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The effect of some amino acids and natural materials on inhibiting the swarming motility of *Proteus mirabilis*

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Proteus mirabilis is a Gram negative bacterium, which exhibits swarming motility, and is characterized by rapid and coordinated surface migration. Therefore it is considered a significant pathogen, a leading cause of catheter-associated urinary tract infection (CaUTI). In this research, the isolation and identification of *Proteus mirabilis* was accomplished from different clinical cases (22) urine, (7) blood, and (1) sample each for an ear discharge and a burn case. All of the samples were cultured primarily on both nutrient agar and MacConkey's agar. After that, ordinary tests (cultural and cellular characteristics as well as biochemical tests) were carried out to diagnose this bacterium. In addition, molecular identification depending on the 16srRNA gene was also followed using the PCR technique after extraction of the DNA. Attempts to inhibit the swarming motility were made using six amino acids including (alanine, valine, aspartic acid, cystine, histidine, and tryptophan) chosen randomly, as well as aqueous extraction of some natural materials, including corn silk (*Zea mays*), parsley (*Petroselinum crispum*), dried orange peel and mallow (*Malva parviflora*), which were selected based on traditional remedies commonly prescribed to the general population by herbalists for the treatment of urinary tract infections. The phenotypic identification of *Proteus mirabilis* exhibited catalase positive, oxidase negative, positive for urase production and this bacterium was able to ferment the lactose and glucose in Kligler medium. In addition, the molecular diagnosis represented by data from 16srRNA sequencing, which was analyzed by the National Center for Biotechnology Information through Local Alignment Search Tool, helped to identify five strains of *Proteus mirabilis* from all samples, two of which were recorded in NCBI with accession numbers (OR976487 and OR976486. The results of the study of the impact of the amino acids on swarming motility of *P. mirabilis* demonstrate that the strains S3 and S4 exhi-bited complete inhibition with all tested amino acids. Strain Ghmosu-1 was affected by all amino acids except valine, which had a minimal effect. Strain Ghsumo-2 showed complete inhibition with valine, alanine, aspartic acid, and cysteine, while histidine and tryptophan had no effect. Finally, strain L was the least affected, as its motility was not entirely inhibited but only slightly weakened by certain amino acids. For natural materials, the strongest substances that primarily inhibited the swarming were orange peels and mallow. Parsley came in the second rank because it inhibited the movement in two strains only.However, the corn silk material did not show any inhibitory effect on the swarming movement for any of the three strains. The natural com-pounds tested did not align with herbalist recommendations for treating urinary tract infections. Therefore, reliance on these com-pounds is discouraged, and physicians should remain the primary authority for prescribing appropriate medications.

Keywords: alanine; aspartic acid; cystine; aspartic acid; corn silks; parsley; orange peel; mallow.

Introduction

Swarming represents a flagella-driven form of collective bacterial movement, enabling a population of cells to spread across a semi-solid surface. This phenomenon has been observed across various bacteria, including genera such as *Bacillus*, *Proteus*, *Pseudomonas*, and *Serratia*. Although both Gram-positive and Gram-negative bacteria are capable of swarming, the specific environmental conditions required for swarming and the biological traits influencing its development vary between species. Under laboratory conditions, swarming is influenced by several environmental factors, including nutrient availability and humidity levels (Srinivasan et al., 2019; Kotian, et al., 2020). In addition, a general requirement for swarming motility is the activity of the flagellum. In many bacterial species, multiple flagella are produced during swarming (Nakamura & Minamino, 2019; Partridge, 2022).

When swimming cells are transferred to solid culture media, they soon come into contact with the solid implant surface and undergo physiological and morphological changes, as they begin to differentiate into more elongated cells, ranging in length from 20–80 µm, and surrounded by a large number of hyperflagellated flagella (Kasallis et al., 2023).

