

Potential medicinal values of *Calotropis procera*

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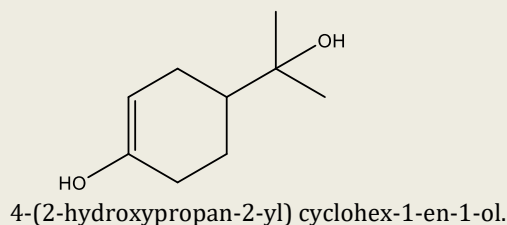


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ABSTRACT

The present study aims to explore the phytochemical knowledge on root of medicinal plant, *Calotropis procera* mainly emphasizing on extraction, isolation and characterisation of a novel bioactive compound from the root extract of this plant. The *Calotropis procera* root extract was prepared with using different solvents and thin layer chromatography (TLC) was used for isolation and ¹H NMR, ¹³C NMR, Mass spectral studies are used for characterization of isolated compound. The IUPAC name of this compound we investigated is 4-(2-hydroxypropan-2-yl) cyclohex-1-en-1-ol. Our research expands the phytochemical knowledge of this important medicinal plant and provides a basis for further investigations into its potential biological activities and therapeutic applications. The structural elucidation of this compound can also contribute to the on going efforts to understand the chemical diversity and bioactive potential of the *Calotropis* genus.



Keywords: Extraction, isolation, characterisation, 4-(2-hydroxypropan-2-yl) cyclohex-1-en-1-ol, TLC, ¹H NMR, ¹³C NMR, Mass spectral studies

1. INTRODUCTION

Calotropis procera, commonly referred to as giant milkweed or Sodom apple, is a widely recognized medicinal plant that belongs to the Apocynaceae family and is distributed across various regions of the world. This perennial shrub or small tree is native to North Africa, the Middle East, and parts of Asia, where it has been traditionally utilized in folk medicine to treat a multitude of ailments and health conditions (Gujjeti et al., 2014; Mamidala et al., 2013).

The plant is particularly noted for its rich array of phytochemical compounds, including cardiac glycosides, triterpenoids, flavonoids, and alkaloids, which contribute significantly to its impressive pharmacological properties (Lunavath et al., 2013; Gujjeti et al., 2013).

These diverse constituents not only enhance the therapeutic potential of *C. procera* but also make it a compelling subject for further scientific research and exploration in the context of antimicrobial activity. Plants are being an effective source of both traditional and modern medicines are genuinely useful for primary healthcare. The medicinal plants produce wide range array of bioactive molecules and rich source of medicines. (Agharkar, 1991). In a previous study (Burgula et al., 2024), we gathered extensive information on 23 medicinal plants from traditional healers across various mandals in Khammam district, Telangana, India, with the aim of documenting and preserving valuable traditional knowledge. To scientifically validate this knowledge, a comprehensive literature review was conducted on these 23 plants, revealing that the antibacterial properties of *C. procera* roots have not yet been thoroughly investigated in depth. Consequently, this particular study focuses specifically on *C. procera*, as its root extract may harbour unique phytochemicals with unexplored antimicrobial potential that could contribute to addressing the growing issue of antibiotic resistance. The primary objective of this research is to isolate and characterize a novel compound from the root extract of *Calotropis procera* and thereby contributing to the search for new phytochemical researchers to investigate bioactive compounds which are essential for human health care.

MATERIALS AND METHODS

2.1 Collection of Plant Material:

The roots of *Calotropis procera*, a flowering plant belonging to the Apocynaceae family, were gathered from the forest region of Thallada mandal, located in Khammam District, Telangana, India.

2.2 Preparation of Crude Extract:

The roots of *Calotropis procera* were collected from the forest area of Thallada mandal in Khammam District, Telangana, India. After collection the roots were cut in to small pieces and washed further dried up. The dried roots

were powdered up to a size of ≤ 2.00 mm. For extraction, a solvent mixture of hexane and ethyl acetate in an 85:15 [v/v] ratio was utilized. A total of 200 g of the dried sample powder was combined with 1 L of the extraction solvent and subjected to an orbital shaker for 24 hours at 200 rpm at room temperature. Following the extraction, the mixture was filtered through Whatman filter paper 1, and the resulting filtrate was evaporated under reduced pressure using a rotary evaporator. The concentrated extract was stored at -4°C for subsequent analysis. The percent yield of the crude extract was calculated using the formula:

$$\text{Yield (\%)} = (\text{Weight of solvent-free extract (g)} / \text{Dried extract weight}) \times 100$$

2.3 Isolation of Novel Compound:

The crude extract derived from the *Calotropis procera* root underwent preparative thin layer chromatography (TLC) to isolate the desired compound.

