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Antimicrobial activities of Fragaria × ananassa leaves against multidrug-resistant *Acinetobacter baumannii*

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KEY WORDS

Strawberry (*Fragaria ananassa*) leaf, Multidrug-resistant (MDR), *Acintobacter baumannii*, Sterilized, Non-sterilized, Extract

ABSTRACT

This research assesses *Fragaria ananassa* leaf efficacy as an antibacterial agent against multidrug resistance (MDR) bacteria. The following cross-sectional research was performed from August 2018 to March 2019. About 120 bacterial isolates were isolated from 600 patients (276 males and 334 females) admitted to the Liver Institute Hospital (LIH) ICU. Antibiotic susceptibility was assessed using the VITEK 2 Compact ID/AST system. Among the biochemically described bacterial isolates, *Acinetobacter baumannii* was found to be multidrug-resistant, and its molecular identification was carried out using the 16S rDNA marker gene. Different Solvents, such as methanol, ethanol, acetone, and ethyl acetate were employed separately to extract the chemicals from the strawberry leaves. These extracts were tested for their antibacterial efficacies against the isolated *Acinetobacter baumannii*. The agar well diffusion results showed that the antibacterial activities were subsequently 25, 21, 12, and 26 mm for ethanol, methanol, ethyl acetate, and acetone. Sterilization at a high, moist temperature did not diminish the extract's antibacterial activity. The leaf extract was efficacious against *Acinetobacter baumannii*, with a minimum inhibitory concentration of 2 mg/mL and a minimum bactericidal concentration of 4 mg/mL. In conclusion, both cold and heat-treated strawberry leaf extracts are efficient as a conventional therapy against multidrug-resistant bacteria.

Introduction

Bacteria that exhibit resistance to at least three distinct types of antimicrobial medicines may be categorised as multidrug-resistant bacteria **(Magiorakos et al., 2012)**. It is possible for a single bacterium harbors numerous unique resistance genes, wherein each gene provides resistance against a particular drug. Phytotherapy may be a realistic option for treating multidrug-resistant microorganisms (MRB). According to the World Health Organisation (WHO), an estimated 80% of individuals residing in developing nations depend on traditional medicine as their major source of healthcare, with a predominant focus on using medicinal plants **(Lindmeier, 2018)**. Several phytotherapy manuals have underscored various of medicinal plants that exhibit potential efficacy in addressing infectious diseases due to their widespread availability, fewer side effects, and reduced toxicity **(Braga et al., 2005)**. Numerous studies have reported the antimicrobial properties of various herbal extracts **(Braga et al., 2005; Boucher et al., 2009)**. There are multiple sources of guidance available on numerous botanical species that have been recognized for their therapeutic properties in treating diseases such as urinary tract infections, gastrointestinal, respiratory, and dermatological infections, alongside various other medical disorders **(Brantner and Grein, 1994; De Boer et al., 2005)**. These secondary metabolites of plants, including alkaloids, flavonoids, and terpenoids, benefit human and animal health **(Roy et al., 2022).** Approximately 12,000 of these chemicals have so far been identified **(Kårlund et al., 2015).**

Antibiotics are the mainstay of treatment for bacterial illnesses. However, antibiotic misuse has become the leading cause of the creation and spread of multidrug-resistant strains of many microorganism families **(Coates et al., 2002).** The global health community is highly concerned about creating and spreading antibiotic resistance and introducing novel disease-causing pathogens. Given the evidence of the fast global spread of antibiotic-resistant clinical isolates, it is crucial to discover novel antimicrobial drugs**.** To combat the rise of multidrug-resistant bacteria, scientists increasingly resort to herbal remedies to find new therapeutic avenues **(Coates et al., 2002).**

The strawberry (*Fragaria ananassa*), a member of the Rosaceae family, is farmed commercially worldwide because of its therapeutic value. The Romans first documented

strawberries' medicinal properties, and from there, they spread across ancient Greece. The berries acquired appeal as a potential gout treatment and gastrointestinal assistance **(Morris and Sistrunk, 2018)**. Like other members of the Rosaceae family, the strawberry plant, such as the apple, peach, plum, raspberry, and pear, has a wealthy secondary metabolites composition consisting of hundreds of non-volatile and volatile chemicals. Strawberries contain polyphenols that may protect the human body against many diseases and illnesses **(Kårlund et al., 2014).**

Strawberry leaves have a high concentration of polyphenolic chemicals **(Kårlund et al., 2014)**. Polyphenols are vast secondary plant metabolites with a broad biological activity range. Due to their potential health benefits, they have been the focus of several scientific investigations. There have been reports of potential health benefits of strawberry polyphenols against cancer, cardiovascular disorders**,** Alzheimer's disease, and inflammation-related diseases **(Edirisinghe et al., 2011)**.

