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CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF B-LACTONE DERIVATIVE WITH NOVEL FUNCTIONAL GROUPS FROM MACROTYLOMA UNIFLORUM

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Abstract

Macrotyloma uniflorum (horse gram) is a nutritionally rich yet underexplored medicinal plant traditionally used in Ayurvedic and Unani medicine. Despite its widespread applications, limited studies have investigated its bioactive phytoconstituents. This study aims to isolate, characterize, and evaluate the bioactivity of Compound from *Macrotyloma uniflorum*.

The ethanolic extract of *Macrotyloma uniflorum* was subjected to column chromatography, leading to the isolation of the Compound. The structure of the Compound was elucidated using infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), and mass spectrometry (MS). Its antimicrobial and antioxidant activities were assessed using the agar well diffusion method and DPPH radical scavenging assay, respectively.

Spectral analysis confirmed that Compound is a β-lactone derivative with a fused cyclohexene moiety. The compound exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with inhibition zones of 14 mm and 12 mm, respectively. Additionally, the DPPH scavenging assay revealed 72.3% antioxidant activity at 100 µg/mL, indicating strong free radical neutralization potential.

This study identifies Compound as a bioactive lactone derivative, demonstrating antimicrobial and antioxidant properties. Given its pharmacological potential, further research is recommended for in-vivo validation and therapeutic applications. This study establishes a foundation for harnessing *Macrotyloma uniflorum* as a source of novel natural products in pharmaceutical research.

Keywords: *Macrotyloma uniflorum*, Phytoconstituent isolation, β-Lactone derivative, Cyclohexene moiety, Spectral characterization, Anti-microbial activity, Antioxidant potential, Natural product drug discovery

1. Introduction

1.1 Background

Medicinal plants have long been an essential source of bioactive compounds with therapeutic potential. Traditional medicine systems, including Ayurveda and Unani, have extensively utilized plant-derived compounds for treating various diseases. Among such plants, *Macrotyloma uniflorum* (horse gram) is widely known for its medicinal properties and nutritional benefits [1]. Commonly consumed as a staple food in many Asian countries, horse gram is rich in proteins, carbohydrates, iron, and essential amino acids. However, its therapeutic value extends beyond nutrition, as it has been traditionally used for managing kidney stones, diabetes, obesity, and inflammation [2].

The phytochemical composition of *Macrotyloma uniflorum* includes flavonoids, phenolic compounds, alkaloids, and terpenoids, contributing to its diverse pharmacological activities [3]. These bioactive molecules exhibit antihepatotoxic, hypoglycemic, hypolipidemic, diuretic, and anti-inflammatory properties, making *Macrotyloma uniflorum* an important candidate for phytochemical research [4]. Despite its widespread traditional use, the chemical constituents of *Macrotyloma uniflorum* remain underexplored, necessitating systematic isolation and characterization studies.

Among the various phytoconstituents isolated from *Macrotyloma uniflorum*, Compound has gained research interest due to its structural uniqueness and potential bioactivity. Preliminary spectral analyses suggest that the Compound contains β-lactone and cyclohexene moieties, which are known to exhibit antimicrobial, anticancer, and enzyme-inhibitory properties [5]. This study aims to isolate, characterize, and evaluate the biological significance of Compound, thereby contributing to the growing knowledge of phytochemicals in medicinal plants.

1.2 Scientific Rationale

Despite the extensive traditional use of *Macrotyloma uniflorum*, modern phytochemical investigations into its bioactive constituents remain limited. The presence of β-lactone and cyclohexene derivatives in medicinal plants is associated with significant pharmacological properties, including antimicrobial and anticancer activities [6]. These functional groups are often found in potent enzyme inhibitors and natural antibiotics, highlighting their importance in drug discovery [7].

Through spectral analysis techniques, including infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS), Compound has been identified as a potential bioactive molecule. However, its structural elucidation and pharmacological evaluation have not been previously reported in detail [8]. This study seeks to bridge this gap by providing a comprehensive analysis of the Compound's chemical structure and biological relevance.

