

## Chemical Composition, Antimicrobial and Cytotoxic Activities of Essential Oils from *Schinus terebinthifolius* Raddi Growing in Egypt

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Received: 17<sup>th</sup> Jun, 18; Revised 29<sup>th</sup> May, 19; Accepted 10<sup>th</sup> Jun, 19; Available Online: 25<sup>th</sup> Jun, 19

### ABSTRACT

*Schinus terebinthifolius* Raddi (Anacardiaceae), has been used traditionally for respiratory ailments, wounds, diarrhea, tumors and leprosy. This study is aimed to analyze the chemical composition of the essential oils from the leaves, fruits and barks of *Schinus terebinthifolius* grown in Egypt and investigate their antimicrobial effect and cytotoxic activities against human liver (HepG2) and colon cancer cells (Caco-2). Gas Chromatography-Mass Spectroscopy (GC-MS) revealed that  $\beta$ -phellandrene was the major component in the fruits and leaves with 32.40% and 19.88%, respectively. The bark contains 53.99% of  $\alpha$ -pinene as major component. Monoterpenes hydrocarbons were the most abundant fraction representing 57.09 % of leaves oil, 78.17%, of fruits oil and 70.44% of barks oil. The essential oils of fruits and barks exerted the most potent inhibitory activity against *Staphylococcus aureus* ATCC 29213 with MIC of 50  $\mu$ g/mL compared to ciprofloxacin. All essential oils exerted potent cytotoxic activities against human liver (HepG2) and colon cancer cells (Caco-2) with IC<sub>50</sub> values ranging from 1.56 to 10.13  $\mu$ g/mL. The essential oils from fruits and barks of *Schinus terebinthifolius* Raddi showed a great importance to be clinically used for various staphylococcal infections. In addition, the tested essential oils have potent abilities to inhibit liver and colon cancer cell viability. Further evaluation is warranted to clarify the mechanism of action.

**Keywords:** Essential oils, *Schinus terebinthifolius*, Anacardiaceae, antimicrobial, cytotoxic.

### INTRODUCTION

*Schinus terebinthifolius* Raddi (family Anacardiaceae), commonly known as pink pepper, is a perennial tree that is native to South America mainly Brazil, Paraguay and Argentina<sup>1</sup>. It has been introduced as an ornamental tree in most tropical and subtropical parts of the world including northern Africa, southern Europe, tropical Asia, New-Zealand and western US<sup>2</sup>. It is widely used in the popular medicine particularly in the native area, as well as it is included in the Brazilian National List of Medicinal Plants of Interest to the Unified Health System<sup>3,4</sup>. In Egypt, *Schinus terebinthifolius* has been cultivated as ornamental species<sup>5</sup>. The leaves has been traditionally utilized as antiseptic poultices for wound healing and skin ulcers<sup>6</sup>. The leaf infusion alleviates the infections of respiratory, digestive, urinary tracts, as well oral candidiasis<sup>7,8</sup>. The macerated roots are considered effective in treating ganglionic tumors<sup>9</sup>. The aqueous methanolic and dichloromethane extracts from *Schinus terebinthifolius* leaves showed an effective inhibitory activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*; the inhibitory activity was attributed to presence of phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones and free steroids<sup>6,10,11</sup>. The bark decoction was used for diarrhea, uterine inflammation, tumors and corneal

diseases<sup>12</sup>. The fruits and their oil showed antimicrobial activity on gram-positive pathogens<sup>13</sup>. The vaginal suppository or the gel containing alcoholic bark extracts are indicated as topical antiseptic for gynecological use<sup>14</sup>. In dentistry, *S. terebinthifolius* is commonly used as effective alternative medicine for oral cavity disorders such as stomatitis, tooth decay and periodontitis<sup>15</sup>. The fruits of *S. terebinthifolius* were used for colds, fungal and bacterial infections, in addition to their essential oil, which imparts a peppery flavor of highly significant economic value<sup>16</sup>. Previous phytochemical studies of this species have revealed the presence of various monoterpenes, sesquiterpenes, triterpenes, flavonoids, tannins, steroidal saponins, sterols, gums, resins and essential oils<sup>17,18,19</sup>.  $\alpha$ -pinene, isolated from the leaves, significantly induced apoptosis on melanoma cells<sup>20</sup>.

