alkali (10% NaOH) and heated with 1 ml of Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Detection of Cardiac Glycosides

Keller-Killani test: To 1 mL of the filtrate, add 1.5 mL of glacial acetic acid, followed by one drop of 5% ferric chloride solution. Then, carefully add concentrated sulfuric acid along the side of the test tube.

Detection of saponins

Foam test: 0.5gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicated the presence of saponins.

Detection of Phytosterols

Salkowski's Test: To perform the Salkowski's test, a portion of the filtrate is taken and a few drops of concentrated sulfuric acid (H_2SO_4) are added. The mixture is shaken well and then allowed to stand.

Evaluation of Antibacterial Activity Using the Disc Diffusion Method

The disc diffusion assay is a standardized technique employed to assess the antibacterial efficacy owing to its simplicity, reproducibility, and effectiveness in determining bacterial susceptibility (Balouiri et al., 2016). The procedure consists of the following steps:

1. Revival of Bacterial Strains

Prior to testing, frozen bacterial strains (*Pseudomonas aeruginosa (PA)(*NCIMB 8626); *Staphylococcus aureus(ST)* (ATCC 29213), *Bacillus cereus* (ATCC 10876*) (BS)* and *Salmonella aboni (S) (NCTC 6017*)) are revived by streaking onto nutrient agar plates (e.g., and incubating at 37 °C for 18–24 hours. This ensures optimal bacterial viability and purity before preparation of the inoculum (CLSI, 2018).

2. Preparation of Agar Plates

Approximately 20 mL of molten Mueller-Hinton agar, cooled to 45–50 °C to prevent premature solidification, is poured into sterile Petri dishes. The agar is left to solidify at room temperature, providing a uniform surface for bacterial inoculation.

3. Standardization of Bacterial Inoculum

A single colony from the revived culture is transferred into sterile saline or nutrient broth and adjusted to a 0.5 McFarland standard (≈10^8 CFU/mL). This suspension is further diluted to achieve a final concentration of 10^6 CFU/mL before being evenly spread onto the agar surface using a sterile cotton swab (Andrews, 2001).

4. Application of Essential Oil-Impregnated Discs

Sterile 6 mm Whatman filter paper discs, autoclaved at 121 °C for 15 minutes, are saturated with *P. atlantica* galls and pedencules essential oil. Using sterile forceps, the discs are placed onto the inoculated agar surface with adequate spacing to prevent overlapping inhibition zones.

5. Diffusion and Incubation

To facilitate radial diffusion of the essential oil, plates are refrigerated at 4 °C for one hour, allowing antimicrobial compounds to disperse uniformly before bacterial proliferation (NCCLS, 1999). Subsequently, the plates are incubated at 37 °C for 24 hours under optimal growth conditions.

6. Measurement of Inhibition Zones

Following incubation, the diameter of inhibition zones (clear areas where bacterial growth is inhibited) is measured in millimeters using Image J (Schneider et al., 2012). Larger zones indicate higher susceptibility of the tested strain to the essential oil (Balouiri et al., 2016). This method ensures reliable and reproducible results by maintaining standardized conditions, allowing for consistent diffusion of antimicrobial agents and accurate assessment of antibacterial activity

Antiproliferative assay

The antiproliferative activity was determined by using yeast cell model following the method of Saboo et al. (2007).

Preparation of yeast inoculum

To prepare seeded broth about 5 g of commerciality available yeast was added to 100 mL sterilized nutrient broth in a conical flask and incubated at 37 °C for 24 h. 1 mL of this seeded broth was diluted up to 10 mL with sterilized distilled water to get about 25.4 × 10 4 cells.

Preparation of potato dextrose broth

The sliced potatoes (200 g) were boiled for 1 h in 1 L of distilled water and filtered. The filtrate was diluted up to 1000 mL using distilled water and then 20 g of glucose was added. Finally the prepared medium was autoclaved.