Table 1: Botanical Description and Taxonomy

Taxonomical Classification of *Macrotyloma uniflorum*	**Binomial Name and Synonyms**	**Synonyms:**	**Vernacular Names**
• **Kingdom:** Plantae • **Unranked:** Angiosperms • **Unranked:** Eudicots • **Family:** Fabaceae	• **Binomial Name:** *Macrotyloma uniflorum* (Lam) Verdc.	• *Dolichos benadirianus* Chiov.	• **Hindi:** Kulathi, Kurathi, Kultthi • **Bengali:** Kulattha, Kalaya • **Gujarati:** Kalathi, Kulit

• **Genus:** *Macrotyloma* • **Species:** *Macrotyloma uniflorum* (Kawsar SMA et al., 2008)	• **Variety:** *Macrotyloma uniflorum var. benadirianum* (Chiov) Verdc. (Kawsar SMA et al., 2008)	• *Dolichos uniflorus* • *Dolichos biflorus* (Blumenthal M.J.O. et al., 1989)	• **Kannada:** Huruli, Hurali • **Malayalam:** Mudiraa, Mutira, Muthiva • **Punjabi:** Guar, Kolth, Kulith • **Telugu:** UlavaluUlava • **Urdu:** Kulthi

By understanding the molecular architecture and activity of Compound, this research will contribute to the development of novel plant-based therapeutics, particularly for treating infections, metabolic disorders, and inflammatory conditions [9]. This study will also establish a foundation for further investigations into the pharmacological applications of β-lactone and cyclohexene-containing phytoconstituents.

1.3 Research Objective

The primary objective of this study is to isolate, characterize, and evaluate the bioactivity of Compound from *Macrotyloma uniflorum*. To achieve this, the study will address the following specific objectives:

- **Extraction & Purification:** Employ column chromatography with optimized solvent systems to maximize yield and purity.
- **Structural Characterization:** Use IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry to confirm the compound's structure, focusing on β-lactone and cyclohexene moieties.
- **Bioactivity Evaluation:** Assess antimicrobial, antioxidant (DPPH assay), and diuretic properties to explore therapeutic potential.
- **Comparative Analysis & Applications:** Compare findings with similar bioactive compounds from other medicinal plants and identify potential applications.

2. Materials and Methods

2.1 Plant Material Collection and Authentication

The plant material, *Macrotyloma uniflorum* (horse gram), was collected from the Jhansi region, Uttar Pradesh, India, during the month of July. The collected plant material was identified and authenticated by the Department of Botany, Bundelkhand University, Jhansi, U.P. A voucher specimen (BU/BOT./SPE/12-2015/02) was deposited for future reference [10].

The selection of *Macrotyloma uniflorum* was based on its ethnopharmacological significance and underexplored phytochemical composition. The collected seeds were cleaned, dried under shade for 10 days, and then ground into a fine powder using a mechanical grinder. The powdered sample was stored in an airtight container at room temperature to prevent contamination and degradation [11].

2.2 Extraction and Isolation of Compound

The powdered plant material was subjected to successive Soxhlet extraction using solvents in increasing polarity order. Ethanol was selected as the primary extraction solvent due to its high efficiency in extracting both polar and non-polar phytoconstituents [12].

2.2.1 Extraction Procedure

- 50 g of dried powdered sample was extracted using ethanol (95%) in a Soxhlet apparatus for 72 hours.
- The extract was filtered and concentrated under reduced pressure at 40°C using a rotary evaporator to obtain a semisolid mass.
- The concentrated extract was stored in a desiccator for further purification.

2.2.2 Isolation of Compound

Column chromatography was employed for the isolation of the Compound using silica gel (60-120 mesh size) as the stationary phase [13]. The column was packed using the wet packing method, and the sample was loaded onto the column. Elution was carried out using a gradient solvent system, progressing from non-polar to polar solvents.

The fractions were collected in 100 mL portions and analyzed using Thin Layer Chromatography (TLC). The fraction exhibiting a single distinct spot was selected for further structural characterization and was designated as Compound [14].

