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Research Article
Cytotoxic Potential of N-hexadecanoic Acid Extracted from *Kigelia pinnata* Leaves

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Abstract

Objective: The aim of the present study was to screen and to identify the cytotoxic compound from the leaves of *Kigelia pinnata*. **Materials and Methods:** Dry leaf powder of *K. pinnata* was extracted with chloroform and the extract was tested for anticancer cytotoxic activity by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2h-tetrazolium bromide (MTT) assay in human colorectal carcinoma (HCT-116) cells. Crude extract was purified by silica gel column chromatography and the active fraction was identified by using spectroscopic techniques such as nuclear magnetic resonance, fourier transform-infrared spectroscopy and gas chromatography-mass spectrometry analysis. The identified compound was docked with cancer drug target protein, DNA topoisomerase-I using AutoDock4. **Results:** Crude extract showed significant anticancer cytotoxic activity with IC_{50} value of 80 µg mL^{-1}. The GC-MS chromatogram showed the presence of more than 15 different phytochemical compounds. The active fraction of the crude was identified as N-hexadecanoic acid with a molecular weight 256.42 Da and molecular formula $C_{16}H_{32}O_2$. Molecular docking analysis showed that N-hexadecanoic acid has high affinity interaction with DNA topoisomerase-I with a free binding energy -6.71 kcal mol^{-1}. The N-hexadecanoic acid demonstrated significant IC_{50} value of 0.8 µg mL^{-1} against HCT-116 cells. **Conclusion:** Based on the results of docking studies, it is proposed that the observed cytotoxic activity of N-hexadecanoic acid is due to its interaction with DNA topoisomerase-I and itcould be explored further for its anticancer cytotoxic potential with other cancer drug target proteins.

Key words: *Kigelia pinnata*, cytotoxic activity, N-hexadecanoic acid, palmitic acid, AutoDock4, HCT-116 cells, MTT assay, TLC, DNA topoisomerase-I

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Kigelia pinnata is an African tree, traditionally used in Africa for its medicinal value and has been widely used to cure many human ailments. Few recent reports on *K. pinnata* tree indicated that several parts of the tree have potential anticancer activity. Medicinal plants have always been used to cure many human diseases and many bioactive phytochemicals have been isolated, identified and studied for its bioactive potential. Medicinal plants serves as an alternative source of drugs for the treatment for wide variety of diseases, bacterial infection, cancer and other human diseases. About 80% of world population still depend on medicinal plants for their medicine. *Kigelia pinnata* also known as *Kigelia africana* is one such medicinal plant, well known for its ability in curing cancer, malaria, skin ailments, sickle cell anaemia and others[1-3].

The biological importance of *K. pinnata* has been reported in many of the recent studies. Verminoside, the anti-inflammatory phytochemical was reported from *K. africana*[4]. The anti-inflammatory activity of extracts prepared from various parts of *K. pinnata*[5]. The bark of *K. pinnata* have also been pharmacologically documented to possess antiamoebic, antifungal, antiulcer, antibacterial and antioxidant activities[6]. The wound healing activity of *K. pinnata* bark aqueous extract was already been reported[7]. The antibacterial, antifungal, antigiardal and anticancer activity of *K. africana* fruit extracts was also reported[8].

Presence of phytohemicals such as naphthoquinonelapachol, phenyl propanoid, stigmasterol, β-sitosterol and small amounts of free ferulic acid, coumaric acid and 6 methoxymelein are reported from the bark of *K. pinnata*. Various other chemical constituents such as naphthaquinones, iridoids, fatty acids, norviburtinal, sterols, lignans, terpenoid and flavonoids are the essential building blocks responsible for its wide range of biological activities. Palmitic acid is one of the most common fatty acid present in animals, plants and microorganisms[9]. Harada *et al.*[10] has reported that, palmitic acid exhibited strong anticancer cytotoxic activity against human fibroblast cells by selectively inhibiting the effect of DNA topoisomerase-I and without affecting the activity of DNA topoisomerase-II. In the present study an attempt was made to isolate and to identify anticancer cytotoxic phytochemical from the leaf extract of *K. pinnata*.