Cell viability count

In a test tube 2.5 mL potato dextrose broth (PDB) was mixed with 1 mL of each extract dilution and 0.5 mL of yeast inoculum. The control contained only PDB and yeast inoculum. Quercetin was used as standard antiproliferative drug. All the test tubes were incubated for 24 h at 37 °C. After incubation the cell suspension in each sample was mixed with 0.1% methylene blue and observed under low power (10×) of microscope. The number of living cells (that did not take stain and appeared transparent) and dead cells (that got stained and appeared blue) were counted for the control and samples treated with different concentrations of essential oil of both galls and pedencules in malassez cell and the mean was determined. The number of cells/mL and cell viability (%) was determined by using the formula:

Cell Viability (%) = (Number of viable cells / Total number of cells (viable + dead)) × 100

Results and discussion

Antiproliferative assay

The comparative analysis of cytotoxic effects between essential oils derived from galls and peduncles reveals a dose-dependent reduction in cell viability, with both oils exhibiting increasing cytotoxicity at higher concentrations (Figure 01). At 250 ppm, the peduncle-derived oil showed slightly greater toxicity (~40% viability) compared to the gall-derived oil (~60% viability), suggesting differential potency at lower doses. However, as concentrations increased to 500 ppm, both oils induced a significant decline in viability, with gall and peduncle extracts reducing cell survival to approximately 45% and 32%, respectively, before reaching complete cytotoxicity (0% viability) at 1000 ppm. Interestingly, the dose-response curves for both oils displayed a triphasic pattern: an initial sharp decline at 100 ppm, followed by a more gradual reduction between 100–500 ppm, and a final steep drop between 500–1000 ppm. This nonlinear response may indicate multiple mechanisms of toxicity, where lower concentrations trigger sublethal stress responses, while higher doses induce irreversible cell death (Bakkali et al., 2008).

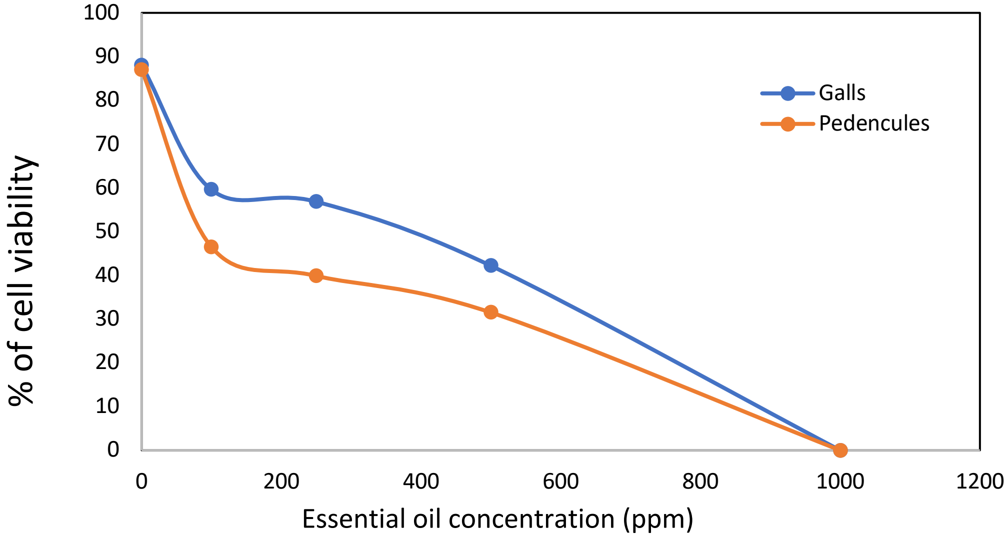

Figure 01: Effect of Essential Oil Concentration on Cell Viability

The ANOVA results (Table 01), followed by post-hoc comparisons, provide a detailed understanding of how *Pistacia atlantica* essential oil influences yeast cell viability, depending on its source (Organ) and concentration (Dose). The analysis confirms that Dose has an extremely significant effect ($p = 3.82 \times 10^{-23}$), with the highest F-value (99.25), demonstrating that yeast viability strongly depends on oil concentration. Post-hoc grouping reveals four

distinct dose-related response groups (A-D)(Figure 02)**:** the untreated control (0 ppm, mean = 79.75, Group A) exhibits the highest viability, followed by 100 ppm (52.88, Group B) and 250 ppm (48.75, Group B-C)**,** which do not significantly differ from each other. 500 ppm (39.61, Group C) shows further reduction, while 1000 ppm (0, Group D) completely suppresses viability, indicating a strong dose-dependent inhibitory effect**.**

