

Comparative Phytochemical Evaluation of Barbaloin in Aloe Socotrina Linn. Derived From Tissue Culture and Wild Sources Using Lc–Ms/Ms And Uv Spectroscopy

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ABSTRACT

Background: Standardisation of homoeopathic medicinal plants is essential to ensure consistency and reproducibility of therapeutic preparations. Aloe socotrina Linn. is widely used in homoeopathy, with barbaloin as its key phytoconstituent. However, variability in phytochemical content due to environmental factors poses challenges. Tissue culture offers a controlled alternative for plant propagation. **Aim:** To compare the presence and concentration of barbaloin in Aloe socotrina derived from tissue culture and wild sources. **Methods:** An experimental comparative study was conducted using LC–MS/MS and UV spectrophotometric analysis. Mother tinctures were prepared as per Homoeopathic Pharmacopoeia standards. Barbaloin identification was performed using a standard reference compound. Quantitative comparison was based on peak area and absorbance values. **Results:** Both samples exhibited identical retention times (~12.3–12.6 min) and m/z values of approximately 417, confirming the presence of barbaloin. Tissue culture samples showed higher peak area (597,879 vs 525,093) and higher UV absorbance. Statistical analysis indicated $p < 0.05$, confirming a significant difference. **Conclusion:** Tissue culture-derived Aloe socotrina is qualitatively identical but quantitatively superior in barbaloin content, supporting its use in homoeopathic standardisation.

Keywords: Aloe socotrina, Barbaloin, Tissue Culture, LC–MS/MS, Homoeopathy, Standardisation.

INTRODUCTION

Medicinal plants constitute the foundation of homoeopathic therapeutics, where the quality, identity, and consistency of the source material play a crucial role in determining the reliability of the final medicinal preparation^{1,2}. Aloe socotrina Linn. is a well-established homoeopathic remedy, widely used in clinical practice for gastrointestinal disturbances such as diarrhoea, dysentery, and conditions associated with portal congestion³⁻⁶. Its pharmacological activity is primarily attributed to barbaloin, an anthraquinone glycoside that serves as a key phytochemical marker for Aloe species⁷⁻¹⁰.

One of the major challenges in homoeopathic pharmacy is the variability in phytochemical composition of medicinal plants obtained from wild sources^{11,12}. Factors such as environmental conditions, soil composition, climatic variations, microbial interactions, and seasonal changes significantly influence the biosynthesis and accumulation of secondary metabolites¹³. This variability may lead to inconsistencies in the preparation of mother tinctures (Ø), which form the basis of homoeopathic potentization¹⁴.

In recent years, plant tissue culture technology has emerged as a promising approach to overcome these limitations. Tissue culture enables the propagation of plants under controlled, aseptic, and reproducible conditions, thereby ensuring uniform growth and genetic stability. Moreover, several studies have demonstrated that in vitro cultivation can enhance the production of secondary metabolites due to optimised nutrient supply and regulated growth conditions¹⁵⁻¹⁸.

Despite these advantages, there is limited experimental evidence comparing the phytochemical content of tissue culture-derived and wild Aloe socotrina, particularly in the context of homoeopathic drug standardisation. Establishing such comparative data is essential to validate tissue culture as a reliable alternative source of medicinal plants.

Therefore, the present study aims to comparatively evaluate the phytoconstituent barbaloin in Aloe socotrina Linn., derived from both tissue culture and wild sources, using advanced analytical techniques such as LC–MS/MS and UV spectroscopy, with a focus on its implications for standardisation in homoeopathic pharmacy.

MATERIALS AND METHODS

Study Design

The present study was conducted as an experimental comparative laboratory investigation aimed at evaluating the phytoconstituent barbaloin in *Aloe socotrina* Linn. derived from two different sources, namely tissue culture and wild varieties. The study was designed to assess both qualitative and quantitative differences using advanced analytical techniques, ensuring reproducibility and scientific validity.

Plant Material and Authentication

Two distinct samples of *Aloe socotrina* Linn. were utilized in this study. The first sample was obtained from tissue culture propagation, grown under controlled in vitro conditions to ensure uniformity and absence of environmental variability. The second sample was collected from wild sources, representing naturally occurring plants exposed to environmental factors. Both samples were authenticated based on standard botanical identification procedures to confirm their taxonomic identity.

Preparation of Mother Tincture

The mother tinctures of both tissue culture-derived and wild *Aloe socotrina* were prepared in accordance with the guidelines of the Homoeopathic Pharmacopoeia of India (HPI). The fresh plant material was subjected to maceration using 95% alcohol in a ratio corresponding to a drug strength of 1/10. The mixture was allowed to stand for a period of 14 days with occasional shaking to ensure proper extraction of phytoconstituents. After completion of the maceration process, the extracts were filtered and stored under appropriate conditions for further analysis.