The present study is conducted to identify the chemical composition of the essential oils isolated from different parts of *Schinus terebinthifolius* (leaves, fruits and bark) from Egyptian origin and investigate their antimicrobial and cytotoxic activities against liver and colon carcinoma cells.

### MATERIAL AND METHODS

#### Plant material

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Table 1: Chemical composition of essential oils isolated from different parts of *Schinus terebinthifolius* Raddi.

No	Compounds	Retention Index		Composition (%)		
		Calculated	Reported	Leaves	Fruits	Barks
1.	Thujene	923	930	0.90	1.08	–
2.	$\alpha$ -pinene	931	939	17.22	16.68	53.99
3.	Camphene	950	954	0.26	0.17	3.47
4.	Sabinene	972	975	2.12	0.68	–
5.	$\beta$ -Pinene	977	979	4.50	4.26	4.12
6.	$\alpha$ -Myrcene	987	990	0.75	0.92	–
7.	Decane	994	1000	0.62	0.56	–
8.	$\alpha$ -Phellandrene	1005	1002	6.03	10.56	2.84
9.	$\alpha$ -Terpinene	1017	1017	0.65	2.35	–
10.	<i>o</i> -Cymene	1027	1026	0.33	0.74	–
11.	<i>dl</i> -Limonene	1029	1029	2.42	3.74	–
12.	$\beta$ -Phellandrene	1030	1029	19.88	32.40	–
13.	$\alpha$ -Ocimene	1045	1039	–	0.25	6.02
14.	$\gamma$ -Terpinene	1057	1059	1.14	3.03	–
15.	<i>cis</i> -Sabinene hydrate	1072	1070	–	0.09	–
16.	$\alpha$ -Terpinolene	1083	1088	0.27	0.75	–
17.	undecane	1093	1100	0.92	0.85	–
18.	<i>cis</i> - <i>p</i> -menth-2-en-1-ol	1122	1121	–	1.66	–
19.	<i>Trans</i> -1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol	1142	1139	–	1.24	–
20.	Isoborneol	1156	1160	–	0.11	–
21.	Terpinen-4-ol	1178	1177	0.72	11.10	–
22.	$\alpha$ -Terpineol	1193	1188	–	2.59	–
23.	Dodecane	1189	1200	0.59	–	–
24.	<i>trans</i> -Piperitol	1206	1208	–	0.48	–
25.	$\alpha$ -Terpinyl acetate	1345	1349	–	0.27	–
26.	$\alpha$ -Copaene	1376	1374	–	–	3.32
27.	$\beta$ -Elemene	1388	1390	–	–	2.23
28.	<i>E</i> -Caryophyllene	1393	1408	3.36	–	6.71
29.	$\alpha$ -Bergamotene	1411	1412	–	–	2.88
30.	$\gamma$ -Elemene	1433	1436	0.53	–	–
31.	$\alpha$ -Humulene	1456	1454	0.26	–	–
32.	Germacrene D	1482	1485	4.27	0.11	2.57
33.	$\alpha$ -Selinene	1491	1498	0.48	–	–
34.	Bicyclogermacrene	1495	1500	4.39	–	2.46
35.	Germacrene A	1507	1509	2.49	–	–
36.	$\delta$ -Cadinene	1517	1523	0.36	–	–
37.	Selina-3,7(11)-diene	1537	1546	0.20	–	–
38.	Germacrene B	1553	1561	16.76	0.24	–
39.	Spathulenol	1573	1578	0.44	–	–
40.	Globulol	1582	1590	1.03	–	–
41.	Viridiflorol	1589	1592	0.55	–	–
42.	Guaiol	1593	1595	0.30	–	–
43.	Rosifoliol	1602	1600	0.18	–	–
44.	10- <i>epi</i> - $\gamma$ -Eudesmol	1619	1623	–	0.31	–
45.	<i>trans</i> -Muurolol	1637	1642	0.29	–	–
46.	$\alpha$ -Cadinol	1646	1654	1.10	–	–
47.	Juniper camphor	1687	1691	0.44	–	–
	Monoterpene Hydrocarbons			57.09	78.17	70.44
	Oxygenated Monoterpenes			0.72	17.54	–
	Sesquiterpene Hydrocarbons			33.1	0.35	20.17
	Oxygenated Sesquiterpenes			4.33	0.31	–
	Alkanes			1.51	0.85	–
	Total Identified			96.75	97.22	90.61

Different parts of *Schinus terebinthifolius* (leaves, fruits and barks) were collected during the month of April 2017

from the Garden of Mohamed Ali Palace, Giza, Egypt. The plant was kindly authenticated by Mrs. Tereize Labib, the

Table 2: The inhibition zone of the essential oils isolated from leaves, fruits and bark of *Schinus terebinthifolius* Raddi using paper disc diffusion method.

