



Exploring the antibacterial potential of ajwa date fruit: A review on combatting antibiotic-resistant bacterial strains

Abdulrahman Shaheen, Faisal Al-Sarraj

Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box, Jeddah, Saudi Arabia

Abstract

One of the most urgent challenges to world health in the twenty-first century is antimicrobial resistance. A major threat to human life, bacterial illnesses associated with multidrug-resistant microorganisms are rapidly growing. Current research pipelines primarily rely on structural derivatives, and the hunt for novel antibiotics to combat resistance has stalled for more than 20 years. Since this method has not changed as quickly as resistance, new therapeutic approaches must be created. Natural medicinal agents are regarded as a good source of new antimicrobials because of their strong antibacterial efficiency and ability to target other targets than conventionally employed antimicrobials. The application of natural compounds with antibacterial qualities is one interesting avenue for research. Ajwa date extract has gained attention among them because of its capacity to combat bacterial strains that are resistant to antibiotics. The nutritional and therapeutic qualities of Ajwa dates, a type of date fruit (*Phoenix dactylifera* L.) grown in Medina, Saudi Arabia, have long been recognized. Ajwa dates' substantial polyphenolic content, which includes flavonoids, glycosides, and phenolic acids and contributes to their strong antibacterial, anti-inflammatory, and antioxidant properties, has been revealed by recent scientific research. In this regard, investigating Ajwa date extract as a possible method to combat bacteria resistant to antibiotics is a viable path for antimicrobial therapy research and development. To better understand the antibacterial qualities of Ajwa date extract, this review will look at its modes of action, effectiveness against different bacterial strains, and possible uses in the fight against antibiotic resistance. The review intends to shed light on the potential of Ajwa date extract as a natural substitute in the continuous fight against bacterial illnesses resistant to antibiotics by combining the existing scientific data.

Keywords: Ajwa dates, antibacterial, antimicrobial, antioxidant, fruit, phoenix dactylifera

Introduction

One of the biggest risks to world health in the twenty-first century is antimicrobial resistance (AMR). A major threat to human life, bacterial illnesses associated with multidrug-resistant microorganisms are rapidly growing. The primary causes of the current state of AMR worldwide are factors such as globalization, technological advancement, environmental contamination, and changes in the socioeconomic environment [1, 2]. In 2019, the WHO listed the top ten rising dangers to human health, with antimicrobial resistance (AMR) at the top. International organizations now acknowledge the need to fight AMR within the framework of the One Health and Global Health strategy. Nonetheless, a significant problem with AMR is the application of efficient therapeutic procedures [3].

The search for new types of antibiotics to overcome resistance has plateaued for over two decades, and current development pipelines rely predominantly on structural derivatives. This approach has not evolved as rapidly as the resistance and therefore; there is need to develop new therapeutic strategies [4, 5]. Natural therapeutic agents are considered as a suitable source of novel antimicrobials because they have high antimicrobial efficacy and they select different targets in comparison to traditionally used antimicrobials, making them beneficial in the treatment of multiple drug-resistant bacterial infections [6, 7, 8, 9].

A potential avenue for research is the application of natural substances that have antibacterial qualities. The ability of Ajwa date extract to combat bacterial strains resistant to antibiotics has made it a topic of attention among these [10]. Dates are monocot plants that are members of the Araceae family. The fruit produced by the date palm tree is known as

date fruit. The fruit is 3–7 cm long and 2–3 cm in diameter, with an oval, cylindrical shape. Saudi Arabia produces prophet dates, also known as Ajwa dates. According to hadiths from Sahih al-Bukhari and Sahih Muslim, which were passed down by Sa'ad bin Abi Waqqash, the Prophet Muhammad SAW is said to have said, "Whoever eats seven Ajwa dates in the morning, on that day they will avoid poison and magic" [10]. Numerous medical ailments have been found to benefit from the use of dates in traditional or folk medicine. The hepatoprotective, nephroprotective, anti-allergic, neuroprotective, anti-genotoxic [11], antimicrobial, antiviral, and antifungal qualities of date fruits have been shown in experimental animal models. By triggering endocrine and antioxidant systems, the pollen extract from dates has been demonstrated to reduce cadmium-induced testicular damage. By altering cytokine levels *in vivo*, oral date extract administration also changed immunological responses [11].

The nutritional and therapeutic qualities of Ajwa dates, a type of date fruit (*Phoenix dactylifera* L.) grown near Medina, Saudi Arabia, have long been recognized. Ajwa dates' substantial polyphenolic content, which includes flavonoids, glycosides, and phenolic acids and contributes to their strong antibacterial, anti-inflammatory, and antioxidant properties, has been revealed by recent scientific research [10]. With the emergence of extensively drug-resistant (XDR) and multidrug-resistant (MDR) bacterial species, commonly known as "superbugs," the need for new antimicrobial drugs has grown more pressing.

Given this, investigating Ajwa date extract as an alternative method to combat bacteria resistant to antibiotics presents an attractive option for antimicrobial therapy research and

development^[12, 13]. The deep black color and delicious flavor of Ajwa dates, which are farmed in Al Madinah, Saudi Arabia, are well known. These opulent fruits are prized for their substantial therapeutic benefits. Ajwa dates are unique among dates because of their unique nutritional makeup, which includes significant amounts of fiber, carbohydrates, amino acids, minerals, and vitamins. Furthermore, Ajwa dates are a rich source of phytochemicals such as phenolics and flavonoids, which have anti-inflammatory, antiviral, antioxidant, and anticancer effects. Because they include fiber and potassium, these dates also help with digestion, ease constipation, and promote muscle function^[14, 15].

