Egypt. J. Bot. **Vol. 63**, No. 2, pp. 621-634 (2023)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Anticancer and Antimicrobial Activity of Jatropha's Leaves Extracts

Zeinab M. Saleh[(1)#], Ahmed Z. Abdel Azeiz[(1)], Ahmed B.M. Mehany[(2)], Zeinab A.S. El-Swaify[(3)]

[(1)]Collage of Biotechnology Misr University for Science and Technology (MUST), 6th October City 12573, Egypt; [(2)] Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt; [(3)]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University (Girl Branch), Cairo 11651, Egypt.

THE GENUS *Jatropha* holds great promise as a potential novel drug source because it have many useful activities and phytochemical constituents. This study evaluated the phytochemical composition, anticancer effects against HepG2 (Hepatocellular carcinoma cell line), and antimicrobial efficiency of methanol leaf extracts from *Jatropha curcas L.*, *Jatropha gossypifolia L.*, and *Jatropha multifidia L.* in vitro. Flavonoids, tannins, alkaloids, saponins, terpenes and sterols were detected in the methanol extracts of both *J. curcas* and *J. gossypifolia*. The leaf extract from *J. curcas* exhibited high effectiveness against the HepG2 cell line, with a selective index of 2.04, as well as effective against Gram-positive and Gram-negative bacteria. Two fractions (JCF1 and JCF2) were collected after the crude methanol extract was purified from *J. curcas* using a silica gel column. Gram (+ve) bacteria were the only ones susceptible to JCF1, but Gram (+ve) and Gram (-ve) bacteria were both susceptible to JCF2. Hexadecanoic acid (18.43%), anethole (18.28%), estragol (18.28%), oleic acid (13.67%), phytol (10.84%), and carvacrol (7.56%) were found to be the most abundant components of JCF1 in a Gas Chromatography–Mass Spectrum (GC-MS) analysis. In contrast, the most abundant chemical components of JCF2 were hexadecanoic acid (18.05 %), hexadecanoic acid methyl ester (9.50 %), octadecanoic acid methyl ester (3.29 %), anethole (3.1 %), estragole (3.1 %), thymol (1.61%), and carvacrol (1.61%).

Keywords: Anticancer, Antimicrobial, Carvacrol, GC-MS analysis, *Jatropha* spp., Thymol.

Introduction

Many active compounds are found in medicinal plants, providing a prosperous resource food industry, cosmetics and medical purposes (Ahmed et al., 2016). The Euphorbiaceae family is regarded as one of the most prominent families of angiosperms. About 7,800 species are found in the Euphorbiaceae, divided into 300 genera and 5 subfamilies. *Jatropha L.* is one of the main genera in this family, which belongs to the Crotonoidea subfamily and the Jatropheae tribe. It has about two hundred species spread across tropical and subtropical zones (Webster, 1994; Félix-Silva et al., 2014a).

Because of the therapeutic potential of Jatropha L., the plant was given the name "*Jatropha*" which comes from the Greek roots "jatros," meaning "doctor," and "trophe," meaning "food" (Sabandar et al., 2013). The medicinal properties, chemical structure, and

#Corresponding author email: zeinab.saleh@must.edu.eg
Received 30/09/2022; Accepted 06/03/2023
DOI: 10.21608/ejbo.2023.162736.2137
Edited by: Prof. Dr. Fawzy M. Salama, Faculty of Science, Assuit University, Assuit, Egypt.

biological activity of several *Jatropha* species including *J. curcas*, *J. integerrima*, *J. elliptica*, *J. gossypiifolia*, and *J. multifida* have been widely reported (Cavalcante et al., 2020).

Antibiotic-resistant bacteria have emerged as a global health crisis, contributing to high yearly mortality. Antibiotic resistance can be attributed to improper use of antibiotics. Natural constituents, such as plant-based active compounds, are one approach to solving these issues (Akhtar & Mirza, 2018; Prastiyanto et al., 2020b). A number of extracts from *Jatropha* plants have been reported to have antimicrobial activity, including *J. curcas* (Félix-Silva et al., 2018b), *J. gossypifolia* (Prastiyanto et al., 2020a), and *J. multifidia* (Rahu et al., 2021).

Cancer is a significant global health issue; drug side effects and the high cost of treatments remain the considerable limitations of conventional therapy. Nowadays, it is crucial to develop innovative therapeutic approaches. Consequently, medicinal plants could be used as novel, safe and effective anticancer drugs through their natural constituents (Merrouni & Elachouri, 2021).

Jatropha curcas has been studied in detail due to its potential pharmacological effects, such as antiviral, anti-inflammatory, antibacterial and anticancer properties due to the presence of active constituents in its extracts (Dahake et al., 2013; Ahmed et al., 2020; Solesi et al., 2020; Bastos et al., 2021).

The present study aimed to evaluate the anticancer and antimicrobial activity of the leaves of different *Jatropha* species.

Materials and Methods

Collection and identification of samples

Jatropha curcas, *Jatropha gossypifolia*, and *Jatropha multifida* fresh leaves were obtained from the National Genetic Resources Bank (Gene Bank), Agricultural Research Center, Giza, Egypt, in June 2021. Prof. Abd Elmigid Ali Abd Elmigid kindly provided identification of three species. He is Professor of Flora and Plant Taxonomy, Department of Flora Research and Plant Taxonomy, Horticultural Institute, Agricultural Research Center. After 7-10 days of drying in the shade of a well-ventilated room, the leaves of the three tested species were crushed into a fine powder and used in various investigations.

Plant material extraction and preparation

Two hundred grams of powdered leaves were extracted with methanol HPLC grade (ADVET CHEMBIO PVT.LTD, India) for 24h while the mixture was continuously shaken. Whitman's filter paper (No.1) was used to filter the extracts. The rotary evaporator was used to concentrate the filtrate at 46°C. Finally, at 4 °C, the crude extracts were stored for further experiments (Omoregie & Folashade, 2013).

Phytochemicals analysis

Methanol leaf extracts were screened for their phytochemical constituents (tannins, saponins, terpenes, sterols, flavonoids, and alkaloids) as described by Wall et al. (1954), Woo et al. (1977), Balbaa (1986) and Tiwari et al. (2011).

Antimicrobial assay

Microbial strains

The American Type Culture Collection (ATCC; Rockville, MD, USA) procured the microbial strains. The assayed microorganisms included Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538). Among fungal strains, *Aspergillus niger* NRRL 326 and *Candida albicans* ATCC 10231 were tested in this study.

The agar diffusion method assay determined the antimicrobial assay as described by (Valgas et al., 2007). An inoculum of a bacterial or fungal strain was spread on the surface of the cooled molten Muller Hinton Agar plate (MHA, for bacteria) or Potato Dextrose Agar (PDA, for fungi). The inoculum-containing plates were then drilled into wells using a sterile cork borer. Then, a solution containing plant extracts (50µL) was added to each well. The plates were then incubated at 37°C for 24 to 48h for bacteria, at 25°C for 3 to 5 days for fungus and at 25°C for 24-48h for yeast (*C. albicans*). After incubation, antimicrobial activity was determined by measuring the size of the inhibition zone around the well. DEMSO was used as a negative control. Conventional antibiotics (polymyxin, novobiocin, and nystatin) were used as positive controls to examine the treatments' effects on Gram-positive, and Gram-negative bacteria and fungi, respectively.

