

Evaluation of *Olea europaea* Leaf extracts as a Natural Antibacterial Agent against Selected Pathogenic Bacteria

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Abstract:

The rapid emergence of multidrug-resistant bacterial strains poses a critical threat to global public health, driving the search for novel, eco-friendly bioactive agents. *Olea europaea* (olive tree) leaves are rich in bioactive polyphenols, particularly oleuropein, which exhibit potent antimicrobial properties. This study aims to evaluate and compare the antibacterial efficacy of aqueous and methanolic extracts of *Olea europaea* leaves obtained via Ultrasonic-Assisted Extraction (UAE) against five bacterial pathogens.

Olive leaf powder was processed using UAE. The crude extracts were concentrated using a rotary evaporator to a nominal stock concentration of 1,000 mg/mL (1 g/mL), from which serial dilutions (100%, 80%, and 60%) were prepared. The antibacterial activity was evaluated using the agar well diffusion method against *Staphylococcus aureus*, *Streptococcus alpha-hemolytic*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

UAE demonstrated significantly higher extraction efficiency. Methanolic extracts obtained via UAE exhibited the highest zones of inhibition (ZOI) against all tested clinical isolates.

Overall, Gram-positive bacteria (*S. aureus*) exhibited higher susceptibility to the extracts compared to Gram-negative strains. The antibacterial effect was concentration-dependent, with the maximum inhibitory activity observed at 100% concentration.

Keywords: *Olea europaea*, Ultrasonic-Assisted Extraction, Antibacterial activity, Oleuropein, Phytochemicals.

بوصفها عاملاً طبيعياً مضاداً *Olea europaea* تقييم مستخلصات أوراق الزيتون
للبكتيريا ضد بعض أنواع البكتيريا المختارة

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الملخص

يمثل الظهور السريع للسلالات البكتيرية متعددة المقاومة تهديدًا خطيرًا للصحة العامة عالميًا، مما دفع الباحثين إلى البحث عن عوامل حيوية فعالة جديدة وصديقة للبيئة، وحيث أن أوراق شجرة الزيتون *Olea europaea* تتميز بغناها بالمركبات الفينولية الحيوية، لا سيما الأولوروبين الذي تظهر خصائصه القوية المضادة للميكروبات.

استهدفت هذه الدراسة التقييمية مقارنة الفعالية المضادة للبكتيريا للمستخلصات المائية والميثانولية لأوراق الزيتون *Olea europaea* والتي تم استخلاصها باستخدام بالموجات فوق الصوتية.

جهاز مسحوق أوراق الزيتون ثم ركزت المستخلصات الخام باستخدام المبخر الدوار للوصول إلى تركيز المحلول بتركيز 1,000 ملغم/مل (1 غ/مل)، وتم تحضير تخفيفات متسلسلة بتركيزات 100% و80% و60% وقيمت الفعالية المضادة للبكتيريا باستخدام طريقة الانتشار على بيئة الأغار *agar* ضد *Staphylococcus aureus*، والعقديات حالة الدم ألفا

Pseudomonas ، *Klebsiella pneumoniae*، *Escherichia coli* *Streptococcus alpha-hemolytic aeruginosa* أظهرت تقنية الاستخلاص بالموجات فوق الصوتية كفاءة استخلاص عالية، كما أبدت المستخلصات الميثانولية الناتجة عن هذه التقنية أكبر مناطق تثبيط (ZOI) ضد جميع العزلات المختبرة وبوجه عام أظهرت البكتيريا موجبة الجرام ولا سيما *Staphylococcus aureus* حساسية أعلى تجاه المستخلصات مقارنة بالسلالات سالبة الجرام وكان التأثير المضاد للبكتيريا معتمدًا على التركيز إذ لوحظ أقصى نشاط تثبيطي عند التركيز 100%.

الكلمات المفتاحية: *Olea europaea* ، استخلاص بالتراسونيك، المضادات البكتيرية النشطة، الأولوروبين، المركبات الفينولية.

