RESEARCH PAPER

Isolation, Characterization and Biological Evaluation of Exopolysaccharide from Brevundimonas spp. DMW10 with Antioxidant and EGFR Inhibitory Potential

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Abstract

Microbially derived exopolysaccharides (EPS) have attracted growing interest owing to their broad spectrum of bifunctional properties. The present work focuses on EPS-producing bacteria were isolated and identified as *Brevundimonas* spp. through 16S rRNA sequencing. The extracted EPS underwent structural analysis through Fourier Transform Infrared (FTIR) spectroscopy, which confirmed the occurrence of key functional groups including hydroxyl and carboxyl groups, indicating its polysaccharide nature. The antioxidant potential of EPS was assessed through hydroxyl radical scavenging and reducing power assays, which exhibited moderate, concentration-dependent activity, demonstrating its ability to neutralize reactive oxygen species. Docking simulations were performed to further analyze the lung cancer target protein EGFR (Epidermal Growth Factor Receptor). The results revealed favorable binding interactions of EPS-derived compounds with the binding site of EGFR, suggesting its potential role in inhibiting cancer-related pathways. Overall, the EPS produced by *Brevundimonas* spp. exhibits significant antioxidant activity and promising anticancer potential. These findings highlight its applicability as a natural bioactive compound in pharmaceutical and nutraceutical applications, warranting further investigation through advanced structural analysis and in vivo studies.

Key word : Brevundimonas spp. Antioxidant activity Molecular docking EGFR FTIR analysis 16S rRNA sequencing

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Introduction

Hunting for novel organically occurring chemicals that could substitutes chemically manufactured compounds is the most emphasizing objective of contemporary biotechnology (Carmen Rizzo et al., 2022). Approximately half of the world's primary generations of organic materials are found in ocean's microorganisms (Field, C.B et.al., 1998). Several microorganisms thrive in high temperature environments such as hot springs, hydrothermal vents, deep marine ecosystems, hyperacid lakes, acid mine drainage, excessive UV exposure, and more (Aparna Banerjee et al., 2021). Numerous places on Earth are occupied by varieties of microbes. In addition, marine environmental forms an extensive and varied ecosystem where numerous microorganisms play a major role in survival of plants ecology. In this system exopolysaccharides are considered as a major component (Abreu N.A et.al., 2016). Extracellular polysaccharides are high molecular weight complex biopolymers, also derived as exopolysaccharides (EPSs). Extracellular polysaccharides are mainly composed of sugars and other organic compounds like carbohydrates, such as proteins, lipids, humic substances, or extracellular DNA (Rana and Upadhyay, 2020). Marine bacteria producing EPSs are composed of three or four diverse monosaccharides which are aligned in repeated units of ten or less monosaccharides collectively known as heteropolysaccharides (Decho, A.W.1990). These polymers are critically involved in factors like particle formation, sedimentation and the cycling of dissolving metals they also form a component of dissolved organic carbon (DOC) found in marine environment (Ding, Y.X et al., 2008; Verdugo, P.2012). Both intrinsic (genetics) and extrinsic (growth media, physiochemical circumstances) factors impact the EPS synthesis. Even the same genus having identical physiochemical needs produce structurally distinct EPS (Felz et al. 2019; Tiwari et al. 2020). Bacteria are the most preferred source of EPS production due to their ability of fast growth and loosely attached mucoid layers which contribute significantly to isolation of EPS as they can be easily separated from the cells. Angelin and Kavitha (2020) reported EPS production from bacteria are biocompatible, biodegradable and are non-toxic in nature. Both Arctic and Antarctic conditions in Taxonomic taxa like *Pseudoalteromonas, Marinobacter, Polaribacter, Shewanella, Colwellia, Winogradskyella,* and *Pseudomonas,* are characterized to produce EPS (Casillo et al. 2018; Caruso et al. 2019). Understanding the undelaying structure of extracted EPS is crucial to grasping their modes of action and granting appropriate