Cytotoxicity assay

Cell line and culture

Human Hepatocellular Carcinoma Cells (HepG2) and Human Amnion Normal Liver Cells (WISH) cell lines were obtained from the VACSERA Tissue Culture Unit. RPMI-1640 medium with 10% FBS (Fetal Bovine Serum) and antibiotics (gentamycin, 50 g/ml from Lonza) was used to culture the cell lines.

MTT assay

The MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) assay was used to determine cell viability (Mosmann, 1983). Human cancer cells were seeded at a density of 5x104 cells/well in 96-well microtiter plates with 100µL of fresh culture RPMI-1640 medium. They were incubated at 37°C for 24h with 5% carbon dioxide in a humidified atmosphere to form a partial monolayer. The old growth medium was discarded before being replaced with a new medium containing the test compound at a different concentration (1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 2, 1 and 0.5µg/mL). After incubation for 2 days at 37°C with 5% CO_2, the growth medium was removed and 10µL of the 12mM MTT stock solution (5mg of MTT in 1mL of PBS) was added to each well. After incubation at 37°C for 4h, the MTT reagent was removed and replaced with 50µL of DMSO, which was then thoroughly mixed with a pipette. Control wells contained only the incubation medium without the test compound. The percentage of viability was calculated as [(ODt/ODc)]x100, where ODt is the mean optical density of wells treated sample and ODc is the mean optical density of the untreated sample, using a microplate reader (SunRise, TECAN, Inc. USA). The positive control for this experiment is the standard anticancer drug, vinblastine.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA). The means of the treatments were compared to those of the controls using a two-tailed Student's t-test. We considered a $P< 0.05$ to be statistically significant. The concentration required to inhibit 50% of cancer cells "inhibitory concentration" (IC50) and the concentration required to cause toxic effects on 50% of normal intact cells "cytotoxicity concentration" (CC50) values were determined using GraphPad Prism software (San Diego, CA, USA).

Purification of the crude extract

TLC analysis

Thin layer chromatography (TLC) determines which solvent most effectively separates the compounds from Jatropha curcas crude extracts. Different mixtures of methanol and dichloromethane (in volumes of 10 ml) with different ratios (10:0), (5:5), (7.5:2.5), (5:7.5), and (0:10) were used to create the solvent systems in this investigation. Ultraviolet (UV) light was used to observe the purified compounds, and each spot's retardation factor (Rf value) was recorded.

Column chromatography

Jatropha curcas crude extract (4.6g) was purified by column chromatography (50cm long, 3cm in diameter) on silica gel (60-200 mesh, LOBA CHEMIE PVT.LTD, India). Fifty mL of dichloromethane was used to elute the compounds, followed by 50mL of methanol. In this experiment, HPLC-grade solvents were used.

TLC analysis for the collected fractions

Six different solvent systems were used as mobile phases to separate the fractions obtained from column fractionation. The first solvent system was a mixture of toluene: ethyl acetate: formic acid (7:2:1). Other systems included combination of different ratios of chloroform to methanol (v/v) 1:9, 2:8, 3:7, 4:6, and 5:5. Retardation factors (Rf values) were calculated by observing the compounds under UV irradiation light and with visualization reagents.

TLC Visualization reagents

a) The Drangendorff's reagent was used to check the existence of alkaloids: Bismuth nitrate (0.4g) was dissolved in glacial acetic acid (5mL) to create a solution (A). The mixture was diluted with distilled water (25mL). Solution B consists of 10g of potassium iodide dissolved in 25mL of distilled water. Five mL of solutions A and B were pipetted into a 50mL volumetric flask, and the volume was adjusted with 10% (v/v) aqueous sulphuric acid solution. A volume of 30% H_2O_2 (0.2mL) was added to this mixture (Kokotkiewicz et al., 2017).

b) Vanillin sulphuric acid: solution (1) is 1% vanillin ethanolic solution, and the second solution is 10% sulphuric acid in ethanol. Solution 1 was sprayed on the plate, and solution 2 was sprayed immediately. There

was a 5-10min heating period during which the TLC was heated to 110°C. For terpenoid, this reagent produces purple color for terpenoids (Wagner & Bladt, 1996).

c) Aluminium chloride Reagent: one g of aluminium chloride was dissolved in 100mL of 95% ethanol. This reagent is for identifying flavonoids (Ghosh et al., 1987).

GC/MS analysis

The Trace GC1310-ISQ mass spectrometer examined the two fractions collected from *J. curcas* extract (Thermo Scientific, Austin, TX, USA). A TG-5MS capillary column (30m x 0.25mm x 0.25µm film thickness) was connected to the mass spectrometer. Helium maintained the carrier gas flow rate at 1mL/min. The temperature in the oven was set (at 50°C for 1min, then at a rate of 5°C per minute from 50 to 28°C) and then held isothermally for 20min. An injector port temperature of 260°C was maintained continuously. To automate the injection of the diluted 1µL samples, an autosampler AS1300 coupled with a GC operating in split mode was used. Full scan mode m/z 40–1000 EI, mass spectra were acquired at ionization voltages of 70eV. A temperature of 200°C was selected for the ion source. Components were determined by cross-referencing retention times and mass spectra with those in the WILEY 09 and NIST 11 mass spectral databases (Rao et al., 2017).

Results and Discussion

Preliminary screening of chemical constituents of Jatropha plants

The results of the phytochemical composition of methanol extract of tested *Jatropha* species showed the presence of flavonoids, alkaloids, saponins, tannins,terpenoids and sterols (Félix-Silva et al., 2014a; Evanjelene & Velu, 2021). Flavonoids were absent in the methanol extract of the leaf of *J. multifidia*. Flavonoids have several biological functions, including antibacterial, anti-inflammatory, and antioxidant effects (Ahmad et al., 2015). Mondal et al. (2019) reported the anticancer activity of alkaloids. Terpenes are the most significant phytochemical group with several functions, including antibacterial, antiviral, antioxidant, and anti-inflammatory (Cox-Georgian et al., 2019). These results support the idea that Jatropha species can be used as a medicinal plant for various medical conditions.

Antimicrobial activity of the crude extracts

The leaf methanol extracts from *J. curcas*, *J. gossypifolia*, and *J. multifidia* were tested for their antimicrobial efficacy against Gram (-ve) *Escherichia coli*, Gram (+ve) *Staphylococcus aureus*, and the fungi *Aspergillus niger* and *Candida albicans* Table 1. Maximum antibacterial activity against *E. coli*, as measured by inhibition zone diameter (19.33 ± 1.15mm) was observed in the methanol extract from *J. gossypifolia* leaves. The extract of *J. gossypifolia* was more active against *E. coli* than the control drug, Novobiocin (15mm). These findings corroborate with Gamal El-Din et al. (2022) that *J. gossypifolia* leaf oil was the most effective against *E. coli*. A number of active constituents, including caryophylline oxide and phytol, may be responsible for the antibacterial properties of *J. gossypifolia* leaves (Schmidt et al., 2010; Ghaneian et al., 2015). Only *J. curcas* leaves extract (10.66 ± 0.77mm) suppressed the growth of *S. aureus*. This result was consistent with Sharma et al. (2012) experiment. The traditional medical application of *J.curcas* is supported because it contains secondary metabolites that contribute to antibacterial action (Mahesh & Kaushiki, 2018). *Jatropha* extracts showed more efficiency against *E. coli* than *S. aureus* in this investigation. However, Sokovic et al. (2008) reported that Gram-positive bacteria are more susceptible than Gram-negative bacteria. There was no antifungal activity against *Aspergillus niger*, and *Candida albicans* in methanol leaf extract from the three *Jatropha* species tested (Aiyelaagbe et al., 2007). However, the antifungal activity of *Jatropha* species was reported in previous studies (Gaikwad et al., 2012; Babahmad et al., 2018). The antibacterial efficiency of these extracts may explain the plant's many uses in ethnomedicine (Table 2).