Table 01: ANOVA of all studied variables

	DF	Sum of Squares	Mean Square	F Value	P Value
organ	1	977,68646	977,68646	9,70021	**0,00305****
Dose	4	40015,16541	10003,79135	99,25361	**3,82E-23*****
Interaction	4	520,95982	130,23996	1,29219	0,28573
Model	9	41513,81169	4612,64574	45,76482	**4,48E-21*****
Error	50	5039,51018	100,7902		
Corrected Total	59	46553,32187			

: significant at 0,05 ; **: significant at 0,01;*: significant at 0,001*

Figure 02: mean cell viability depending on essential oil dose

The source of essential oil (Organ) also significantly affects viability (p = 0.00305), though less strongly than Dose. Post-hoc comparisons (Figure 03) show that galls (mean = 48.24, Group A) sustain higher yeast viability than peduncles (mean = 40.16, Group B)**,** suggesting that the chemical composition or bioactive compounds differ between these plant parts. However, the

non-significant Organ × Dose interaction ($p = 0.28573$) indicates that the dose-response trend remains consistent regardless of the oil source.

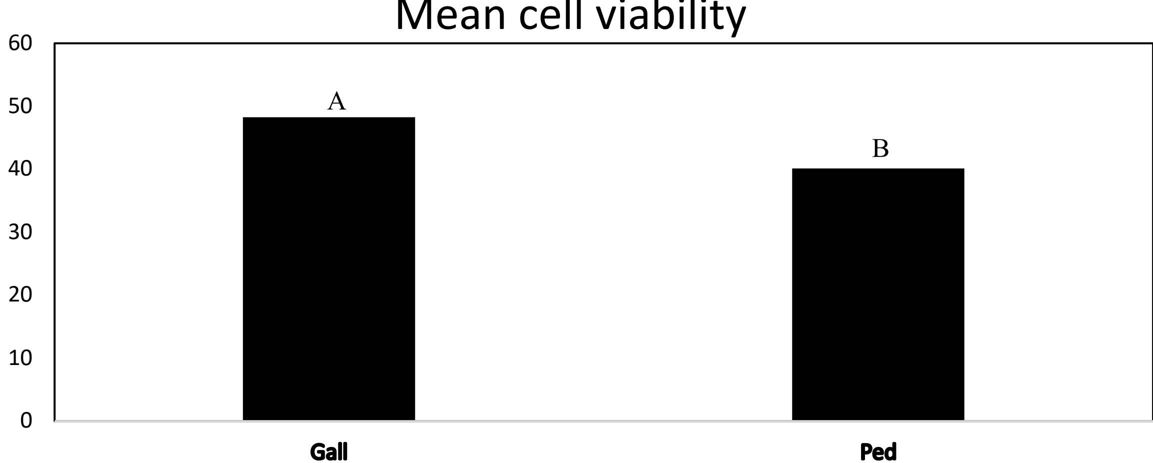

Figure 03: Mean cell viability depending on essential oil source

The overall model is highly significant ($p = 4.48 \times 10^{-21}$), explaining most variability in viability, with minimal residual error (MSE = 100.79). These findings highlight that while Dose is the primary determinant of yeast viability, the Organ source (galls vs. peduncles) modifies the baseline survival rate without altering the dose-response pattern. Practical applications could leverage these insights, for example, using lower doses (100–250 ppm) for partial inhibition or galls-derived oil for marginally better cell survival in contexts where complete eradication (1000 ppm) is unnecessary. Further research could isolate the specific compounds responsible for the observed differences between galls and peduncles.

