RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 3: 447-454 (March 2026)

Network Pharmacology and Molecular Docking Analysis of GC-MS Identified Compounds from Ethyl Acetate Extract of *Kocuria* sp. against Oral Cancer

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10.18805/IJAR.BF-2016

ABSTRACT

Background: Oral cancer poses a significant global health challenge due to its poor prognosis and limited therapeutic advancements. **Methods:** This study investigates the therapeutic potential of *Kocuria sp. NAT1*, a bacterial strain isolated from *Pachycondyla sennaarensis* collected in Riyadh, Saudi Arabia, for oral cancer treatment. Bacterial isolates were cultured in nutrient broth and metabolites were extracted using ethyl acetate. Identification was conducted using MALDI-TOF MS. **Result:** GC-MS analysis identified 39 bioactive compounds, including 2,5-piperazinedione and pyrrolo[1,2-a]pyrazine-1,4-dione. Network pharmacology was employed to assess drug-likeness and target interactions, integrating Swiss ADME and Swiss Target Prediction. Oral cancer-related targets were retrieved from multiple databases and a protein-protein interaction (PPI) network was constructed using STRING and analyzed with Cytoscape. Functional pathway enrichment (GO/KEGG) revealed significant involvement in apoptosis resistance, cancer cell migration and metastasis regulation. Molecular docking using Auto Dock Tools confirmed strong binding affinities of the key bioactive compounds to oncogenic targets, including TNF, SRC, KRAS and EGFR. These findings highlight the potential of *Kocuria sp. NAT1*-derived metabolites as novel candidates for oral cancer therapy, warranting further *in vitro* and *in vivo* validation to explore their clinical applicability.

Key words: Gc-Ms analysis, *Kocuria* sp., Molecular docking, Network pharmacology, Oral cancer.

Abbreviations: ADME: Absorption, distribution, metabolism and excretion; ATP: Adenosine triphosphate; BP: Biological process; CC: Cellular component; EGFR: Epidermal growth factor receptor; GC-MS: Gas chromatography-mass spectrometry; GO: Gene ontology; GUI: Graphical user interface; HBA: Hydrogen bond acceptor(s); HBD: Hydrogen bond donor(s); HIF-1: Hypoxia-inducible factor 1; HPLC: High-performance liquid chromatography; KEGG: Kyoto encyclopedia of genes and genomes; MALDI-TOF: Matrix-assisted laser desorption/Ionization-Time of flight; MAPK: Mitogen-activated protein kinase; MF: Molecular function; MolWt: Molecular weight; NIST: National institute of standards and technology; PBS: Phosphate-buffered saline; PDB: Protein data bank; PI3K-Akt: Phosphoinositide 3-kinase-protein kinase B; PPI: Protein-protein interaction; RPM: Revolutions per minute; SDF: Structure data file; SMILES: Simplified molecular input line entry system; TNF: Tumor necrosis factor.

INTRODUCTION

Oral cancer, which accounts for approximately 48% of head and neck cancer cases, is predominantly diagnosed as oral squamous cell carcinoma (OSCC), with a global 5-year survival rate below 50% despite significant advancements in treatment (Irani, 2020). OSCC progresses through a multistep process involving complex genetic and chromosomal alterations (Jensen *et al*., 2012), making early detection and prevention critical (Irani, 2016). Key risk factors include tobacco use and alcohol consumption (Ram *et al*., 2011), chronic inflammation (Alnuaimi *et al*., 2015), human papillomavirus (HPV) and Candida infections (Ram *et al*., 2011), ultraviolet (UV) radiation, genetic predisposition and immunosuppression, which increases the risk in renal and bone marrow transplant patients (Sarode *et al*., 2015). Preventive strategies focus on minimizing tobacco and alcohol use detecting. Despite advancements in treatment, such as surgery, radiation therapy and chemotherapy, oral carcinoma remains challenging to manage due to late diagnosis, high recurrence rates, resistance to chemotherapy and lymph node metastasis. The tumor microenvironment, immune evasion and the lack of effective targeted therapies further contribute to treatment failure (Melo-Alvim *et al*., 2022); (Liu *et al*., 2022).