applications and it forms backbone for research based on EPS (Sushmitha et al. 2022). Fourier Transform Infrared (FTIR) spectroscopy is a rapid, non-destructive analytical technique that offers information on molecular bonding patterns and functional groups of polysaccharides (Kacuráková & Wilson, 2001). Research on EPS has recently received an immense amount of attention because of their numerous unique intricate chemical structures and functionalities (Castellane TCL et al., 2014). Research on molecular arrangements and chemical compositions is essential to determine the EPSs structure-function interactions (Thejaswi Bhandary and Paari Kuppusamy Alagesan 2024). The length of the polymer chain affects the EPSs structure and physiochemical characteristics. The tertiary structure and physical behavior of the polymer are influenced by complicated chain entanglement anf intra molecular interactions, which become more likely as the polymer's length rises (Zhang et al., 2024). Additionally, prebiotic qualities, immunomodulatory, anticancer, and antioxidant effects, in addition to the ability of lower blood cholesterol, are one among the potential health benefits of bacteria; exopolysaccharides (Yildiz & Karatas, 2018). Excellent antioxidant qualities are demonstrated by EPSs synthesized from *L. plantarum YW32*, which also proven to limit increase in tumor cells in vitro and pathogenic microorganisms (Wang, J et al.,2015). In addition, each strain of bacteria produces unique EPS with different biological activity, therefore bacterial EPS can be incorporated either individually or in combination with some other materials for various applications in the biomedical and pharmaceutical industries (Abdelhamid et al., 2020). The EPS precise underlaying mechanism is yet unknown, and its complicated and discerning structure makes it difficult to investigate the composition activity relationship (Wei Wang et al.,2023). Molecular docking analysis has been carried out to study the EPS-protein interaction process in complex biological settings. Computational research investigating the binding of carbohydrate-rich EPS, such as kefiran, with whey proteins revealed favorable binding affinities and hydrophobic interaction patterns. These results shed light on functional relationships that are crucial to protein-polysaccharides complexes and food sciences (Jiménez-Pérez C et al., 2023). Docking analyses indicated that docking is useful beyond biochemical activity in EPS research. For example, it provide insight into how antibiotics and other ligands bind to extracellular polymeric substances in environmental systems (Kun Li et al.,2024). Consequently, this study concentrates on the isolation, identification and partial characterization of EPS from a marine isolate, as well as the evaluation of its biological activity, including antioxidant and antidiabetic effects.

Materials and method

Collection of water sample:

Seawater sample were obtained from a coastal region of Tuticorin, South India,

and maintained in an ice box until it was transported to the laboratory under a sterile plastic container.

Isolation of marine organism:

Marine water sample was properly mixed, and 1 mL of the collected sample was transferred aseptically into 9 mL of sterile physiological saline (0.85% NaCl) to obtain a 10^{-1} dilution. Serial dilutions were prepared up to 10^{-5} using the same diluent under aseptic settings (Nwosu et al., 2019).

Plating and Incubation

Aliquots (0.1 mL) from suitable dilutions were uniformly spread onto Zobell Marine Agar (ZMA) plates using a sterile glass spreader. The inoculated plates were incubated at 28–30 °C for 24–72 hrs in an inverted position. Marine agar was used to ensure proper salt concentration and nutrient composition suitable for marine bacterial growth (Dash et al., 2013).

Selection and Purification of Isolates

Following incubation, the plates were observed for the presence of bacterial. Colonies differing in size, shape, color, elevation, margin, and surface texture were selected. Individual colonies were picked and streaked repeatedly on fresh marine agar plates to obtain pure cultures. (Cappuccino & Sherman, 2014).

EPS production Screening

For exopolysaccharide (EPS) production, isolated colonies were subjected to screening using media composed of (g/L): glucose 20, CaCO3 0.1, NH4NO3 0.8, K2HPO4 0.6, KH2PO4 0.05, MnSO4□4H2O 0.1 and yeast extract 1.0 (Kim et al., 1998). Isolates showing mucoidal growth on marine agar media were further screened for EPS production.

Morphological and biochemical identification

Bacterial isolates were characterized based on their colony morphology, including parameters such as shape, size, color, elevation, and surface texture. Subsequently, a set of

biochemical test, including catalase, oxidase and carbohydrate fermentation tests, were performed to evaluate their metabolic properties.

Molecular identification of *Brevundimonas sp. strain DMW10*

The EPS produced bacterial isolate was identified by 16S rDNA sequencing. Genomic DNA was extracted using the GeneJET Genomic DNA Isolation Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The 16S rDNA gene was amplified using universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') under the following PCR conditions: initial denaturation at 95°C for 4 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 10 min. The PCR product was

verified by agarose gel electrophoresis and purified prior to sequencing. Sequencing was performed by Eurofins Genomics, India Pvt. Ltd. (Bangalore, India). The resulting sequences were analyzed using the BLASTn program against GenBank to determine sequence similarity, submitted to NCBI GenBank for accession, and a phylogenetic tree was constructed using the neighbor-joining method in MEGA7 software to infer evolutionary relationships.

Isolation and purification of EPS

With slight modifications, the acetone precipitation procedure was employed to isolate EPS (Sudhamani et al., 2004). To eliminate cells, bacterial cultures were harvested and centrifuged for seven minutes at 9000 rpm. After adding two volumes of cold acetone to the supernatant, it was incubated at 4 °C for the whole night. For further examination, the precipitate was collected after centrifugation at 12,000 rpm for 15 minutes at 4 °C.

