JOURNAL OF DYNAMICS AND CONTROL

VOLUME 8 ISSUE 8

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ABSTRACT: *The rising incidence of drug-resistant bacteria and viruses has increased the demand for novel treatment strategies. This study explores the antioxidant and antibacterial properties of Pantoea septica MOP, isolated from the medicinal flower Hibiscus rosa-sinensis. The objective of this research was to evaluate whether P. septica MOP could provide a natural alternative to synthetic antibiotics and mitigate oxidative damage. P. septica MOP was tested against human pathogens, including Gram-positive and Gram-negative bacteria, to assess its antibacterial efficacy. The results revealed that P. septica MOP significantly inhibited several pathogenic bacteria, including Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae, with notable inhibition zones. With an inhibitory zone of 18 ± 0.84 mm, Staphylococcus aureus showed the greatest sensitivity at 80 µg. The antioxidant activity study demonstrated significant efficacy, with the sample reaching 72.54 ± 0.62% and the standard ascorbic acid showed 95.03 ± 0.75% at the maximum concentration of 100 µg. These findings suggest that P. septica MOP from Hibiscus rosa-sinensis possesses both antibacterial and antioxidant properties, making it a promising candidate for natural antibiotic alternatives. The study highlights the potential of plant-associated microbes in developing novel therapeutic agents and highlights the importance of exploring natural resources to address antibiotic resistance and oxidative stress-related diseases. Further research is needed to fully understand the mechanisms of these bioactivities and their therapeutic potential.*

KEYWORDS: *Pantoea septica; Hibiscus rosa-sinensis; Drug-Resistant Pathogens; Bioactive Compounds; Optimization; Antimicrobial; Antioxidant*

1. INTRODUCTION

The spread of infectious diseases has become a major public health concern due to the dramatic increase in their incidence and fatal injury rates across the world. Several drugs have been used to treat these infections within the last ten years [1]. However multidrug-resistant (MDR) bacteria have emerged as a result of antibiotic overuse and abuse; these bacteria are fast expanding throughout the globe and represent a major risk to the general population on an international level [2, 3]. Based on the results of laboratory-based susceptibility tests, multi-drug-resistant bacteria are usually able to withstand the effects of

multiple classes of antibiotics [4, 5]. In particular, diseases that successfully "escape" the effects of antibacterial drugs are typically caused by multidrug-resistant bacteria, also known as "ESKAPE" pathogens [6]. These bacteria include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. It is critical to investigate potential based on plant antibiotic alternatives to tackle antibiotic-resistant bacteria because of the adverse effects of drugs and the growing problem of resistant microbes [7].

Herbal remedies have long been used to cure a variety of human illnesses. Antimicrobial agents often come from plant-based chemicals due to their many desirable qualities, including being natural, cheap, harmless for the human body, and efficient even at low doses [8]. Globally, 80% of the population utilizes botanical extracts or chemical compounds in conventional drugs, based on estimations from the World Health Organization (WHO) [9]. Flavonoids, phenol compounds, alkaloids, terpenoids, tannins, essential oils, lectins, polypeptides, and polyacetylenes are only some of the bioactive substances found in medicinal herbs that have been shown to effectively treat infectious diseases in multiple research studies [10].

Conventional medicine has made extensive use of the blossoming plant *Hibiscus rosa-sinensis*, most often known as the Chinese hibiscus. Research into its possible medicinal uses, especially its antioxidant and antibacterial effects, has recently dominated the scientific community. Researchers have tested the plant's compounds on several Gram-negative and Gram-positive bacteria that might cause illness in humans. Bacteria cause a lot of illnesses and infections, and the fact that they're becoming more resistant to traditional antibiotics has prompted researchers to look for new ones [11]. Considered to contribute to *Hibiscus rosa-sinensis's* medical characteristics are its bioactive components, which include anthocyanins, flavonoids, and phenolic acids. Discovering how *Hibiscus rosa-sinensis* compounds function

as antioxidants and antibacterial *in vitro* may provide light on this plant's possible use as a natural treatment for oxidative damage and infections caused by bacteria. Alternatively, plant-based medicinal drugs may be feasible as a result of the research, providing an indefinite, viable option for combating the epidemic of resistance to antibiotics [12].

The bacterium *Pantoea septica* has been found in Hibiscus plants. This bacterium belongs to the Pantoea genus, which contains multiple species that are potentially pathogens or components of the plant microbiome and are recognized for their associations with flowers. The discovery of *P. septica* in hibiscus highlights the importance of studying medicinal plant microbiota relationships, which impact plant development and health and determine the safety and effectiveness of drugs derived from plants [13]. The genus Pantoea was established by *Pantoea agglomerans*, a member of the family Enterobacteriaceae. The number of Pantoea species that have been recognized has been steadily rising over the last several years. Differentiating closely linked organisms from the genus Pantoea using methods like the API 20E, Biolog systems, or traditional PCR of 16S rRNA fragments proves difficult because the 25 species that have been identified possess numerous morphological characteristics and demonstrate a high degree of homology [14].

Pantoea septica, a naturally occurring antibacterial and antioxidant agent, was investigated in this novel study and it was extracted from *Hibiscus rosa-sinensis*. *Pantoea septica* has mostly been investigated for its roles in plant biology, but this study sheds light on a hitherto unexplored aspect of its medicinal potential. *Pantoea septica* has the potential to be a great substitute for synthetic medications due to its potent antioxidant properties and antimicrobial efficiency against human infections and oxidative damage. This research provides novel possibilities for more effective and sustainable treatment approaches to tackle antibiotic resistance and chronic illnesses. It also increases the potential application of microbes found in plants in drug development.