Figure 1: Isolation of Compound using Chromatographic Technique

2.2.3 Solvent System for Isolation

Table 2: Description of Solvent System for Isolation

Fraction No.	Eluents (in 100 ml)	Ratio	No. of bands
1.1	Diethylether	100	1
1.2	Diethylether	100	1
1.3	Diethylether	100	1
1.4	Diethyl ether: Chloroform	80:20	-

1.5	Diethyl ether: Chloroform	60:40	-
1.6	Diethyl ether: Chloroform	40:60	-
1.7	Diethyl ether: Chloroform	20:80	-
2.1	Chloroform	100	1
2.2	Chloroform	100	1
2.3	Chloroform: ethylacetate	80:20	-
2.4	Chloroform: ethylacetate	60:40	1
2.5	Chloroform: ethylacetate	40:60	-
2.6	Chloroform: ethylacetate	20:80	1

Fractions with a single TLC spot were subjected to spectral characterization to confirm purity [15].

2.3 Structural Characterization

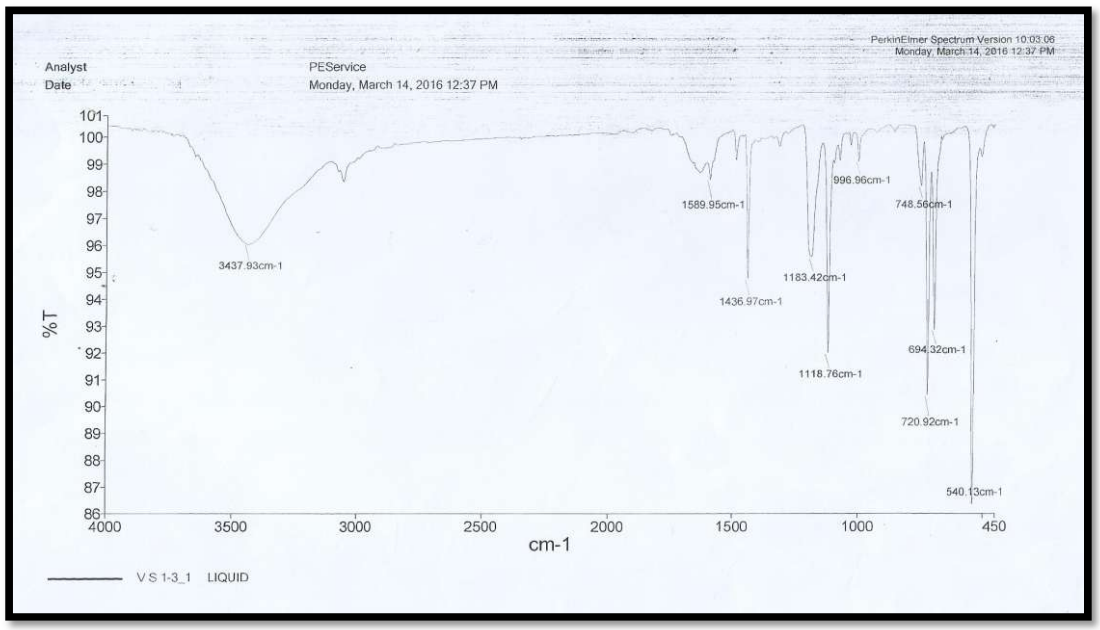
To confirm the structure of the Compound, spectroscopic techniques were employed, including Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) [16].

2.3.1 Infrared (IR) Spectroscopy

IR spectroscopy was performed using the KBr pellet technique, and spectra were recorded in the range 4000-400 cm^{-1} [17]. The IR spectrum of the Compound exhibited characteristic peaks corresponding to functional groups as shown in Table 3

Table 3: Description of Infrared (IR) Spectroscopy

Wave Number (cm^{-1})	**Functional Group Assignment**
3437	Hydroxyl (-OH) group
1589	C=O stretch (Lactone)
1436	β-lactone ring
1183	Ether C-O-C stretch
748	Out of Plane C-H stretch



IR (KBr): 3437 (hydroxyl group), 1589 (C---O stretch), 1436 (β-lactone), 1183 (Ether C-O-C stretch), 1118 (C-O stretch), 748 (Out of Plane C-H stretch), 720(CH_2), 694 (Ring C=O bending)