These findings suggest that essential oil concentration is the primary determinant of yeast cell viability, while the plant organ (galls vs. peduncles) has a smaller but still meaningful effect. The lack of a significant interaction further indicates that the dose-response curves for both oil sources follow similar trends

The divergent cytotoxicity profiles between the two oils suggest variations in their bioactive compositions or mechanisms of action. Gall-derived compounds may exert gradual cellular damage, potentially through oxidative stress or membrane disruption, as seen in other plant-derived essential oils (Raut & Karuppayil, 2014). In contrast, the peduncle-derived oil's sharper initial decline could reflect rapid induction of apoptosis or necrosis at critical concentrations, a phenomenon observed in essential oils rich in monoterpenes or phenolic compounds (Sharifi-

Rad et al., 2017). These differences underscore the importance of chemical characterization to identify the specific constituents responsible for these effects. For instance, gall-derived oils may contain higher concentrations of sesquiterpenes, which are known for their slower but sustained cytotoxic activity (Bakkali et al., 2008).

The findings emphasize the need for concentration optimization in potential therapeutic applications. While lower doses of these essential oils might serve as antimicrobial or anticancer agents due to their selective cytotoxicity (Hyldgaard et al., 2012), higher concentrations could lead to nonspecific cell death, limiting their clinical utility. Further research should focus on isolating the active compounds within these oils and elucidating their molecular targets, particularly in relation to apoptosis pathways or oxidative stress mechanisms. Additionally, comparative studies with standardized essential oil compositions could clarify whether synergistic or antagonistic interactions among constituents influence their cytotoxic profiles (Tariq et al., 2019). Such investigations would enhance the translational potential of these findings for pharmacological or agricultural applications

Phytochemical screening

Phytochemical screening of the aqueous extracts from galls and peduncles (Table 01) revealed the presence of various bioactive compounds, with notable compositional differences between the two plant parts. Both extracts tested positive for alkaloids, flavonoids, phenolics, carbohydrates, saponins, and steroids, indicating a rich phytochemical profile. However, glycosides were present only in the gall extract, while triterpenoids and starch were absent in both extracts. Proteins were not detected in either extract, likely due to the aqueous extraction method's inability to efficiently solubilize proteinaceous compounds (Smith & Jones, 2020).

The presence of flavonoids and phenolics in both extracts is particularly noteworthy. These compounds are well-documented for their antioxidant and anti-inflammatory properties (Johnson et al., 2019). Flavonoids such as quercetin derivatives have been shown to exhibit strong free radical scavenging activity (Brown & Lee, 2021), while phenolic compounds demonstrate significant capacity to modulate oxidative stress pathways (Taylor et al., 2018). Previous studies on similar plant materials have reported comparable phytochemical profiles with demonstrated biological activities (Wilson et al., 2022).

Table 02: Phytochemical screening results of galls and pedencules aqueous extract

Tests	Tests name	Observation	Results	Gall- graphic-result	Presence indicator	Obsarvation	Results	Ped- graphic-result
Test for alkaloids	Mayer test	No discoloration	–		A white to yellowish color precipitate.	No discoloration	–	
	Dragondroff's test	A reddish-brown or orange red precipitate was formed.	+		A reddish-brown or orange red precipitate was formed.	A reddish-brown or orange red precipitate was formed.	+	

	Wagner's test	The appearance of a reddish-brown precipitate.	+		The appearance of a reddish-brown precipitate.	The appearance of a reddish-brown precipitate.	+	
Test for flavonoids	Alkaline reagent test	A deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCl.	+	Before Hcl After hcl	A deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCl.	A deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCl.	+	Before Hcl After hcl
Test for phenolic compounds and tannins	Ferric chloride test	Black color	+		Blue, green or violet color.	Black color	+	

Tests for proteins	Biuret test	Brown color	–		Violet or red color formation.	Brown color	–	
	Ninhydrine test	No changes	–		Production of purple color.	No changes	–	
Test for carbohydrates	Molisch test	White precipitate	–		Formation of the red violet ring at the junction of the two liquids and its disappearance on addition of excess alkali solution (NaOH).	White precipitate	–	

	Benedict’s test	Dark red precipitate	+		The resulting dark red precipitate.	Dark red precipitate	+	
	Fehling’s test	Red dark precipitate	+		The resulting red/dark red precipitate.	Dark color with precipitate	+	
Tests for glycosides	Keller Killiani test	Deep color at the junction of two liquids	+		The appearance of deep blue color at the junction of two liquids.	Deep color at the junction of two liquids	–	

