International Journal of Secondary Metabolite
2025, Vol. 12, No. 4, 861–867
https://doi.org/10.21448/ijsm.1618012
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

HRLC-MS analysis of methanolic leaf extract of *Morus macroura* Miq.

Priyanka T. Sutar[1*], **Dattatraya. K. Gaikwad**[1]

[1]Department of Botany, Shivaji University, Kolhapur, Maharashtra, India

ARTICLE HISTORY
Received: Jan. 11, 2025
Accepted: June 3, 2025

KEYWORDS
Moraceae,
Morus macroura,
HRLC-MS,
methanolic extract,
phytochemicals.

Abstract: The present study aimed to analyze the key chemical constituents of the methanolic leaf extract of *Morus macroura* using high-resolution liquid chromatography-mass spectrometry (HR-LCMS). Commonly known as king white mulberry, *M. macroura* is a medicinal herb traditionally used to treat various ailments. The HR-LCMS analysis revealed the presence of organic compounds, including amino acids and phenols, in both positive and negative electrospray ionization (ESI) chromatograms. Notable compounds identified in the positive ESI chromatogram included *m*-coumaric acid, gibberellin A-105, neomethymycin, and iproniazide. Similarly, the negative ESI chromatogram detected compounds such as 1,25-dihydroxy vitamin D3-26,23-lactone, alangimarckine, tyrosyl-isoleucine, hypoglycin B, tubulosine, and 2-phenylaminoadenosine. The presence of these bioactive compounds suggests that *M. macroura* leaf extract holds potential for use in future pharmaceutical applications.

1. INTRODUCTION

The genus *Morus* (Moraceae) belongs to the family Moraceae, the tribe Moreae. Mainly Moraceae is a family of flowering plants which concludes 40 genera and 1000 species. *Morus* is a genus that consists of various species of deciduous trees, commonly known as mulberries, which are widely distributed in North India, Pakistan, Egypt, and Iran (Yang *et al*., 2023). *M. macroura* has a range of therapeutic uses, and its plant extract is well-known for treating gastric ulcers. It has been shown to be successful in lowering ulcer indices that measure the quantity and severity of lesions. The fruit extract has anti-gastric ulcer and anti-cancer properties (Farrag *et al*., 2017).

Several studies have highlighted the therapeutic potential of *Morus macroura*. Among the six phenolic compounds identified in the bark of *M. macroura*, moracins B and mulberrofuran K demonstrated antibacterial activity against gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*, based on NMR and mass spectrometry data (Jasmansyah *et al*., 2019). The fruit juice of *M. macroura* possesses a wide range of medicinal properties, such as antiphlogistic, emollient, bactericidal, antiviral, astringent, diaphoretic, diuretic, escharotic, fungicidal, laxative, nervine, refrigerant, restorative, sedative, tonic, and vermifuge effects (Wu *et al*., 2025). The bark juice is traditionally used for treating cuts and wounds (Duke & Wain, 1981). Additionally, the leaves of *M. macroura* are known for their therapeutic benefits,

*CONTACT: Priyanka T. SUTAR priyankasutar.research@gmail.com Department of Botany, Shivaji University, Kolhapur, Maharashtra, India

e-ISSN: 2148-6905

including diuretic effects and the ability to lower blood sugar and blood pressure levels (Nazim *et al*., 2017).

Medicinal plants not only aid in treating various illnesses but also play a significant role in boosting immunity. Research in ethnopharmacology and ethnomedicine offers valuable insights for drug development. As natural products, plants serve as a rich source of alternative compounds for modern drug discovery. The *Morus* genus is native to regions including the Indian subcontinent, southern Europe, the Middle East, and northern Africa. These plants hold economic importance due to their leaves being a primary food source for the silkworm (*Bombyx mori* L.), which is essential for silk production (Das & Krishnaswami, 1965; Hăbeanu *et al*., 2023; Vijayan, 2009). Additionally, *Morus* species are widely recognized for their medicinal properties, including antioxidant and antibacterial effects (Budiman *et al*., 2017). This study aims to conduct HR-LCMS analysis to identify the chemical constituents present in the methanolic leaf extract of *Morus macroura*.