LC–MS/MS Analysis

The identification and comparative evaluation of barbaloin were performed using Liquid Chromatography–Mass Spectrometry (LC–MS/MS). A standard solution of barbaloin (5 ppm) was used as a reference for establishing retention time and fragmentation pattern. The analysis was carried out using a Q-TOF LC–MS/MS system operated in negative electrospray ionization mode, with a mass scan range of m/z 50–1700. The chromatographic separation allowed the detection of barbaloin based on its retention time, while mass spectrometry confirmed its identity through characteristic ion peaks and fragmentation patterns. Both tissue culture and wild samples were analyzed under identical conditions to ensure comparability.

UV Spectrophotometric Analysis

To complement the LC–MS/MS findings, UV spectrophotometric analysis was performed in the wavelength range of 200–400 nm. This method was employed to evaluate the absorbance characteristics of anthraquinone compounds, including barbaloin. The absorbance values at specific wavelengths were recorded for both samples, allowing a comparative assessment of phytoconstituent concentration based on intensity of absorption.

Statistical Analysis

The data obtained from LC–MS/MS and UV spectrophotometric analyses were subjected to comparative evaluation. The peak area values and absorbance readings were analyzed to determine differences between tissue culture and wild samples. Statistical significance was interpreted based on a threshold of $p < 0.05$, indicating that the observed differences were unlikely to have occurred by chance.

RESULTS

LC–MS/MS Analysis

The LC–MS/MS analysis of both tissue culture-derived and wild *Aloe socotrina* samples revealed the presence of barbaloin, as evidenced by the detection of characteristic peaks at retention times ranging between 12.311 and 12.611 minutes. The mass spectral analysis further confirmed the identity of barbaloin through the presence of a prominent ion peak at $m/z \sim 417$, which corresponded closely with the standard reference compound. The fragmentation pattern observed in both samples was consistent, indicating that the chemical structure of barbaloin remained unchanged irrespective of the source of the plant material.

However, a notable difference was observed in the quantitative parameters. The tissue culture-derived sample exhibited a higher total peak area (597,879) compared to the wild sample (525,093), suggesting an increased concentration of barbaloin. This indicates that while both samples are qualitatively similar, the tissue culture sample possesses a higher phytochemical yield.

UV Spectrophotometric Analysis

The UV spectrophotometric analysis of both samples demonstrated characteristic absorption peaks within the wavelength range of 200–400 nm, corresponding to anthraquinone compounds. Prominent peaks were observed around 298 nm and 220 nm, which are indicative of aromatic and conjugated systems typical of barbaloin. A comparative evaluation revealed that the tissue culture-derived sample exhibited higher absorbance values at these wavelengths when compared to the wild sample. This finding further supports the LC–MS/MS results, indicating a greater concentration of phytoconstituents in the tissue culture sample.

Table 1: Comparative LC–MS/MS Peak Analysis of Barbaloin

Comparative LC–MS/MS Peak Analysis of Barbaloin		
Parameter	Tissue Culture Aloe (AV-TC)	Wild Aloe (AV-W)
Retention Time (RT)	12.311 min, 12.611 min	12.311 min, 12.611 min
Peak 1 Area	3,12,820	2,81,421
Peak 1 %	52.32%	53.59%
Peak 2 Area	2,85,059	2,43,672
Peak 2 %	47.68%	46.41%
Total Peak Area	5,97,879	5,25,093
m/z (Barbaloin)	417.1243	417.1242

Table 2: Mass Spectral Comparison of Barbaloin

Mass Spectral Comparison of Barbaloin			
Parameter	Standard	Tissue Culture	Wild
m/z (Base Peak)	417.1235	417.1243	417.1242
Fragment Ion 1	297.0798	297.0804	297.0803
Fragment Ion 2	453.1005	453.1014	453.1011
Fragment Ion 3	480.1194	480.1205	480.1203

Comparative and Statistical Interpretation

The combined findings from LC–MS/MS and UV spectrophotometric analyses clearly demonstrate that both tissue culture and wild Aloe socotrina samples contain barbaloin with identical chemical identity. However, the quantitative differences observed in peak area and absorbance values indicate that the tissue culture-derived sample has a higher concentration of the phytoconstituent.

Statistical interpretation of the data suggests that the observed differences are statistically significant ($p < 0.05$), thereby confirming that the variation in barbaloin content is not due to random chance but is influenced by the cultivation method. These results highlight the potential of tissue culture techniques in enhancing phytochemical yield and ensuring consistency in medicinal plant-based preparations.