Overcoming chemotherapy resistance is essential to improving patient outcomes, prompting the search for novel therapeutic agents from natural sources (Huang *et al*., 2021). Natural bioactive compounds, including alkaloids,

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How to cite this article: Aljaser, M., Abutaha, N. and Wadaan, M.A. (2026). Network Pharmacology and Molecular Docking Analysis of GC-MS Identified Compounds from Ethyl Acetate Extract of *Kocuria* sp. against Oral Cancer. *Indian Journal of Animal Research.* **60(3):** 447-454. doi: 10.18805/IJAR.BF-2016.

Submitted: 04-05-2025 **Accepted:** 30-08-2025 **Online:** 24-10-2025

flavonoids and terpenoids derived from plants, have demonstrated the ability to modulate apoptotic pathways, inhibit efflux pumps and enhance chemotherapy efficacy (Fernandes *et al*., 2022). Marine organisms such as sponges, corals and algae produce secondary metabolites with potent anticancer properties that counteract tumor resistance mechanisms (El-Seedi *et al*., 2025). However, an emerging yet underexplored source of bioactive compounds is the insect microbiome, which may offer novel anticancer applications.

The insect microbiome comprises different microbial communities, including archaea, fungi, viruses and bacteria, which contribute to digestion, immunity, reproduction and environmental adaptation (Engel and Moran, 2013). These microbes can be classified into gut microbiota (Engel and Moran, 2013), cuticular microbiota (Duplais *et al*., 2021) and endosymbionts (Eleftherianos *et al*., 2013). While some symbiotic bacteria aid in nutrient digestion, vitamin synthesis and host defense, others produce bioactive metabolites that enhance insect defense mechanisms (Douglas, 2014) (Eleftherianos *et al*., 2013). Notably, certain gut microbes exhibit biotransformation capabilities, such as detoxifying pesticides (Blanton and Peterson, 2020), suggesting potential applications in anticancer drug discovery. Advances in metagenomics and sequencing technologies are expanding our understanding of insect-microbe interactions, with significant implications for agriculture, health and biotechnology. Thus, investigating gut bacteria could uncover novel bioactive resources for drug development.

The genus Kocuria emerged from the reclassification of Micrococcus following comprehensive phylogenetic and chemotaxonomic analyses. Species within Kocuria are characterized as Gram-positive, coccoid, aerobic, non-endospore-forming and non-halophilic microorganisms (Reddy *et al*., 2003). In this study, a Kocuria strain isolated from *Pachycondyla sennaarensis* was identified using the MALDI Biotyper® Sirius System (Bruker). However, limited research exists on Kocuria species within insect microbiomes and their possible contributions to drug discovery. Given their metabolic versatility and ability to produce bioactive secondary metabolites, we hypothesize that Kocuria species from insect microbiomes represent a promising source of novel anticancer agents.

This study employs an integrative approach combining network pharmacology and molecular docking to identify bioactive compounds from Kocuria species with potential oral anticancer properties. Network pharmacology offers a systematic framework to understand the interactions between microbial-derived compounds and key molecular targets involved in oral carcinoma progression. Molecular docking further validates the binding affinities and potential mechanisms of action of these bioactive secondary metabolites, offering insights into their therapeutic relevance. This combined strategy facilitates the identification of promising drug candidates that may overcome chemotherapy resistance and enhance treatment strategies for oral carcinoma.

MATERILAS AND METHODS

Isolation of bacteria

The *Pachycondyla sennaarensis* specimens utilized in this study were collected from areas surrounding King Saud University and immediately transported to the laboratory for processing. Before microbiome analysis, the insects underwent surface sterilization using 70% ethanol for three minutes, followed by rinsing with sterile phosphate-buffered saline (PBS) to eliminate external contaminants. Each specimen was then individually crushed and homogenized in PBS. The homogenates were serially diluted in sterile PBS from 10^{-1} to 10^{-5} and the dilutions were spread onto nutrient agar plates. These plates were incubated for 48 h at 30°C, allowing bacterial colonies to develop. Emerging colonies were subjected to two sequential purification steps on fresh nutrient agar to obtain pure cultures. The purified bacterium was inoculated into 5 mL of nutrient broth and incubated at 37°C for 24 h. Following incubation, cultures were centrifuged and the obtained pellets were preserved for further investigation.

MALDI-TOF mass spectrometry

We utilized the MALDI Biotyper® Sirius System (Bruker, USA) for the rapid identification of bacterial colonies. The isolate was applied onto a MALDI-TOF target in duplicate and each colony was overlaid with 2 µL of matrix solution (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) without additional supplements. Bacterial spectra were automatically acquired using flex Control 3.0 software and analysis was performed with Biotyper 2.0 software.

