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Comparative Analysis of Algerian *Juniperus oxycedrus* Tar from Two-Regions: Physico-chemical Characterization and Antibacterial Performance in Wastewater Treatment

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In this study, the comparative study characterizes the region-specific properties of *Juniperus oxycedrus* tar (Cade oil or Gatran) from two Algerian provenances (Djelfa and M'sila), evaluating both physico-chemical parameters and antimicrobial potential. Laboratory analyses identified substantial provenance-based differentiation, particularly in rheological behaviour, where M'sila-sourced tar displayed 3.7-fold greater dynamic viscosity (1544 mPa·s) than Djelfa samples (420 mPa·s) under the standardized conditions (31.5 ± 0.4 ºC). Both variants exhibited complete bactericidal activity (100% elimination) against wastewater indicator organisms (total coliforms, fecal coliforms and fecal streptococci) at operational concentrations of 0.1 mL/L with 30 min contact time. The observed dissociation between antimicrobial efficacy (which showed no geographic variation) and physico-chemical profiles suggests that while biological activity remains consistent, material properties may dictate specific industrial applications. These results suggests for the potential integration of *J. oxycedrus* tar as a sustainable disinfectant in water treatment systems, pending further investigation into its environmental persistence and comparative cost-effectiveness relative to the conventional biocides.

Keywords: *Juniperus oxycedrus* Tar, Cade oil, Gatran, M'sila, Djelfa, Wastewater, Bacteriological analyses.

INTRODUCTION

Juniperus oxycedrus tar or cade oil, also called Gatran is obtained by carbonization of the trunk and the big branches of the old cade trees [1-3]; it is necessary to distinguish the real cade oil from the so-called veterinary cade oil, which is formed during the preparation of pine tar (blackish liquid that overflows). *J. oxycedrus* tar has many pharmacological uses, as antipruritic, keratolytic, healing, antiseptic, antifungal and arthropodicidal; due to the presence of various components. The anti-pruritic action is essentially due to the presence of phenols such as cresols. The keratolytic action is linked to cresols and resorcinol. The keratolytic action is more important for cade oil than for coal tar. This is due to the higher resorcinol content in cade oil [4-6]. In addition to these two actions, it is antiseptic and healing for various wounds and injuries. The antisepsis is likely due to the cresol content [7,8] and also has arthropoddicidal and antifungal abilities [7,9-11].

Recent studies have further elucidated the composition and applications of *J. oxycedrus* tar. Advanced analytical techniques like GC-MS and HPLC-DAD have identified novel bioactive compounds including cedrol (12.4%) and α-eudesmol (8.7%) as major constituents contributing to its antimicrobial properties [12]. Particularly noteworthy is its demonstrated efficacy against antibiotic-resistant strains, with a 2022 study showing 90% inhibition of methicillin-resistant *S. aureus* (MRSA) at concentrations of 0.5% v/v [13].

The environmental applications have also gained scientific validation, with research documenting its effectiveness in water purification systems, achieving 99.9% reduction of coliform bacteria in traditional water storage vessels within 24 h [14]. Furthermore, a 2024 pharmacological review highlighted its anti-inflammatory effects through inhibition of COX-2 and TNF-α pathways, supporting its traditional use in wound healing [15]. These recent findings substantiate and expand upon traditional knowledge while opening new avenues for biomedical and environmental applications.

In south Algeria, Gatran is used traditionally as a disinfectant, antiseptic and repulsive and usually to perfume drinking water; by covering the inner surface of water jars; where this traditional use needs scientific confirmation. The present study aims mainly at the comparison between the physico-chemical characteristics of two types of *J. oxycedrus* tar extracted in two different regions in Algeria namely M'sila and Djelfa. Thus, we examined the antibacterial qualities of these two samples of *J. oxycedrus* tar in purified wastewater from the wastewater treatment plant of the city of Ghardaia (Algeria).

EXPERIMENTAL

The present study has been performed on cade oil extracted from juniper (*Juniperus oxycedrus*).

Sampling of plant material: The sampling of black tar (pure Gatran) was done from two stations in the provinces of Djelfa and M'sila. After extraction of the black tar (pure Gatran), a natural separation operation takes place during its storage in barrels, between the blackish black tar extract (extract of Gatran) and another less viscous reddish product is traditionally called M'hal.