2. MATERIAL and METHODS

2.1. Collection of Plant Material and Preparation of Methanolic Extract

Plant material of *Morus macroura* was collected from the campus of Shivaji University, Kolhapur. The leaves were thoroughly washed, air-dried under laboratory conditions to prevent contamination, and then oven-dried for 48 hours. After drying, the leaves were ground into a fine powder using a mixer grinder. The powdered leaves were subjected to methanol extraction using the Soxhlet extraction method, with 400 mL of methanol for 8–10 hours at a temperature of 50–60°C. The resulting extract was filtered using Whatman No. 1 filter paper, concentrated using an evaporator at 40°C, and stored in sterile amber-colored glass bottles in a refrigerator at 4°C. From this prepared solution, 2 mL of the methanolic leaf extract was sent to the Sophisticated Analytical Instrument Facility (SAIF) at the Indian Institute of Technology Bombay (IIT Bombay) for qualitative analysis of its chemical constituents using the HRLC-MS technique.

2.2. *HRLC-MS Analysis*

The HRLC-MS analysis was performed using a Thermo Fisher Scientific Q-TOF High-Resolution Orbitrap Liquid Chromatograph equipped with a Q Exactive Plus mass spectrometer and Proteome Discoverer Analyst software (version 1.42). The system featured a Hypersil GOLD C18 column with dimensions of 100 × 2.1 mm and a particle size of 3 microns, along with a dual AIS ESI (Electrospray Ionization) source. This instrument enables high-performance liquid chromatography (HPLC) and advanced mass spectrometry analysis. It provides excellent chromatographic separation and precise detection of compounds with a mass-to-charge (m/z) ratio ranging from 50 to 8000 atomic mass units (amu). The system offers a resolution of up to 280,000, a scan speed of 12 Hz, and a mass accuracy of less than 1 ppm.

3. FINDINGS

High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) was employed to identify bioactive phytochemicals in the methanolic leaf extract of *Morus macroura*. Identification was based on retention time, metabolite classification, database comparisons, and proposed compounds. HR-LCMS analysis revealed numerous medicinally significant components, with data acquired in both positive and negative ionization modes (Figures 1 and 2). A total of 59 compounds were identified in positive ESI mode and 32 in negative ESI mode. The retention times, molecular formulas, and database matches for these active compounds are presented in Tables 1 and 2. These identified compounds likely contribute to the medicinal properties of *M. macroura*, as indicated by HR-LCMS results in both ionization modes.

Table 1. HRLC-MS +ve ESI chromatogram revealed the presence of bioactive compounds in methanolic leaves extract of *M. macroura.*