Proteus mirabilis is an opportunistic bacterium which causes urinary tract infections (UTIs) (Talebi et al., 2023), and catheter-associated UTIs (Chakkour et al., 2024), as well as a variety of infections such as infection stones, otitis externa, bacteremia and endocarditis when host immunity is weakened (Ioannou & Vougiouklakis 2020). *Proteus mirabilis* has virulence factors which include flagella, fimbriae, capsule polysaccharide efflux pump and production of urease enzymes (Wasfi et al., 2020). Urease hydrolyzes the urea in the urine to form ammonia, which raises the pH, producing an alkaline urine. This encourages the formation of stones (calculi) called "struvite" composed of magnesium ammonium phosphate. Because alkaline urine also favors growth of organisms and more extensive renal damage, treatment involves keeping the urine at a low pH.

Proteus mirabilis can migrate in groups of cells driven by flagella on semi-solid surfaces by swarming behavior. Differentiation of motile swarm cells is strictly regulated and involves multilayer signaling networks. Controlling swarming behavior is of crucial interest in developing new infection control strategies (Rütschlin & Böttcher, 2019; Altaey et al., 2025).

This research aimed to investigate the use of specific compounds, including amino acids and natural substances, to evaluate their efficacy in inhibiting swarming motility in *P. mirabilis*. Such inhibition is proposed as a preliminary strategy to prevent bacterial adhesion to host cells. This study represents an initial *in vitro* exploration, with the potential for future *in vivo* applications if proven successful.

Materials and methods

Sample collection. Thirty one different clinical samples were collected from hospitals in Mosul city (Iraq) in the period from September 2023 to October 2023 from both sexes and all age groups. The samples included: urine (22), blood (7), and one sample each for ear discharge and burns. All samples were cultured on nutrient agar (Himedia, India) and MacConkey's agar (Oxoid, England).

Phenotypic identification. The expected bacterial colonies were initially diagnosed as *Proteus* on the basis of their cultural properties of this bacterium like the swarming phenomenon on nutrient agar and pale colonies on MacConkey's agar. Then a Gram stain (BDH, England) was achieved for suspected isolates to detect the rod form and negative reaction represented by cells with red color. After that, some chemical tests were performed depending on (Cappuccino and Welsh 2020), such as oxidase activity using the indicator: N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride; catalase examination to observe the bubble formation produced by the bacterial cells using 3% H_2O_2; as well as detection of urease activity and sugar fermentation on Kligler medium.

Molecular identification. Firstly, DNA was extracted for all 31 isolates by following the instruction of the DNA extraction kit (Geneaid, Taiwan); the primers for detection of 16srRNA gene were 27F 5-AGAGTTTGATCCTGGCTCAG-3 and 1492 R 5-TACGG TTACCTTGTTACGACTT-3 depending on (Nagara et al., 2017), and Green Master Mix (2X) (Promega, USA) was used. Polymerase Chain Reaction (PCR) protocol was followed according to (Adnan et al., 2014), with DNA less than 250 ng/total volume of PCR reaction and 0.1 µmol for both primers as well as 1X from the master mix. Electrophoresis was achieved on agarose 2%, finally the PCR products were sent to Psomagen sequencing company (USA), when the results were received as sequences of the nitrogenous bases, the data was analyzed by the National Center for Biotechnology Information (NCBI) through Local Alignment Search Tool (BLAST) to compare the sequence of our bacterial strains with other global registered strains.

Inhibition the swarming. Two types of substances were used in attempt to inhibit the swarming motility of *P. mirabilis*, they were:

1) amino acids:

– six amino acids chosen randomely were used in this experiment including: alanine, valine, aspartic acid, cysteine, histidine and tryptophan;

– 0.1 g of each amino acid was added to 100 mL of melted nutrient agar medium with 0.1% as final concentration. the mixture was sterilized by autoclave (Gallenkamp, England) at 121 ºC with pressure: 15 pounds per square inch for 20 minutes. Then the mixture was poured into Petri dishes;

– five bacterial strains of *P. mirabilis* were inoculated on the medium supplemented with amino acids separately and incubated at 37 ºC for 24 hours as spot in the center;

– another nutrient medium plate was prepared without adding any amino acid to the culturing to be considered as a control of the work;

2) natural materials:

– four common natural materials were used to detect whether their aqueous extraction was able to inhibit swarming phenomena or not, including: corn silk (*Zea mays*), parsley (*Petroselinum crispum*), dried orange peel and mallow (*Malva parviflora*); all the materials were first dried at room temperature and crushed;

– the materials were selected based on the traditional use as described by herbalists treating the general public as an ancient remedy for treating kidney issues and urinary tract infections; this was done to evaluate the validity of these claims and their effectiveness in addressing such conditions;

– the aqueous extraction prepared depending on Sasirekha et al. (2014) by infusion 10 g from each material in 10 mL of water for 24 hr., the filtered volume of the soaking solution was completed to 100 mL of melted nutrient medium, then autoclaved in standard condition then poured into the plates;

– the medium supplemented with the substances was inoculated with *P. mirabilis* strains in the form of central spots and incubated under standard conditions.

Results

Isolation percentage and the phenotypic identification. Five strains of *P. mirabilis* were obtained from thirty one clinical samples, i.e., with percentage equal to (16%) and were diagnosed using traditional methods starting from the colonies' shape as follows. The bacterial colonies appeared pale pink in color, convex, with smooth edges on MacConkey's agar as shown in Figure 1. The bacteria also smelled like rotting fish. The remarkable movement of bacteria appeared when they were grown on nutrient agar medium and with semisolid agar, and they were in the form of concentric rings, as shown in Figure 2.

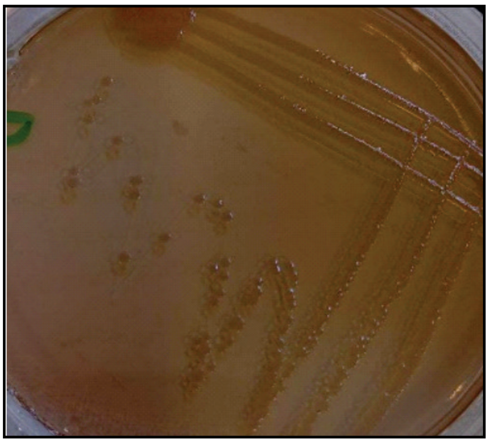

Fig. 1. The colonies of *P. mirabilis* on MacConkey's agar

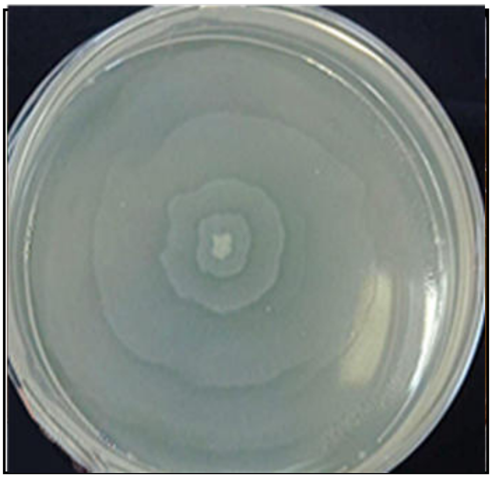

Fig. 2. Swarming phenomenon on nutrient agar

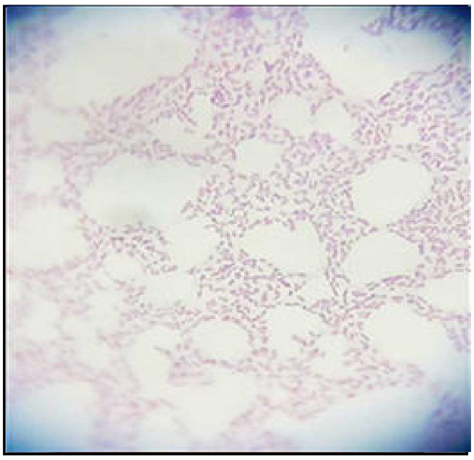

Fig. 3. Microscopic examination of *P. mirabilis* cells

Fig. 4. The positive result (pink) of urase enzyme production

Fig. 5. The result of sugar fermentation on Kligler iron agar, right tube (no culture): middle and left tubes are positive sugar fermentation

After performing the Gram stain, cell morphology was examined with a microscope, the cells appeared in the form of negative bacilli as demonstrated in Figure 3.

