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RESEARCH ARTICLE
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ANTICANCER POTENTIAL OF CRUDE EXTRACT AND TRITERPENOID ISOLATED FROM DATURA METEL LINNAEUS

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Abstract

Objective: This research aims to investigate the anticancer properties of a crude extract and a triterpenoid compound isolated from *Datura metel* Linnaeus. The study explores the diverse chemical composition of D. metel and evaluates the efficacy of the isolated compound against various cancer cell lines.

Methods: **Plant Collection:** Seeds of *D. metel* were obtained, and the plant specimen was identified and deposited.

Extraction and Isolation: Crude extract was obtained using methanol, followed by fractionation. Compound 1 was isolated through thin-layer chromatography and column chromatography.

Anticancer Activity: The MTT procedure was employed to assess cytotoxicity on various cancer cell lines (HepG2, A498, NCI-H226, and MDR 2780AD) using the isolated compound and crude extracts.

Results: Compound 1 exhibited significant anticancer activity compared to the crude extracts across various cell lines. The standard drug, Paclitaxel, demonstrated universal effectiveness. The results show varying IC_{50} values for hexane, chloroform, methanol extracts, daturaolone **1**, and Paclitaxel against different cell lines.

Discussion: The study emphasizes the increasing importance of natural products in pharmaceutical research, with *D. metel* identified as a potential source of novel anticancer agents. Compound **1**, isolated from *D. metel*, stands out for its noteworthy efficacy. The findings align with existing literature, suggesting the potential of *D. metel* extracts in cancer treatment.

Conclusion: The research concludes that Compound **1** from *D. metel* exhibits significant anticancer potential against various cancer cell lines. The study encourages further exploration of *D. metel* as a

potential reservoir for anticancer compounds, with Compound 1 emerging as a promising candidate for further investigation and pharmaceutical development.

Keywords: *Datura metel*, extract, Amyrin type triterpenoid, Anticancer

1.0 Introduction

The extensive use of plants in treating various diseases across different systems of medicine, regardless of their philosophical foundations, serves as a testament to their universal role. Utilizing individual, chemically pure compounds, such as synthetic drugs, is not devoid of inherent constraints. Consequently, there has been a notable resurgence in recent scholarly attention ttowardthe herbal and homoeopathic systems of medicine. These systems depend on botanical sources for therapeutic interventions [1]. According to the World Health Organisation (WHO), approximately 4 billion individuals, constituting 80% of the global population, use herbal medicine to address various aspects of primary health care. Herbal medicine holds significant prominence within traditional medicinal practices worldwide as a fundamental component in diverse systems such as homeopathy, naturopathy, traditional oriental medicine, and Indian medicine. Plants synthesize various secondary metabolites, recognized as a significant reservoir for numerous pharmaceutical compounds [2]. Multiple prior studies have elucidated the extensive spectrum of pharmacological and therapeutic effects exhibited by different medicinal plants [3]. *Datura metel* L., or *D. metel*, is a plant species characterized by its erect shrub-like growth habit and branches that spread outwards. In the local Bengali language, it is known as "Dhutura." It is a perennial herbaceous plant that falls under the taxonomic classification of the Solanaceae family [4]. During an extended temporal interval, specifically in the year 37 A.D., it has been documented that various species belonging to this family exhibited noteworthy therapeutic properties [5, 6]. Various researchers have conducted extensive investigations and studies on *D. metel*, confirming the existence of significant chemical compounds within this plant species. These compounds include amino acids, alkaloids, flavonoids, cardiac glycosides, tannins, carbohydrates, saponins, and phenolic compounds. B-sitosterol has also been identified in the plant [7]. According to a study conducted by Afsharypuor et al. in 1995, it was found that the root of *D. metel* contains a significant amount of atropine. In contrast, the aerial parts of the plant are abundant in scopolamine [8]. The isolation of seven glycosides, specifically daturglycosides 1–7, from the leaves of *D. metel* [9].