Strawberry polyphenols showed a variety of antibacterial properties against *Streptococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Acinetobacter* sp., *Listeria monocytogenes, Micrococcus sp., Proteus sp.,* and *Bacillus sp.* **(Othman et al., 2019)**. Consuming polyphenols can decrease the likelihood of developing multidrug-resistant bacterial infections **(Satchanska, 2022).** *A. baumannii* belongs to the family *Moraxellaceae,* order *Pseudomonadales,* class *Gammaproteobacteria,* and phylum *Proteobacteria* **(Peleg et al., 2008)**. *Acinetobacter baumannii* has long been considered a benign microorganism, especially in the context of healthcare facilities **(Howard et al., 2012)**. The remarkable inclination of *A. baumannii* to acquire and develop resistance against nearly all drugs in our antibiotic arsenal has become a distressing challenge for healthcare communities worldwide **(Vázquez-López et al., 2020)**. The World Health Organisation (WHO) has classified it as one of the "priority pathogens" that pose the greatest threat to human health **(Lindmeier, 2018)**.

The incidence of fatalities and impairments resulting from *A. baumannii* infections is increasing. Retrospective research indicates that the death rates associated with *A. baumannii* infections vary from 29% to 71.6% in Europe and 22.8% to 49.6% in the US **(Patel et al., 2019)**. The death rates linked with hospital-acquired and

ventilator-associated pneumonia were higher in Western Asia (56.2%), Southern Europe (55.7%), and Northern Africa (53.3%) compared to other locations. The countries in the Mediterranean region with the highest recorded mortality rates were Greece (68.2%), Turkey (61.4%), and Egypt (53.3%) **(Mohd Sazlly Lim et al., 2019)**. In Egypt, the prevalence of carbapenem-resistant *A. baumannii* isolates ranges from 26.6% to 100%, whereas the proportion of *A. baumannii* isolates that are multidrug-resistant (MDR) is estimated to be between 30% and 100% **(El-Kholy et al., 2021)**.

The mechanisms underlying antimicrobial resistance in *A. baumannii* can be broadly classified into three categories: 1) decreased accessibility to bacterial targets due to reduced outer membrane permeability resulting from the loss or decreased expression of porins, as well as the overexpression of multidrug efflux pumps, 2) production of antimicrobial-inactivating enzymes, and 3) mutations in penicillin-binding proteins (PBPs) that modify targets or cellular processes **(Rice, 2006)**. Due to its virulence and its resistance to many antibiotics, various medicinal plants, such as ginger (*Zingiber officinale*), lime (*Citrus aurantifolia*), and galangal (*Alpinia galanga Linn*.), were tested against *Acinetobacter baumannii* **(Intorasoot et al., 2017)**.

In addition, various solvent extracts were used to assess the antibacterial affects. For instance, the methanol extract of *Hibiscus sabdariffa* calyces, commonly used by Sudanese people showed efficacy against MDR *Acinetobacter baumanii* (Abdallah, 2016). Also, strawberry fruits have been tested as an antibacterial agent against bacteria causing urinary tract infection, 100% strawberry extract concentration was used to inhibit both monospecies and polyspecies *Porphyromonas gingivalis* and *E. faecalis* biofilm formation (Liya and Siddique, 2018). This study establishes the antimicrobial efficacy of *Fragaria ananassa* leaves against *Acinetobacter baumanii* a virulent multidrug-resistant bacterium that causes serious nosocomial infections in Egyptian hospitals.

