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RESEARCH ARTICLE

Evaluation of Antibacterial and Antifungal Activity of *Pistacia atlantica Subsp. kurdica* Oil Gum Extract from Halabja Province/ Kurdistan Region of Iraq

Hassan H. Hama Amin[1], Peshawa Y. Aziz[2], Shokhan H. Azeez[3]

[1]*Department of Biology College of Science, University of Sulaimani , Sulaymaniyah, Kurdistan Region, Iraq*
[2] *Department of Medical Laboratory Science, Technical College of Applied Science, Sulaimani Polytechnic University, Halabja, Kurdistan Region, Iraq*
[3]*Department of Dental Nursing, Sulaimani Technical Institute, Sulaimani Polytechnic University , Sulaymaniyah, Kurdistan Region, Iraq*

***Corresponding author:**
Peshawa Y. Aziz, Department of Medical Laboratory Science, Technical College of Applied Science, Sulaimani Polytechnic University, Sulaymaniyah, Kurdistan Region, Iraq, Iraq. **E-mail** peshawa.aziz@spu.edu.iq

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ABSTRACT

The *Pistica atlantica subsp. kurdica*, or Ghazwan in Kurdish, which is widely distributed in Kurdistan region, Northern Iraq, is a native plant of this region and the major source of a Kurdish gum. This study investigates the antibacterial and antifungal effect of essential oil extracted from the gum. The gum was collected in June-July 2019 from the region of Halabja, a city in the north of Iraq. Essential oil was extracted by using hydro distillation method; the antibacterial activity was evaluated by agar well diffusion test and the inhibition zone (in mm) was determined against six selected bacterial strains which included *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus leuteus*, *Escherichia coli* and *Klebsiella pneumonia*. The antifungal activity was assessed by testing different concentrations of oil gum against *Aspergillus brasiliensis*. Results showed the oil gum extract to have significant antibacterial activity against both Gram positive and negative bacteria and also to have strong antifungal activity because no growth was identified at different concentrations of the extract and the extract significantly inhibited growth of the *Aspergillus brasiliensis*. The data of this study suggested that *Pistica atlantica subsp. kurdica* oil gum has potential for application in pharmaceutical drugs against bacteria and fungi.

Keywords: Antibacterial, Antifungal, Inhibition zone, *Pistica atlantica subsp. kurdica*, oil gum

1. INTRODUCTION

The *Pistica Atlantica* subsp. *kurdica* is known as Ghazwan in Kurdish (Fathollahi et al., 2019). *P. a. kurdica* is widely distributed and native in Kurdistan around the Zagros Mountains and especially in Eastern and Northern Iraq, Western and Northern Iran, Southern Turkey and Northern Syria (Sharifi and Hazell, 2011). *Pistacia* is a genus of eleven species of the Anacardiaceae family that is distributed in the Mediterranean and Middle Eastern areas (Hosseini et al., 2013). *P. atlantica* is used to make chewing gum, also hull and kernel oil are used as frying oil by natives (Nachvak et al., 2018). *P. a. Kurdica* is the major source of a gum that is not widely known around the world (Sharifi and Hazell, 2011). The unripe fruits of *P. atlantica* are used to make pickle and the ripe fruits are used as food by local people after mixing with other ingredients (Nachvak et al., 2018). The fruits of *P. Atlantica* sub sp. *kurdica* are used in different forms by aboriginal people of various parts of Iran including as a flavoring agent in doogh (a cold yogurt beverage mixed with salt), as foods after milling and mixing with other compounds and as a nut after roasting (Fathollahi et al., 2019).

The various components of *Pistacia atlantica* have antioxidant, antimutagenic, antimicrobial, anti-inflammatory, anti-cancer, anti-cholinesterase and anti-diabetic properties (Hosseini et al., 2013).

Pistacia plants have been known for their medicinal properties since antiquity. They have played important roles in folk medicine and are used to treat eczema, throat infections, renal stones, asthma and stomach ache, and for their astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral and stimulant properties (Meftahizade, 2011). *Pistica atlantica* and its chemical components produce a variety of pharmacological actions such as anticancer, antioxidant, anti-inflammatory and antimicrobial activities (Nachvak et al., 2018). Subsp. *kurdica*, among other subsp. of *Pistacia atlantica*, has great importance in folk medicine as a therapeutic agent for treatment of infections (Khodavaisy et al., 2016). Trees and their derivatives such as fruit, leaves and resin have been used in a broad range of applications in traditional medicine, including as a preservative and breath sweetener as well as to treat gastric ailments and digestive, hepatic and renal diseases (Fathollahi et al., 2019). The traditional and folk uses of Gazhwan indicate the positive effects of the entire plant and its gum on digestive problems and gastrointestinal ailments such as peptic ulcers, diarrhea, gastritis and intestinal upsets (Minaiyan et al., 2014). The gum of this plant is used in medicine, with many researchers reporting that it exerts huge antimicrobial activity (Khodavaisy et al., 2016). Exudate gum of the tree contains resins and volatile oil, the main ingredients of its oil being pinenes, sabinene and limonene (Minaiyan et al., 2014).

