

The Synthesis, Isolated and Characterization Conjugated Linoleic Acid (CLA) from *Datura Innoxia* Mill Seed

Dr. Veerendra S. Yadav, Dr. Sanjay Gaikwad, Dr. Devidas Patil, Mr. Dinesh Gaikwad,

Department of Chemistry: BJS ASC College Wagholi Pune

Department of Botany: BJS ASC College Wagholi Pune

ABSTRACT

The identification of conjugated linoleic acid (CLA) in *Datura* species represents a novel and promising avenue for biomedical exploration. CLA comprises a group of positional and geometric isomers of linoleic acid, renowned for their wide-ranging health benefits, including anti-obesity, anti-diabetogenic, and anti-carcinogenic properties. While CLA is conventionally obtained from ruminant-derived dairy and meat products, the discovery of its potential occurrence in *Datura* offers a compelling plant-based alternative source. Preclinical studies have established CLA's ability to inhibit tumorigenesis via multiple mechanisms, including the induction of apoptosis, suppression of cell proliferation, modulation of immune responses, and inhibition of angiogenesis. However, clinical trials in human subjects have yielded inconsistent outcomes, highlighting the need for continued investigation into its efficacy and therapeutic scope. Building upon these insights, the present study aims to extract, purify, and characterize CLA from *Datura* seeds, followed by evaluation of its anticancer potential. This will involve *in vitro* cytotoxicity assays against human cancer cell lines and mechanistic studies to elucidate its molecular mode of action. The overarching goal is to develop a CLA-based natural formulation with potential application as an anticancer therapeutic agent

KEY WORD: *Datura Innoxia* Mill, CLA, Reflex reaction, Magnetic steroid,

INTRODUCTION

Datura is a wild weed belonging to family Solanaceae, its name derived from Sanskrit word "Dhutra" (divine inebriation) is used for its healing properties. Various species of *Datura* are known and widely employed for their medicinal and toxic properties that are based upon more than 30 alkaloids. Because of their funnel form, fragrant nocturnal blooms, species such as *Datura innoxia*, *Datura metel*, *Datura stramonium* and *Datura wrightii* are cultivated as ornamental plants with all but *D. metel* known from wild populations (DeWolf, 1956). Navajos chewed dried roots to reduce fever. Zunis made poultice from it to treat inflammation and bruises (Del Pozo, 1966). In China, it is known as "Yangjinhua" and used for the treatment of asthma, convulsions, pain, and rheumatism (Pan et al., 2007). Flos *Daturae* has an effect on the treatment of psoriasis for clinical use in China (Guarrera, 1999). It is used in Italy to remove lice from hen bundles. *D. innoxia* Mill seeds are used for acne and bronchitis in Sakarya province of North-west Turkey and locally called "Tatala", while the petroleum ether extract is also found to possess antimicrobial activities against *Escherichia coli* and *Trachystemon orientalis* by Uzun et al. (2004). It is also used commonly in ethno veterinary practices in Nepal (Raut and Shreshtha, 2012) and by Gujjar community in India (Gaur et al., 2010). In Ayurveda, *Datura* plant parts are used to treat various disorders including asthma, skin disorders, jaundice, piles, and diabetes (Dash and Kashyap, 1991). *Datura* seeds have been used as a prophylactic measure to treat animal bites, especially dog bites. Person bitten by mad dog is administered with juice of *Datura* along with butter milk and jaggery. Also, the site of bite is smeared with a paste of the fruit (Papadoyannis, 1995). Extensive research has been carried out since 1925 for the isolation and characterization of the total alkaloid contents in *Datura* species (Berkov et al., 2006; Dovelana et al., 2006). Vitale et al. (1995) has shown that the total alkaloid content in *Datura* varies from 0.02 to 0.52% and scopolamine from 0.0029 to 0.32% relative to the dried material, depending on the geographical area, the part of the plant studied and the stage of growth [1].

Natural product chemistry is one of the most remarkable fields of study on health sciences; it is usually regarded by the layman as one of the most abstruse and remote from everyday life and thought. The term natural product is commonly reserved for those organic compounds of natural origin that are unique to one organism, or common to a small number of closely related organisms. The term secondary metabolite is typically used to refer to an organic compound of limited

distribution in nature (Williams & Lemke, 2002). The study of natural products has had a number of rewards. It has led to the discovery of a variety of useful drugs for the treatment of diverse ailments and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basics of analytical organic chemistry (Taura et al., 2014). Herbal remedies have been used for centuries but more recently the compounds that are active have been identified and this has enabled them to be extracted and purified.



