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PURIFICATION AND STRUCTURAL CHARACTERIZATION OF A BIOACTIVE MOLECULE, 13DOCOSENAMIDE,(Z) FROM THE LEAVES OF ERYTHRINA INDICA

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ABSTRACT

The aim of the study was to isolate a promising anticancer molecule from the leaves of *Erythrina indica*. The dried leave of this herb was ethanol extracted and fraction separated using HPLC. The fractions were studied for anticancer activity against Adenocarcinomic human alveolar basal epithelial cells (A549). The fraction showed bioactivity was extensively studied for its structural characterization which was identified as 13Docosenamide,(Z) based on the results of FT-IR, NMR, GC and MS/MS studies. Based on the above observation, 13Docosenamide,(Z) is an ideal product for the anticancer therapy which can be easily procured using the lab conditions explored in this investigation.

KEYWORDS: *Erythrina indica;* 13Docosenamide, (Z); Bioactive; Purification; Structure.

INTRODUCTION

The genus *Erythrina* comprises of about 110 species of trees and shrubs. The name “coral tree” is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas).[1] It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement

tree (it fixes nitrogen) for other tree crops such as coffee and cacao.[2] *Erythrina indica* belonging to the family Leguminosae also known as Tiger's claw or Moochy wood tree or Variegated coral tree, Sunshine tree, Coral bean, etc. *Erythrina indica* is a compact shrub with knobby stems.[3] It posses dense clusters of deep crimson flowers, that spread broadly open. *E. indica* is a medium-sized, spiny, deciduous tree normally growing to 6-9 m tall. It is called as Kalyana Murungai in Tamil Language.

Young stems and branches are thickly armed with stout conical spines up to 8 mm long, which fall off after 2-4 years rarely; a few spines persist and are retained with the corky bark. Bark is smooth and green when young, exfoliating in papery flakes, becoming thick, corky and deeply fissured with age. Leaves are trifoliate, alternate, bright emerald-green, petioles are long about 6-15 cm, rachis 5-30 cm long, prickly; leaflets smooth, shiny, broader than long, 8-20 by 5-15 cm, ovate to acuminate with an obtusely pointed end. Leaf petiole and rachis are spiny.[4] Flowers are bright red to scarlet, erect terminal racemes 15-20 cm long. Stamens are slightly protruding from the flower. Fruit is a cylindrical torulose pod, green, turning black and wrinkly as they ripen and thin-walled and constricted around the seeds. There are 1- 8 smooth, oblong, dark red to almost black seeds per pod. Erythrina comes from the Greek word 'eruthros' meaning red, shows red flowers of the *Erythrina* species.[5, 6]

In India, China, and Southeast Asia, the bark and leaves are used in many traditional medicines, including one said to destroy pathogenic parasites and relieve joint pain; the juice from the leaves is mixed with honey and ingested to treat tapeworm, roundworm, and threadworm in India; women take this juice to stimulate lactation and menstruation; it is commonly mixed with castor oil to treat dysentery; a warm poultice of the leaves is applied externally to relieve rheumatic joints; and the bark is used as a laxative, diuretic, and expectorant.[7,8] In the present article, we have isolated a bioactive molecule from the ethanol leave extract of *Erythrina indica* and the isolated bioactive compound was extensively characterized.

MATERIALS AND METHODS

Collection and preparation of leaves extract

The leaves of the plant, *E. indica* were collected wildly from the Thanjavur district, Tamil Nadu, India. Collected plant samples were washed by distilled water (DW) to remove undesirable waste materials and excess of water was drained off. The leaves were separated from each other and they were sliced into small pieces. The sliced leaves were shade dried for

few days. The shade dried leaves were powdered separately by grinding machine and the powdered sample were extracted three times repeatedly using 99.9% ethanol and the insoluble contents during the sample extraction were separated using centrifugation at 3000 rpm for 15 min. The resulting supernatant was rotary vacuum evaporated at 40°C and further dried under lyophilization. The dried sample content was stored under dark dried conditions and further sample preparation procedures were applied with respect to individual analysis carried out below. All the experimental values were represented from the mean of triplicate experimental procedures.