Name of Compound	Retention Time	Mass	MFG Formula	DB Diff (ppm)	Hits (DB)
2-Nitroanisole	3.432	153.042	C7H7NO3	2.32	10
Iproniazide	5.508	179.105	C9H13N3O	2.12	1
Hexyl 2-furoate	7.494	196.109	C11H16O3	1.66	4
2-Phenylethyl 3-methylbutanoate	7.59	206.130	C13H18O2	2.64	10
m-coumaric acid	8.505	164.046	C9H8O3	1.83	10
(+)-cis-5,6-Dihydro-5-hydroxy- 4-methoxy-6-(2-phenylethyl)- 2H-pyran-2-one	8.572	248.104	C14H16O4	2.8	10
Isoyatein	9.125	400.152	C22H24O7	-0.14	10
Prenyl caffeate	9.352	248.105	C14H16O4	0.33	10
Dihydroeuparin	9.474	218.094	C13H14O3	-0.94	10
Gibberellin A105	9.953	330.146	C19H22O5	-1.16	10
Aminoglutethimide	10.285	232.121	C13H16N2O2	0.31	4
6-(1,2,3,4-Tetrahydro-6- methoxy-2-naphthyl)-2(1H)-pyridone	11.274	255.125	C16H17NO2	-2.3	8
2-phenylethyl 3-methylbutanoate	11.388	206.130	C13H18O2	0.08	10
3-tert-Butyl-5-methylcatechol	11.392	180.115	C11H16O2	0.73	10
1-Acetoxypinoresinol	11.562	416.147	C22H24O8	-1.1	7
Acrophyline	12.548	283.120	C17H17NO3	-0.35	10
Cirsimaritin	12.6	314.079	C17H14O6	0.33	10
3-Phenoxypropionic acid	12.827	166.063	C9H10O3	-1.26	10
Allyl cinnamate	13.541	188.083	C12H12O2	-0.61	10
C16 Sphinganine	13.932	273.267	C16H35O2	-0.73	1
16b-Hydroxyestradiol	14.284	288.172	C18H24O3	-0.68	10
(9Z,11E,13E,15Z)-4-oxo-9,1113,15-octadecatetraenoic acid	14.459	290.1884	C18H26O3	-1	2
Mitoxantrone	14.71	444.204	C22H28N4O6	-0.56	6
Mammea B/AD cyclo D	14.956	356.162	C21H24O5	-9.11	10
LysoPE(0:0/20:3(8Z,11Z,14Z))	15.004	499.289	C25H46NO7P	-0.09	10
Tricornine	15.151	509.287	C27H43NO8	8035:1	4
Scutigeral	15.202	372.230	C23H32O4	22.69	10
Epothilone D	15.298	491.279	C27H41NO5S	-0.88	4
Lucidenic acid N	15.353	460.282	C27H40O6	-18.98	3
17-methyl-18-norandrosta-4,13(17)-dien-3-one	15.539	270.198	C19H26O	-0.95	4
Fluticasone propionate	15.582	500.184	C25H31F3O5S	-1.53	6
Neomethymycin	15.671	469.297	C25H43NO7	-0.01	4
Adlupulone	15.812	414.277	C26H38O4	14.46	10
Alangimarckine	15.817	475.283	C29H37N3O3	-0.43	5
Cycloate	15.979	215.133	C11H21NOS	-0.91	1
Kanamycin	15.997	484.239	C18H36N4O11	3.02	5
Archangein	16.239	484.239	C21H22O04	-2.99	10
2-Aminoethylphosphocholate	16.49	515.303	C26H46NO7P	-0.65	5
3-oxo-delta5-steriod	16.691	272.214	C19H28O	-4.07	7
2-Angeloyl-9-(3-methyl-2E-pentenoyl)-2b,9a-Dihydroxy-4z,10(14)-oplopadien 3-one	16.891	428.256	C26H36O5	-0.82	10
MG(22:6(4Z,7Z10Z,13Z,16Z,19Z)/0:0/0:0	17.034	402.280	C25H38O4	-1.32	3
Terfenadline	17.034	469.302	C32H41NO2	-9.58	4
10-Oxo-11-Octadecen-13-olide	17.058	294.219	C18H30O3	4286.49	10
19-Noretiocholanolone	17.107	276.209	C18H28O2	-0.02	10
Azafrin	17.18	426.277	C27H38O4	-0.22	6
Deoxytubulosine	17.184	459.289	C29H37N3O2	-1.07	2
Terfenadine	17.377	471.312	C32H41NO2	-1.76	4
Hydroxyprogesterone caproate	17.617	424.280	C27H40O4	2.42	6
Browniine	17.622	467.281	C25H41NO7	9457.21	4
17-beta-Hydroxyestr-4en-3-one cyclopentanepropionate	17.705	398.282	C26H38O3	15.5	10
Terfenadine	17.844	471.312	C32H41NO2	-1.83	4
Taurodeoxycholate	18.016	499.308	C26H45NO6S	1.68	3
Harderoporphyrin	19.399	608.263	C35H36N4O6	-23.71	6

