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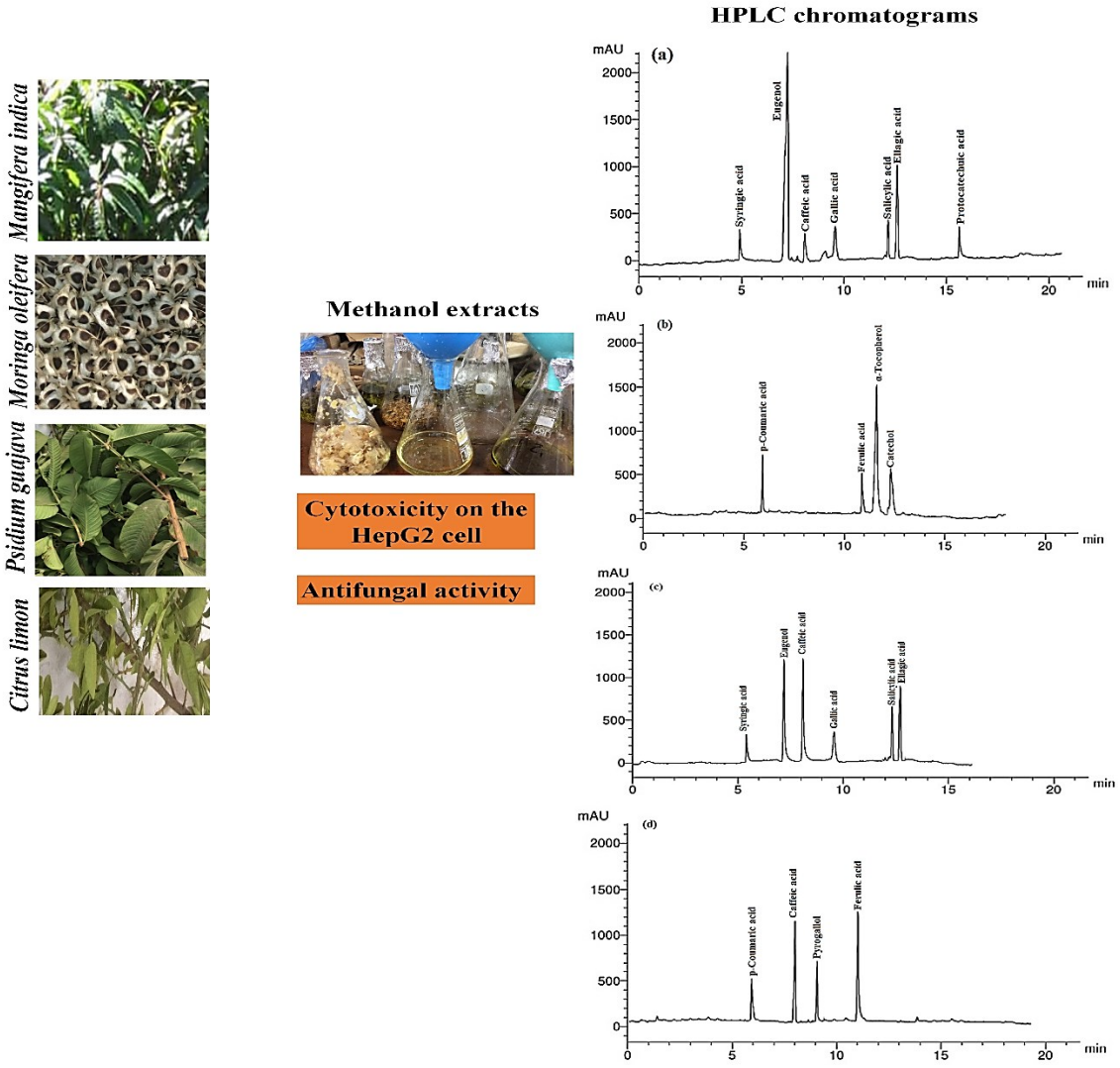
DOI: 10.15376/biores.21.1.1603-1626

GRAPHICAL ABSTRACT



Extracts from Some Tree Pruning Residues and their Cytotoxicity on the HepG2 Cell Line Using the Sulforhodamine-B Assay and their Antifungal Activity

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Methanol extracts (MEs) were obtained from pruning parts of some cultivated plants in Egypt, namely *Mangifera indica* (leaves), *Moringa oleifera* (seeds), *Psidium guajava* (leaves), and *Citrus limon* (branches). The chemical components present in the MEs were identified by HPLC. The cytotoxic activity of the four natural MEs was assessed for the HepG2 cell line after 24, 48, and 72 h of incubation using the sulforhodamine-B (SRB) assay. The antifungal effect was assessed against *Aspergillus flavus* and *Aspergillus terreus*. Based on HPLC, the MEs from *M. indica* leaf extract contained eugenol and ellagic acid; α-tocopherol, and *p*-coumaric acid in *M. oleifera* seeds; eugenol and caffeic acid in *P. guava* leaves; and ferulic acid and caffeic acid in *C. limon* branches. After 24 h of treatment, the EC_{50} for cell growth was 205 µg/mL from *M. indica* leaf ME. At 1000 µg/mL, *M. oleifera* seed ME, *M. indica* leaf ME, and *C. limon* branch ME showed the greatest fungal growth inhibition (FGI) percentages against *A. flavus*. At 1000 and 500 µg/mL *M. oleifera*, *M. indica* at 1000 µg/mL, and *M. oleifera* at 250 µg/mL, MEs exhibited the highest FGI% values against *A. terreus*.

DOI: 10.15376/biores.21.1.1603-1626

Keywords: Anticancer; Antifungal activity; HepG2 cell line; Methanol extracts; Pruning tree residues; Phenolic compounds

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INTRODUCTION

Pruned fruit, ornamental timber, and street trees that are pruned produce large amounts of agricultural leftovers, primarily leaves, branches, and bark, which are rich in biologically active compounds (Aliaño-González *et al.* 2022; Salem *et al.* 2025a,b). Because of their biological activity in preventing cancer and heart disease, bioactive, chemicals such as phenolics, flavonoids, saponins, and essential oils in food have drawn much attention (Kris-Etherton *et al.* 2002).

One important medicinal tree species in the Moringaceae family is the native Indian tree *Moringa oleifera* Lam, and its common name is mango. Mango is one of Egypt's most

valuable fruit crops, grown in large quantities for both the domestic market and export. Research has helped in improving yield and fruit quality, which are vital for the agricultural economy. Mango has spread throughout many nations' tropical and subtropical regions due to its numerous uses as a medicinal and nutraceutical herb (Lin *et al.* 2019; Xu *et al.* 2019; Oldoni *et al.* 2021). In countries including India, Pakistan, and Uganda, *M. oleifera* has long been widely used traditionally to cure a range of diseases, including diabetes, obesity, hysteria, scurvy, and even cancer (Gupta *et al.* 2018). *M. oleifera* was found to exhibit potential bioactivities, including anticancer properties (Al-Asmari *et al.* 2015; Abbassy *et al.* 2020; Pedraza-Hernández *et al.* 2021). Methanolic leaf and seed extracts contain several chemical components that have been shown to help decrease gum bleeding and reduce stress, oxidative damage, wounds, and cancer (Aja *et al.* 2014).