FTIR analysis of EPS

The dried DMW11- EPS was investigated with an FTIR spectrophotometer (Bruker Tensor 27, Germany) to identify the main functional groups. The E3-EPS powder (10 mg) was mixed with potassium bromide (KBr) to form a pellet, and then it was loaded onto the single- crystal germanium of the FTIR spectrometer. The FTIR spectra were recorded within the frequency range of 4000-400 cm−1 with a resolution of 4.0 cm−1 and 64 scans (Ji X et al., 2022).

Molecular Docking Analysis

Molecular docking was performed to evaluate the anticancer potential of marine-derived exopolysaccharides (EPS) by analyzing their interactions with lung cancer-related protein target (EGFR) Three-dimensional structures of target proteins were retrieved from the Protein Data Bank (Berman et al., 2000) and prepared using AutoDock Tools by removing water molecules, adding polar hydrogens, and assigning Kollman charges (Morris et al., 2009). Ligand structures were generated using ChemDraw, converted to 3D conformations, and energy-minimized using Open Babel (O'Boyle et al., 2011). Due to their high molecular weight, EPS were represented as oligosaccharide fragments for docking. Active sites were identified based on literature and validated using CASTp (Tian et al., 2018). Docking simulations were conducted using AutoDock Vina, and binding affinities were expressed in kcal/mol, with the best conformations selected based on lowest binding energy (Trott and Olson, 2010). Protein–ligand interactions were analyzed using PyMOL and Discovery Studio Visualizer (DeLano, 2002). Pharmacokinetic properties were predicted using SwissADME to assess drug-likeness and bioavailability (Daina et al., 2017).

Scavenging activity of DPPH radical

The scavenging capacity of 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) radical by EPS samples was determined according to Zhang et al. (2013) with modifications. Briefly, 1 mL of an aqueous solution of EPS samples of various concentrations (0.2–1 mg/mL) was added to 2 mL of the ethanolic solution of the DPPH radical (0.05 mmol/L). The mixture was vigorously homogenized and incubated at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured in triplicate at 517 nm after centrifugation at 5000 x g for 5 min. The DPPH radical scavenging capacity was defined as:

Scavenging activity (%) = [1− (Asample – Ablank) / Acontrol] × 100

Where, A sample represents the absorbance of the solution with DPPH and EPS; Ablank represents the absorbance of the solution with EPS and ethanol (ethanol instead of DPPH) and; A control represents the absorbance of the solution with DPPH and water (water instead of EPS).

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay was performed following the Liu et al. (2010) method. FRAP reagent was obtained by mixing acetate buffer (300 mmol/L at pH 3.6), 2,4,6-Tris (2-pyridyl)-striazine (TPTZ) (10 mmol/L) dissolved in hydrochloric acid (40 mmol/L) and ferric chloride (20 mmol/L) in a ratio of 10:1:1. The 2.7 mL mixture of FRAP reagent with 0.3 mL EPS (0.2–1 mg/mL) was incubated under heating at 37 °C for 10 min and absorbance was monitored at 593 nm using FRAP reagent as blank. Ferrous sulfate (100– 2000 mmol/ L) was used to perform the calibration curve and the results were expressed in μmol FeSO4/g EPS.

Hydrogen peroxide scavenging activity of EPS

Hydrogen peroxide scavenging activity of EPS was analysed according to the method of (Kanamarlapudi & Muddada, 2017). A solution of hydrogen peroxide (10 mM) was prepared in 0.1 M phosphate buffer saline (pH 7.4). Various concentrations (200–1000 □g) of EPS were rapidly mixed with 2 mL of hydrogen peroxide solution. After an incubation of 10 min, the absorbance of hydrogen peroxide was measured at 230 nm. Ascorbic acid was used as the standard.

Hydroxyl Radical Scavenging (HRS) Activity

HRS assesses the antioxidant capacity to neutralize hydroxyl radicals by disrupting their reactions. The HRS of exopolysaccharide was evaluated based on the method outlined Previously (Wang, J. et.al., 2012) A mixture was prepared by combining 1 mL of 1,10- phenanthroline (0.75 mmol/L), 2 mL of PBS and 1 mL of FeSO4 (0.75 mmol/L). Subsequently, 1 mL of H2O2 (0.12%) and 1 mL of the test samples were introduced. The resulting mixtures were incubated at 37 ◦C for 90 min, while the HRS activity was tracked by

measuring the rise in absorbance at 536 nm. The control sample comprised 1,10- phenanthroline, FeSO4 and H2O2, while the blank samples consisted of 1,10-phenanthroline, FeSO4 and deionized water. The scavenging activity was represented as a percentage, calculated using the following formula:

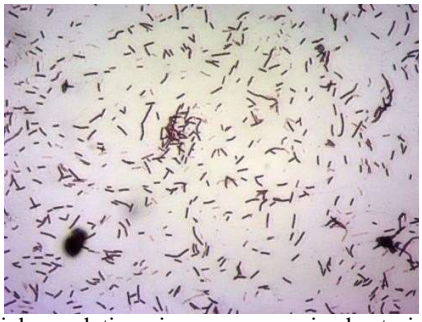
$$OH\ scavenging\ (\%) = (As - Ac)/(Ab - Ac) \times 100$$

where As, Ac and Ab represent the absorbance of the test sample, control and blank, respectively.