Figure 1 – *Datura innoxia* Mill

Synthetic organic chemists have then been able to produce the molecules *in vitro* and so produce them on larger scales.[2]

Datura mainly contains the tropane alkaloids atropine, scopolamine, and hyoscyamine. Uses of *datura* have long histories of hallucinogenic use and have been connected with sorcery, witchcraft, native medicine, and magico-religious rites dating back to 1500 BC and Homer's *Odyssey*. (Homer's use of the plant *moly* as an antidote to Circe's poisonous anticholinergic drugs may have been the first recorded use of an anticholinesterase to reverse central anticholinergic intoxication). Chinese herbal medicines containing tropane alkaloids have been used to treat asthma, chronic bronchitis, pain, and flu symptoms. In Mexico, *Datura* is taken by Yaqui women to lessen pain of childbirth. In Africa, a common use is to smoke leaves from *Datura* to relieve asthma and pulmonary problems. Many cultures worldwide add plants with tropane alkaloids (particularly *Datura* species) to alcoholic beverages to increase intoxication. Recently, *Datura* has been used as a recreational hallucinogen in the US, resulting in sporadic cases of anticholinergic poisoning and death[3].

Datura innoxia Mill., a lesser-studied species within the *Datura* genus, has received limited scientific attention regarding its phytochemical profile and therapeutic potential. In the present study, we aimed to identify and analyze the bioactive chemical constituents present in the fruit of *Datura innoxia*. Preliminary investigations have revealed the presence of several biologically active compounds with potential pharmacological relevance.

The primary objective of this research is to explore the chemical composition of *Datura innoxia* Mill with a focus on isolating compounds that may serve as candidates for anticancer drug development. By identifying and characterizing these compounds, we aim to lay the groundwork for the formulation of a novel anticancer agent derived from *Datura innoxia* Mill.

METHODS AND MATERIAL

Collection of Material: We collected a sample of *Datura* from the ground area of Bakori Phata in Wagholi. We prepared a herbarium specimen of this *Datura* plant and obtained an identification & Authentication certificate from BSI Pune, which identified its species as *Datura innoxia* Mill.

Botanical Description of *Datura innoxia* Mill: *Datura innoxia* Mill., commonly known as pricklyburr, downy thorn-apple, or sacred *datura*, is an herbaceous annual or short-lived perennial belonging to the Solanaceae (nightshade) family. Habit: Typically grows as an erect or spreading, bushy herb, reaching heights of 0.3 to 1.5 meters and can spread up to 2 meters in diameter. Stem: The stems are smooth, often purplish, and covered with dense, short, grayish hairs (pubescent), sometimes glandular. Leaves: The leaves are simple, alternate, and supported by long stalks (petioles). The leaf blade is typically ovate to elliptic, 5-18 cm long and 4-15 cm wide, with an asymmetric, broadly wedge-shaped to truncate base and an acute apex. The margins are usually entire or irregularly toothed and both surfaces are covered in

fine, grayish hairs. Crushed leaves emit an unpleasant odor. Flowers: The flowers are solitary, large (12-19 cm long), and trumpet-shaped (tubular) with a white or creamy corolla, sometimes tinged with purple. They are erect initially and may become nodding later. The calyx is long and tube-like (8-10 cm). They have a pleasant fragrance, especially at night. Fruit: The fruit is a nodding, globose to ovoid capsule, about 3-5 cm in diameter, densely covered with slender, soft spines. It is initially purple-green to brownish when ripe and splits open irregularly at the apex to release numerous black seeds (4-5 mm long). Roots: May develop a tuberous root system.

Materials and chemicals: Round bottom flask, rota evaporator, digital measuring balance, Heating Mantle, Beaker, conical flask, Condensers, Funnel, Glass Road, Whatman filter paper, Thin layer chromatography (TLC) plate (precoated aluminum sheet, 20 x 20 cm), UV-Spectrometer, FTIR, H-NMR, C-NMR, GC-MS, Water, Acetone, Petroleum ether, n-Hexane, Ethyl acetate, Ethanol (95%), DMSO. All characterization test are obtained from department of chemistry SPPU pune.

Methods:

Seed Preparation and Extraction: Datura seeds were separated from mature fruits and thoroughly dried under ambient conditions. The dried seeds (100 g) were finely ground using a mortar and pestle. The resulting powder was transferred to a 1 L round-bottom flask containing 500 mL of distilled water. The mixture was subjected to reflux for 3.5 hours. After cooling, the setup was allowed to stand overnight. On the following day, the mixture was again refluxed for an additional 3.5 hours. Upon completion, an oily layer formed on the surface of the aqueous phase. This oil layer was carefully separated and collected for further use.