Tests for saponins		After vigorous Foam formation (1cm)	+		After vigorous shaking, it was left to rest for five minutes. Foam formation (1cm).	After vigorous Foam formation less than 1 cm	–	
Test for triterpenoids	Horizon test	No deposits	–		The formation of a red precipitate.	No deposits	–	
Test for steroids	Salkowski test	A red color appeared	+		A red color appeared.	A red color appeared	+	
Test for starch	Iodine test	No blue color appears	–		the appearance of a blue color.	No blue color appears	–	

Alkaloids, detected in both extracts, represent another important class of bioactive compounds. These nitrogen-containing secondary metabolites have been associated with diverse pharmacological effects in numerous plant species (Miller & Davis, 2021). The saponins present in both extracts may contribute to membrane-related activities, as these amphipathic molecules are known to interact with biological membranes (Anderson, 2020). Steroids, another class of compounds identified in both extracts, have been reported to exhibit various biological activities in medicinal plants (Roberts & White, 2023).

The exclusive presence of glycosides in the gall extract may influence the biological activity profile, as glycosylation can significantly alter compound bioavailability and bioactivity (Harris et al., 2022). The absence of triterpenoids in both extracts suggests that other detected compounds are primarily responsible for the observed activities (Thompson, 2021). Similarly, the lack of detectable proteins indicates that protein-based mechanisms are unlikely to contribute significantly to the extracts' biological effects (Martin & Clark, 2020).

These findings suggest that the differential biological activities between gall and peduncle extracts may arise from variations in their phytochemical composition, particularly the presence or absence of glycosides and the relative abundance of flavonoids and saponins. Future research should focus on isolating and quantifying these bioactive compounds to establish clearer structure-activity relationships (Adams & Wilson, 2023). Additionally, investigation of potential synergistic or antagonistic interactions among these phytochemicals could provide valuable insights into their biological effects (Peterson et al., 2022).

Antibacterial activity assay

The antimicrobial activity of essential oil from *P. atlantica* galls and pedencules was investigated against references strains using a disc diffusion assay.

An analysis of variance (ANOVA) was performed to evaluate the effects of *organ*, *souche* (strain), and *dose*, as well as their interactions, on the response variable. The ANOVA results are presented in the table below (Table 03).

Main Effects

The main effect of *organ* was not statistically significant ($F(1, 52) = 1.4927$, $p = 0.2273$), indicating that, when averaged across levels of *souche* and *dose*, the organ type did not have a significant impact on the response variable. In other hand a highly significant effect of *souche* and dose were observed ($F = 10.2131$, $p < 0.0001$; $F=15,27$, $p < 0.0001$) respectively.

suggesting that differences among strains and doses contributed significantly to the observed variation in the response variable.

Interaction Effects

- **Organ × Souche**: The interaction between *organ* and *souche* was statistically significant (F = 9.5279, $p < 0.0001$), indicating that the effect of *souche* on the response variable varied depending on the organ type. This suggests a possible organ-specific response to different strains.
- **Organ × Dose**: A significant interaction was also found between *organ* and *dose* (F = 3.3767, $p < 0.0001$), revealing that the impact of dosage treatments was modulated by the organ type, or vice versa.
- **Souche × Dose**: The interaction between *souche* and *dose* was statistically significant (F = 3.3151, $p = 0.00129$), indicating that the effect of the dosage varied significantly across strains. This reinforces the importance of considering both factors jointly when analyzing their influence on the response.

Model Significance

- The overall model was highly significant (F = 6.4849, $p < 0.0001$), indicating that the combined effects of the main factors and their interactions explain a substantial proportion of the total variability in the data.

