

Antimicrobial Activity of *Acacia tortilis* (Talha of Tindouf) against Bacteria and Fungi Isolated from Various Water Sources in the Elbayadh Region (Algeria)

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ABSTRACT

Background: In El Bayadh province, water is an essential resource for survival, prompting residents to settle in areas next to water springs that provide drinking water and support livestock. Water contamination poses a significant threat to humanity, especially in the El Bayadh region. Biological contamination is a substantial risk to water sources. Our research focused on the utilization of medicinal plants to alleviate pollution. Phytochemicals have numerous medical applications and have antibacterial and antifungal characteristics. **Materials and Methods:** This study sought to assess the antimicrobial properties of Acacia gum sourced from Tindouf (Algeria) against microbes isolated from various water sources in El Bayadh (Algeria) via physical and chemical analyses (pH and temperature were measured using a Whitman PHA 260 pH meter) and microbiological analyses (the membrane filtration method was employed to quantify and compare microbial counts). To assess the biological impact of acacia gum, we formulated five concentrations of the substance (0%, 25%, 50%, 75%, and 100%). We integrated it with forty water samples (10 from the valley, 10 from the dam, 10 from taps, and 10 from mineral sources) and subsequently performed a series of physical, chemical, and microbiological tests. **Results:** The cytotoxicity assay of acacia gum exhibited no activity at any concentration tested for cytotoxic effects (active nauplii). Regarding the direct impact on aquatic microbes, we observed a marginal reduction in microbial colonies relative to the findings from the direct analysis and the assessment performed 24 hr post-application of the plant extracts to the valley water. Acacia gum exhibited a more pronounced biological effect on bacteria and molds. Their presence in the water is due to fecal pollution. **Conclusion:** Acacia gum exhibited a more pronounced biological effect on bacteria and molds. Enterococci are microorganisms with a notable capacity for environmental adaptation. They inhabit the intestines of around 75% of humans, and their presence in water results from fecal pollution.

Keywords: Water, Acacia gum, Antibiological effect, Physico-chemical and Microbiological analysis.

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INTRODUCTION

Plants are an open treasure of diverse pharmacologically active. The *Acacia tortilis* (talha) is a secretion from trees of the acacia family, found mainly in Africa. Soluble in water, its composition gives it technological properties that have been known since

Antiquity.^{1,2} Access to clean water remains a crucial problem in Africa, particularly in rural areas, due to poor hygiene and sanitation and the lack of appropriate disinfection methods at the household level.³

Therefore, biological processes and the integration to treat drinking water could be a sustainable alternative to improve its quality due to the availability and non-toxicity of the substances.⁴

The aim of our work is to evaluate the antibiological effect of gum *Acacia tortilis* (talha) on different water sources in the Elbayadh region using the same method as that used in wastewater treatment plants.



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MATERIALS AND METHODS

Water sampling

Correct sampling is essential to obtain meaningful results. It must be considered a primary analysis phase (Table 1). It is performed under regulatory conditions of hygiene and asepsis. Sampling by ISO 5667-13:2011.

Harvesting the gum

The gum must be harvested without destroying the gum tree (*Acacia tortilis*). There are two ways to collect the gum properly: pick it up with your hands if it is close enough, or use an iron hook on the tree.

In the Tindouf region (27.7929038, -7.9774661, 79 m), the gum was gathered under aseptic conditions (this choice was based on its frequent use by the local population in traditional medicine).

Preparation of the gum solutions

We will prepare five solutions of gum with different concentrations (0%, 25%, 50%, 75% and 100%). The gum solutions were concentrated and placed in desiccators at that point of subjection to preliminary screening. A preliminary phytochemical analysis of the prepared extract was conducted to assort the presence of various classes of phytochemicals.⁵

Cytotoxicity assay

The cytotoxic activity of water extracts from both gum *Acacia tortilis* samples was determined by the brine shrimp method.⁶ After incubation, active nauplii were selected for cytotoxicity testing activity of gum acacia. Six different concentrations of the extract: 0, 25, 75 and 100 g/L were prepared using distilled water. 1 mL of each test solution was added to pre-marked test tubes containing 5 mL of seawater and ten nauplii. After 24 hr, the number of surviving nauplii in each test tube was counted using a magnifying glass and recorded.