Fermentation

The bacterial isolates were cultured in nutrient broth for fermentation. The isolate was inoculated into an Erlenmeyer flask containing 500 mL of nutrient broth (prepared in quadruplicate) and incubated under standardized conditions in a shaker incubator at 150 RPM and 30°C for 5 days. Following incubation, the bacterial cultures were filtered using Whatman No. 1 filter paper to remove cellular debris. Metabolite extraction was performed by adding an equal volume (500 mL, 2X) of ethyl acetate (HPLC grade, Sigma-Aldrich) to each flask, followed by stirring for 15 minutes to ensure efficient extraction. The organic phase was subsequently concentrated to dryness using a rotary evaporator (Heidolph, Germany) maintained at 45°C. The resulting dry extract was weighed and stored in glass vials at -80°C for subsequent analyses.

GC-MS analysis of extract

A 1 µL sample was injected *via* an autosampler into an Agilent 7890B GC-MS system (Agilent Technologies, USA). Compound identification was performed using the NIST

MS database, following a previously described method (Abutaha and AL-Mekhlafi, 2024).

Network pharmacology analysis

Prediction of drug-like potential of Ethyl acetate compounds

The SMILES notations for various chemical compounds were obtained from PubChem. These notations were then analyzed using the SwissADME platform to evaluate their drug likeness potential. Key molecular descriptors, such as hydrogen bond donors (HBD), molecular weight (MolWt), oral bioavailability (OB) and hydrogen bond acceptors (HBA) were assessed to determine their suitability for further investigation. This systematic screening process was designed to identify compounds with optimal druglike characteristics, providing a foundation for subsequent research and analysis.

Target prediction

The Swiss Target Prediction platform (http://www.swisstargetprediction.ch/) was employed to identify possible molecular targets for the compounds, with an emphasis on predictions relevant to the human species (*Homo sapiens*). By using the SMILES notations of the compounds, the platform employed a reverse pharmacophore mapping approach to identify prospective molecular targets. Similarly, the therapeutic targets associated with oral cancer were predicted using multiple databases, including DisGeNET, GeneCards, OMIM, PharmGKB and TTD. Following the integration of results from each database and the removal of duplicate entries, a consolidated list of oral cancer-related therapeutic targets was generated. This comprehensive approach ensured the identification of key molecular targets for further investigation. The intersection of therapeutic targets for oral cancer (OC) and the predicted targets of the compounds was analyzed using Venny 2.1 software. This process helped identify shared targets and create Venn diagrams (Abutaha *et al*., 2024).

Construction of a common target PPI network

To construct the protein-protein interaction (PPI) network for the shared targets of the compounds and oral cancer, we input the common target data into the STRING database (http://www.string-db.org/), selecting *Homo sapiens* as the organism and applying a confidence threshold of >0.7. The resulting PPI data and network diagrams were then visualized and analyzed using Cytoscape software (version 3.7.2) (https://cytoscape.org/).

Functional analysis of target proteins

Gene ontology (GO) is widely used to annotate genes and their expression products. GO functional analysis was conducted using the ShinyGO v0.741 database. Significant gene enrichment was determined at P<0.05. The Kyoto Encyclopedia of Genes and Genomes (KEGG) was utilized to explore the signaling pathways of drug targets.

Molecular docking and visualization

The 3D molecular structures of the compounds were sourced from the PubChem database, while receptor protein structures were obtained from the RCSB PDB database. Open Babel GUI was used to convert SDF files to PDB format. Protein and ligand preparations were performed using AutoDock Tools 1.5.7 by removing water molecules, extracting original ligands and assigning Gasteiger charges. Non-polar hydrogens were added and flexible bonds in small molecules were set to be rotatable. The docking grid was adjusted to enclose the active site based on the reference ligand's coordinates. Docking simulations were executed using AutoDock Vina 1.1.2 to predict binding affinities, generating binding energy values for each interaction. PyMOL 4.3.0 and PLIP were employed for visual analysis, assessing binding conformations and intermolecular interactions (Abutaha *et al*., 2025).

