

Isolation, structural characterization and *in silico* drug-like properties prediction of a natural compound from the ethanolic extract of *Cayratia trifolia* (L.)

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ABSTRACT

Background: Natural products have continually played an important role in drug discovery because it serves as active principles in drugs as well as templates for synthesis of new drugs. *Cayratia trifolia* (L.) is a medicinal plant, which has been reported to have antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities. **Objective:** Therefore, the objective of this study is to isolate and identify the natural compound from the ethanolic extract of *Cayratia trifolia* (L.) and to predict the Absorption, Distribution, Metabolism and Excretion (ADME) properties of isolated natural compound. **Materials and Methods:** Column chromatography and thin layer chromatography were used to isolate the natural compound and Fourier-transform infrared (FTIR) spectroscopy was used to predict the functional groups present in the isolated natural compound. The structural characterization studies were functionally carried out using ¹H, ¹³C, two-dimensional nuclear magnetic resonance (NMR) and mass spectrometry methods. **Results:** FTIR showed that, the groups of OH, C-H, C = C may be present in the isolated natural compound. ¹H, ¹³C, two-dimensional NMR and mass spectrometry data suggests that the isolated natural compound probably like linoleic acid. *In silico* ADME properties, prediction of the compound was under acceptable range. **Conclusion:** Based on the results, it can be concluded that, the isolated natural compound of linoleic acid that has been exhibited good medicinal properties.

Key words: *Cayratia trifolia* (L.), Chromatography techniques, Spectroscopy methods, Linoleic acid and ADME properties

INTRODUCTION

Medicinal plants provide an inexhaustible resource of raw materials for the pharmaceutical, cosmetics and food industries and more recently in agriculture for pest control. People have learned to increase the power of medicinal plants by preparing medicinal compounds from them.^[1,2] In traditional societies, nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes.^[3] Natural products from medicinal plants provide ultimate opportunities for new drug leads because of the unmatched availability of chemical diversity.^[4-6] Due to the demand of chemical

diversity in screening programs, seeking therapeutic drugs from natural products has grown throughout the world. In addition, a good proportion of drugs that have been approved for clinical trials, are either Natural products or their analogues.^[7,8] The active compounds do not play an important role in the metabolism of plants and hence it is often referred to as secondary metabolites.^[9] Finding new secondary metabolites is a prerequisite for the development of novel pharmaceuticals. This thematic series on the biosynthesis and function of secondary metabolites deals with the discovery of new biologically active compounds from all kinds of sources such as medicinal plants.^[10]

Cayratia trifolia (L.) is the medicinal plant of the family Vitaceae. It is commonly known as Fox grape in English, it's a native to India, Asia and Australia. Whole plant of *Cayratia trifolia* (L.) has been reported to contain yellow waxy oil, steroids, terpenoids, flavonoids and tannins by preliminary phytochemical screening.^[11,12] Leaves contain

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stilbenes, piceid, reveratrol, viniferin and ampelopsin.^[13] Stem, leaves and roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves.^[14] Root paste mixed with coconut oil can be used as a decoction. Roots grounded with black pepper can be used as a poultice on boils. Infusion of seeds along with an extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood.^[15] Paste of tubers is applied on the affected part in the treatment of snake bite. Whole plant is used as diuretic, in tumors, neuralgia and splenopathy.^[16] The bark extract has been reported to have antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities in animal models.^[17] Therefore, the main aim of the present study is to isolate and identify the natural compound from ethanolic extract of *Cayratia trifoliola* (L.) and to analyze their drug like properties.

MATERIALS AND METHODS

Collection of plant material

Cayratia trifoliola (L.) was collected from in and around the area of Kumbakonam, Thanjavur District, Tamil Nadu, India. The plant was authenticated by Dr. P. Sathyanarayanan, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2010-2011/Tech. 1527.^[18] Fresh whole plant material was washed under running tap water, air-dried and powdered.

Preparation of extract

Based on the previous studies, 300 g of plant powder was extracted with 1500 ml of ethanol for 72 h in occasional shaker at room temperature. The extract was collected and concentrated at 40°C under reduced pressure using a rotary evaporator. The dried extract was stored at 4°C until further compound isolation process.

Compound isolation

The ethanolic extract of *Cayratia trifoliola* (L.) 5 g was fractionated on silica gel column (3 × 30 cm) and successfully eluted with petroleum ether (100%) followed by petroleum ether: Chloroform (8:2, 6:4, 4:4, 2:8 v/v). The column fractions were collected in 20 ml of test tubes. Totally 72 fractions were collected and each fraction was analyzed by thin layer chromatography (TLC) plates for a single spot.