Moringa oleifera is highly resilient and fast-growing, thriving in Egypt's arid and hot climate. Research on this crop promotes sustainable farming practices, especially the use of poor and marginal lands. The plant contains a broad array of phenolic chemicals, primarily glycosides, flavonoids, and phenolic acids. The leaves of *M. oleifera* also include alkaloids, tannins, saponins, glucosinolates, and isothiocyanates (Rocchetti *et al.* 2020; Giuberti *et al.* 2021). It has been established that the content of phenolic compounds in *M. oleifera* leaves was higher than the levels found in the flower and seed (do Nascimento *et al.* 2017). The seeds exhibit distinct antimicrobial, antidiabetic, and anti-inflammatory activities (El-Fakharany *et al.* 2024; Shahbaz *et al.* 2024). Every component of this plant contains tocopherols (both γ- and α-forms), phenolic compounds, *β*-carotene, and vitamin C. As for the proteins, the sulfur amino acids cysteine and methionine are included (Ferreira *et al.* 2008). The largest concentration of potentially helpful substances that could aid in the treatment of specific disorders was discovered to be present in ethyl acetate, ethanol, and methanol extracts from various parts of *M. oleifera* (Prabakaran *et al.* 2018).

Guava (*Psidium guajava* L.) can reach a height of 15 m as a massive spreading shrub or a little tree. Guava is a leading fruit tree for newly reclaimed desert soils in Egypt due to its high adaptability to these challenging conditions. Studying it provides insights into how to successfully cultivate fruit trees in arid environments. The plant is used to cure and prevent scurvy in both Africa and Asia (Jaiarj *et al.* 1999). Guava leaves are widely used in Mexico to prevent diarrhea, and the active components are believed to include quercetin and its glycosides (Lozoya *et al.* 1994). The *P. guajava* leaves have the largest storage capacity of any plant organ for bioactive compounds, including secondary metabolites. Numerous studies have documented the phytochemical profiles and biological activities of guava leaf extracts from various plants (Díaz-de-Cerio *et al.* 2017; Tousif *et al.* 2022; Möwes *et al.* 2025).

Promising antibacterial properties were found in the aqueous and ethanol extracts, particularly in water extracts of *P. guajava*'s leaves, roots, and stem bark (Sanches *et al.* 2005). The leaves of *P. guajava* contain an essential oil rich in cineol, tannins, triterpenes, and flavonoids (Kumar *et al.* 2021b). It has been demonstrated that several of the chemical constituents of *P. guajava* impart cytotoxic, antioxidant, antibacterial, antidiarrheal, antimycobacterial, antihyperglycemic, and antimalarial effects (Roy *et al.* 2006). Therefore, plant leaves, although generally considered agricultural waste, can be valorized as nutraceuticals.

Lemon, or *Citrus limon* (*C. limon*, family: Rutaceae), is a fragrant evergreen shrub that grows worldwide in tropical and temperate climates (Johann *et al.* 2007). Usually, its branches are thorny. As part of Egypt's strong citrus industry, lemon is a commercially important fruit crop that contributes to the national economy through domestic sales and

exports. Lemon cultivation in Egypt faces significant environmental threats, including water scarcity, increased soil salinity, climate change, and various pests and diseases. Studying this resource provides a case study for overcoming these challenges through improved farming practices, genetic selection, and resilient production systems. In traditional medicine, ailments including diabetes, obesity, blood lipid reduction, cardiovascular disease, mental diseases, and certain types of cancer are treated using infusions produced from the aerial (leaf) sections of lemons (Campêlo *et al.* 2011). Instead of focusing on branches, most studies have examined the bioactivity of leaf extracts and peels. Lemon peel extracts are also claimed to possess antibacterial and antifungal characteristics (Saleem and Saeed 2020). The fruit of *C. limon* is rich in bioactive substances, including phenolic acids, flavonoids, terpenes, tannins, fibers, vitamin C, and essential oils, especially limonene (Bruno *et al.* 2014; Rauf *et al.* 2014).

Specifically, phenolic compounds have potential uses in dietary supplements, drugs, pharmaceutical products, *etc*. Syringic acid has been reported to provide a broad spectrum of pharmacological effects, including anti-inflammatory, hepatoprotective, cardioprotective, neuroprotective, antibacterial, antidiabetic, antioxidant, and antiendotoxic effects, and it has extensive therapeutic applications, such as antimicrobial activity and fungitoxicity (Chong *et. al.* 2009; Shi *et al.* 2016; Srinivasulu *et al.* 2018; Shimsa *et al.* 2024). p-Coumaric acid has been observed to act as an antioxidant, anticancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis agent, and its mitigatory effects against diabetes, obesity, and hyperlipaemia have been documented (Pei *et al.* 2016; Wang *et al.* 2022; Tehami *et al.* 2023).

Eugenol is known for its antifungal activity (Salem *et al.* 2025c). Inducing cell death, arresting the cell cycle, and inhibiting migration, metastasis, and angiogenesis in several cancer cell lines are some of the ways eugenol achieves its anticancer actions. Additionally, patients receiving traditional chemotherapy may benefit from eugenol as an adjuvant treatment (Zari *et al.* 2021; Wadood *et al.* 2024). Caffeic acid is an excellent antioxidant, with potential anti-inflammatory and anti-cancer properties (Maurya and Devasagayam 2010; Khan *et al.* 2016; Espíndola *et al.* 2019). Its potential to prevent diabetes, neurological disorders, and enhance sports performance has been studied (Alam *et al.* 2022; Muhammad Abdul Kadar *et al.* 2022). p-Hydroxybenzoic acid is known for several uses, such as a UV stabilizer in plastics, a building block for polymers, and a preservative in medications and cosmetics (Safarova 2022), with various biological properties, *viz.*, antimicrobial, antialgal, antimutagenic, antiestrogenic, hypoglycemic, anti-inflammatory, anti-platelet aggregating, nematicidal, antiviral, antibacterial, and antioxidant (Manuja *et al.* 2013; Wang and Jiang 2022). Pyrogallol has been shown to possess antiproliferative properties against various cancer types (Maheshwari and Sharma 2023). Its primary anticancer mechanism is the stimulation of apoptosis by O_2 overproduction and cell inhibition through the induction of DNA breakage and apoptosis (Rohmawaty *et al.* 2025).