RESULTS AND DISCUSSION

In this study, an orange-pigmented bacterial strain was isolated from *P. sennaarensis* near the university in Riyadh, Saudi Arabia. The strain was purified and cultured on nutrient agar, forming round, raised, smooth, convex and mucoid colonies with non-diffusible orange pigmentation. Microscopic analysis revealed that the cells were Gram-positive cocci. MALDI-TOF MS, a commonly used technique for bacterial identification, classified the strain as *Kocuria sp. NAT1*. *Kocuria sp. NAT1* was fermented using nutrient broth and extracted with ethyl acetate. The extract was analyzed using GC-MS, leading to the identification of 39 compounds (Table 1) that belong to different classes of compounds. The analyzed phytochemicals exhibited varying solubility, gastrointestinal absorption, blood-brain barrier, permeability, interactions with P-glycoprotein and cytochrome P450 enzymes. Most compounds had high GIA, with a few exceptions showing low solubility. BBB permeability was observed in several compounds, while some acted as CYP inhibitors, particularly against CYP3A4 and CYP1A2. Many compounds adhered to Lipinski's rule of five, but some, such as long-chain fatty acids (*e.g.*, n-hexadecanoic acid, erucic acid and squalene), exceeded the MLOGP threshold (>4.15), indicating potential bioavailability concerns. Additionally, certain molecules, including ethyl oleate and dodecanoic acid esters, showed low solubility, potentially affecting their pharmacokinetic properties. Overall, while most phytochemicals exhibited favourable absorption and drug-likeness properties, a few had limitations in solubility and metabolic interactions, which may impact their therapeutic applications. This result indicates that these compounds exhibit high oral bioavailability, a crucial factor in developing new medicines (Tasleem *et al*., 2021)

A total of 39 compounds were identified through GC-MS analysis. The most abundant compound, 2, 5-

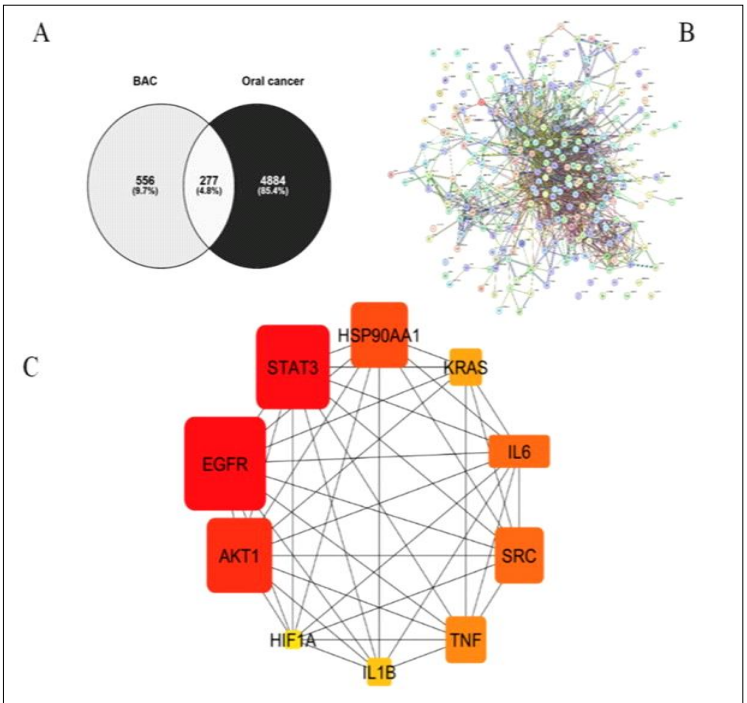
Piperazinedione, 3, 6-bis(2-methylpropyl)- (18.5%), reported to possess antimicrobial activity against multidrug-resistant and biofilm-forming bacteria, suggesting its potential as a lead compound in treating antibiotic-resistant infections (Driche *et al*., 2024). Similarly, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (13.2%) from Staphylococcus sp. strain MB30 exhibited significant anticancer activity against lung (A549) and cervical (HeLa) cancer cells, with IC_{50} values of 19.94±1.23 and 16.73± 1.78 µg/mL, respectively. The compound induced apoptosis,

Table 1: Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the Ethyl Acetate Extract from *Kocuria* sp.