2. MATERIALS AND METHODS

Sample Collection and Isolation of Bacterial Strains

Flowering *Hibiscus rosa-sinensis* plants were gathered from a garden in Woraiyur, Tiruchirapalli, Tamil Nadu, India. Before isolating *Pantoea septica*, the blossoms were surface-sterilized with 70% ethanol as the solvent and rinsed with distilled water. After sterilization, the flowers were minced and mixed with sterile salt water to produce a homogeneous mixture. After that, the resulting mixture was spread out on a selective medium, such as Nutrient Agar (NA) and Mac Conkey Agar (MA), and incubated at 37°C for one to two days so that bacterial colonies could develop [15].

Morphological and Molecular Identification

Pantoea septica was identified using a two-step process that included morphology and molecular analysis. Subsequently, process begins with cultivating the bacterium on a special medium and examining its morphology under a light microscope, focusing special attention on the size, color, form, and Gram staining of the colonies. Polymerase chain reaction (PCR) using primers that targeted the 16S rRNA gene were used to identify the bacterium as a molecular. Genomic DNA was obtained from the bacterial cells. *Pantoea septica* was confirmed by sequencing the PCR products and comparing the resultant sequences to known databases using BLAST analysis. The bacterial strain was successfully identified and classified using this hybrid approach of morphological differentiation and molecular sequence [16].

Antibacterial efficacy

Pantoea septica bacteria were grown in Luria-Bertani (LB) broth and incubated at 37°C with 180 rpm agitation for 24 hours to prepare bacterial extracts. Following a 15-minute centrifugation run at 10,000 rpm, the resulting culture was strained to remove debris. The resulting solution was then condensed using a rotary evaporator and reconstituted in a solvent

suitable for further testing. The agar diffusion technique was used to evaluate the effectiveness of antibacterial agents. To accomplish this, pathogenic bacteria were placed into Mueller-Hinton Agar (MHA) plates, and wells were formed with microbial extracts. The zones of inhibition were determined after incubation at 37°C for 24 hours [17].

Antioxidant Potential

The antioxidant capacity of *Pantoea septica* was assessed by cultivating colonies of bacteria in nutrient broth and harvesting cells at the end of the log phase. For the antioxidant experiments, the supernatant that was free of cells was extracted by centrifugation. To determine the antioxidant capacity, tests were conducted utilizing methods such as the DPPH radical scavenging assay with different concentrations (20, 40, 60, 80, and 100 µg). Spectrophotometric measurements of variations in absorption were used to determine the capacity for radical scavenging %. The relative antioxidant capacity of the *Pantoea septica* supernatant was determined by comparing the findings to standard antioxidants. The approach used in this study quantifies the bacterial strain's capacity to decrease oxidative damage and neutralize free radicals [18].

Screening of Selected Strains

To verify that certain bacterial strains could extract microbial oils from *Hibiscus rosa-sinensis*, a screening method was used. This technique identified microorganisms that produce oil utilizing the saponification process.

Screening Technique for Bacteria

To identify bacteria that may produce oil, the saponification process was employed. The bacterial strains were cultured in a 3 mL tube and then centrifuge at 5000 rpm for 10 minutes to extract the liquid portion. Then 2-3 drops of phenolphthalein and 3-5 drops of 0.5N alcoholic potassium hydroxide (KOH) to the liquid above the sediment. After the mixture had been purpled by phenolphthalein for 30 minutes, it was allowed to cool to room

temperature. When the tubes formed foam or soap after heating, it indicated that there was fixed oil present. It was shown that the bacterial strains were producing microbial oil in the presence of this foam-like substance [19].

Submerged Fermentation

A mineral salt medium (MSM) was used for submerged fermentation to evaluate lipid synthesis by certain bacterial strains. In 100 mL of mineral salt medium, which included magnesium chloride ($MgCl_2$), sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), peptone, yeast extract, and glucose, 2 mL of bacterial strain cultures were administered. The media's pH was set to 7. The medium was sterilized, mixed with distilled water, and then left to cool before the culture of bacteria was inoculated. To promote proliferation and lipid synthesis, the cell cultures were kept in an incubation chamber at 28°C for three days. The lipid content was ascertained after fermentation by using the suitable separation procedure. [20].

Lipid extraction

Lipid components were extracted according to Anahas and Muralitharan [21, 22]. Centrifugation was applied to the bacterial culture at 5,000 rpm for 5 minutes. The particle was measured after its supernatant was removed. Centrifugation at 5,000 rpm for 5 minutes proceeded with the addition of 1% SDS to the pellet, which assisted in breaking up the cell walls. Following the destruction of cells, the pellet of cells was extracted and the resulting solution was mixed with chloroform ($CHCl_3$) and ethanol in a 1:1 ratio. Following that, for 10 minutes, the resulting mixture was spun in a centrifuge at 10,000 rpm. The top layer was removed once the two separate levels were differentiated. After twenty minutes of evaporation, the chloroform (bottom) layer that contained the lipids was measured.

The lipid content's total dry weight was calculated using the Bligh and Dyer technique. We used the following formula to determine the lipid content:

$$LC\ (\%\ dwt) = (W_2 - W_1) / W \times 100$$

Growth Parameters Optimization

a) Optimization of Incubation Time

By altering the incubation period, the production of microbial oil was optimized. The overnight culture was added to 0.5 mL of MSM broth media and allowed to incubate at 37°C for 1 to 5 days. Chloroform was used to separate the microbial oil from the MSM broth after incubation. Following that, we measured the microbial yield of oil to determine precisely how prolonged an incubation period would maximize oil production.

b) Optimization of pH

To test if changing the pH of the MSM broth medium has any influence on the generation of bacterial oil, the pH was adjusted to 5 to 9. After sterilizing the MSM broth, 0.5 mL of overnight inoculum was added and left to incubate at 37°C for three days. The ideal pH for oil synthesis was determined by extracting bacterial oil with chloroform after incubation and quantifying the amount produced.

c) Optimization of Carbon Concentration

The optimal amount of oil was achieved by adjusting the carbon source concentration in the MSM broth medium range from 10 to 50 g/L. After incubating at 37°C for three days, the broth was inoculated with 0.5 mL of overnight culture. The ideal carbon content for producing bacterial oil was determined by extraction of the oil with chloroform after incubation and quantification of its yield.

d) Optimization of Carbon to Nitrogen Ratio (C/N)

Increasing the MSM broth medium's carbon to nitrogen (C/N) ratio to 4:1, 5:1, 6:1, 7:1, 8:1, and 9:1 enhanced microbial oil generation. About 1 ml of the overnight culture was added to the medium, and then it was incubated at 37°C for three days. To determine the

optimal C/N ratio for optimizing the production of oil, an incubation step was followed by solvent extraction of bacterial oil. The final product was subsequently determined [23].