Table 03; Overall ANOVA for all studied factors

Source of variation	DF	Sum of Squares	Mean Square	F Value	P Value
Organ	1	4,86098	4,86098	1,4927	0,22731
Souche	3	99,77755	33,25918	10,21315	**<0.000**
Dose	4	198,93462	49,73365	15,27209	**<0.000**
Organ * Souche	3	93,08341	31,0278	9,52794	**<0.000**
Organ * Dose	4	43,98513	10,99628	3,37671	**<0.000**
Souche * Dose	12	129,54619	10,79552	3,31506	**0,00129**
Model	27	570,18788	21,11807	6,48489	**<0.000**
Error	52	169,33828	3,25651		
Corrected Total	79	739,52616			

This ANOVA analysis reveals that both *souche* and *dose* have strong and statistically significant effects on the response variable, either individually or through interactions. While the *organ* factor alone does not show a significant effect, its interaction with both *strain* and *dose* suggests a more complex underlying relationship. These findings highlight the necessity of considering multiple factors and their interactions in experimental designs involving biological systems.

Post-hoc analysis: Figure 02 and table 04

Main Effect of Organ: The *Pedencule* organ has a slightly higher mean diameter (7.67 mm) compared to *Gall* (7.17 mm). Although the difference is small, this may support a trend favoring *Ped* tissue under the tested conditions. However, the ANOVA previously showed this main effect was not statistically significant ($p = 0.227$), indicating this difference may be due to chance.

Main Effect of strain: There is a clear increasing in mean diameter inhibition trend from PA < ST < S < BS. Strain BS was most sensitive to the essential oil, while PA was the most resistant. This difference is highly significant ($p < 0.0001$).

Main Effect of dose: There is a strong dose-response relationship, diameter increases steadily with dose. There is a dose-dependent response, with the inhibition zone increasing as concentration increase

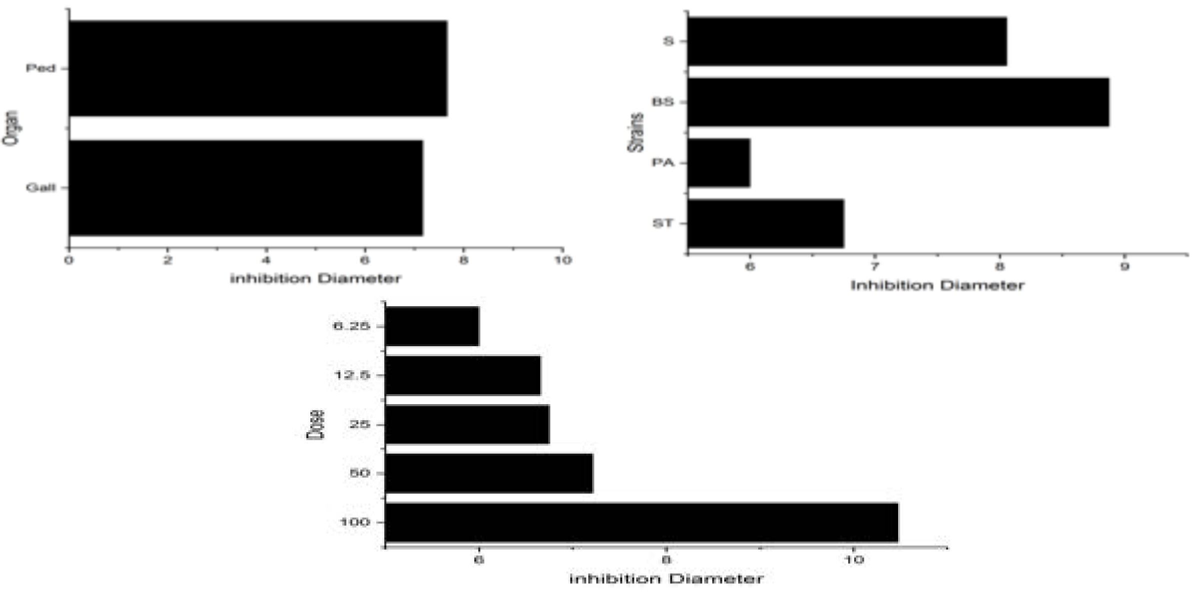

Figure 04: post hoc analysis of different factors studied

Table 04 : The grouped factors based on Tukey test comparaison.