Functional group analysis and structure elucidation

The Shimadzu FTIR-8400S Fourier Transform Infrared Spectrometer instrument used for analyzes the presence of functional groups in the isolated compound. The spectrometer works under purged condition. Solid sample of an isolated compound was dispersed in polyethylene

pellets depending on the region of interest. This instrument has a typical resolution of 1.0/cm. Signal averaging, signal enhancement, baseline correction and other spectral manipulations were possible. Structure elucidations of the isolated compound were carried out using spectroscopic techniques: Mass spectrometry, ¹H and ¹³C NMR together with two-dimensional experiments (correlation spectroscopy [COSY] and heteronuclear single quantum coherence [HSQC]). Optical rotation was determined with a Perkin-Elmer polarimeter (model 341). NMR spectra on solutions in CDCl₃ were recorded on a Bruker DRX-500 NMR Spectrometer at 500 MHz (¹H) and 125 MHz (¹³C); the signals of the deuterated solvent were taken as a reference. The molecular mass of the isolated compound was analyzed by mass spectrometry.

Absorption, distribution, metabolism and excretion properties prediction (ADME)

ADME properties prediction were carried out using QikProp 2.3 module (Schrodinger Suite 2012).^[19] QikProp helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Significant ADME properties such as molecular weight (MW), H-bond donor, H-bond acceptor and log P (O/W) were predicted.

RESULTS

The ethanolic extract of *Cayratia trifoliola* (L.) was subjected to column chromatography by using different solvents such as petroleum ether and chloroform in the increasing order of polarity and the fractions were collected. Totally, 72 fractions were collected which is shown in Table 1. Out of these, 10th fraction identified as a single spot [Figure 1] with the R_f value (0.72 cm) using TLC analysis; it may indicate the presence of a single compound in this fraction. About 20 mg of the pure compound was obtained from the single fraction and it was used for further studies.

FTIR analysis of identified functional groups were broad band at 3412/cm for the hydroxyl group, 2928/cm for

Table 1: Collected fractions details by column chromatography

| Solvents | Fractions collected | Number of fractions |
|-----------------------------------|---------------------|---------------------|
| Petroleum ether (100%) | 1-5 | 5 |
| Petroleum ether: chloroform (8:2) | 6-9 | 4 |
| Petroleum ether: chloroform (6:4) | 10-18 | 9 |
| Petroleum ether: chloroform (4:6) | 19-42 | 24 |
| Petroleum ether: chloroform (2:8) | 43-64 | 22 |
| Chloroform (100%) | 65-72 | 8 |

C–H group, 1728/cm and 1623/cm to show the presence of the carbonyl group and C = C presence in the single fraction were shown in Table 2 and Figure 2.

The absorptions of the NMR spectrometry have made a tremendous impact in many areas of chemistry, biology and medicine. In the ¹H NMR [Figure 3] spectrum the triplet at δ 0.81 is due to a terminal methyl group, the strong singlet at δ 1.28 is due to long chain methylene groups. The strong signals at δ 1.59, 1.79 and 1.98 are due to methylene protons attached to unsaturated systems and the signal at δ 2.30 are due to two bis allylic protons. The signals at δ 4.56 (1H), 5.12 (2H) and at 5.36 (1H) suggest that the compound contains two double bonds. In the ¹H–¹H COSY NMR [Figure 4] spectrums cross peaks are observed between double bonded protons and the allylic methylene protons. The protons of one double bond are not coupled with the protons of the

other double bond. The coupling between the methyl group at δ 0.81 and methylene protons at δ 1.59, 1.79 and 1.98 were observed. In the ¹³C NMR [Figure 5] spectrum the signal at δ 15.9 is due to the methyl group, a single at δ 39.4 is due to the α-carbon atom to the carbonyl group, the bunch of signals between 17.6 and 34.4 are due to long chain methylene carbons. The signals at 124.6, 129.3, 134.8 and 135.6 are due to four unsaturated carbon atoms. In the HSQC [Figure 6] spectrum the



Figure 1: Thin layer chromatography (TLC) analysis of the isolated natural compound

Table 2: Functional group analysis of 10th fraction using FTIR

| Functional groups | Type of vibration | Characteristic absorptions (1/cm) | |
|-------------------|-------------------|-----------------------------------|---------|
| O-H | Alcohol | Stretch | 3412.58 |
| C-H | Alkane | Stretch | 2928.87 |
| C=O | Carbonyl | Stretch | 1728.22 |
| C=C | Aromatic | Stretch | 1623.50 |
| C-N | Amine | Stretch | 1170.79 |