Characterization of Microbial Oil by GC-MS Analysis

Gas chromatography-mass spectrometry (GC–MS) was used to characterize the chloroform-extracted bacterial oil and determine the chemical compounds. A gas chromatograph coupled with a mass spectrometer (GC-MS) and an AOC-20i auto-sampler were parts of the SHIMADZU QP2020 GC system that was used to analyze the chloroform mixture extract. The injector temperature was 250 °C, the ion-source temperature was 200 °C, and the subsequent conditions were used to perform the assessment: 1.2 mL/min injection velocity. The GC oven was set to begin at 50 °C (isothermal for 2 minutes) and then raised to 280 °C (maintained at the same level for a further 2 minutes [24, 25].

Considering masses-to-charge ratio (*m/z*) from 50 to 500 Da, mass spectra were obtained at a scan time of 0.3 seconds. We used the online library system of Wiley 8th Edition (WILEY8LIB) to analyze the mass spectra that we received. To find the molecular mass and structures of the unidentified components, their spectra were correlated with the spectrum of previously identified compounds contained in the WILEY8LIB database. The findings component provided the table data, which included the detection and quantification of the chemical components.

3. RESULTS

Isolation and Screenin3g of oil-producing bacteria

The target of isolating the 53 bacterial strains from *Hibiscus rosa-sinensis* flowers was to find those that could accumulate lipids within the cells. Submerged fermentation with a carbon source in a nitrogen-limited substrate was used to produce these strains. Thirteen of the fifty-three original isolates showed evidence of lipid bodies within their cells. Moreover, the ability of these thirteen isolates to accumulate lipids was tested. Out of the thirteen

isolates tested, five showed significant lipid formation, as shown by the existence of prominent lipid bodies. Additional profiling and evaluation were conducted on these interesting strains to assess their suitability for industrial uses and their capacity for generating microbial oils. The findings highlight the possibility that, provided suitable cultural conditions, some strains of *Pantoea* spp. might produce substances rich in lipids.

Identification of Bacterial Strains

Pantoea septica has been confirmed by morphological and genetic methods. On nutrient agar plates, the bacterial colonies were smooth, round, and yellow-pigmented. These microbes were identified as rod-shaped, Gram-negative bacteria. The isolate's species was further validated by molecular identification using 16S rRNA gene sequencing. Further sequencing and BLAST searches revealed a 99% match with previously identified *Pantoea septica* sequences in the NCBI database, and the PCR amplification generated a distinct band equal to the predicted size of the 16S rRNA gene. The bacterial isolate was confirmed as *Pantoea septica* by combining morphological and molecular evidence and sequence submitted to GenBank with Accession Number PP990720.

In Figure 1, phylogenetic tree construction shows how the newly identified bacterial strain evolved with other species of bacteria. This tree was built using data from the 16S rRNA gene sequence, which shows where the particular isolate falls in the taxonomic arrangement that exists relative to other bacterial genera and species. In addition to confirming the strain's categorization, the phylogenetic analysis provides insight into its possible inherent and unique features with other microbial strains.

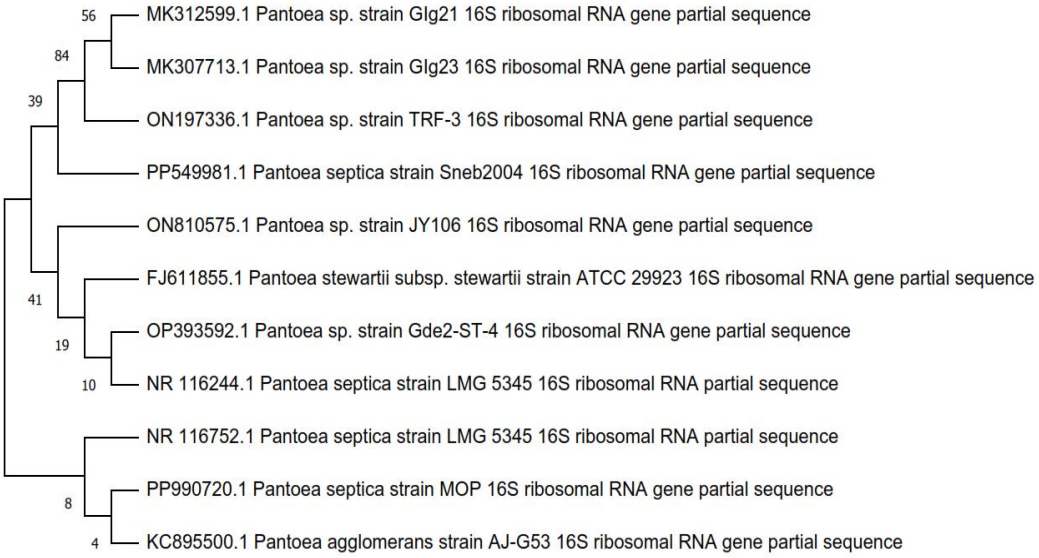

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the close relationship of the *Pantoea septica* strain MOP isolated from *Hibiscus rosa-sinensis* with other *Pantoea septica* strains in the NCBI database.