MATERIALS AND METHODS

Preparation of leaf extract of *K. pinnata*: Fresh leaves of *K. pinnata* were collected from the Wood Stock Park, VIT University, Vellore, Tamil Nadu, India. Leaves were then shade dried and grounded using mortar and pestle. About 10 g of ground leaves was soaked in 100 mL chloroform for 48 h in room temperature. Chloroform was then filtered in Whatman No. 1 filter paper and condensed in rota vacuum evaporator. The obtained extract is stored in 4°C for further study[11].

MTT assay: Five thousand HCT-116 cells were seeded in a 96-well plate. About 24 h later cells were treated with various concentrations of crude extract (40, 80, 160, 240, 320 and 400 μg mL^{-1}) prepared from the leaves of *K. pinnata* for 24 h. After drug treatment cells were washed twice with drug-free medium. As soon as drug treatment incubation period was completed 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium-bromide (MTT, 5 mg mL^{-1} in PBS) was added to each well, the plate was incubated for 4 h at 37°C and 100 μL of 0.04 mol L^{-1} HCl in isopropanol was added. Within 1 h the absorbance was measured on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm. The percentage of surviving cells at each concentration relative to the control group was plotted[12,13].

Thin layer column chromatography: Crude extract was spotted on TLC sheet (Merck, India) and petroleum ether:acetone (6:4) was used as a solvent system. The separated bands are visualized by using Deep Vision-TLC Chamber, at UV 254 and 365 nm and visible light.

GCMS analysis: The extracts was analysed in a Perkin Elmer Clarus 680 equipped with Mass spectrometer Clarus 600 (EI) fitted with Elite-5MS capillary column (30 m, 0.25 mm ID, 250 μm df). The GC oven was maintained with the initial temperature of 60°C for 2 min, ramp 10°C min^{-1} to 300°C, hold 6 min. The injector temperature was 250°C and the oven temperature was maintained at 300°C for 6 min. Helium was used as a carrier gas with constant flow rate of 1 mL min^{-1}. Mass transfer line and source temperature were set at 240°C. Turbo Mass version 5.4.2 software was used for the spectral analysis. Structure determination was done by comparison of mass spectral patterns to NIST-2008 library[14].

Silica gel column chromatography: Crude extract was purified by silica gel column chromatography (Silica 60-120, HiMedia, India). Petroleum ether:acetone (6:4) was used as a solvent system. Fractions were collected at a flow rate of 1 mL min^{-1}. A total of 58 fractions were collected with 5 mL each and all fractions was screed for cytotoxicity on HCT-116 cells.

FT-IR analysis and NMR analysis: The FT-IR spectrum of the pure compound was recorded in Perkin Elmer Spectrum 1 FTIR spectrometer at a resolution of 1 cm^{-1} at a scan range[15] of 450-4000 cm^{-1}. The H^1NMR analysis of the pure fraction was analyzed using AV500 FT-NMR spectrometer[16].

Docking studies: The ligand molecule (N-hexadecanoic acid) was downloaded from PubChem website (CID985). The structure of DNA topoisomerase-I was downloaded from protein data bank website (www.rcsb.org) with the PDB ID: 1TL8. The protein molecule was cleaned and viewed in PyMol software. The interaction between the ligand and drug target protein molecule was studied using AutoDock 4.2 (MGL tools). The result was viewed again in PyMol to analyze the interactions of aminoacids.

RESULTS

Cytotoxicity of crude extract: The crude extract demonstrated significant cytotoxicity against human colon cancer cell line (HCT-116), with an IC_{50} value of 80 µg mL^{-1}. The cytotoxicity exhibited by the crude extract at different concentrations (40-400 µg mL^{-1}) are shown in Fig. 1. Cell viability was decreased to 15% when the crude concentration was increased to 400 µg mL^{-1}.

Thin layer chromatography: The TLC separation of the crude extract revealed the presence of 12 different bands when petroleum ether:acetone (6:4) used as a solvent system. The separated bands of crude extract are shown in Fig. 2a. The TLC separation of the purified compound separated as a single band (Fig. 2b).

GC-MS analysis: Gas chromatography analysis revealed the presence of almost 15 different phytochemicals in the chloroform extract of *K. pinnata* leaves. The chromatogram obtained from GC-MS analysis is shown in Fig. 3.

