TCABSE-J Communications
ISSN: 2583- 2557, *Volume 1, Issue 8, pp6-8. 2024.*

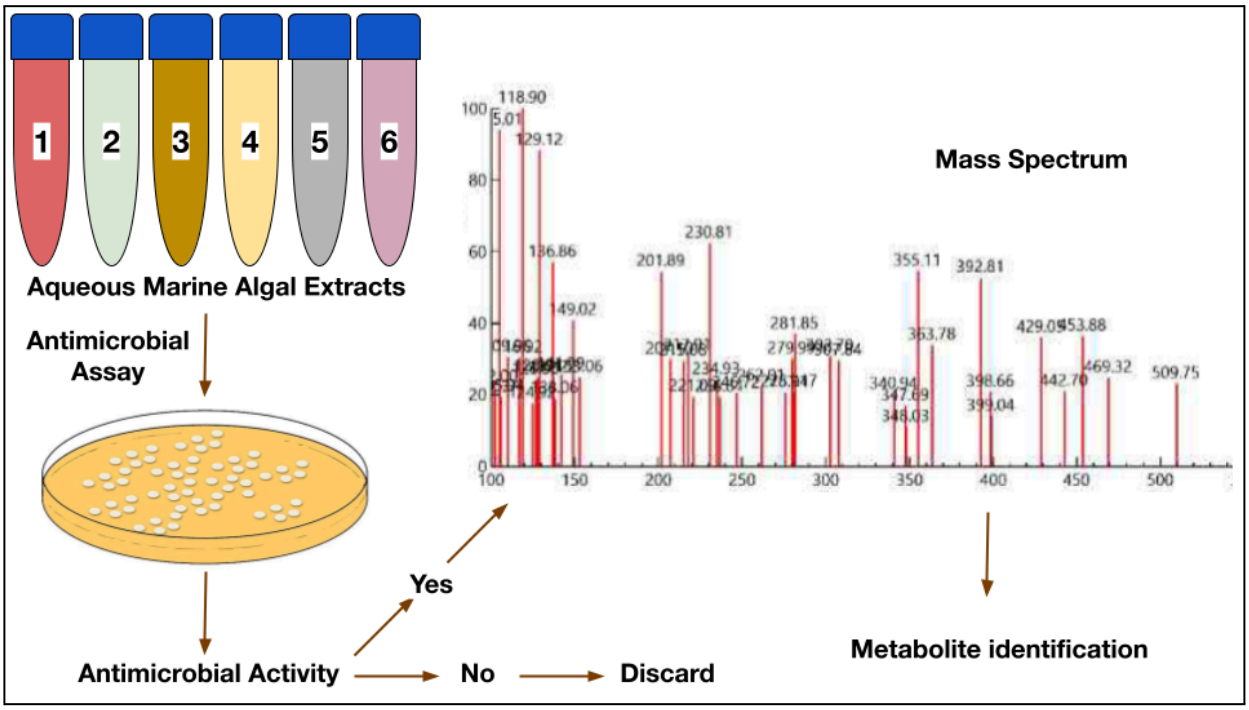
Mass spectroscopy-based metabolite profiling of marine algal extract that exhibited *in vitro* antibacterial activity against laboratory *E. coli* cells

Kiranmani Sampadarao[1, 2], Manjunadh Mallapureddi[1, 3], Prasanthi Valluri[1, 4], Anuhya Kolluri[1, 5], Sreesirisha Kolli[1, 6], Manikanta Sodasani[1], Madhuri Vissapragada[1] and Ravikiran S. Yedidi[1, 3, 4, *]

[1]Department of Intramural research core, The Center for Advanced-Applied Biological Sciences & Entrepreneurship (TCABS-E), Visakhapatnam 530003, A.P. India; [2]Department of Biotechnology, GITAM deemed to be University, Visakhapatnam 530045, A.P. India; [3]Department of Zoology, Andhra University, Visakhapatnam 530003, A.P. India; [4]Department of Biotechnology, Andhra University, Visakhapatnam 530003, A.P. India; [5]Department of Applied Microbiology, Sri Padmavati Mahila Visvavidyalayam, Tirupati 517502 A.P. India; [6]Department of Biotechnology, Dr. Lankapalli Bullayya College, Visakhapatnam 530016, A. P. India. (*Correspondence to R.S.Y.: tcabse.india@gmail.com).

Keywords: Marine algal extracts, antimicrobial activity, LC-MS, metabolite profiling, metabolites.