Regarding the results of biochemical tests: it was found for the oxidase test that *P. mirabilis* gave a negative reaction. As for the test of catalase: the result was positive for this enzyme. Also, the result was positive for urease production as shown in Figure 4 by the change in the the color of medium from yellow to pink. Also the fermentation of sugars on Kligler iron agar (KIA) was investigated. After the tubes were incubated at 37 °C for 24 hours, the color of the medium converted from red to yellow, which indicates the fermentation of lactose and glucose, and the deposition of a black color at the bottom of the tube indicates production of hydrogen sulfide H_2S, the result is represented in Figure 5.

Molecular diagnosis. Gel electrophoresis with 2% agarose for the PCR product was performed to detect the 16SrRNA gene, and the bands corresponding to the gene appeared at approximately 1500 base pairs as demonstrated in Figure 6 for some isolated bacteria using the ladder (M).

After receiving the sequencing data, the strains were molecularly identified as *P. mirabilis* in five strains utilizing the international site NCBI, two of strains were registered in the Global GenBank under the names: GHSUMO-1，GHMOSU-2 with accession numbers: OR976486 and OR976487 respectively.

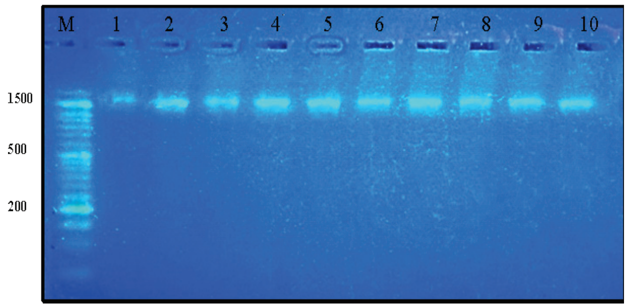

Fig. 6. The electrophoresis of PCR product for the 16S rRNA gene of some bacterial isolates, 2% agarose gel, voltage of 60 volts for 75 minutes: the observed bands correspond to an approximate molecular size of 1500 bp; the columns M is the DNA ladder (1500 bp),and the numbers 1–10 represent the bacterial isolates

The effect of different amino acids on swarming motility. The results of the effect of the various amino acids which include: valine, alanine, histidine, aspartic acid, cysteine and tryptophan on swarming phenomenon for five strains of *P. mirabilis* revealed that there are clear differences in the swarm inhibition as shown in Table 1. The table demonstrates that three amino acids (alanine, aspartic acid and cystine) had the higher ability to inhibit the swarming for four strains out of five. The remaining amino acids (valine, histidine, tryptophan) inhibited the movement of three strains, both histidine and tryptophan had the same effect on same three strains of *P. mirabilis*.

Table 1
The effect of different amino acids on swarming motility of *P. mirabilis*

The medium	Ghmosu-1	Ghsumo-2	L	S3	S4
Nutrient agar (NA)	+++	+++	+++	+	+
NA + valine	++	–	+	–	–
NA + alanine	–	–	++	–	–
NA + histidine	–	+++	++	–	–
NA + aspartic acid	–	–	+	–	–
NA + cystine	–	–	++	–	–
NA +tryptophan	–	+++	++	–	–

Table 1 illustrates the differential impact of amino acids on bacterial swarming motility among the tested strains. Strains S3 and S4 exhibited the most pronounced inhibition, with swarming completely suppressed in response to all tested amino acids. In contrast, strain GHMOSU-1 demonstrated impaired swarming motility in the presence of all amino acids except valine, which exerted only a minimal effect compared to the control. For strain GHSUMO-2, swarming motility was entirely inhibited by valine, alanine, aspartic acid, and cysteine, whereas histidine and tryptophan had no discernible impact. Lastly, strain L exhibited the least susceptibility to amino acid-mediated inhibition, as its swarming motility was not completely inhibited. However, slight reductions in motility were observed in response to alanine, histidine, cysteine, and tryptophan, while valine and aspartic acid exerted a more pronounced inhibitory effect.The inhibition of P. mirabillis GHMOSU-1 with the amino acid cystine (a) where only bacterial growth is observed at the point of cultivation and no expansion of growth or any central ring indicating the appearance of swarming movement,compared with control sample (b) where the rings of swarming were very clear

The strains S3 and S4 were the most affected by all the amino acids, responding significantly to their influence. They were followed by strain Ghmosu-1 and 2, while strain L did not respond to any amino acid. However, it retained the ability to induce swarming, though this ability was weakened by valine and aspartic acid.