High Pressure Liquid chromatography (HPLC) purification of bioactive compound

The ethanol extracted crude dry powder was dissolved in 5 ml of methanol, filtered through a 0.2 µm syringe filter and separated by Reverse Phase (RP)-HPLC. RP-HPLC was performed using a Waters 600 HPLC system (Waters, USA) equipped with an Xterra Prep RP18 OBD column (Waters, USA; 5 µl, 18 × 100 mm) held at 40°C. The solvent system consisted of distilled water (solvent A) and acetonitrile (solvent B). The compounds were eluted at a flow rate of 4 ml/min with a linear gradient from the mixture A:B (100:0, vol/vol) to A:B (0:100, vol/vol) in 12 min. The absorbance of the eluate was measured at 210 nm. All the collected fractions were dried and stored at - 20°C for further studies. All the fractions were collected separately, rotary evaporated and tested for anticancer activity against Adenocarcinomic human alveolar basal epithelial cells (A549) as per the methods described by Masters.[9]

Identification of the purified bioactive molecule

The dried fraction showing anticancer activity against A549 was identified for the bioactive molecule present in it. The molecule was chemical studied and identified as per the following instrumentation procedures.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared absorption spectrum was recorded on an IR affinity-1 FTIR system (Shimadzu, Japan) at a spectral resolution of 4 $cm^{-1}$with an average of 10scans in the wavenumber range of 400–4000cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H & ^{13}C NMR spectrum of the purified bioactive fraction was recorded on a Bruker AV600NMR spectrometer (Germany) using deuterated $CDCl_3$ as the sol-vent. Chemical

shifts were expressed in parts per million (ppm) downfield from an internal standard of tetramethylsilane (TMS).

Gas Chromatography Mass Spectroscopy (GC-MS)

The fourth fraction having anticancer activity was analyzed for GC-MS on a Thermo Trace GC Ultra coupled with Polaris Q MS and TriPlus auto-sampler using a DB-5 (0.25 mm × 30 m × 0.22 µm) column in which helium was used as carrier gas. The temperature was set between 50°C to 250°C at a rate of 10°C min^{-1}. The initial temperature was held for 2 min and final temperature of 250°C was held for 10 min. The GC flow rate was 1 ml min^{-1} and the total run time was 32 min. MS was performed at scan mode between 0 – 600 m/z with an Ion trap EI+.

RESULTS AND DISCUSSION

Erythrina indica contain several phenolic metabolites, such as pterocarpans, isoflavones, flavanones and chalcones, some of which displayed antiplasmodial activity, antimycobacterial activity and cytotoxic activity against various cancer cell lines.[6,8,10] This present study explored the isolation, purification and identification of a bioactive compound which was primarily isolated based on its biological activity against the cancer cell line, Adenocarcinomic human alveolar basal epithelial cells (A549). Initially, the crude leaves preparation from *E. indica* was extracted with ethanol and fractionated using HPLC (Fig. 1). The fourth fraction eluted at the retention time of 4.743 min only showed anticancer activity with A549 cell line. This fraction was about 15.5 % of the total ethanol extracted compounds which was rotary evaporated and lyophilized for further structural characterization.

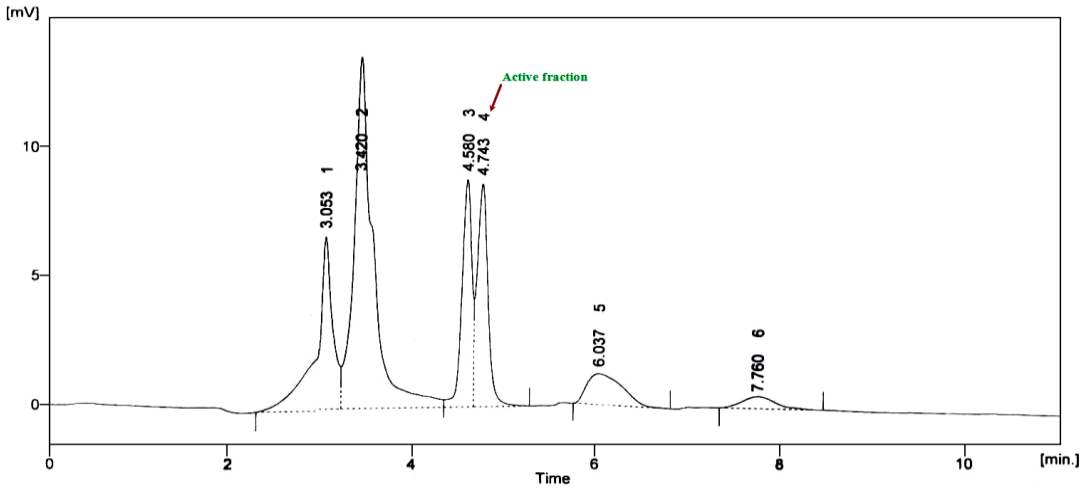


Fig 1: HPLC chromatogram of the crude ethanol leaves extract of *Erythrina indica*.

