International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

In Vitro Callus Induction, Phytochemical Characterization and GC-MS Profiling of Bioactive Compounds in *Pterocarpus marsupium* (Roxb.)

Pallavi G. Tandlepatil[1], Sangeeta R. Ahuja[2]

[1]Department of Botany, Maulana Azad college of Arts, Science and Commerce, Chh.Sambhajinagar (MS) India.

[2]Department of Botany, Sir Sayyed College, Chh.Sambhajinagar (MS) India.
Email: *pallavitandlepatil12[at]gmail.com*

Abstract: ***Pterocarpus marsupium (Roxb.), a plant of significant medicinal value, has traditionally been used in the treatment of various ailments. The present study investigates the phytochemical profile of a callus extract of P. marsupium using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The analysis revealed the presence of 29 compounds, several of which, including taurine, a pregnan-20-one derivative, and a colchicine derivative, are reported to possess bioactive properties. The findings provide a detailed phytochemical fingerprint of the P. marsupium callus extract and establish a basis for further investigation of the therapeutic potential of the identified compounds. The chromatogram demonstrates the separation of the constituent compounds as a function of retention time, with individual peaks corresponding to different detected substances. The Peak Report based on the Total Ion Chromatogram (TIC) provides quantitative information on the relative abundance of each identified compound.***

Keywords: P. marsupium, micropropagation, in vitro regeneration, GC-MS Chromatogram, Callus Extract

1. Introduction

The medicinal value of plants is primarily attributed to the presence of low-molecular-weight organic compounds known as secondary metabolites, including flavonoids, phenols, alkaloids, saponins, and terpenoids, which exert specific physiological effects in the human body [1]. Numerous plant species occurring in nature remain unexplored for their medicinal potential. Among these, *Pterocarpus marsupium* Roxb. is one of the most valuable multipurpose forest trees, commonly known as Indian Kino and Bijasal in Hindi. It is a large deciduous tree widely distributed across the central, western, and southern regions of India and has a long history of traditional and ethnobotanical applications in various cultures [2].

Spectrometric and chromatographic screening methods provide valuable preliminary information for the selection of crude plant extracts with potentially useful biological properties, thereby facilitating further chemical and pharmacological investigations [3]. Gas Chromatography–Mass Spectrometry (GC–MS), a hyphenated analytical technique, is highly compatible and widely employed for the identification and quantification of chemical constituents. Unknown organic compounds present in complex mixtures can be identified through interpretation of their mass spectra and comparison with reference spectra available in established spectral libraries [4].

The present study reports the identification of bioactive compounds in the aqueous callus extract of *Pterocarpus marsupium* Roxb. using Gas Chromatography–Mass Spectrometry (GC–MS) analysis, which may provide valuable insights into its potential applications in traditional medicine.

2. Material and Methods

Plant material
The explant of *Pterocarpus marsupium* Roxb. was collected from Chaukka, Chhatrapati Sambhajinagar district, Maharashtra, India. The plant material was authenticated by the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University (B.A.M.U.) Herbarium, Chhatrapati Sambhajinagar. A voucher specimen (Accession No. 01187) was deposited in the B.A.M.U. Herbarium for future reference.

Explant Preparation and Sterilization
The standard protocol of MS medium for micropropagation of plants was followed, which is earlier used by Misal and Chavan, 2024 [5]. The nodal segments used as an explant were collected and washed with running tap water for 5 minutes, followed by 70% ethanol for 2 minute and finally with distilled water for 5 minutes. Surface sterilization of explant was carried out by washing with sterile distilled water for 5 minutes followed by 0.2% of mercuric chloride ($HgCl_2$). Two more rinses in laminar airflow with sterilized double distill water came after it. All explants were cut into small sections and inoculated onto the appropriate medium.

Preparation of culture medium
All experiments in the present study were conducted using Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), supplemented with varying concentrations of auxins and cytokinins. The culture medium was supplemented with 3% sucrose and 2.5–3.0 g/L Clerigar as a gelling agent, and the pH was adjusted to 5.6–5.8. The prepared media were sterilized by autoclaving at 121 °C and 15 psi pressure.

3. Culture Conditions

Following inoculation, the culture bottles were transferred to

a culture room maintained at a temperature of 25 ± 2 °C under a 16-hour photoperiod provided by cool-white fluorescent tubes.

Preparation of Plant Extract

Freshly collected callus of *Pterocarpus marsupium* was thoroughly washed with running water, shade-dried, and subsequently powdered to the required particle size. The powdered callus (2 g) was subjected to hot Soxhlet extraction using methanol as the solvent. The resulting extract was concentrated by evaporating the solvent in a hot-air oven at 30–40 °C. The dried extract was collected and stored in a refrigerator at 4 °C until further use.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

A 2 µL aliquot of the aqueous extract of *Pterocarpus marsupium* was used for GC–MS analysis [6]. The extract was dissolved in HPLC-grade methanol and subjected to GC–MS analysis using a JEOL GCMATE II GC–MS system equipped with a secondary electron multiplier and an Agilent Technologies 6890N Network GC system for gas chromatographic separation. Separation was performed using an HP-5 fused-silica capillary column (50 m × 0.25 mm I.D.). The column temperature was maintained at 100 °C for 20 min and subsequently increased to 235 °C for 3 min. The injector temperature was maintained at 240 °C. Helium was used as the carrier gas, with a split ratio of 5:4. A 1 µL sample was injected into the injector, and the sample was evaporated in splitless mode at 300 °C. The total GC–MS run time was 30 min [7].