Reaction with Hydrobromic Acid: A 20 mL portion of the extracted oil was mixed with 100 mL of aqueous hydrobromic acid (HBr) in a 200 mL beaker. The mixture was stirred continuously using a magnetic stirrer for 5 hours at room temperature. Following the reaction, a white, sticky paste formed, which was isolated as the product.



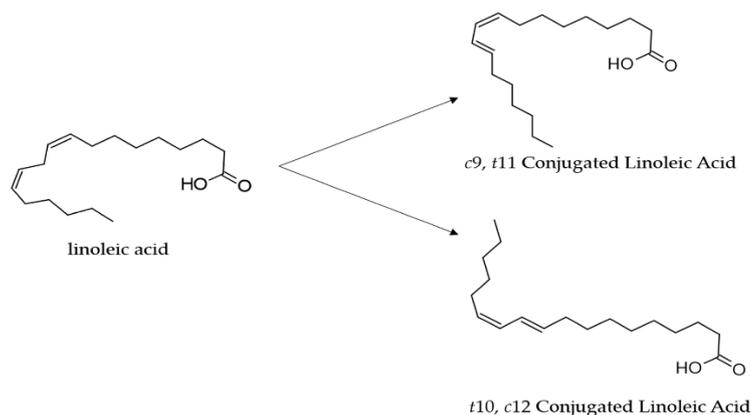
Figure 2 – Reflex Reaction



Figure 3 – Oily Layer



Figure 3 – Product



RESULT AND DISCUSSION:

1. TLC

The Purity of the product is checked with the help of TLC by using coated aluminium foil. The product is loaded by preparation of solution in DMSO. The loaded TLC allowed to run in solvent n-hexane (7) : ethyl acetate (3). Rf values is then calculated.



Figure 4: TLC Plate product

$$\begin{aligned}
 R_f &= \frac{\text{Distance travelled Product}}{\text{Distance travelled by Solvent}} \\
 &= \frac{2}{7} \\
 &= 0.740
 \end{aligned}$$

2. IR SPECTRA

The Solid Infrared spectral study were done by BRUKER FTIR Instruments from Department of Chemistry, SPPU Pune in the range 500 cm⁻¹ to 3500 cm⁻¹ range.

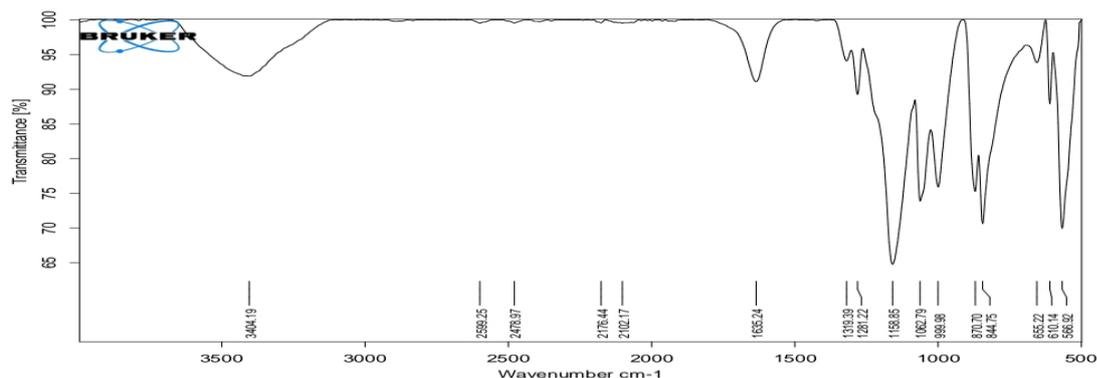


Figure 5 : IR Spectra product

3404.19cm ⁻¹	O-H	Alcohol group OH
1635.24cm ⁻¹	C=O	Carbonyl group
1319.39cm ⁻¹	C-O	C-O-C
1281.22cm ⁻¹		C=O-O-R
1062.79cm ⁻¹	C-O	Alcohol group OH
999.98cm ⁻¹		Ether group C-O-C
870.70cm ⁻¹	C-H	Aromatic ring
844.75cm ⁻¹		Alken group

Table 1 : IR Spectral values of Product

3. H¹-NMR SPECTRA

The H¹-NMR SPECTRA study were done by BRUKER Instruments from Department of Chemistry, SPPU Pune in the range 0 to 15 ppm range.