Cytotoxicity activity of crude extracts of Jatropha species

The results of the cytotoxicity activity of *J. curcas, J. gossypifolia* and *J. multifidia* leaf extracts were displayed in Fig. 1. The IC_{50}, CC_{50}, and SI values of extracts used in this study were listed in Table 2. The IC_{50} values for *J. curcas*, *J. gossypifolia*, and *J. multifidia* extracts against HepG2 cell line were 47.2 ± 2.48µg/mL, 15.3 ± 0.95µg/mL and 29.6 ± 1.27µg/mL, respectively. The cytotoxic effects (CC_{50}) of *J. curcas*, *J. gossypifolia*, and *J. multifidia* against WISH cells were 96.5 3.17g/mL, 22.7 1.84g/mL, and 39.5 2.80g/mL, respectively. The leaf extract of

J. curcas appeared to significantly reduce the cell viabilities in the HepG2 cell lines, with the highest selective index (SI value) of 2.04 among the tested extracts. Therefore, this extract was selected for further investigation. This result was compared with vinblastine, which is an alkaloid substance used to treat various types of cancer, most notably Hodgkin's lymphoma, but also non-small cell lung cancer, brain cancer, testicular cancer, melanoma, bladder cancer, and other types of cancer (Cox-Georgian et al., 2019). Like paclitaxel, vinblastine interrupts the cell cycle of cancer cells. In contrast to paclitaxel, which inhibits microtubule assembly, vinblastine prevents microtubule formation (Long & Fairchild, 1994). Vinblastine showed a selective index of (4.4). The HepG2 cell line was more sensitive to *J. curcas* extract than *J. gossypifolia* and *J. multifidia* extracts (Fig. 1, Table 3). The findings lined up with the previous study that reported the potential anticancer activity of methanolic extracts obtained from *J. curcas* root against HT-29 human colon adenocarcinoma and Chang liver cell lines (Ehsan et al., 2011). Zhang et al. (2012) isolated bioactive compounds Curcusone C, D and 4E-jatrogrossidentadion from *J. curcas* root extract; these compounds were responsible for cytotoxic activity toward HepG2. Due to the cytotoxicity efficiency of leaf methanol extract of *J. curcas*, isolation and identification of active constituents were recommended.

TLC analysis

Seven bands with Rf values of 0.125, 0.25, 0.35, 0.475, 0.5, 0.625, and 0.7 cm were clearly separated in a TLC analysis of a methanol extract of *J. curcas* leaves using 100% dichloromethane as the mobile phase. Two fractions were collected by column chromatography. JCF1 was most effectively separated by TLC using toluene, ethyl acetate, and formic acid (7:2:1) as the mobile phase, yielding five bands at Rf values of 0.38, 0.44, 0.52, 0.7, and 0.74 cm. The most efficient separation for JCF2 was obtained using chloroform: methanol (1:1) as a mobile phase, where two bands were obtained at Rf values of 0.72 and 0.88 cm. The spray reagents showed the presence of terpenoids and flavonoids in JCF1, while terpenoids, alkaloids and flavonoids were detected in JCF2.

TABLE 1. Antimicrobial activity of the methanol leaves extract of *J. curcas*, *J. gossypifolia*, and *J. multifidia*

Test strains	Diameter of Inhibition Zones in mm*						
	J. curcas	***J. gossypifolia***	***J. multifidia***	**Polymyxin**	**Novobiocin**	**Nystatin**	**DEMSO**
E. coli	12.66 ± 0.57	19.33 ± 1.15	10	NT	15	NT	NA
S. aureus	10.66 ± 0.77	NA	NA	20	NT	NT	NA
C. albicans	NA	NA	NA	NT	NT	25	NA
A. niger	NA	NA	NA	NT	NT	10	NA

*The values represent average for triplicate analyses ± standard deviation, NA. not active, NT. not tested.

TABLE 2. CC50, IC50, and selective index (SI) for the *J. curcas*, *J. gossypifolia,* and *J. multifidia* extracts as compared with vinblastine*

Treatment	CC_{50}	IC_{50}	SI
J. curcas	96.5 ± 3.17*	47.2 ± 2.48*	2.044
J. gossypifolia	22.7 ± 1.84	15.3 ± 0.95*	1.48
J. multifidia	39.5 ± 2.86	29.6 ± 1.27*	1.33
Vinblastine	8.61± 0.39	1.94 ± 0.17	4.43

CC_{50}: the concentration required to cause toxic effects on 50% of intact normal cells "cytotoxicity concentration", IC_{50}: the concentration required to inhibit 50% of cancer cells "inhibitory concentration", IS: The selective index represents the ratio of CC_{50}/ IC_{50}. All values represent mean ± standard deviation, *P < 0.005 indicating a significant difference compared to the control (Vinblastine).