Materials and Methods

Sample collection and isolation of pathogenic bacteria

This cross-sectional research took place from August 2018 to March 2019 after securing informed consent from all the participating patients. The National Liver Institute (NLI) and Menoufia University's local Ethics Committee accepted the study protocol. Six-

hundreds infected patients (276 males and 334 females) hospitalized in the ICU of Liver Institute Hospital (LIH) provided various clinical samples, which included blood, sputum, urine, T. tube (a silicone stent for the trachea with an external limb), ascitic fluid and drains. All the patients considered had developed nosocomial infections at least 48 hours after being admitted to the hospital. Within 2 hours, all the samples had been delivered to the NLI's microbiological lab for analysis. Nutrient agar, blood agar, MacConkey agar, and mannitol salt agar (Oxoid, UK) were used to cultivate the samples, which were then incubated at 37^{o}C under aerobic conditions for 24 to 48 hours. Blood Samples were inoculated in blood culture bottles and incubated in the BACT/ALERT system (Biomeriuex, France). After 24 hours of incubation at 37 degrees Celsius, positive cultures were subcultured on standard microbiology media such as blood agar, MacConkey agar, and nutrient broth. The purified pathogenic bacteria were identified primarily depending on their morphological and biochemical characteristics **(Lagier et al., 2015)**.

Identification of *Acinetobacter* isolates

Acinetobacter isolates were identified using standard microbiological methods **(Lagier et al., 2015)**. *Acinetobacter* forms smooth, sometimes mucoid, pale yellow to greyish white colonies, about 1–2 mm in diameter. Therefore, presumptive identification of *Acinetobacter* is based on colony appearance and biochemical characteristics. Species identification was further confirmed using the VITEK 2 Compact automated ID/AST instrument (Biomerieux, France) **(Percival et al., 2014)**.

Molecular identification

In sum, according to the supplier's instructions, the microbe was cultured for genomic DNA extraction by GeneJet genomic DNA Kit (Thermo K0721). The 16S rDNA was amplified using 27F and 1492R by PCR. Then, the PCR product was purified by QIAquick, Qiagen Inc. kit. Next, the DNA was sequenced by 27F/518R primers through the applied biosystems 3130 automated DNA sequencer (ABI, 3130, USA). The obtained sequence was uploaded to the NCBI database under the sequence identifier MW661233.1, which was used as a query in the blastn algorithm (e-value 1e-11 and a word size of 128) to retrieve the authentic homologs **(Zayed and Badawi, 2020; Zayed et al., 2022)**. The retrieved sequences were aligned using MAFFT version 7 **(Katoh et al., 2019)**, and the maximum likelihood tree was inferred assuming the lowest BIC

(Bayesian Information Criterion) score model, which is K2P+I via the iqtree software **(Hoang et al., 2018)**.

Detection of antibiotic resistance of *Acinetobacter baumannii*

The following antibiotics were used to detect the antibiotic resistance of *Acinetobacter baumannii*: Ampicillin/sulbactam (10/10 µg), cefepime (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), ceftriaxone (30 µg), meropenem (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), levofloxacin (5 µg), colostin (10 µg), and trimethoprim/sulphamethoxazole (1.25/23.75 µg). The bacterial suspension was inoculated and uniformly distributed on the surface of HM agar plates; then, the antibiotic discs were placed in the center of the plates. The plates were incubated at 37^{o}C for 24 hours. The antibiotics that produced an inhibition zone were considered effective against the studied bacterial isolates, while those that recorded no inhibition zone were classified as ineffective against the bacterial isolates **(Jorgensen and Ferraro, 2009)**.

Plant collections and preservation

Leaves of *Fragaria ananassa* were gathered in the village of Met Asem, Menofuia, Egypt. A plant taxonomist from the Botany and Microbiology Department, Faculty of Sciences at Menofuia University, Egypt, identified and authenticated the three samples. The leaves were rinsed under a continuous tap water flow and subsequently treated with distilled water to remove any accumulated dirt particles. They were then completely dried at room temperature and finely ground using an electrical grinder (Monilex mixer grinder, France) at a speed of 5 for three minutes **(Tayel et al., 2012)**.

Bioactive compounds extraction

30g of each ground sample was soaked for three days in 100 mL of each solvent: 70% methanol, ethanol, acetone, ethyl acetate, and distilled water at room temperature. After three days, the mixture was filtered through a filter paper (Whatman No. 2), and then the supernatant part was taken. The solvent was then evaporated at room temperature until reaching a constant weight. The residual dried extract was dissolved in 20% DMSO as a preservative to obtain extract with a concentration of 100 mg/mL as a stock solution, put in dark amber glass vials, keeping it from destruction by light, and kept at 4 degrees Celsius until use **(Tayel et al., 2012)**.