In order to better understand the antibacterial qualities of Ajwa date extract, this review will look at its modes of action, effectiveness against different bacterial strains, and possible uses in the fight against antibiotic resistance. This study aims to shed light on the potential of Ajwa date extract as a natural substitute in the ongoing fight against infections caused by bacteria resistant to antibiotics by combining the existing scientific data.

Historical Significance of Ajwa Dates

Historically, several plant species have been studied and utilized to treat or stop the onset of different illnesses. One of the most significant fruits in North Africa and the Middle East is date fruit. Both nutritional and functional benefits are

offered by it. Date fruit has been suggested by numerous *in vitro* and *in vivo* research because it is rich in different nutrients and physiologically active substances like antioxidants that have a direct effect on human health. It therefore aids in the prevention or treatment of numerous illnesses, including infections^[16]. According to the earliest evidence from Mesopotamia (Iraq), date cultivation most likely started around 3000 BCE. Given the lengthy history of date agriculture and the extensive distribution and interchange of its variants, the date palm's precise origin is still unknown. Nonetheless, it is largely accepted to have spread throughout the Arabian Peninsula, North Africa, and the Middle East after originating mostly in western India or the ancient Mesopotamia region (southern Iraq)^[17]. Date palms are currently common in many arid Old World locations, and to a lesser degree in temperate zones. Middle Eastern nations including Saudi Arabia, Iran, Iraq, and the United Arab Emirates produce the majority of dates, as do African nations where the plant thrives in dry climates (Fig.1)^[18]. Due to varietal diversity and growth conditions, hundreds of distinct date cultivars with varying colors, tastes, textures, and sizes are grown throughout the world^[18]. Cultivated in Medina, Saudi Arabia, ajwa dates are historically and culturally significant, especially in Islamic and traditional medicine. These unusual fruits have been used for ages in traditional medicine for a variety of therapeutic purposes^[14, 19].

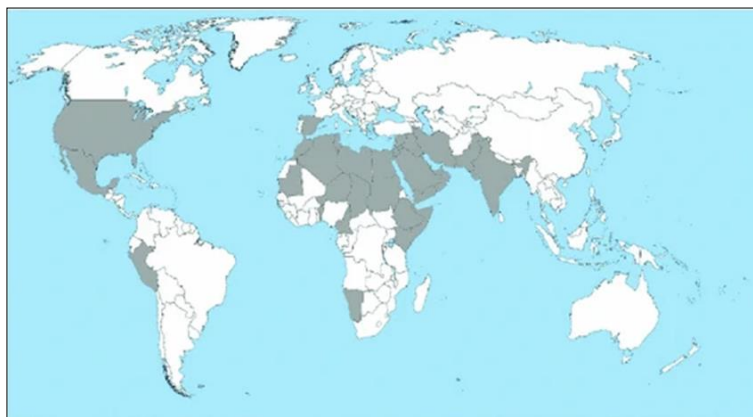


Fig 1: The global distribution of date palms^[18].

Growth stages of date fruits

Date fruits are classified based on shape, size, and color, varying significantly with maturity stages that impact taste and quality. The fruits undergo five stages (Figure 2) of development until they ripen, each stage named differently across cultures^[16].

1. **Hababouk Stage:** This initial stage post-fertilization lasts four to five weeks, with the immature fruit covered by the calyx, weighing under 1g^[20].
2. **Kimri Stage:** Also known as the green stage, it's the longest in fruit development, lasting nine to fourteen weeks. The solid green fruit remains inedible, weighing around 6g^[21].
3. **Khalal Stage:** The fruit's color shifts from green to yellow or red, and certain varieties like Halawi, Barhi, and Zaghoul become edible^[21].
4. **Rutab Stage:** Referred to as the soft ripe stage lasting two to four weeks, the fruit's top ripens, texture softens, and color darkens. Sugar content rises, making it sweet and suitable for harvesting and storage^[22].
5. **Tamr Stage:** The final stage of date fruit maturity where they reach full ripeness. Dates turn black or brown, with dry dates having a hard texture and Rutab dates being soft. The fruit loses moisture, maintaining low weight to prevent spoilage. Semi-dry and dry dates contain about 50% sucrose^[16, 23].

