

Original Article

Spectral analysis, *in vitro* cytotoxicity and antibacterial studies of bioactive principles from the leaves of *Conocarpus lancifolius*, a common tree of Jazan, Saudi Arabia

Análise espectral, citotoxicidade *in vitro* e estudos antibacterianos de princípios bioativos das folhas de *Conocarpus lancifolius*, uma árvore comum de Jazan, Arábia Saudita

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Abstract

The objective of the present study was to analyse the bioactive compounds of the leaves of *Conocarpus lancifolius* (*C. lancifolius*). The GC-MS analysis of the hot methanolic extract of the leaves (HME) of *C. lancifolius* exhibited the bioactive compounds such as 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, morphin-4-ol-6,7-dione, 1-bromo-N-methyl-, phytol, hexadecanoic acid, 2,3-dihydroxypropyl ester, 2,2':4',2''-terthiophene, ethyl iso-allocholate, caryophyllene oxide, campesterol, epiglobulol, cholestan-3-ol, 2-methylene-, (3 α ,5 α)-, dasycarpidan-1-methanol, acetate (ester) and oleic acid, eicosyl ester. The FT-IR analysis of HME of *C. lancifolius* showed a unique peak at 3184, 2413, 1657 cm⁻¹ representing coumaric acid, chlorogenic acid and ferulic acid. The HME of *C. lancifolius* was actively inhibiting the proliferation of breast cancer cells MCF-7 ATCC at the concentration of 72.66 \pm 8.21 μ g/ml as IC50 value. The HME of *C. lancifolius* also revealed a good spectrum of activity against Gram-positive and Gram-negative bacterial cultures screened in this work. The activity observed has shown more or less similar effects against screened bacteria. However, the magnitude of potentiality was significantly lesser compared to standard ciprofloxacin disc at p < 0.001 level (99% confidence intervals). Furthermore, the study demonstrating the bioactive compounds can be isolated from the leaves of *C. lancifolius*.

Keywords: plants, leaves, bioactive molecules, multiple drug resistance, anticancer, antibacterial.

Resumo

O objetivo do presente estudo foi analisar os compostos bioativos das folhas de *Conocarpus lancifolius* (*C. lancifolius*). A análise por GC-MS do extrato metanólico quente das folhas (HME) de *C. lancifolius* exibiu os compostos bioativos como 1-(3-Metoxi-2-nitrobenzil) isoquinolina, morfina-4-ol-6,7- diona, 1-bromo-N-metil-, fitol, ácido hexadecanoico, 2,3-di-hidroxipropil éster, 2,2 ': 4', 2 '' - tertiofeno, isoalcolato de etil, óxido de cariofileno, campesterol, epiglobulol, colestano -3-ol, 2-metileno-, (3 α , 5 α) -, dasycarpidan-1-metanol, acetato (éster) e ácido oleico, éster eicosílico. A análise FT-IR de HME de *C. lancifolius* mostrou um pico único em 3184, 2413, 1657 cm⁻¹ representando ácido cumárico, ácido clorogênico e ácido ferúlico. O HME de *C. lancifolius* inibiu ativamente a proliferação de células de câncer de mama MCF-7 ATCC na concentração de 72,66 \pm 8,21 μ g/ml como valor de IC50. O HME de *C. lancifolius* também revelou bom espectro de atividade contra culturas de bactérias Gram-positivas e Gram-negativas rastreadas neste trabalho. A atividade observada mostrou efeitos mais ou menos semelhantes contra bactérias rastreadas. No entanto, a magnitude da potencialidade foi significativamente menor em comparação com o disco de ciprofloxacina padrão em nível de p < 0,001 (intervalos de confiança de 99%). Além disso, o estudo demonstrando os compostos bioativos pode ser isolado das folhas de *C. lancifolius*.

Palavras-chave: plantas, folhas, moléculas bioativas, resistência a múltiplos medicamentos, anticâncer, antibacteriano.

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1. Introduction

Multiple drug resistance (MDR) is one of the foremost therapeutic problems associated with anticancer drug therapy and antibiotics / antibacterial therapy. Therefore, several researchers across the world aimed to develop novel bioactive molecules against cancer and infectious bacterial organisms. Nature is blessed with abundant medicinal plants and trees with unique medicinal values. Traditionally, herbs and medicinal plants were the main resources for curing many diseases across the world in ancient times. Therefore, natural products play a significant role in maintaining a healthy life (Moni et al., 2019). *C. lancifolius* is a greenish tree that grows around 10 to 30 meters and is widely distributed in Jazan, Saudi Arabia (Figure 1). *C. lancifolius*, one of the two species in

the genus *Conocarpus*, is a tree that belongs to the family Combretaceae. The tree is fast-growing, heat tolerant, and therefore has been grown in most of the parks and roadsides in Jazan region. It has been reported for medicinal value, especially anti-diabetic and cytotoxicity property (Al-Taweel et al., 2016). Studies suggested that *C. lancifolius* is used as a food for cattle goats, sheep, and camels in some countries; its wood is used to produce burning fuel, coal and build ships (Wensvoort, 2008). It has been reported as a medicinal plant for the therapy of diabetes mellitus, anaemia, flu, fever, skin ulcers, and syphilis (El-Sayed et al., 2012; Malik et al., 2014). However, there are very limited reports showing the medicinal value of *C. lancifolius* grown in Jazan, Saudi Arabia. This made us to analyse the bio constituents and their medicinal properties to document its therapeutic value.