Results and Discussion

Collection of Water Sample and Isolation of Marine Bacteria

Seawater samples obtained from the coastal region of Tuticorin exhibited a substantial microbial load, a feature commonly associated with nutrient-enhanced marine environments. Following serial dilution and plating on Zobell Marine Agar (ZMA), a substantial quantity of bacterial colonies was observed following incubation. The colony-forming units (CFU) showed dense microbial populations in seawater samples, which is in agreement with previous research by Nwosu et al., (2019), who reported microbial counts in marine water ranged from 10^6–10^7 CFU/mL (Nwosu et al., 2019). The use of marine agar supported the development of halophilic and marine-adapted bacteria owing to its appropriate salt composition and nutrients (Dash et al., 2013).



Morphological Diversity and Purification of Isolates

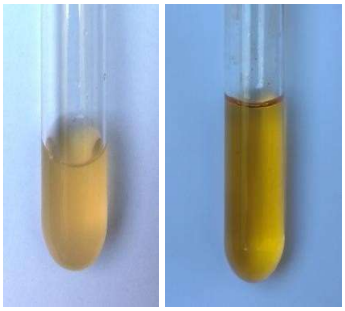
Morphologically distinct colonies, differing in size, shape, color, elevation, margin and texture representing the diverse bacterial populations, were selected for synthesis of EPS. Studies on marine bacterial isolation having similar morphological diversity has also been reported in previous research (Zhang et al., 2015). As it is customary in microbiology that repeated streaking yields pure cultures, ensuring the isolation of pure bacterial strains for further research (Cappuccino and Sherman, 2014).

Screening for Exopolysaccharide (EPS) Production

Numerous strains displayed mucoidal and slimy colony morphology, which indicates the extracellular polysaccharide production. The accumulation of polysaccharides on the cell surface is a well-known sign of EPS secretion (Kim et al., 1998). This is precisely why the mucoid character of colonies is very important. These findings align with the previous studies demonstrating that glucose-rich media enhance EPS production in marine bacteria (Freitas et al., 2011).

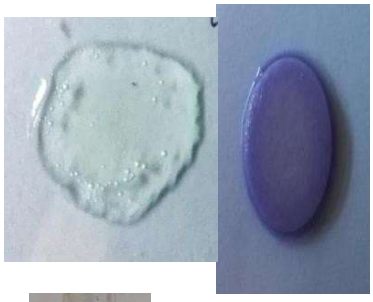
Morphological and Biochemical Identification

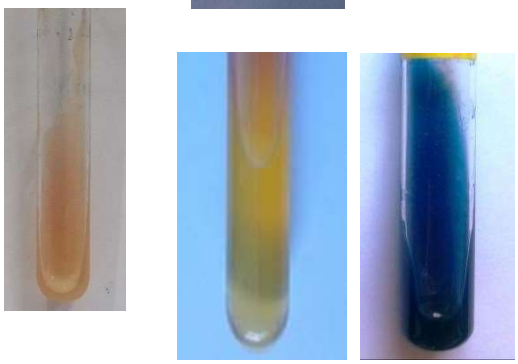
On marine agar, the isolate *Bravidomonas* sp. DMW1 displayed a unique colony morphology with a circular shape, smooth surface and mucoidal texture. These features are indicative of typical marine bacterial adaptation and suggests the potential production of extracellular polymeric substances (Poli et al., 2010; Zhang et al., 2015). *Bravidomonas sp*. DMW10 aerobic metabolic nature was confirmed by biochemical characterization revealing catalase positive an oxidase positive (Cappuccino and Sherman, 2014). Additionally, the isolate showed metabolic versatility by fermenting specific carbohydrates which is typical to marine bacteria (Holt et al., 1994).