Lipid Production in Submerged Fermentation

Pantoea septica, an oleaginous species, was used in submerged fermentation, and the bacterial biomass and lipid production was recorded during times. Preliminary fermentation data show a dramatic increase in lipid synthesis and biomass growth within the first three days. However, bacterial biomass remained increasing, and lipid yield continued to decline after three days. A possible explanation for the decrease in lipid production after three days, continuing biomass synthesis using stored lipids. In the first five days, the biomass concentration peaked at 810 mg/L after that, it started decreasing, may be because nutrients were exhausted or metabolic processes were occurring.

Pantoea septica can accumulate lipids extensively during the first stage of fermentation, according to the data, reaching a maximum production level in 3 days. Lipid production decreases that follow, even as biomass growth persists, demonstrating the

complex processes that occur in this bacterium's lipid breakdown and utilization. Optimizing and analyzing the process further will help determine the factors that impact lipid accumulation as well as how to maximize lipid synthesis.

Characterization of Microbial Oils using GC-MS Analysis

Six major compounds were found in the GC-MS analysis of microbial oil and Figure 2 and Table 1 contain the names of the bioactive components BENZOIC ACID, 2,5-BIS(TRIMETHYLSILOXY)-, TRIMETHYLSILYL ESTER -8.47%, (1S, 2S)-CIS-2-PHENYLCYCLOPENTANOL (R)-O-ACETYLMANDELATE ESTER 3.55%, 1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER-8.85%, PROPANOIC ACID, 2-METHYL-, 5-(2,3-DIHYDRO-3,3-DIMETHYL-2-OXO-1H-INDOL-1-YL)-10,11-DIHYDRO-5H-DIBENZO[A, D]CYCLOHEPTEN-5-YL ESTER- 0.8%, 2-FLUORO-5-TRIFLUOROMETHYL BENZOIC ACID, PENTYL ESTER-1.17%, PIMELIC ACID, DI(2-NITRO-5-FLUOROPHENYL) ESTER-6.17%, 1H-FURO[3,4-C]PYRROLE-4-CARBOXYLIC ACID, 6-(2- FURANYL)HEXAHYDRO-1,3-DIOXO-4-PHENYL-, METHYL ESTER, (3A.ALPHA.,4.BETA.,6.BETA.,6-) 3.26% with their retention times (RT), chemical formulas, heights, and area percentage is shown in Figure 2 and Table 1.

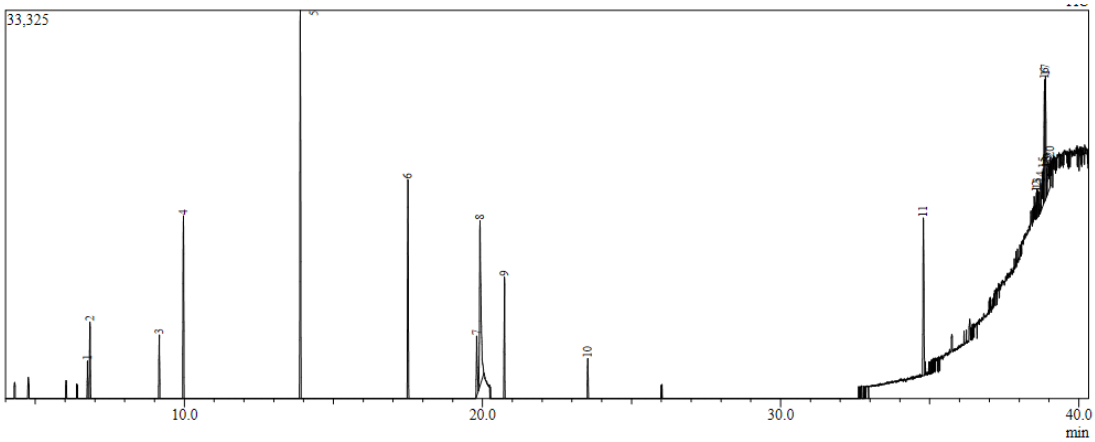

Fig. 2. GC-MS chromatogram showing the lipid profile of *Pantoea septica* isolated from *Hibiscus rosa-sinensis*, with distinct peaks corresponding to various lipid compounds detected in the sample.

The gas chromatography-mass spectrometry (GC-MS) found that the sample mostly consisted of ester chemicals, which existed in much greater concentrations. The mass spectra's fragment characteristics allow research to definitively confirm the compounds' molecular positions. Esters were the most common kind of unsaturated fatty acid, accounting for around 75% of the total. On the other hand, very little levels of saturated fatty acids, such as palmitic and stearic acids, were found. This research shows that unsaturated fatty acids and esters were far more abundant in the sample than saturated fatty acids, which were present in much smaller amounts. Finding both major and minor esters with distinct retention characteristics, these data provide insight into the sample's ester composition and distribution, which might be useful for future structural and compositional studies.

Table 1. Identification of lipid compounds from *Pantoea septica* isolated from *Hibiscus rosa-sinensis* using GC-MS analysis, detailing the compound names, molecular formulas, molecular weights, relative abundances (Area%), and retention times.

S. No	Name	Molecular Formula	Molecular Weight	Area%	Retention Time
1.	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	8.47	9.967
2.	(1S,2S)-CIS-2-Phenylcyclopentanol (r)-o-acetylmandelate ester	$C_{21}H_{22}O_4$	338	3.55	19.807
3.	1,2-Benzenedicarboxylic acid, dioctyl ester	$C_{24}H_{38}O_4$	390	8.85	34.786
4.	Propanoic acid, 2-methyl-, 5-(2,3-dihydro-3,3-	$C_{29}H_{29}NO_3$	439	0.8	38.605

	dimethyl-2-oxo-1h-indol-1-yl)-10,11-dihydro-5h-dibenzo[a,d]cyclohepten-5-yl ester				
5.	2-Fluoro-5-trifluoromethyl benzoic acid, pentyl ester	$C_{13}H_{14}F_4O_2$	278	1.17	38.723
6.	1H-furo[3,4-c]pyrrole-4-carboxylic acid, 6-(2-furanyl)hexahydro-1,3-dioxo-4-phenyl-, methyl ester, (3a.alpha.,4.beta.,6.beta.,6	$C_{18}H_{15}NO_6$	341	3.26	38.965

Optimization of Microbial Oil Synthesis Process

Several parameters were systematically modified to increase lipid production in an experiment that aimed at optimizing the bacterial oil synthesis mechanism. To find out how various carbon sources, including glucose, starch, maltose, sucrose, and xylose affected lipid formation, we experimented extensively with pH, incubation period, carbon concentration, and other critical variables.