Infectious diseases are the main cause of death worldwide and bacterial resistance to antibiotics has become a global concern now that the clinical efficacy of many existent antibiotics is being threatened by the development of multidrug-resistant pathogens (Ghalem and Mohamed, 2009). The utilization of plant compounds to treat infections is an ancient practice followed in different areas of the world, and plants with antimicrobial properties could potentially solve the current problems related to the use of antibiotics (Meftahizade, 2011). Hence, there is ongoing research to discover new antimicrobial agents, either by the design and synthesis of new agents or through the search for new natural sources for antimicrobial agents (Ghalem and Mohamed, 2009). Essential oils as antimicrobial agents are defined as safe natural substances for their users and for the environment and they are presumed to be low risk for resistance development by bacteria and other pathogenic microorganisms (Hosseini et al., 2013). Essential oils of plants and their other products from secondary metabolism have been widely used in folk medicine, food flavoring, fragrance, and pharmaceutical industries. Some biological activities of essential oils have been known for a long time (Alma et al., 2004).

Staphylococcus aureus is a pathogenic bacteria that causes wound infection after surgery, endocarditis, toxic shock syndrome, osteomyelitis and food poisoning (Mylotte et al., 1987). Meanwhile, *Escherichia coli* exists in the human intestine and is the main cause of lower urinary tract infection, coleocystis, septicaemia. (Ghalem and Mohamed, 2009).

The essential oils present in different parts of *P. atlantica*subsp. *kurdica* (oxygenated monoterpenes and hydrocarbons) are the main constituents of its ripe and unripe fruits, flowers, leaf-buds, leaves, twigs, and resins (Alma et al., 2004). Phenolic and flavonoid compounds (epicatechin, catechin, and gallic acid) are present in its various parts (Sulieman et al., 2012, Minaiyan et al., 2014). Fatty acids present in Ghazwan include Eicosanoic, palmitic, linolenic, lignoceric, palmitoleic, pentadecanoic, hexadecanoic and octadecanoic acids (Tahir et al., 2019).

While different studies have examined the composition, antioxidants and antibacterial activity of the extracts of different parts of this tree, including the fruit, leaves, and gum, few studies have explored the antibacterial and antifungal activities of *P. atlantica subsp. kurdica* oil gum against selected species of bacteria and fungi. Therefore, this study was performed to evaluate its *in vitro* antibacterial and antifungal effects against selected bacteria and fungi that could have direct impact on human health.

2. Materials and Methods

2.1 Plant Material and Essential Oil Extraction:

The newly harvested gum of *Pistacia atlantica kurdica* (pistachio tree of the Atlas) was collected in June-July 2019 from the Balambo range in the south of Halabja city in the north of Iraq, 14 km from the Iranian border. The plant was identified and compared with voucher specimens in the College of Agriculture/ University of Sulaimani. Hydro distillation method was performed for extraction of essential oil from the gum as follows: 100g of gum was soaked in 350 ml of distilled water in a conical flask for 3 hours according to published procedure (Azeez and Gaphor, 2019). Finally, the essential oil was collected after filtration.

2.2 Antibacterial activity of oil gum:

The antibacterial test was conducted using an agar well diffusion test against some bacterial strains as shown in Table (1). Muller-Hinton agar was seeded with 100 µl of 0.5 McFarland bacterial strains which included *Bacillus subtilis* (ATCC® 6633™), *Staphylococcus aureus* (ATCC® 6538P™), methicillin-resistant *Staphylococcus aureus* (MRSA: Clinical isolates), *Micrococcus leuteus* (ATCC®9341™), *Escherichia coli* (ATCC®8739™) and *Klebsiella pneumonia* (MDR) from Shar Hospital. This was made five wells (1 control and 4 tests) by cork borer, and 75 µl of the oil gum was added to each, which was prepared using the tween 20 by 1:10 ratio. The plates were then incubated at 37 degrees Celsius for 24 hours. The inhibition zone was estimated based on diameter (in mm).