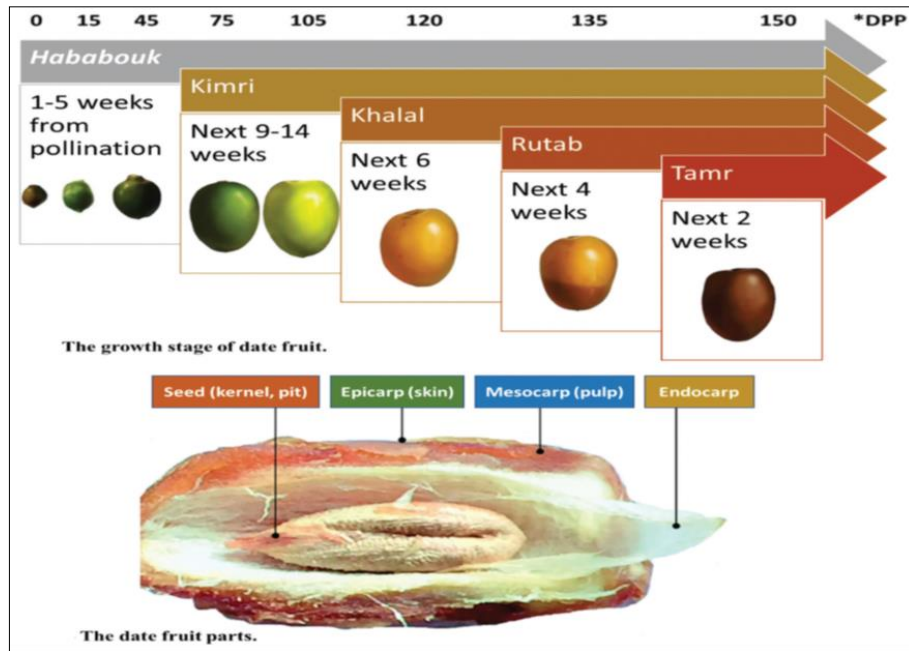


Fig 2: the date fruit's stage of growth [16].

The nutritional significance of fruit part of Ajwa date

The second expert committee report from the American Institute for Cancer Research (AICR) and World Cancer Research Fund International (WCRF) recommended that instead of using dietary supplements to prevent cancer and other chronic diseases, people should meet their nutritional needs through a regular diet [24]. Since ancient times, millions of people around the world have relied on Ajwa date fruit as a staple diet. In order to increase the intake of Ajwa dates and prevent the advent of numerous diet-related disorders, it is crucial to describe their nutritional profile. Numerous investigations have reported on the nutritional makeup of Ajwa date pit and flesh. Ajwa flesh was

discovered to contain 80% reducing sugars, as well as proteins, lipids, and other amino acids [14, 19, 25]. However, compared to Ajwa flesh, Ajwa date pits contain a greater quantity of proteins, crude fat, and crude fiber. Ajwa date flesh's proximate composition, pits, and pointed richness in moisture, ash, glucose, fructose, galactose, and maltose were recently investigated. In contrast, the Ajwa pits are rich in protein, fibre, and crude fat. Additionally, their analysis revealed that crude fiber and fat in Ajwa pits had a positive connection (0.90 and 0.94) with crude protein. Along with calcium, zinc, and other minerals, Ajwa dates are particularly rich in potassium [19, 25].

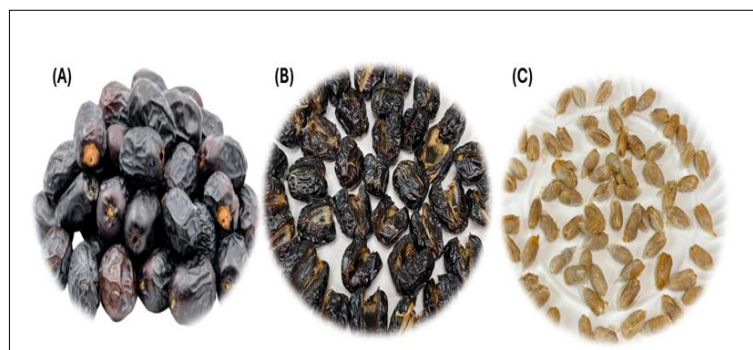


Fig 3: Part of Ajwa Dates: A) Fruit, B) Flesh, C) Pit

Phytochemicals in Ajwa date fruit

Intercellular plant metabolites known as phytochemicals with high antioxidant properties may help prevent diabetes, heart disease, and cancer. Rich in polyphenols, Phenolic acid (Gallic acid, caffeic acid, chlorogenic acid, syringic acid, p-coumeric acid, m-coumeric acid and ferulic acid, protocatechuic acid, hydroxybenzoic acid, vanillic acid, isovanillic acid, chlorogenic acid, ferulic acid, isoferulic acid, hydroxycinnamic acid and chlorogenic acid), flavonoids, including Quercetin, Luteolin, Iso-quercetin, Apigenin, Rutin, Catechins, Iso-Flavonoids, Sterols, quercetin, myricetin, naringenin, apigenin, luteolin, kaempferol, and Lignans [14]. Ajwa date fruit helps decrease

cholesterol and prevent cardiovascular diseases [26, 27]. Ajwa fruit portions may have various amounts of these phenolic and antioxidant chemicals based on factors such as genetic composition, analytical circumstances, and degree of hydration [28]. Date phytochemical quality and quantity can also be influenced by the extraction process, fruit condition (fresh or dried), and extraction solvent [29].

Ajwa date's pharmacological and biological properties

The development of tolerance and resistance in the current medications has reduced their effectiveness. Consequently, the pharmaceutical sector has seen a growing trend of substituting natural pharmaceuticals (derived from plants)

for manufactured drugs. Phytochemicals with greater disease-preventive qualities are abundant in these natural sources^[30]. Due to its anti-inflammatory, hepatotoxic, anti-cancer, antimicrobial, antidiabetic, nephrotoxic, and hepatoprotective properties, as well as its ability to improve cardiac function, ajwa dates have been used traditionally and historically to treat a variety of illnesses^[11, 14, 31, 32].