The extract is divided into two sections. The first portion was sterilized in an autoclave at 121°C for 20 minutes

(autoclave-treated extract (AE)), while the second portion was used without autoclaving (non-autoclave-treated extract (NAE)). The effect of sterilization on bioactive compounds was then evaluated between the two.

Detection of the antibacterial activity of strawberry leaf extract

Extract of leaves was produced just before the test. The extracts' antibacterial activity was tested using the agar well diffusion method according to **Hegazy et al., (2020)** on the Mueller-Hinton Agar (MHA). The bacterial suspensions ($1.5x10^8$ CFU/mL) were distributed well on MHA plates. Each plate was drilled using a cork borer to create two wells (7mm). Approximately 100 µl (sterilized and non-sterilized) of each extract from our 10 mg/mL stock solution was applied to each well. The negative control was DMSO, however, colistin was used as the positive control. Each plate was incubated for 24 hours at 37°C. Bioactivity was determined after incubation by measuring the zone of inhibition. The entire experiment was carried out in triplicate.

Detection of MIC and MBC of the strawberry leaf extract

The minimum inhibitory concentration (MIC) is the lowest concentration known to inhibit the growth of *Acintobacter banumanni*. DMSO was utilized as a preservative since it is regarded as a promising agent for preserving the efficacy and vitality of plant extracts **(Tayel et al., 2012)**. MIC was determined using a 96-well microplate with a U-shape by adding 20 µl of a 24h-old nutrient broth bacterial culture (around $1.5x10^6$ CFU), followed by 100 µL of plant extract at concentrations of 2, 4, 6, 8, and 10 mg/mL **(Tayel et al., 2010)**. The plate was loosely covered with cling film to prevent dehydration of the bacteria, following overnight incubation at 37°C. The plates were then covered loosely with fresh, clean cling film to prevent dehydration of the bacteria and incubated at 37°C for 18-20 h. Observing the turbidity and recording the concentration that inhibits bacterial growth in the absence of turbidity is known as MIC. Minimum bactericidal concentration (MBC) refers to the lowest concentration capable of killing microorganisms. The MBC test was calculated by transferring 100 µL from MIC wells and higher concentrations (4, 6, 8, 10, 12) mg/mL to MH agar. The inoculum was uniformly distributed and incubated on the plates for 24 h. at 37°C; MBC is confirmed when there is no bacterial growth on Muller Hinton agar. The experiment was done three times for each solvent.

Results

Biochemical and morphological identification of isolated pathogenic bacteria

Our research sought to isolate bacteria that cause nosocomial infections in hospitalized patients at LIH, Egypt. Approximately 120 samples of pathogenic bacteria were isolated from intensive and moderate ICU, internal medicine, and other units during the period of our study, during which the incidence of nosocomial infections was approximately 20%. The bacterial isolates were identified using gram stains and biochemical tests (Figs. 1&2). From a total of 120 bacterial samples, 52 (43%) are gram-negative, and 68 (57%) are gram-positive; additionally, females have a higher infection rate (54%) than men (Fig. 1).

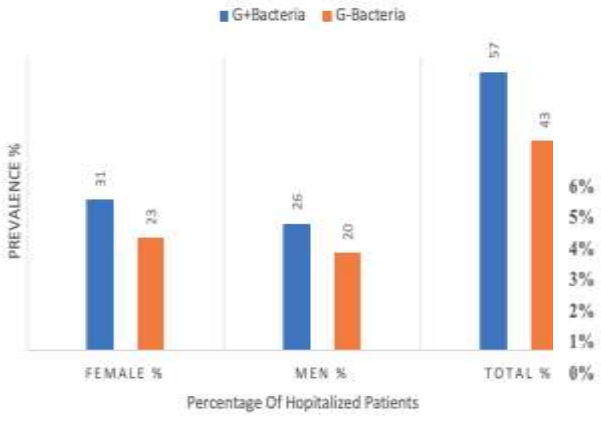

Fig. (1): Gram-positive (G+) and Gram-negative (G-) bacteria prevalence in hospitalized patients