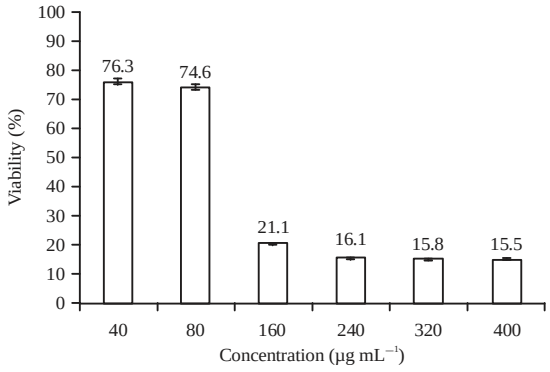

Fig. 1: Cytotoxic activity of crude extract from the leaves of *Kigelia pinnata* on HCT-116 cells

Column chromatography: Petroleum ether:acetone (6:4) was used as a solvent system for separating the active fraction. Fractions having similar colour were pooled and tested for cytotoxic activity. The active fractions 34-39 were concentrated and subjected to spectroscopic studies to identify the individual phytochemicals present.

FT-IR analysis: The FT-IR spectrum recorded for the active compound is given in Fig. 4. Peak at 3543.23 cm^{-1} indicates the presence of O-H functional group. About 1710.86 cm^{-1} peak indicates the presence of (C=O) carbonyl group. Peaks at, 1220.94 and 1091.71 cm^{-1} indicates presence of (C-O) acidic group.

H^1NMR shifts: The H^1NMR analysis revealed the positioning and number of hydrogen atoms within the molecule. There are 32 hydrogen atoms in the compound (Fig. 5). Absence of aromatic hydrogen atoms in the spectrum, suggests that the compound is aliphatic in nature.

Mass spectral studies: The pure compound was analyzed in GC-MS to identify the molecular mass of the compound. The mass spectrum demonstrated a molecular weight of 256.32 g mol^{-1} (Fig. 6).

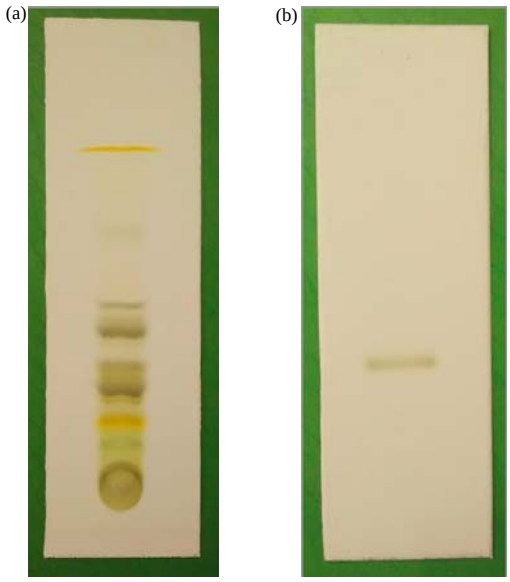

Fig. 2(a-b): TLC of crude chloroform extract and pure bioactive fraction (a) Crude extract and (b) Pure compound