Figure 1. The tree *Conocarpus lancifolius* located in Jazan city, Kingdom of Saudi Arabia (A) Aerial view of the tree shows many bunches of stems with leaves (B) Landscape view of the tree which shows the bunches of the leaves arises from the stem of tree.

2. Materials and methods

2.1. Collection, identification, and extraction

Jazan city of Jizan province is located in the southwest part of Saudi Arabia, situated in the Red sea's coastal region. It has a natural habitat of *C. lancifolius* and is planted around the city. The tree branches of *C. lancifolius* was collected by breaking from its main branch and pooled. The collected samples were packed in polythene biohazard yellow bags, tied, and transported to the working laboratory. The leaves of *C. lancifolius* were plucked out from the collected branches and washed thoroughly in normal tap water to remove the impurities. Later the leaves were further washed in Millipore water and air-dried by spreading on the clean floor at room temperature under the shade for 3 weeks. A herbarium curator identified the washed specimens in the Herbarium of Jazan University (JZUH) and the reference number was 1213 JZUH. The voucher specimen was also deposited at the herbarium of Jazan University for future reference.

The air-dried leaves were cut into small pieces and powdered using a grinder. The coarsely powdered samples obtained were pooled and kept in an airtight container. Extraction was carried out using a continuous percolation technique with methanol as a solvent. 200g of dried leaves powder was subjected to Soxhlet extraction with methanol at 60° C for 4 hours. After extraction, the extracts were transferred in a separate glass beaker and kept open for the evaporation of the solvent via an air-drying process. After complete air drying of the leaves extract, dried samples were collected by scraping from the glass beakers and pooled and weighed. The dried powder of the methanolic extract of the leaves (HMEL) of *C. lancifolius* was subjected to various phytochemical analysis, FT-IR and GC-MS studies to determine the various bio compounds present in *C. lancifolius*.

2.2. Spectral analysis of the HMEL

2.2.1. Gas chromatography-Mass spectrometry (GC-MS) analysis

A Thermo Scientific GC-MS, AS 3000 autosampler, trace ultra-GC, and ISQ detector were used to identify the structural aspects of the methanolic extract of bioactive compounds. The instrument equipped with a TR 5MS capillary column with dimensions of 30 m length x 0.25 mm diameter (internal diameter) x 0.25 µm (film thickness) was used for the separation of the components. Helium, at a flow rate of 1.2 mL/min (constant flow mode), was used as the carrier gas. A volume of 2 µL of sample extract was injected in splitless mode. The injection port was set at 320 °C, and the temperature of the oven was initially set at 70 °C for 5 minutes. The oven temperature was subsequently ramped to 205° C at the rate of 5° C/min for 5 minutes; 280° C at the rate of 5° C/min for 5 minutes, 290° C at the rate of 5° C/min for 5 minutes, and finally to 300° C at the rate of 5°C/min for 5 minutes. The maximum oven temperature was set at 320° C. The mass spectrometer was operated in an electron ionization (EI) mode within the

mass range of 60-900 amu with 0.6 scan times (min). The MS transfer line temperature and ion source temperature were kept at 320° C and 350°C, respectively, with an electron multiplier voltage of 1 Kv. The mass spectrophotometer was operated and spectral analysis was performed using Xcalibur software. Interpretation of the mass spectrum was determined by using inbuilt software libraries such as NIST, REPLIB, and MAINLIB. The constituent percentages were measured based on the peak area.

2.2.2. Fourier transform infrared spectroscopy studies (FT-IR)

The leaf powder was analyzed through FT-IR spectroscopy (Nicolet iS10 FT-IR spectrophotometer) using the pressed pellet technique. The pellet sample's spectra were obtained through the FT-IR spectrophotometer against reference KBr pellet in the range of 400 - 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

2.3. Cytotoxic effect of the HMEL

In this work, the human breast cancer cell line MCF-7 cell line was cultured and maintained in RPMI-1640 medium at pH 7.4. Further, 10% fetal bovine serum (FBS) was added with RPMI-1640 medium as a supplement. Penicillin (100 U/ml) and streptomycin (100 g/ml) were added to the medium. The cells were incubated in a CO₂ incubator (Heraeus, GmbH, Germany) at 37°C with 90% humidity and 5% CO₂. The Cells were treated with HMEL of *C. lancifolius* by dissolving in DMSO while the untreated control cultures received only the vehicle (DMSO < 1%).