Test microorganisms	Inhibition zone of Essential oil (mm)			
	STL	STF	STB	Ciprofloxacin (5 µg/mL)
<b>Bacterial Strains</b>				
<i>Staphylococcus aureus</i> ATCC 33591	7	6	8	22
<i>Staphylococcus aureus</i> ATCC 29213	8	18	12	25
<i>Escherichia coli</i> ATCC 25922	9	8	8	22
<i>Pseudomonas aeruginosa</i> ATCC 10145	6	6	6	20
<i>Staphylococcus aureus</i> Clinic isolate 1	NIZ	NIZ	6	15
<i>Staphylococcus aureus</i> Clinic isolate 2	NIZ	NIZ	9	18
<b>Fungal strains</b>	STL	STF	STB	Nystatin (25 µg/mL)
<i>Candida albicans</i> MTCC183	NIZ	NIZ	6	18
<i>Aspergillus niger</i> NRRL 595	NIZ	NIZ	NIZ	10

Values are the mean of two replicate, NIZ=No Inhibition Zone, STL=*Schinus terebinthifolius* leaves, STF= *Schinus terebinthifolius* Fruit, STB=*Schinus terebinthifolius* Bark

Taxonomy Specialist at El-Orman Botanical Garden, Giza, Egypt. A Voucher specimen, PHG-P-ST-186, has been deposited at Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University.

#### Essential oil isolation

The fresh parts of examined plant (1kg of each part) were immediately hydro-distilled for 4 hr using Clevenger type apparatus. The resulting essential oil of leaves, fruits and barks were dried separately over anhydrous sodium sulfate, then each pure oil was kept at - 4°C in the dark until further analyzed.

#### Gas Chromatography/Mass Spectrometry (GC/MS)

The GC-MS analyses of the resulting oils were carried out at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25µm film thickness). Analysis was carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 80 C for 2 min; rising at 5.0 C/min to 300 C and held for 5 min. The injector and detector were held at 280° C. 0.2 µL of diluted samples (1:10 hexane, v/v) were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 35-500.

#### Identification of oil components

The components of essential oils were identified by comparison of their Kovats indices with those of reference compounds and by comparison with the reported Kovats indices in the literature data<sup>21,22</sup>.

#### Antimicrobial activity

##### Microbial strains

Six microbial standard strains *Staphylococcus aureus* ATCC 33591, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Candida albicans* MTCC183 and *Aspergillus niger* NRRL 595 were obtained from the Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Egypt. In addition, two

clinical isolate *Staphylococcus aureus* were kindly obtained from Arab Contractors Medical Center.

#### Paper disk diffusion method

The broth cultures of test organisms were freshly prepared for each assay according to the previously mentioned method<sup>23</sup>. Nutrient agar plates 15 ml were prepared and then surface dried at 37 °C for 30 min. The bacterial or fungal inoculum were spread over the surface of the dried agar plates using a sterile glass spreader. The plates were dried, inverted at 37 °C approximately 30 min until the bacterial overlay had dried. 10 µL of essential oils were pipetted on to a 6 mm sterile disc (Whatman filter paper No. 1), then the disc was placed onto the agar plate and incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungal species. Ciprofloxacin (5µg/mL) and nystatin (25µg/mL) were used as positive control for bacteria and fungi, respectively. The diameter of the inhibition zone for each was recorded in mm.

#### Minimum inhibitory concentration (MIC) using dilution method

The series of double fold were performed in 96-well microtitre plates with U shaped wells, using two-fold serial dilutions. 100 µl of essential oil were added to the first well and mixed. The series of double-fold dilutions were done until the last well of the microwell plate and then 100 µl of bacterial suspension was added in respective wells and in control wells. The plates were sealed, placed in plastic bags and incubated at 37°C for 24 h. The MIC is the lowest concentration of extract that exhibited no growth by visual reading.