1. Antioxidant activity

In Arabia, ajwa date fruits are consumed in large quantities. Their high levels of phenolics, melatonin, carotenoids, and vitamins give them a potent antioxidant effect^[33]. The majority of studies evaluating the antioxidant activity of Ajwa fruit have used alcoholic and aqueous extracts. Ajwa fruit's antioxidants are primarily hydrophilic and exhibit potent antioxidant action in the lipid membrane system^[29, 34]. Ajwa fruit aqueous extract had considerable antioxidant activity when compared to alcoholic extracts, according to Saleh^[35]. Using ethyl acetate, methanolic, and aqueous extracts of Ajwa dates at 250 mg/mL, the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay reduced lipid peroxidation by 88, 70, and 91%^[15].

In rabbits poisoned with lead, the ethanolic extract of Ajwa dates demonstrated a decrease in lipid hydroperoxides and an increase in blood antioxidant enzyme levels^[32]. The inhibition of free radicals, which in turn slows the spread of illness, is one potential mechanism by which ajwa dates produce their antioxidant effect. Ajwa dates' potent antioxidant properties have been supported by additional research^[11, 36, 37]. According to a recent study by Al-Yahya *et al.* (2016)^[11], Ajwa date extracts stop the depletion of important antioxidants such as carnitine acyltransferase, glutathione peroxidase, and superoxide dismutase. Arshad *et al.*, (2015)^[38] demonstrated a potent antioxidant in a recent study using various extracts of Ajwa fruit and pit, with 74.19 mg/mL of gallic acid equivalents in methanolic extracts. Additionally, they demonstrated that acetone extracts of Ajwa dates exhibited potent radical scavenging action in DPPH and lipid peroxidation tests. In contrast to conventional solvents, they noted that aqueous extracts of Ajwa pits exhibited exceptional radical scavenging activity^[38].

2. Anti-inflammatory activity

One of the body's key defenses against a variety of threats, including infections, burns, poisonous substances, allergies, and other stimuli, is inflammation^[39]. Numerous issues, including diabetes and arthritis, are brought on by activated inflammatory leukocytes producing too many free radicals^[37]. Numerous illnesses emerge and worsen as a result of disruptions in the inflammatory mechanism. Inflammation, diabetes, cancer, and other illnesses are significantly influenced by transcription factors (LOX and NF-κB). Therefore, appropriate transcription factor regulation is a crucial step in disease prevention. Plant components including flavonoids and phenolics have been demonstrated in earlier research to be potent anti-inflammatory agents^[40]. Ajwa date methanolic and aqueous extracts were found to have anti-inflammatory effects in albino rats via raising COX 1 and 2 enzyme levels. The lipid peroxidation cyclooxygenase enzymes COX-1 and COX-2 are inhibited by Ajwa date extracts in ethyl acetate, methanol, and water. Additionally, these research have demonstrated the anti-inflammatory properties of polyphenols, fiber, steroids, and

minerals found in Ajwa dates^[15, 37]. In both *ex vivo* and *in vivo* models, lyophilized extract of Ajwa dates at a concentration of 250 mg/mL inhibits the development of apoptotic markers like caspase-3 and Bax as well as pro-inflammatory cytokines like IL-6, IL-10, and TNFα (Al-Yahya 2015). Similarly, in a mouse model, lyophilized extract of Ajwa dates at a concentration of 250 mg/mL has strong cardioprotective properties and decreases edema, myonecrosis, and infiltration of inflammatory cells in cardiomyocyte architecture^[11].

3. Antidiabetic activity

Through the modification of metabolic and molecular pathways, plants are a key component in the treatment of diabetes and its complications, such as diabetic retinopathy^[41]. By increasing the generation of insulin and decreasing the intestinal wall's absorption of glucose, phytochemicals can regulate the actions of pancreatic tissues. The antidiabetic properties of Ajwa extracts may be attributed to flavonoids, steroids, phenol, and saponins, all of which are important in preventing diabetes^[31]. Agglucosidase enzymes, which affect how well glucose is absorbed in the kidneys and small intestine, are inhibited by phenolic substances. Furthermore, the phenolic chemicals also affect how much insulin the body secretes. Ajwa fruit's potent antioxidant profile can scavenge free radicals and have a potent antidiabetic effect^[37]. When streptozotocin-induced diabetic rats are given 100 g/L of aqueous Ajwa seed extract, their blood glucose levels decrease. Long-term usage of Ajwa seed extract also balances oxidative stress conditions and improves liver and kidney function in streptozotocin-induced diabetic rats^[42].

4. Anticancer activity

Ajwa date extracts, both aqueous and methanolic, have been shown to suppress marginal cell growth against human gastric, lung, breast, and colon cell lines at concentrations of 100 mg/mL^[37]. Ajwa dates' flavonoids, particularly quercetin, steroids, and polyphenols are what give them their positive anti-tumor effects^[31]. Ajwa dates' bioactives boost the body's production of antioxidant enzymes like SOD, GST, and catalase, which prevents injured cells from proliferating and lowers the risk of mutagenesis. The cyclooxygenase inhibitory activity of ajwa dates is comparable to that of commercial anti-inflammatory medications such as aspirin, ibuprofen, celebrex, and naproxen^[15]. According to Eid *et al.* (2014), ajwa date extract and ajwa polyphenols are helpful at preventing the formation of colon cancer cells and preserving intestinal health^[43]. Additionally, according to Eid *et al.* (2015), Ajwa date extract preserves and withstands alterations in microbiota^[43].