The cytotoxic profile of the HMEL of *C. lancifolius*, was assessed by MTT assay (Syam et al., 2012; Gloria et al., 2019). Briefly, 1 × 10⁶ cells/ml was seeded in 96 well microtiter plates for both, and various gradient concentrations of samples were plated out in triplicates and incubated in a CO₂ incubator for 48 h. Each plate included untreated cell controls and blank cell-free control. After incubation, MTT (5 mg/ml) was added to each well, and the plates were incubated for a further 4 h after which the media was removed. DMSO (100 µl) was added into each well to solubilize the formazan crystals. The absorbance was read at a wavelength of 570 nm using a microtiter plate reader (BioTek Instruments, USA). The percentage of cellular viability was calculated with the appropriate controls taken into account. The experiment was done in triplicate, and the following formula was used to calculate the inhibitory rate of cell proliferation: Growth inhibition = (OD control - OD treated) / OD control × 100. The cytotoxicity of the sample on cancer cells was expressed as IC50 values, the sample concentration reducing the absorbance of treated cells by 50% concerning untreated cells.

2.4. Morphological changes in treated cells

Human breast cancer cells MCF-7 was cultured in RPMI-1640 medium at pH 7.4 by placing in 24 well culture plates and incubated overnight in a CO₂ incubator. The cells were then treated in two batches with methanolic extract of *C. lancifolius* at IC50 and kept for 48 hr. After

the treatment, the first batch treated cells were directly photographed and observed for morphological changes under 20 & 40X magnifications using Nikon Eclipse TS 100 inverted phase-contrast microscope, Nikon cooperation, Japan. Eclipse TS 100 microscope uses eco illumination that provides sufficient brightness for imaging through phase contrast. The morphological characters were confirmed in the second batch by performing a neutral red dye test. The test was performed by removing the media, and cells were washed with cold, sterile PBS. The washed cells were treated with 0.5% neutral red at a volume of 100 µl to each well and stained for 2 minutes. The stains were washed out with PBS and immediately observed under phase contrast inverted microscope under 20 & 40X magnification using Nikon Eclipse TS 100 inverted phase-contrast microscope, Nikon cooperation, Japan.

2.5. Antibacterial studies

2.5.1. Bacterial strains used and standardization

An equal set of Gram-positive and Gram-negative bacteria was employed in this study, in line with the earlier report (Okhundredaev et al., 2019). Briefly, 24 h cultures of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* were prepared in nutrient broth and incubated at 37 °C. The cultures were standardized using serial dilution-agar plate analysis. The cultures were briefly diluted serially using sterilized nutrient broth, resulting in working bacterial culture with a concentration gradient of 10⁻¹ to 10⁻⁹. Spread plate technique was followed, 100 µl of the working bacterial cultures were spread on agar plates with individual organisms in their respective plates. The working bacterial cultures' potential viability was determined in terms of colony-forming unit per ml (CFU/ml) and tabulated.

2.5.2. Determination of Minimum inhibitory concentration

The minimum inhibitory concentration was performed by the broth tube dilution method. The gradient concentrations of samples were prepared by diluting the Millipore water samples to get a predetermined concentration of 1250, 1000, 750, 500, 250, 125 µg/100 ml. The minimum concentration of inhibiting the bacterial growth was determined based on the visibility of bacterial growth in the broth, and the concentration was noted and used for antibacterial sensitivity studies.

2.5.3. Antibacterial sensitivity studies

A specified quantity of Muller Hinton (MH) agar was dissolved in Millipore water and sterilized in an autoclave. The agar plates were prepared aseptically and the spread plate technique was followed to seed the organisms on individual MH agar plates. The plates were covered with their lids, and the culture was allowed to diffuse through the media for 30 min. Wells of diameter 10 mm were made using a sterile standard borer for the individual organism, the extracts were placed in the respective wells and plates

were incubated at 37°C for 24 h. The antibacterial effects of bioactive extracts were quantified by measuring the zone of inhibition around each well in millimetres (mm).

The Kirby-Bauer technique was employed with standard ciprofloxacin disc (5µg/disc). The technique was performed as described earlier in agar well diffusion procedure, but the ciprofloxacin disc was placed on the agar surface rather than in punched well and the plates were incubated at 37 °C for 24 h. The spectrum of the antibacterial effect of ciprofloxacin disc was determined by measuring the zone of inhibition around the disc and the values were expressed in millimetres (mm).