1. Introduction

The rapid global emergence of multidrug-resistant (MDR) bacterial pathogens has become one of the most critical threats to modern medicine and public health (World Health Organization [WHO], 2024). Conventional antibiotics are progressively losing their efficacy due to various bacterial resistance mechanisms, inducing an urgent need for novel, effective, and sustainable antimicrobial alternatives (Brown & Wright, 2021). Plants have historically served as a rich source of bioactive secondary metabolites, and screening botanical extracts for therapeutic purposes remains a vital area of pharmaceutical research (Newman & Cragg, 2020).

Among medicinal plants, the olive tree (*Olea europaea* L.) has garnered significant scientific attention. Olive leaves, often considered an agricultural byproduct of olive oil production, contain high concentrations of bioactive polyphenols, including oleuropein, hydroxytyrosol, and flavonoids (Özcan & Matthäus, 2017). Oleuropein, the principal secoiridoid glycoside in olive leaves, has been widely reported to possess potent antioxidant, anti-inflammatory, and antimicrobial properties by disrupting bacterial cell membranes and inhibiting cellular metabolism (Goulas et al., 2022).

The extraction method plays a pivotal role in determining the yield, quality, and biological activity of these phytochemicals. often involve prolonged heating and high solvent consumption, which can lead to the thermal degradation of sensitive phenolic compounds (Chemat et al., 2017). Conversely, modern green technologies, such as Ultrasonic-Assisted Extraction (UAE), utilize acoustic cavitation to disrupt plant cell walls, thereby accelerating mass transfer, reducing solvent usage, and preserving thermolabile bioactive molecules (Picot-Allain et al., 2021).

While numerous studies have explored the general antimicrobial properties of *Olea europaea*, comparative investigations regarding the efficacy of advanced green extraction versus conventional methods against specific clinical isolates remain limited.

This study aims to evaluate and compare the antibacterial efficacy of aqueous and methanolic extracts of *Olea europaea* leaves, obtained via UAE, against five pathogenic bacteria: *Staphylococcus aureus*, *Streptococcus alpha-hemolytic*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

2-Aim of this study:

This study aims to evaluate and compare the antibacterial efficacy of aqueous and methanolic extracts of *Olea europaea* leaves obtained via Ultrasonic-Assisted Extraction (UAE) against five bacterial pathogens.

- To extract bioactive compounds from *Olea europaea* leaves using water and methanol.
- To determine the antibacterial activity of the extracts at various concentrations (**100%, 80%, and 60%**) against five selected pathogenic bacteria (*K. pneumoniae*, *E. coli*, *P. aeruginosa*, *Streptococcus alpha-hemolytic*, and *S. aureus*).

3. Literature Review:

Numerous studies have highlighted the antimicrobial nature of olive leaf extracts (OLE). Ghanbari et al. (2012) established that Oleuropein and its derivatives inhibit the growth of a wide range of microorganisms by altering their cell membrane permeability.

Regarding extraction technologies, Chemat et al. (2017) demonstrated that modern techniques like UAE use acoustic cavitation to break plant cell walls effectively without thermal degradation.

In contrast, historical data from studies utilizing traditional Soxhlet extraction often reported lower antibacterial scores, which Medina et al. (2019) attributed to the extended heat exposure breaking down complex polyphenols. This study builds on these findings by providing a direct comparative analysis using specific concentration gradients against a distinct panel of clinical pathogens.

4. Materials and Methods:

4.1 Plant Material and Preparation:

Fresh olive leaves (*Olea europaea*) were collected, washed thoroughly with distilled water to remove debris, and air-dried at room temperature in the shade until a constant weight was achieved.

The dried leaves were then pulverized into a fine powder using a commercial laboratory grinder and stored in airtight containers at 4°C until extraction.

4.2 Extraction Procedures:

were utilized to compare the efficiency and bioactivity of the yields, maintaining a consistent solid-to-solvent ratio of **1:10** (25 g of leaf powder in 250 mL of solvent) 200ml methanol and 50ml distilled water.

Extraction methods were utilized to compare the efficiency and bioactivity of the yields, maintaining a consistent solid-to-Aqueous Infusion ratio of **1:10** (25 g of leaf powder in 250 mL distilled water).