5. Antimicrobial Activity

5.1. Antiviral Activity

Ajwa date fruit (*Phoenix dactylifera* L.) is a promising natural remedy for infections since it has shown strong antimicrobial action against a variety of antiviral, antifungal, and antibacterial pathogens^[14]. Ajwa date fruit has garnered attention for its potential antiviral activity, which is believed to be part of its broader spectrum of health benefits. This antiviral property is likely attributed to the fruit's rich composition of bioactive compounds, including phenolic compounds and flavonoids. Acetone-extracted Ajwa date pit's antiviral activity against *Pseudomonas* phage was

examined by Jassim and Naji (2010) [44]. Antiviral activity with MIC <10 mg/mL was demonstrated by the results. Using *Pseudomonas aeruginosa* ATCC 25668 as the host cell, a crude acetone extract of the pit of the date palm (*Phoenix dactylifera* L.) was made, and its antiviral activity was assessed against lytic *Pseudomonas* phage ATCC 14209-B1. With a minimum inhibitory concentration (MIC) of less than 10 µg ml⁻¹, it was shown that date pits' antiviral action was mediated by their binding to the phage. Phage inactivation kinetics, concentration exponent (η), and decimal reduction time (D-values) were all calculated. Pit extract's antiviral potency is significantly indicated by parameters such as concentration exponent, phage inactivation kinetics, and decimal reduction time. *Pseudomonas* phage ATCC 14209-B1's infectivity is strongly inhibited by date pit extracts, which also totally stopped bacterial lysis. future research should be conducted to investigate the potential of extracts for HIV treatment. By blocking the infectivity of phage lysis, the Ajwa date extract may be a low-cost method of protecting patients from viral infections [44]. All things considered, it is anticipated to encourage further investigation into its potential as a novel antiviral agent against harmful human viruses.

5.2. Antifungal Activity

The fruit of the Ajwa date plant has been shown to have antifungal action in addition to its general antibacterial qualities. Its overall antibacterial benefits are linked to the fruit's bioactive constituents, such as flavonoids and other phenolic compounds, which also have antifungal activity. The broad-spectrum antibacterial action and potent antioxidant capacity of Ajwa dates are thought to be the cause of their antifungal activity, which may help the body's defenses against fungal infections. Although there are few particular studies on the antifungal activity of Ajwa dates, there is some evidence to support this feature. For example, a study that evaluated various Emirati date types, including Ajwa, discovered that date pit extracts exhibited efficacy against the common fungal pathogen *Candida albicans* [45].

According to Bokhari *et al.* (2011), the growth of *F. solani*, *Fusarium sp.*, *Alternaria spp.*, *F. oxysporum*, *A. alternata*, *A. flavus*, and *Trichoderma sp.* was suppressed by aqueous, methanol, and acetone extracts of Ajwa date leaves and pits. The leaf extract exhibited 51.5% inhibitory action against *A. Alternata*, whereas *F. solani*, *F. fusarium*, and *F. oxysporium* showed inhibitory activity of 29.4%, 38.5%, and 6.3%, respectively. Additionally, the Ajwa pits exhibited modest efficacy against *F. solani* and *F. oxysporium*, as well as 40.9% and 38.5% inhibitory activity against *A. Alternata* and *F. Fusarium*, respectively. Additionally, according to Bokhari and Perveen (2012), the activity pattern was as follows: methanolic pit extract > methanolic leaves extract > acetone pits extract > acetone leaves extract [46].

Ajwa dates' antifungal and antibacterial qualities have been mentioned in relation to their general health advantages, indicating that the fruit may have antifungal potential. Even though antifungal activity has been noted, more study is necessary to completely comprehend the scope and processes underlying this characteristic. The majority of studies have focused on the fruit's antibacterial effects, and future experiments especially targeting its antifungal qualities would be good to show its efficacy against various fungus species.

5.3. Antibacterial activities

One of the main obstacles to antimicrobial medications is bacterial resistance. Antibiotic resistance in bacteria can be acquired, adaptive, or innate. Intrinsic resistance is the resistance exhibited as a result of the bacterium's natural traits [12]. Numerous complex resistance mechanisms are adopted by bacteria, including reduced permeability, target site changes, efflux pump systems, and the destruction of antibacterial medications by the organism's own enzymes (Figure 4). Since natural goods and their ingredients are cheap and have no negative side effects, using them to combat the issue of resistance is an excellent way to control the infection.

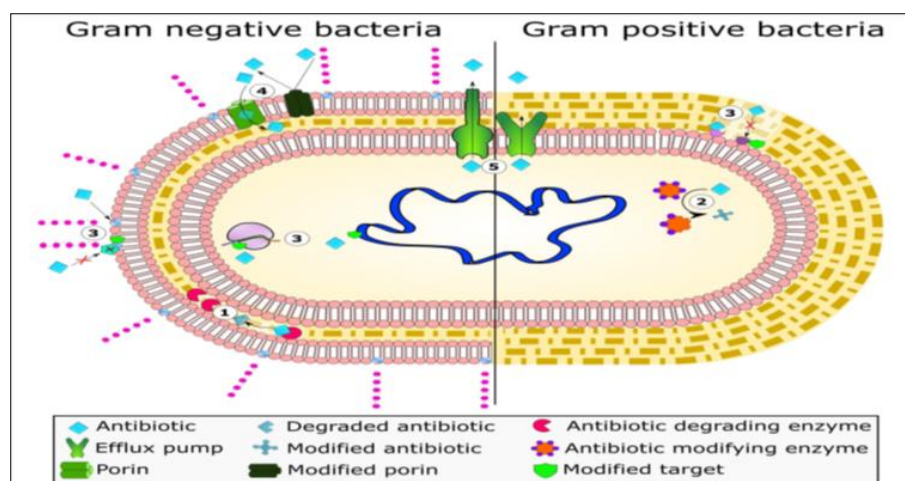


Fig 4: Mechanisms by which bacteria fight antibiotics. Enzymatic hydrolysis (1), enzymatic antibiotic modifications through group transfer and redox process (2), changes to antibiotic targets (3), reduced antibiotic permeability through porin alteration (4), and active antibiotic extrusion via membrane efflux pumps (5) are among the common mechanisms of antibiotic resistance seen in bacteria [12].