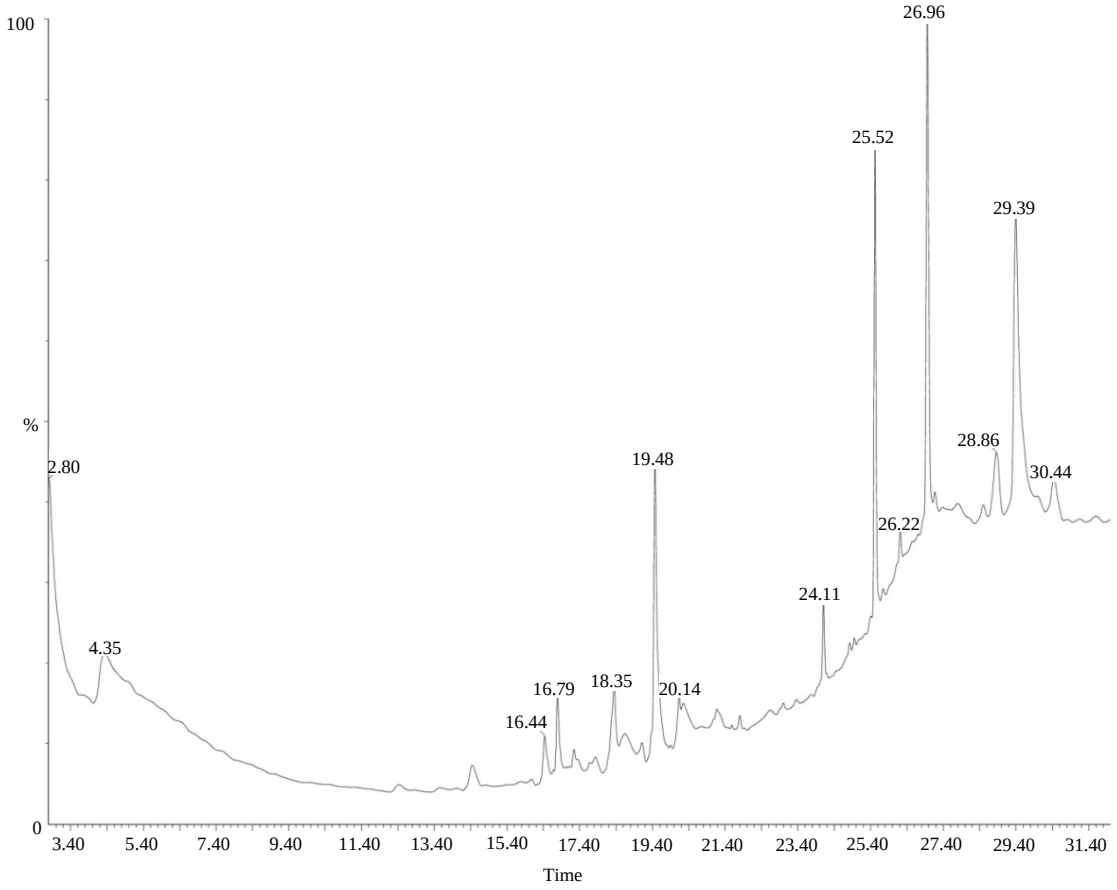

Fig. 3: GC-MS chromatogram of chloroform extract from *Kigelia pinnata* leaves

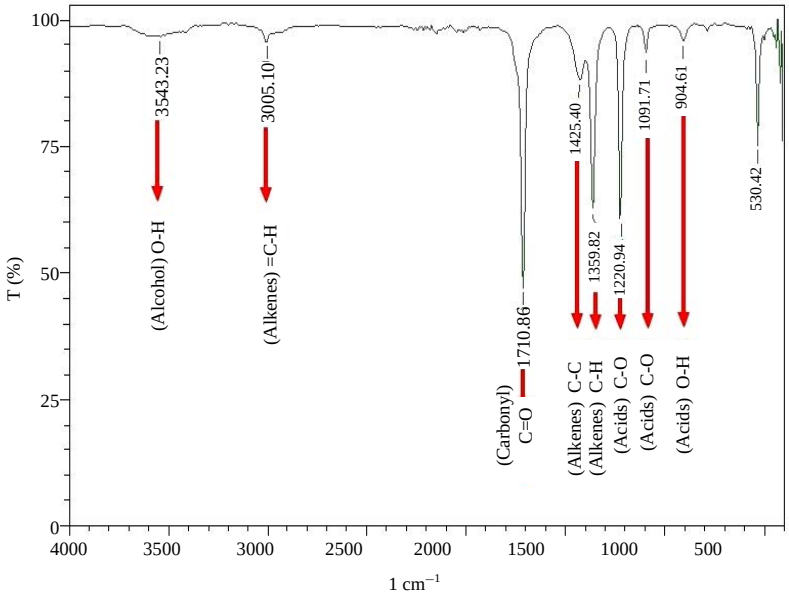

Fig. 4: FT-IR spectrum of the pure compound

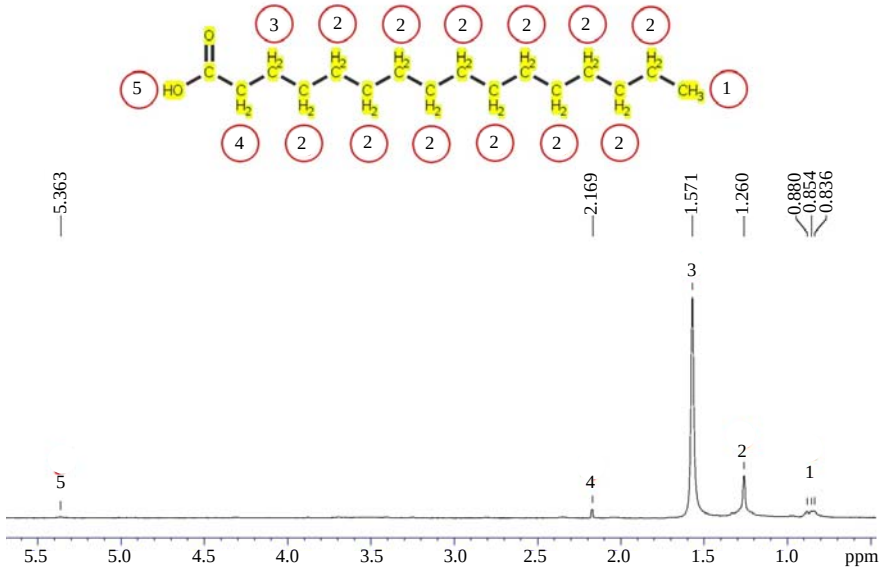

Fig. 5: H[1]NMR analysis of pure compound