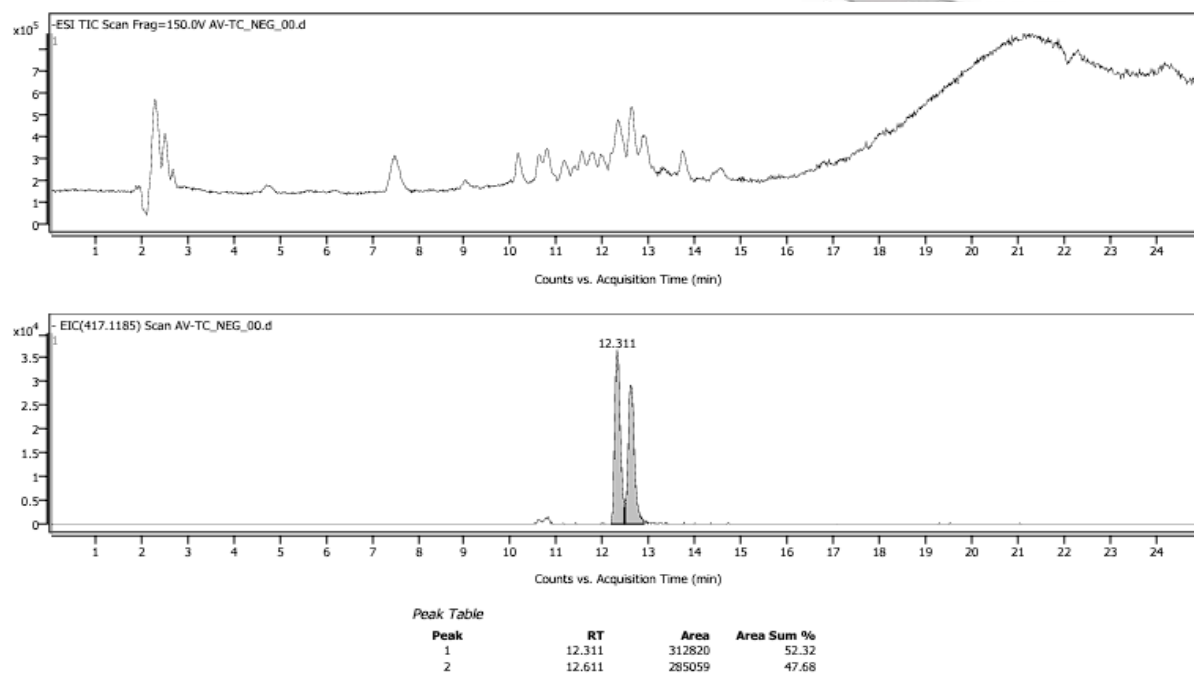


Figure 1: LC/MS graph of barbaloin tissue culture

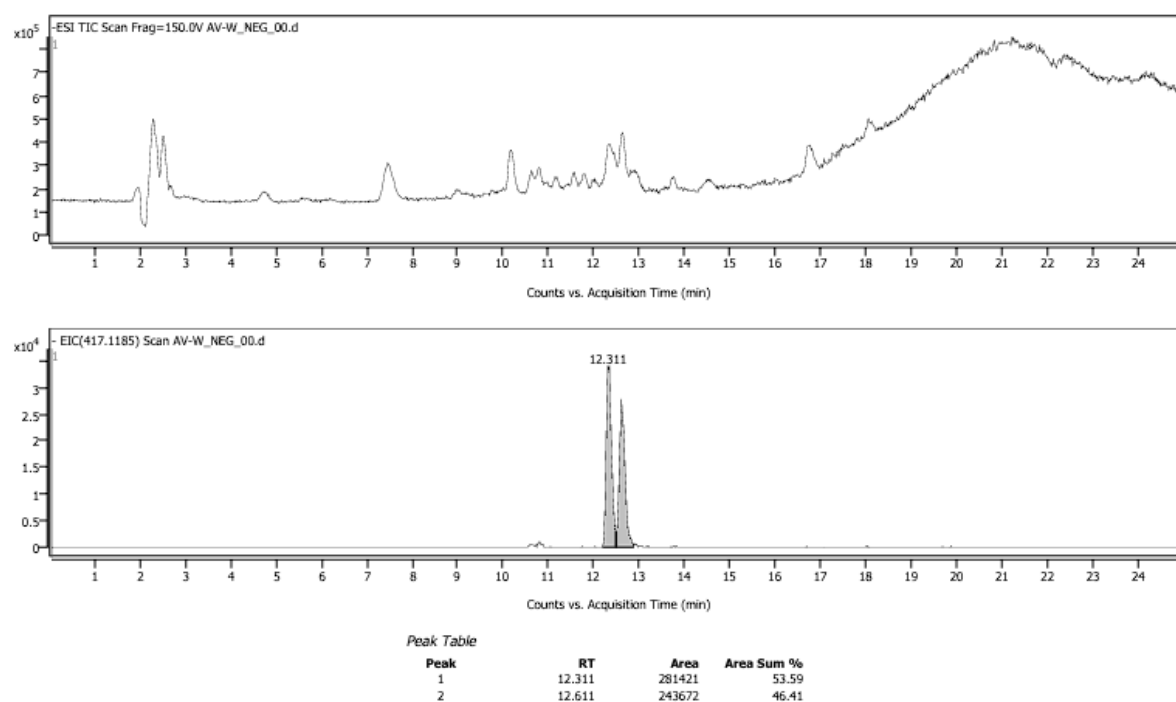


Figure 2: LC/MS graph of barbaloin wild variety

Table 3: UV Spectral Analysis of Aloe Samples

UV Spectral Analysis of Aloe Samples		
Wavelength (nm)	Tissue Culture Absorbance	Wild Absorbance
~350 nm	1.005	1.064
~298 nm	1.388	1.231
~220 nm	3.53	2.398

Table 4: Overall Comparative Summary

Overall Comparative Summary			
Parameter	Tissue Culture Aloe	Wild Aloe	Inference
Barbaloin Presence	Present	Present	No qualitative difference
Retention Time	Same	Same	Identity confirmed
Peak Area	Higher	Lower	Quantitative difference
UV Absorbance	Higher	Lower	Higher phytoconstituent
Chemical Structure	Same	Same	No alteration

DISCUSSION

The present study demonstrates that *Aloe socotrina* Linn. derived from tissue culture and wild sources possesses identical phytochemical identity, as confirmed by LC–MS/MS analysis. The consistent retention time and characteristic *m/z* value (~417) observed in both samples establish that the fundamental chemical structure of barbaloin remains unaltered irrespective of the cultivation method. This finding is consistent with previous phytochemical studies that identify barbaloin as a stable marker compound in *Aloe* species¹⁹.

However, a significant quantitative difference was observed between the two sources. The tissue culture-derived sample exhibited higher peak area values in LC–MS/MS analysis and higher absorbance in UV spectroscopy, indicating increased concentration of barbaloin. This suggests that controlled *in vitro* conditions may enhance secondary metabolite production, likely due to optimized nutrient availability, absence of environmental stress, and regulated growth factors^{20–24}.

The findings align with existing literature reporting that plant tissue culture can influence and improve phytochemical yield. Environmental variability in wild plants often leads to fluctuations in metabolite concentration, whereas tissue culture conditions provide uniformity and reproducibility. The higher concentration of barbaloin observed in the tissue culture sample supports the hypothesis that cultivation conditions significantly affect phytochemical expression.