Significant antibacterial action is demonstrated by Ajwa date fruit against a range of bacterial species, especially those that cause gastroenteritis. The fruit's abundance of bioactive substances, such as polyphenols, flavonoids, and

phenolic acids, is responsible for this activity [10, 47]. According to a study by Eid *et al.* (2014) [43], eating whole date fruit extract (the digested Ajwa date extract; DDE) and extract high in polyphenols (date polyphenol extract; DPE)

causes alterations in the beneficial bacteria in the colon. The findings were based on fecal cultures that resembled the human large intestine and were pH-regulated. Both extracts clearly increased the growth of bifidobacteria, according to the fluorescence microscopic count. The bacterial growth increase within 24 hours was higher for those who took whole date extract (DDE) than for those who took the extract rich in polyphenols (DPE). Bacterial metabolism also produced flavonoids aglycones (myricetin, luteolin, quercetin, and apigenin) in less than an hour. These findings demonstrate that Ajwa date-derived DDE and DPE extracts can both dramatically boost the growth of good bacteria^[43], as well as their capacity to suppress pathogens and increase lactate and acetate production^[48], all of which support colon health^[16].

However, in a study by Hussain Mallhi *et al.* (2014), the impact of extracts (water, methanol, and acetone) from Ajwa date fruit, seeds, bark, and leaves on strains of Gram-positive bacteria (*S. Aureus*, *S. Pyogenes*) and strains of Gram-negative bacteria (*E. Coli*, *P. Aeruginosa*) was tested. Based on disc diffusion in inhibition, the study found that all plant parts exhibited antibacterial activity, with fruits and leaves exhibiting the highest antibacterial values compared to seeds and bark. The aqueous extract was the least active because methanol and acetone were able to dissolve a wide range of botanical ingredients more readily than water could. However, several substances that have been shown to have antibacterial qualities, such as tannins, alkaloids, and flavonoids, are responsible for the antibacterial action^[31].

Bacterial illnesses can be effectively treated with the Ajwa date and its components. The growth of both Gram-positive (*Staphylococcus aureus*, and *Enterococcus faecalis*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia.coli*, *Salmonella typhi*, *Pseudomon aeruginosa*, and *Salmonella paratyphi*) was somewhat reduced by the methanol and acetone extracts of the pits of Ajwa dates^[44, 49]. *Escherichia coli* and *Klebsiella pneumonia* activity are inhibited by ajwa dates, which also prevent methylprednisolone's lowering effect^[49]. Because it inhibits *Enterococcus fecalis* activity, methanolic extract of Ajwa dates is also useful against enteric illnesses^[49]. In study carried out by Samad *et al.*, (2016) demonstrated that *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Serratia marcescens* are all effectively combatted by the methanolic extract of Ajwa dates^[27].

Ajwa dates were extracted using two different techniques in another investigation by Abdullah *et al.* (2019)^[47] methanol extraction and hot aqueous extraction. The purpose of the study was to evaluate the extracts' ability to combat harmful microorganisms that cause gastroenteritis. *Vibrio cholerae*, *Shigella flexneri* (ATCC 12022), *Salmonella typhi*, *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* were among the bacterial species. The results showed that at a concentration of 100 mg/mL of Ajwa date extracts, no antibacterial activity was detected. It was discovered that 200 mg/mL of hot aqueous and methanol extract of Ajwa date fruit was the minimal concentration needed to stop bacterial growth. This suggests that the hot aqueous extract's effectiveness against every tested bacterium was nearly equal to that of the methanol extract. In contrast to the hot aqueous extract, which showed average zones of inhibition between 13 and 17 mm, the methanol extract showed greater inhibitory zones, with average zones of inhibition spanning between 15 and 34 mm^[47]. The zones of

inhibition expanded as extract concentrations rose. *S. flexneri* was the most susceptible of the five bacteria under study to hot aqueous and methanol extract, as evidenced by the biggest zone of inhibition for almost all concentrations. It was discovered that the growth of the microorganisms under investigation may be inhibited by both Ajwa date extraction methods. Potential bactericidal activity was shown by both extraction techniques. With an MBC range of 250 to 500 mg/mL in practically all examined bacteria, the methanol extract showed a greater capacity to kill bacteria. However, when employing hot aqueous extract, the MBC range was between 500 and 1000 mg/mL. *V. cholerae* was the most susceptible organism to methanol extract, as indicated by both the MIC and MBC of 250 mg/mL. *E. Coli* and *S. Typhimurium*, on the other hand, were the least sensitive bacteria when utilizing the hot aqueous extract, with an MBC of 1000 mg/mL. The combined findings of the MIC and MBC tests showed that ajwa date extract has bacteriostatic qualities at lower dosages and bactericidal qualities at higher concentrations^[47].