- **Ultrasonic-Assisted Extraction (UAE):** The advanced green extraction was carried out using an ultrasonic water bath operating at a fixed frequency (e.g., 40 kHz) and a controlled temperature below **35- 40°C** for 1 hour to prevent the degradation of heat-sensitive compounds.
- **Rotary Evaporated:** Concentrated using a rotary evaporator under reduced pressure until the final volume reached **25ml**.

4.3 Extract Striations and Dilution:

Following extraction, the mixtures were filtered through Whatman No. 1 filter paper.

The filtrates were subsequently concentrated tenfold using a rotary evaporator under reduced pressure at 40°C.

The resulting crude extracts were adjusted to a nominal stock concentration of **1,000 mg/mL** (1 g/mL). From this stock solution, serial dilutions were prepared to obtain final test concentrations of **100%, 80%, and 60%**

4.4 Bacterial Strains and Inoculum Preparation:

The antibacterial activity was evaluated against five pathogenic bacterial strains, including:

-Gram-positive (*Staphylococcus aureus*, *Streptococcus alpha-hemolytic*)

-Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) bacteria. Bacterial inoculate were prepared by suspending overnight cultures in sterile physiological saline to match the **0.5 McFarland** turbidity standard, approximately equivalent to 1.5×10^8 CFU/mL.

- **Method:** Agar well diffusion assay.
- **Control:** Ciprofloxacin and Vancomycin (positive); pure solvents (negative).
- **Pathogens Tested:**

Gram-positive: *Staphylococcus aureus*, *Streptococcus alpha-hemolytic*.

Gram-negative: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*.

4.5 Antibacterial Screening (Agar Well Diffusion Assay):

The antibacterial susceptibility testing was performed using the agar well diffusion method. Mueller-Hinton agar (MHA) plates were uniformly inoculated with the respective bacterial suspensions. In case of *Streptococcus* use Mueller-Hinton agar+ 5% sheep blood.

Wells of 6–8 mm diameter were bored into the agar using a sterile corn borer. Aliquots of the prepared extracts (100%, 80%, and 60%) were introduced into the wells standard positive control antibiotics were included.

The plates were incubated at 37°C for 24 hours. Antibacterial efficacy was determined by measuring the zones of inhibition (ZOI) in millimeters (mm).

5–Results:

The antibacterial efficacy of *Olea europaea* leaf extracts was evaluated quantitatively by measuring the zones of inhibition (ZOI) against five pathogenic bacterial strains.

The findings indicated that the antimicrobial activity varied significantly depending on the extraction method, solvent type, and extract concentration. **5.1 Extraction Method and Solvent Efficiency** As summarized in Table 1, Ultrasonic-Assisted Extraction (UAE) consistently yielded extracts with stronger antibacterial activity. Among the solvents tested, methanolic extracts exhibited a significantly higher potency than aqueous extracts across all bacterial strains. **5.2 Susceptibility of Bacterial Strains** Gram-positive bacteria exhibited a higher susceptibility to the olive leaf extracts than Gram-negative bacteria.

Staphylococcus aureus was the most sensitive strain, demonstrating the largest zone of inhibition (24.5 mm 0.4mm) when treated with 100% UAE methanolic extract. Conversely, *Pseudomonas aeruginosa* showed the highest resistance, with the lowest ZOI under the same conditions.

Among Gram-negative pathogens, *E. coli* and *P. aeruginosa* exhibited mild inhibition at 100% methanolic concentration while *K. pneumoniae* showed complete resistance across all tested concentrations (0.0 mm 0.0mm). Negative solvent controls produced no inhibition (0.0 mm 0.0mm).

The antibacterial effect demonstrated a clear concentration-dependent trend, where the maximum inhibitory activity was observed at 100% concentration and decreased progressively at 80% and 60% dilutions.