Gram's Stain Indole

MR VP





Citrate Urease

TSI

Oxidase

Catalase

Fig 1 : Morphological and Biochemical identification

Table 1 : Observation of morphological and biochemical characteristics of *Bravidomonas* sp. DMW10

Morphological features		Cultural features	
Size	Small (1–2 mm)	Gram's staining	Gram-positive
Shape	Circular	Shape	Cocci (tetrads/clusters)
Elevation	Convex	Capsule staining	– (non-capsulated)
Texture	Smooth, butyrous	Spore staining	– (non-sporing)
Colour	Yellow to creamy	Motility	Non-motile
Margin	Entire (smooth)	Flagella	Absent

Biochemical test	
Indole	–
Methyl Red (MR)	–
Voges–Proskauer (VP)	–
Citrate Utilization	–
Catalase	+
Oxidase	+
Urease	–

Molecular identification of *Brevundimonas sp. strain DMW10*

The EPS-producing strain (DMW10) 16S rDNA sequence (~1400 bp) was successfully amplified and sequenced. The isolates taxonomica affiliation was confirmed by BLASTn analysis, which confirmed the highest sequence similarity (≥99%) with members of the genus Brevundimonas. The sequence was submitted to the NCBI GenBank database under the accession number (OQ772259.1). Using the neighbor-joining method for phylogenetic analysis, strain DMW10 was in close relationship with other Brevundimonas species, resulting in distinct clade with robust bootstrap support, indicating its evolutionary kinship within the genus. The isolates morphological and biochemical traits were confirmed by molecular identification. *Brevundimonas* species are renowned for their metabolic versatility, including the generations of exopolysaccharides with potential biotechnological applications production and they have been widely

reported from marine and aquatic habitats (Ryan and

Pembroke, 2018). The genus *Brevundimonas* is confirmed by the strong sequence similarity, indicating its possible involvement in the generation of marine EPS and related bioactivities.

>AGGGCAGCTACACATGCAAGTCGAACGGA
CCCTTCGGGGTTAGTGGCGGACGGGTGAGT
AACACGTGGGAACGTGCCTTTAGGTTCGGA
ATAGCTCCTGGAAACGGGTGGTAATGCCGA
ATGTGCCCTTCGGGGGAAAGATTTATCGCCT
TTAGAGCGGCCCGCGTCTGATTAGCTAGTTG
GTGAGGTAACGGCTCCACCCAAGGCGACGA
TCAGTAGGCTGTCTGAGAGATGACCAGACA
CACTGGGACTGAGACACGATCCAGACTCTTA
CGGGAGGCAGCAGTGGGGGAATCTGCGCAA
TGGGCGAAAGCCTGACGCAGCCATGCCGCG
TGAATGATGAAGGTCTTAGGATTGTAAAATT
CTTCACCGGGGACGATAATGACGGTACCCG
GAGAAGAAGCCCCGGCTAACTTCGTGCCAG
CAGCCGCGGTAATACGAAGGGGGCTAGCGT
TGCTCGGAATTACTGGGCGTAAAGGGCGCG
TAGGCGGATCGTTAAGTCAGAGGTGAAATC
CCAGGGCTCAACCCTGGAACTGCCTTTGATA
CTGGCGATCTTGAGTATGAGAGAGGTATGTG
GAACTCCGAGTGTAGAGGTGAAATTCGTAG
ATATTCGGAAGAACACCAGTGGCGAAGGCG
ACATACTGGCTCATTACTGACGCTGAGGCGC
GAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGATTG
CTAGTTGTCGGGCTGCATGCAGTTCGGTGAC
GCAGCTAACGCATTAAGCAATCCGC
CTGGGGAGTACGGTCGCAAGATTAAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCG
CAGAACCTTACCACCTTTTGACATGCCTGGA
CCGCCACGGAGACGTGGCTTTCCCTTCGGGG
ACTAGGACACAGGTGCTGCATGGCTGTCGTC
AGCTCGTGTCGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCTCGCCATTAGTTGC
CATCATTTAGTTGGGAACTCTAATGGGACTG
CCGGTGCTAAGCCGGAGGAAGGTGGGGATG
ACGTCAAGTCCTCATGGCCCTTACAGGGTGG
GCTACACACGTGCTACAATGGCAACTACAG
AGGGTTAATCCTTAAAAGTTGTCTCAGTTCG
GATTGTCCTCTGCAACTCGAGGGCATGAAGT
TGGAATCGCTAGTAATCGCGGATCAGCACC
GCGGTGAATACGTTCCCGGGCCTTGTACACA
CCGCCCGTCACACCATGGGAGTTGGTTCTAC
CGGAGGCAGCCGACCACGCGTACCC
CGAAGGCGGTGCGCTAACCAGCAAT