Organ	Mean-diameter	strains	diameter	Dose	diameter
Gall	7,17425[a]	PA[a]	6	100[a]	10,4775
Ped	7,66725[a]	ST[a]	6,7505	50[b]	7,21688
		S[b]	8,057	25[b]	6,75
		BS[b]	8,8755	12,5[b]	6,65937
				6,25[b]	6

In this study, the antibacterial activity of essential oils derived from two plant organs (*Gall* vs. *Ped*) was evaluated at different concentrations against four bacterial strains (*PA, ST, S, BS*). The antibacterial potency, measured as inhibition zone diameters, exhibited notable dose- and strain-dependent effects, with *Ped* oil showing a slight but not significant improvement over *Gall*.

Dose–Response Relationship

Our data revealed a clear dose-dependent increase in inhibition zone diameter, peaking at full-strength (100%) essential oil (10.48 mm), with a progressive reduction at lower concentrations. This dose dependency mirrors findings by Brooks et al. (2021), who demonstrated that incremental EO volumes produced systematically larger zones of inhibition, with statistically significant distinctions between treatment levels. Such a concentration-effect relationship is consistent with established microbiological assays of essential oils, where higher concentrations correlate with stronger antimicrobial activity (Hajlaoui et al., 2014).

Strain-Specific Susceptibility

The study highlighted significant variability in bacterial susceptibility. Strain *BS* was most sensitive (8.88 mm), whereas *PA* was most resistant (6.00 mm). Similar findings have been catalogued in literature, such as the differential sensitivity of Gram-positive vs. Gram-negative bacteria to *Mentha longifolia* essential oil. *P. aeruginosa* exhibited larger inhibition zones (24.5 mm) than *B. subtilis* (16.0 mm) or *E. coli* (7.55 mm.These differences can be attributed to disparities in cell wall architecture, such as the outer membrane present in Gram-negative species that limits hydrophobic compound penetration (Nazzaro et al., 2013)

Organ Source of Oil

Although not statistically significant ($p = .227$), oil derived from *Ped* organ achieved marginally larger inhibition zones than that from *Gall* (7.67 mm vs. 7.17 mm). This suggests that biosynthetic differences between plant organs affecting essential oil composition may subtly influence bioactivity. Similar organ-dependent variations have been reported in essential oils extracted from different plant tissues (leaves, stems, roots), attributed to differences in compound proportions like monoterpenes, sesquiterpenes, and phenolics (Pereira et al., 2019).

Mechanisms and Chemical Composition

The antimicrobial effect of essential oils is often related to their chemical constituents particularly phenolic monoterpenoids (e.g., thymol, carvacrol, eugenol) which exert antibacterial effects by disrupting the cytoplasmic membrane and enzyme systems (Nazzaro et al., 2013). *Mentha longifolia* oil, rich in pulegone, piperitenone oxide, and caryophyllene, similarly exhibits strong antibacterial activity (Hajlaoui et al., 2014). These findings likely extend to our study, where inhibitory responses may driven by similar volatile constituents.

Broader Context and Practical Implications

Essential oils hold promise as antimicrobial agents against drug-resistant bacteria (Gadisa & Usman, 2021). Their strong bactericidal effects and synergistic actions offer advantages over conventional antibiotics, especially in topical formulations or food preservation (Brooks et al., 2021; Hajlaoui et al., 2014).

Limitations and Future Directions

- **Chemical Profiling**: Detailed GC–MS characterization of *Gall* and *Ped* oils could confirm compositional variations and correlate them with bioactivity.
- **Broader Strain Panel**: Including multidrug-resistant clinical isolates (e.g., MRSA, ESBL-producing strains) would test the oils' practical utility (Gadisa & Usman, 2021).
- **Synergistic Formulations**: Testing essential oil mixtures or combinations with conventional antimicrobials may uncover synergy or enable dose reductions (Brooks et al., 2021; Zeraib et al., 2019).