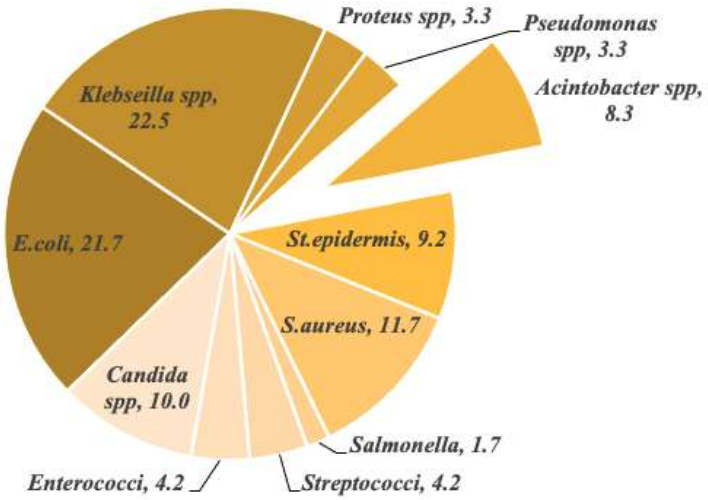

Fig. (2): Total percentage of detected pathogenic microorganisms in all clinical samples collected.

The infection rate was highest for *Klebsiella* spp. at 22.5%, followed by *E. coli* at 21.7%, *S. aureus* at 11.7%, *Candida* sp. at 10%, *St. epidermis* at 9.7%, and *Acinetobacter* spp. at 8.3%. (Fig. 2). Also, *Klebsiella* sp., *E. coli, S. aureus,* and *St. epidermis* were predominant in almost all clinical sample groups (Fig. 3).

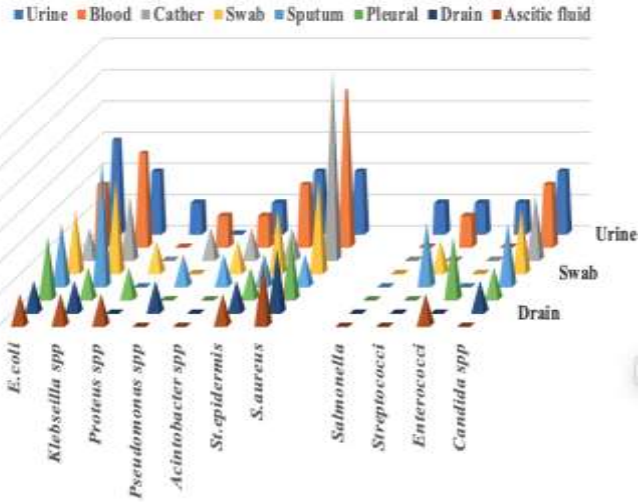

Fig. (3): Distribution of nosocomial pathogenic microorganisms that were isolated from various clinical samples

Table (1): Biochemical characteristics of the selected pathogenic bacteria using VITEK 2 Compact automated ID/AST equipment.

Biochemical test	*Acinetobacter baumannii* ASM21
Catalase	Positive
Citrate	Positive
Coagulase	Negative
Gas	Negative
Gelatin Hydrolysis	Negative
Gram Staining	Negative
H_2S	Negative
Hemolysis	Negative
Indole	Negative
MR (Methyl Red)	Negative
Nitrate Reduction	Negative
Oxidase	Negative
Pigment	Negative
Urease	Negative
VP (Voges Proskauer)	Negative
Galactose	Positive
Glucose	Positive
Lactose	Negative
Malonate	Positive
Mannitol	Negative
Mannose	Positive
Rhamnose	Positive
Sucrose	Negative
Xylose	Positive

Based on preliminary antibiotic sensitivity analysis, *Acinetobacter baumannii* has been selected as this study's focal point of exploration. *A. baumannii* is a gram-negative aerobic coccobacillus. Its identification was confirmed using VITEK 2 Compact automated ID/AST equipment (Table 1).

Phylogenetic analysis

A phylogenetic tree constructed using partial 16S rDNA sequences (V_{1-3}) is shown in Fig. 4. This tree utilizes the maximum likelihood analysis and incorporates 1000 bootstrap replicates to show the evolutionary relationships between our *Acinetobacter baumannii* strain ASM21 and other bacterial-type strains. The phylogenetic tree is rooted using the *Pseudomonas argentinensis* CH01 homologous sequence. The tree was visualized and presented using the iTOL (Interactive Tree of Life) webserver **(Letunic and Bork 2016)**.