Peak	Rt	Area%	Name	MF	Mw
1	13.99	0.289892073	2-Coumaranone	$C_8H_9O_2$	134
2	15.843	0.340106524	3-Aminoacetophenone	C_8H_9NO	135
3	21.576	0.321948799	1,4-Benzoguinone, 2,6-diethyl-, 1-imine	$C_{10}H_{13}NO$	163
4	22.67	0.882921241	3-Aminoacetophenone	C_8H_9NO	135
5	23.26	0.342706891	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	228
6	25.139	0.298959675	2-Naphthalenol, 5-amino-	$C_{10}H_9NO$	159
7	26.988	0.387151615	(3S,6S)-3-Butyl-6-methylpiperazine-2,5-dione	$C_9H_{16}N_2O_2$	184
8	27.22	2.499503878	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154
9	28.761	1.552445316	Benzenemethanol, 3-hydroxy-5-methoxy-	$C_8H_{10}O_3$	154
10	29.099	0.998116328	7-Ethyl-4,6-pentadecandione	$C_{17}H_{32}O_2$	268
11	29.635	0.978388783	3,6-Diisopropylpiperazin-2,5-dione	$C_{10}H_{18}N_2O_2$	198
12	30.191	6.420422262	Hexahydro-2H-pyrido(1,2-a)pyrazin-3(4H)-one	$C_8H_{14}N_2O$	154
13	30.69	16.31813696	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210
14	30.878	5.219830572	(2S,6R)-2,6-Dibutyl-4-methylpiperidine	$C_{14}H_{29}N$	211
15	31.253	0.629700933	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
16	31.773	0.197305531	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
17	33.59	0.408380071	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258
18	34.521	1.284892629	Oleic Acid	$C_{18}H_{34}O_2$	282
19	34.863	1.327697736	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
20	34.976	1.002729394	Ethyl Oleate	$C_{20}H_{38}O_2$	310
21	35.45	17.54620019	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226
22	36.172	3.486481253	2-Cyclohexen-3-ol-1-one, 2-dodecanoyl-	$C_{18}H_{30}O_3$	294
23	36.665	0.474776698	2,5-Piperazinedione, 3-benzyl-6-isopropyl-	$C_{14}H_{18}N_2O_2$	246
24	37.226	0.430948507	Glycidyl palmitate	$C_{19}H_{36}O_3$	312
25	37.387	0.226880805	Erucic acid	$C_{22}H_{42}O_2$	338
26	37.86	5.43362528	4-Benzyloxy-3-methoxybenzyl alcohol	$C_{15}H_{16}O_3$	244
27	38.022	0.204735391	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286
28	38.154	0.707614133	2,5-Piperazinedione, 3-(hydroxymethyl)-6-(phenylmethyl)-	$C_{12}H_{14}N_2O_3$	234
29	38.312	0.469381828	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione	$C_{15}H_{20}O_4$	264
30	38.609	12.48638994	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	244
31	38.896	0.456008686	1-Tricosanol	$C_{23}H_{48}O$	340
32	39.045	0.468217112	1-Hexacosene	$C_{26}H_{52}$	364
33	39.193	0.285855233	1-Heptacosanol	$C_{27}H_{56}O$	396
34	40.135	0.909156722	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356
35	41.003	0.252393957	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	487
36	41.91	0.908672912	1,3-Dioxolo[4,5-c]pyran, tetrahydro-	$C_{18}H_{28}O_8$	372
37	42.701	4.220169115	Dodecanoic acid, hexadecyl ester	$C_{28}H_{56}O_2$	424
38	45.816	1.106961483	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	456
39.	46.996	2.656299576	Squalene	$C_{30}H_{50}$	410

as evidenced by nuclear condensation, cell shrinkage and DNA fragmentation. Flow cytometry analysis revealed G1 phase cell cycle arrest, while Western blotting confirmed the downregulation of cyclin-D1, CDK-2 and anti-apoptotic proteins (Bcl-2 and Bcl-xL), along with the activation of caspase-9 and caspase-3, leading to PARP cleavage. Additionally, it inhibited cancer cell migration and invasion, suggesting its potential as a promising anticancer agent (Lalitha *et al*., 2016). Furthermore, squalene (2.81%) exhibited significant anticancer potential through multiple mechanisms. Acting as a potent antioxidant, squalene prevents oxidative DNA damage and lipid peroxidation (Valgimigli, 2023), thereby reducing cancer risk. It is believed to exert anticancer effects by preventing the farnesylation of Ras oncoproteins and blocking the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) into mevalonate, disrupting key pathways in tumor development. Additionally, squalene regulates enzymes involved in xenobiotic metabolism and acts as a free radical scavenger to reduce oxidative stress and DNA damage (Smith, 2000). Furthermore, squalene interferes with cholesterol metabolism, which is crucial for tumor growth (Xiao *et al*., 2023). Recent studies highlight its ability to enhance the efficacy of anti-cancer drugs such as adriamycin, 5-fluorouracil, bleomycin and cisplatin (Yarkoni and Rapp, 1979) (Pimm *et al*., 1980) (Nakagawa *et al*., 1985). Moreover, 3′,8,8′-Trimethoxy-3-piperidyl-2,2′-binaphthalene-1,1′,4,4′-tetrone (0.26%) demonstrated a wide-ranging effect and potential action in anticancer, antimicrobial, immunomodulatory and anti-inflammatory activities (Okasha *et al*., 2024) (Youssef *et al*., 2023) (Al-Askar *et al*., 2024). Lastly, oleic acid has multiple health benefits, including regulating cellular functions, suppressing cancer growth, reducing inflammation, controlling oncogene expression, lowering blood pressure and aiding wound healing (Olowofolahan *et al*., 2024).