Phaeophorbide b	19.455	606.248	C35H34N4O6	-0.54	7
Oxidized dinoflagellate luciferin	19.729	602.276	C33H38N4O7	-0.98	3
3beta-hydroxy-5alpha-cholest-7-ene-4alpha-carboxylate	20.967	430.345	C28H46O3	-4.73	9
(6beta,24R)-6-Hydroxystigmast-4-en-3-one	21.069	428.365	C29H48O2	-2.28	10
22E,24x)-Ergosta-4,6,8,22-tetraen-3-one	21.217	392.308	C28H40O	-1.13	4
Fucosterol9	21.784	412.370	C29H48O	-2.22	10

Table 2. HRLC-MS -ve ESI chromatogram revealed the presence of bioactive compounds in methanolic leaves extract of *M. macroura.*

Name of Compound	Retention Time	Mass	MFG Formula	DB Diff (ppm)	Hits (DB)
Melibiose	1.086	342.1128	C12H22O11	10	10
Hexyl 2-furoate	8.645	196.1076	C11H16O3	12.17	10
2-Phenylaminoadenosine	9.056	358.1375	C16H18N6O4	4.06	10
Ligustroside	11.055	524.1847	C25H32O12	8.9	9
Hypoglycin B	11.058	270.1222	C12H18N2O5	-2.19	10
Portulacaxanthin III	12.551	268.0706	C11H12N2O6	-3.99	10
Tubulosine	15.486	475.2778	C29H37N3O3	11.9	6
Salviaflaside methyl ester	15.554	536.1552	C25H28O13	-4.1	8
Tyrosyl-Isoleucine	15.558	294.1585	C15H22N2O4	-1.77	10
Melleolide D	15.943	482.1724	C24H31ClO8	-3.37	5
Alpha-(p-Methoxphenyl)-6methyl-2-pyridineacrylic acid	16.013	269.1032	C16H15NO3	7.43	10
Limonoate	16.014	506.217	C26H34O10	-3.56	10
Prupaside	16.015	552.2223	C27H36O12	-2.9	5
1,25-Dihydroxyvitamin D3-26,23-lactone	16.334	444.2839	C27H40O5	8.31	4
Epithilone D	16.414	491.2741	C27H41NO5S	-7.19	4
(2S, 4S)-Monatin	16.656	292.1075	C14H16N2O5	-5.26	10
9-HOTE	17.066	294.2175	C18H30O3	6.95	10
8S-HODE	17.605	296.2332	C18H32O3	6.69	10
Alangimarckine	17.713	475.2802	C29H37N3O3	6.89	8
beta-citraurin	17.946	432.3019	C30H40O2	2.05	4
Deoxytubulosine	18.593	459.2861	C29H37N3O2	5.3	1
α-Linolenic Acid	19.264	278.223	C18H30O2	5.82	10
Linalyl caprylate	19.661	280.2382	C18H32O2	7.12	10
2S-hydroxy-octadecanoic acid	19.733	300.2644	C18H36O3	6.82	9
Octyl octanoate	19.947	256.2383	C16H32O2	7.34	10
Dioctyl hexanedioate	19.961	370.3059	C22H42O4	6.43	10
Omega-hydroxybehenic	20.082	356.3265	C22H44O3	7.01	4
Azukisapogenol	20.691	472.3525	C30H48O4	5.93	10
Triparinarin	21.645	866.65	C57H86O6	-8.69	1
Buxamine E	21.655	384.3579	C26H44N2	-19.4	1
PE(24:1(15Z)/20:3(8Z,11Z,14Z))	21.945	851.6215	C49H90NO8P	22.25	10
DG(24:1(15Z)/18:1(11Z)/0:0)	22.557	703.6184	C45H84O5	1440.37	10