3. Results

The phytochemical analysis of the HMEL of *C. lancifolius* showed various compounds such as carbohydrates, amino acids, alkaloids, tannins, steroids, and saponins. The GC-MS spectral chromatogram (Figure 2) showed possible bioactive compounds in the HMEL of *C. lancifolius*. Figure 3 depicting the structure of bioactive constituents in the HMEL of *C. lancifolius*. The potential bioactive compounds are 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, Morphine-4-ol-6,7-dione, 1-bromo-N-methyl-, Phytol, Hexadecanoic acid, 2,3-dihydroxypropyl ester, Ethyl iso-allocholate, Caryophyllene oxide, Epiglobulol, Cholestan-3-ol, 2-methylene-, (3á,5â)-, Dasycarpidan-1-methanol, acetate (ester), Campesterol and Oleic acid, eicosyl ester. The FT-IR spectroscopy of the HMEL of *C. lancifolius* was displayed with unique fingerprint regions of various bioactive compounds (Figure 4). The respective compounds of FT-IR analysis were tabulated (Table 1) and represents the presence of possible compounds amino acids, coumaric acid, ferulic acid, Fatty acids, aromatic hydrocarbons, quercetin, tannins, cutins and polysaccharides.

The results of Table 2 suggest that the HMEL of *C. lancifolius* was able to inhibit the proliferation of MCF-7 breast cancer cells. The IC50 value of the HMEL of *C. lancifolius* showed growth inhibition at the concentration of 72.66 ± 8.21 µg/ml (Figure 5). The morphological changes further confirmed the anticancer activity of HMEL of *C. lancifolius* in MCF-7. The non-treated cells had shown typical morphology of healthy MCF-7 cells (Figure 6A & C). The morphology of control cells was intact, showing clear nucleation and cell differentiation. In contrast, the cells that were treated with extracts had shown detached cells, dead cells, and shrunken cells with condensed or fragmented apoptotic nuclei (Figure 6B & D). The spectrum of antibacterial effect of HMEL of *C. lancifolius* against selected human pathogenic bacteria including Gram-positive *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*; Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* was measured. The study revealed that HMEL of *C. lancifolius* exhibited an equal spectrum of activity against Gram-positive and Gram-negative bacteria. The zone of inhibition was expressed in millimetre (mm), which was recorded in Table 3.

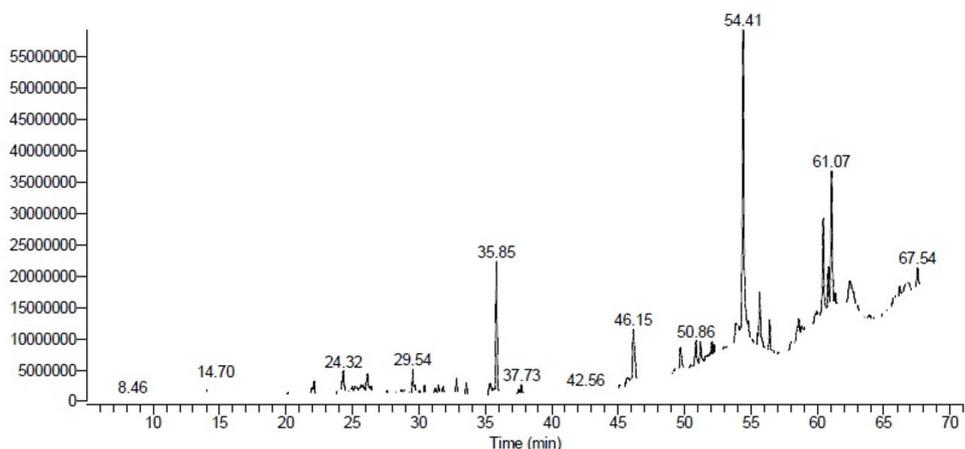


Figure 2. GC-MS spectral chromatogram of the methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius*.