Fig 2 : Phylogenetic tree

FTIR analysis of EPS

The EPS from *Brevundimonas* sp. DMW10 displayed distinctive absorption peaks in the 4000–500 cm^{-1} range in FTIR spectrum. O–H stretching vibrations are representated by a wide peak at 3524.67 cm^{-1}, whereas C–H stretching is represented by peak 2979.82, 2885.31, and 2821.66 cm^{-1}. The presence of hydroxyl groups characteristic of polysaccharides was indicated by a large and powerful peak at 3524.67 cm^{-1}, which was attributed to O–H stretching vibrations (Synytsya and Novák, 2014). The C-H stretching vibrations of aliphatic groups are represented by the peaks at 2979.82, 2885.31, and 2821.66 cm^{-1}, indicating the existence of a carbohydrate backbone (Kumar et al., 2020). Among all the peaks strong bands were observed at 2383.85 cm^{-1} and 2346.24 cm^{-1}. Carbonyl (C=O) stretching is depicted by peaks at 1767.64 cm^{-1} and 1680.85 cm^{-1}. C=O stretching vibrations were found as the source of the absorption bands at 1767.64 cm^{-1} and 1680.85 cm^{-1}, confirming the presence of carbonyl containing compounds such as uronic acids or esterified components (Wang et al., 2019). Peaks observed at 1545.84 cm^{-1} and 1510.16 cm^{-1} depicted the presence of N–H bending and C–N stretching vibrations, indicating the potential presence of proteinaceous components or amino sugars (Li et al., 2016). C–O–C stretching vibrations, whjch are confined to glycosidic connections were identified as a pronounced peak at 1243.04 cm^{-1}, whereas C–O stretching vibrations of polysaccharide units are associated with the peak at 1057.88 cm^{-1} (Coates, 2000). The absorption band at 951.81 cm^{-1} indicates the presence of β-glycosidic linkages (Synytsya and Novák, 2014). Furthermore, skeletal and ring vibrations of carbohydrate structures were linked to the peaks at 688.54 cm^{-1} and 517.85 cm^{-1}. Additional peaks were found at 1545.84 cm^{-1} and 1510.16 cm^{-1}. The fingerprint region showed lower wavenumber peaks at 688.54 cm^{-1} and 517.85 cm^{-1}, and also substantial peaks at 1243.04 cm^{-1} and 1057.88 cm^{-1}, along with a clear band at 951.81 cm^{-1}. When taken as a whole, these peaks indicates that the EPS structure contains several functional groups.

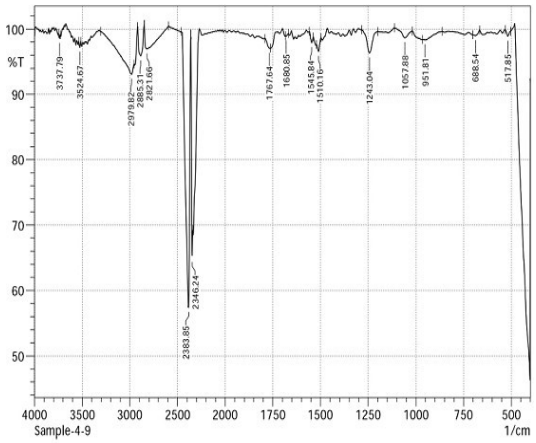
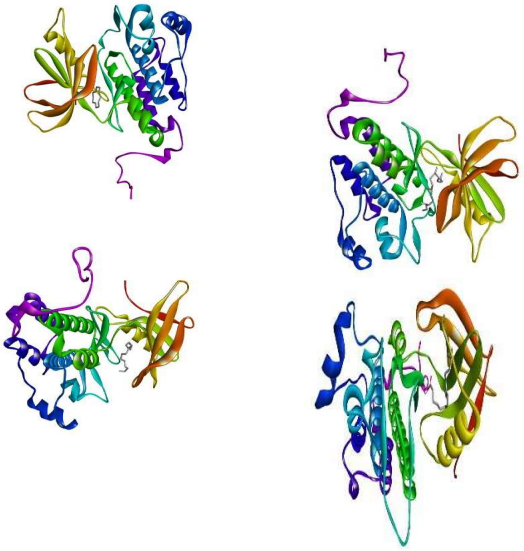


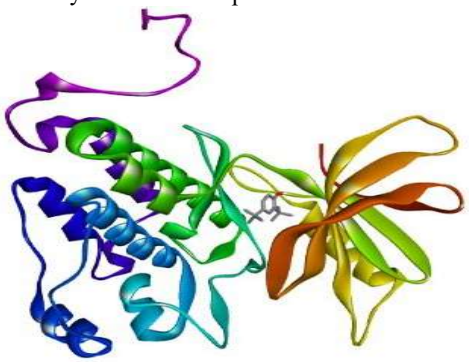
Fig : 3 FTIR analysis of Exopolysaccharide from *Brevundimonas* sp. DMW10