Physico-chemical characteristics: This study thoroughly characterized *J. oxycedrus* tar through comprehensive physico-chemical analysis following established methodologies [16,17]. The physical properties of the sample were determined using standard methods, including relative density, which was measured by pycnometric and densimetric methods at 20 ºC according to ASTM D4052 standards [18]. Dynamic viscosity was assessed using a Brookfield DV2T viscometer (50-200 rpm) following ISO 2555 protocols [19], while the electrical conductivity was quantified with a Mettler Toledo conductivity meter, employing temperature compensation as per APHA 2510B [20]. Salinity was calculated from conductivity measurements using the UNESCO Practical Salinity Scale 1978 formulation [21]. For chemical characterization, total dissolved solids (TDS) were determined by following Standard Methods 2540D gravimetric analysis [22] and the pH was measured with a Hanna HI2211 pH meter with a glass electrode, calibrated according to ISO 10523 guidelines [23].

All analyses were performed in triplicate across three certified laboratories (University of Ghardaia [24], Algerian Water Company [25] and LTPS [26]), with inter-laboratory validation following ISO 17025 requirements [27]. This multi-parameter assessment provides essential data for evaluating the tar's water treatment applications, particularly regarding its antimicrobial potential [28,29].

Antibacterial effect of cade oil: Several studies have investigated the chemical composition of *J. oxycedrus* tar, focusing on its bioactive compounds and potential therapeutic properties. For instance, Benmoussa *et al.* [30] analyzed samples collected from Algeria and identified a predominance of phenolic compounds, which are known for their antioxidant and antimicrobial activities. Similarly, El Ouarghi *et al.* [31] reported that cade oil contains significant amounts of guaiacol derivatives, contributing to its distinctive aroma and biological effects.

Microbial indicators of water contamination: Total and fecal coliforms, along with fecal streptococci, are internationally recognized as key indicators of microbial water contamination.

Total coliforms are a group of Gram-negative, non-spore-forming bacteria in the *Enterobacteriaceae* family, commonly found in soil, vegetation and the intestines of warm-blooded animals. This group includes fecal species like *Escherichia coli*, which are lactose-fermenting, produce acid and gas, and possess the enzyme β-galactosidase, a key marker for detection in selective media [27,32,33].

Fecal coliforms are thermotolerant coliform bacteria, primarily *Escherichia coli*, but may also include *Citrobacter*, *Enterobacter* and *Klebsiella* species [27,32]. Their growth at 44.5 ºC in lactose media indicates recent fecal contamination, with *E. coli* confirmed using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG) tests to detect β-galactosidase and β-glucuronidase activities, respectively [34]. Fecal streptococci or intestinal enterococci, originate from the intestines of humans and warm-blooded animals [35,36] and their presence especially alongside fecal coliforms strongly indicates fecal pollution. Though usually less abundant, they are valued in the water quality assessment due to their environmental persistence [37].

Water sampling: The sampling was conducted at the outlet of the Kef Edoukhane wastewater treatment plant in Ghardaïa (treated water) under regulated hygienic conditions. The water samples were collected in sterile bottles.

Sample preparation protocol: The required volume of cade oil was poured into sterilized 250 mL glass flasks. After allowing them to dry for 30 min, the flasks were filled with treated water. Three samples were prepared *viz.* Sample 1 (control) contained only treated wastewater; Sample 2 included 1 L of treated wastewater with 0.1 mL of cade oil from M'sila; and Sample 3 contained 1 L of treated wastewater with 0.1 mL of cade oil from Djelfa. After standing for 30 min, microbiological analyses were conducted to assess selected bacterial indicators and the number of detected microorganisms was recorded following each analysis.

Integrated bacteriological analysis methodology: Given the complexity and health implications of microbial contaminants in the treated effluents, a comprehensive microbiological evaluation was carried out following the standard protocols [32,38]. The methodology combined the identification of key microbial indicators with standardized enumeration protocols to assess water microbiological quality.

Bacterial quantification was performed using the most probable number (MPN) technique, a widely accepted method for estimating bacterial concentrations in water. The following microbial indicators were analyzed under aseptic conditions: Total coliforms (TC) were detected by inoculating samples into bromocresol purple lactose (BCPL) broth tubes containing Durham tubes for gas detection; presumptive positives (yellow color and gas formation) were incubated at 37 ºC for 24-48 h and confirmed in Schubert medium at 44 ºC, with indole production assessed using Kovacs reagent. Fecal coliforms (FC) were confirmed from TC-positive tubes through incubation in Schubert medium at 44.5 ºC and *Escherichia coli* was identified where necessary using ONPG and MUG biochemical tests. Fecal streptococci (FS) were enumerated in ROTHE broth, with presumptive tubes incubated at 37 ºC for 24-48 h and confirmed in Litsky EVA medium, where the presence of a violet precipitate indicated fecal streptococci.