2.3 Antifungal susceptibility test:

The antifungal activity was assessed using different concentrations of oil gum (100, 50, 25 µl /ml) as final concentrations in the medium. These different concentrations were mixed separately with autoclaved Potato Dextrose Agar (PDA) medium at 55 degrees Celsius and poured into a petri-dish. The *Aspergillus brasiliensis* (ATCC® 16404™) was inoculated on PDA medium and after 5 days of incubation a small piece of culture was cut off with a cork borer and transferred to the medium two plates with and without oil gum extract and after that incubated at 28 degrees Celsius for 5 days.

3. Results

3.1 Antibacterial activity

The susceptibility of the oil gum was tested by well diffusion method. Its antibacterial activity was determined by measuring the diameter of the inhibition zone. The oil gum extract had significantly broad antibacterial activity against Gram positive and negative bacteria. It was highly active against the Gram-positive bacteria, especially against *Bacillus subtilis* (ATCC® 6633™), for which the inhibition zone was about 19 mm, and *Staphylococcus aureus* (ATCC® 6538P™) which inhibited apparently. Interestingly, the extract was observed to inhibit the MRSA and *Klebsiella pneumonia* (MDR), as illustrated in Table (1) and Figure (1, 2).

Table (1) Antibacterial activity of Oil Gum against selected bacterial spp.

Extract	Inhibition zone (mm)					
	Bacillussubtilis (ATCC® 6633™)	*Staphylococcusaureus* (ATCC® 6538P™)	*MRSA*	*Micrococcus leteus*(ATCC®9341™)	*Escherichiacoli* (ATCC®8739™)	*Klebsiella pneumonae*
Oil gum	19	11	4	6	4	3
	19	9	5	5	6	3
	19	11	4	7	6	3
	19	11	4	6	6	3

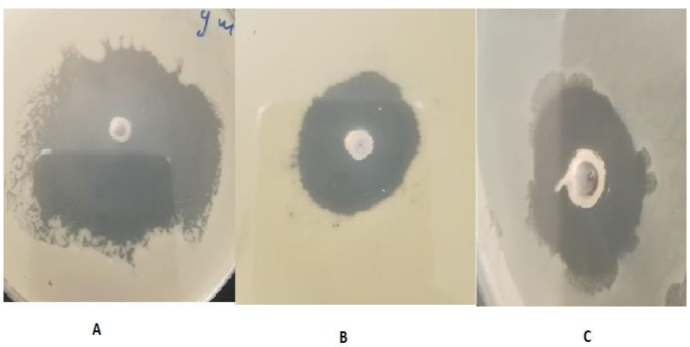

Figure (1) shows the inhibition zone of oil gum extract against: A- Bacillussubtilis (ATCC® 6633™), B- Staphylococcusaureus (ATCC® 6538P™), C- Escherichiacoli (ATCC®8739™).

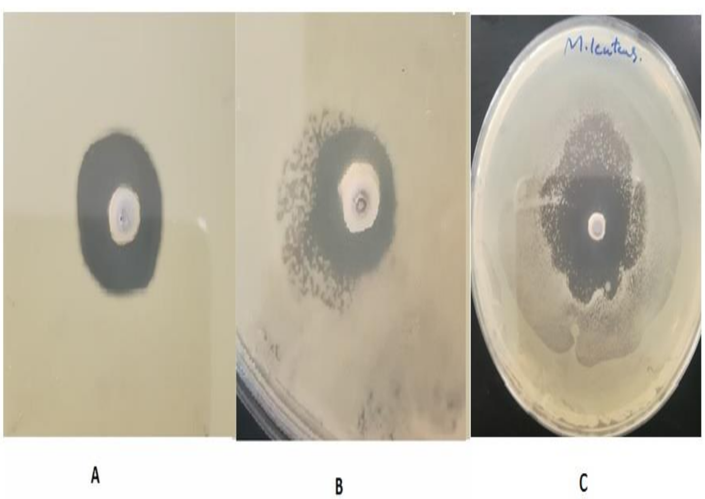

Figure (2) shows the inhibition zone of oil gum extract against some clinical isolates: A- MRSA, B- Klebsiella pneumonia (MDR) and C- Micrococcus leuteus (ATCC®9341™).

3.2 Antifungal activity

The results, as shown in Figure (3), indicate that the extract significantly inhibited growth of the *Aspergillus brasiliensis* (ATCC® 16404™) because no growth was observed in the different concentrations of the extract added to the inoculated medium.