Inhibition by natural materials. Four plant materials were used to inhibit the movement of swarming including: corn silk, parsley, dried orange peel and mallow. The strongest substances which primarily inhibited the swarming were orange peels and mallow. The parsley came in the second rank because it inhibited the movement in two strains only.

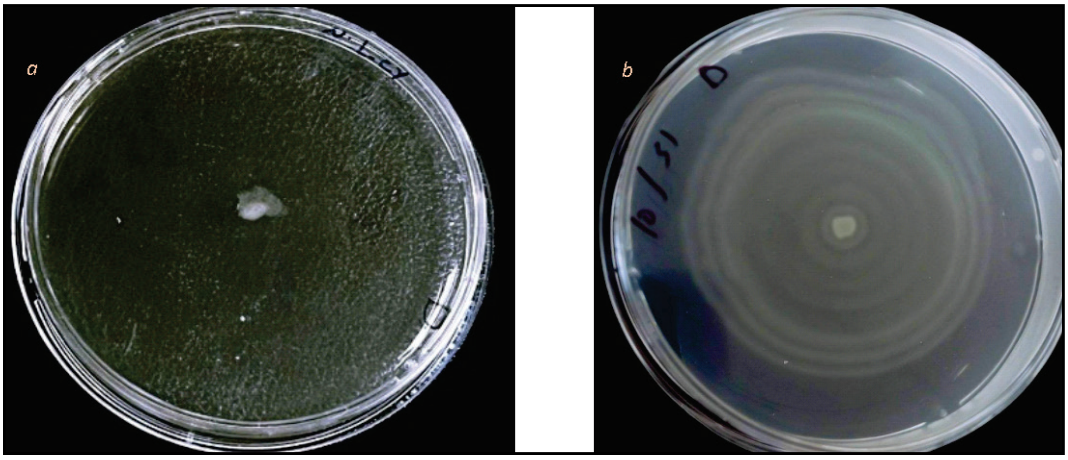

Fig. 7. The inhibition of *P. mirabillis* GHMOSU-1 with the amino acid cystine (*a*) where only bacterial growth is observed at the point of cultivation and no expansion of growth or any central ring indicating the appearance of swarming movement, compared with control sample (*b*) where the rings of swarming were very clear

Table 2
The effect of natural material on swarming phenomenona

	Ghmosu-1	Ghsumo-2	L	S3	S4
Corn Silks	++	++	+++	ND	ND
Parsley	++	+	–	–	++
Orange peel	+	–	+++	–	–
Mallow	–	+	++	–	–

Note: ND – not done; "–" – no swarming; "+" – weak swarming; "++" – moderate swarming; "+++" – strong swarming.

In comparison to Table 1, Table 2 demonstrates that strains 3 and 4 also clearly exhibited the most significant inhibition of swarming motility. Strains Ghmosu-1 and Ghsumo-2 displayed variable responses to natural compounds, ranging from strong to weak inhibition which was obviously the case with corn silks. In contrast, strain L exhibited the loweat inhibition of swarming motility relative to the control sample, as also shown in Table 1, with the exception of parsley, which was the only natural compound that had a noticeable effect on its motility.

Discussion

Proteus species are facultatively anaerobic bacteria belonging to the Enterobacteriaceae. They are widely distributed in the environment and are commonly associated with human and animal infections. Accurate identification and characterization of *Proteus* species are critical for effective diagnosis and treatment. Traditional diagnostic methods, including culture-based techniques and biochemical assays, remain the cornerstone for identifying these bacteria.

The colonies of *P. mirabilis* appeared pale pink because they were not fermenting lactose (Hezam et al., 2023). Also, the swarming phenomenon did not show on the MacConkey's medium due to the presence of bile and crystal salts in the medium (Saleh et al., 2020).