D. metel has been demonstrated to exhibit a wide range of biological activities, including analgesic, insecticidal, anti-inflammatory, antibacterial, cytotoxic, antidiabetic, antioxidant, antipyretic, wound healing, reproductive, neuro- and nephron-protective, and antispasmodic properties [10, 11]. *D. metel* has historically been utilized in India due to its alleged medicinal properties for treating various health conditions. This particular substance has been widely employed as a therapeutic intervention for multiple ailments. These include but are not limited to mental disorders such as madness or insanity, gastrointestinal disturbances like indigestion, cerebral afflictions, dermatological conditions, catarrhal infections, aural discharge, elephantiasis, and even incidents of being bitten by a rabid canine [12]. In Bangladesh, various components of the *D. metel* plant have been observed to be employed for therapeutic purposes in managing allergies, eczema, and scabies [13]. Haploid embryos were successfully generated through anther culture technique, thereby facilitating the production of a haploid *D. metel* plant [14]. In Chinese traditional medicine, the flower of *D. metel*, known as biamantuoluo, has been documented as having potential benefits for psoriasis and skin inflammation [15]. The utilization of seeds from the *D. metel* plant as a constituent in tea preparations has been observed in Brazil. This particular application is known to induce a sedative effect. Additionally, the dried flowers of the *D. metel* plant have been reported to be employed for smoking [4]. According to a recent study conducted by Ishola and co-workers, it has been observed that young individuals in Nigeria also perceive *D. metel* as a substance with hallucinogenic properties [13].

Besides its multiple medicinal uses, it is important to note that *D. metel* has also been acknowledged as a plant with toxic properties, capable of exerting detrimental effects on vital organs such as the liver and kidney [16]. According to research findings, it has been determined that all components of this particular herb possess toxic properties. The presence of poisonous tropane alkaloids and

anticholinergic agents, such as hyoscyamine, scopolamine, and atropine, is responsible for its toxicity [17]. Convulsions, mouth dryness, fever, tachycardia, hot-flushed dry skin, headache, hallucinations, acute confusion, coma, delirium, dilated pupils, urine retention, a weak and rapid pulse, and potentially fatal outcomes have been identified as prevalent manifestations associated with Datura poisoning [18]. Tropane alkaloids, which possess toxic properties, are widely distributed within various parts of the plant, with a notable concentration in the leaves and seeds. An anticholinergic syndrome is caused by tropane alkaloids inhibiting central and peripheral muscarinic neurotransmission [14, 19]. Significantly, *D. metel* demonstrated safety at a dosage of 2000 mg/kg body weight, as no observable signs of toxicity or mortality were observed. Histological examinations revealed that the organs had lost mass and that the liver had necrotic changes, followed by elevated ALP, GPT, and SGOT [20, 21]. The current investigation deals with the isolation and anticancer potential of a bioactive triterpenoid isolated from *Datura metel* L.

2.0 Material and Methods

2.1 Plant Collection

The seed of D. *metel* was obtained from Village Razagram tehsil Khall Dir Lower, KP, Pakistan. The fresh specimen of the plant was brought to the University of Swabi and was identified by Dr. Muhammad Ilyas, Assistant Professor. The voucher specimen number BOT(UOS-521) was deposited at the Botany Department of the University of Swabi, KP, Pakistan.

2.2 Extraction and Isolation

The seeds (5.21 kg) were washed with water and then dried in the shade for 18 days. The dried plant material was subjected to cold extraction with methanol, which yielded 102.22 g of crude extract. The extract was subjected to fractionation by using a flask that afforded hexane (8.21g), chloroform (28.34), and methanol extract (20.22g). All these obtained fractions were subjected to thin-layer chromatography (TLC), among which chloroform fraction comprised the maximum number of compounds selected for isolation. The chloroform extract was subjected to column chromatography, and the evaluation of the column was done with hexane and ethyl acetate (0:100) by increasing polarity. Sub-fraction AF1-AF-10 was obtained, among which sub-fraction AF-7 was subjected to repeated column chromatography, which yielded compound 1. Our research group previously elucidated the chemical structure of compound **1** (**Fig. 1**) based on advanced spectroscopic analysis [22].