Conclusion

In conclusion, research into the antibacterial properties of Ajwa date fruit indicates a promising strategy for battling bacterial strains that are resistant to antibiotics. Ajwa dates' many characteristics, including their growth stages and historical significance, highlight their nutritional value. Antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, antiviral, antifungal, and antibacterial activity are only a few of the pharmacological and biological characteristics of Ajwa dates that demonstrate their varied therapeutic potential. The effectiveness of Ajwa Date fruit against resistant forms of bacteria can be clarified by comprehending the mechanisms of action behind its antibacterial properties. Furthermore, Ajwa Dates' polyphenols' pharmacokinetics and synergistic effects highlight how effective they are at fighting bacterial infections. This analysis opens the door for more investigation and its incorporation into antibacterial treatments against resistant diseases by highlighting Ajwa Date fruit as a natural resource with strong antibacterial properties.

References

1. Coque TM, *et al.* Antimicrobial resistance in the global health network: known unknowns and challenges for efficient responses in the 21st century. *Microorganisms*,2023;11(4):1050.
2. Al-Maaqar SM, *et al.* IDENTIFICATION OF ANTIBACTERIAL AGENTS AGAINST KLEBSIELLA PNEUMONIAE TARGETING THE CTX-M-15 PROTEIN USING INTEGRATED STRUCTURE MODEL-BASED VIRTUAL SCREENING METHODS. *Journal of microbiology, biotechnology and food sciences*,2024;14(2):e11876-e11876.
3. Kumar S. Antimicrobial resistance: A top ten global public health threat. *EClinicalMedicine*,2021;41:101221.
4. MacNair CR, Rutherford ST, Tan MW. Alternative therapeutic strategies to treat antibiotic-resistant pathogens. *Nature Reviews Microbiology*,2024;22(5):262-275.

5. Qaeed MA, *et al.* Effect of different ratios of mentha spicata aqueous solution based on a biosolvent on the synthesis of agnps for inhibiting bacteria. *Journal of Nanomaterials*,2023;2023(1):3599501.
6. Qaeed MA, *et al.* The effect of different aqueous solutions ratios of Ocimum basilicum utilized in AgNPs synthesis on the inhibition of bacterial growth. *Scientific Reports*,2023;13(1):5866.
7. Al-Masaudi SB, *et al.* *In vitro* antibacterial activity of honey against multidrug-resistant Shigella sonnei. *Complementary Therapies in Clinical Practice*,2020;41:101257.
8. Al-Masaudi SB, Al-Maaqar SMS. Susceptibility of multidrug-resistant enteric pathogenic diarrheal Bacteria to Saudi Honey. *Journal of King Abdulaziz University: Science*,2020;32(1):47-64.
9. Alshammari FA, Al-Maaqar SM. Effects of environmental factors and nutritional requirements on EPS production and bioactivity. *Biologia*,2024:1-14.
10. Mauludiyana S, Dachlan YP, Saputro ID. Anti-inflammatory and antibacterial potential of Ajwa date (Phoenix dactylifera L.) extract in burn infection. *Journal of Advanced Pharmaceutical Technology & Research*,2023;14(3):161-165.
11. Al-Yahya M, *et al.* 'Ajwa' dates (Phoenix dactylifera L.) extract ameliorates isoproterenol-induced cardiomyopathy through downregulation of oxidative, inflammatory and apoptotic molecules in rodent model. *Phytomedicine*,2016;23(11):1240-1248.
12. Almutairy B. Extensively and multidrug-resistant bacterial strains: case studies of antibiotics resistance. *Frontiers in Microbiology*,2024;15:1381511.
13. Anwar S, *et al.* Role of Ajwa date fruit pulp and seed in the management of diseases through *in vitro* and *in silico* analysis. *Biology*,2022;11(1):78.
14. Khalid S, *et al.* A review on chemistry and pharmacology of Ajwa date fruit and pit. *Trends in food science & technology*,2017;63:60-69.
15. Zhang C-R, *et al.* Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa date fruit. *Journal of agricultural and food chemistry*,2013;61(24):5834-5840.
16. Alsarayrah NA, *et al.* The health values of Phoenix dactylifera (dates): A review. *Emirates Journal of Food and Agriculture*,2023;1:1-16.
17. Chao CT, Krueger RR. The date palm (Phoenix dactylifera L.): overview of biology, uses, and cultivation. *HortScience*,2007;42(5):1077-1082.
18. Al-Khayri JM, *et al.* Advances in date palm (Phoenix dactylifera L.) breeding. *Advances in Plant Breeding Strategies: Fruits: Volume 3*,2018:727-771.
19. Assirey EAR. Nutritional composition of fruit of 10 date palm (Phoenix dactylifera L.) cultivars grown in Saudi Arabia. *Journal of Taibah University for science*,2015;9(1):75-79.
20. Hussain MI, Farooq M, Syed QA. Nutritional and biological characteristics of the date palm fruit (Phoenix dactylifera L.)—A review. *Food Bioscience*,2020;34:100509.
21. Tafti AG, Fooladi M. A study on the physico-chemical properties of Iranian Shamsaei date at different stages of maturity,2006.
22. Baliga MS, *et al.* A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.). *Food research international*,2011;44(7):1812-1822.
23. Al Udhaib R. Solvent Extraction of Antioxidants, Phenols and Flavonoids from Saudi Arabia Dates,2015.
24. Wild CP, Stewart BW, Wild C. *World cancer report 2014*,2014 World Health Organization Geneva, Switzerland.
25. Khalid S, *et al.* Nutritional assessment of ajwa date flesh and pits in comparison to local varieties. *JAPS: Journal of Animal & Plant Sciences*,2016;26(4).
26. Hamad I, *et al.* Metabolic analysis of various date palm fruit (Phoenix dactylifera L.) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules*,2015;20(8):13620-13641.
27. Samad MA, *et al.* Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (Phoenix dactylifera) cultivars. *Molecules*,2016;21(4):419.
28. Al-Turki S, Shahba MA, Stushnoff C. Diversity of antioxidant properties and phenolic content of date palm (Phoenix dactylifera L.) fruits as affected by cultivar and location. *J. Food Agric. Environ*,2010;8(1):253-260.
29. Al-Farsi M, *et al.* Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. *Journal of agricultural and food chemistry*,2005;53(19):7592-7599.
30. Chirumbolo S. Plant phytochemicals as new potential drugs for immune disorders and cancer therapy: really a promising path? *Journal of the Science of Food and Agriculture*,2012;92(8):1573-1577.
31. Hussain Mallhi T, *et al.* Ajwa date (Phoenix dactylifera): an emerging plant in pharmacological research. *Pakistan journal of pharmaceutical sciences*,2014;27(3).
32. Ragab AR, *et al.* Antioxidant and tissue-protective studies on Ajwa extract: dates from Al Madinah Al-Monwarah, Saudia Arabia. *J Environ Anal Toxicol*,2013;3(163):2161-0525.1000163.
33. Shahbaz K, *et al.* Cytotoxic and antioxidant effects of Phoenix dactylifera L. (Ajwa date extract) on oral squamous cell carcinoma cell line. *BioMed Research International*,2022;2022(1):5792830.
34. Chaira N, *et al.* Simple phenolic composition, flavonoid contents and antioxidant capacities in water-methanol extracts of Tunisian common date cultivars (Phoenix dactylifera L.). *International journal of food sciences and nutrition*,2009;60(sup7):316-329.
35. Saleh EA, Tawfik MS, Abu-Tarboush HM. Phenolic contents and antioxidant activity of various date palm (Phoenix dactylifera L.) fruits from Saudi Arabia. *Food and Nutrition Sciences*,2011:2011.
36. Ahmed A, *et al.* Nutritional probing and HPLC profiling of roasted date pit powder. *Pakistan Journal of Nutrition*,2016;15(3):229.
37. Zhang C-R, *et al.* Health-benefits of date fruits produced in Saudi Arabia based on *in vitro* antioxidant, anti-inflammatory and human tumor cell proliferation inhibitory assays. *Journal of the Saudi Society of Agricultural Sciences*,2017;16(3):287-293.