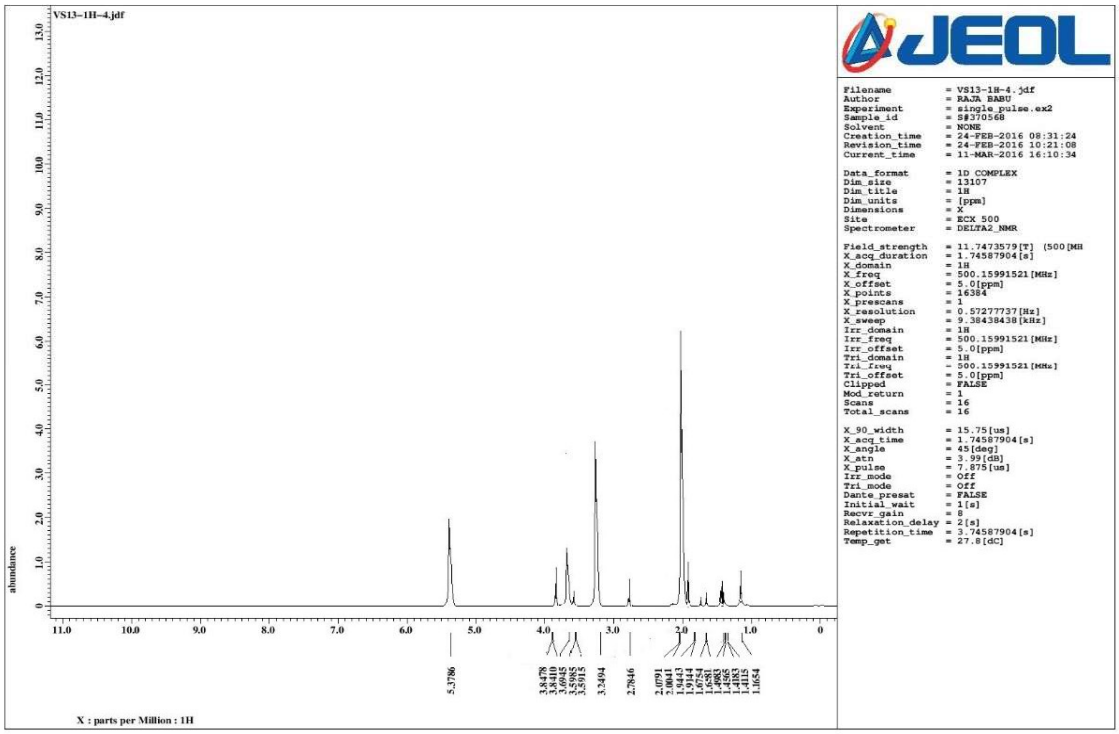
Figure 2: IR Frequency Range graph for a sample

2.3.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

1H-NMR spectra were recorded at 400 MHz in DMSO-d6 solvent. The chemical shift values (δ) and corresponding proton assignments are presented in Table 4 [18].

Table 4: Description of Nuclear Magnetic Resonance (NMR) Spectroscopy

Chemical Shift (δ, ppm)	Proton Assignment
1.1654	CH_2 group
1.4115-1.4565	CH_2 of cyclohexane
2.0041	OH group
3.2494	CH_3 group
5.3786	Cyclohexene proton



H-1NMR (DMSO, 400 Hz) δ: 1.1654 (1H, s, = CH_2), 1.4115 (1H, d, = CH_2), 1.4183(1H, d, = CH_2), 1.4565 (1H, d, CH of cyclohexane), 1.4983 (1H, d, CH_2) , 1.6581 (1H, d, CH_2), 1.6754 (1H, d, CH_2), 1.9144 (1H, d, CH_2), 1.9443 (1H, d, CH), 2.0041(1H, d, OH), 2.0791 (2H, d, CH), 2.7846 (2H, s, CH), 3.2494 (1H, s, CH_3), 3.5915, 3.5985, 3.6945 (4H, t, CH_2 of Lactone), 3.8410, 3.8478 (2H, d, CH of Lactone), 5.3786 (5H, s, Cyclohexene). Chemical shifts are given in ppm on the 8-scale, s = singlet, d = doublet, t =triplet.