Physicochemical analyses

Color

The sample taken in a glass vial is examined with the naked eye: If there is a deposit, floating solids, opale scence.⁷ Good quality drinking water must not present any of these characteristics (it must be colorless).

Odor and flavor

The smell is evaluated by simple olfactory sensation, while the flavor is detected by tasting, which requires rinsing the mouth with distilled water before each tasting (PR NF EN ISO 9697).

Temperature measurement

The temperature of the water plays a non-negligible role in the intensity of the sensation of the water.

pH

Take about 100 mL of water to be analyzed in a beaker, stir gently and dip the electrode in the chalice. Let it stabilize for a while with a low speed of agitation and note pH.⁸

Electrical conductivity

A beaker is filled with sufficient water for immersion of the conductivity meter electrode and the conductivity meter is tromped into the chalice. The conductivity value is noted with a unit of micro Siemens per centimetre ($\mu\text{S}/\text{cm}$).⁹

Phytochemical screening

Phytochemical screening using detections of phytochemical compounds of plant parts by color reactions and thin-layer chromatography was carried out according to the analytical techniques described in many works.¹⁰⁻¹⁷

Evaluation of antibiological effect (antibacterial and antifungal effect)

To analyze the water sample before and after treatment with different concentrations of acacia gum, we used the filtration ramp and disparate culture media according to Table 2.

Microorganisms and culture media used

With this method of membrane filtration, we performed four (Figure 1, Table 2) analyses for each water sample.

RESULTS

Cytotoxicity assay

In Table 3, our cytotoxicity experiment, Gum of Acacia did not show any activity at any concentration prepared cytotoxic activity.

Organoleptic parameters

Regarding Table 4, we noticed that the organoleptic characteristics of wadi water and dam water are similar (green to brown color, bitter taste and earthy smell). Contrary to tap water and mineral water characteristics. An insignificant difference in flavor between tap water and mineral water.

Physical parameters

T°C

From the results displayed in Figure 2, we noticed a slight variation in temperature for the four water samples, with values ranging from 18°C to 21°C and an average of 19.5°C±1.5°C. Seasonal temperatures do not exceed the estimated Algerian standards of 25°C.¹⁸

Hydrogen potential

This parameter measures the concentration of H⁺ protons contained in water. It influences most chemical and biological mechanisms in water.

The pH values of the different water samples shown in Figure 2 demonstrate that all samples have a slightly base-neutral pH (between 7 and 8) with a minimum of 7.27 for wadi water and, a maximum of 7.96 for tap water and an average of 7.60.

Electrical conductivity

Based on the results in Figure 2, the conductivity values of our samples range from 1011 μS/cm to 1520 μS/cm with an average of 1265.5 μS/cm.

Phytochemical screening

The results of the chemical screening performed on gum *Acacia tortilis* are represented in Table 5.

Microbiological analyses

Wadi water

Direct analysis: According to the results displayed in Figure 3, we noticed the presence of some microorganisms in the wadi water, such as thermotolerant coliforms with a concentration of 10⁵ CFU/250 mL, molds and a small number of *Pseudomonas* sp.

This histogram also reveals that the wadi water does not contain certain germs such as enterococci and microorganisms that are revivable at 22°C and 37°C.

Analysis after 24 hr: The results displayed in Figure 3 show an increase in the number of colonies compared to the direct analysis results, especially thermo-tolerant coliforms and molds, with a limited increase in *Pseudomonas* sp.