Optimization of pH

Figure 3 shows the relationship between pH and lipid yield, which is expressed as g/L. At a pH of 5, the lipid production reaches a maximum of about 12.56 ± 0.125 g/L and following that declines as it raises pH. There is a significant reduction in lipid production from 9.43 ± 0.09 g/L at pH 7 to 7.47 ± 0.07 g/L at pH 6. At pH 8, the production rate decreases to about 5.53 ± 0.05 g/L, at pH 9, it falls to 4.05 ± 0.04 g/L, and at pH 10, it reduces to its lowest value of around 3.16 ± 0.03 g/L. This trend indicates that lipid synthesis is most efficient in slightly acidic conditions (about pH5) and that it decreases substantially in high alkaline conditions.

Optimization of incubation time

The optimization of incubation time for intracellular lipid production was evaluated over various days of incubation. Figure 4 shows the relationship between incubation time and lipid yield, which is expressed as g/L. At 3-day incubation, the lipid production reaches a maximum of about 3.56 ± 0.07 g/L and following that declines as it increases incubation day. There is a significant reduction in lipid production from 1.98 ± 0.03 g/L at 4th day to 1.26 ± 0.02 g/L at pH 6. At 1st day of incubation, the production rate decreases to about 0.91 ± 0.01 g/L, at 2nd incubation period; it increases to 2.14 ± 0.04 g/L. This trend indicates that lipid synthesis is most efficient in increasing the incubation time (about 3rd day) and that it decreases substantially in further incubation time.

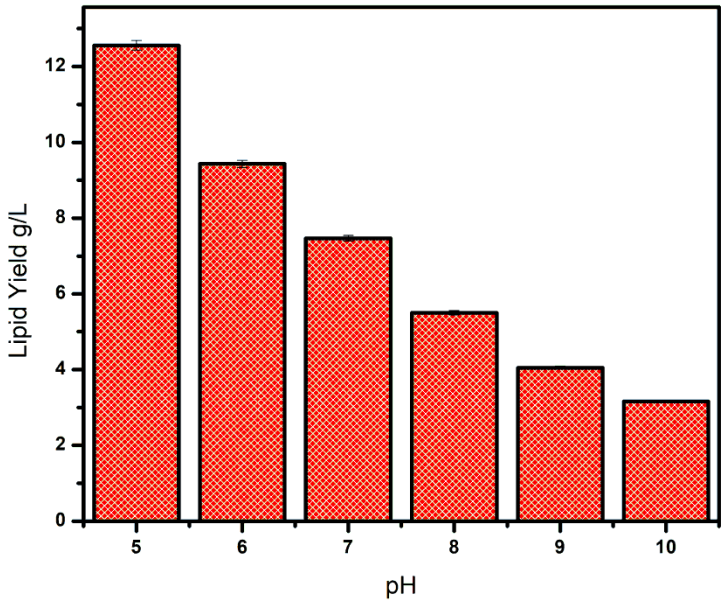

Fig.3. Effect of pH on lipid yield (g/L) from *Pantoea septica* isolated from *Hibiscus rosa-sinensis*. The values are presented as the Mean ± SD.

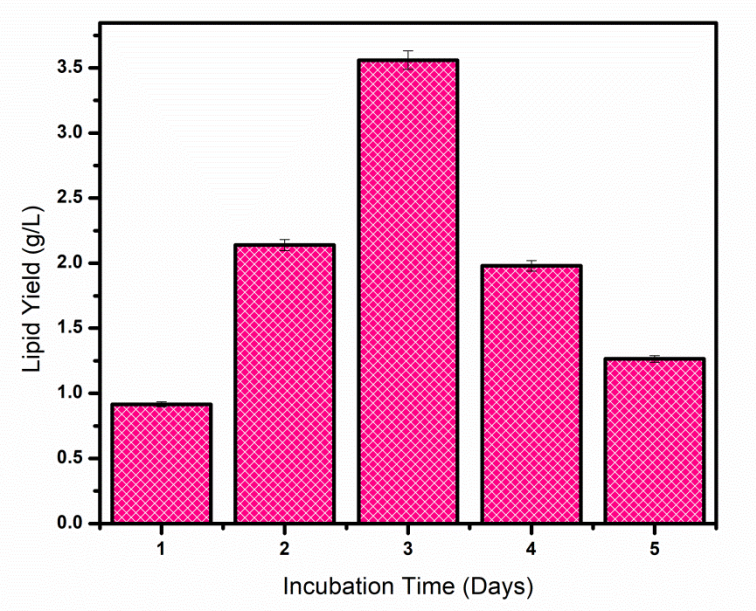

Fig.4. Effect of incubation time (days) on lipid yield (g/L) from *Pantoea septica* isolated from *Hibiscus rosa-sinensis*. The values are presented as the Mean ± SD.