Molecular docking analysis

The interaction of EPS-derived chemicals with important cancer-related proteins, including EGFR, Bcl-2 and Caspase-3 was assessed using molecular docking research. It showed moderate to substantial interactions showing binding affinities ranging between -2.9 to −6.4 kcal/mol. The chemicals that affinity were Phenol, 2,4-bis(1,1-dimethylethyl) showed the strongest binding affinity (−6.4 kcal/mol), followed by Ethyl 2-(2-chloroacetamido)-3,3 and Cyclododecane (−5.9 kcal/mol), as well as Hexadecane, 2,6,10,14-tetramethyl and 11- Hexadecenoic acid, 15-methyl (−5.8 kcal/mol) were among the other chemicals with intermediate binding affinities, whereas Pyrrolidine



(−2.9 kcal/mol) had the lowest interaction. In general, the docking results indicate that some chemicals generated from EPS have a significant potential for binding to important proteins associated with cancer. Phenol, 2,4-bis(1,1-dimethylethyl) had the greatest binding affinity (−6.4 kcal/mol) among the chemicals examined, indicating a significant interaction with the target proteins active sites, perhaps through hydrophobic and hydrogen bonding interactions (Sliwoski et al., 2014). Significant binding was further demonstrated by compounds such Ethyl 2-(2-chloroacetamido)-3,3 and Cyclododecane (−5.9 kcal/mol), as well as Hexadecane, 2,6,10,14-tetramethyl and 11-Hexadecenoic acid, 15-methyl (−5.8 kcal/mol), suggesting their possible contribution to bioactivity. While binding with Bcl-2 and Caspase-3 suggests likely stimulation of apoptosis, a crucial factor in anticancer activity, the interaction with ECFR suggests potential suppression of cell growth pathways (Elmore, 2007; Nicholson, 2000). Overall, the docking results show that compounds generated from EPS have excellent anticancer potential, especially since they have long-chain hydrocarbon and phenolic structures.

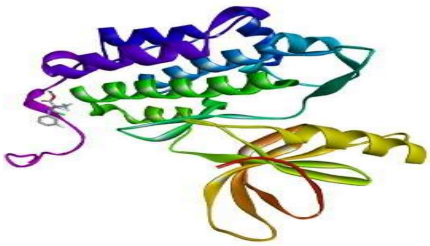


(Phenol,2,4-bis(1,1-dimethylethyl

Ethyl 2-(2-chloroacetamido)-3,3

Cyclododecane

Hexadecane 11-Hexadecenoic acid



2,6,10,14-tetramethyl

Fig 4 : 3D structures of selected bioactive compounds identified from EPS.

Antioxidant Activity of EPSc DPPH

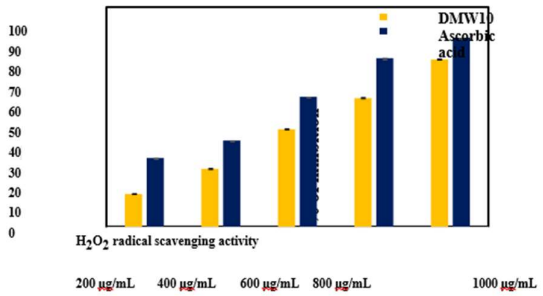
Radical Scavenging Activity

Exopolysaccharide (EPS) demonstrated a concentration- dependent increase in antioxidant activity, according to the DPPH radical scavenging assay. While the conventional ascorbic acid exhibited greater activity ranging from 14.32 ± 0.30% to 73.31 ± 0.14%, the percentage inhibition ascended from 6.69 ± 0.18% at 200 µg/mL to 49.95

± 0.18% at 1000 µg/mL. The progressive inclination shows that the EPS can contribute hydrogen atoms to stabilize DPPH radicals, even if it showed relatively lesser activity that the standard. The EPS structures functional groups, such as hydroxyl and carboxyl groups, are contributing to its scavenging activity. Microbial polysaccharides have been reported to similar dose dependent antioxidant activity (Wang et al., 2017; Zheng et al., 2020).