Multidrug-resistance (MDR) among various bacterial species has become a global medical issue in recent years. MDR bacterial strains are challenging to control especially in an outbreak situation such as the recent COVID-19 pandemic. A constant search for new sources of antimicrobial compounds has been going on for years. Marine organisms have been fascinatingly interesting in the context of antimicrobial agents. In this study we picked one out of the six marine algal extracts (MAEs) that showed reasonable antibacterial activity. A full LC-MS profiling was performed in order to understand the possible metabolites that may contribute to its antibacterial activity. Our results identified more than 10 metabolites in the MAE out of which anyone might be a causative for the antibacterial activity. A thorough search for the antibacterial properties for each of the identified metabolites was performed using standard metabolite databases. Surprisingly, all the metabolites that were identified in this study were either directly/indirectly involved in antibacterial properties suggesting that the current MAE has to be further investigated for its medicinal benefits.



Citation: Sampadarao, K., Mallapureddi, M., Valluri, P., Kolluri, A., Kolli, S., Sodasani, M., Vissapragada, M. and Yedidi, R.S. (2024). Mass spectroscopy-based metabolic profiling of marine algal extract that exhibited *in vitro* antibacterial activity against laboratory *E. coli* cells. *TCABSE-J*, Vol. 1, Issue 8:6-10. Epub: July 24th, 2024.