Fruits and vegetables contain gallic acid, a phenolic acid with antiviral, antibacterial, anticancer, and anti-inflammatory properties (Hadidi *et al.* 2024). Gallic acid can neutralize free radicals, reduce oxidative stress, and protect cells from harm due to its strong antioxidant properties (Behera *et al.* 2023; Obafemi *et al.* 2023; Harwansh *et al.* 2024). Ferulic acid is a low-toxicity phenolic acid that the human body may readily absorb and metabolize. Numerous physiological roles of ferulic acid have been documented, including anti-inflammatory, antimicrobial, antithrombotic, anticancer, and antioxidant

properties. Additionally, it reduces cholesterol, boosts sperm viability, and guards against coronary heart disease (Ou and Kwok 2004; Saleem *et al.* 2022). The most powerful and well-known type of vitamin E, a fat-soluble antioxidant vital to human health, is α-tocopherol. It is involved in numerous biological processes and is essential for protecting cells against harm from free radicals (Ungurianu *et al.* 2021; Dasgupta *et al.* 2023). Salicylic acid has been demonstrated to possess antibacterial activity (Song *et al.* 2022) as well as analgesic and antioxidant properties (Sahoo and Paidesetty 2015). Ellagic acid has been investigated for its possible anti-cancer effects and advantages in other areas, such as brain function and skin whitening, in addition to its well-known antioxidant and anti-inflammatory properties (Baradaran Rahimi *et al.* 2020; Golmei *et al.* 2024).

These extracts were investigated for their cytotoxicity on the HepG2 cell line using the sulforhodamine-B (SRB) assay. Additionally, the antifungal effect of these extracts *vs*. *Aspergillus flavus* and *Aspergillus terreus* was assessed. Furthermore, this study sought to propose an approach for reducing agro-waste for bio-based chemicals as analyzed by the HPLC method.

EXPERIMENTAL

Materials

Plant materials and extracts

Pruning parts from four trees were used in the present work, namely, *Mangifera indica* L. (leaves), *Moringa oleifera* Lam. (seeds), *Psidium guajava* L. (leaves), and *Citrus limon* L. (branches). The plants were collected from Cairo, Egypt. These plants were identified and deposited at the Herbarium of the Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, by a botanist and vouched under numbers M457, M458, and M459, for *M. indica*, *P. guajava*, and *C. limon*, respectively, while *M. oleifera* was Zidan0077 (Salem *et al.* 2021).

A small laboratory mill was used to grind all of the components into powder once they had been air-dried. For a week at room temperature, around 50 g of each plant was extracted using the soaking method in 150 mL of methanol (Olayaki *et al.* 2015)

Methods

HPLC analysis of extracts

Phenolic components from the methanol extracts (MEs) were tested by HPLC (Agilent 1100, USA). The analysis consisted of a C18 column (125 mm, 4.60 mm, 5 µm), a binary LC pump, and a UV/Vis detector. The chromatograms were examined using the Agilent Chem-Station. A gradient mobile phase of the two solvents, methanol (solvent A) and 1 part acetic acid in 25 parts of water (solvent B), was chosen for the separation of phenolic acids. The gradient program remained at 100% B for three minutes. After that, the eluent A concentration increased to 80 % for the next 2 min, and then it dropped to 50 % once more for the next 5 min at the 250 nm detection wavelength. Five minutes of 50% eluent A came next. Consequently, this mobile phase was used to confirm standard compounds and define the elution order of phenolic compounds (El-Hefny *et al.* 2023; Alkharpotly *et al.* 2024; Lackner *et al.* 2025). All chemical standards (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cytotoxicity testing

The determination of the cytotoxicity of natural compounds on the HepG2 cell line was conducted using the sulforhodamine-B (SRB) assay (Vichai and Kirtikara 2006; Vajrabhaya and Korsuwannawong 2018). In the cytotoxicity test, the SRB (0.4%) was utilized as a protein dye after dissolution in 1% acetic acid. A 50% trichloroacetic acid (TCA) fixation stock solution was made and kept at 4 °C. A quantity of 50 µL of the stock solution was added to 200 µL of DMEM (Dulbecco's Modified Eagle Medium) in each microplate well. For SRB dye solubility, tris base [tris (hydroxymethyl) aminomethane] was used at a concentration of 10 mM, pH 10.5.

Alternatively, to enable cell attachment to the 96-well plate, cells were seeded at a density of 5×10^3 cells/well in DMEM medium. They were then left for 24 h before being treated with the MEs of *Citrus limon* (branches), *Psidium guajava* (leaves), *Moringa oleifera* (seeds), and *Mangifera indica* (leaves). 1 mL from each of the following prepared extract concentrations (125, 250, 500, and 1000 µg/mL) was applied to the cell monolayer after 24 h. Each concentration was tested in three wells. Using fresh DMEM complete media, the wells were filled to a level of 200 µL per well and incubated for 24, 48, and 72 h. No treatment was given to the control cells. 50 µL of cold 50% TCA was used to fix the cells after they had been incubated for one hour at 4 °C. Following a single wash with deionized water, 50 µL of 0.4% SRB diluted in 1% acetic acid was applied to the wells for 30 min at ambient temperature. After cleaning the wells once with 1% acetic acid, the plates were left to air dry. To remove the protein-bound dye, a 10 mM unbuffered Tris base (100 µL/well) was used. The optical density (OD) of each well was measured at 570 nm by an ELISA (Bio-Rad, Hercules, CA, USA) microplate reader. The cell survival (%) was calculated using Eq. 1 as follows (OD = optical density):

$$\text{Survival fraction} = \frac{OD\ (treated\ cells)}{OD\ (control\ cells)} \quad (1)$$

Dose-response curve-fitting models were used to determine the IC_{50} values, or the concentrations of the natural substance needed to generate 50% inhibition of cell growth (GraphPad Prism software, version 5). The survival curves were used to calculate the IC_{50} for two distinct experiments that were run in triplicate.

Antifungal activity of the extracts

The four extracts were made at concentrations of 1000, 500, 250, 125, 62, and 32 µg/mL by dissolving them in 10% DMSO (dimethyl sulfoxide). Potato dextrose agar (PDA) medium was used for the growth of two common mold fungi, *Aspergillus flavus* AFl375 and *Aspergillus terreus* Y.H. Yeh V0103, with their accession numbers being MH355,958 and MH355,953, respectively (Taha *et al.* 2019). The bioassay was evaluated using the radial growth method at 26 °C with a relative humidity of 65±5 % (Hamad *et al.* 2019). After sterilizing the PDA medium, the concentrated extracts were added and transferred into Petri dishes that had also been sanitized. Fungal mycelial discs of 5 mm diameter and a culture of 7 days of age were placed on the surface of the treated medium for each kind of fungus. All the inoculated plates were incubated at 26 °C, and after the control treatment had finished growing (inoculated plates did not contain plant extracts), the fungal diameter growth was measured in triplicate (Salem *et al.* 2017). Fungal growth inhibition (FGI) was computed as shown in Eq. 2,

$$FGI\ (\%) = [(G_1 - G_2)/G_1] \times 100 \quad (2)$$

where G_1 and G_2 are the average diameters (mm) of the fungal colonies of the treatment and control (10% DMSO), respectively, and FGI is the fungal growth inhibition (%).

Statistical Analysis

Statistical analysis was carried out with GraphPad Prism V6.02 (GraphPad Software Inc., Boston, USA). Tukey post-hoc tests and one-way ANOVA were deployed to examine the data. The mean ± SD of two separate experiments is used to present the data. Statistical significance was defined as a P-value of less than 0.05. For the antifungal activity of the extracts, the two-way ANOVA test was applied with two factors (the source of the methanol extract and the concentration of the methanol extract). The comparison among means was done using Duncan's Multiple Range Test at a 0.05 level of probability.