The compounds present in the extract were identified by comparing their respective mass spectra with those in the NIST14.LIB spectral library.

4. Results and Discussion

GC–MS Analysis of *Pterocarpus marsupium* Callus Extract

GC–MS analysis of the Pterocarpus marsupium callus extract revealed a complex phytochemical profile comprising 29 chromatographic peaks, indicating the presence of diverse chemical constituents. The Total Ion Chromatogram (TIC) showed distinct peaks distributed across the chromatographic run, with retention times ranging from 0.125 to 35.831 min. Among the detected constituents, Ethane, 1-chloro-1-fluoro- exhibited the highest relative peak area (27.2431%), followed by Phosphine, methyl- (11.3533%), Ethyl 5,8-dihydroxy-1,3-dimethylisoquinoline-4-carboxylate (8.6817%), Succinic acid, (4-methoxyphenyl) ester (8.0481%), Sulfoxide, decyl methyl (6.3063%), and 3-Methylthiobutyraldehyde (4.5328%). The remaining compounds occurred at comparatively lower relative abundances, generally contributing less than 4% of the total peak area.

Of particular interest, several compounds identified in the callus extract have structurally diverse chemical characteristics. Taurine was detected at a retention time of 0.520 min with a relative peak area of 1.5221%. In addition, a pregnan-20-one derivative was identified at 21.741 min with a peak area of 1.0173%, while a colchicine derivative, Colchicine,N-desacetyl-N-[4-hydroxy-3,5 dimethoxycinnamoyl]-, was detected at 29.970 min with a relative peak area of 1.0051%. The mass spectral library matching indicated these compound identities based on comparison with the NIST14.LIB database.

Overall, the GC–MS profile demonstrates that the aqueous callus extract of *P. marsupium* contains a broad spectrum of detectable organic constituents, including compounds belonging to different chemical classes. The detection of taurine, pregnan-20-one derivative, and colchicine derivative provides an important preliminary phytochemical fingerprint of the callus extract and supports its potential for further phytochemical and pharmacological investigation.

Table 1: GC–MS Phytochemical Profile and Peak Identification of Callus Extract

Peak	Retention Time	Final Time	Area%	Height	Name
1	0.125	0.135	6.3063	32410	Sulfoxide, decyl methyl
2	0.16	0.17	4.5328	31084	3-Methylthiobutyraldehyde
3	0.19	0.24	8.6817	31085	Ethyl 5,8-dihydroxy-1,3-dimethylisoquinoline-4-carboxylate
4	0.255	0.275	3.8936	27984	S-Methylpropanethiosulfonate
5	0.32	0.36	8.0481	23453	Succinic acid, (4-methoxyphenyl) ester
6	0.375	0.51	11.3533	22724	Phosphine, methyl-
7	0.52	0.54	1.5221	12518	Taurine
8	0.553	0.575	1.6427	12199	Tetrahydrofuran-D8
9	0.605	0.615	1.2326	6801	Butane, 1-methoxy-2-methyl-
10	0.705	0.725	1.9095	4594	Methional
11	1.422	1.495	27.2431	114938	Ethane, 1-chloro-1-fluoro-
12	1.527	1.595	1.5202	5167	Phosphine, methyl-
13	1.689	1.78	1.1393	3667	1-Propanol, 2-methyl-2-nitro-
14	4.981	5.005	1.0001	3396	2-Butyne-1,4-diol, diformate
15	9.582	9.7	1.2795	3259	Carbamate, N-(2-naphthyl)-, 3-pentynyl ester
16	11.866	12.03	2.2074	4436	25,25-Dinor-9,19-cyclolanost-24-yne-, 3-(t-butyldimethylsilyloxy)-
17	17.545	17.65	1.4703	2524	14,16-Hentriacontanedione, 25-hydroxy-
18	21.741	21.8	1.0173	4131	Pregnan-20-one, 5, 6-epoxy-3-hydroxy-, (3.beta, 5.beta, 6.beta.)-
19	26.257	26.305	0.9996	2749	Ni(ii)-1,6-bis(2-hydroxy-1,5,5-trimethyl-4,5-dihydro-1H-pyrrol-4-on-3-yl)-2,5-diazahexa-