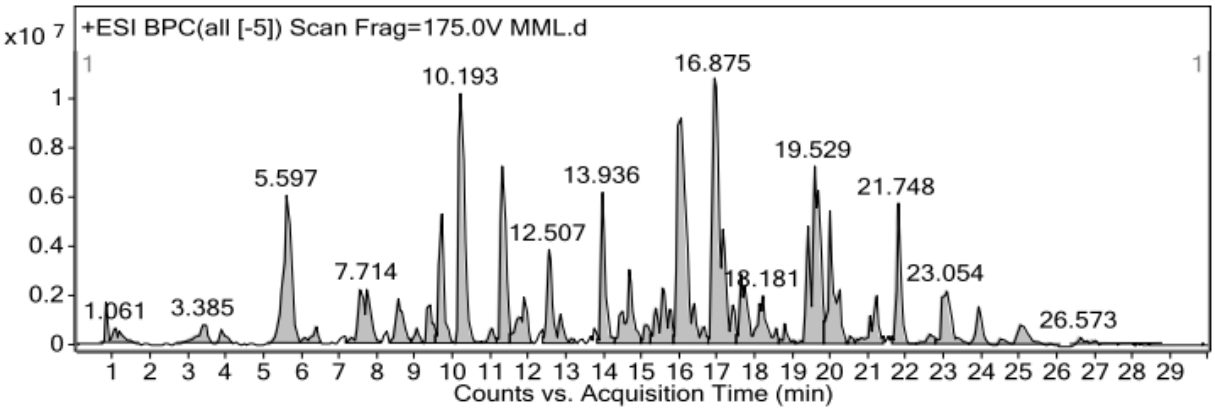

Figure 1. +ve ESI chromatogram of methanolic leaves extract of *M. macroura*.

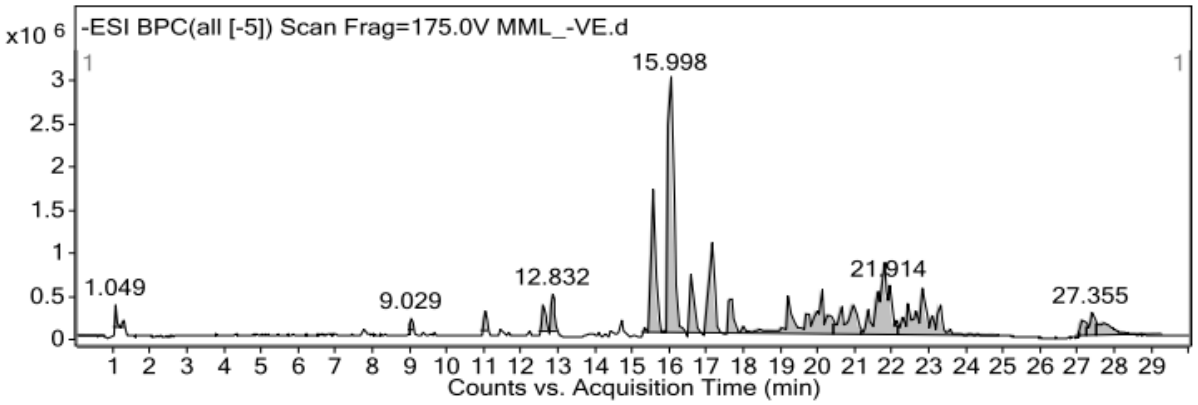

Figure 2. -ve ESI chromatogram of methanolic leaves extract of *M. macroura.*

4. DISCUSSION and CONCLUSION

In the 1950s, iproniazid was the first drug utilized for depression treatment and was classified as an antidepressant with antimicrobial properties (Macedo *et al*., 2017). Recognized as the first clinically effective Monoamine Oxidase Inhibitor (MAOI), iproniazid was initially developed for tuberculosis treatment (Entzeroth & Ratty, 2017). However, in 1952, researchers observed its antidepressant effects when tuberculosis patients reported unexpected euphoria while taking isoniazid, a structurally similar compound. Isoniazid combats bacteria by generating isonicotinyl radicals, which disrupt bacterial cell-wall formation (Jena *et al*., 2014; Lei *et al*., 2000). Due to its antimicrobial properties, iproniazid and related MAOIs have demonstrated efficacy in treating infections such as aphthous ulcers, labial and genital herpes, and respiratory infections (Liang *et al*., 2013).