RESULTS AND DISCUSSION

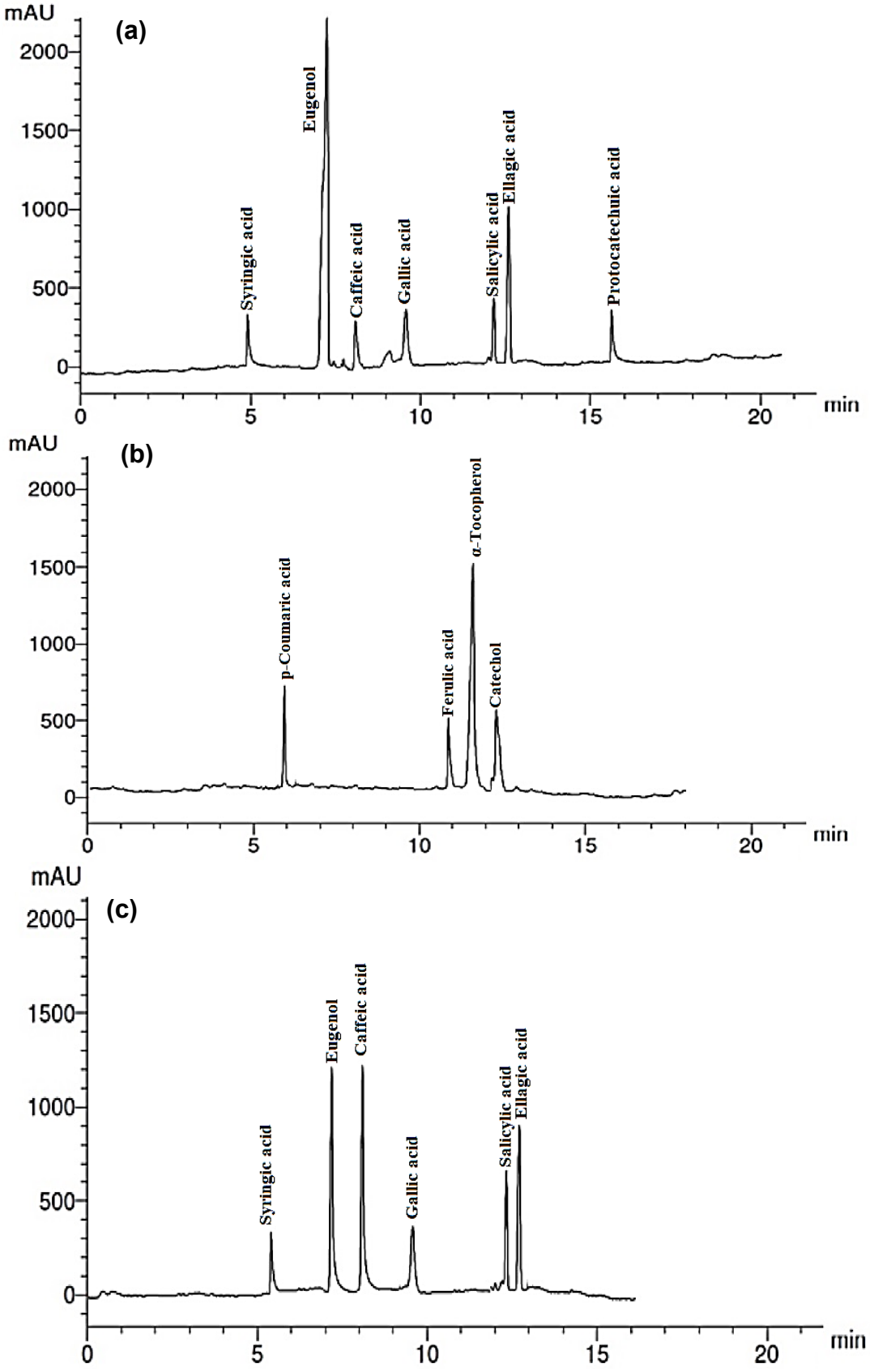
Phenolic Compounds in the Extracts by HPLC

The HPLC analysis (Table 1) revealed that eugenol (28.6 µg/mL), ellagic acid (14.36 µg/mL), salicylic acid (7.12 µg/mL), and gallic acid (6.14 µg/mL) were the most prevalent phenolic components found in *M. indica* leaf ME. The two chemicals with the highest percentages in the ME from *M. oleifera* seeds were *p*-coumaric acid (10.2 µg/mL) and *α*-tocopherol (24.1 µg/mL). Eugenol (16.4 µg/mL), caffeic acid (15.4 µg/mL), ellagic acid (9.66 µg/mL), and salicylic acid (8.47 µg/mL) were among the several phenolic components found in the ME from *P. guava* leaves. Ferulic acid (17.0 µg/mL), caffeic acid (15.3 µg/mL), and pyrogallol (11.4 µg/mL) were the primary chemicals found in the *C. limon* branch ME. Figure 1 displays the HPLC chromatograms of the chemicals found in the four MEs.

Table 1. Phenolic Compounds Identified in the Methanol Extracts

Retention Time (min)	Compound	Concentration (µg/mL)			
		M. indica Leaves	*M. oleifera* Seeds	*P. guava* Leaves	*C. limon* Branches
5.0	Syringic acid	5.12	-	4.33	-
6.0	*p*-Coumaric acid	-	10.23	-	6.12
7.0	Eugenol	28.6	-	16.40	-
8.0	Caffeic acid	5.66	-	15.36	15.34
8.5	*p*-Hydroxybenzoic acid	-	-	-	-
9.02	Pyrogallol	-	-	-	11.42
9.8	Gallic acid	6.14	-	6.78	-
10.5	Ascorbic acid	-	-	-	-
11.0	Ferulic acid	-	5.61	-	17.05
11.5	α-Tocopherol	-	24.13	-	-
12.0	Salicylic acid	7.12	-	8.47	-
13.0	Ellagic acid	14.36	-	9.66	-
12.3	Catechol	-	5.34	-	-
15.7	Protocatechuic acid	3.68	-	-	-