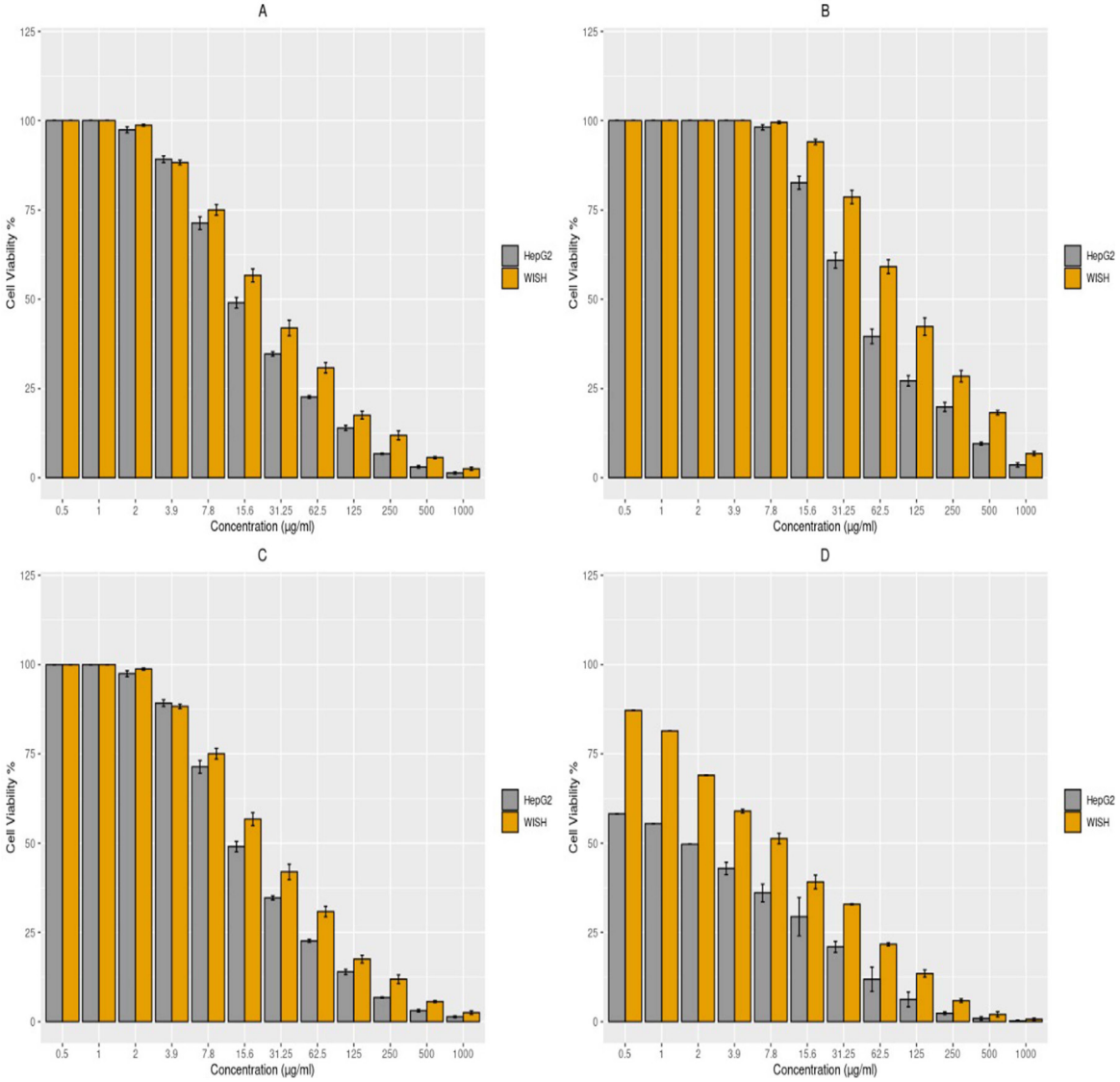

Fig. 1. Effect of methanol leaves extracts of *Jatropha* spp. against HepG2 and WISH cell lines viability: A) *J. gossypifolia*, B) *J. curcas*, C) *J. multifidia* and D) Vinblastine

TABLE 3. Antimicrobial activity of the JCF1 and JCF2 fractions separated from *J. curcas* methanol leaves extract

Microbial strains	Diameter of inhibition zone mm*					
	JCF1	JCF2	Polymyxin	Novobiocin	Nystatin	DEMSO
E. coli	NA	15	NT	15	NT	NA
S. aureus	15	14	20	NT	NT	NA
C. albicans	NA	NA	NT	NT	25	NA
A. niger	NA	NA	NT	NT	10	NA

*The values represent the average for triplicate analyses, NA: not active, NT: Not tested.

Antimicrobial activity of the fractions

The results of antimicrobial activities of JCF1 and JCF2 are displayed in Table 3. Both JCF1 and JCF2 showed antibacterial activity against *S. aureus* with an inhibition zone of 15mm and 14mm, respectively (Fig. 2). However, only JCF2 showed antibacterial activity against *E. coli* with an inhibition zone of 15mm. Our results are in agreement with previous literature that reported the methanolic fraction obtained from latex

and seed cake of *J. curcas* exhibited marked antibacterial activities against Gram-positive and Gram-negative bacteria (Sharma et al., 2016). The findings can be explained by different bacterial strains exhibiting different resistance levels to compounds in the various solvent fractions (Bajpai et al., 2007).

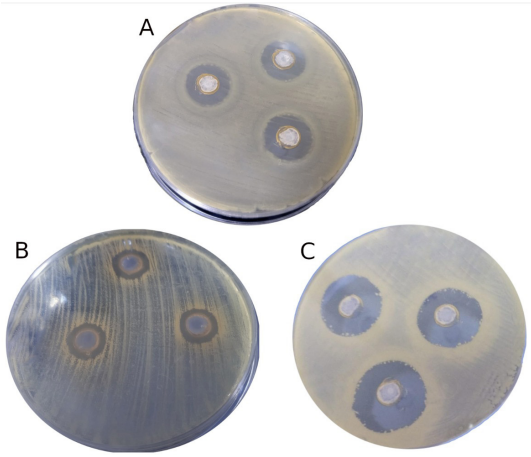

Fig. 2. Antibacterial activity of the fractions of *J. curcas* against *Staphylococcus aureus*, (A): JCF1, (B): JCF2, (C): Polymyxin (positive control)

Cytotoxicity activity of the fractions

The cytotoxicity results for the two fractions of *J. curcas* leaf extract are presented in Fig. 3. Both fractions demonstrated various anticancer effects on the studied cell lines. The cytotoxic activities (IC_{50}) of JCF1 and JCF2 fractions against HepG2 were 25.81± 0.97µg/mL and 119.71± 2.46µg/mL, respectively. The cytotoxic effects (CC_{50}) against WISH cells of JCF1 and JCF2 fractions were 40.59 ± 2.44µg/mL and 208.7 ± 6.63µg/mL, respectively (Table 4). Our findings were consistent with previous studies that reported the crude methanolic extract of *J.curcas* latex had cytotoxic effects against HepG2 with IC_{50} 19.11 ±1.21µg/mL (Ahmed et al., 2020). The seed fractions of *J. curcas* had a growth-inhibiting effect on human hepatocellular carcinoma (HuH-7) and breast cancer (MCF-7) cell lines (Katagi et al., 2017). Nazeema & Girija (2013) reported that the crude methanolic fractions of *J. curcas* stem had antiproliferative activity against the HeLa cancer cell line with an IC_{50} value of 98.18µg/mL.

GC/MS analysis of JCF1 fraction

Twelve compounds were detected in the GC/MS chromatogram of the JCF1 (Fig. 4). Hexadecanoic acid (18.43%), Anethole (18.28%), Estragole (18.28%), Oleic acid (13.67%) and phytol (10.84%) were the major compounds identified in JCF1. Moreover, Carvacrol (7.56%), pentadecanoic acid, 14-methyl-, methyl ester (6.47%), Hexadecanoic acid methyl ester (6.47%), octadecanoic acid, methyl ester (3.42%), α'-Amyrin (2.71%), 9-Eicosyne (2.88%) and elaidic acid (0.79%) were detected in JCF1 (Table. 5). Phytol was found previously as a major component of the *J. gossypifolia* and *J. roseae* leaves oil, representing 10.33%, and 15.25%, respectively (Gamal El-Din et al., 2022). Antimicrobial, antioxidant, and anticancer activities of phytol were reported (Song & Cho, 2015). Anethole and estragole are isomers of monoterpenes; they can be found in the essential oils of aromatic plants and have antimicrobial and anticancer properties (De Vincenzi et al., 2000; Kubo et al., 2008; Carvalho et al., 2015; Lashkari et al., 2020). This study is the first to report the detection of anethole in a methanol extract of *J. curcas* leaf. The hexane extract of *J. curcas* stem bark contained oleic acid as the major compound (Namuli et al., 2011). Carvacrol occurs naturally as a component of essential oils and aromatic plant materials. Carvacrol showed antiviral, antimicrobial and anticancer properties (De Vincenzi et al., 2004; Pilau et al., 2011; Lima et al., 2013; Fan et al., 2015). Several studies have reported antioxidant and antimicrobial properties of the methyl ester of octadecanoic acid and oleic acid (Meechaona et al., 2007; Chen et al., 2011).

GC/MS analysis of JCF2 fraction

GC/MS analysis of JCF2 revealed the presence of 9 compounds (Fig. 4). The compounds detected in JCF2; Hexadecanoic acid (18.05%), Hexadecanoic acid methyl ester (9.50%), Octadecanoic acid methyl ester (3.29%), Anethole (3.02%), Estragole (3.02%), Thymol (1.61%), Carvacrol (1.61%), Benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5 isopropenyl-, trans (1.00%), 4,4'-Dimethyl-2,2'-dimethylenebicyc lohexyl-3,3'-diene (1.44%) (Table. 5). Previous literature reported that hexadecanoic acid methyl ester, 9-octadecanoic acid methyl ester and octadecanoic acid were the major volatile components in the hexane partition of *J. curcas* root (Othman et al., 2015). Thymol and carvacrol were the basic elements of thymus compounds and specific to certain genera of the Lamiaceae family (Kasrati et al., 2014), identified in our study in low concentrations.