#### Cytotoxic activity

##### Mammalian cell lines

Human Liver cancer cells (HepG-2) and Human Colon cancer cells (Caco2) were purchased from VACSERA, Egypt.

##### Media of cell line culture

Dulbecco's Modified Eagle's Medium (DMEM) (Bio Wittaker @ Lonza, Belgium) was used for perpetration of growth medium, the liquid medium was supplemented with 10 % heat inactive fetal bovine serum, 10 mM HEPES

Table 3: The Minimum Inhibitory concentration (MIC) of the essential oils isolated from leaves, fruits and bark of *Schinus terebinthifolius* Raddi using dilution method.

The tested Microorganism	Minimum Inhibitory concentration (µg /mL)			
	STL	STF	STB	Ciprofloxacin
<b>Bacterial Strains</b>				
<i>Staphylococcus aureus</i> ATCC 33591	100	100	100	1.56
<i>Staphylococcus aureus</i> ATCC 29213	100	50	50	1.56
<i>Escherichia coli</i> ATCC 25922	100	100	100	3.12
<i>Pseudomonas aeruginosa</i> ATCC 10145	100	100	100	3.12
<i>Staphylococcus aureus</i> Clinic isolate 1	NIZ	NIZ	200	3.12
<i>Staphylococcus aureus</i> Clinic isolate 2	NIZ	NIZ	200	3.12
<b>Fungal strains</b>				
<i>Candida albicans</i> MTCC183	STL	STF	STB	Nystatin
<i>Aspergillus niger</i> NRRL 595	NIZ	NIZ	300	12.5
	NIZ	NIZ	NIZ	25

Table 4: IC<sub>50</sub> (µg/mL) values to essential oil from the leaves, Fruits and barks of *Schinus terebinthifolius* Raddi against hepatic cancer cells (Hep-G2) and colon cancer cells (Caco-2).

	IC <sub>50</sub> of isolated essential oils (µg/mL)		
	STL	STF	STB
Human Cancer Cells			
HepG-2 Cells	9.14	1.56	6.77
Caco-2 Cells	10.13	3.773.25	

buffer (pH 7.3), 2 mM glutamine and (50 µg/ml) gentamycin.

*Evaluation of cytotoxic activity*

The essential oils were evaluated for their cytotoxic activity against two tumor cell lines Human Liver cancer cells (HepG-2) and Human Colon cancer cells (caco-2) using MTT assay<sup>24</sup>. When the cells grown on 75 cm<sup>2</sup> tissue culture flasks reached confluence (usually 24 hr), the cell suspension of the two tumor cell lines were prepared in complete growth medium (DMEM). Aliquots of 100 µl of cell suspension were added to each well on a 96- well tissue culture plate. The blank wells contained medium in place of cell suspension. The cells were incubated for 24 h at 37 °C in humidified atmosphere of 5 % CO<sub>2</sub>. After the formation of a complete monolayer cell sheet in each well of the plate, the medium was aspirated and replaced with DMEM with 2% fetal bovine serum. Then, the volatile oil was dispensed into 96- tissue culture plate at 100 µl/well. Another set of well was kept for including wells of cell controls as negative control. Serial three fold dilutions of the volatile oil was added into a 96-well sterile tissue culture plate using multichannel pipette (eppendorff, Germany). The treated and untreated cells were covered with a plate sealer then allowed to grow and proliferate by further incubation of the plate for 24 hrs at 37 °C in humidified atmosphere of 5 % CO<sub>2</sub>. At the end of incubation the plate was examined using the inverted microscope.

*Determination of viable cells*

The number of survival cells was determined by staining with MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) which reduced by metabolically active cells. After the end of incubation period, the plates containing the treated and untreated cells were inverted to remove the medium. Then, the wells were

washed by 100 µl of PBS and then the cells were fixed with 10 % formalin for 15 min. at room temperature. The cells were then stained with 100 µl of MTT for 20 min. the excess of stain was removed and the plates were rinsed with de-ionized water, then dried. To obtain quantitative data the dye was extracted from the cells by adding glacial acetic acid (33%) to each well and mixed the contents of each well before reading the absorbance on the ELIZA reader (SunRise TECAN, Inc.®, USA) at 490 nm. The absorbance is proportional to the number of survival cells in the culture plate [43]. The percentage cell viability was calculated using the Microsoft Excel ® version 2010. Percentage cell viability was calculated as follows:

% Cell viability =

$$\frac{\text{Mean Abs} - \text{Mean Abs plant extract}}{\text{Mean Abs control}} \times 100$$

Where: Abs absorbance at 490 nm cell quantity

**RESULTS**

*Chemical composition of the essential oils*

The chemical composition of the essential oils from the leaves, fruits and barks of *Schinus terebinthifolius* are shown in Table 1. The total identified components of leaves, fruits and bark were 96.75%, 97.22% and 90.61%, respectively. The monoterpene hydrocarbons showed predominance in all parts of the plant such as α-pinene, camphene, β-pinene, α-ocimene, germacrene D and α-phellandrene, in addition to minor sesquiterpenes. The major components of leaf essential oil were β-phellandrene (19.88%) followed by α-pinene (17.22%), then germacrene B (16.76%) while β-phellandrene and α-pinene were found as major components with 32.40% and 16.68 %, respectively followed by terpinen-4-ol (11.01%) in essential oil of fruits. The essential oil of bark contains higher quantity of α-pinene (53.99%) and lacks β-phellandrene.

*Antimicrobial activity*

The antimicrobial evaluation of *Shinus terebinthifolius* leaves, bark and fruits volatile oils are shown in Table 2 and 3. The results indicated that the essential oil isolated from *S. terebinthifolius* fruits (STF) exhibited the most potent antimicrobial activity against *Staphylococcus aureus* ATCC 29213 with inhibition zone of 18 mm compared with ciprofloxacin (25 mm). In addition, the oil isolated from *S. terebinthifolius* bark (STB) showed the

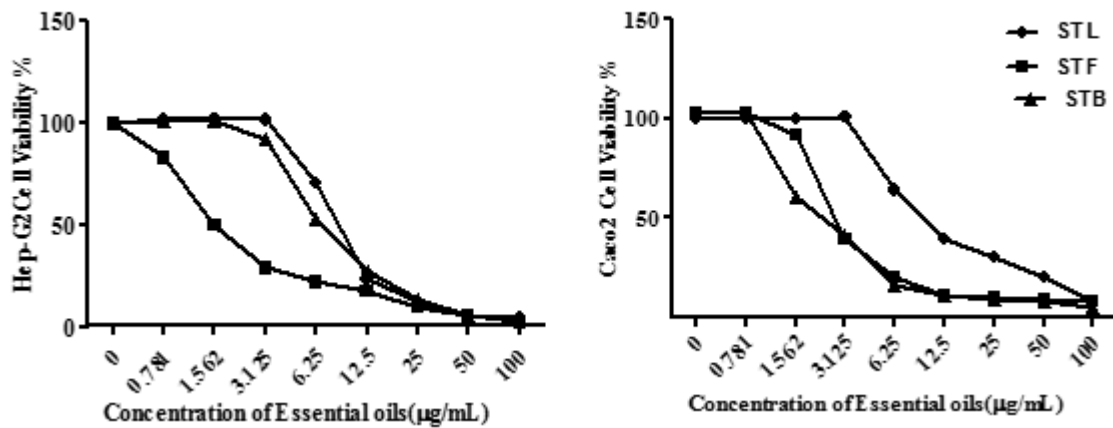


Figure 1: The effect of different concentrations of essential oils from the leaves, fruits and barks of *Schinus terebinthifolius* on the cell viability of Hep-G2 and Caco2 cells.