After removing redundant targets, SWISS Target Prediction identified 833 potential targets for the chemical compounds. To integrate oral cancer-related targets, different databases were utilized, resulting in 5,111 oral cancer-related target proteins closely associated with oral cancer. The intersection of chemical compound targets with oral cancer-related targets yielded 227 target genes (Fig 1). The STRING database analysis, conducted with a confidence threshold of ≥ 0.7, revealed a highly interconnected PPI network consisting of 276 nodes and 1485 edges (Fig 1B). This strong enrichment suggests that the proteins in the network are functionally associated rather than randomly connected, highlighting their potential biological relevance. The top ten hub genes were selected from the PPI network using the CytoHubba plugin. They are considered the key molecular targets of compounds for

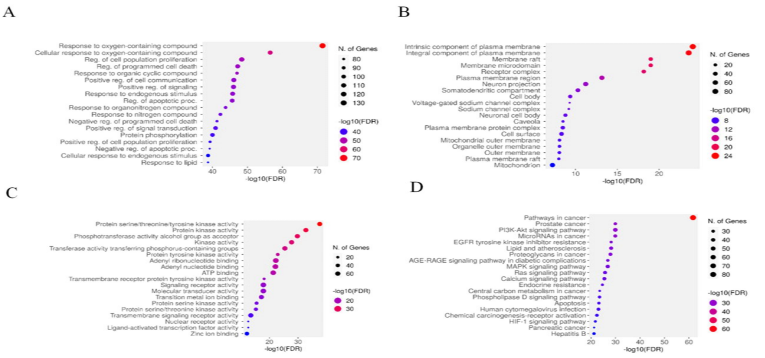


(A) Venn diagram: This panel illustrates the overlapping genes between compound-related and oral cancer-related targets, revealing potential intersections. (B) Compound-Target Network: Highlights key interactions within the network. (C) Top 10 Hub Genes: Displays the ten most significant genes ranked using the degree algorithm, emphasizing their importance in the network.

Fig 1: Analysis of target genes and compound interactions.

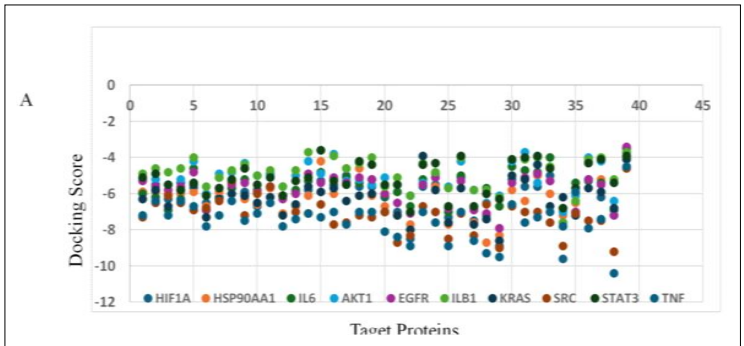
the inhibition of oral cancer cell growth (Fig 1C). The hub genes, from the highest degree score to the least, include TNF, SRC, KRAS, EGFR, STAT3, HSP90AA1, AKT1, IL6, IL1B and HIF1A. These proteins regulate apoptosis and drive metastasis through MMPs, integrins, cytoskeletal remodeling and key signaling pathways. Their role in apoptosis resistance and migration makes them vital targets for cancer therapy (Szczygielski *et al*., 2024) (Debnath and Kundu, 2025) (Lee *et al.*, 2025)

The KEGG pathway enrichment analysis aligns with the Gene Ontology (GO) results, which classify enriched terms into three main categories: molecular functions (MF),



Additionally, the chart includes the outcomes of the KEGG pathway analysis (D), highlighting significantly enriched pathways.