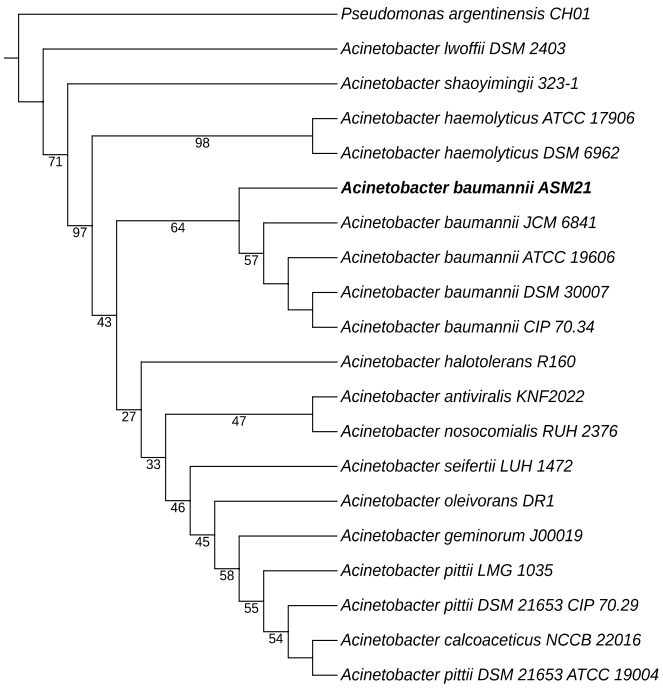

Fig. (4): A phylogenetic tree of *Acinetobacter baumanii* ASM21 using partial 16S rDNA sequences.

Antibiotic resistance of Acinetobacter baumannii

A. benoumonia's antibiotic resistance and susceptibility against nine antibiotics are mentioned in Table 2. According to the result, *Acinetobacter baumanii* was classified as an MDR strain as it resists three classes of the

studied antibiotics **(Falagas and Karageorgopoulos, 2008)**.

Table (2): Antibiotic susceptibility and resistance of the selected nosocomial bacteria *Acinetobacter baumannii*.

Antibiotic	Conc. (µg)	Resistance
Ampicillin/sulbactam	10/10	+
Amikacin	30	+
Ceftriaxone	30	+
Cefepime	30	+
Meropenam	10	+
Imipenem	10	+
Gentamicin	10	+
Tobramycin	10	+
Ciprofloxacin	5	-
levofloxacin	5	-
Piperacillin/ Tazobactam	100/10	-
Trimethoprim/ Sulfamethoxazole	1.25/23.75	-
Colistin	10	-

The bacterial isolate was resistant to the antibiotic = +. However, the bacterial isolate was sensitive to the tested antibiotic = -.

Antibacterial activity of the strawberry leaf extract.

The antibacterial activity of strawberry leaves has not been thoroughly studied using a variety of organic solvents. Hence, the antibacterial efficacy of *Fragaria ananassa* leaf extract against *Acinetobacter* bacteria was evaluated. Herby, acetone was the best organic solvent, resulting in the largest inhibition zone (26 mm), followed by ethanol (25 mm). Moreover, heat sterilization of the extract was discovered to increase its antibacterial activity (Table 3), as flavonoids in strawberry leaves hold up well in hot conditions. **Elhamirad and Zamanipoor, (2012)** found that glycosylated substances undergo thermal hydrolysis at higher temperatures, increasing their solubility and altering the diffusion coefficient, raising the concentration of flavonoids and increasing the antimicrobial activity of the sterilized extract.

Table (3): Effect of strawberry leaf extract dissolved in different organic solvents on the *A. baumannii* growth. These results are the means of three replicas.

	Organic solvent		Inhibition Zone (mm)
Fragaria x annanasa	Acetone	AE	26±1.00
		NAE	24±1.00
	Methanol	AE	21±0.57
		NAE	20±0.57
	Ethanol	AE	25±1.00
		NAE	24±0.57
	Ethyl acetate	AE	12±1.00
		NAE	11±0.57
Colostin (10µg)			13±0.57

AE; autoclaved extract, NAE; means Non autoclaved extract

Table (4): Minimal inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) of *Fragaria ananassa* leaf extract on the *Acinetobacter* bacteria

	Solvents	MIC (mg/mL)	MBC (mg/mL)
***Fragaria-X-annanasa* (AE)**	**Methanol**	2.00±0.00	4.00±0.00
	Ethanol	2.00±0.57	4.00±0.57
	Acetone	2.00±0.57	4.00±0.57
***Fragaria-X-annanasa* (NAE)**	**Methanol**	10.0±0.00	12.0±0.57
	Ethanol	10.0±0.57	12.0±0.57
	Acetone	8.00±0.57	10.0±0.57