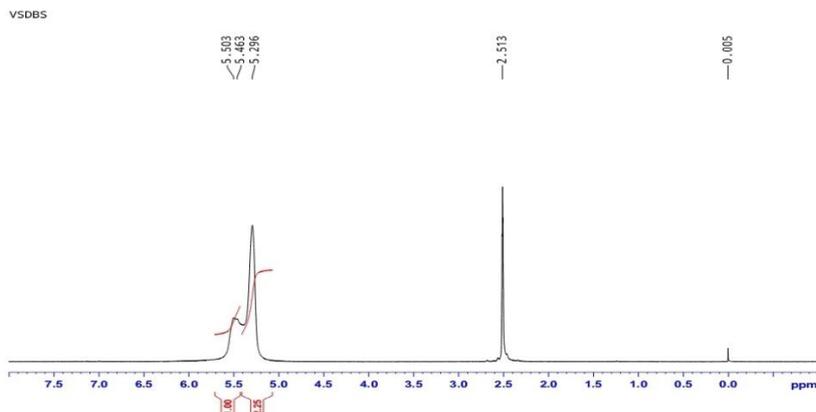


Figure 6 : H1NMR Spectra product

5.503ppm	C=C	I I R-C=C-H
5.463ppm	Alkene protons	
5.296ppm	Alkene protons	
2.513ppm	C=O	O II I RO-C-C-H I
0.005ppm	This peak is likely from a solvent residual peak.	

Table 2 : H-NMR Spectral values of Product.

4. GC-MS SPECTRA

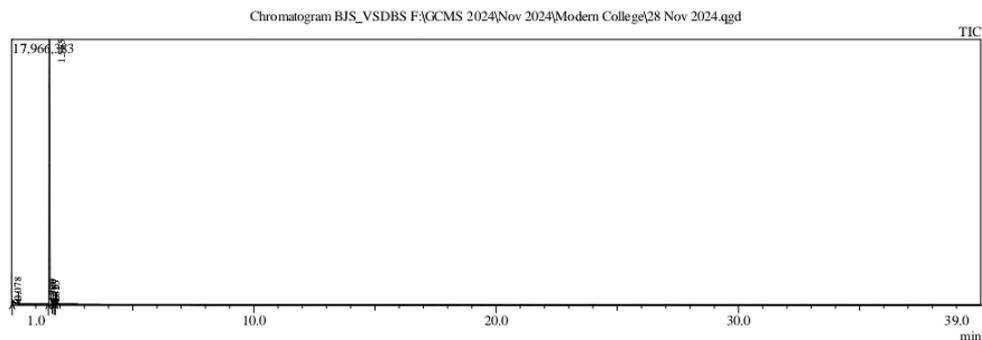
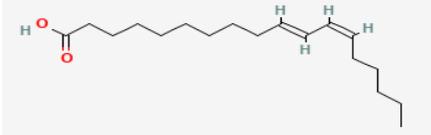


Figure 7 : GC-MS Spectra product

Determining the molecular and structural formula of a compound using Infrared (IR) spectroscopy, Proton Nuclear Magnetic Resonance (¹H NMR) spectroscopy, Carbon-13 Nuclear Magnetic Resonance (¹³C NMR) spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS) involves a systematic approach

Name	IUPAC Name	Molecular formula	Structural formula
CLA (Conjugated Linoleic acid)	(10E,12Z)-octadeca-10,12-dienoic acid	C ₁₈ H ₃₀ O ₂	

The identification of conjugated linoleic acid (CLA) in *Datura* species presents a promising avenue for biomedical research. CLA is a group of positional and geometric isomers of linoleic acid, known for its diverse health-promoting properties, including anti-obesity, anti-diabetogenic, and anti-carcinogenic effects. Although CLA is traditionally sourced from ruminant-derived dairy and meat products, the potential presence of CLA in *Datura* suggests an alternative plant-based source worth investigating.

Preclinical studies have demonstrated CLA's capacity to suppress tumorigenesis through mechanisms such as induction of apoptosis, inhibition of cell proliferation, modulation of immune responses, and interference with angiogenesis. Despite encouraging results in animal and in vitro models, clinical trials in humans have shown variable outcomes, indicating that further research is necessary to clarify CLA's efficacy and therapeutic window in cancer prevention and treatment.