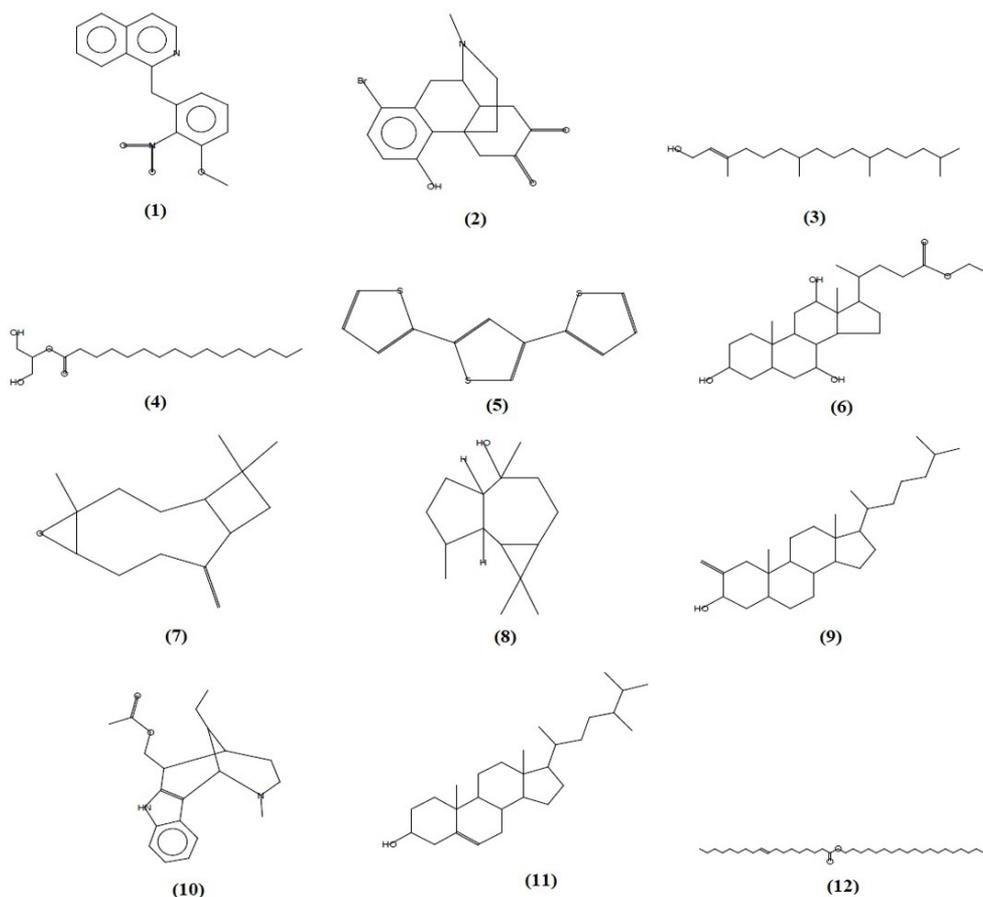


Figure 3. Bioactive components of the hot methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius*. **(1)** 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, MF: $C_{17}H_{14}N_2O_3$; MW: 294; RT: 54.41 **(2)** Morphin-4-ol-6,7-dione, 1-bromo-N-methyl-, MF: $C_{17}H_{18}BrNO_3$; MW: 363; RT: 61.07 **(3)** Phytol, MF: $C_{20}H_{40}O$; MW: 296; RT: 35.85 **(4)** Hexadecanoic acid, 2,3-dihydroxypropyl ester, MF: $C_{19}H_{38}O_4$; MW: 320; RT: 46.15 **(5)** 2,2':4,2''-Terthiophene, MF: $C_{12}H_8S_3$; MW: 248; RT: 50.45 **(6)** Ethyl iso-allocholate, MF: $C_{26}H_{44}O_5$; MW: 436; RT: 56.67 **(7)** Caryophyllene oxide, MF: $C_{15}H_{24}O$; MW: 220; RT: 24.32 **(8)** Epiglobulol, MF: $C_{15}H_{26}O$; MW: 222; RT: 25.19 **(9)** Cholestan-3-ol, 2-methylene-, (3á,5á)-, MF: $C_{28}H_{48}O$; MW: 400; RT: 27.22 **(10)** Dasycarpidan-1-methanol, acetate (ester), MF: $C_{20}H_{26}N_2O_2$; MW: 326; RT: 32.05 **(11)** Campesterol, MF: $C_{28}H_{48}O$; MW: 400; RT: 58.60 **(12)** Oleic acid, eicosyl ester, MF: $C_{38}H_{74}O_2$; MW: 562; RT: 58.08. **MF – Molecular Formula; MW – Molecular Weight; RT – Retention Time (Minutes).**

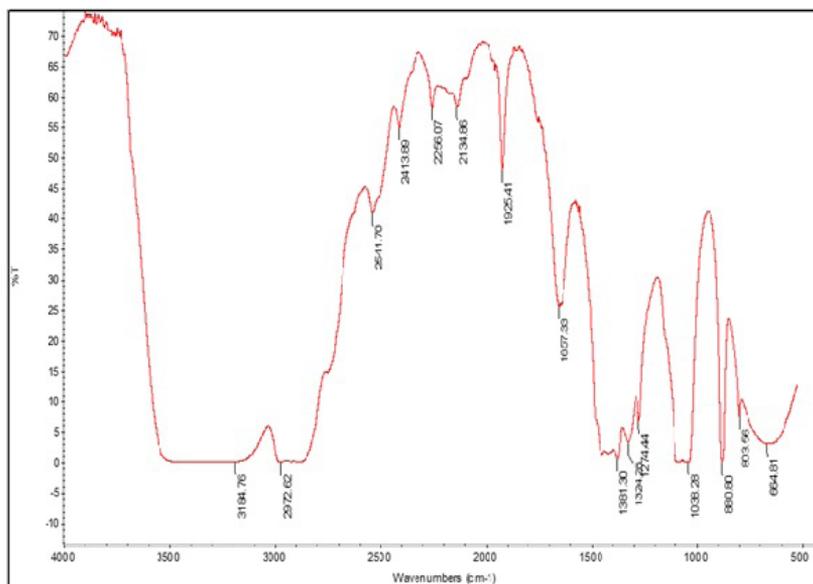


Figure 4. FT-IR spectroscopy of the hot methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius*.