The FT-IR spectrum of the isolated bioactive compounds showed all the functional groups of 13Docosenamide,(Z) compound as shown in the Figure 2. The amine group (NH_2) functional group present at the 13Docosenamide,(Z) was predicted at the wavelengths of 1630, 1386 and 1133cm^{-1}. The aliphatic alkane ((CH_2)n) group was depicted at wavelength of 3387; 2856; 2352; 2320; 1392; 1385; 774 cm^{-1} as well as the terminal alkane (CH_3) was observed at 2923 cm^{-1}. Similarly, the significant important group of Alkene group (HC=CH) and Carbonyl group (C=O) was located at 928 cm^{-1} and 1640 cm^{-1}.

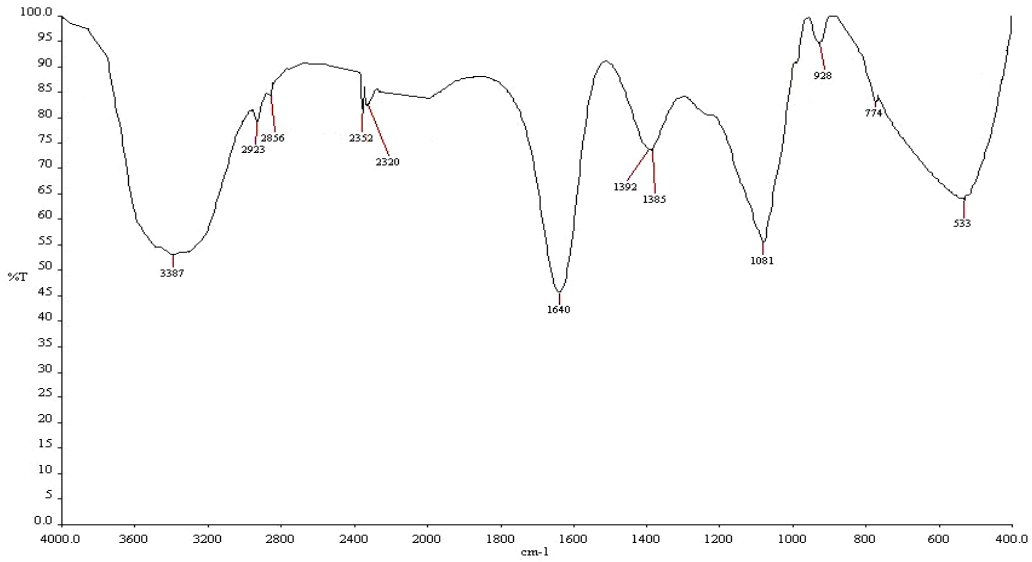


Fig. 2: FT-IR Spectrum of the purified bioactive compound, 13Docosenamide,(Z).

^{1}H and ^{13}C NMR spectrum of the isolated bioactive compound also representing the functional groups of 13Docosenamide,(Z) was shown in the figures 3 and 4. The presence of chemical shifts ranges from 0.8288 - 0.8965 was due to the presence of hydrogen atom in functional group of CH_3 (Terminal alkane hydrogen) as well as the chemical shifts between 0.9061 - 2.3707 was responsible for (CH_2)n (Aliphatic alkane hydrogen). Moreover, the protons in the region of 3.5493 - 3.6450 corresponds to the presence of HC=CH (Alkene hydrogen) and the protons ranged within 4.0830 – 7.2959 chemical shifts was due to the CO-NH_2 (Amide hydrogen) which were the significant characteristic groups of 13Docosenamide,(Z).