Fig 2: Bar chart depicting the results of the gene ontology (GO) analysis, categorizing enriched terms into three primary domains: Biological processes (A), cellular components (B) and molecular functions (C).



Furthermore, molecular docking (MD) analysis presents the 2D and 3D interaction profiles of two significant compounds: B: Squalene, which interacts with TNF (-10.4 kcal/mol) (PDB ID: 7JRA) and C : 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone, which binds with HIF1A (-9.6 kcal/mol) (PDB ID: 4Z1V).

Fig 3: The heatmap illustrates the binding affinities of 39 compounds (numbered 1 to 39) and glycerol (control, numbered 39) along the x-axis, assessed against 10 target proteins displayed on the y-axis (A).

cellular components (CC) and biological processes (BP) (Fig 2). The BP category reveals significant enrichment in pathways related to cell population proliferation, apoptotic regulation and responses to organic and nitrogen-containing compounds, indicating that these processes are crucial in cellular adaptation and survival. This is consistent with the KEGG analysis, which highlights key signalling pathways such as PI3K-Akt, MAPK and HIF-1, known to regulate tumour progression, apoptosis resistance and metabolic stress adaptation. The CC category shows enrichment of genes associated with the plasma membrane, receptor complexes and membrane microdomains, which suggests a pivotal role in transmem-brane signalling, receptor-mediated interactions and cellular communication. This finding aligns with KEGG pathway analysis results, particularly with the involvement of EGFR and TNF signalling, both of which are critical for cancer cell survival, immune evasion and inflammatory responses.

Furthermore, the MF category reveals enrichment in protein kinase activity, ATP binding and receptor signalling, reinforcing the idea that kinase-driven oncogenic pathways play a crucial role in cancer progression. This is further supported by molecular docking results (Fig 3A), where squalene showed high binding affinity to TNF (-10.4 kcal/mol) (Fig 3B) and 3′,8,8′-Trimethoxy-3-piperidyl-2,2′-binaphthalene-1, 1′,4,4′-tetrone exhibited strong interactions with key oncogenic targets such as HIF1A (-9.6 kcal/mol) (Fig 3C). The negative control displayed weaker interactions (-3.4 to -4.6), confirming that the tested compounds exhibited stronger binding, with TNF, SRC and HIF1A showing the most significant interactions. These interactions suggest that the compounds may function as potential inhibitors of critical transcription factors and kinases involved in oncogenic signalling, inflammation and metastasis. Additionally, the presence of ligand-activated transcription factor activity and nuclear receptor activity in the GO analysis further supports the hypothesis that these compounds might interfere with nuclear signalling pathways that regulate gene expression, cellular differentiation and metabolic reprogramming in cancer cells.

SUMMARY

This study investigated the anti-oral cancer potential of metabolites from *Kocuria* sp. NAT1.

- GC-MS identified 39 bioactive compounds from the bacterial extract.
- Network pharmacology predicted strong drug-likeness and interactions with key cancer targets like TNF and EGFR.
- Functional analysis showed involvement in apoptosis and metastasis pathways.
- Molecular docking confirmed high binding affinity to oncogenic proteins.
- The results propose these bacterial metabolites as promising candidates for oral cancer therapy.

CONCLUSION

Our integrated computational approach demonstrates that the tested compounds possess significant anticancer potential by modulating key oncogenic processes. The convergence of GO enrichment, KEGG pathway and molecular docking analyses indicates that these effects are mediated through the inhibition of kinase activity, disruption of receptor signalling, and alteration of apoptotic pathways, thereby targeting critical mechanisms of tumor growth, angiogenesis and therapy resistance. Subsequent *in vitro* and *in vivo* validation will be essential to translate these promising computational findings into potential therapeutic applications.

ACKNOWLEDGEMENT

The authors express their sincere appreciation to the Ongoing Research Funding Program (ORF-2025-757), King Saud University, Riyadh, Saudi Arabia.

Funding

This project was funded by the Ongoing Research Funding Program (ORF-2025-757), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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