(-): Not detected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(a)
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1500
1000
500
0
Syringic acid
Eugenol
Caffeic acid
Gallic acid
Salicylic acid
Ellagic acid
Protocatechuic acid
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p-Coumaric acid
Ferulic acid
α-Tocopherol
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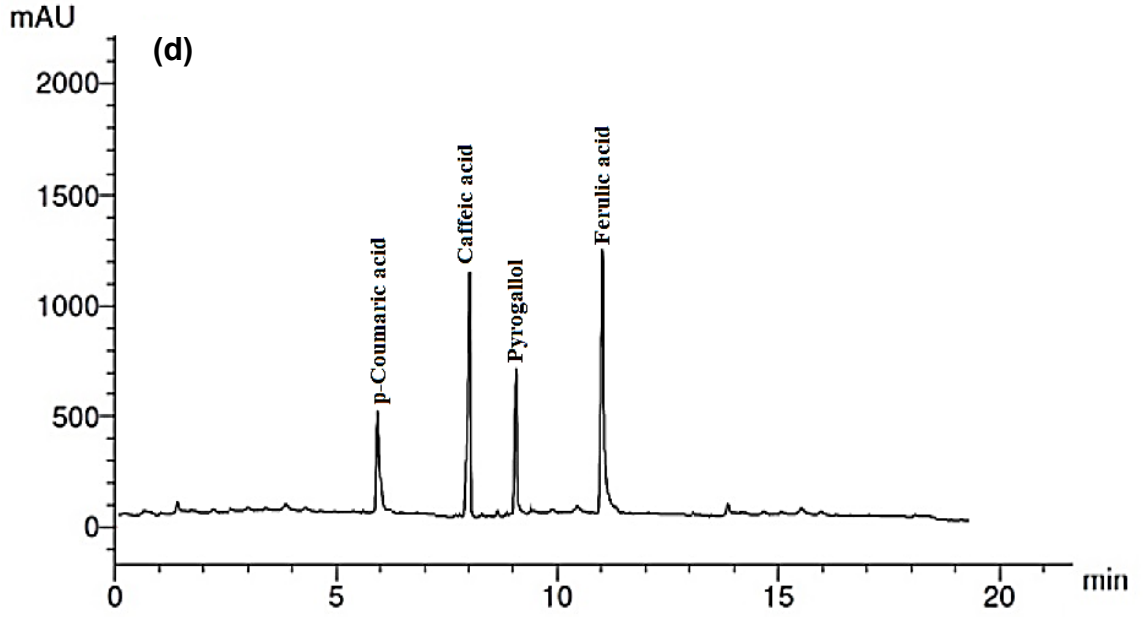

Fig. 1. HPLC chromatograms of the phenolic compounds in the methanol extracts: Extract from *Mangifera indica* leaves (a); *Moringa oleifera* seeds (b); *Psidium guava leaves* (c); *Citrus limon* branches (d). mAU = milli-Absorbance Units (arbitrary units - Y-axis); Retention time (min -X-axis)

Cytotoxicity of Extracts on the HepG2 Cell Line over 24 h to 72 h

The cytotoxic activity of the four natural extracts was determined for the HepG2 cell line after 24, 48, and 72 h of incubation using the SRB assay (Table 2). The surviving fraction was obtained for the cancer cell lines treated by 0, 125, 250, 500, and 1000 µg/mL. The concentration that best caused 50% inhibition of cell growth (IC_{50}) after 24 h of treatment was *M. indica* leaves (205 µg/mL) (Fig. 2a), compared to non-treated cells, followed by *M. oleifera* seeds (305 µg/mL) (Fig. 2b).

M. oleifera seeds (185 µg/mL) and *P. guava* leaves (195 µg/mL) were the extracts that had the greatest effect of cytotoxicity on the HepG2 cell line during a 48-h incubation period (Figs. 3b, 3c). The lemon extract showed no sign of selective cytotoxicity on the HepG2 cancer cell line compared to the other plant extracts.

At the 72-h incubation period (Fig. 4), extracts from *M. indica* leaves and *M. oleifera* seeds exhibited moderate cytotoxicity on HepG2, and *P. guava* leaves showed a weak cytotoxic effect. Lemon extract did not show any cytotoxic effect on the HepG2 cell line.

Table 2. Inhibitory Concentration 50 (IC_{50}) Cytotoxicity Assay for the Four Natural Extracts

Methanol Extract	IC50 (µg/mL) Extracts after Three Time Periods		
	24 h	48 h	72 h
Mangifera indica leaves	205±0.004	220±0.004	445±0.008
Moringa oleifera seeds	305±0.03	185±0.002	490±0.03
Psidium guava leaves	380±0.04	195±0.07	765±0.013
Citrus limon branches	410±0.003	No IC50	No IC50

The absorbance was read at 570 nm. Values are expressed as the mean ± SD of two independent experiments

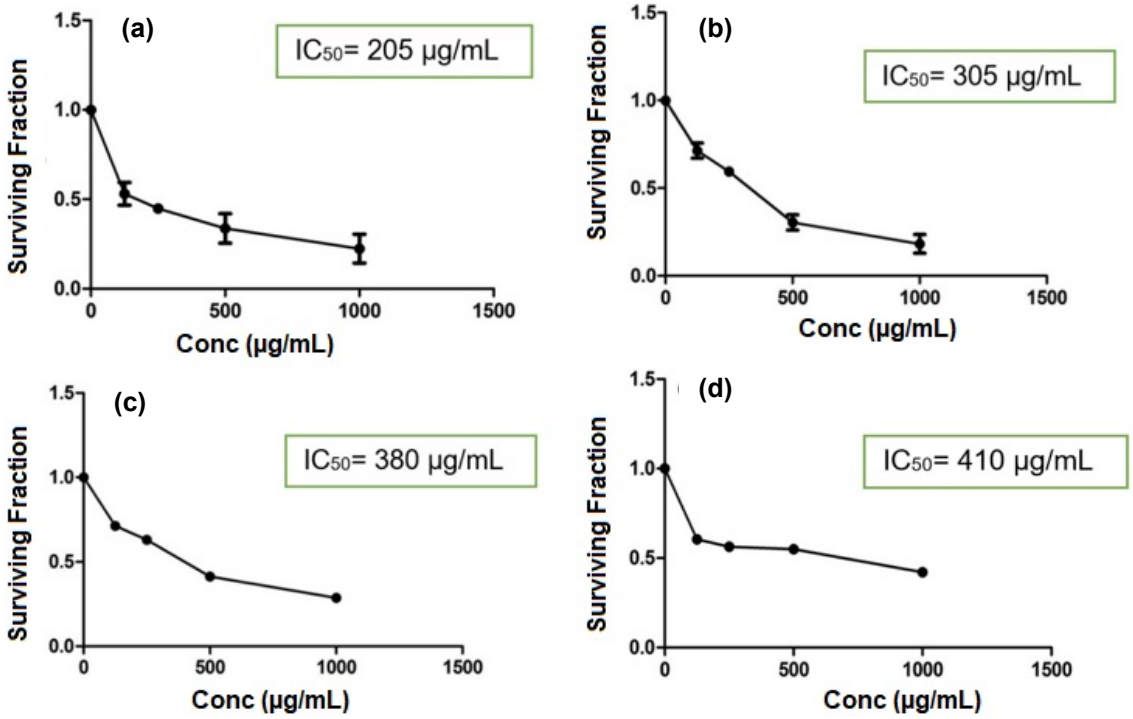

Fig. 2. Cytotoxicity of extracts on HepG2 cell line after 24 h of incubation. a: Extract from *Mangifera indica* leaves (a); Moringa *oleifera* seeds (b); *Psidium guava* leaves (c); *Citrus limon* branches (d)