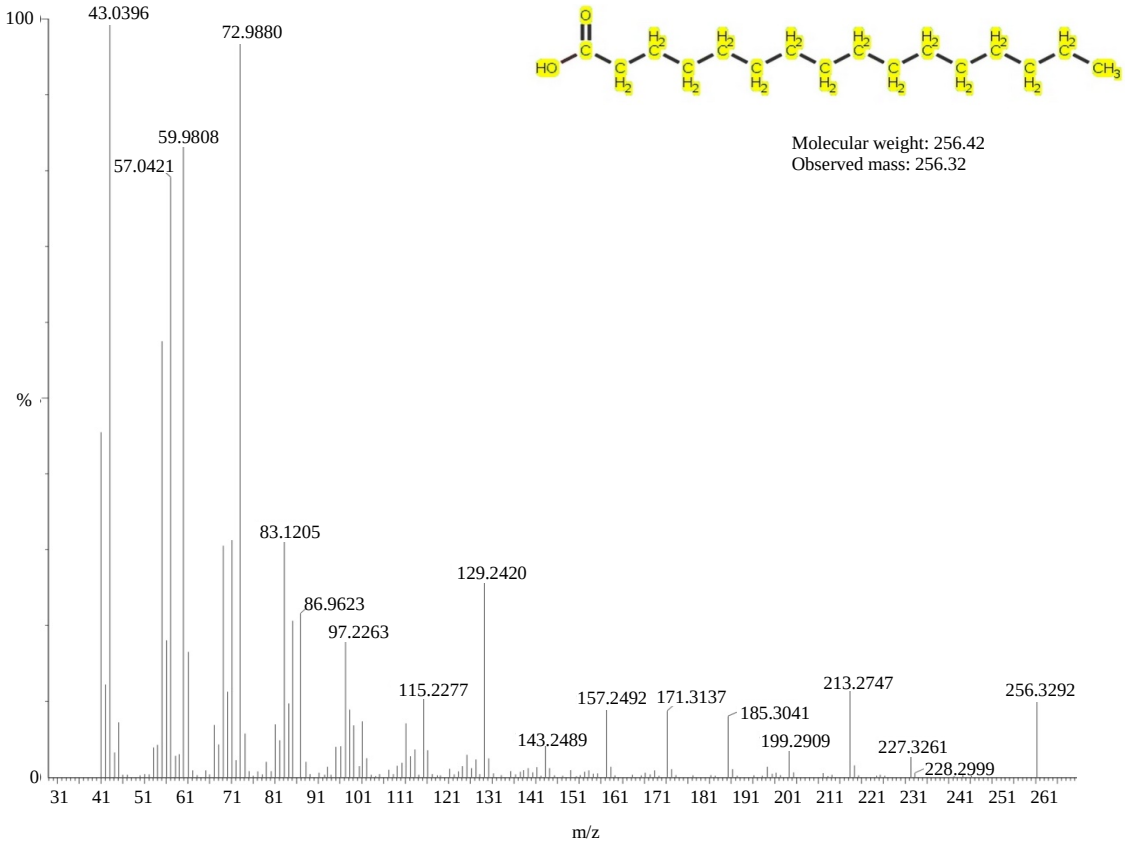

Fig. 6: Mass spectrum of the pure compound (GC-MS)

Structure elucidation: The spectral data obtained from H[1]NMR, FT-IR and GC-MS were used to interpretate the structure of the compound. The pure compound was identified as N-hexadecanoic acid (Fig. 7).