Table 1. Phytochemical composition of the hot methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius* through FT-IR analysis.

Wave number (cm ⁻¹)	Intensity Estimation	Group or Functional Class	Compounds
3184	W	C-H str. (Aromatic) C-H str. (Alkane)	Coumaric acid, Aliphatic compounds Steroids, Tannins, Saponins
2972	M	C-H str. (Alkene) C-H str. (Aldehyde/ketones)	Coumaric acid, Aliphatic compounds
2541	S	-SH str. (Thiol)	Amino acids
2413	M	O-H (Carboxylic acid)	Coumaric acid, Ferulic acid ¹ , Chlorogenic acid ¹
2256	M	CN str.	Fatty acids
2134	M	C≡C, (Alkyne)	Aromatic hydrocarbons
1925	S	CH (Arene)	Aromatic hydrocarbons
1657	S	C=O str (Carboxylic group) C=C str(Aromatic) C=C str(Alkenes)	Quercetin, Coumaric acid, Ferulic acid, Chlorogenic acid, Ester, Tannins, Pectin
1324	M	S=O Stretching (sulfone)	Alkanes
1274	M	C-O-C Stretching (Ethers)	Quercetin, Tannins
1033	M	S=Ostr.	Cutin
880	S	Out of plane bending	Polysaccharides
803	M	=C-H bending vibration NH (Amines) Out of plane Wagging	Quercetin, Fatty acids
664	M	C-S Stretching	Sulfates

W: Weak; M: Medium; S: Strong

Table 2. Cytotoxic effect of methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius* against treated MCF-7 cells.

Drug analyte (Test Sample)	IC ₅₀
HMEL*	72.66 ± 8.21 µg/ml

* Hot methanolic extract of the leaves *Conocarpus lancifolius* MCF-7: Human breast cancer cells. Each value is the mean of n=3 batches with standard deviation by performing Dunnett multiple comparison test.

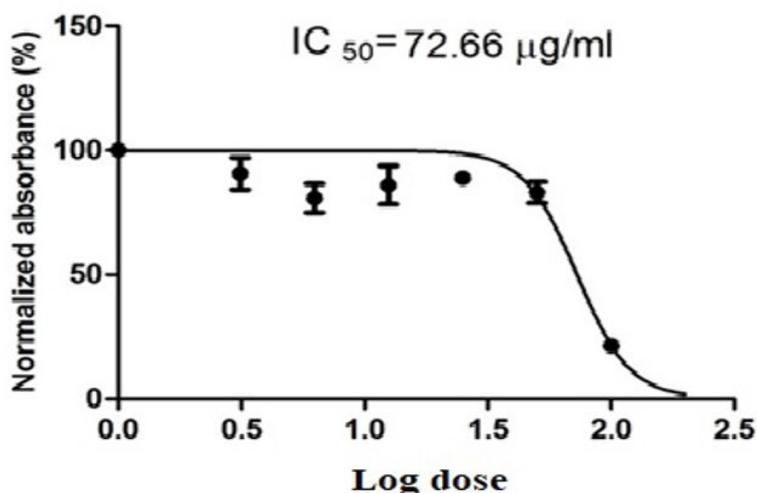
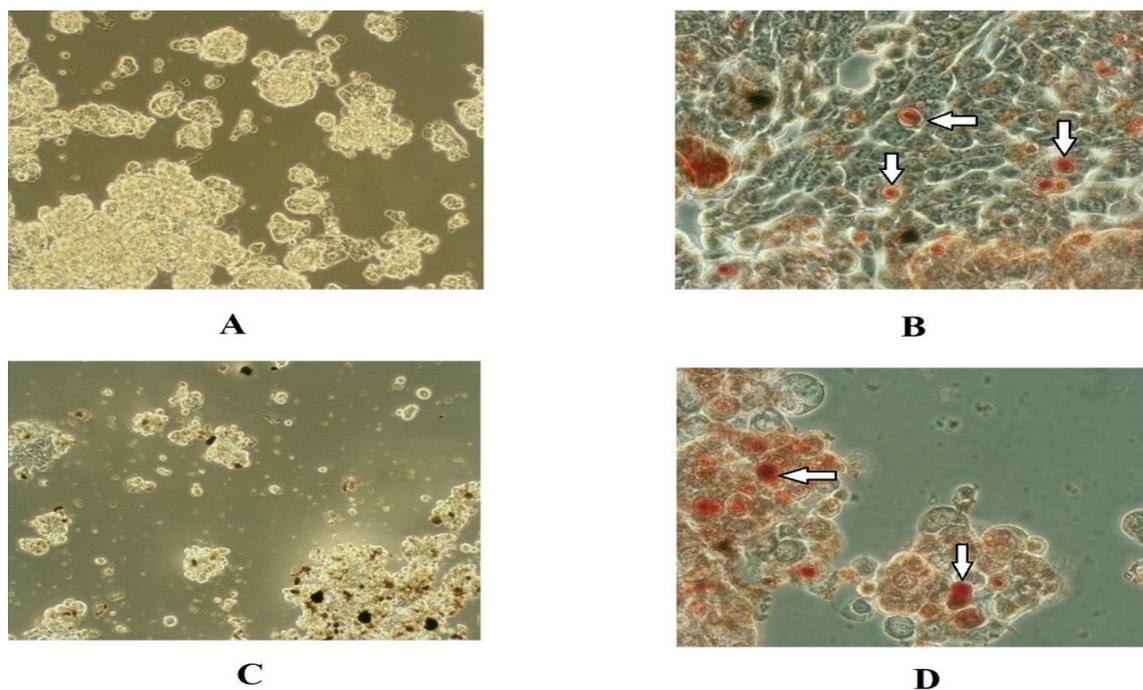
**Figure 5.** The dose response curve of the hot methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius* against MCF-7 cells.***Figure 6.** Morphology of MCF-7 cells unstained and stained with neutral red after 48 h treatment with of the hot methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius*. (A) Microscopical view of unstained healthy cells at 1×10^6 cells / ml concentration mounted at $20 \times$ magnification under phase contrast microscope (B) Microscopical view of stained healthy cells at 1×10^6 cells / ml concentration mounted at $20 \times$ magnification under phase contrast microscope (C) Microscopical view of unstained cells after the treatment at 1×10^6 cells / ml concentration mounted at $40 \times$ magnification under phase contrast microscope (D) Microscopical view of stained cells after the treatment at 1×10^6 cells / ml concentration mounted at $40 \times$ magnification under phase contrast microscope The arrow arrows of the figure B & D indicate the nuclei with apoptotic nuclear morphology in both un treated and drug treated cells.