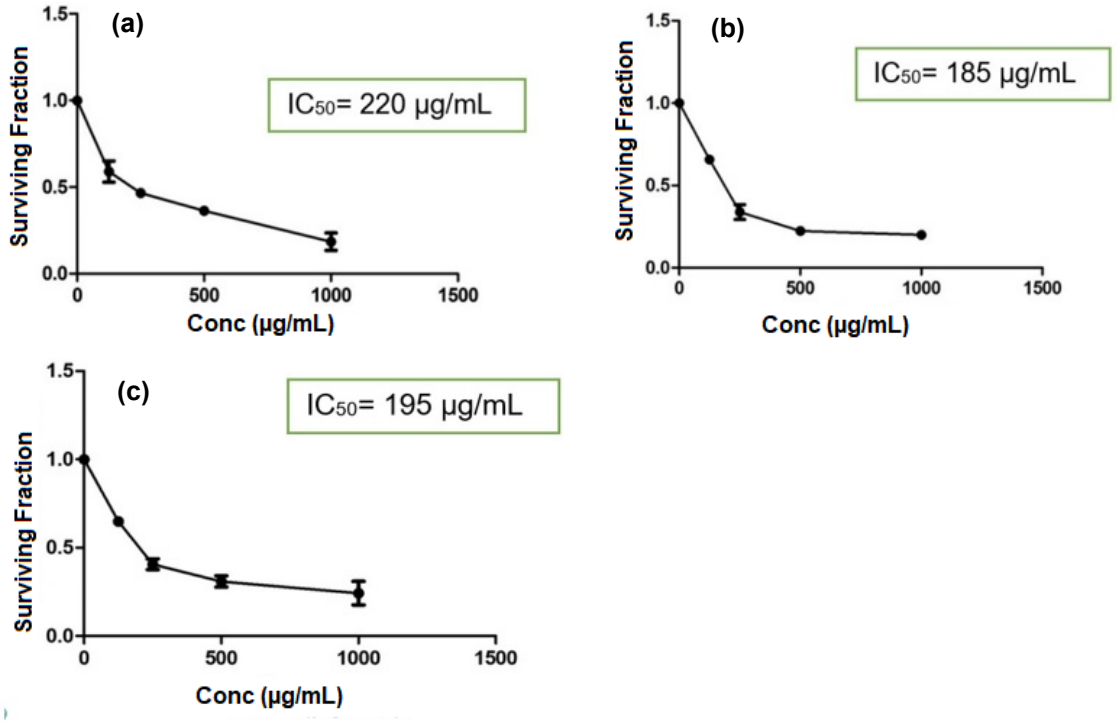

Fig. 3. Cytotoxicity of extracts on the HepG2 cell line after 48 h of incubation. a: Extract from *Mangifera indica* leaves (a); *Moringa oleifera* seeds (b); *Psidium guava* leaves (c)

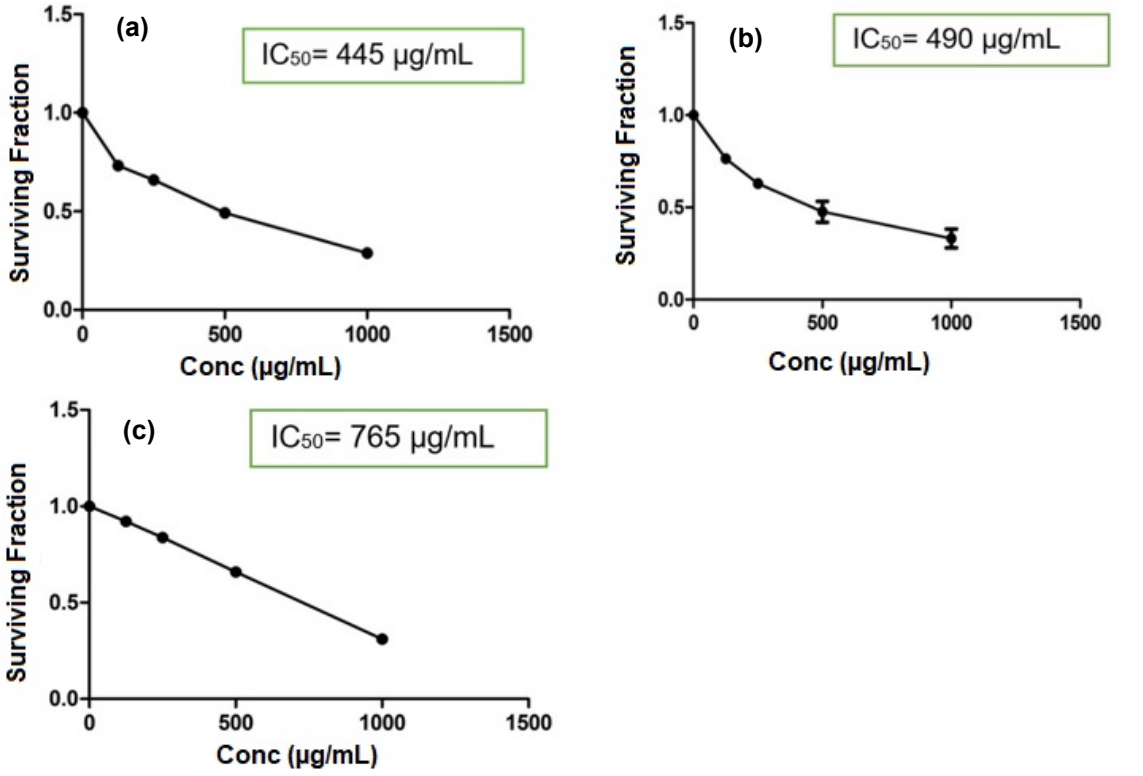

Fig. 4. Cytotoxicity of extracts on the HepG2 cell line after 72 h of incubation. a: Extract from *Mangifera indica* leaves (a); Moringa *oleifera* seeds (b); *Psidium guava* leaves (c)

Antifungal Activity of Extracts

Table 3 shows the percentage of fungal growth inhibition (FGI %) of the MEs *vs*. *Aspergillus flavus* and *Aspergillus terreus*. The strongest FGI from the MEs against *A. flavus* was observed at 1000 µg/mL by *M oleifera* ME from seeds (74.8%), *M. indica* leaf ME (64.8%), and *C. limon* branch ME (64.4%). These were followed by 500 µg/mL from *M. oleifera* seed ME (62.2%), and *C. limon* branch ME (54.4%), as well as *P. guava* leaf ME at 1000 µg/mL (53.7%).

Moringa oleifera seed ME showed the highest FGI percentage values against *A. terreus* growth at 1000 and 500 µg/mL (79.6% and 77.3%, respectively), followed by *M. indica* leaf ME at 1000 µg/mL (75.9%), and *M. oleifera* seed ME at 250 µg/mL (72.6%). These values were followed by *P. guava* leaf ME at 1000 µg/mL (71.1%), *C. limon* branch ME at 1000 µg/mL (70.4%), *M. indica* leaf ME at 500 µg/mL (70.0%), *P. guava* leaf ME at 500 µg/mL (65.2%), and *C. limon* branch ME at 500 µg/mL (61.1%).

Several phenolic compounds were found in the ME of *Citrus limon* (branches), *Psidium guajava* (leaves), *Moringa oleifera* (seeds), and *Mangifera indica* (leaves), including ascorbic acid, pyrogallol, ferulic acid, gallic acid, α-tocopherol, ellagic acid, salicylic acid, syringic acid, eugenol, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid, and catechol. The MEs at 500 and 1000 µg/mL yielded good antifungal activities for *Aspergillus flavus* and *Aspergillus terreus*.