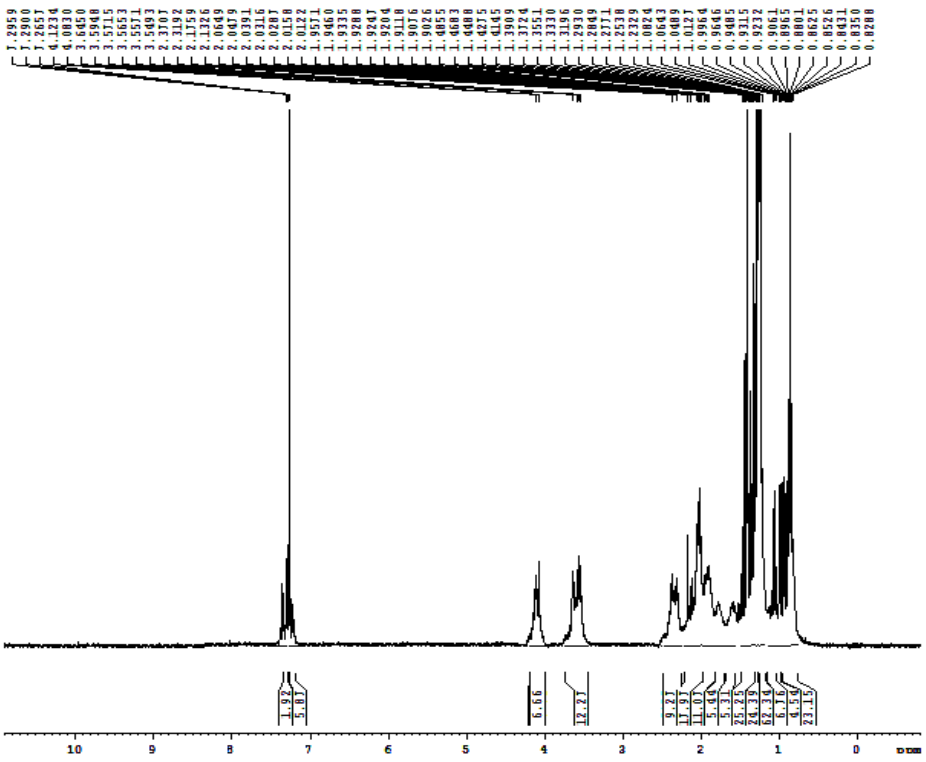


Fig. 3: ^{1}H NMR Spectrum of the purified bioactive compound, 13Docosenamide,(Z).

Moreover, ^{13}C NMR spectrum of this bioactive fraction showed the presence of same functional groups of CH_3 (Terminal alkane carbon) observed between 11.1367 - 17.7370, further, the aliphatic $(CH_2)n$ (Aliphatic alkane carbon) was depicted within 22.4317 - 49.2317. Furthermore, the significant functional groups of HC=CH (Alkene carbon) was represented within 116.9571 – 130.1763 and R-CO-N (Amide carbon) was ranged within 168.5973 - 174.4380.

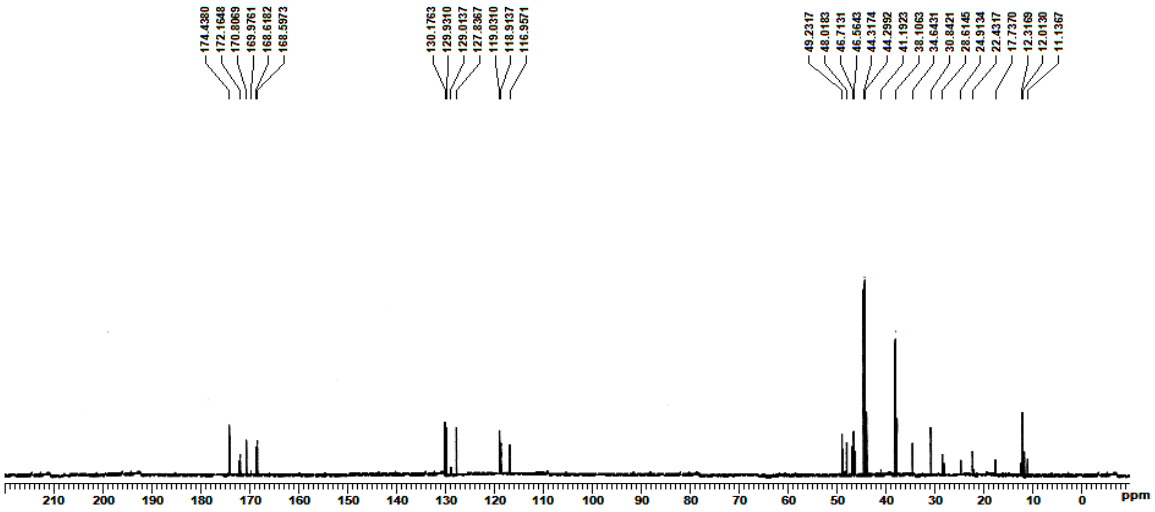
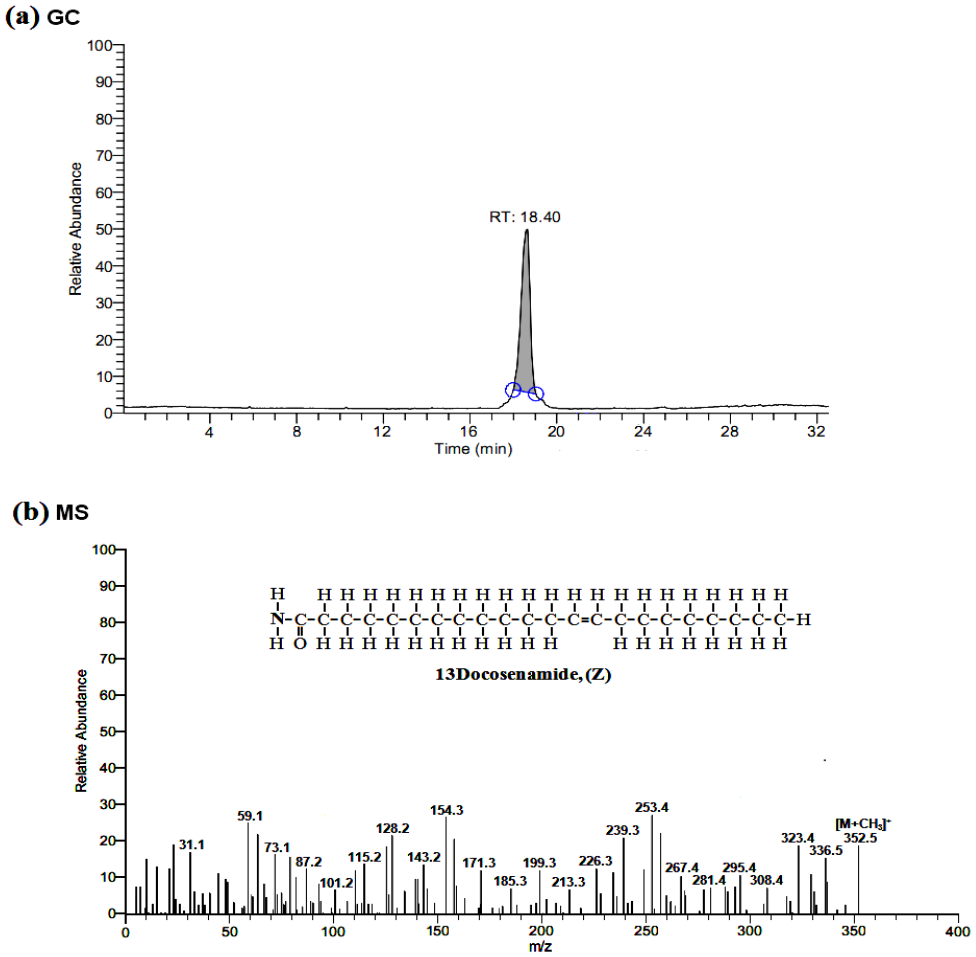