Table 3. The antibacterial activity of the hot methanolic extract of the leaves (HMEL) of *Conocarpus lancifolius* against selected human pathogenic bacteria.

Human pathogenic bacteria	Concentration CFU/mL	Zone of inhibition (mm)		
		HMEL [§] (Test analyte)	Ciprofloxacin disc (5µg/disc)	Methanol (Solvent control)
<i>Staphylococcus aureus</i>	2 × 10 ⁻⁵	18.3 ± 0.8	27.3 ± 0.8	-
<i>Streptococcus pyogenes</i>	2 × 10 ⁻⁴	20.3 ± 1.2	26.6 ± 0.8	-
<i>Bacillus subtilis</i>	3 × 10 ⁻⁶	18.3 ± 0.87	28.6 ± 2.1	-
<i>Escherichia coli</i>	4 × 10 ⁻⁶	21.6 ± 0.8	31.8 ± 2.2	-
<i>Klebsiella pneumonia</i>	2 × 10 ⁻³	19 ± 1	28.6 ± 2	-
<i>Pseudomonas aeruginosa</i>	2 × 10 ⁻⁴	18 ± 0.9	27 ± 0.9	-

(-) No activity; *CFU - Colony forming unit;

[§]HMEL – Hot methanolic extract of the leaves; Each value is the mean of n=6 batches with standard deviation by performing Dunnett multiple comparison test, (post hoc test). All the test values are significantly lesser than standard ciprofloxacin disc at P < 0.001 (999% confidence interval) level.

4. Discussion

In the present study, HMEL of *C. lancifolius* exhibited 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, a derivative of the alkaloid isoquinoline. In 2013 researchers reported the antibacterial efficacy of novel isoquinoline derivatives (Galán et al., 2013). Phytol is acyclic diterpene alcohol that has been detected in this study. Phytol is produced by all photosynthetic plants closely associated with chlorophyll (Islam et al., 2018). Phytol is also reported as a major constituent of plant-derived essential oils, and it has potent antimicrobial properties (Murbach Teles Andrade et al., 2014). Hexadecanoic acid, 2,3-dihydroxypropyl ester is a fatty acid derivative that has been detected in HMEL of *C. lancifolius*. Hexadecanoic acid, commonly termed palmitate, has been reported for its antibacterial and antifungal properties (Chandrasekaran et al., 2011; Rahuman et al., 2000). The presence of palmitic acid has been reported in the leaves of *C. lancifolius* in 2011 (Redha et al., 2011). 2,2':4',2''-terthiophene is an oligomer of the heterocycle thiophene reported for optical properties and anticancer effect (Chiara et al., 2010; Zhang et al., 2009). Ethyl isoallocholate, otherwise called ethyl cholate, was observed in the HMEL of *C. lancifolius*, reported for the antibacterial property (Malathi et al., 2016). However, a recent study has demonstrated reduced tumor growth (Thakur and Ahirwar, 2019). Caryophyllene oxide is a bicyclic sesquiterpene observed in the HMEL of *C. lancifolius* and has been shown as an anticancer molecule (Pan et al., 2016). Epiglobulol is a member of the class of compounds known as 5,10-cycloaromadendrane sesquiterpenoids that have been observed in the HMEL of *C. lancifolius*, which has been reported for antimicrobial activity (Tan et al., 2008). Cholestan-3-ol, 2-methylene-, (3 α ,5 α)-, a saturated tetracyclitriterpene have been determined in the HMEL of *C. lancifolius*. Cholestan-3-ol has already been reported in *Canscora perfoliata* Lam. (Gentianaceae)