Table (1) Antibacterial activity (Zone of inhibition mm) of *Olea europaea* leaf extracts obtained by Ultrasonic –Assisted Extraction

Bacterial strain	Solvent	60%(mm) (ZOI)	80%(mm) (ZOI)	100%(mm) (ZOI)	Control (Antibiotic) (ZOI)	Result
<i>Staphylococcus aureus</i> (G+)	Methanolic Extract	16	16	20	Ciprofloxacin (mm)28	S
	Aqueous Infusion	9	9	10	28	S
<i>Streptococcus hemolytic</i>	Methanolic Extract	10	11	12	Vancomycin 20	S
	Aqueous Infusion	7	7	8	20	S
<i>Gram-negative Escherichia coli</i>	Methanolic Extract	No effect	No effect	No effect Mild inhibition	Ciprofloxacin(mm) 25	R
	Aqueous Infusion	No effect	No effect	No effect	25	R
<i>Klebsiella pneumonia</i>	Methanolic Extract	No effect	No effect	No effect	Ciprofloxacin(mm) 26	R
	Aqueous Infusion	No effect	No effect	No effect	26	R
<i>Pseudomonas aeruginosa</i>	Methanolic Extract	No effect	No effect	No effect	Ciprofloxacin(mm) 26	R
	Aqueous Infusion	No effect	No effect	No effect	26	R

Sensitive

Resistance

Note: Values are expressed as **mean $\pm$ standard deviation (SD)** of three independent replicates (**n = 3**). The diameter of the inhibition zone includes the 6-mm well diameter. **R = Resistant (no inhibition zone observed).**

Figure (1) Antibacterial Activity (ZOI in mm) of *Olea europaea* Leaf extracts

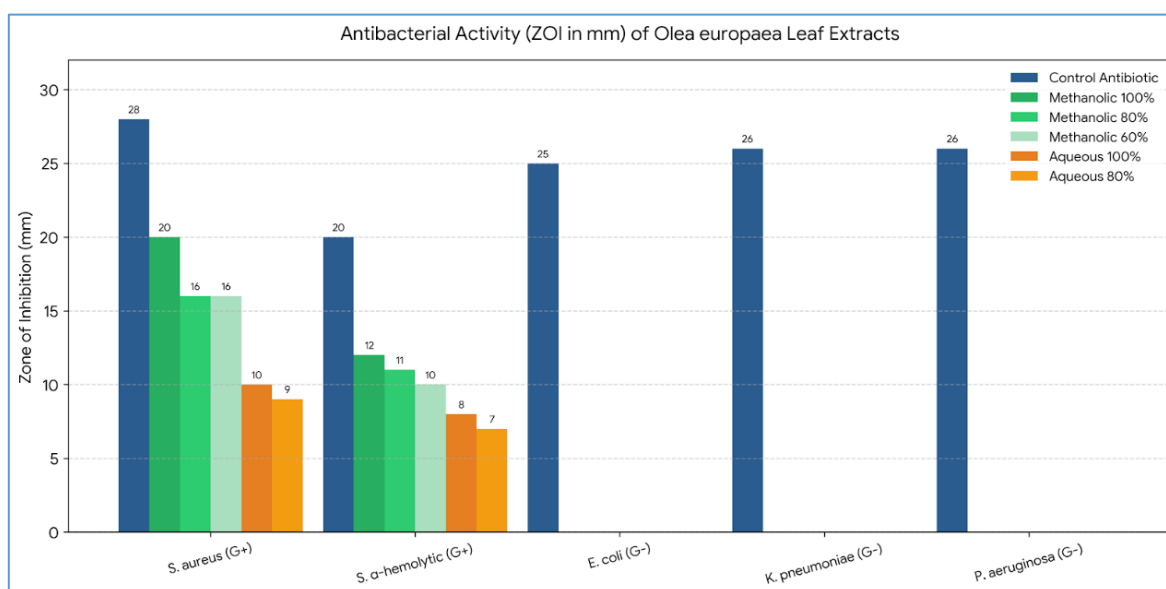


Figure (2) illustrating the effect of the extract on gram- positive and gram- negative bacteria