This result is in agreement with an investigation by Babahmad et al. (2018) that reported the essential oil of *J. curcas* leaves contains thymol and carvacrol. Antimicrobial, anticancer, and antioxidant properties are just a few of thymol's and carvacrol's biological functions (Kumar & Rawat, 2013; Kang et al., 2016; Miladi et al., 2016). Estragole was previously detected in the essential oil of betel leaf as the major compound (Madhumita et al., 2019), which was detected in our study in low concentration. Francis et al. (2021) reported that hexadecanoic acid, hexadecanoic acid methyl ester and phytol were major compounds identified in the leaf extract of *J. curcas* with antifungal activity.

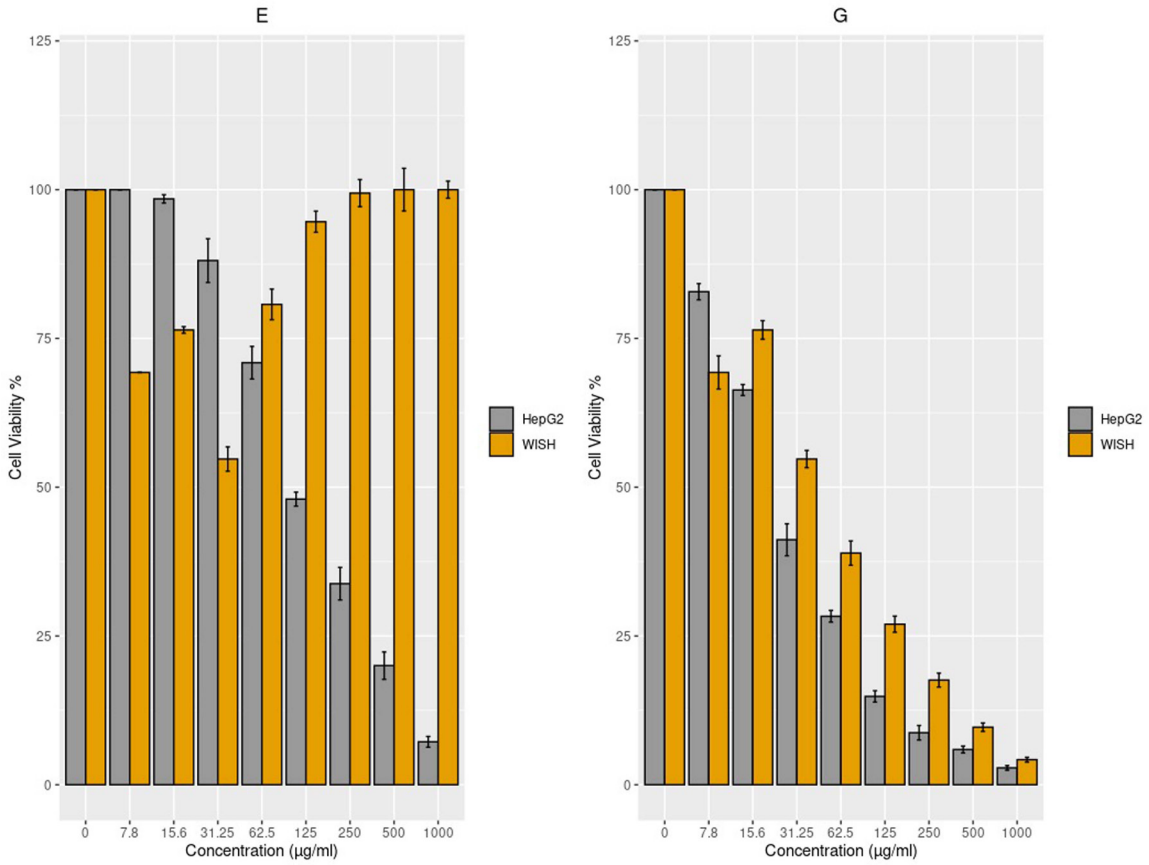

Fig. 3. Effect of fractions obtained from *J. curcas* on HepG2 and WISH cell lines viability. (G): JCF1, (E): JCF2

TABLE 4. CC50, IC50, and selective index (SI) for the JCF1 and JCF2 fractions obtained from *J. curcas* methanol extract as compared with vinblastine *

Treatment	CC_{50}	IC_{50}	SI
JCF1	40.59 ± 2.44	25.81± 0.97	1.57
JCF2	208.7 ± 6.63	119.71± 2.46	1.74
Vinblastine sulfate	8.61± 0.39	1.94 ± 0.17	4.43

CC50: the concentration required to cause toxic effects on 50% of intact normal cells "cytotoxicity concentration", IC50: the concentration required to inhibit 50% of cancer cells "inhibitory concentration", IS: The selective index, represents the ratio of CC50/ IC50. All values represent mean ± standard deviation, *P < 0.005 indicate significant difference compared to the control (Vinblastine sulfate).

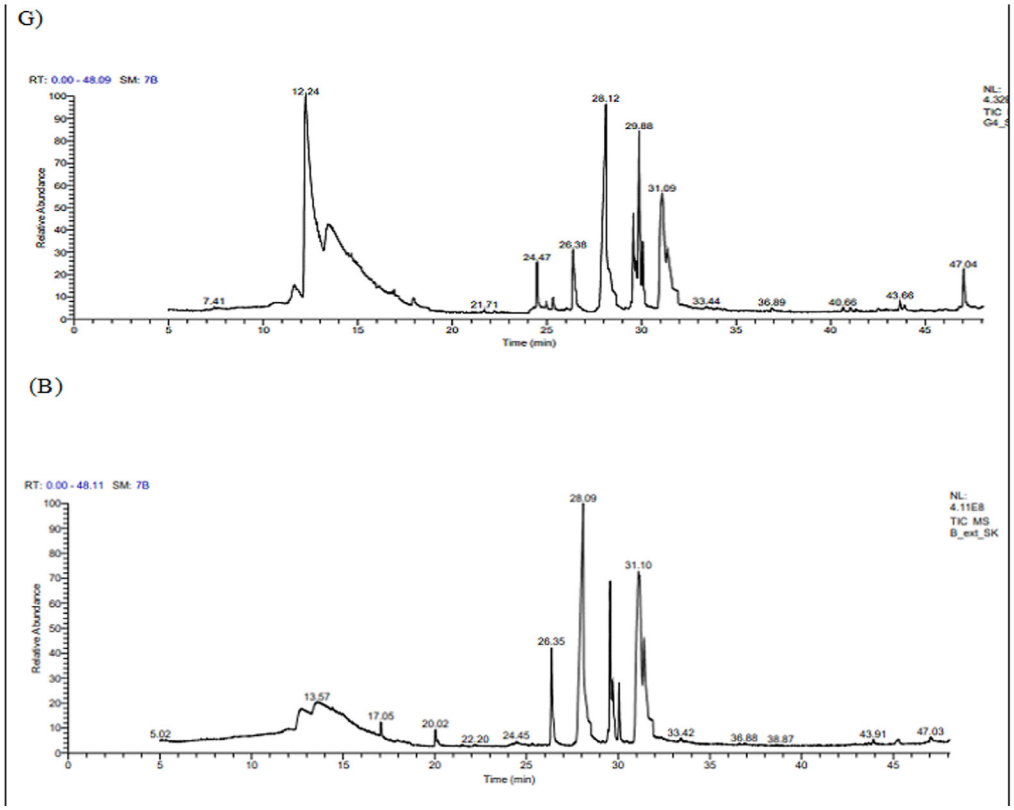

Fig. 4. GC-MS chromatogram of fractions obtained from *J. curcas* methanol leaf extract. (G): JCF1, (B): JCF2