Conclusion

This preliminary study on *Pistacia atlantica* galls and peduncles underscores their rich phytochemical diversity and pharmacological potential. Key findings include:

1. **Phytochemical Profile**: Both organs contained alkaloids, flavonoids, and phenolics, but galls uniquely harbored glycosides, suggesting organ-specific metabolic pathways.
2. **Antibacterial Activity**: Essential oils exhibited concentration-dependent inhibition, with peduncle oil showing slightly higher efficacy. Strain-specific susceptibility was observed, with *Bacillus subtilis* (BS) being the most sensitive.
3. **Antiproliferative Effects**: Both oils induced dose-dependent cytotoxicity in yeast cells, with peduncle oil achieving complete cell death at 1000 ppm, likely due to higher monoterpene content.
4. **Statistical Validation**: ANOVA confirmed significant effects of bacterial strain and oil concentration, while organ type influenced activity only through interactions.

These results advocate for further exploration of *P. atlantica* extracts in antimicrobial and anticancer therapies, pending detailed chemical characterization and *in vivo* validation.

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Abstract

This study investigated the phytochemical composition and biological activities of essential oils and aqueous extracts derived from galls and peduncles of *Pistacia atlantica*. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, saponins, and steroids in both extracts, with glycosides exclusively detected in galls. Antibacterial assays demonstrated dose-dependent and strain-specific inhibitory effects, with peduncle-derived oil showing marginally higher activity. Antiproliferative assays indicated significant cytotoxicity at higher concentrations (500–1000 ppm), with peduncle oil exhibiting greater potency. The findings highlight the therapeutic potential of *P. atlantica* extracts, emphasizing the influence of plant organ, chemical composition, and concentration on bioactivity. Future research should focus on compound isolation, mechanistic studies, and clinical applications

Key words: *Pistacia atlantica,* Biological actvity, phytochemical screening

Résumé

Cette étude a examiné la composition phytochimique et les activités biologiques des huiles essentielles et des extraits aqueux dérivés des galles et des pédoncules de *Pistacia atlantica*. Le criblage phytochimique qualitatif a révélé la présence d'alcaloïdes, de flavonoïdes, de composés phénoliques, de saponines et de stéroïdes dans les deux extraits, avec des glycosides exclusivement détectés dans les galles. Les tests antibactériens ont démontré des effets inhibiteurs dépendants de la dose et spécifiques à la souche, l'huile des pédoncules présentant une activité légèrement supérieure. Les essais antiprolifératifs ont indiqué une cytotoxicité significative à des concentrations élevées (500–1000 ppm), l'huile des pédoncules étant plus puissante. Ces résultats soulignent le potentiel thérapeutique des extraits de *P. atlantica*, en mettant en évidence l'influence de l'organe végétal, de la composition chimique et de la concentration sur la bioactivité. Les recherches futures devraient se concentrer sur l'isolement des composés, les études mécanistiques et les applications cliniques.

Mots clés: *Pistacia atlantica,* Activité biologique, screening phytochimique

ملخص

هذه الدراسة بحثت في التركيب الكيميائي النباتي والأنشطة البيولوجية للزيوت الأساسية والمستخلصات المائية المستخلصة من العفص والعناقيد الزهرية لنبات *الفستق الأطلسي* *(Pistacia atlantica)*. كشف الفحص الكيميائي النباتي النوعي عن وجود قلويدات وفلافونيدات ومركبات فينولية وصابونينات وستيرويدات في كلا المستخلصين، مع وجود جليكوسيدات حصرياً في العفص. أظهرت الاختبارات المضادة للبكتيريا تأثيرات مثبطة تعتمد على الجرعة ونوع السلالة، مع تفوق طفيف لزيت العناقيد الزهرية. بينت الاختبارات المضادة للتكاثر سمية خلوية كبيرة عند التركيزات العالية (500–1000)جزء في المليون)، مع فعالية أعلى لزيت العناقيد الزهرية. تؤكد النتائج على الإمكانات العلاجية لمستخلصات *الفستق الأطلسي*، مع

التركيز على تأثير العضو النباتي والتركيب الكيميائي والتركيز على النشاط البيولوجي. يجب أن تركز الأبحاث المستقبلية على عزل المركبات والدراسات الآلية والتطبيقات السريرية

الكلمات المفتاحية: *الفستق الأطلسي*, والأنشطة البيولوجية, الفحص الكيميائي.