Analysis with gum acacia

The higher the acacia plant gum extract concentration, the fewer the microorganism colonies. We noticed a minimal decrease in microbial colonies compared to the results of direct analysis and analysis after 24 hr of wadi water, especially on Hektoen, PDA and King B culture medium.

Dam water

Direct analysis: from the results in Figure 4, we noticed that the dam water contains some microorganisms such as enterococci, molds and a reduced number of *Pseudomonas* sp with a load of 02 CFU/250 mL.

This histogram also reveals the absence of certain germs such as thermotolerant (faecal) coliforms and microorganisms revivable at 22°C and 37°C.

Analysis after 24 hr: the results displayed in Figure 4 show a limited increase in the number of colonies compared to the results of the direct analysis of the dam water.

Analysis with gum of Acacia

The higher the acacia plant gum extract concentration, the lower the number of microorganism colonies. From the results in Figure 4, we noticed an effective decrease in the number of microbial

Table 1: Samples of water.

Samples	Regions and GPS
Water of wadi	33.6945645,0.9988709,1193 m El Bayadh Algeria.
Dam water	33.1604743,1.26575,600 m Brezina, Algeria.
Tap water	33.6588799,1.0711676,298 m El Bayadh Algeria.
Mineral water	Commercial water (testimonial).

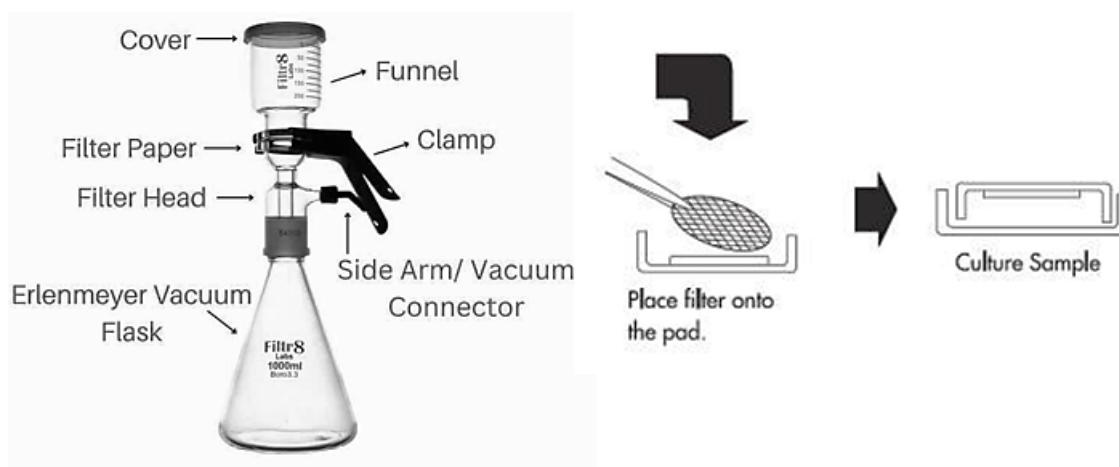


Figure 1: Diagram of the principle of the membrane culture technique.