					1,5-dien
20	29.97	30.1	1.0051	2607	Colchicine, N-desacetyl-N-(4-hydroxy-3, 5-dimethoxycinnamoyl)-
21	31.895	31.98	1.5082	4022	5-Bromo-4-nitroimidazole-2-(2-thioacetic acid)
22	32.162	32.26	1.45	3284	l-Felinine
23	33.068	33.08	1.3821	4165	Ethyl 4,4,6,6-tetramethyl-9-oxo-3,5,7,10-tetraoxa-4,6-disiladodecan-1-oate
24	33.165	33.215	1.02	4015	Trimethylsilyl 3-methyl-4-((trimethylsilyl)oxy)benzoate
25	34.261	34.37	1.6059	2841	[2-(4-methylphenyl)-(benzo(f)(1-nickela-2, 3-diazaindene))]-cyclopentadienyl
26	34.53	34.615	1.0139	3922	Tartronic acid, 4-(dimethylethylsilyl)phenyl-, dimethyl ester
27	34.89	34.98	1.472	4211	5H-Thiazolo(2, 3-a)pyridine-8-carboxamide, 3-(1-adamanthyl)-7-methyl-5-oxo-
28	35.635	35.76	1.2709	2615	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane
29	35.831	35.98	1.2727	4049	4-Hydroxybenzoic acid, 2TMS derivative

Table 2: Bioactive Compounds and Their Reported Uses

Compound Name	Peak	Retention Time	Bioactivity / Potential Uses
Taurine	7	0.52	Antioxidant, anti-inflammatory, antibacterial, and neuroprotective properties. Has potential in preventing and managing various diseases.
Carbamate, N-(2-naphthyl)-, 3-pentynyl ester	15	9.582	Carbamate derivatives have been investigated as potential acetylcholinesterase inhibitors, which are used to treat certain neurological disorders.
Pregnan-20-one, 5,6-epoxy-3-hydroxy-, (3.beta.,5.beta.,6.beta.)-	18	21.741	A sterol derivative. Sterols are a class of bioactive compounds with reported anti-inflammatory, antimicrobial, and cytotoxic properties.
Colchicine, N-desacetyl-N-(4-hydroxy-3, 5-dimethoxycinnamoyl)-	20	29.97	An alkaloid derivative. Alkaloids are known for their potent biological activities, and some, like colchicine, are used as chemotherapeutic agents.
l-Felinine	22	32.162	L-Felinine is an amino acid derivative. Amino acids and their derivatives are fundamental to many biological processes and can have various physiological effects.

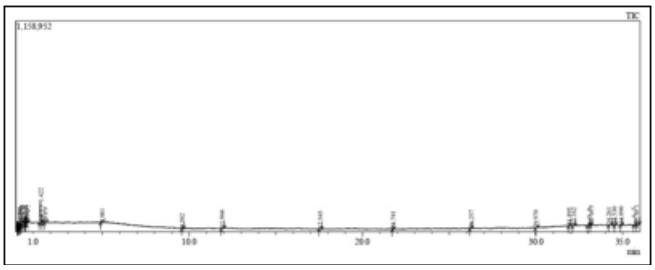

Figure 1: Chromatogram for GC–MS Profiling of Bioactive Compounds in *Pterocarpus marsupium*.

5. Conclusion

Gas chromatography–mass spectrometry (GC–MS) analysis of *Pterocarpus marsupium* callus extract revealed a comprehensive metabolite profile characterized by diverse chemical constituents. The detection of prominent bioactive metabolites—including taurine, a pregnane derivative, and a colchicine analogue—highlights the potential of *in vitro* callus cultures as a sustainable source of high-value phytochemicals. Consequently, this preliminary metabolic fingerprint provides a robust foundation for future pharmacological investigations targeting these specific chemical scaffolds. Subsequent studies focusing on chromatographic isolation, structural validation, and targeted *in vitro* and *in vivo* bioassays are required to confirm their therapeutic efficacy and underlying mechanisms of action.

Acknowledgements
The authors are very much thankful to Department of Botany, Maulana Azad college of Arts, Science and Commerce, Chh. Sambhajinagar and Department of Botany, Sir Sayyed College, Chh. Sambhajinagar for providing necessary laboratory facilities.

References

[1] Mohammedi Z, Atik F. Impact of solvent extraction type on total polyphenols content and biological Activity from Tamarixa phylla(L) karst. International Journal of Pharma and Bio Sciences 2011; 2(1):609-615.
[2] Chauhan B, Chaudhary AK. Memory enhancing activity of methanolic extract of Pterocarpus marsupiumRoxb.Phytopharmacology2012; 2(1):72-80.
[3] Mathekaga AD, Meyer JJM. Antibacterial activity of South African *Helichrysum species*. South African Journal of Botany1998; 64: 293-295.
[4] Balamurugan V, Balakrishnan V. Preliminary phytochemicals, pharmacognostic evaluation and Antimicrobial activity of *Moringa Concanensis* Nimmoleaf. Global Journal of Bio-science & Biotechnology 2013; 2(2):243-247.
[5] V.D. Misal, & A.M. Chavan, "In vitro micropropagation and mass multiplication of Stevia rebaudiana Bertoni," World Journal of Advanced Research and Reviews, 23(1), pp. 250-254, 2024.
[6] Gopala Krishnan S, Vadivel E. GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* Linn. International Journal of Pharma and Bio Sciences 2011; 2(1):313-320.
[7] Deepa P, Kaleena PK, Valivittan K.GC-MS analysis and antibacterial activity of chromatographically
[8] Separated purefractions of leaves of *Sansevieria roxburghiana.* Asian Journal of Pharmaceutical and Clinical Research 2011; 4(4):130-133.