On culture media, the concentric rings of the swarming motility resulted from a succession of two stages: the consolidation stage, in which the cells stop moving and lose their differentiation into swimming cells, and the swarming stage, in which cells differentiate and move. This results from repetition of this process. However, swarming motility of *Proteus* on solid media can pose challenges to laboratory diagnostics, leading to misinterpretation or contamination of adjacent cultures (Jose & Singh, 2020).

After cell morphology was examined by Gram stain, the cells appeared in the form of negative bacilli. About the findings of biochemical tests: firstly, the oxidase test of this bacterium gave negative reaction to the indicator (N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride). The cells did not give the purple color of the reagent, which means that the bacterium does not possesses the oxidase enzyme. The positivity of the catalase enzyme was demonstrated by the bubbles on the slide. The catalase enzyme works to get rid of the toxicity of H_2O_2 by breaking the bond into oxygen and water. In addition, for urease production, the color of the medium changed from yellow to pink. This result means that the bacteria secreted the urease enzyme, which decomposed the protein materials and removed the amine group from the amino acids, leading to the production of ammonia gas, which led to a rise in the pH level from neutral to alkaline, thus changing the color to pink and giving a positive result (Cappuccino & Welsh, 2020). Finally, the color of the Kligler iron agar at the bottom of the tube changed, and the color of the medium converted from red to yellow, which indicates the fermentation of lactose and glucose. The changing in the color of the medium at the bottom of the tube only from red to yellow indicates the fermentation of glucose only, and the deposition of a black color at the bottom of the tube indicates production. Hydrogen sulfide H_2S, collects gas at the bottom of the tube below the culture medium, or forms air pockets, evidence of gas production.

In recent years, advancements in molecular techniques, such as (PCR) and whole-genome sequencing, have provided more precise tools for detecting and characterizing *Proteus* species. This is because molecular identification nowadays is considered the right way to identify the bacteria, which is dependent on the universal gene 16srRNA, which is considered a good tool for correct diagnosis, due to the rare exposure of its nucleotide sequences to mutations (Alzahrani & Ghaleb, 2023).

The 16S rRNA gene plays a crucial role in bacterial diagnostics due to its presence in all bacteria and its highly conserved regions, which allow accurate identification and classification of bacterial species. Its variable regions provide enough genetic diversity to differentiate between species, making it an invaluable tool for microbial identification, especially when conventional methods may be insufficient. The use of 16S rRNA gene sequencing in PCR-based assays has become a standard approach in clinical microbiology, enabling more precise and rapid detection of bacterial pathogens.

Each bacterial flagellum consists of a long helical protein filament which connects through a hook to the basal body in the cell envelope. Rotation of the motor complex in the membrane is powered by the transport of protons or sodium ions across the membrane. The rotor is surrounded by a ring of membrane-anchored stator complexes that comprise the corresponding ion channels and their interactions with the rotor generate the torque for the rotation of the flagellum. Most bacterial species possess multiple stator systems which can engage in highly dynamic rotor–stator interactions tuning the flagellar motor, The incorporation and exchange of stators in the motor complex depends on diverse environmental factors, such as the level of drag or sodium ion concentration (Rütschlin & Böttcher 2019).

In our opinion, the inhibitory effect of alanine on swarming motility may be attributed to its impact on the metabolic balance of the bacterial cell, thereby suppressing this phenomenon. Aspartic acid may increase the acidity of the medium, which could negatively affect the flagellar activity. Similarly, cysteine metabolism within the cell may lead to the accumulation of sulfide, which is considered toxic material.

Tryptophan is used in production of the signaling molecules that regulate flagellar movement. If indole is produced at high concentrations from tryptophan, it will inhibit collective motility or disrupt the regulation of molecular signaling.

As for histidine, it plays a role in regulating the pH inside the cells (pH buffering) and is involved in vital metabolic pathways. It also helps stabilize motility proteins and swarming activity under optimal conditions. The accumulation of histidine metabolites, such as histamine, may lead to the inhibition of collective motility due to their impact on molecular signaling (Quorum Sensing).