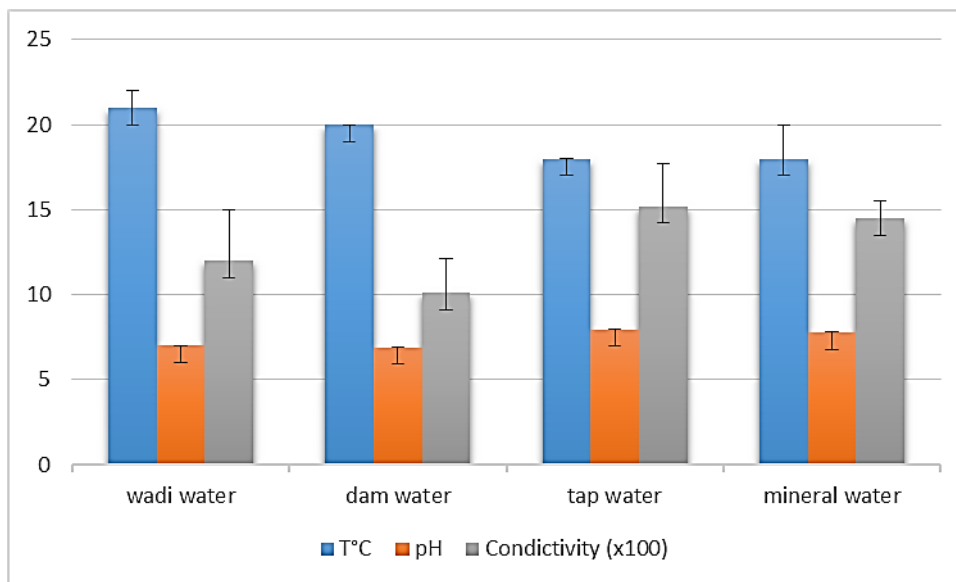


Figure 2: Histogram shows the results of the physical parameters.

Table 2: Microorganisms and culture media used.

Microorganisms sought	The volume of water filter	Culture medium	Incubation
<i>Thermotolerant coliforms</i> (faecal)	250 mL	Hectoen	48 hr at 44°C
<i>Enterococci</i>	250 mL	Slanetz and Bartley	48 hr at 37°C
<i>Pseudomonas aeruginosa</i>	250 mL	King B	48 hr at 37°C
Micro-organisms Revivifiable at 22°C	100 mL	GN	72 hr at 22°C
Micro-organisms Revivifiable at 37°C	100 mL	GN	48 hr at 37°C
Moulds and yeasts	100 mL	PDA	72 hr at 22°C

colonies compared to the results of direct analysis and analysis after 24 hr, especially on PDA and King B culture medium.

Tap water

Direct analysis: according to the results shown in Figure 5, we observed the presence of some microorganisms in the tap water that are generally bacteria (thermotolerant coliforms, *Pseudomonas* sp) and a small number of molds.

We also noticed that the tap water does not present some germs such as enterococci and microorganisms revivable at 22°C and 37°C.

Analysis after 24 hr: the results displayed in Figure 5 show an insignificant increase in enterococci number compared to the direct tap water analysis.

Analysis with *Acacia* gum: we notice the absence of enterococci, thermo-tolerant coliforms and *Pseudomonas* sp, with an intense decrease in molds.

Table 3: Cytotoxicity experiment.

Concentrations	Dead	Alive
0%	0	10
25%	0	10
50%	0	10
75%	0	10
100%	0	10

Mineral water

Direct analysis: According to the results in Figure 5, we noticed the absence of microorganisms in the mineral water except for a single bacterial colony in PDA.

Analysis after 24 hr: According to the results in Figure 5, we noticed the absence of microorganisms in the mineral water except for 5 bacterial colonies: one in the Slanetz medium and 4 in PDA.

Analysis with *Acacia* gum: We noticed the absence of microorganisms in the mineral water treated with the gum of *Acacia*.