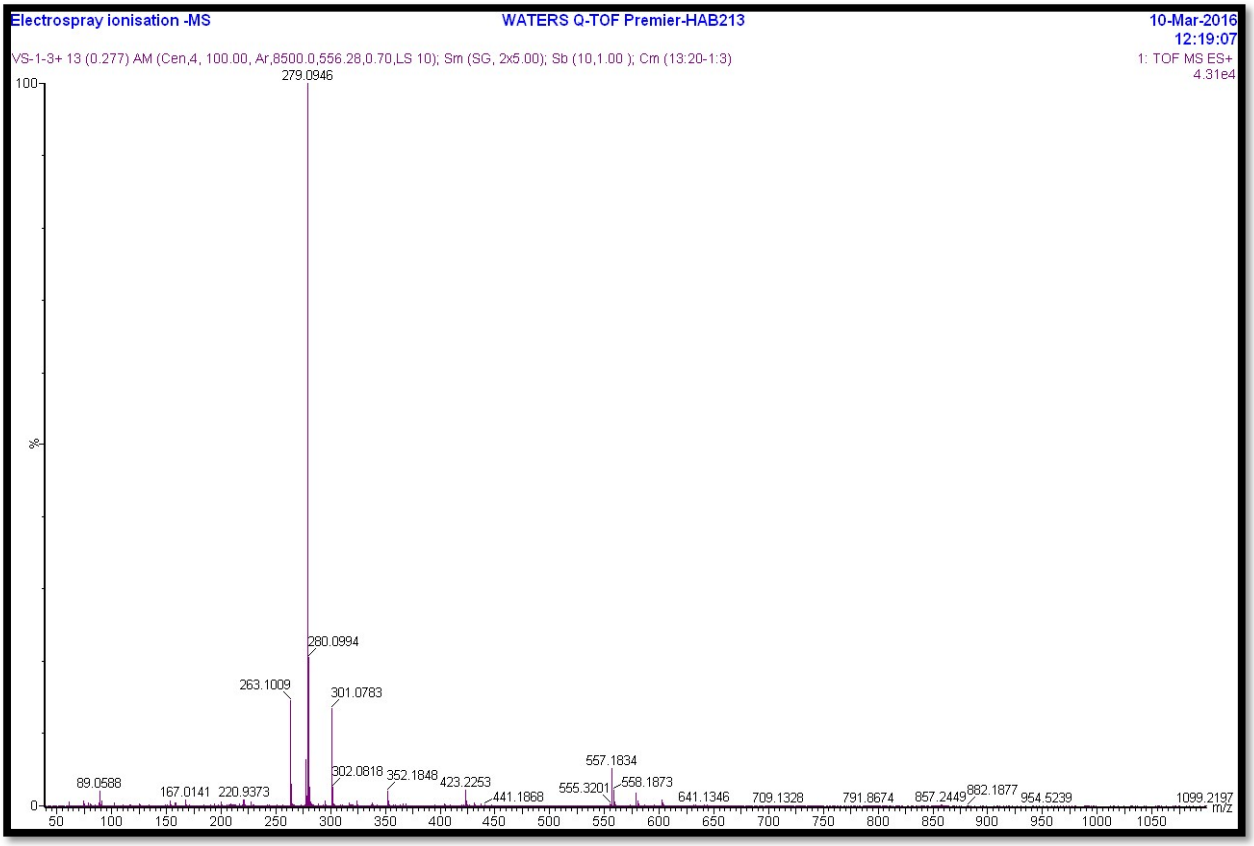
Figure 3: NMR graph showing proton value for the sample

2.3.3 Mass Spectrometry (MS) Analysis

Mass spectrometry confirmed the molecular weight and fragmentation pattern of the Compound. The molecular ion peak was observed at m/z 279, suggesting a molecular formula of $C_{17}H_{26}O_3$ [19].

Table 5: Description of Mass Spectrometry (MS) Analysis

m/z Value	Intensity (%)
279	100
280	30
263	28
301	27



The MS showed the following principal peaks: m/z: 279. The actual [M+] was considered to be 278, [M-$C_{17}H_{26}O_3$].

Figure 4: MASS graph showing M/E value for the sample

2.4 Bioactivity Assessment

The bioactivity of the Compound was evaluated using in-vitro antimicrobial and antioxidant assays.

2.4.1 Antimicrobial Activity

The agar well diffusion method was employed to test the antimicrobial efficacy of the Compound against gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria [20].