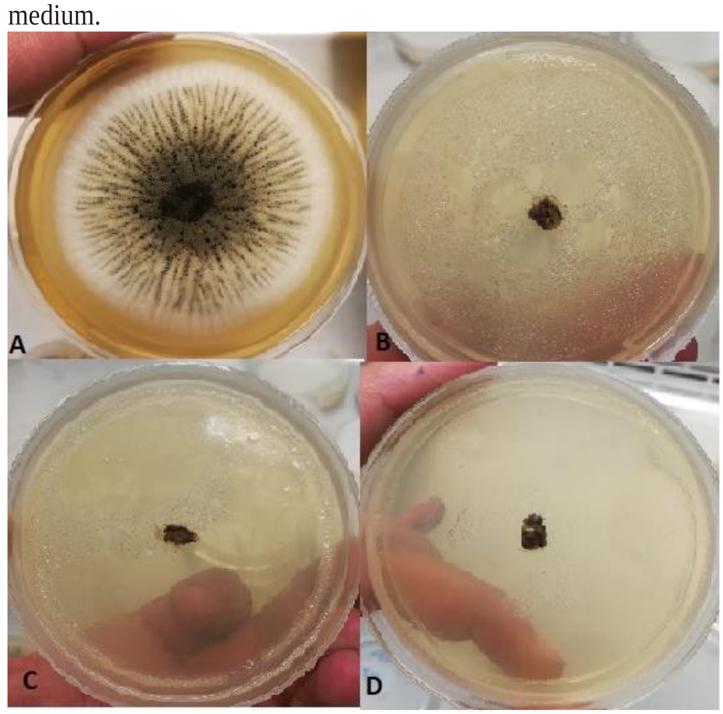

Figure (3) shows the antifungal activity of oil gum extract against Aspergillus brasiliensis (ATCC® 16404™) at different concentrations: A-without extract, B- 100 µl /ml, C-50 µl /ml and D- 25 µl /ml.

4. Discussion

Today the multidrug resistance of microorganisms is increasing rapidly and discovery of new antibacterial products is required in order to overcome this challenge. This generally involves the extraction from the plant of essential oil which is composed of different families of chemicals such as terpenes, aldehydes, alcohols, esters, phenolic, ethers, and ketones (Swamy et al., 2016). *Pistacia atlantica* subsp. *kurdica* (Gum) contains α-pinene, β- pinene, myrcene, limonene and terpineol (Fathollahi et al., 2019). GC-MS analyses of the essential oil of *Pistacia* *atlantica kurdica* gum showed that 79.76% of essential oil of *Pistacia atlantica kurdica* gum grown in Kurdistan, Iraq was alpha-Pinene (Azeez and Gaphor, 2019). Exudate gums of the *Pistacia atlantica kurdica* tree contain resins and volatile oil, with pinenes, sabinene and limonene being the main ingredients of its oil (Minaiyan et al., 2014).

This study represents the first reported investigation of the antibacterial effects of essential oil of *Pistacia atlantica kurdica* gum from Kurdistan, Iraq against the selected bacterial species. Gram negative bacteria were more resistant to the oil gum when compared to the Gram positive; this may be due to the greater complexity of the double membrane-containing cell envelope in comparison with the single membrane structures of Gram-positive bacteria (Ghalem and Mohamed, 2009). Several mechanisms can lead to inhibiting bacterial growth, such as destruction of the cell membrane, damage to the protein content of the cell membrane and proton motive force depletion. In addition, there are many other ways in which bacterial cells can be altered by essential oil, such as through the effect on ATP and ATPase, protein membrane, fatty acid (Nazzaro et al., 2013). Overall, the essential oil can lead to damage of the cell membrane, release of cell content, and then death of the bacterial cell.

Pistacia atlantica kurdica acts as a potential natural inhibitor to prevent the growth of Botrytis cinerea fungi (Hesami et al., 2013). The main finding of this study was identifying the significant antifungal activity of oil gum against *Aspergillus brasiliensis* because no growth was observed at different concentrations of oil gum extract added to the inoculated medium. These results agree with another study, by Khodavaisy et al. (2016), which demonstrated that *Pistacia atlantica kurdica* had an inhibitory effect on *A. parasiticus*. The data of this study suggest that *P. atlantica subsp. kurdica* oil gum has potential for application in pharmaceutical drugs against bacteria and fungi. The recommendation for future studies is to detect the active components of the oil gum and determine the mechanisms by which the gum acts as an antifungal and antibacterial agent *in vitro*.

Conclusion

The aim of this study was to assess the *in vitro* antimicrobial and antifungal activities of the *Pistacia atlantica kurdica* oil gum extract. The work showed that the extract had a potential antibacterial effect against selected bacterial species and potential antifungal activity against *Aspergillus brasiliensis*.

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