MIC and MBC of the Fragaria ananassa leaf extract aganist *Acinetobacter baumanii* ASM21

The MIC and MBC of *Fragaria ananassa* leaf extract against *Acinetobacter* bacteria are determined in Table 4. The findings demonstrate that the leaf extract of *Fragaria ananassa* possessed potent inhibitory and bactericidal properties against the pathogenic activity. *A. baumannii* was completely eradicated at 4 mg/mL with heated treated extract and 12 mg/mL with unheated extract, regardless of the organic solvents used.

Discussions

A nosocomial infection, or healthcare-associated infection (HAI), is caused by infectious agents or their toxins. The infection develops after a minimum of 48 hours of admission to a healthcare facility when the infection is not present or in its incubation stage upon admission. The infection can be localized or systemic **(Razine et al., 2012)**. We effectively isolated approximately 120 samples of pathogenic bacteria from our study from diverse units, including the intensive and moderate ICU, internal medicine, and other departments. The most predominant strains were identified as *Klebsiella* sp., *E. coli*, *S. aureus*, *Candida* sp., *St. epidermis*, and *Acinetobacter* sp. (Fig. 2). *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were found to be present in almost all of the clinical samples selected for analysis, as depicted in Fig. 3. Several other studies have also identified the same bacterial strains as the primary pathogens associated with nosocomial infections (NIs) **(Davoudi et al., 2014)**.

Contrary to the findings reported in our research, a recent investigation revealed that the occurrence of multidrug-resistant (MDR) bacteria among isolated pathogenic bacteria amounted to 42.6% (23 out of 54). The two most observed multidrug-resistant (MDR) organisms were *Pseudomonas aeruginosa*, accounting for 30.4% of cases, and *Staphylococcus aureus*,

accounting for 21.7% **(Tolera et al., 2018)**. The study focused on *Acinetobacter baumannii*, a highly pathogenic bacterium known for its resistance to many drugs, and its role in causing serious nosocomial infections among hospitalized patients in Egypt. In addition, the World Health Organization (WHO) included it in its compilation of "priority pathogens", referring to bacteria that present the most significant risk to human health **(Lindmeier, 2018)**.

In the human body, due to morphological modifications, *A. baumannii* has a natural resilience to desiccation that allows it to remain viable for months. Due to the bacterium's capacity to avoid the host's immune system and develop biofilm, infections caused by *A. baumannii* may be exceedingly challenging to cure. In addition, *A. baumannii* has an array of resistance strategies, such as multidrug efflux pumps, β-lactamases, permeability deficiencies, aminoglycoside-modifying enzymes, and alterations of target sites **(Wong et al., 2017)**. The current literature highlights a significant gap in research exploring the antibacterial effects of strawberry leaf extract against Acinetobacter.

Based on the findings of our study, the bacterial strain under investigation was determined to possess multidrug resistance. The popularity of traditional medicines, particularly those derived from botanical sources, is rising due to their environmentally sustainable characteristics and reduced incidence of adverse effects, which starkly contrast to modern synthetic and chemical medications, which have experienced a decrease in public favor as a result of an increasing number of side events **(Farnsworth et al., 1985)**. The antibacterial effectiveness of *Fragaria ananassa* leaf extract against *Acinetobacter* bacteria was assessed using a range of organic solvents. Acetone was identified as the most effective organic solvent, exhibiting the biggest inhibitory zone with a diameter of 26 mm. Furthermore, it has been observed that applying heat sterilization to the extract leads to an augmentation in its antibacterial efficacy, as seen in Table 3. Considering parallel research, the ethanolic extract of strawberries had stronger antibacterial activity against pathogenic microorganisms than the methanolic extract **(Liya and Siddique, 2018)**. The antibacterial activity of the strawberry extract was highest against *P. aeruginosa* and lowest against *K. pneumoniae*.

The findings of our study indicate that the leaf extract of *Fragaria ananassa* exhibited a noteworthy

inhibitory impact on the development of *Acinetobacter*. The minimum inhibitory concentration (MIC) was determined to be 2 mg/mL when using the AE, while NAE demonstrated a MIC of 8 mg/mL. The AE and NAE have minimum bactericidal concentrations (MBCs) of 4 and 10 mg/mL, respectively. The results of this study indicate that the leaf extract of *Fragaria ananassa* can be a potential antibacterial agent against *Acinetobacter baumanniii*.