Hydrogen peroxide radical scavenging activity

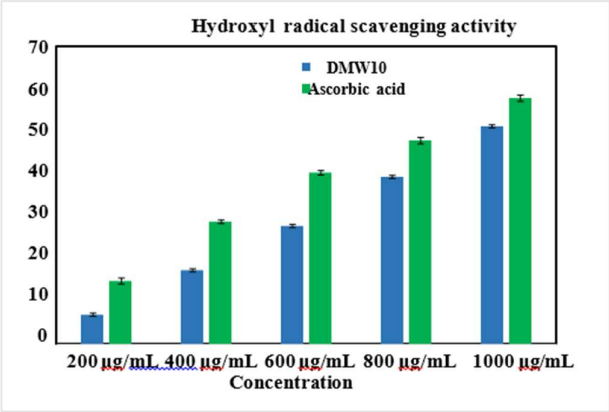
The hydrogen peroxide (H_2O_2) scavenging activity was assessed at different concentrations (200-1000 µg/ml) and relative to ascorbic acid, a common antioxidant. EPS percentage inhibition was observed to be increased from 14.86 ± 0.16% at 200 µg/ml to 76.20 ± 0.24% at 1000 µg/ml indicating the moderate level of antioxidant activity. In contrast, ascorbic acid had much greater activity at lower doses, ranging from 31.18 ± 0.32% to 85.84 ± 0.24% throughout the same concentration range. Significant scavenging activity was demonstrated by the EPS at higher doses, especially at 800 µg/ml and above. The presence of functional groups like hydroxyl and carboxyl groups, which may donate electrons to neutralize hydrogen peroxide and stop the production of dangerous hydroxyl radicals (•OH), may be account for the reported antioxidant potential of EPS. In spite of being relatively weak, hydrogen peroxide may produce very reactive species inside the cells, which may lead to oxidative stress and cellular damage (Ruch et al., 1989).

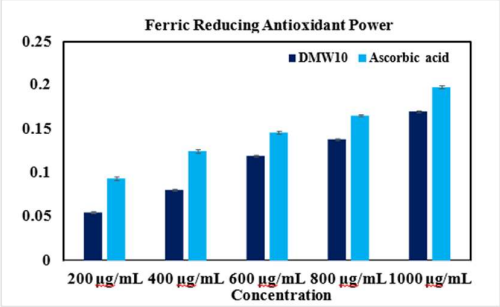


Concentration

Hydroxyl scavenging activity of EPS

The exopolysaccharide (EPS) capacity to scavenge hydroxyl radicals notably increased with concentration. The % inhibition ranged from 6.85 ± 0.36% to 51.26 ± 0.36%, suggesting that the EPS has a moderate antioxidant capacity. The existence of functional groups capable of donating donate electrons or hydrogen atoms to neutralize hydroxyl radicals is suggests by the progressive increase in scavenging activity with concentration. In comparison with the standard ascorbic acid exhibited higher activity (14.80 ± 0.72% to 57.88 ± 0.75%), confirming its strong antioxidant capacity (Smirnoff, 2001). According to Halliwell & Gutteridge (2015), hydroxyl radicals are highly reactive species that cause oxidative damage to biomolecules including protein, lipids and DNA (Halliwell & Gutteridge, 2015). The scavenging activity of EPS is due to hydroxyl, which enhance radical quenching through hydrogen atom transfer or electron donation mechanisms (Wang et al., 2008). The current findings are supported by reports of comparable mild antioxidant activity for microbial exopolysaccharides (Li & Shah, 2014; Liu et al., 2009).





Ferric Reducing Antioxidant Power

The reducing power of exopolysaccharide (EPS) increased in a concentration-dependent manner, showing absorbance values in the range of 0.055 ± 0.001 to 0.171 ± 0.001 (200-1000 µg/mL), showing its electron donation capacity. Ascorbic acid on the other hand, had greater reducing power (0.094 ± 0.002 to 0.198 ± 0.002), demonstrating its potent antioxidant ability (Smirnoff, 2001). The gradual increase in the absorbance indicates that EPS may successfully break free radical chain events by reducing Fe^{3+} to Fe^{2+} (Oyaizu, 1986). Functional groups like hydroxyl and carboxyl groups in EPS, which promote electron transport and radical stabilization, may be responsible for this action (Wang et al., 2008). The current results are validated by reports of similar moderate lowering power for microbial exopolysaccharides (Li & Shah, 2014; Liu et al., 2009).

Conclusion

The present study utilized 16S rRNA sequencing to validate the bioactive potential of exopolysaccharide (EPS) produced by *Brevundimonas* spp. During structural characterization by FTIR, key functional groups such as hydroxyl and carboxyl groups were identified, confirming the polysaccharide composition of the EPS. The antioxidant analysis indicated its ability to mitigate oxidative stress, revealing moderate yet significant activity, including hydroxyl radical scavenging and reducing power, in a concentration-dependent fashion. Molecular docking experiments against the lung cancer target protein EGFR (Epidermal Growth Factor Receptor) revealed favourable binding interactions, implying the drugs derived from EPS may possess anticancer properties. These interactions suggest the possible inhibition of EGFR-mediated signaling pathways, which are vital for cancer development. Overall, the study shows that EPS from *Brevundimonas* species could be a natural biomolecule that fights cancer and acts as an antioxidant. To identify the active components responsible for bioactivity, subsequent research should focus on the detailed structural elucidation of EPS using techniques such as NMR. More research is needed in vitro and in vivo to make sure it is safe and works against cancer. Research on the mechanism of action, large-scale manufacturing and formulation development is also important to find out how it could be used in the pharmaceutical and nutraceutical industries.

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