TCABSE-J Communications
ISSN: 2583- 2557, *Volume 1, Issue 8, pp6-8. 2024.*

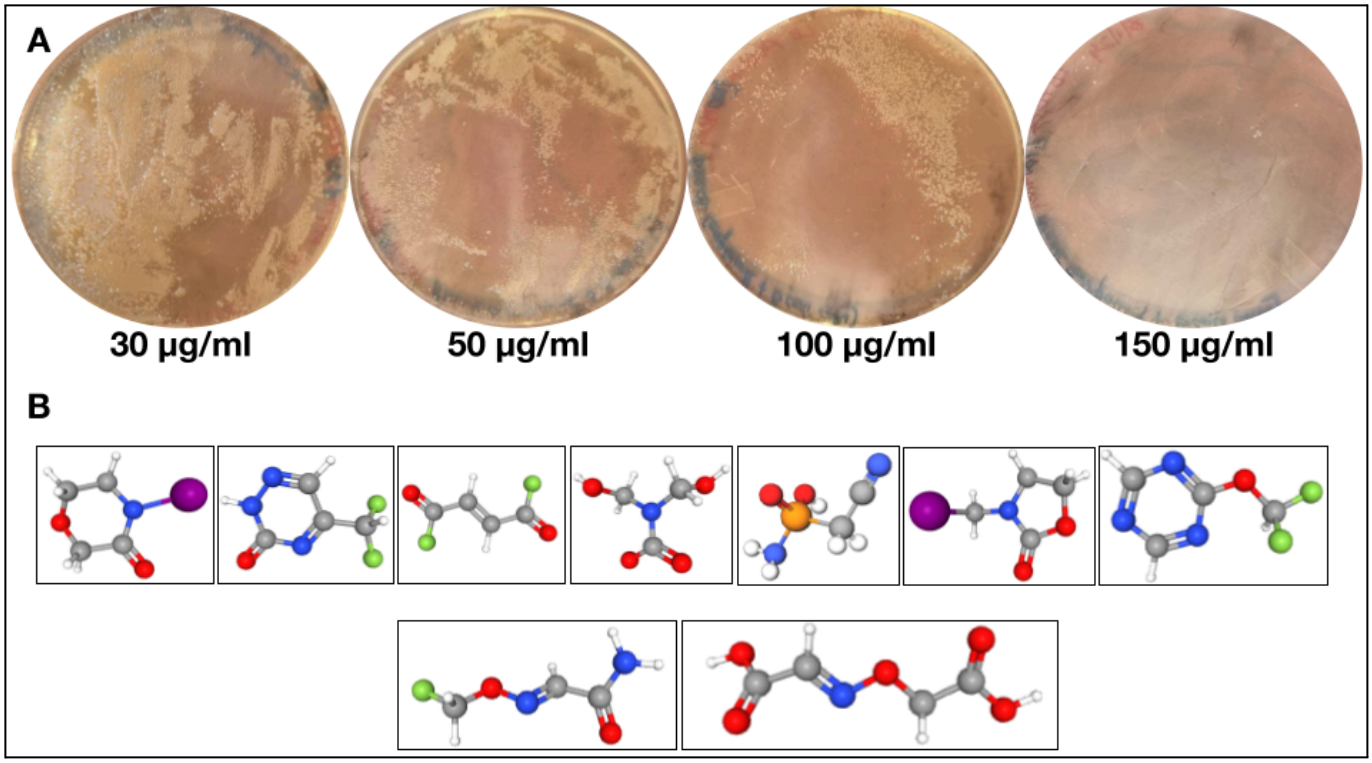


Figure 1. Antibacterial activity of MAE5 against laboratory *E. coli* and metabolites identified in MAE5 using LC-MS. (**A**) LB agar plates containing different concentrations of MAE5 against a red color background are shown here. Three plates from the left contain >100 CFUs each and the plate at the right end contains <20 CFUs indicating that MAE5 is most effective at 150 µg/ml concentration. (**B**) Nine metabolites with antimicrobial activity. Top row from left to right are: iodomorpholinone; 5-(difluoromethyl)-2H-1,2,4-triazin -3-one; fumaroyl fluoride; N,N-bis(hydroxymethyl)carbamate; cyanomethylphosphonamidic acid; 3-(Iodomethyl)-1,3-oxazolidin-2 -one; 2-(difluoromethoxy)-1,3,5-triazine; and bottom row from left to right are: 2-(fluoromethoxyimino) acetamide and 2-(carboxy methylideneamino)oxyacetic acid.

Antimicrobials, from ancient herbal remedies to modern pharmaceuticals, protect public health by combating harmful microorganisms. However, as microorganisms evolve, they develop defenses, rendering antimicrobial treatments ineffective [1, 2]. Misuse and overuse of these medications have led to antimicrobial resistance (AMR), a global public health and development threat [3]. The One Health concept emphasizes the interdependence of human, animal, and environmental parameters for controlling antimicrobial resistance (AMR) [3, 4]. In India, AMR rates have risen disproportionately in the past decades, with a lack of sufficient research and data preventing a nation-wide comparison [5]. Out of 2152 studies published, 48.3% were on humans, while only 3.3% were on animals, 4.2% on environment and 0.5% on One Health. The current magnitude of AMR in India is significant, with over 70% of Gram-negative bacteria resistant to fluoroquinolones and third generation cephalosporins [6]. The country also faces resistance to colistin, with high mortality rates associated with colistin-resistant *K. pneumoniae* [7]. It is estimated that bacterial antimicrobial resistance was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths [8]. By 2050, up to 10 million deaths could occur annually. Currently, as most of the bacteria show resistance towards the major class of antimicrobials there is still a need for new antibiotics for the upcoming diseases [9]. In this study, to develop new novel antibiotics six aqueous marine algal extracts (MAEs) were obtained through a research collaboration and were used to evaluate their antibacterial activity *in vitro*.

The MAEs 1 to 4 and MAE6 did not show any detectable antibacterial activity within the concentration range of 30 µg/ml to 150 µg/ml tested in this study. However, MAE5 showed a decrease in the number of CFUs at 100 µg/ml compared to the lower concentrations (30 µg/ml and 50 µg/ml) of the same. As shown in Figure 1, significant decrease in the number of CFUs (~10 CFUs) at 150 µg/ml concentration. MAE5 was outsourced to Biofact Research Pvt Ltd., Visakhapatnam for LC-MS analysis. The sample was analyzed in both positive $[M+H]^+$ and negative $[M-H]^-$ modes and multiple mass spectra were obtained for all LC

peaks in the chromatogram. Peaks with intensity of at least 50% or above were particularly analyzed in order to avoid any data misinterpretation due to background noise. The m/z ratios of the peaks were noted and were used for further analysis. The m/z values for the peaks that were identified above were directly taken as their mass values and were used for searching the possible metabolites. The advanced search option within the PubChem database [10, 11] was used to search (https://pubchem.ncbi.nlm.nih.gov/) for the potential metabolites. The antibacterial activity of each metabolite was identified based on the patents filed for that particular metabolite.