(Thanga Krishna et al., 2012). Dasycarpidan-1-methanol, acetate (ester) an alkaloidal compound has been identified in the HMEL of *C. lancifolius* in this work which is not yet reported. This compound has been reported for exhibiting anti-inflammatory, antibacterial, antifungal, and anticancer properties (Abeer Fauzi Al et al., 2017). Campesterol and Oleic acid, eicosyl ester were identified in the HMEL of *C. lancifolius* with high retention time. Campesterol is a phytosterol that has not been reported in *C. lancifolius*. However, the compound has been reported for antioxidant properties (Kmieciak et al., 2011). Oleic acid, eicosyl ester is a monounsaturated omega-9 fatty acid, that has been reported for having mosquito repellent activity (Gurunathan et al., 2016). The FT-IR spectrum depicted a large parabola-shaped peak observed at 3184 cm⁻¹ with aromatic C-H stretching, vibrations, with weak intensity representing the presence of Coumaric acid, aliphatic compounds (Fachriyah et al., 2019). However, alkane stretching indicated the presence of steroids, tannins, saponins. The peak at 2413 cm⁻¹ with medium intensity showed O-H (Carboxylic acid), which indicates the presence of Coumaric acid, Chlorogenic acid, and ferulic acid (Gurunathan et al., 2016). The peak at 1657 cm⁻¹ with strong intensity showed C=O (Carboxylic group) with stretching vibration, which demonstrated Quercetin, Coumaric acid, and ferulic acid (Gurunathan et al., 2016).

The cytotoxic effect of HMEL of *C. lancifolius* may be due to the presence of hexadecanoic acid, 2,3-dihydroxypropyl. Earlier research findings suggested that the hexadecanoic acid was exhibiting cytotoxicity to human leukemia cells at the concentration of 12.5 to 50 µg/ml, but did not show any cytotoxicity to normal human dermal fibroblast (Al-Wahaibi et al., 2020). The anticancer property may also be due to the presence of ethyl isoallocholate, 2,2':4',2''-terthiophene, caryophyllene oxide, and dasycarpidan-1-methanol, acetate (ester). Ethyl isoallocholate has been reported for its potent anticancer

property and showed the highest cytotoxicity against A549 lung cancer cells in vitro and in vivo (Pan et al., 2016; Fachriyah et al., 2019). Studies have demonstrated the cytotoxic effect of thiophenes. Studies also suggested that caryophyllene oxide has proved to have an anticancer effect against MG-63 human osteosarcoma cells (Pan et al., 2016). The spectrum of antibacterial activity was due to the presence of phytol, hexadecanoic acid, 2,3-dihydroxypropyl ester, and dasycarpidan-1-methanol, acetate (ester). An earlier study suggested that alkaloidal extract from Leaves of *C. lancifolius* exhibited an antibacterial effect (Hayssam et al., 2013). The HMEI of *C. lancifolius* has exhibited several potentially bioactive compounds that were reflected in the cytotoxicity and antibacterial studies.

Conclusion

The need to develop novel drug molecules is increasing in demand gradually and steadily because of the development of MDR against cancer and bacterial infections. Plant resources are remarkable sources for the development of medicinal agents from time immortal. The pharmaceutical importance of *C. lancifolius* is not yet explored properly. The present study suggested the presence of certain significant compounds in HMEI of *C. lancifolius*. The bioactive compounds are hexadecanoic acid, sesquiterpenoids, phytol and dasycarpidan-1-methanol, ethyl iso-allocholate, 2,2':4',2''-terthiophene and caryophyllene oxide. The respective compounds were responsible for antimicrobial and cytotoxicity activities. The results obtained in this study are highly promising to develop novel anticancer and antibacterial drug molecules, which will be a significant advancement towards welfare of human society.

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Abbreviations

HMEL, hot methanolic extract of the leaves; **GC-MS**, Gas chromatography–mass spectrometry; **FT-IR**, Fourier transform - infrared spectroscopy; **MDR**, Multiple drug resistance; **MTT**, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide; **IC**, Inhibitory concentration