TABLE 5. The phytochemical compounds detected in the JCF1 and JCF2 fractions were obtained from the methanol extract of *J. curcas* using GC/MS Chromatography

Compounds	% Peak area		RT	
	F1	F2	F1	F2
Hexadecanoic acid	18.43	18.05	28.12	27.82
Anethole	18.28	3.02	12.21	12.65
Estragole	18.28	3.02	12.21	12.65
Oleic Acid	13.67	___	31.93	___
Phytol	10.84	___	29.88	___
Carvacrol	7.56	1.61	13.43	13.50
Pentadecanoic acid, 14-methyl-, methyl ester 6.47	6.47	___	26.38	___
Octadecanoic acid, methyl ester	3.42	3.29	30.06	30.04
α'-Amyrin	2.71	___	47.04	___
9-Eicosyne	2.88	___	24.47	___
Elaidic acid	0.79	___	31.93	___
Hexadecanoic acid methyl ester	6.47	9.50	26.38	26.35
Thymol	___	1.61	___	13.50
Benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5isopropenyl-, trans	___	1.00	___	17.05
4,4'-Dimethyl-2,2'-dimethylenebicyc lohexyl-3,3'-diene	___	1.44	___	20.02

A dash (–) indicated no detected , RT: retention time.

Conclusion

The highest anticancer activity was obtained from the methanol extract of *Jatropha curcas* leaves with a selective index of 2.04. This extract also showed the most increased antimicrobial activities. GC/MS analysis of this extract showed the presence of anethole, estragole, phytol, and some fatty acids. Also, it detected the presence of thymol and carvacrol, which were basic elements specific to certain genera of the Lamiaceae family, not the Euphorbiaceae family. Hence, it is necessary to separate the active ingredients from the leaves of *J. curcas* and test each compound for antimicrobial and anticancer activity. The present study suggests using *Jatropha curcas* leaves as a source of antimicrobial and anticancer agents and can be tested as a preservative food source in further investigations.

Acknowledgments: We would like to thank Dr. Nermin G. Mohamed and Dr. Moataz Tarek El Safoury (Faculty of Biotechnology, Misr University For Science and Technology) for their valuable contribution to this work.

Conflicts of interest: There are no conflicts to declare.

Authors' contributions: Zeinab A.S. El-Swaify proposed the idea and the study plan and participated in the data interpretation, manuscript writing and revision. Zeinab M. Saleh participated in all experiments performance, data interpretation, manuscript writing, revision and submission process. Ahmed Z. Abdel Azeiz participated in the chemical analysis of the plants extracts, data interpretation, manuscript writing and revision. Ahmed B.M. Mehany participated in the study plan proposal and the manuscript writing and revision.

Ethics approval: Not applicable.

References

Ahmad, A., Kaleem, M., Ahmed, Z., Shafiq, H. (2015) Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—A review. *Food Research International,* **77**, 221-235.

Ahmed, M., Phul, A.R., Bibi, G., Mazhar, K., Ur-Rehman, T., Zia, M., Mirza, B. (2016) Antioxidant, anticancer and antibacterial potential of Zakhm-e-hayat rhizomes crude extract and fractions. *Pakistan Journal of Pharmaceutical Sciences*, **29**, 895-902.

Ahmed, A.M., Ibrahim, M.M., El-said, M.A.A.E., Elsadek, B.E. (2020) Anti-Cancer Activity of Curcin and Latex Isolated from Jatropha Plant *(Jatropha Curcas* L.). *Journal of Agricultural Chemistry and Biotechnology*, **11**, 339-344.

Aiyelaagbe, O.O., Adeniyi, B.A., Fatunsin, O.F., Arimah, B.D. (2007) In vitro antimicrobial activity and phytochemical analysis of *Jatropha curcas* roots. *International Journal of Pharmacology*, **3**, 106-110.

Akhtar, N., Mirza, B. (2018) Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian Journal of Chemistry,* **11**, 1223-1235.

Balbaa, S.I. (1986) Chemistry of crude drugs. Laboratory Manual. Faculty of Pharmacy, Cairo University, 195p.

Bajpai, V.K., Rahman, A., Kang, S.C. (2007) Chemical composition and anti-fungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Miki ex Hu. *Industrial Crops and Products,* **26**(1), 28-35.

Babahmad, R.A., Aghraz, A., Boutafda, A., Papazoglou, E.G., Tarantilis, P.A., Kanakis, C., et al. (2018) Chemical composition of essential oil of *Jatropha curcas* L. leaves and its antioxidant and antimicrobial activities. *Industrial Crops and Products*, **121**, 405-410.

Bastos, E.M.S., da Silva, A.B., Coelho, P.L.C., Borges, J.M.P., da Silva, V.D.A., da Cunha, V.H.M., Costa, S.L. (2021) Anti-inflammatory activity of *Jatropha curcas* L. in brain glial cells primary cultures. *Journal of Ethnopharmacology,* **264**, 1-12.

Carvalho, A.A., Andrade, L.N., de Sousa, É.B.V., de Sousa, D.P. (2015) Antitumor phenylpropanoids found in essential oils. *BioMed Research International*, **2015**, 1-21.

Cavalcante, N.B., da Conceição Santos, A.D., da Silva Almeida, J.R.G. (2020) The genus Jatropha (Euphorbiaceae): A review on secondary chemical metabolites and biological aspects. *Chemico-*

biological Interactions, **318**, 1-18.

Chen, C.H., Wang, Y., Nakatsuji, T., Liu, Y.T., Zouboulis, C.C., Gallo, R.L., et al. (2011) An innate bactericidal oleic acid effective against skin infection of methicillin-resistant *Staphylococcus aureus*: a therapy concordant with evolutionary medicine. *Journal of Microbiology and Biotechnology*, **21**, 391-399.

Cox-Georgian, D., Ramadoss, N., Dona, C., Basu, C. (2019) Therapeutic and medicinal uses of terpenes. In: "*Medicinal Plants*", pp. 333-359. Springer Cham.

Dahake, R., Roy, S., Patil, D., Rajopadhye, S., Chowdhary, A. (2013) Potential anti-HIV activity of *Jatropha curcas* Linn. Leaf extracts. *Journal Antivirals & Antiretrovirals*, **5**, 160-165.

De Vincenzi, M., Silano, M., Maialetti, F., Scazzocchio, B. (2000) Constituents of aromatic plants: II. Estragole. *Fitoterapia*, **71**(6), 725-729.

De Vincenzi, M., Stammati, A., De Vincenzi, A., Silano, M. (2004) Constituents of aromatic plants: carvacrol. *Fitoterapia*, **75**(7-8), 801-804.

Ehsan, O., Norhani, A., Wan, Z.S., Abdul, R.O., Syahida, A., Wen, B.K., Nor, A.Z., Rudi, H., Yin, W.H. (2011) Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. *Journal of Medicinal Plants Research,* **5**(1), 49-57.