Fig. 4:[13] C NMR Spectrum of the purified bioactive compound, 13Docosenamide,(Z).

Mass spectral analysis of 13Docosenamide,(Z) compound is illustrated in Figure 5. It exhibited a molecular ion peak at the retention time of 18.40 min (Fig. 5a) with a molecular mass of methylated 13Docosenamide,(Z) with 352.5 MW (Fig. 5b). Further, the MS/MS spectrum showed exact sequential pattern of 13Docosenamide,(Z) which were 731.1, 59.1, 128.2, 154.3, 213.3, 239.3, 308.4, 336.5 and 352.5 (Fig. 5c). This pattern showed exact pattern of 13Docosenamide,(Z) as predicted from the NIST database for the molecular identification of chemical compounds. Earlier study showed that five different compounds isolated from the methanol extract of stem bark of *Erythrina* sp viz., epilupeol, 6-hydroxygenistein, 3ß, 28- dihydroxyolean-12-ene, epilupeol, and stigmasterol showed varying degrees of anticancer potential.[11] Similarly, previous studies explored ethanol leave extract of *E. indica* showed anthelmintic, antiulcer, diuretic, analgesic activities etc.[12, 13] 13Docosenamide,(Z) was less understood from *E. indica* which was characterized based on its anticancer potential and the present results holds as a baseline data for the effective isolation and purification of this bioactive compound from this medicinal plant.



(c) Interpretation

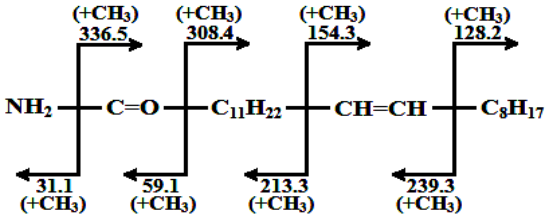


Fig. 5: (a) Gas Chromatography, (b) Mass spectrum and molecular weight interpretations of the purified bioactive compound, 13Docosenamide,(Z).

CONCLUSION

From the above findings, the isolated bioactive compound was fractionated at its purified state and the purified compound was identified as 13Docosenamide,(Z). The present investigation proved that the ethanolic extract of *E. indica* leaves contains 13Docosenamide,(Z) as its major secondary metabolites which are known for their broad range of biomedical properties.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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