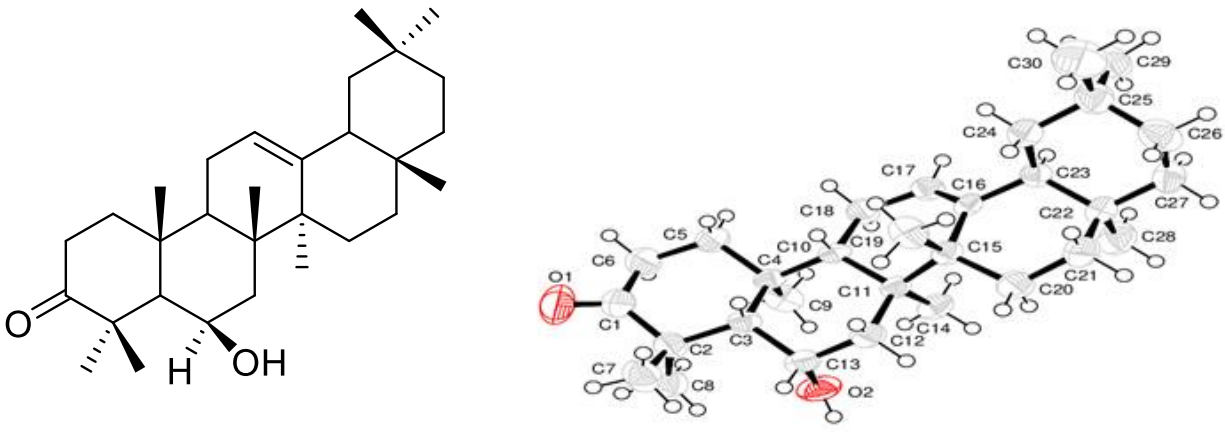

Figure 1: Chemical structure and x-rays crystallographic image of compound **1** isolated from *Datura metel* Linnaeus

2.3 Anticancer Activity

The cytotoxicity of the isolated compounds was assessed through the utilization of the MTT procedure. The RPMI 1640 medium, which was obtained from Gibco BRL, was supplemented with streptomycin sulfate at a concentration of 100 µg/ml, penicillin sodium salt at a concentration of 100 µg/ml, Na_2CO_3 at a concentration of 2 mg/ml, and fetal bovine serum (FBS) at a concentration of

10%. The FBS used in this study was obtained from Gibco, affiliated with the Institute of Bioinformatics at the National Chiao-Tung University in Hsinchu, Taiwan. The medium that had been prepared was used for preserving three distinct human cancer cell lines. These cell lines included human A498 cells obtained from renal tissue, human hepatoma cells (HepG2), non-small cell lung cancer cells (NCI-H226), and MDR human ovarian cancer 2780AD cells. The HepG-R and HepG2 cell lines, consisting of 2 × 104 and 9 × 103 cells, respectively, were utilized in the study. The hepatocytes of mice were observed to remain adhered to the surface of 96-well plates. The cells were preserved using various compounds at concentrations ranging from 1.5 to 100 μM or a control group treated with a vehicle solution containing 0.2% DMSO. Following preservation, the cells were incubated for 48 hours. The examination used an MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) tracer obtained from Sigma in St. Louis, MO, USA. An identical experimental procedure was conducted on the remaining cell lines. The IC50 values of the test compound were determined by analyzing the concentration-effect curves across various cell lines. The positive control in the current research was Paclitaxel (Sigma) [23, 24].

The in vitro cytotoxicity effect was assessed by employing LCMK-2 monkey kidney epithelial cells and mice hepatocytes. The compounds underwent a 24-hour incubation period, during which the viability of the cells was evaluated using MTT procedures. The cells were stored in RPMI 1640 medium, a commonly used cell culture medium, supplemented with 10% fetal bovine serum (FBS) from Gibco BRL, a well-known brand in the field. To prevent bacterial contamination, the medium also contained 110 μg/ml of penicillin sodium salt, a commonly used antibiotic. Additionally, a 2 mg/ml solution of sodium bicarbonate was added to maintain the pH balance of the medium. Lastly, another antibiotic, 100 μg/ml of streptomycin sulfate, was included to prevent further bacterial growth and preserve the cell culture's integrity. The initial inoculation involved the placement of 7.1 × 103 LCMK-2 cells and 8.6 × 103 mice hepatocytes into individual wells of a 96-well plate. The cells were subjected to preservation alongside a test sample at various concentrations and a vehicle solution containing 0.2% DMSO. Following this, the cells were incubated for 48 hours, after which the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, provided by Sigma, was performed [25].