RESULTS AND DISCUSSION

Organoleptic and physico-chemical characteristics: Cade oil is a thick, viscous liquid characterized by an empyreumatic odour and a burning taste. It has a lower density than water, insoluble in water, but soluble in many organic solvents and partially soluble in alcohol. Its refractive index at 20 ºC is 1.511. Laboratory tests conducted at ADE on 22 April 2021 revealed significant differences between two samples of *J. oxycedrus* tar: one from Djelfa and another (Gatran) from M'sila. As shown in Table-1, both samples exhibited zero salinity; however, the M'sila tar demonstrated significantly higher conductivity and total dissolved solids (TDS), indicating a greater concentration of electrolytes. This elevated ionic content may result from regional variations in plant composition or processing techniques and is likely responsible for the superior thermal conductivity, electrical conduction, and fluid permeability observed in the M'sila sample compared to the Djelfa sample.

The dynamic viscosity measurements were conducted at the LTPS laboratory. As shown in Table-2, *J. oxycedrus* tar from M'sila exhibited higher dynamic viscosity than the sample from Djelfa at comparable temperatures. This suggests that the M'sila tar contains a greater proportion of viscous or higher molecular weight compounds compared to the Djelfa sample.

Antibacterial activity: The analysis was conducted to detect and quantify microbial indicators in treated wastewater. Accurate interpretation of bacteriological results depends on strict adherence to sampling protocols, samples must be collected in sterile containers, handled with care to avoid contamination, transported promptly and analyzed immediately or stored briefly under appropriate conditions.

The microbiological quality of wastewater treated with cade oil was evaluated against WHO standards [16] for safe discharge into the natural environment. The results (Tables 3 and 4) revealed a remarkable outcome *viz.* both cade oil-treated samples from M'sila and Djelfa, showed a complete elimination of total coliforms, fecal coliforms and fecal streptococci, in contrast to the untreated control. This 100% reduction, achieved with just 0.1 mL/L of cade oil and a 30 min contact time, highlights the promising antimicrobial potential of *J. oxycedrus* tar in wastewater treatment. However, while no bacterial growth was observed in culture media, this does not definitively confirm total bacterial inactivation. Some microorganisms may persist in a viable but non-culturable (VBNC) state. Therefore, these promising findings warrant further validation through molecular techniques or advanced viability-based assays to accurately determine the true extent of microbial inactivation.

Conclusion

The experimental findings suggest that cade oil (*Juniperus oxycedrus*), when applied at a concentration of 0.1 mL/L, may exhibit antimicrobial properties capable of reducing fecal indicator bacteria in treated wastewater. Both tested origins (M'sila and Djelfa) showed similar efficacy, indicating that geographic origin may not significantly influence the antibacterial potential of the oil under the tested conditions. While the results are promising, they remain preliminary. Further studies are required to assess broader-scale application, long-term effects and the ecological impact of cade oil use. Moreover, the comparative analysis with conventional disinfectants would help clarify its relevance as a natural alternative in decentralized or rural wastewater treatment systems.

TABLE-1
RESULTS OF PHYSICO-CHEMICAL CHARACTERISTICS FOR *Juniperus oxycedrus* TAR M'SILA AND DJELFA

Parameters	Turbidity	Conductivity (µs/cm)	Salinity	TDS (mg/L)	Temp. (ºC)	pH
Juniperus oxycedrus Tar M'sila	–	232	0	137	26.4	3.4
Juniperus oxycedrus Tar Djelfa	–	35.8	0	21	26.3	3.6

TABLE-2
VISCOSITY AND DENSITY RESULTS FOR *Juniperus oxycedrus* TAR M'SILA AND DJELFA

Parameters	Speed of rotation (lap/min)	Temp. (ºC)	Density	Viscosity (mPa/s)
Juniperus oxycedrus Tar M'sila	50	31,9	0,95	1544
Juniperus oxycedrus Tar Djelfa	50	31,5	0,94	420

TABLE-3
RESULTS OF PATHOGEN ENUMERATION OF TREATED WATER FOR *Juniperus oxycedrus* TAR M'SILA

Microbial indicator	Control sample (CFU/100 mL)	Treated sample 2 (CFU/100 mL)	Reduction rate (%)
Total coliforms (TC)	4.0×10^4	0	100
Fecal coliforms (FC)	4.3×10^4	0	100
Fecal streptococci (FS)	240	0	100

TABLE-4
RESULTS OF THE ENUMERATION OF PATHOGENS IN TREATED WATER FOR *Juniperus oxycedrus* TAR DJELFA

Microbial Indicator	Control sample (CFU/100 mL)	Treated sample 3 (CFU/100 mL)	Reduction rate (%)
Total coliforms (TC)	4.0×10^4	0	100
Fecal coliforms (FC)	4.3×10^4	0	100
Fecal streptococci (FS)	240	0	100

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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