Data from studies conducted in other regions indicate variability in how polar and non-polar amino acids influence swarming motility in bacteria (Ulitzer, 1975; Eberl et al., 1999). Our finding about aspartic acid, which is similar to glutamic acid, agreed with some studies which mentioned that glutamic acid did not promote swarming, and that arginine repressed swarming, indicating that there are clear differences in swarming cues between species (Kohler et al., 2000; Bernier et al., 2011). Non-permissive minimum medium, a combination of 22 amino acids induced swarming, according to an old investigation (Jones & Park, 1967) into the nutritional needs for swarming. Additionally, glutamic acid, aspartic acid, serine, proline, alanine, asparagine, and glutamine were found to be sufficient for promoting swarming when added separately to the base medium.

Allison and coworkers demonstrated that the invasion of urothelial cells by *P. mirabilis* depends on motility and swarming differentiation (Allison et al., 1993).

Other researchers revealed that an increase was observed in swarming motility with glutamine, serine, and threonine supplementation suggests that these amino acids play a significant role in the differentiation of swarm cells in these strains. Glutamine has previously been identified as a swarming-enhancing agent in *Serratia liquefaciens*, *Aeromonas* species (Kirov et al., 1986), and *Proteus* species from other environments (Allison et al., 1993).

The results in Tables 1 and 2, particularly the latter one. The natural materials used were not successful in inhibiting the swarming motility in *P. mirabilis* (which greatly aids the bacterium in invading the urinary tract), and were thus not consistent with those described by herbalists. Therefore, it is advised not to rely on these natural compounds, and that physicians should remain the trusted source for prescribing appropriate medications. This agrees with (Mohammad et al., 2024) who emphasized that physicians should remain the primary authority for prescribing medications. Additionally, public awareness should be enhanced to discourage individuals from seeking infection treatment from herbalists.

Plants produce an enormous diversity of secondary metabolites and a great deal of research has focused on natural products and their effects on bacterial population behaviors including swarming motility (Mohammad & Al-Wattar, 2023; Mohammad et al., 2024).

Due to the cell density requirements for swarming, it seems probable that swarm cell differentiation is controlled by some natural substances, *P. mirabilis* may need a certain threshold level of these substances to promote metabolic pathways required for swarming or for changes in cell wall composition required for different swarm cells (Rather, 2005).

Another study demonstrated that red pepper induced swarming inhibition of uro-pathogenic *P. mirabilis in vitro* and could be employed in the treatment of patients that suffer from UTI caused by *P. mirabilis*. Also, the findings of their study recommends avoidance of the consumption of indomie noodles with their additives due to their stimulatory action on swarming of *P. mirabilis*, which may allow the microorganism to reach and colonize other sites of the urinary tract, causing infections (Saleh et al., 2020; Altaay & Al-Haaik, 2022).

Conclusion

This study highlights the inhibitory effects of specific amino acids and natural plant materials on the swarming motility of *P. mirabilis*, a bacterium known for its role in urinary tract infections and biofilm formation. The results demonstrate that amino acids such as alanine, aspartic acid, and cysteine significantly suppress swarming, while the natural materials, dried orange peel and mallow exhibit promising anti-swarming properties. In general, our results may give a relative impression of the lack of benefit to patients from natural materials dispensed by herbalists. However, due to the small number of samples used we recommend conducting broader research on the therapeutic role of natural materials against *P. mirabilis* bacteria, especially their effect on motility.

These findings provide a foundation for developing novel, eco-friendly strategies to control bacterial motility, particularly in clinical and environmental settings where biofilm-associated infections pose significant challenges. Also we suggest using swarming inhibitors in culture media to obtain with the help of antimicrobial agents (antibiotics) a more bacteriostatic or bactericidal effect on dangerous opportunistic *Proteus* spp.

Future work will concentrate on the mechanisms of action of each swarming cue to know how *P. mirabilis* uses the factors to sense and respond to the environment, consequently gaining new insight into the regulation of swarming and identifying new targets to prevent.

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There are no conflicts of interest.

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