Building on these findings, our forthcoming research will focus on the extraction, purification, and characterization of CLA from *Datura* seeds, followed by evaluation of its anticancer activity. This will include in vitro cytotoxicity assays against human cancer cell lines and mechanistic studies to elucidate its mode of action. The ultimate objective is to develop a CLA-based formulation with potential application as a natural anticancer therapeutic

REFERENCE

1. Neeraj O. Maheshwari, Ayesha Khan* and Balu A. Chopade, Rediscovering the medicinal properties of *Datura* sp.: A review Vol. 7(39), pp. 2885-2897, 17 October, 2013 DOI: 10.5897/JMPR11.1657 ISSN 1996-0875 ©2013 Academic Journals <http://www.academicjournals.org/JMPR>.
2. Muhammed Assen Ali and Sisay Awoke Endalew*, Chemical Constituents of *Datura stramonium* L. Leaves and Its Antibacterial Activity against Human Pathogenic Bacteria, *Abyss. J. Sci. Technol.* Vol. 6, No. 2, 2021, 15-21 ISSN 2616 – 4728 (Online); 2616 – 471X (Print) © 2021 Wollo University.
3. Vishal V Koulapur, Prasanna S. Jirli, Ravindra S. Honnugar, S. S. Pujar, Santosh Patil, POISONING DUE TO *DATURA* – A RARE CASE REPORT, *Indian Journal of Forensic and Community Medicine*, January – March 2015;2(1):64-66.
4. Singh OM and Singh TP. Phytochemistry and pharmacological profile of *Zanthoxylum armatum*: *Indian Journal of Natural Products & Resource.* 2; 2011: 275-285.
5. Singh OM, Singh TP and Singh HB. *Adhatoda vasica* Nees: A review on its phytochemical and pharmacological profile. *The Natural Products Journal.*1; 2011: 29-39.
6. Rasekh H R, Khoshnood-Mansourkhani M J and Kamalinejad M, Hypolipidemic effects of *Teucrium polium* in rats, *Fitoterapia.* 72; 2001: 937-939.
7. Friedman M and Levin CE, Composition of Jimson Weed (*Datura stramonium*) seeds, *Journal of Agricultural and Food Chemistry.* 37; 1989: 998-1005.
8. Witte, Muller K and Arfmann HA, Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid chromatography-mass-spectrometry, *Planta* 1987:192-197.
9. Sharma M C and Sharma S, Phytochemical, Preliminary
10. Pharmacognostical and Antimicrobial Evaluation of Combined Crude Aqueous Extract. *International Journal of Microbiological Research.* 1(3); 2010: 166-170.
11. Habib A, Mohammad M, Fahimeh R and Mohammad HH. Bioactivities of jimsonweed extract, *Datura stramonium* L (Solanaceae), against *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Turkish Journal of Agriculture and Forestry.* 35; 2011: 623-629.
12. Dugan GM, Gumbmann MR and Friedman M, Toxicological evaluation of jimson weed (*Datura stramonium*) seed, *Food and Chemical Toxicology.* 27(8); 1989, 501-510.
13. Usha K, Singh B, Praseetha P, Deepa N, Agarwal DK, Agarwal R, Nagaraja A. Antifungal activity of *Datura stramonium*, *Calotropis gigantea* and *Azadirachta indica* against *Fusarium mangiferae* and malformation in mango, *European Journal of Plant Pathology.* 124; 2009: 637-657.
14. erkov S, Pavlov A, Kovacheva P, Stanimirova P and Philipov S. Alkaloid spectrum in diploid and tetraploid hairy root cultures of *Datura stramonium*, *Z Naturforsch C.* 58; 2003, 42- 46.
15. Doncheva T, Philipov S and Kostova N, Alkaloids from *Datura stramonium* L *Comptes Rendus de 'l' Academic Bulgare de Sciences.* 57; 2004, 41-44.
16. Taye B, Giday M, Animut A and Seid J, Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pacific Journal of Tropical Biomedicine.* 2011: 370-375.
17. Puyvelde V, Geysen LI, Ayobangira FX, Hakizamungu E and Nshimiyimana A, Kalisha A. Screening of medical plants of Rwanda for acaricidal activity. *Ethnopharmacology.* 13; 1985: 209-215.
18. Robins RG, Parr AG, Payne J, Walton NJ and Rhodes MG, Factors regulating tropane-alkaloids production in transformed root culture of a *Datura candida* x *D. aurea* hybrid. *Planta.*181; 1990: 414-422.
19. Binev R, Valchev I and Nikolov J, Clinical and Pathological studies of Jimson weed (*Datura stramonium*) poisoning in horses, *Trakia Journal of Sciences.* 4(3); 2006: 56 63.
20. You LX and Wang SJ, Chemical Composition and Allelopathic Potential of the Essential Oil from *Datura stramonium* L, *Advanced Materials Research.* 233-235; 2011: 2472-2475.