Several phenolic compounds were found in the ME of *Citrus limon* (branches), *Psidium guajava* (leaves), *Moringa oleifera* (seeds), and *Mangifera indica* (leaves), including ascorbic acid, pyrogallol, ferulic acid, gallic acid, α-tocopherol, ellagic acid, salicylic acid, syringic acid, eugenol, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid,

and catechol. The MEs at 500 and 1000 µg/mL yielded good antifungal activities for *Aspergillus flavus* and *Aspergillus terreus*.

Table 3. The Antifungal Activity of the Methanol Extracts against the Growth of *Aspergillus flavus* and *Aspergillus terreus*

Extract	Concentration (µg/mL)	Fungal Inhibition Percentage (%)	
		Aspergillus flavus	*Aspergillus terreus*
Control	0	0.00l	0.00p
Mangifera indica leaves	32	0.00l	0.00p
	62	6.66±2.93k	40.37±4.20k
	125	17.03±2.79j	50.74±1.69ij
	250	28.14±1.69i	51.85±2.79hi
	500	45.92±0.64f	70.00±2.93c
	1000	64.81±1.69b	75.92±0.64b
Moringa oleifera seeds	32	0.00l	0.00p
	62	0.00l	0.00p
	125	30.00±1.11i	56.66±1.11fg
	250	42.96±1.69g	72.59±1.69c
	500	62.22±1.11c	77.41±0.64ab
	1000	74.81±0.64a	79.63±1.28a
Psidium guava leaves	32	4.81±1.69k	36.66±2.22l
	62	17.77±1.11j	48.88±2.22j
	125	28.14±1.28i	52.59±1.69hi
	250	29.62±0.64i	58.14±1.28f
	500	40.74±1.69h	65.18±0.64d
	1000	53.70±0.64d	71.11±2.93c
Citrus limon branches	32	0.00l	6.29±1.28o
	62	0.00l	16.29±0.64n
	125	30.37±0.64i	20.00±1.11m
	250	49.62±1.69e	54.07±1.28gh
	500	54.44±1.11d	61.11±1.11e
	1000	64.44±1.11b	70.37±1.28c

Values are means±SD. Means with the same letter/s within the same column are not significantly different according to Duncan's Multiple Range Test at a 0.05 level of probability.

Several phenolic compounds were found in the ME of *Citrus limon* (branches), *Psidium guajava* (leaves), *Moringa oleifera* (seeds), and *Mangifera indica* (leaves), including ascorbic acid, pyrogallol, ferulic acid, gallic acid, α-tocopherol, ellagic acid, salicylic acid, syringic acid, eugenol, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid, and catechol. The MEs at 500 and 1000 µg/mL yielded good antifungal activities for *Aspergillus flavus* and *Aspergillus terreus*.

The ME from the leaves of mango identified the following phenolic compounds: eugenol, syringic acid, gallic acid, caffeic acid, salicylic acid, ellagic acid, and protocatechuic acid. Mango is a rich source of various polyphenolic compounds, as gallic acid and methyl gallate were detected in young leaves (Barreto *et al.* 2008). Mangiferin, catechins, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid are the main polyphenols in mangos in terms of their antioxidative potential and/or amount (Masibo and He 2008). Phenolic compounds, including gallic acid, sodium gallate, ellagic acid, protocatechuic acid, and methyl gallate, were identified in the crude methanol extract from the leaves of mango (Jhaumeer Laulloo *et al.* 2018).

Mango leaf ME showed promising cytotoxicity on HepG2 after a 24-h exposure to IC_{50} (205 µg/mL). A colon cancer cell line (SW-620), a renal cancer cell line (786-0), and the breast cancer cell lines MCF 7, MDA-MB-435, and MDA-N have all been shown to be significantly cytotoxically affected by the stem bark extract of mango (Muanza *et al.* 1995). The L929 cell lines showed cytotoxic activity in response to different doses of *M. indica* leaf hexane-ethyl acetate extract (Helen *et al.* 2013).

Because of their anti-inflammatory and antioxidant properties, polyphenols found in mango leaf extracts, such as gallotannins, phenolic acids, quercetin, and mangiferin, have chemopreventive actions against a variety of cancer types (Jung *et al.* 2012). Mangiferin is mostly in charge of the anticancer effects of mango leaf extract. It has been shown that these substances inhibit the invasion, migration, and proliferation of some cancers (Klein-Júnior *et al.* 2020). Extracts from mango leaves demonstrated low harm to non-carcinogenic cells and protection against oxidative stress and cytotoxicity. The extracts also demonstrated antitumor effects on (MDA-MB-231) highly and (MCF7) minimally invasive breast cancer cells and (MCF10) non-tumorigenic cells at IC50 >200 µg/mL (Fernández-Ponce *et al.* 2017). In a different investigation, the ethanolic extract of mango leaves had shown cytotoxic effects against liver hepatoblastoma (ATCC HB8065, Hep-G2) at an IC_{50} >200 µg/mL (Ganogpichayagrai *et al*. 2017). At ≥200 µg/mL, mango leaf extract demonstrated cytotoxic capability against every examined cancer cell line, indicating anticancer activity (Ganogpichayagrai *et al.* 2017). Proto-catechuic acid, gallic acid, hyperin, catechin, quercetin, kainic acid, ethyl digallate, ellagic acid, and shikimic acid are among the polyphenols and phenolic acids found in mango leaf extract that can stop the growth of infections (Ediriweera *et al.* 2017). The growth of target fungal species, such as *Aspergillus fumigatus*, *Aspergillus niger*, and *Alternaria alternata*, has been reduced from 56% to 97 % by a compound isolated from mango leaf extract at a concentration of 1000 ppm (Kanwal *et al.* 2010). Strong antifungal activity was demonstrated by the crude leaf extracts against a few specific fungus species, including *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* (Disegha and Akani 2017).

p-Coumaric acid, ferulic acid, α-tocopherol (vitamin E), and catechol were identified in the ME of *M. oleifera* seeds. The seed extract from *M. oleifera* contained *o*-coumaric acid, myricetin, quercetin, kaempferol, resveratrol, naringenin, biochanin A, naringin, and catechin in varying amounts (Prabakaran *et al.* 2018). As previously noted, *M. oleifera*'s antioxidant capability was mainly attributed to polyphenols (Bholah *et al.* 2015). α-tocopherol was the most prevalent isomer in *M. oleifera* and *M. stenopetala* oil, with 75.71 mg/kg and 115.60 mg/kg, respectively (Pluháčková *et al.* 2023). Extracts from *Moringa* seeds, which are a strong source of antioxidants and antibacterial properties, were found to include ten phenolic compounds: gallic acid, *p*-coumaric acid, ferulic acid, caffeic acid, protocatechuic acid, cinnamic acid, catechin, epicatechin, vanillin, and quercetin (Govardhan Singh *et al.* 2013). Derived from hydroxybenzoic and hydroxycinnamic acids, phenolic acids presented in *M. oleifera* extracts are a subgroup of phenolic chemicals with numerous beneficial characteristics, including anti-inflammatory, antioxidant, and anti-cancer effects (Verma *et al.* 2009). Additionally, it was discovered that the seed oil included substantial levels of tocopherols, with α-tocopherol accounting for 51% of the total (Gharsallah *et al.* 2021).