Optimization of carbon concentration

The optimization of carbon concentration for intracellular lipid production was investigated using glucose as the carbon source at varying concentrations in a Minimal Salt Medium (MSM). The study revealed that a glucose concentration of 50 g/L resulted in the highest lipid yield of 5.43 ± 0.27 g/L. In contrast, lower concentrations, such as 10 g/L, were associated with reduced lipid content of 1.52 ± 0.07. There is a significant reduction in lipid production from 4.86 ± 0.24 g/L at 40 g/L to 3.57 ± 0.178 g/L at 30 g/L of carbon concentration. Figure 5 illustrates the relationship between glucose concentration and lipid yield, highlighting a maximum lipid production at 50 g/L. These results suggest that a higher glucose concentration enhances lipid production, whereas minimum glucose levels limit lipid accumulation. Thus, employing 50 g/L of glucose in MSM medium is optimal for maximizing lipid yield in *Pantoea septica* cultures.

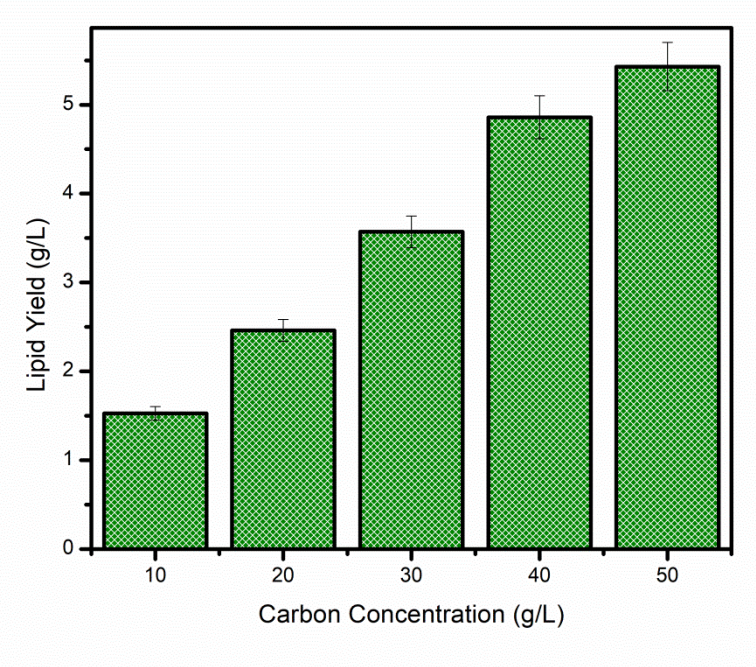

Fig.5. Effect of carbon concentration on lipid yield (g/L) from *Pantoea septica* isolated from *Hibiscus rosa-sinensis*. The values are presented as the Mean ± SD.

Optimization of the carbon-to-nitrogen (C/N) ratio

The optimization of the carbon-to-nitrogen (C/N) ratio for microbial oil production was assessed using various ratios in Minimal Salt Medium (MSM). The study identified that a C/N ratio of 9:1, achieved with 6.53 ± 0.272 g/L of glucose and nitrogen source, resulted in the highest microbial oil yield. The Figure 6 illustrates that microbial oil production was significantly improved at this specific C/N ratio while decreasing the ratio led to a noticeable decline in oil yield. There is a significant reduction in lipid production from 4.18 ± 0.19 g/L at 7:1 ratio to 3.83 ± 0.14 g/L at 6:1 ratio of carbon and nitrogen. Figure 6 illustrates the relationship between glucose concentration and lipid yield, highlighting a maximum lipid production at 50 g/L. These findings emphasize the importance of maintaining an optimal C/N ratio for maximizing microbial oil production, with a C/N ratio of 9:1 proving to be the most effective for enhancing yield in MSM medium.

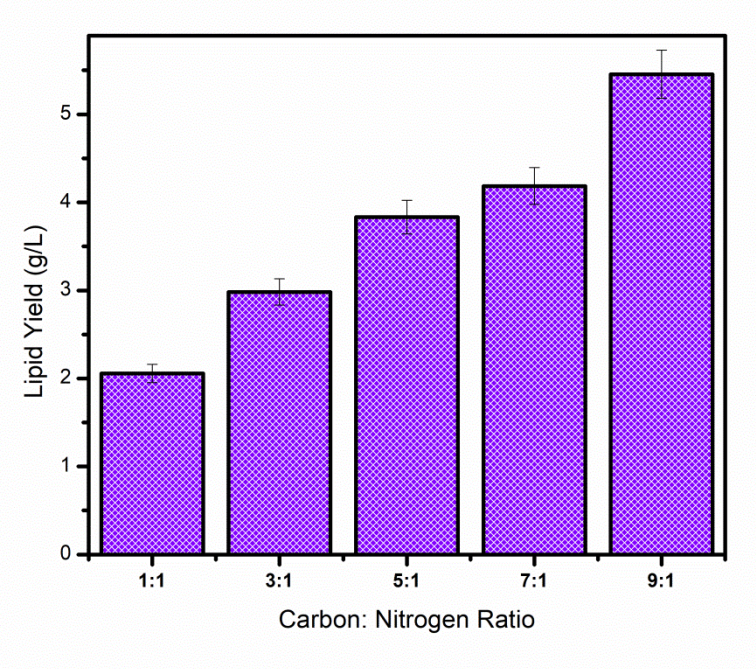

Fig.6. Effect of carbon and nitrogen ratio on lipid yield (g/L) from *Pantoea septica* isolated from *Hibiscus rosa-sinensis*. The values are presented as the Mean ± SD.

Optimization of Carbon sources

The optimization of carbon sources for lipid production was investigated by employing different substrates, including glucose, starch, maltose, sucrose, and xylose in a Minimal Salt Medium (MSM). The results demonstrated that glucose was the most effective carbon source, leading to the highest lipid yield of 15.52 ± 0.0.77 g/L followed by starch 12.64 ± 0.63 g/L, sucrose 11.46 ± 0.57 g/L. In contrast, the use of xylose resulted in a lower lipid content of 7.84 ± 0.39 g/L. Figure 7 illustrates the comparative performance of glucose, starch, maltose, sucrose, and xylose, highlighting the superior lipid production achieved with glucose. These findings indicate that glucose is a more suitable carbon source for maximizing lipid accumulation in MSM medium, whereas starch is less effective in promoting lipid biosynthesis in MB colony cultures.