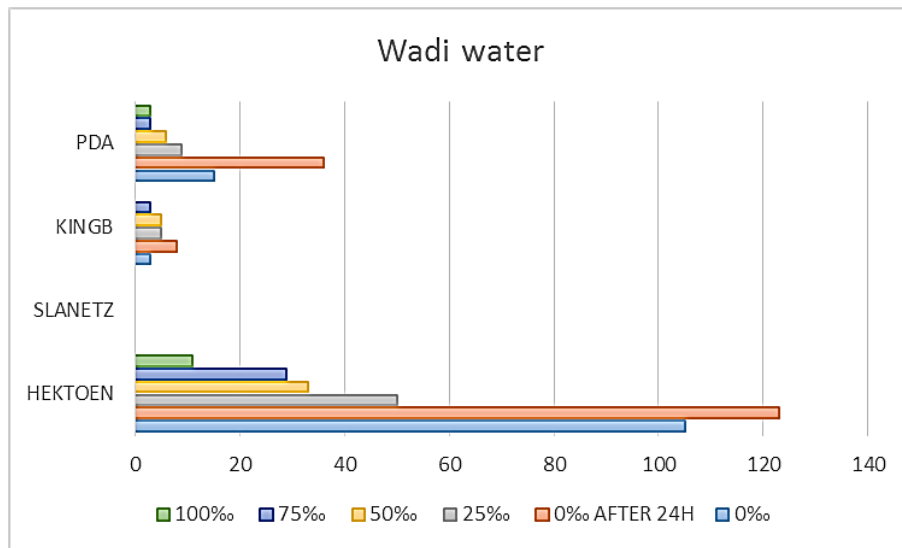


Figure 3: Microbiological analyses of Wadi water.

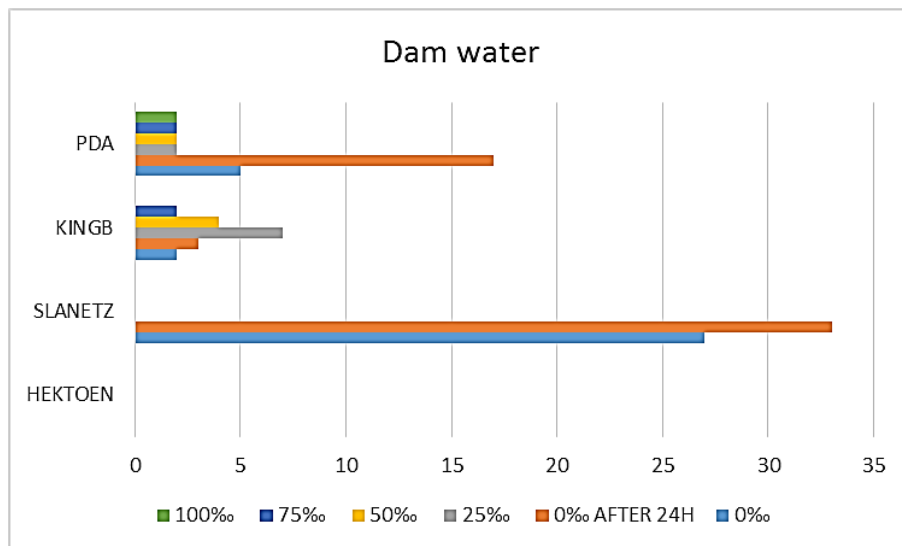


Figure 4: Microbiological analyses of Dam water.

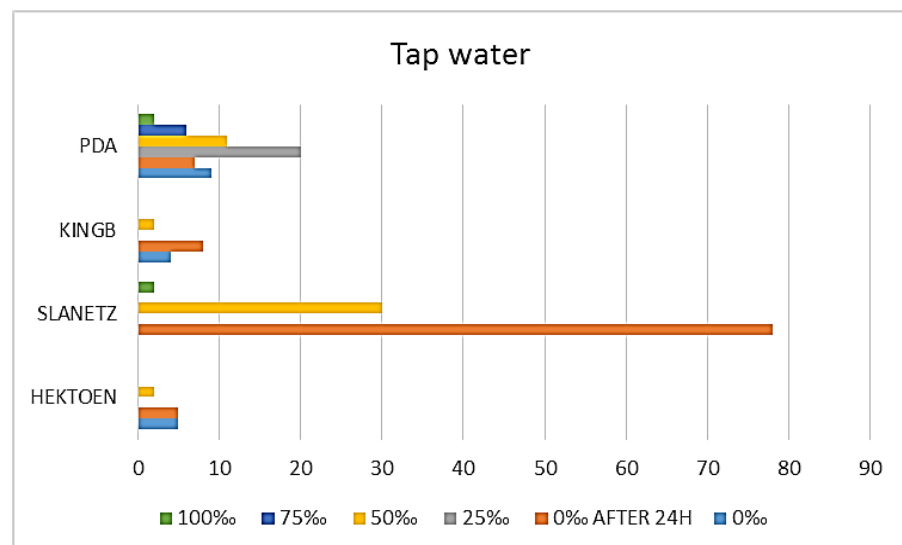


Figure 5: Microbiological analyses of Tap water.