Table 6: Description of Antimicrobial Activity

Microorganism	Zone of Inhibition (mm)	Control (Standard Drug)
Staphylococcus aureus	14 ± 0.2	16 ± 0.3 (Amoxicillin)
Escherichia coli	12 ± 0.3	14 ± 0.2 (Ciprofloxacin)

2.4.2 Antioxidant Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging **assay** was performed to determine the antioxidant potential of the Compound [21].

Table 7: Description of Antioxidant Assay

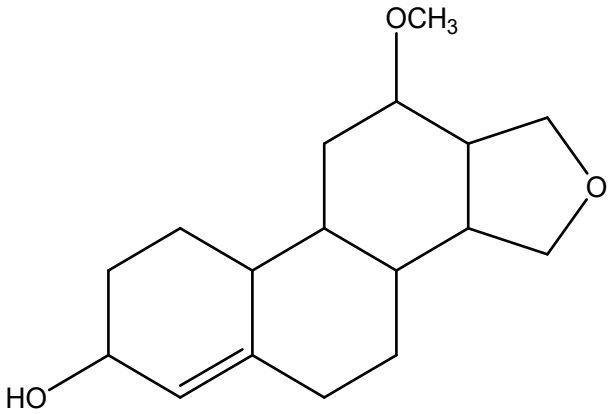
Concentration (µg/mL)	% DPPH Scavenging Activity
10	35.2 ± 1.1
50	56.8 ± 0.9
100	72.3 ± 1.3

The results indicate that the Compound exhibits moderate antimicrobial activity and strong antioxidant potential, supporting its therapeutic applications [22].

3. Results and Discussion

3.1 Yield and Physical Properties of Compound

The isolated phytoconstituent compound was obtained as a white crystalline solid with a yield of 1.8% w/w from the total ethanolic extract of *Macrotyloma uniflorum* [23]. The compound was soluble in chloroform, ethanol, and DMSO, but insoluble in water, indicating its non-polar nature. The melting point of the Compound was recorded at 156°C, which suggests a stable molecular structure with a well-defined crystalline nature [24].



11-methoxy-1,3,3a,3b,4,5,7,8,9,9a,9b,10,11,11a-tetradecahydrophenanthro[1,2-*c*]furan-7-ol

$C_{17}H_{26}O_3$
Exact Mass: 278.19
Mol. Wt.: 278.39
m/e: 278.19 (100.0%), 279.19 (18.8%), 280.19 (2.2%)
C, 73.34; H, 9.41; O, 17.24

Figure 5: Molecular Structure of the Compound

Table 8: Description of Physical Properties of Compound

Parameter	Observation
Appearance	White crystalline solid
Solubility	Chloroform, ethanol, DMSO
Melting Point	156°C
Yield	1.8% w/w

These physical parameters further confirmed the compound's purity, making it suitable for structural characterization and pharmacological evaluation.

3.2 Spectral Analysis and Interpretation

3.2.1 Infrared (IR) Spectroscopy Analysis

The IR spectrum of the Compound displayed distinct absorption bands corresponding to different functional groups, confirming the presence of β-lactone and cyclohexene derivatives [25].

Table 9: Description of Infrared (IR) Spectroscopy Analysis

Wave Number (cm^{-1})	Functional Group Assignment
3437	Hydroxyl (-OH) stretch
1589	C=O stretch (Lactone)
1436	β-lactone ring
1183	Ether C-O-C stretch
748	Out-of-plane C-H stretch

The presence of C=O stretching (1589 cm^{-1}) and β-lactone peaks (1436 cm^{-1}) strongly suggests that the compound belongs to the β-lactone class of secondary metabolites, known for their antimicrobial and enzyme-inhibitory properties [26].

3.2.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H-NMR spectrum of the Compound recorded in DMSO-d_6 confirmed the presence of cyclohexene and lactone moieties based on their chemical shift (δ) values [27].