FTIR: Fourier-transform infrared

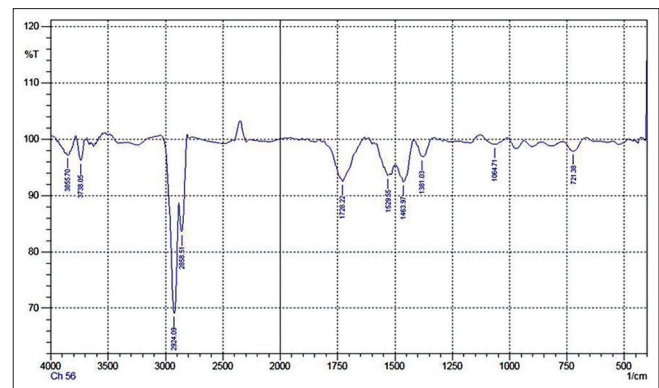


Figure 2: Fourier Transform infra-red spectroscopy analysis of the isolated natural compound

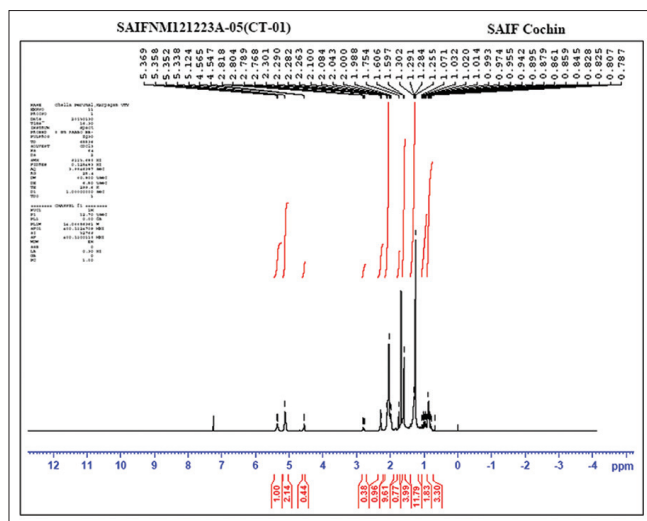


Figure 3: ¹H NMR spectrum analysis of the isolated natural compound

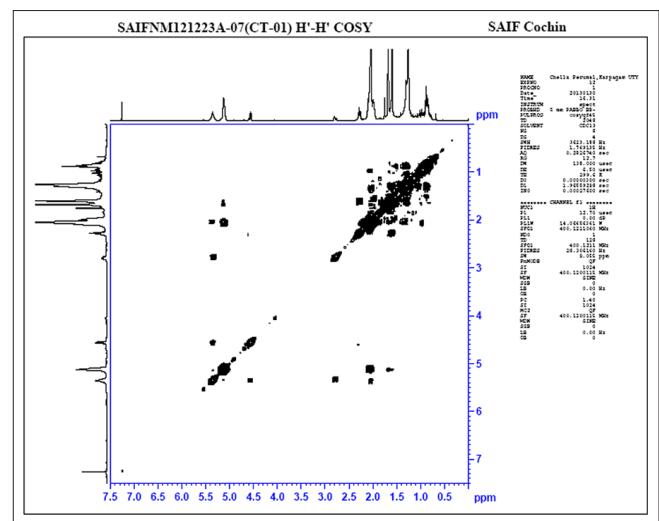


Figure 4: H–H COSY spectrum analysis of the isolated natural compound

unsaturated protons and the unsaturated carbons are correlated. Being a long chain fatty acid, mostly it will be a mixture with its homologues fatty acids. The weak signal at δ 173.0 is due to a quaternary carbon (carbonyl carbon). Mass spectrometry analysis revealed [Figure 7] that, molecular weight of the isolated compound was 280.44 (g/mol). All the data suggests that, the compound may be an unsaturated long chain fatty acid probably like linoleic acid [Figure 8 and Table 3]. ADME properties of the isolated natural compound of linoleic acid shown in Table 4 and it was under acceptable range.

DISCUSSION

Natural compounds are present in crude plant extract, but they might not be extracted using a single solvent. Different compounds according to their polarity elute out in different solvents.^[20] The vital role of these natural constituents of medicinal plants is alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate and phenolic

compounds.^[21] The identification of natural compounds using chromatography and spectroscopic techniques may provide efficient information regarding qualitative and quantitative composition of herbal medicines.^[22]

Table 3: Isolated natural compound of linoleic acid details

| | |
|-------------------|----------------------------------------------------------------------------------------------------------------|
| Molecular formula | C ₁₈ H ₃₂ O ₂ |
| Molecular weight | 280.44 (g/mol) |
| IUPAC name | Cis, cis-9,12-octadecadienoic acid |
| Density | 900.00 kg/m ³ |
| Smilies | CH ³ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH |

IUPAC=International Union of Pure and Applied Chemistry

Table 4: ADME properties of linoleic acid

| Ligand | Molecular weight (g/mol) | H-Bond donor | H-Bond acceptor | Log P (OW) |
|---------------|--------------------------|--------------|-----------------|------------|
| Linoleic acid | 280.44 | 1 | 2 | 5.846 |