Aspergillus, *Penicillium*, *Rhizopus*, and *Trichoderma* fungal growth species were all somewhat suppressed by all of the *M. oleifera* extracts tested; however, the aqueous extract showed greater inhibitory effects than the organic extracts (Oniha *et al.* 2021). *M.*

oleifera plant parts extracts highly affected the growth of *Fusarium oxysporum*, *Fusarium solani,* and *Alternaria solani*, and *Alternaria alternata* (El-Mohamedy and Abdalla 2014). Depending on the extract dose, seed extracts of *M. oleifera* inhibited the growth of *Fusarium solani* and *Rhizopus solani* (Jabeen *et al.* 2008). In comparison to the seed extract (16.5 mm against *A. flavus* and 16.33 mm against *A. niger*), the leaves provided larger zones of inhibition (18.33 mm against *A. flavus* and 17.2 mm against *A. niger*) at 100 mg/mL for ME (Ayirezang *et al.* 2020). Seed extracts showed potential effects against *Botrytis cinerea,* causing gray mold disease of tomato (*Solanum lycopersicum* L.) (Ahmadu *et al.* 2020).

Syringic acid, eugenol, gallic acid, caffeic acid, ellagic acid, and salicylic acid were found in the leaf ME of *P. guajava*. Guava leaves' bioactive and medicinal qualities are mostly determined by the presence of special bioactive polyphenolic chemicals, including quercetin, other flavonoids, and ferulic, caffeic, and gallic acids (Chen and Yen 2007; Farag *et al.* 2020). Certain phenolic components, including gallic acid, galocatechin, catechin, ellagic acid, naringenin, and quercetin, were detected in the *P. guajava* leaf extract (Bezerra *et al.* 2020; Tousif *et al.* 2022). According to the chromatogram results, phenolic acids including ferulic acid were present in guava leaf extracts and are believed to be the cause of their antioxidant properties (Chen and Yen 2007).

Psidium guajava leaf extract showed promising antifungal activity against the growth of three fungal strains of *Candida* (Piña-Vázquez *et al.* 2017). Flavonoids, steroids, and tannins were among the antimicrobial substances found in the methanolic extract; they could be involved in *P. guajava*'s ability to combat *A. niger* and *Candida albicans* (Dhiman *et al.* 2011). As *P. guajava* leaves are a rich source of phenols, flavonoids, and tannins, the extract showed potent activity against the growth of *A. niger* (Das and Goswami 2019). Phenolic compounds from *P. guajava* leaves showed promising inhibition against the fungal growth of strains of *Candida albicans* and *Candida tropicalis* (Morais-Braga *et al.* 2017). At 50 µg/mL, guava leaf extracts had an over 95% inhibitory effect on the synthesis of α-dicarbonyl compounds, whereas the presence of phenolic components such as gallic acid, catechin, and quercetin showed over 80% inhibitory effects (Wu *et al.* 2009). The isolated compounds from the crude extract were observed to have anticancer and anti-inflammatory activity on NO, TNF-α, and PEG2 production in RAW264.7 cells (Huang *et al.* 2021).

In the present work, phenolic compounds (*p*-coumaric acid, pyrogallol, caffeic acid, and ferulic acid were detected in the *C. limon* branch ME. After 24 h of treatment, this extract showed modest cytotoxicity on the HepG2 cell line; however, after 42 and 72 h, it had no cytotoxic effect. The main phenolic components found in lemon fruits were hesperidin, ferulic acid, eriocitrin, and *ρ*-coumaric acid (Dong *et al.* 2019). The methanol extract of roasted lemon peel powder showed anticancer activity with IC_{50} values to be 240 and 148 µg/mL toward Hep-G2 and MCF-7, respectively (Al-Okbi *et al.* 2024). The plant leaf ethanol extract was discovered to have cytotoxicity against HeLa cell lines and inhibit *Pseudomonas aeruginosa* and *Microvirga aerilata* growth at 50 µg (Raji *et al.* 2017). Most of the previous works about the antimicrobial activities of *C. limon* extracts were carried out using the peels' essential oils and leaves, and observed promising effects (Segaran *et al.* 2020; Mosa *et al.* 2022).

The plant's ability to combat pathogens is based on its compounds in leaf extracts. Different solvents have demonstrated varying solubility capabilities regarding different plant-based constituents (Prabakaran *et al.* 2018). Numerous phenolics, alkaloids, and terpenoids from plants, including gallic acid, chlorogenic acid, caffeic acid, ferulic acid,

quercetin, and kaempferol, are known to exhibit strong antimicrobial effects (Fu *et al.* 2016; Paz *et al.* 2018). Since phenolics interact with proteins and enzymes in microbial cell membranes, their presence may suggest that these substances contribute to antimicrobial activity (Mostafa *et al.* 2018). Furthermore, they deliberately damage the functionality of cell membranes, which inhibits several cellular functions and eventually causes microbes to die (Mostafa *et al.* 2018). The mechanism by which phenolic compounds exert antimicrobial activity involves depleting intracellular ATP levels, depolarizing the plasma membrane, causing cytoplasm leakage, damaging genetic material, and reducing the concentration of microbial protein (Guo *et al.* 2019).

CONCLUSIONS

1. Quantitative results were found the cytotoxicity of methanol extracts from the pruning parts of four trees: *M. indica* (leaves), *M. oleifera* (seeds), *P. guajava* (leaves), and *C. limon* (branches), on the HepG2 cell line using sulforhodamine-B after 24, 48, and 72 h of treatment. After 24 h, the methanol extract from *M. indica* and *M. oleifera* showed the highest cytotoxicity effect with IC_{50} values of 205 and 305 µg/mL, respectively. After 42 h, the methanol extract from *P. guava* and *M. oleifera* showed the highest cytotoxicity effect with IC_{50} values of 195 and 185 µg/mL, respectively.
2. All the extracts worked against *Aspergillus flavus* and *Aspergillus terreus* at 500 and 1000 µg/mL. The efficacy may be explained through bioactive phenolic compounds, as measured by high-performance liquid chromatography (HPLC).
3. Based on HPLC, the main phenolic compounds in the methanol extracts (MEs) from *M. indica* leaf extract were eugenol and ellagic acid; in *M. oleifera* seeds, they were α-tocopherol and *p*-coumaric acid; in *P. guava* leaves, they were eugenol and caffeic acid; and in *C. limon* branches, they were ferulic and caffeic acids.
4. The extracts studied have great potential to serve as a forerunner to newer, safer, and more effective antibacterial drugs. The study's findings aid in investigating the potential for using certain tree wastes for medicinal applications in the future.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Mamoun S. M. Abd El-Kareem, Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Egypt, for his help in HPLC analysis.

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Article submitted: May 16, 2025; Peer review completed: July 25, 2025; Revised version received: August 11, 2025; Further revised version received: December 13, 2025; Accepted: December 25, 2025; Published: January 7, 2026.
DOI: 10.15376/biores.21.1.1603-1626