Conclusions

In the light of the recent proliferation of MDR bacteria, it is not surprising that traditional treatments based on plant extracts are gaining favor as an alternative to modern pharmaceuticals. Strawberry fruits exhibit potential health benefits, but further research is still needed to elucidate the power of the strawberry leaf chemicals against MDR *Acinetobacter*. This study tested the effectiveness of *Fragaria ananassa* leaf extract against multidrug-resistant *Acinetobacter* bacteria and looked at the usage of several organic solvents to prepare a strawberry leaf extract. The results showed acetone was the most effective organic solvent, producing an inhibitory zone 26 mm in diameter, followed closely by ethanol at 25 mm. In addition, it was found that the extract's antibacterial activity is enhanced through heat sterilization. The efficacy of the leaf extract against *Acinetobacter baumannii* has been observed, with a minimum inhibitory concentration of 2 mg/mL and a minimum bactericidal concentration of 4 mg/mL for the heat-treated extract. In contrast, the non-heat-treated extract exhibited minimum inhibitory and bactericidal concentrations of 8 mg/L and 10 mg/L, as this confirms that sterilization has a great effect on bioactive compounds of extract and gives a higher antibacterial effect than non-sterilized ones, respectively. In conclusion, strawberry leaf extract is effective as a traditional therapy against multidrug-resistant bacteria, whether under cold or heat extraction.

Competing interests

The authors declare no competing interests.

Funding

The authors declare that they did not receive any fund to conduct this study.

Ethics declarations

The study was approved by the Institutional Review Board of the National Liver Institute (NLI), Menoufia University, Egypt (NLI IRB Protocola Number 00474/2023) under IRB Name: NLI IRB 00003413 FWA0000227. The NLI follow the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Guideline for Good Clinical Practice (GCP).

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النشاط المضاد للميكروبات لأوراق الفراوله على بكتيريا الاسينيتوبكتر بنيومنيا المقاومه للمضادات الحيويه

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يقيم هذا البحث فاعليه اوراق الفراوله كعامل مضاد للميكروبات ضد البكتيريا المقاومه للمضادات الحيويه .،تم اجراء الدراسه المعمليه في الفتره من اغسطس ٢٠١٨ حتي مارس٢٠١٩. تم عزل حوالي ١٢٠ عزله بكتيريه من ٦٠٠ مريض (٢٧٦ ذكرًا و٣٣٤ أنثى) تم إدخالهم إلى وحدة العناية المركزة بمستشفى معهد الكبد القومي). تم تقييم اختبارالحساسية الخاص بالمضادات الحيوية. من بين العزلات البكتيرية الموصوفة كيميائيًا، وجد أن بكتيريا الاسينتوبكتر مقاومة للعديد من المضادات الحيويه، وتم تحديد هويتها الجزيئية باستخدام التحليل الجيني للبكتيريا. تم استخدام مذيبات مختلفة بشكل منفصل لاستخلاص المواد الكيميائية من أوراق الفراولة و دراسه تأثيرتها المختلفه، مثل الميثانول والإيثانول والأسيتون وخلات الإيثيل. تم اختبار هذه المستخلصات لفعاليتها المضادة للبكتيريا ضد بكتيريا الاسينتوبكتر المعزولة. أظهرت نتائج انتشار بيئه الاجار أن الأنشطة المضادة للبكتيريا كانت فيما بعد ٢٥، ٢١، ١٢، و ٢٦ ملم للإيثانول، الميثانول، أسيتات الإيثيل، والأسيتون. التعقيم في الاوتوكليف عند درجة حرارة عالية ورطبة لم يقلل من نشاط المستخلص المضاد للبكتيريا. كان مستخلص اوراق الفراوله فعالاً ضد بكتيريا الاسينتوبكتر، وجد ان اقل تركيز من المستخلص النباتي له القدره علي ايقاف نشاط البكتيريه هو ٢ مل جم/مل وتركيز مبيد البكتيريا لا يقل عن ٤ مل جم/مل.

في الختام: تعتبر مستخلصات أوراق الفراولة المعالجة بالحرارة وغير المعالجه فعال كعلاج تقليدي ضد البكتيريا المقاومة للمضادات الحيويه.