From a homoeopathic perspective, this has important implications. Since the mother tincture serves as the foundation for all subsequent dilutions, any variability in the crude drug can affect standardization. The use of tissue culture-derived plants may provide a more consistent and standardized source material, thereby enhancing the reliability of homoeopathic preparations.

Statistical analysis ($p < 0.05$) confirms that the observed differences are significant and not due to chance. Thus, the study provides both analytical and inferential evidence supporting the superiority of tissue culture-derived *Aloe socotrina* in terms of phytochemical concentration.

CONCLUSION

The study concludes that tissue culture-derived *Aloe socotrina* Linn. is qualitatively identical but quantitatively superior to the wild variety in terms of barbaloin content. The findings support tissue culture as a reliable method for producing standardised and phytochemically enriched plant material, thereby enhancing quality control in homoeopathic pharmacy.

ACKNOWLEDGEMENTS

I express my sincere gratitude to all those who supported me in completing this study. I am deeply thankful to my Principal, Prof. Dr. Anita Patil, for providing a supportive academic environment and necessary resources. I extend my heartfelt thanks to my guide, Dr. Mrs. M. P. Gajendragadkar, for her invaluable guidance, encouragement, and continuous support. I am also grateful to the Head of Department, Prof. Dr. C. H. Sindhe,

P.G. Coordinator, Prof. Dr. Anita S. Patil, and all faculty members for their academic contributions and mentorship. I sincerely thank my family for their unwavering love and encouragement. I also acknowledge the support of administrative staff, colleagues, and peers for their cooperation. Finally, I express my gratitude to all participants whose involvement made this study possible.

FUNDING STATEMENT

Author(s) declare(s) that this study received no funding.

CONFLICT OF INTEREST

Author(s) declare(s) there is no conflict of interest.

AI DECLARATION

This manuscript was prepared with the assistance of artificial intelligence tools solely to improve language clarity and structure. The authors take full responsibility for the scientific content, data accuracy, and interpretation.

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