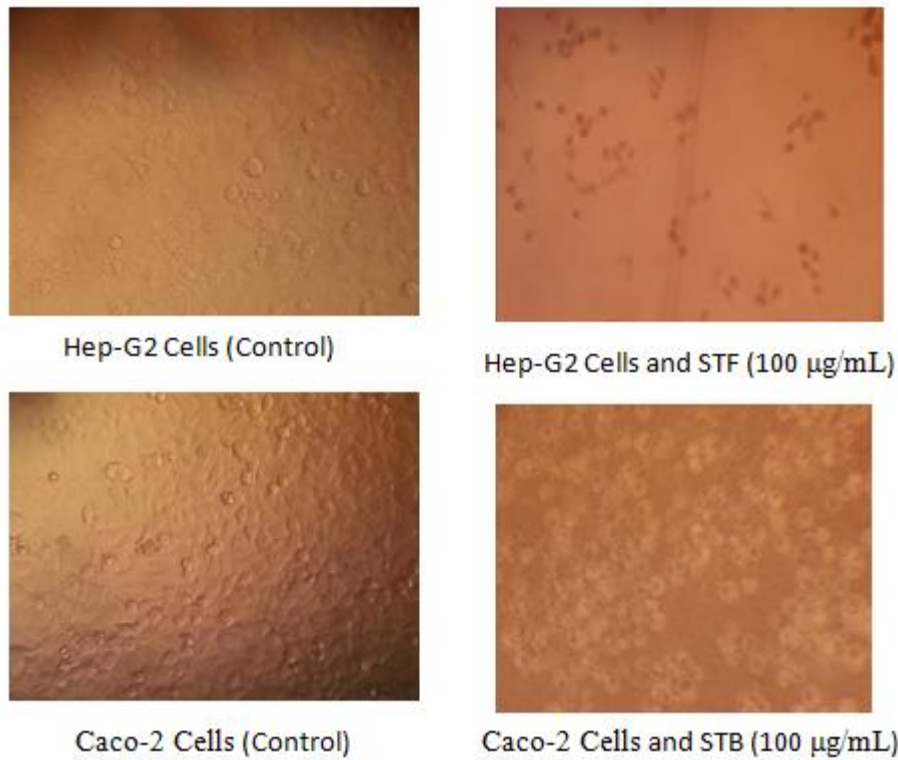


Figure 2: The structural changes in the HepG2 and Caco2 cancer cells due to the cytotoxic effect of essential oil.

most potent inhibitory effect on the tested bacteria including the two clinical isolates of *Staphylococcus aureus* and fungal organisms of *C. albicans* MTCC183 with inhibition zone of 6 mm, 9 mm and 6 mm, respectively. Also, the results of dilution method showed that the essential oils from STF and STB were the most potent as antibacterial effect against *Staphylococcus aureus* ATCC 29213 with MIC 50 µg/mL. Also, the results showed that the oil of STB exhibited antifungal activity against *C. albicans* with MIC of 300 µg/mL compared with nystatin 12.5 µg/mL while other essential oils did not show any antifungal activity.

*Cytotoxic activity against human liver cancer cells (HepG-2) and human colon cancer cells (Caco-2)*

The detected HepG2 and Caco-2 cell line viabilities are shown in Table 4 and Figure 1. The results indicated that the essential oil from the fruits of *Schinus terebinthifolius* fruit (STF) inhibited 97.7% of HepG2 cancer cells at a concentration of 100 µg/mL. Similarly, the essential oils from *Schinus terebinthifolius* bark (STB) and leaves (STL) inhibited HepG2 cell line viability by 96.93% and 95.23%, respectively, at the same concentration. Regarding the results of IC<sub>50</sub> in HepG2 cell line, the essential oil from *Schinus terebinthifolius* fruit (STF) showed the highest cytotoxic effect with IC<sub>50</sub> of 1.562 µg/mL followed by STB and STL with IC<sub>50</sub> of 6.77 and 9.14 µg/mL, respectively. Concerning to Caco-2 cell line, the results showed that STB exerted the highest

cytotoxic effect with  $IC_{50}$  of 3.25  $\mu\text{g}/\text{mL}$  followed by STF and STL with  $IC_{50}$  of 3.77 and 10.13, respectively. These results were confirmed by structural changes in the HepG2 and coca-2 cell lines as shown in figure 2 where incubation of HepG2 cells with STF caused vacuolization of cells and shrinkage of cell size. Also, the cytopathic effects were apparent in coca-2 cells with STB evidenced by distortion, degeneration of cells and cell death pattern.