3.0 Results

The anticancer potential of the hexane, chloroform, methanol extract, and compound 1 was evaluated using various cancer cell lines, namely HepG2, A498, NCI-H226, and MDR 2780AD. According to the data presented in **Table 1**, it can be observed that both the extract and compound 1 exhibited differing levels of efficacy in terms of their anticancer properties. Compound 1 shows a notable level of anticancer activity compared to the crude extract. The effectiveness of the standard drug, Paclitaxel, was observed to be universally effective against all tested cancer cell lines.

Table 1: Anticancer activity of extracts and isolated compound from *D. metel*

Compounds	**IC_{50}**			
	HepG2	A498	NCI-H226	MDR 2780AD
Hexane	>100	>100	>100	>100
Chloroform	55.09±0.48	155.65±0.44	98.34±0.19	0.49±0.32
Methanol	98.45±0.59	197.34±0.34	122.43±0.22	0.88±0.29
Compound 1	16.22±0.33	112.32±0.43	75.76±0.32	0.47±0.23
Paclitaxel	7.23±0.30	97.00±0.25	60.54±0.12	0.19±0.09

4.0 Discussion

The utilization of natural products has become increasingly prominent in the continuous exploration of groundbreaking pharmaceutical agents that aim to combat a range of diseases, with a particular emphasis on cancer [26-28]. These natural products offer a valuable source of novel chemical compounds, which can be the foundation for developing therapeutic drugs targeting diverse pharmacological targets [29, 30]. Several plant-derived compounds, such as vinblastine, vincristine, podophyllotoxin

derivatives (including etoposide), and camptothecin and its derivatives, have been commercially introduced as significant anticancer medications [31]. According to Islam *et al.*, *D. metel* has been identified as possessing various pharmacological properties, including antitussive, bronchodilator, antiasthmatic, anaesthetic, hallucinogenic, and anticancerous effects [19]. The antitumor properties of the hexane, chloroform, methanol extract, and isolated compound 1 were assessed in this study. The evaluation was conducted using the MTT bioassay, and various cell lines, including HepG2, A498, NCI-H226, and MDR 2780AD, were utilized for this purpose. The findings of our study indicate that both the extract and compound 1 exhibited varying levels of efficacy in terms of their anticancer properties. Compound 1 shows noteworthy anticancer properties compared to the crude extract, while the standard drug, Paclitaxel, demonstrates activity against all tested cancer cell lines. Various researchers conducted several investigations have yielded similar findings, suggesting that numerous extracts of *D. metel* may hold promise as potential sources of anticancer agents [29, 32, 33].

5.0 Conclusion

Throughout history, numerous civilizations have used traditional herbal remedies to address various health conditions. These esoteric practices frequently employed botanical species possessing intrinsic therapeutic attributes. Botanical sources provide the majority of novel therapeutic compounds that have the potential to alleviate human afflictions and improve well-being. *D metel* is a botanical species with numerous phytochemical compounds that confer various pharmacological effects. Despite its intrinsic toxicity, it is important to emphasize that the plant demonstrates substantial promise as a feasible source of therapeutic compounds derived from plants. After conducting this research, it has been conclusively established that *D. metel* possesses favorable attributes that have the potential to contribute significantly to the field of cancer medicine. Furthermore, the implementation of *D. metel* can improve the standard of living for a substantial demographic consisting of millions of people. Therefore, future research must prioritize thoroughly analyzing the therapeutic properties intrinsic to the plant being evaluated, specifically its potential as an anticancer agent. However, it is critical to simultaneously underscore the significance of implementing stringent safety protocols to protect against any possible negative consequences.

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