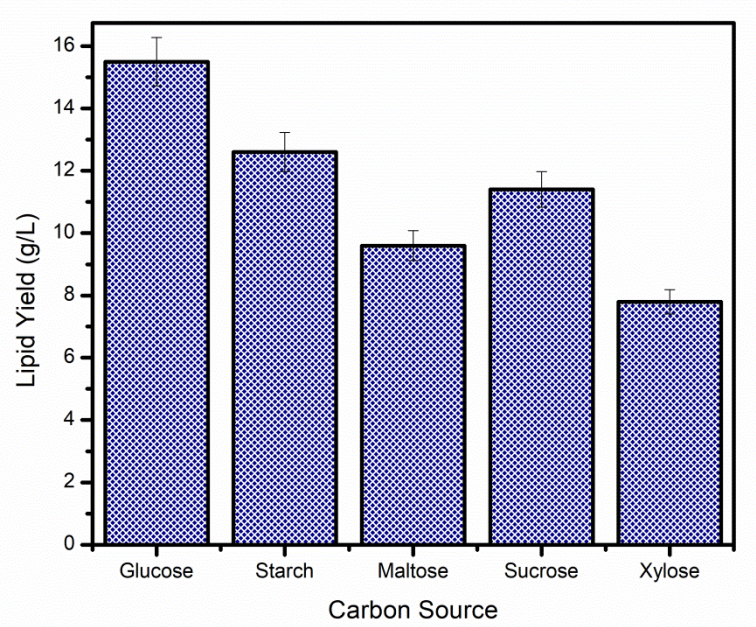

Fig.7. Effect of carbon source on lipid yield (g/L) from *Pantoea septica* isolated from *Hibiscus rosa-sinensis*. The values are presented as the Mean ± SD.

Antimicrobial efficacy

In a recent study, the antibacterial activities of *Pantoea septica* extracts were investigated using the agar well diffusion technique against several pathogenic microbes. The zone of inhibition increased across several doses (20 µg, 40 µg, 60 µg, 80 µg) as the research showed, which was proportional to the dosage. With an inhibitory zone of 18 ± 0.84 mm, *Staphylococcus aureus* showed the greatest sensitivity at 80 µg. Similarly, at the same dose, *Pseudomonas aeruginosa* exhibited a zone of 17 ± 0.25 mm, indicating significant suppression. Zones of 17 ± 0.09 mm and 16 ± 0.10 mm, respectively, were shown by *Klebsiella pneumoniae* and *Escherichia coli*, which followed. The zone of inhibition for *Candida albicans* was 13 ± 0.27 mm at the maximum dose, making it the least inhibited (Table 2). In particular, these results show that extracts of *Pantoea septica* can effectively combat fungi, Gram-positive and Gram-negative bacteria, and other microorganisms.

Table 2. Antimicrobial activity of *Pantoea septica* extracts isolated from *Hibiscus rosa-sinensis* against selected pathogenic organisms. The values are presented as the Mean ± SD.

S. No	**Organisms**	**Zone of Inhibition (mm) (Agar well Diffusion) Various concentrations of *Pantoea septica* extracts**			
		20 µg	**40 µg**	**60 µg**	**80 µg**
1	*Escherichia coli*	9 ± 0.07	12 ± 0.06	14 ± 0.08	17 ± 0.09
2	*Klebsiella pneumoniae*	8 ± 0.07	11 ± 0.08	13 ± 0.09	16 ± 0.10
3	*Pseudomonas aeruginosa*	11 ± 0.18	13 ± 0.75	15 ± 0.43	17 ± 0.25
4	*Staphylococcus aureus*	10 ± 0.06	12 ± 0.04	14 ± 0.07	18 ± 0.84
5	*Candida albicans*	9 ± 0.29	10 ± 0.14	11 ± 0.53	13 ± 0.27

Antioxidant Potential

Increasing concentrations consistently improved the antioxidant effectiveness, according to recent research that evaluated the antioxidant activity of *Pantoea septica* extracts isolated from *Hibiscus rosa-sinensis*. The antioxidant activity was found to be 35.87 ± 1.79 for the sample and 55.47 ± 0.77 for the ascorbic acid standard at an initial concentration of 20 µg. Both the samples and standard showed significant improvement when the concentration reached 40 µg, with the samples increasing to 41.58 ± 0.79 (% inhibition) and the standard to 63.27 ± 0.16 (% inhibition). As the concentration was increased to 60 µg, the sample was 48.37 ± 0.41 (%) and the standard was 72.65 ± 0.63 (%). The sample for antioxidant activity reached 55.69 ± 0.78 and the standard reached 81.82 ± 0.91 (%) at 80 µg, while the antioxidant activity increased. With a sample of 72.54 ± 0.62 (%) and a standard of 95.03 ± 0.75 (%), the most substantial antioxidant activity was seen at the maximum concentration of 100 µg (Fig.8). The results show that the antioxidant agent's activity is dose-

dependent; hence, it has great therapeutic potential as a tool to fight oxidative stress, since larger concentrations of the agent greatly increase its effectiveness.