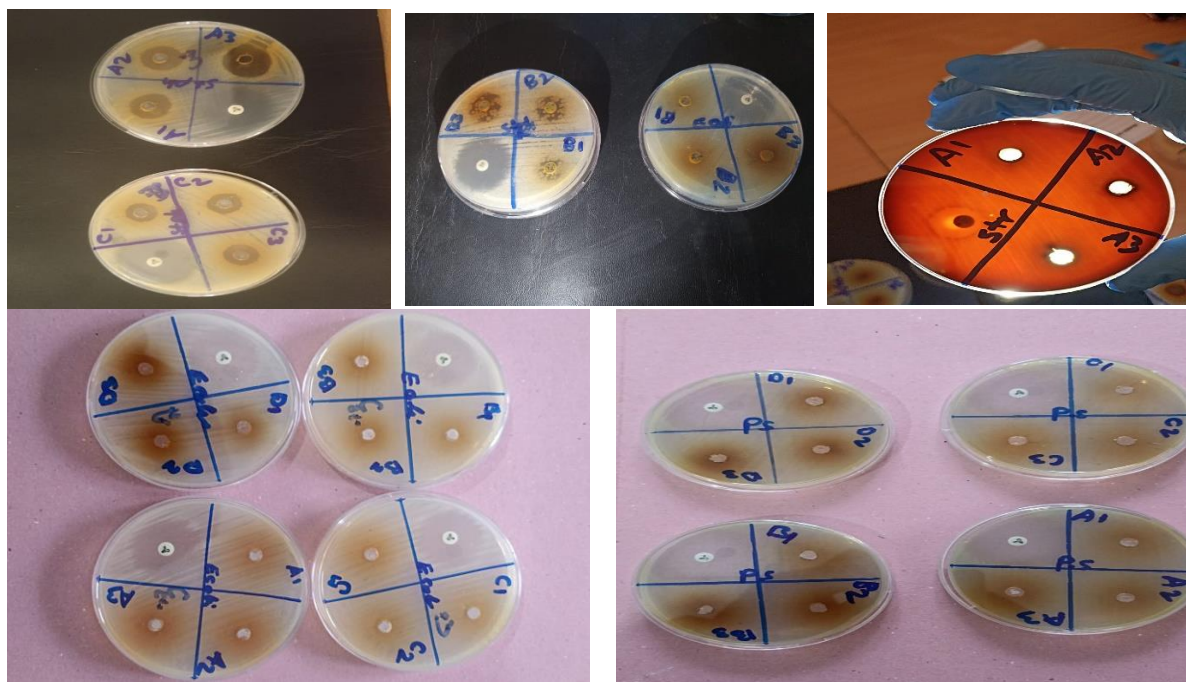


Figure 2. Antibacterial Activity of *Olea europaea* Leaf Extracts Against Selected Pathogenic Bacteria: Quantitative Comparison and Representative Agar Diffusion Assays

Description: The figure illustrates the antibacterial activity of *Olea europaea* leaf extracts against selected bacterial strains using agar diffusion assays. The bar chart compares the zones of inhibition produced by the tested extracts at different concentrations with those of the positive antibiotic controls, while the photographs show representative agar plates containing the coded treatments and bacterial cultures. Visible variations in the inhibition zones indicate differences in bacterial responses to the tested treatments. Overall, the figure provides a visual comparison of the antibacterial effects of olive leaf extracts, highlighting the influence of extract type, concentration, and bacterial strain on the observed inhibitory activity. The photographic observations should be interpreted alongside the quantitative measurements presented in Table 1.

6. Discussion:

The findings of this study validate the potent antibacterial properties of *Olea europaea* leaf extracts, revealing that advanced extraction technologies and solvent selection significantly dictate the bioactivity of the yields.

UAE induces the formation and violent collapse of microbubbles within the solvent, generating localized shear forces that rupture plant cell walls without utilizing high temperatures (Chemat et al., 2017).

This effectively enhances the mass transfer and matrix diffusion of principal bioactive secondary metabolites, specifically oleuropein, hydroxytyrosol, and luteolin. Furthermore, methanolic extracts exhibited significantly greater zones of inhibition compared to aqueous extracts.

This aligns with the chemical principle of differential solubility, where organic solvents like methanol possess an intermediate polarity that is highly effective at dissolving lipophilic and hydrophilic phenolic structures simultaneously (Özcan & Matthäus, 2017).

Oleuropein, which holds high solubility in alcoholic solutions, acts primarily by disrupting the lipophilic cell envelope of microorganisms. The distinct variations in susceptibility between **Gram-positive** and **Gram-negative** bacteria observed in this study corroborate classic microbiological architectures.