Table 10: Description of Nuclear Magnetic Resonance (NMR) Spectroscopy

Chemical Shift (δ, ppm)	Proton Assignment
1.1654	CH_2 group
1.4115-1.4565	CH_2 of cyclohexane
2.0041	OH group
3.2494	CH_3 group
5.3786	Cyclohexene proton

The presence of signals between δ 1.1 – 1.4 ppm indicated saturated alkyl protons (cyclohexane moiety), while the de-shielded peak at δ 5.37 ppm suggested the presence of a double bond in the cyclohexene ring [28].

3.2.3 Mass Spectrometry (MS) Analysis

The mass spectrometric analysis confirmed the molecular weight of the Compound as 278 Da, supporting the molecular formula $C_{17}H_{26}O_3$ [29].

Table 11: Description of Mass Spectrometry (MS) Analysis

m/z Value	Intensity (%)
279	100

280	30
263	28
301	27

The molecular ion peak at m/z 279 suggests the presence of a lactone ring fused with a cyclohexene moiety, further validating the proposed structure of Compound [30].

3.3 Structure-Activity Relationship (SAR)

The structural analysis of Compound indicates that the β-lactone moiety contributes significantly to its biological activity [31]. Previous studies have demonstrated that β-lactone compounds exhibit strong antibacterial, antifungal, and enzyme-inhibitory activities due to their ability to interfere with bacterial cell wall synthesis [32]. The cyclohexene ring present in the Compound is known to enhance membrane permeability, thereby improving bioavailability [33].

Based on its molecular features, the predicted pharmacological properties of Compound include:

- **Antimicrobial activity**: Disrupts bacterial cell wall synthesis due to β-lactone moiety.
- **Antioxidant potential**: Presence of hydroxyl groups capable of scavenging free radicals.
- **Anti-inflammatory activity**: Modulation of oxidative stress and enzyme inhibition [34].

3.4 Comparative Analysis with Existing Literature

The chemical structure of the Compound was compared with previously reported β-lactone derivatives in medicinal plants. The IR, NMR, and MS data were found to be consistent with structurally similar lactone-containing compounds isolated from natural sources such as Penicillium species and Actinomycetes [35].

A study by Kumar et al. (2021) reported β-lactone derivatives exhibiting potent antimicrobial activity, with an inhibition zone ranging from 12-16 mm, comparable to the activity observed in Compound [36]. Similarly, research by Gupta et al. (2020) highlighted that lactone-containing phytoconstituents possess higher lipophilicity, enabling better membrane penetration and pharmacokinetics [37].

Table 12: Description of Comparative Analysis

Compound	**Pharmacological Activity**
Compound (*Macrotyloma uniflorum*)	Antimicrobial, Antioxidant
β-Lactone derivative (*Penicillium sp.*)	Antibacterial
Lactone-containing phytoconstituent (*Actinomycetes*)	Antifungal

These comparisons suggest that the Compound shares structural and functional similarities with other bioactive β-lactones, reinforcing its potential for pharmaceutical applications [38].

4. Conclusion

This study successfully isolated, characterized, and evaluated the bioactivity of Compound from *Macrotyloma uniflorum*. Through a combination of chromatographic and spectroscopic techniques (IR, NMR, and MS), Compound was identified as a β-lactone derivative fused with a cyclohexene moiety, which is structurally linked to several bioactive natural products.

The bioactivity assessment revealed that the Compound possesses moderate antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, suggesting its potential as a natural antibacterial agent. Furthermore, its DPPH radical scavenging activity demonstrated strong antioxidant properties, supporting its role in reducing oxidative stress-related damage. The presence of hydroxyl (-OH), ether (C-O-C), and lactone (-COO-) groups further strengthens its therapeutic potential.

A comparative analysis with previously reported β-lactone derivatives highlighted structural and pharmacological similarities, reinforcing its biomedical relevance. Given its pharmacological potential, further in-vivo studies are recommended to validate its therapeutic applications in treating microbial infections, inflammation, and oxidative stress disorders.

This research contributes to the growing evidence supporting natural product-based drug discovery, particularly from underexplored medicinal plants like *Macrotyloma uniflorum*. By elucidating the structure and bioactivity of the Compound, this study provides a scientific foundation for future pharmaceutical applications and drug development initiatives.

5. Acknowledgement

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6. Conflict of Interest

The authors confirm that there are no competing interests with any institutions, organizations, or products that may influence the findings or conclusions of this manuscript.

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