A total of 5 mass spectra were analyzed based on the significant peaks identified in the LC. Each spectrum contained multiple peaks with m/z values ranging from 100 to 700 suggesting that the majority of the metabolites were small molecules with molecular weight less than 500 Da. Small molecules that are less than 500 Da are more likely to possess the drug likeness and are potential candidates for preclinical drug discovery. More than 100 metabolites were identified among which, the final metabolites with antibacterial or antimicrobial activity were filtered. A total of 9 metabolites were identified that were previously validated and patented for their antimicrobial activity. As shown in Figure 1, the 3-dimensional structures of these metabolites contain diversity with various functional groups. Each of these 9 metabolites have been patented. For example, the iodomorpholinone has been patented for HIV integrase inhibitor, topical microbicidal solution and contact lens sterilizers [12-14]. Similarly, 3-(Iodomethyl)-1,3-oxazolidin -2-one has been patented as an antibiotic and also as an inhibitor of factor XIA [15, 16]. All the other 7 identified metabolites have similar antibacterial profiles that were previously published. Based on these patented studies, it is safe to conclude that this study sheds light on the metabolic profile of the MAE5 that showed antibacterial activity and confirms the presence of antibacterial metabolites.

Acknowledgements: We thank The Yedidi Institute of Discovery and Education, Toronto for scientific collaborations.

Conflict of interest: The authors declare no conflict of interest in this study.

Author contributions: K.S. and S.K. performed antibacterial assays; M.M., P.V. and A.K. performed LC-MS analysis and metabolite identification. M.S. and M.V. supervised K.S., M.M., P.V., S.K. and A.K. R.S.Y. is the principal investigator who designed the project, trained all authors, secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. edited and finalized the manuscript.

References

1. Sophie S. Abby, Eric tannier, Monola Gouy, and Vincent Daubin. Lateral gene transfer as a support for tree of life. Proc Natl Acad Sci USA. 2012 March 27: 109(13):4962-4967.
2. Lan, R and Reeves, P.R. Gene transfer is a major factor in bacterial evolution. Mol Biol Evol. 1996 January; 13(1):47-55.
3. Rokon-Uz-Zaman M, Bushra A, Pospo TA, et al. Detection of antimicrobial resistance genes in Lactobacillus spp. from poultry probiotic products and their horizontal transfer among Escherichia coli. Vet Anim Sci. 2023;20:100292. Published 2023 Mar 10.
4. Velazquez-Meza ME *et al*. Antimicrobial resistance: One Health approach. *Vet World*. 2022;15(3):743-749.
5. Dixit A *et al.* Antimicrobial Resistance: Progress in the Decade since Emergence of New Delhi Metallo-β-Lactamase in India. *Indian J Community Med*. 2019;44(1):4-8.
6. Taneja N, Sharma M. Antimicrobial resistance in the environment: The Indian scenario. *Indian J Med Res*. 2019;149(2):119-128.
7. Arjun R, Gopalakrishnan R, Nambi PS *et al*. A Study of 24 Patients with Colistin-Resistant Gram-negative Isolates in a Tertiary Care Hospital in South India. *Indian J Crit Care Med*. 2017;21(5):317-321.
8. Murray, Christopher J L *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet, Volume 399, Issue 10325, 629 - 655.
9. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
10. Kim S, Chen J, Cheng T, et al. PubChem 2023 update. *Nucleic Acids Res.* 2023;51(D1):D1373–D1380.
11. Bolton EE, Chen J, Kim S, Han L, He S, Shi W, Simonyan V, Sun Y, Thiessen PA, Wang J, Yu B, Zhang J, Bryant SH. PubChem3D: A new resource for scientists. J Cheminform. 2011 Sep 20; 3(1):32.
12. National Center for Biotechnology Information (2024). PubChem Patent Summary for JP-4625838-B2, [Translated] HIV integrase inhibitor. Retrieved July 23, 2024 from https://pubchem.ncbi.nlm.nih.gov/patent/JP-4625838-B2.
13. National Center for Biotechnology Information (2024). PubChem Patent Summary for US-2003228376-A1, Novel topical microbicidal compositions. Retrieved July 23, 2024 from https://pubchem.ncbi.nlm.nih.gov/patent/US-2003228376-A1.
14. National Center for Biotechnology Information (2024). PubChem Patent Summary for US-5925317-A, Dual neutralization system for iodine treatment of contact lenses. Retrieved July 23, 2024 from https://pubchem.ncbi.nlm.nih.gov/patent/US-5925317-A.
15. National Center for Biotechnology Information (2024). PubChem Patent Summary for TW-201018697-A, Tricyclic oxazolidinone antibiotic compounds. Retrieved July 23, 2024 from https://pubchem.ncbi.nlm.nih.gov/patent/TW-201018697-A.
16. National Center for Biotechnology Information (2024). PubChem Patent Summary for CN-102026996-A, [Translated] Pyridazine derivatives as factor XIA inhibitors. Retrieved July 23, 2024 from https://pubchem.ncbi.nlm.nih.gov/patent/CN-102026996-A.