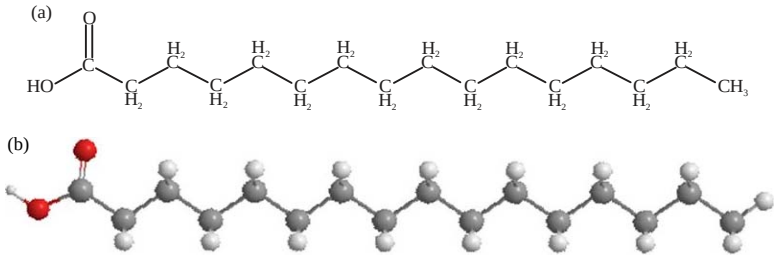

Fig. 7: Structure of N-hexadecanoic acid

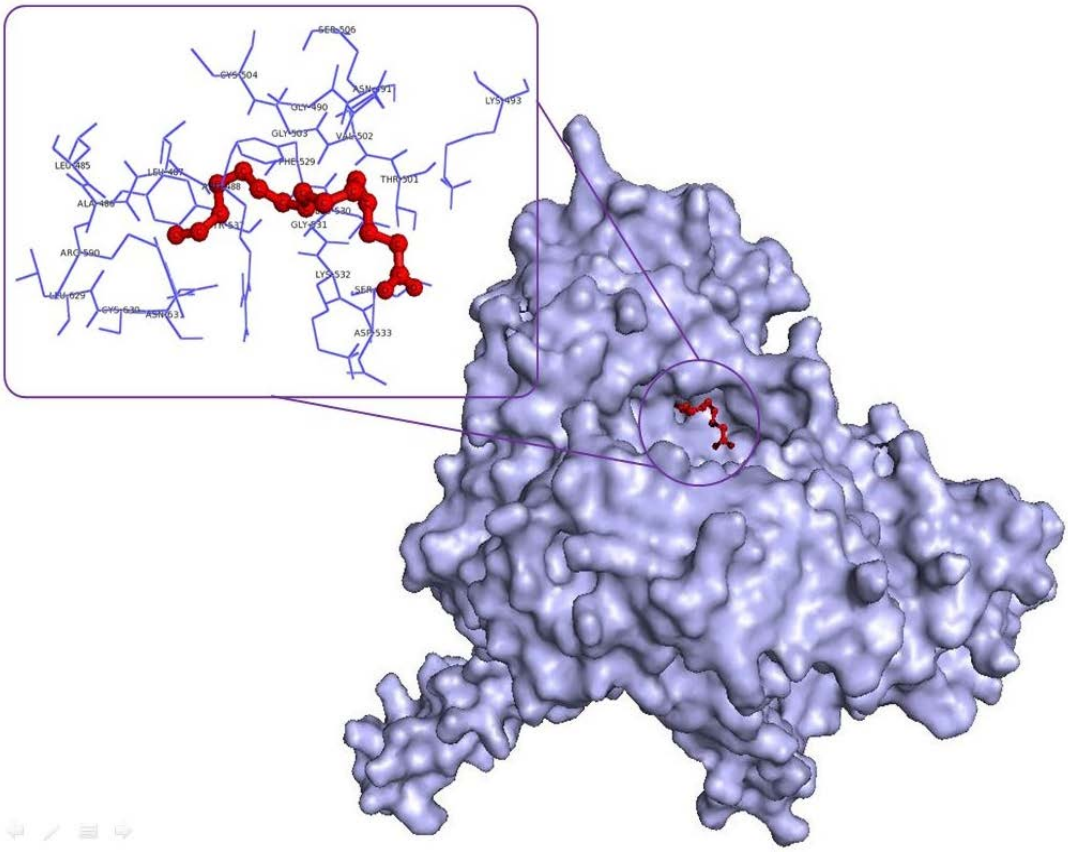

Fig. 8: Interactions of N-hexadecanoic acid with DNA topoisomerase-I

Docking studies: The interaction of N-hexadecanoic acid with DNA topoisomerase-I was analyzed using docking studies. It showed high affinity towards DNA topoisomerase-I with a free binding energy of -6.71 kcal mol^{-1} and a Ki value of 12.03 µM (Fig. 8).