ADME=Absorption, Distribution, Metabolism and Excretion

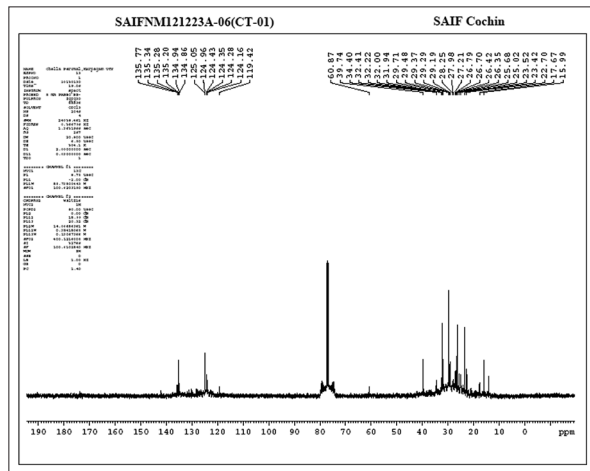


Figure 5: ¹³C NMR spectrum analysis of the isolated natural compound

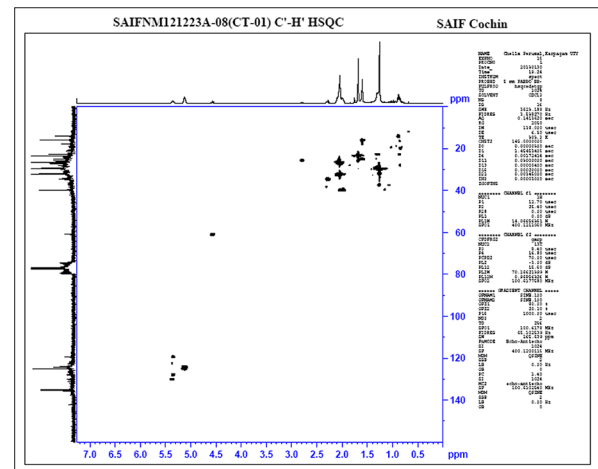


Figure 6: C-H HSQC spectrum analysis of the isolated natural compound

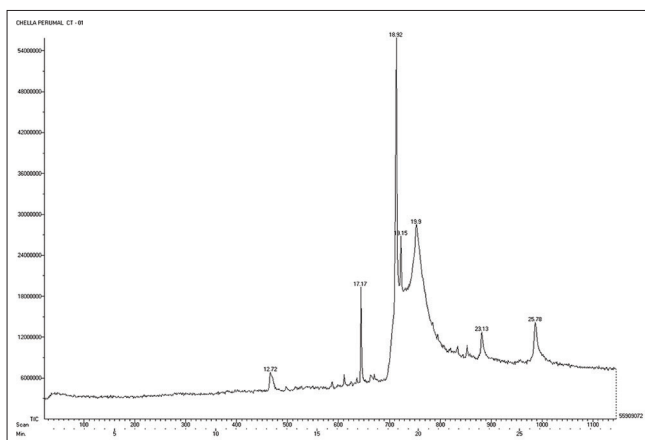


Figure 7: Mass spectrometry result of isolated natural compound

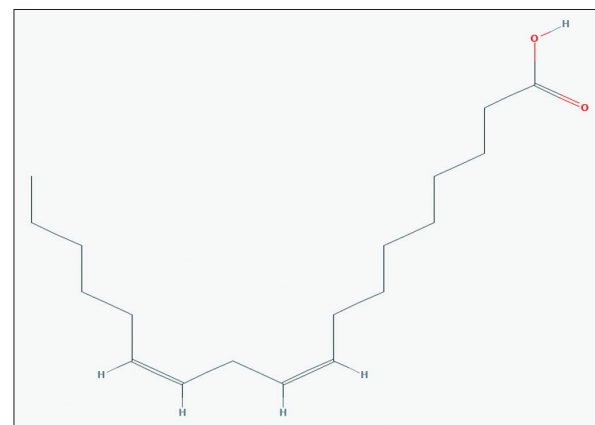


Figure 8: Structure of linoleic acid

In the present study, column chromatography and TLC eluted a natural compound of linoleic acid is an essential fatty acid that must be consumed for proper health. A diet only deficient in linoleate causes mild skin scaling, hair loss and poor wound healing in rats.^[23] Linoleic acid has become increasingly popular in the beauty products industry because of its beneficial properties on the skin. Research points to linoleic acid's anti-inflammatory, acne reductive and moisture retentive properties when applied topically on the skin.^[24-27]

CONCLUSION

In the present study, the natural compound of linoleic acid was isolated and identified from the ethanolic extract of *Cayratia trifolia* (L.). The ADME properties of the linoleic acid were under acceptable range. Therefore, it can be concluded that this natural compound possess many biological activity. In future, this compound may lead to drug design and development for curing various illness and disorder.

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