Adenosine and its analog, CV-1808, interfere with neutrophil-mediated bacterial killing by reducing superoxide production, a key component of the oxidative burst. At high concentrations, both compounds significantly enhance the survival of *Staphylococcus aureus*. In contrast, inosine, a metabolic product of adenosine, does not influence bactericidal function. While natural adenosine levels are unlikely to impact immune responses, pharmacological doses of adenosine and its derivatives could impair neutrophil activity, potentially increasing susceptibility to infections. Adenosine reduces superoxide production in polymorphonuclear neutrophils (PMNs) when stimulated by *S. aureus*, with CV-1808 exhibiting a similar effect. Although adenosine decreases the oxidative burst by 33% in response to *S. aureus*, this reduction does not compromise PMNs' bactericidal capacity. Even at high concentrations (1 mM), adenosine does not significantly affect PMN chemiluminescence or bactericidal activity, as it undergoes minimal metabolism by PMNs. Overall, adenosine exerts a more pronounced effect on the oxidative burst than on bacterial killing, with its impact at higher concentrations remaining limited (Hardart *et al*., 1991).

Tubulosine, a compound with a well-characterized structure since the 1960s, has been investigated for its potential in cancer therapy, particularly in breast cancer. Studies indicate that tubulosine exhibits significant anticancer activity in human breast cancer cells stimulated by interleukin-6 (IL-6). It effectively disrupts IL-6-induced JAK2/STAT3 signaling by inhibiting the interaction between the IL-6 receptor (IL-6R) and glycoprotein 130 (gp130). This disruption reduces STAT protein phosphorylation and transcriptional activity, ultimately decreasing cancer cell viability and promoting apoptotic cell death. Additionally, tubulosine mitigates IL-6-induced signaling in both human breast cancer cell lines and *Drosophila* cells, demonstrating its broader influence on these pathways. These findings underscore tubulosine's potential as a novel therapeutic agent for inflammation-related cancers, offering a targeted approach to disrupting key signaling pathways involved in cancer progression (Byung *et al*., 2019).

The HRLC-MS analysis of the methanolic leaf extract of *Morus macroura* revealed a range of bioactive compounds with potential therapeutic properties. The analysis identified numerous medicinally significant components in both positive and negative ESI ionization modes, contributing to the understanding of the plant's chemical profile. The presence of compounds such as moracins B and mulberrofuran K in the bark, and various bioactive compounds in the leaves, supports the traditional use of *M. macroura* in treating ailments. These findings highlight its potential as a source of bioactive phytochemicals with antioxidant, antibacterial, and anticancer activities. The presence of compounds like tubulosine further underscores its promise in treating inflammation-related cancers, particularly through modulation of key signaling pathways such as the JAK2/STAT3 pathway. Overall, the results suggest that *M. macroura* could serve as a valuable candidate for future pharmaceutical developments, offering both antimicrobial and anticancer therapeutic potentials.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Priyanka T. Sutar: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing original draft. **Dattatraya. K. Gaikwad**: Methodology, Supervision, and Validation. Authors may edit this part based on their case.

Orcid

Priyanka T. Sutar https://orcid.org/0009-0001-2536-5087
Dattatraya. K. Gaikwad https://orcid.org/0000-0002-2122-2783

REFERENCES

Budiman, A., Aulifa, D.L., Kusuma, A.S., & Sulastri, A. (2017). Antibacterial and antioxidant activity of black mulberry (*Morus nigra* L.) extract for acne treatment. *Pharmacognosy Journal*, *9(5)*, 611-614.