Cytotoxicity of the pure compound: The anticancer cytotoxic activity of the pure compound was studied against HCT-116 cell lines, using MTT assay. The compound demonstrated an IC_{50} value of 0.8 µg mL^{-1} concentration (Fig. 9).

DISCUSSION

Recent studies have shown anticancer and cytotoxic property of extracts prepared from the bark, root, leaf, fruit and seed of *K. pinnata*. *Kigelia pinnata* seed oil obtained from n-hexane extract showed greater cell growth suppression of Caco-2 cells than HEK-293 cells[17]. Dichloromethane extract of *K. pinnata* leaves and root showed moderate *in vitro* anticancer activity against renal cells (TK10), ER positive breast cancer (MCF-7) and melanoma

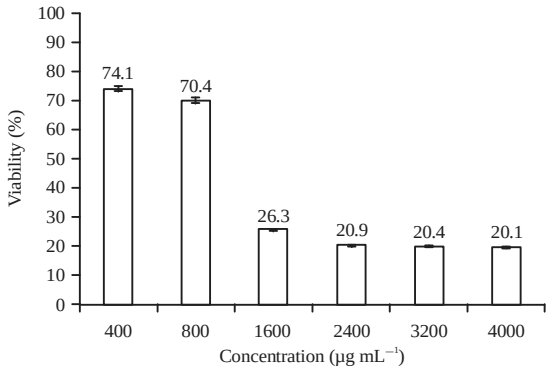

Fig. 9: Cytotoxic activity of N-hexadecanoic acid on HCT-116 cell line

cancer (UACC62) cell lines[18]. Hexane, ethyl acetate and methanolic extracts of the root showed cytotoxicity on human rhabdomyosarcoma (RD) cancer cell lines. Ethyl acetate extract (IC_{50}, 142.2±1.1 ng mL^{-1}) gave the highest activity compared to the reference drug cyclophosphamide (IC_{50}, 143.4±0.5 ng mL^{-1})[19]. Ethyl acetate extract of the *K. pinnata* root oil exhibited significant cytotoxicity against human breast cancer cell lines (IC_{50} 5 µg mL^{-1})[20].

Several cytotoxic agents have also been isolated from *K. pinnata* by bioactivity-guided bioassay methods. Antiproliferative activity of compounds, hydro-xyethyl-naphtho[2,3-b]furan-4,9-dione, ferulic acid isolated from *K. pinnata* was reported against the human melanoma cell lines (SK-MEL-28 and Malme-3M) and breast cancer cells (MCF-7 and MDA-MB-468)[21]. The N-hexane stem bark extract of *K. pinnata* and two of its products, atranorinand-2-beta, 3 beta, 19 alpha-trihydroxy-urs-12-en-28-oicacid showed cytotoxicity at high concentrations[22]. Lapachol (regarded as a potential anti-cancer drugs)[21], norviburtinal and iso-pinnatal were reported to be active against melanoma cell lines[23,24]. The N-hexadecanoic acid is a common fatty acid, found in plants[9]. Harada *et al.*[10] has reported that N-hexadecanoic acid selectively inhibit DNA topoisomerase-I and thereby prevents the proliferation of human fibroblast cells.

The results of our study were supported by previous reports that the cytotoxic activity of the leaf extract is due to the presence of several phytochemicals in the leaf. Docking study also predict the DNA topoisomerase-I inhibitory activity of N-hexadecanoic acid. Hence, the proposed mechanism of cytotoxic activity of N-hexadecanoic acid is due to its interaction with DNA topoisomerase-I and thereby prevents proliferation of cells. These findings further confirm the medicinal value of *K. pinnata* and its anticancer cytotoxic potential.

CONCLUSION

Bioactivity guided extraction of *K. pinnata* leaves resulted in identification of the lead compound, N-hexadecanoic acid and it showed significant cytotoxicity against human colorectal carcinoma cells (HCT-116) with an IC_{50} value of 0.8 µg mL^{-1}.

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