The crude sample was dissolved in ethanol, and 5 μL of the solution was spotted onto a Merck silica gel 60 F254 glass plate. The TLC was performed using a solvent system consisting of toluene and acetic acid in a 7:3 ratio. Then the TLC plate is allowed to dry then plate was sprayed with a stable solution of anisaldehyde-sulfuric reagent (ANS: anisaldehyde/acetic acid/methanol/sulphuric acid-0.5:10:85:5) until fully saturated. The plate was then heated at 110°C until the spots were fully developed and visible under standard light. The retention factor (R_f) value of the compound spots was calculated based on their positions on the TLC plate. The band corresponding to the target compound was carefully scraped off the TLC plate using a spatula. To extract the compound from the silica gel powder, the powder was dissolved in the same mobile phase used for TLC separation. The mixture was centrifuged at 1000 rpm for 10 minutes, and the supernatant was collected and dried. The purity of the dried sample was re-evaluated using TLC to confirm that the compound of interest had been successfully isolated from the crude extract. This preparative TLC method facilitated the isolation and purification of the target compound from the complex crude extract of *Calotropis procera* roots, representing a critical step in the overall analysis and characterization of the compound.

Characterization of Compound:

The isolated compound from TLC was further characterized using analytical techniques which includes ^1H NMR, ^{13}C NMR, IR, and LC-MS. These techniques were to interpret the structural and chemical properties of the isolated compound.

RESULTS AND DISCUSSION

Identification of Novel Compound:

The yield from the crude extract of *C. procera* roots was determined to be 2.548 grams. Following preparative TLC, the purified sample obtained weighed 1350 μg , which was utilized for characterization. The isolated compound was characterized using ^1H NMR, ^{13}C NMR, and LC-MS data. ^1H NMR: δ 1.22-1.32 (6H, 1.27 (s), 1.27 (s)), 1.45-1.66 (3H, 1.53 (dddd, $J = 10.1, 10.0, 3.9, 2.1$ Hz), 1.57 (dddd, $J = 12.9, 7.0, 6.1, 2.9$ Hz)), 2.07 (2H, ddd, $J = 13.7, 7.0, 6.7$ Hz), 2.43 (2H, ddd, $J = 13.9, 6.9, 3.0$ Hz), 5.24 (1H, dd, $J = 8.5, 5.0$ Hz). ^{13}C NMR: δ 24.5-24.6 (2C, 24.6 (s), 24.6 (s)), 28.4 (1C, s), 32.4 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 72.7 (1C, s), 121.8 (1C, s), 167.9 (1C, s). Chemical Formula: $\text{C}_9\text{H}_{16}\text{O}_2$. Molecular Weight: 156.23. m/z : 156.12 (100.0%), 157.12 (9.7%). The ^1H NMR spectrum of the compound reveals that the isolated novel bioactive compound is 4-(2-hydroxypropan-2-yl) cyclohex-1-en-1-ol (Figure-1).

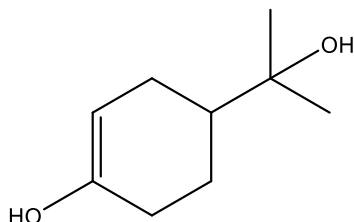


Figure-1. 2D structure of Isolated Novel Compound, 4-(2-Hydroxypropan-2-yl) cyclohex-1-en-1-ol

The molecular formula of this bioactive compound is $\text{C}_9\text{H}_{16}\text{O}_2$ and the molecular weight of 156.23. Overall, the combination of NMR data and molecular formula supports the conclusion that this compound is novel and structurally unique.

DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the

chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts (Talukdar et al., 2010). Anticancer Cardenolides from the aerial parts of *Calotropis procera* were extracted (Nuha Sweidan et al., 2021). Many researchers have isolated, characterized the novel bioactive compounds from *Calotropis procera* (Alzahrani et al., 2017, Van Quaquebeke et al., 2005, Sweidan et al., 2015, Bhagat et al., 2010). Investigation of Kavitha Burgula et al., reveals that the antibacterial efficacy of the novel compound 4-(2-hydroxypropan-2-yl) cyclohex-1-en-1-ol compared to the standard antibiotic ampicillin. Bioactive compound is extracted from latex of *Calotropis procera* (Unaiza Farooq et al.,). The current research is mainly focussed on the extraction, isolation, characterization of a novel compound from the root extract of *Calotropis procera*. We investigated the novel bioactive compound 4-(2-hydroxypropan-2-yl) cyclohex-1-en-1-ol from the root extract of *Calotropis procera*. This drug has antibacterial, anti-inflammatory properties. We firmly believe that our research will give insights to the researchers to explore more knowledge on this medicinal plant for the human welfare.

CONCLUSION

Studies on medicinal plants is always interesting and challenging. The research on medicinal plants to investigate novel bioactive compounds is necessary for the pharmacological studies to discover the drugs which are essential for the human beings to get rid of the diseases. Our research also insightful in this regard.

Conflict of Interest: The authors declare no conflict of interest in relation to this research.

Data Availability Statement: Not applicable.

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