Evanjelene, V.K., Velu, G. (2021) Preliminary phytochemical, proximate and nutrient analysis indifferent leaf extracts of *Jatropha curcas* L. *Indian Journal of Pharmacy & Drugs Studies,* **2**, 7-14.

Fan, K., Li, X., Cao, Y., Qi, H., Li, L., Zhang, Q., Sun, H. (2015) Carvacrol inhibits proliferation and induces apoptosis in human colon cancer cells. *Anti-cancer Drugs*, **26**(8), 813-823.

Félix-Silva, J., Gomes, J.A.S., Barbosa, L.M.Q., Pinheiro, I.T.M.G., Soares, L.A.L., et al. (2014) Systemic and local anti-inflammatory activity of aqueous leaf extract from *Jatropha gossypiifolia* L. (Euphorbiaceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**(6), 142-5.

Félix-Silva, J., Gomes, J.A., Fernandes, J.M., Moura, A.K., Menezes, Y.A., et al. (2018) Comparison of two Jatropha species (Euphorbiaceae) used popularly to treat snakebites in Northeastern Brazil: Chemical profile, inhibitory activity against *Bothrops erythromelas* venom and antibacterial activity. *Journal of Ethnopharmacology,* **213**, 12-20.

Francis, M., Chacha, M., Ndakidemi, P.A., Mbega, E. (2021) Phytochemical analysis and in vitro antifungal evaluation of *Jatropha curcas* against Late Leaf Spot disease on groundnut. *Journal of Animal & Plant Sciences,* **47**(1), 8358-8371.

Gaikwad, R.S., Kakde, R.B., Kulkarni, A.U., Gaikwad, D.R., Panchal, V.H. (2012) In vitro antimicrobial activity of crude extracts of *Jatropha* species. *Current Botany*, **3**(3), 9-15.

Gamal El-Din, M.I., Youssef, F.S., Altyar, A.E., Ashour, M.L. (2022) GC/MS analyses of the essential oils obtained from different *Jatropha* species, their discrimination using chemometric analysis and assessment of their antibacterial and anti-Biofilm activities. *Plants*. **11**(9), 1-18.

Ghosh, P., Sil, P., Thakur, S. (1987) Spray reagent for the detection of coumarins and flavonoids on thin-layer plates. *Journal of Chromatography*, **403**, 285-287.

Ghaneian, M.T., Ehrampoush, M.H., Jebali, A., Hekmatimoghaddam, S., Mahmoudi, M. (2015) Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. *Environmental Health Engineering and Management Journal*, **2**(1), 13-16.

Kang, S.H., Kim, Y.S., Kim, E.K., Hwang, J.W., Jeong, J.H., Dong, X., Lee, J.W., Moon, S.H., Jeon, B.T., Park, P.J. (2016) Anticancer effect of thymol on AGS human gastric carcinoma cells. *Journal of Microbiology and Biotechnology*, **26**(1), 28-37.

Kasrati, A., Jamali, C.A., Fadli, M., Bekkouche, K., Hassani, L., Wohlmuth, H., Leach, D., Abbad, A. (2014) Antioxidative activity and synergistic effect of Thymus saturejoides Coss. essential oils with cefixime against selected food-borne bacteria. *Industrial Crops and Products*, **61**, 338-344.

Katagi, A., Sui, L., Kamitori, K., Suzuki, T., Katayama, T., Hossain, A., Noguchi, C., Dong, Y., Yamaguchi,

F. and Tokuda, M. (2017) High anticancer properties of defatted *Jatropha curcus* seed residue and its active compound, isoamericanol A. *Natural Product Communications*, **12**(12), 1881-1884.

Kokotkiewicz, A., Migas, P., Stefanowicz, J., Luczkiewicz, M., Krauze-Baranowska, M. (2017) Densitometric TLC analysis for the control of tropane and steroidal alkaloids in *Lycium barbarum*. *Food Chemistry*, **221**, 535-540.

Kubo, I., Fujita, K.I., Nihei, K.I. (2008) Antimicrobial activity of anethole and related compounds from aniseed. *Journal of the Science of Food and Agriculture*, **88**(2), 242-247.

Kumar, D., Rawat, D.S. (2013) Synthesis and antioxidant activity of thymol and carvacrol based Schiff bases. *Bioorganic & Medicinal Chemistry Letters*, **23**, 641-645.

Lashkari, A., Najafi, F., Kavoosi, G., Niazi, S. (2020) Evaluating the In vitro anti-cancer potential of estragole from the essential oil of *Agastache foeniculum* [Pursh.] Kuntze. *Biocatalysis and Agricultural Biotechnology*, **27**, 1-7.

Lima, I.O., Pereira, F.D.O., Oliveira, W.A.D., Lima, E.D.O., Menezes, E.A., Cunha, F.A., Diniz, M.D.F.F.M. (2013) Antifungal activity and mode of action of carvacrol against *Candida albicans* strains. *Journal of Essential Oil Research,* **25**, 138-142.

Long, B.H., Fairchild, C.R. (1994) Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase. *Cancer Research,* **54**, 4355–4361

Madhumita, M., Guha, P., Nag, A. (2019) Extraction of betel leaves (Piper betle L.) essential oil and its bio-actives identification: Process optimization, GC-MS analysis and anti-microbial activity. *Industrial Crops and Products,* **138**, 1-12.

Mahesh, P., Kaushiki, O. (2018) Evaluation of antibacterial activity of *Jatropha curcus* seed oil. *International Journal of Life Sciences*, **6**, 335-342.

Meechaona, R., Sengpracha, W., Banditpuritat, J., Kawaree, R., Phutdhawong, W. (2007) Fatty acid content and antioxidant activity of Thai bananas. *Maejo International Journal of Science and Technology,* **1**, 222-228.

Merrouni, I.A., Elachouri, M. (2021) Anticancer medicinal plants used by Moroccan people: Ethnobotanical, preclinical, phytochemical and clinical evidence. *Journal of Ethnopharmacology,* **266**, 1-35.

Miladi, H., Zmantar, T., Chaabouni, Y., Fedhila, K., Bakhrouf, A., Mahdouani, K., Chaieb, K. (2016) Antibacterial and efflux pump inhibitors of thymol and carvacrol against food-borne pathogens. *Microbial Pathogenesis*, **99**, 95-100.

Mondal, A., Gandhi, A., Fimognari, C., Atanasov, A.G., Bishayee, A. (2019) Alkaloids for cancer prevention and therapy: Current progress and future perspectives. *European Journal of Pharmacology,* **858**, 1-16.

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods,* **65**, 55-63.

Namuli, A., Abdullah, N., Sieo, C.C., Zuhainis, S.W., Oskoueian, E. (2011) Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn. extracts. *Journal of Medicinal Plants Research*, **5**, 1-9.

Nazeema, T.H., Girija, S. (2013) Characterisation of the active antiproliferative principles *of Jatropha curcus* and *Jatropha gossippifolia* on Hela cell lines. *International Journal of Pharmacy and Pharmaceutical Sciences,* **5**, 346-355.

Omoregie, E.H., Folashade, K.O. (2013) Broad spectrum antimicrobial activity of extracts of *Jatropha curcas*. *Journal of Applied Pharmaceutical Science*, **3**, 1-5.

Othman, A.R., Abdullah, N., Ahmad, S., Ismail, I.S., Zakaria, M.P. (2015) Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. *BMC Complementary and Alternative Medicine*, **15**, 1-10.