Byung, H.K., Eun, H.Y., & Yu, C.L. (2019). Anticancer activity of Tubulosine through suppression of Interleukin-6- Induced Janus kinase 2/Signal Transducer and Activation of Transcription 3 signaling. *Journal of Breast Cancer, 22(3)*, 362-374.

Das, B.C., & Krishnaswami (1965). Some observations on interspecific hybridization in mulberry. *Indian J. Sericulture*, *4*, 1-8.

Duke, J.A., & Wain, K.K. (1981). *Medicinal Plants of the World*. Computer Index with More than 85,000 Entries, 3 Vols.

Entzeroth, M., & Ratty, A. (2017). Monoamine oxidase inhibitors—revisiting a therapeutic principle. *Open Journal of Depression*, 631-68. https://doi.org/10.4236/ojd.2017.62004

Farrag, E.K., Kassem, M.E.S., Bayoumi, D., Shake, S.E., Afifi, M.S. (2017). Phytochemical study, phenolic profile and antigastric ulcer activity of *Morus macroura* Miq. fruits extract. *J App Pharm Sci.*, *7(05)* 152-160. https://doi.org/10.7324/JAPS.2017.70527

Hăbeanu, M., Gheorghe, A., & Mihalcea, T. (2023). Silkworm *Bombyx mori*—sustainability and economic opportunity, particularly for Romania. *Agriculture, 13(6)*, 1209. https://doi.org/10.3390/agriculture13061209

Hardart, G.E., Sullivan, G.W., Carper, H.T., & Mandell, G.L., (1991). Adenosine and 2-phenyl aminoadenosine (cv-1808) inhiboit human neutropnil bactericidal function. *Infection and Immunity, 59(3)*, 885-889.

Jasmansyah, Hakim, E.H., & Syah, Y.M. (2019). Antibacterial constituents from *Morus macroura*. *Res. J. Chem. Environ*., *23(11)*, 37-40.

Jena, L., Waghmare, P., Kashikar, S., Kumar, S., & Harinath, B.C., (2014). Compulational approach to understanding the mechanism of action of isoniazid, an anti-TB drug. *International Journal of Mycobacteriology, 3*, 276-282.

Lei, B., Wei, C.J., & Tu, S.C. (2000). Action mechanism of antitubercular isoniazid. Activation by mycobacterium tuberculosis Kat G, isolation, and characterization of inha inhibitor. *Journal of Biology and chemistry, 275*, 2520-2526.

Liang, Y., Quenelle, D, Vogel, J.L., Mascaro, C., Ortega, A., & Kristie, T.M. (2013). A novel selective LSD1/KDM1A inhibitor epigenetically blocks herpes simplex virus lytic replication and reactivation from latency. *mBio*. *5*, 4(1), e00558-12. https://doi.org/10.1128/mBio.00558-12

Macedo, D., Filho, J., Sousa, J.M.C., Nadia, C., Soares de., Quevedo, J., Barichello, T., Junior, H.V.N. (2017). Antidepressant, antimicrobial or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. *Journal of Affective Disorders*, *208*, 22-32.

Vijayan, K. (2009). Approaches for enhancing salt tolerance in mulberry (*Morus* L.) *A review Plant Omics J, 2(1)*, 41-59.

Wu, H., Sun, N., Lou, D., Geng, T., Lu, F., Li, Y., Wang, S., & Zhu, G. (2025). Unveiling the unique aroma of *Morus macroura* through integrated volatile metabolome and transcriptome analysis, *Scientia Horticulturae, 339*, 113814. https://doi.org/10.1016/j.scienta.2024.113814

Yang, C.X., Liu, S.Y., Zerega, N.J.C., Stull, G.W., Gardner, E.M., Tian, Q., Gu, W., Lu, Q., Folk, R.A., Kates, H.R., Guralnick, R.P., Soltis, D.E., Soltis, P.S., Wang, Y.H., & Yi, T.S. (2023). Phylogeny and biogeography of *Morus* (Moraceae). *Agronomy 13(8)*, 2021. https://doi.org/10.3390/agronomy13082021