Table 4: Organoleptic parameters.

Samples	Color	Odor	Flavor
Water of wadi	Green to brown	- Earthy - Slushy -Not very unpleasant.	Not very bitter and not very unpleasant.
Dam water	Green to brown	- Earthy -Not very unpleasant.	Not very bitter and not very unpleasant.
Tap water	Limpid	Odourless	has no taste.
Mineral water	Limpid	Odourless.	has no taste.

DISCUSSION

The organoleptic factors (color, odor and flavor) are often the warning factors for pollution without presenting a risk to health.¹⁹

The results attributed to the organoleptic quality indicate that the water of Oued El-Bayadh and Brézina dam do not conform to the Algerian discharge standards.²⁰ This fetid smell is due to the existence of either chemical products or decomposed matter (organic or inorganic) or bacteria that release H₂S.²¹ The increase in temperature favours the development of microorganisms, thus consuming oxygen and consequently reducing the dissolved oxygen content.^{16,22} The pH values of all our samples are slightly base-neutral and conform to the Algerian standard recommended by Jora,²⁵ which is 6.5≤pH≤8.5. The decrease in pH (wadi water) may result from bacterial activity and decomposition of organic matter.^{23,24}

Electrical conductivity assesses the number of dissolved salts in water.²¹ The value must remain below the standard set by Jora,²⁵ which is 2800 μS/cm. Therefore, the conductivity of our samples is within the standards.

According to the results of microbiological analysis, the number of microorganisms in Wadi water is more than in other waters. After adding gum acacia to different concentrations, we observe that germs decrease progressively according to the gum acacia concentration.

The data obtained from phytochemical screening by a methodology are based on the detection of compounds through protocols oriented according to the results obtained from the biological screening. They are often consistent with those in the literature.²⁵⁻²⁸

As shown in Table 2, all concentration prepared cytotoxic activity showed no moderate toxicity to active nauplii. These results confirmed in the work of Weli⁶ on the effect of different polarities leaves crude extracts of *Omani juniperus* excels in antioxidant, antimicrobial and cytotoxic activities and their biochemical screening.²⁹

Gum of Acacia has shown a biologically more active effect on bacteria and molds.³⁰ Therefore, the compounds responsible for these biological properties could be used as active ingredients of

Table 5: Chemical screening of gum *A. tortilis*.

Phytochemical compounds	Gum of <i>A. tortilis</i>
Alkaloids	+
Flavonoïds	++
Saponosides	+++
Coumarins	+
Free Quinones	-
Tannins	+

- : Total absence or not detected; + : Presence in trace amounts. ++ : Presence in abundant quantity; +++: Presence in very abundant quantity.

natural preservative formulation in the food and bio-pesticide industries.³¹⁻³³

Enterococci are microorganisms that have a remarkable ability to adapt to their environment. They are present in the intestines of about 75% of humans³⁴ and their presence in water is due to contamination of fecal origin.³⁵

CONCLUSION

Acacia tortilis gum from Tindouf (Algeria) has low antimicrobial activity against waterborne microbes, due to its antimicrobial plant compounds. This is likely due to its lower phytochemical composition compared to *Acacia senegal* and *Acacia seyal* (gum arabic).

ACKNOWLEDGEMENT

The mode and mechanism of action of the active phytochemical constituents in gum still need to be fully understood and research is ongoing. Some authors have proposed several hypotheses based on their observations: it could be an action on energy metabolism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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