Pilau, M.R., Alves, S.H., Weiblen, R., Arenhart, S., Cueto, A.P., Lovato, L.T. (2011) Antiviral activity of the *Lippia graveolens* (Mexican oregano) essential oil and its main compound carvacrol against human and animal viruses. *Brazilian Journal of Microbiology*, **42**, 1-9.

Prastiyanto, M.E., Tama, P.D., Ananda, N., Wilson, W., Mukaromah, A.H. (2020a) Antibacterial potential

of *Jatropha* sp. latex against multidrug-resistant bacteria. *International Journal of Microbiology,* **2020**, 1-6.

Prastiyanto, M.E., Wardoyo, F.A., Wilson, W., Darmawati, S. (2020b) Antibacterial activity of various extracts of *Averrhoa bilimbi* against multidrug resistant bacteria. *Journal of Biology & Biology Education,* **12**, 163-168.

Rahu, M.I., Naqvi, S.H.A., Memon, N.H., Idrees, M., Kandhro, F., Pathan, N.L., Sarker, M.N.I., Bhutto, M.A. (2021) Determination of antimicrobial and phytochemical compounds of *Jatropha curcas* plant. *Saudi Journal of Biological Sciences*, **28**, 2867-2876.

Rao, K.V.R., Mani, P., Satyanarayana, B., Rao, T.R. (2017) Purification and structural elucidation of three bioactive compounds isolated from *Streptomyces coelicoflavus* BC 01 and their biological activity. *3 Biotech Journal*, **7**, 1-12.

Sabandar, C.W., Ahmat, N., Jaafar, F.M., Sahidin, I. (2013) Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): a review. *Phytochemistry*, **85**, 7-29.

Schmidt, E., Bail, S., Friedl, S.M., Jirovetz, L., Buchbauer, G., Wanner, J., et al. (2010) Antimicrobial activities of single aroma compounds. *Natural Product Communications,* **5**, 1365-1368.

Sharma, A.K., Gangwar, M., Tilak, R., Nath, G., Sinha, A.S.K., Tripathi, Y.B., Kumar, D. (2012) Comparative in vitro antimicrobial and phytochemical evaluation of methanolic extract of root, stem and leaf of *Jatropha curcas* Linn. *Pharmacognosy Journal,* **4**, 34-40.

Sharma, A.K., Gangwar, M., Kumar, D., Nath, G., Sinha, A.S.K., Tripathi, Y.B. (2016) Phytochemical characterization, antimicrobial activity and reducing potential of seed oil, latex, machine oil and presscake of *Jatropha curcas*. *Avicenna Journal of Phytomedicine*, **6**, 366-375.

Sokovic, M., Marin, P.D., Brkic, D., van Griensven, L.J. (2008) Chemical composition and antibacterial activity of essential oils against human pathogenic bacteria. *Food*, **1**, 220-226.

Solesi, O.A., Adesina, F.C., Adebayo-Tayo, B.C., Abiodun, A.S. (2020) Gas chromatography/mass spectrometry (GC-MS) analysis of *Jatropha curcas* latex and its antimicrobial activity on clinical isolates. *World Journal of Advanced Research and Reviews*, **8**, 12-18.

Song, Y., Cho, S.K. (2015) Phytol induces apoptosis and ROS-mediated protective autophagy in human gastric adenocarcinoma AGS cells. *Biochemistry and Analytical Biochemistry*, **4**, 1-7.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H. (2011) Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia,* **1**, 98-106.

Valgas, C., Souza, S.M.D., Smânia, E.F., Smânia Jr, A. (2007) Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology,* **38**, 369-380.

Wagner, H., Bladt, Sabine (1996) Plant Drug Analysis A Thin Layer Chromatography Atlas Second Edition. Springer. 38, 304p.

Wall, M.E., Krider, M.M., Krewson, C.F., Eddy, C.R., Willaman, J.J., Corell, D.S., Gentry, H.S. (1954) Steroidal sapogenins VII. Survey of plants for steroidal sapogenins and other constituents. *Journal of the American Pharmaceutical Association*, **43**, 1-7.

Webster, G.L. (1994) Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden*, **81**, 33-144.

Woo, W.S., Chi, H.J., YunChoi, H.S. (1977) Alkaloid screening of some Saudi Arabian plants. *Korean Journal of Pharmacognosy,* **8**, 109-113.

Zhang, X.Q., Li, F., Zhao, Z.G., Liu, X.L., Tang, Y.X., Wang, M.K. (2012) Diterpenoids from the root bark of Jatropha curcas and their cytotoxic activities. *Phytochemistry Letters,* **5**, 721-724.

النشاط المضاد للسرطان و الميكروبات من مستخلص اوراق نبات *Jatropha*

زينب محمد صالح[1]، احمد زين عبد العزيز[1]، أحمد بلال مهنى[2]، زينب احمد سيد السويفي[3]
[1]كلية التكنولوجيا الحيوية جامعة مصر للعلوم والتكنولوجيا - مدينة ٦ أكتوبر- مصر، [2]قسم النبات والميكروبيولوجي - كلية العلوم جامعة الازهر- القاهرة - مصر، [3]قسم النبات والميكروبيولوجي - كلية العلوم جامعة الازهر(فرع البنات) - القاهرة - مصر.

يعتبر نبات الجاتروفا مصدراً هاماً للعديد من المركبات ذات الانشطة البيولوجية المتنوعة. تهدف هذه الدراسة إلى معرفة التركيب الكيميائي وإلى اختبار النشاط المضاد للسرطان والميكروبات لمستخلص الميثانول لاوراق ثلاث انواع من نبات الجاتروفا *Jatropha curcas* L., *Jatropha gossypifolia* L. and *Jatropha multifidia*. وأظهرت النتائج أن مستخلص أوراق نباتات *J. curcas* and *J. gossypifolia* كانت تحتوي على مركبات تانين، صابونين، قلويدات، فلافونيدات، تربينات وستيرولات. مستخلص أوراق *J. curcas* أظهر أعلى نشاط مضاد لسرطان الكبد وكان معامل الإختيار 2.04 وكذلك نشاط مضاد للبكتريا الموجبة والسالبة لصبغة جرام. تم تنقية هذا المستخلص بواسطة عمود الكروماتوجرافي وتم فصل مستخلصين وتم إختبار النشاط المضاد للسرطان والمضاد للبكتريا لكلا المستخلصين. أظهر كلا من المستخلصين نشاط مضاد للسرطان حيث كان معامل الإختيار 1.74 و 1.57 للمسخلص الأول والثاني على التوالي، بينما أظهر المستخلص الأول نشاط مضاد للبمتريا الموجبة فقط، وأظهر المستخلص الثاني نشاط مضاد للبكتريا الموجبة والسالبة. تم إجراء عمليات الفصل الكروماتوجرافي لمستخلصين باستخدام GCMS. اتضح في المستخلص الأول وجود المركبات التالية: حمض هكساديانويك (18.43%)، أنيثول (18.28%)، إستراجول (18.28%)، حمض أوليك (13.67%)، فيتول (10.84%)، كارفاكرول (7.56%)، كمركبات رئيسية. بينما المستخلص الثاني كان يحتوي على: حمض هكساديانويك (18.05%)، هكساديكانويك ميثيل إستر (9.50%)، أوكتاديكانويك ميثيل إستر(3.29%)، أنيثول (3.02%)، إستراجول (3.02%) ، ثيمول (1.61%)، كارفاكرول (1.61%)، كمركبات رئيسية.