## DISCUSSION

Plants are globally known as a natural therapeutic source for the treatment and prevention of diseases where more than 35,000 plant species are utilized for medicinal purposes around the world<sup>25</sup>. The family Anacardiaceae comprises about 82 genera and 700 species that are used traditionally as a healing, stomachic and anti-diarrheal agents, due to tannins and essential oil contents<sup>26</sup>. *Schinus terebinthifolius* Raddi (Anacardiaceae) is widely found in Northeast region of Brazil and has been used in folk medicine to alleviate respiratory infections<sup>10</sup>. The essential oils obtained from leaves collected in several areas of Brazil are composed of monoterpene hydrocarbons, mainly  $\alpha$ -pinene, limonene and p-cymene, as well as of sesquiterpene hydrocarbons with germacrene D and bicyclogermacrene as major components which are similar to the analyzed oil<sup>27, 28</sup>. The chemical composition of fruits oil is similar to that of Tunisian species which contains  $\alpha$ -phellandrene (34.38%),  $\beta$ -phellandrene (10.61%),  $\alpha$ -pinene (6.4%)<sup>29</sup> while it is different from that of Brazilian species where  $\delta$ -3-carene represented the higher quantity<sup>30</sup>. A previous study on the Egyptian fruits revealed presence of elixene (15.18%),  $\alpha$ -pinene (15.01%), germacrene D (14.31%) as major constituents<sup>31</sup>. The nature of essential oils and their quantities are significantly affected by abiotic factors such as light intensity, temperature, nutrition level and water availability which differ from country to another<sup>32</sup>. This is the first study to investigate the chemical composition of bark oil of *Schinus terebinthifolius* Raddi in Egypt. The results indicated that the oil of STF exhibited the most potent antimicrobial activity against *Staphylococcus aureus* ATCC 29213, these results corroborate with other previous studies in which the alcoholic fruit extracts of *S. terebinthifolius* Raddi exhibited inhibitory effect on *S. aureus* ATCC 6538 and *Bacillus cereus* ATCC 11778<sup>33</sup>. Also, it was found that 70% hydroalcoholic extract of *S. terebinthifolius* barks significantly inhibited growth of *S. aureus* ATCC 6538 with MIC of 35.3 mg/mL<sup>34</sup>. Furthermore, the essential oil of leaves from Brazil exhibited potent antibacterial activity against staphylococcal isolates from dogs with otitis externa with MIC of 78.1 mg/mL<sup>35</sup>. In addition, the essential oil of fresh leaves from Zimbabwe significantly inhibited *Yersinia enterocolitica*, *P. aeruginosa*, *E. coli*, *Acinetobacter calcoaceticus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Bacillus subtilis* with at least 58% inhibition compared to the positive control<sup>36</sup>. The monoterpenes of essential oils causes expansion of the bacterial cell membrane increasing the fluidity and permeability leading to disturbance in membrane proteins, inhibition of cell respiration and dysregulation of ion

transport process<sup>37,38</sup>. This is in agreement with our studies that showed that STL, STF and STB are rich in monoterpenes hydrocarbons with 57.09%, 78.17% and 70.44%, respectively. The monoterpenes are most likely responsible for the antimicrobial activity presented by the essential oil tested, either by acting alone or acting synergistically with other oil constituents<sup>39,40,41</sup>.

Concerning to the cytotoxic activity, the essential oil of fruits originating from Tunisia was effective against human breast cancer cells (MCF-7) with  $IC_{50}$  of 47  $\mu\text{g}/\text{mL}$ <sup>29</sup>. The previous studies confirmed the apoptotic and antimetastatic activity of  $\alpha$ -pinene isolated from fruits of *Schinus terebinthifolius* Raddi that significantly induced cell apoptosis causing disruption of the mitochondrial potential, production of reactive oxygen species, increase in caspase-3 activity, heterochromatin aggregation, DNA fragmentation and exposure of phosphatidyl serine on the cell surface<sup>20</sup>. Thus, the cytotoxic activity is attributed to presence of  $\alpha$ -pinene as a major component. Also, the essential oil of leaves showed cytotoxic effect against murine melanoma cell line (B16F10-Nex2) and human melanoma (A2058), breast adenocarcinoma (MCF7), human leukemia (HL-60) and cervical carcinoma (HeLa) cell lines attributing to  $\alpha$ - and  $\beta$ -pinene<sup>42</sup>. The literature reported that the juice of pressed root and bark oils were an effective alternative medicine for the treatment of tumors and leprosy<sup>9</sup>. The above results indicated that all the isolated essential oils on HepG-2 and Caco-2 cell may be promised for further studies as new antitumor drugs and for isolation of bioactive compounds from these oils.

## CONCLUSION

The essential oils from fruits and barks of *Schinus terebinthifolius* Raddi showed a great importance to be clinically used in the treatment of various staphylococcal infections. In addition, the tested essential oils have potent abilities to inhibit liver and colon cancer cell viability. Further evaluation is warranted to clarify the mechanism of action.

## CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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