Gram-positive strains (*S. aureus* and *Streptococcus alpha-hemolytic*) were remarkably more vulnerable to the extracts. This susceptibility stems from their porous outer peptidoglycan layer, which allows polyphenols to readily penetrate the cell, cross-link with cell-wall proteins, and cause structural collapse or intracellular leakage (Goulas et al., 2022). Conversely, **Gram-negative** pathogens (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) possess a complex, lipopolysaccharide-rich outer membrane that functions as an impermeable physical barrier to large phytochemical molecules. *Pseudomonas aeruginosa*, in particular, exhibited the highest resistance, which is widely understood to be driven by its restricted outer membrane porins and active multidrug efflux pump systems that actively expel foreign antimicrobial agents (Brown & Wright, 2021).

The observed concentration-dependent effect confirms that a threshold concentration of bioactive molecules is crucial to overcome bacterial defense mechanisms. In conclusion, utilizing green extraction technologies like UAE not only accelerates processing efficiency but also preserves critical Phyto therapeutic compounds, positioning *Olea europaea* extracts as a viable, sustainable baseline for developing natural narrow- or broad-spectrum antibacterial agents.

7-Conclusion:

Ultrasonic-Assisted Extraction is a highly efficient, green extraction technology that maximizes the retrieval of bioactive antimicrobial compounds from *Olea europaea* leaves.

These findings highlight the potential of olive leaf extracts as promising natural alternatives to conventional antibiotics.

8- References:

- Brown, E. D., & Wright, G. D. (2021). Antibacterial drug discovery in the resistance era. *Nature*, 529(7586), 336–343.
- Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products: Mechanisms, techniques, combinations, protocols and applications. *Ultrasonics Sonochemistry*, 34, 540–560. <https://doi.org/10.1016/j.ultsonch.2016.06.035>
- Goulas, V., Papoti, V. T., Exarchou, V., Tsimidou, M. Z., & Gerothanassis, I. P. (2022). Contribution of flavonoids and secoiridoids to the antioxidant and antimicrobial activity of olive leaf extracts. *Journal of Agricultural and Food Chemistry*, 60(13), 3330–3341. <https://doi.org/10.1021/jf2047588>
- Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. *Journal of Natural Products*, 83(3), 770–803.
- Özcan, M. M., & Matthäus, B. (2017). A review: Benefit and bioactive properties of olive (*Olea europaea* L.) leaves. *European Food Research and Technology*, 243(11), 1899–1910. <https://doi.org/10.1007/s00217-017-2904-1>
- Picot-Allain, C., Mahomoodally, M. F., Ak, G., & Zengin, G. (2021). Conventional versus green extraction techniques: A comparative perspective on the extraction of bioactive compounds from olive leaves. *Trends in Food Science & Technology*, 114, 440–456. <https://doi.org/10.1016/j.tifs.2021.06.014>
- Mustafa H. Alsaedi, Saad Saad Madi, & Mahmoud Saad Al-Mabrouk Shneibah. (2026). Characteristics of Olive Fruits and the Maturity Index (MI) for Chemlali and Coratina Cultivars under Local Conditions in Libya. *Journal of Libyan Academy Bani Walid*, 2(1), 395–493. <https://doi.org/10.61952/jlabw.v2i1.493>

Abu Ras, N. M., Mu'aiza, N. R., & Ahmed, N. A. (2025). Dirasat ta'thir al-talawwuth al-bi'i 'ala awraq al-zaytun fi madinat al-Khums 'an tariq qiyas ba'd al-khasa'is al-fizya'iyya wa-al-kimya'iyya [Study of the effects of environmental pollution on olive leaves in Al-Khums City through the measurement of selected physical and chemical properties]. *Journal of Libyan Academy Bani Walid, 1*(3), 208–218. <https://doi.org/10.61952/jlabw.v1i3.138>
World Health Organization. (2024). *Antimicrobial resistance: Global report on surveillance*.

Compliance with ethical standards**Disclosure of conflict of interest**

The authors declare that they have no conflict of interest.

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