38. Arshad FK, *et al.* A relative *in vitro* evaluation of antioxidant potential profile of extracts from pits of Phoenix dactylifera L.(Ajwa and Zahedi dates). Int J Adv Inf Sci Technol,2015;35(35):28-37.
39. Sharma G, *et al.* Anti-inflammatory activity and total flavonoid content of Aegle marmelos seeds,2011.
40. Talhouk R, *et al.* Anti-inflammatory bioactivities in plant extracts. Journal of medicinal food,2007;10(1):1-10.
41. Gupta SK, *et al.* Curcumin prevents experimental diabetic retinopathy in rats through its hypoglycemic, antioxidant, and anti-inflammatory mechanisms. Journal of Ocular Pharmacology and Therapeutics,2011;27(2):123-130.
42. Hasan M, Mohieldein A. *In vivo* evaluation of anti diabetic, hypolipidemic, antioxidative activities of Saudi date seed extract on streptozotocin induced diabetic rats. Journal of clinical and diagnostic research: JCDR,2016;10(3):FF06.
43. Eid N, *et al.* The impact of date palm fruits and their component polyphenols, on gut microbial ecology, bacterial metabolites and colon cancer cell proliferation. Journal of nutritional science,2014;3:e46.
44. Jassim SA, Naji MA. *In vitro* evaluation of the antiviral activity of an extract of date palm (Phoenix dactylifera L.) pits on a Pseudomonas phage. Evidence-Based Complementary and Alternative Medicine,2010;7(1):57-62.
45. Hussain MI, *et al.* Phenolic composition and antimicrobial activity of different Emirati date (Phoenix dactylifera L.) pits: a comparative study. Plants,2019;8(11):497.
46. Bokhari NA, Perveen K. *In vitro* inhibition potential of Phoenix dactylifera L. extracts on the growth of pathogenic fungi. Journal of Medicinal Plants Research,2012;6(6):1083-1088.
47. Abdullah N, Ishak NM, Shahida WW. *In-vitro* antibacterial activities of Ajwa date fruit (Phoenix dactylifera L.) extract against selected gram-negative bacteria causing gastroenteritis. Int. J. Pharm. Sci. Res,2019;10(6):2951-2955.
48. Gibson M, *et al.* Solvent Extraction of Antioxidants, Phenols and Flavonoids from Saudi Arabia Dates,2015.
49. Aamir J, *et al.* Evaluation of the combinational antimicrobial effect of Annona Squamosa and Phoenix Dactylifera seeds methanolic extract on standard microbial strains. International Research Journal of Biological Sciences,2013;2(5):68-73.