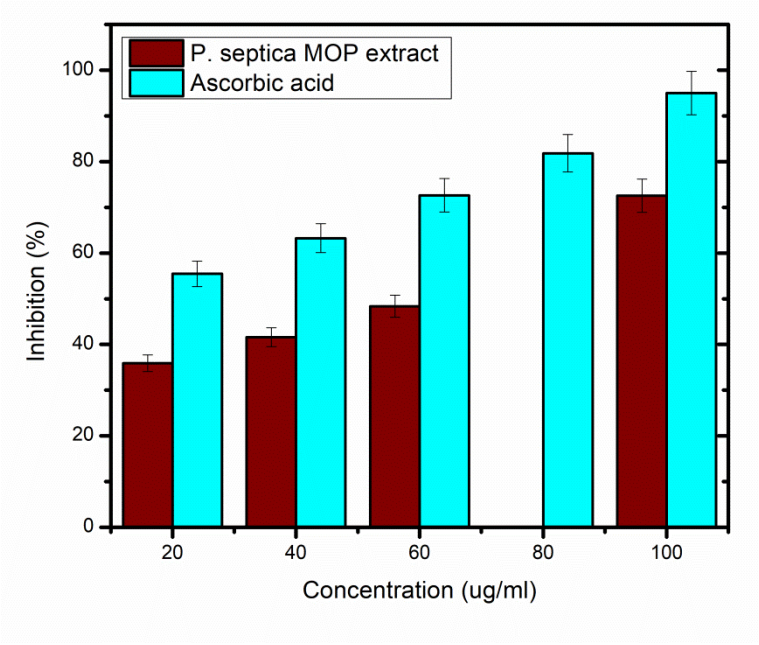

Fig. 8. Free radical scavenging activity of *Pantoea septica* extracts isolated from *Hibiscus rosa-sinensis*. The values are presented as the Mean ± SD.

4. DISCUSSION

Antibiotic resistance and disorders associated with oxidative stress are on the rise, which renders it all the more important to identify alternative sources of antioxidants and antibacterial agents. This research investigated *Pantoea septica*, a microbe isolated from *Hibiscus rosa-sinensis,* to determine if it could substitute for conventional antioxidant and antibacterial drugs. *Pantoea septica* has powerful antibacterial effects against many human diseases, according to the findings. This strain certainly produces bioactive compounds that may hinder bacterial development and reproduction, as shown by the zones of inhibition have been identified.

Pantoea septica may produce bioactive compounds, such as antimicrobial peptides or secondary metabolites, which are responsible for its antibacterial action. Its wide-spectrum

capability is shown by *Pantoea septica's* potency against both Gram-positive and Gram-negative bacteria. The challenges involved in treating illnesses caused by microorganisms that are resistant to many drugs make this discovery all the more important. *Pantoea septica* provides a potential natural alternative for therapeutic uses; its efficacy may be attributable to its enzyme-producing capabilities, which either break down bacterial cell walls or disrupt crucial bacterial activities [26].

The antibacterial and antioxidant activities of *Pantoea septica* were found to be rather strong. To determine the bacterial extract's antioxidant capability, the research used an array of tests, including the DPPH radical scavenging assays. The presence of *Pantoea septica* chemicals that may neutralize free radicals and reduce oxidative damage is supported by their significant scavenging activity. Since oxidative damage is associated with a wide range of chronic illnesses, including cancer, cardiovascular disease, and neurological problems, this antioxidant action is very important. *Pantoea septica's* capacity to synthesize phenolic compounds, flavonoids, and other naturally occurring antioxidants may be responsible for its antioxidant components. These chemicals, which are present in the bacterial extract, may have health advantages and might be used to produce natural antioxidant supplements.

Pantoea septica showed promise as a natural medicine due to its antibacterial and antioxidant properties. *Pantoea septica* has the potential to replace synthetic drugs which may cause side effects and resistance in pharmacological and nutritional contexts. New antibacterial drugs or antioxidant supplements might be developed using the natural chemicals obtained from *Pantoea septica* [27].

The antibacterial efficacy of microbial oils from *Pantoea septica* is primarily due to the presence of fatty acids and other bioactive compounds that disrupt bacterial cell membranes. The oil's composition, particularly the chain length and saturation level of the fatty acids, plays a crucial role in its effectiveness against different bacterial strains. Dynamic

modeling helps in predicting and controlling the production of these key components, ensuring the oil maintains a potent antibacterial profile [28]. The antioxidant properties of microbial oils are linked to the presence of unsaturated fatty acids, phenolic compounds, and other secondary metabolites that neutralize free radicals. By optimizing the fermentation process and employing control strategies, the production of these antioxidant compounds can be maximized. This enhances the oil's ability to protect cells from oxidative stress, which is crucial for its application in preventing and treating diseases associated with oxidative damage [29].

Pantoea septica MOP also offers a natural defense against infections and oxidative damage, so adding it to functional meals or nutritional supplements might improve health even more. *Pantoea septica's* antibacterial and antioxidant properties should be considered in the context of other bacterial and plant-derived treatments when compared to other natural sources or other bacterial strains. Isolating and characterizing the particular bioactive chemicals causing these effects should be the primary goal of future studies. These compounds might be identified and quantified using modern techniques like mass spectrometry and chromatographic procedures. *Pantoea septica* extracts may have therapeutic promise, but only after rigorous testing in *in vivo* models can we say for sure if they are safe and efficacious.

The safety and clinical evaluation of microbial oil from *Pantoea septica* strain MOP, optimized through dynamic modeling, involves rigorous toxicological assessments and preclinical trials to ensure its biocompatibility and efficacy. The oil's antibacterial and antioxidant properties are tested in phased clinical trials to establish therapeutic potential and safety. Successful trials pave the way for regulatory approval and potential medical applications.

5. CONCLUSION

In conclusion, the study on *Pantoea septica* isolated from *Hibiscus rosa-sinensis* demonstrates its significant potential as a natural source of antibacterial and antioxidant agents. The findings reveal that *Pantoea septica* possesses considerable antimicrobial activity against a range of human pathogens and exhibits robust antioxidant properties, making it a promising candidate for developing natural alternatives to synthetic drugs. The dual efficacy in combating bacterial infections and reducing oxidative stress highlights the therapeutic potential of this bacterial strain. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for these activities and assessing their safety and effectiveness in clinical applications.

ACKNOWLEDGMENTS

The authors would like to thank Vivekanandha College of Arts and Sciences for Women, Tiruchengode for their kind support during characterization and all other lab studies.

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