Journal of Pharmacy and Pharmacology 9 (2021) 378-386
doi: 10.17265/2328-2150/2021.11.004

Chemical Composition,Cytotoxic and Anti-arthritic Activities of Hexane Extracts of Certain *Schinus* Species

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Abstract: The genus *Schinus* (Anacardiaceae) comprises about 29 species that are utilized in both traditional and current medicine to alleviate various disorders such as rheumatism, bronchial infections, stomach upset, menstrual disturbance, bronchitis, and conjunctivitis. In this study we aimed to investigate the chemical composition of hexane extracts obtained from *Schinus polygamous* and *Schinus terebinthifolius* leaves as well as their cytotoxic and anti-arthritic activities. The GC-MS analysis of *S. polygamus* leaves revealed identification of fifty-five components: being betulin (6.54%), phytol acetate (5.27%), lupeol (3.96%), palmitic acid methyl ester (3.58%) and heptacosane (3.25%). While, seventeen compounds were identified in *S. terebinthifolius* leaves, predominately characterized by phytol (14.70%), lupeol (13.33%), linolenic acid methyl ester (11.17%), palmitic acid methyl ester (7.74%), and 28-oxours-12-en-3-yl acetate (7.44%). Both extracts showed moderate cytotoxic activities against hepatocellular carcinoma cells with IC_{50} values of 102.65 and 65.55 µg/mL, respectively. While colorectal carcinoma cell line presented IC_{50} values of 217.78 and 83.55 µg/mL, respectively. Further, both extracts provided moderate anti-arthritic effects (IC_{50}: 80.12 and 63.09 µg/mL, respectively) using protein denaturation test, compared to diclofenac sodium (IC_{50} = 15.12 µg/mL).

Key words: *Schinus polygamous*, *Schinus terebinthifolius*, Betulin, Phytol, Cytotoxic, Anti-arthritic.

1. Introduction

The genus *Schinus* L. (Anacardiaceae) covers about 29 species found in Mexico, Argentina, South America, Bolivia, Chile, Argentina, and Uruguay [1]. Small brilliant pink fruits grouped in bunches on pendulous stems distinguish the dioecious trees of these plants [2]. Several *Schinus* plants are utilized in both traditional and current medicine to alleviate a variety of ailments, including rheumatism, bronchial infections, stomach upset, menstrual disorders, gonorrhoea, bronchitis, and conjunctivitis [3, 4]. Among these plants, two known species, namely *S. polygamous* (Cav.) Cabrera and *S. terebinthifolius* Raddi were cultivated indifferent counties of North Africa, especially Egypt and Tunisia [5]. *Schinus polygamus* (Cav.) Cabrera is characterized by being medicinal shrub found in Chile, Argentina, Bolivia, Uruguay and Brazil [6]. Traditionally, the native population have been used the infusion of *Schinus polygamus* leaves and stems for treatment of arthritis and wound cleaning [7]. In addition, a recent study proved the hepatoprotective and antioxidant properties of the leaves grown in Egypt [8]. Another species named *Schinus terebinthifolius* Raddi has been expanded in South America, particularly Brazil, because of its characteristic spice taste to the demand of the national market [9]. Because of its anti-inflammatory and bactericidal effects on oral, respiratory and gynecological infections, the Brazilian National Health Surveillance Agency has approved its bark for inclusion in the national list of essential medicines [10, 11]. Besides, recent research reports have investigated the anticholinesterase, antioxidant, antibacterial, and antifungal effects of *Schinus terebinthifolius* Raddi [12].

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Rheumatoid arthritis is a chronic autoimmune inflammatory disease characterized by pathological changes, including permanent synovitis, inflammatory cell infiltration, cartilage attack and bone disorders [13]. The current therapy regimen for arthritis consists of steroidal and non-steroidal anti-inflammatory drugs (SAIDs and NSAIDs), and disease-modifying anti-rheumatic chemotherapeutics (such as methotrexate and cyclophosphamide) [14]. However, long-term use of these drugs can lead to serious adverse reactions, manifested as gastrointestinal tract (GIT) ailments, immunodeficiency, liver dysfunction, kidney damage and humoral disturbances [15]. Therefore, the need for safe, effective, natural and more potent anti-inflammatory agent has increased [16].

This research was designed to characterize the phytochemical components of *n*-hexane extracts of *S. polygamus* (cav.) Cabrera and *Schinus terebinthifolius* Raddi leaves via GC/MS analysis and investigate the *in vitro* cytotoxic and anti-arthritic activities.

2. Experimental

2.1 Plant Materials and Extracts

Schinus polygamus (Cav.) Cabrera and *Schinus terebinthifolius* Raddi leaves were obtained from Manial palace garden, Giza, Egypt on March 2020. The botanical samples were taxonomically authenticated by taxonomy specialist called Mrs. Tereize Labibat El-Orman plant Garden, Giza, Egypt. The voucher specimens, PHG-P-SP-185 and PHG-P-ST-185 have been placed at Pharmacognosy Department, College of Pharmacy-Ain Shams University.

The dried plant materials of both species are converted to powder by a mechanical grinder. The powdered materials of both species (250 g) were exhaustively percolated in *n*-hexane (4.5 L) until depletion. The solvents were evaporated using a rotavapor under vacuum to get 11.7 g *Schinus polygamus* extract and 9.9 g *Schinus terebinthifolius* extract.

2.2 Gas Chromatography/Mass Spectrometry (GC/MS)

The GC/MS investigation of both extracts were done at the Department of Pharmacognosy, College of Pharmacy, Ain Shams University, with the following specifications, instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), conjugated with a thermo-mass detector. The system of GC-MS was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 μm 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 ℃ for 2 min; rising at 5.0 ℃/min to 300 ℃ and held for 5 min. The injector and detector were held at 280 ℃. 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.

2.3 Identification of Hexane Components

The phytochemical constituents of hexane extracts were characterized by matching of their GC/MS spectra, fragmentation profile of compounds and retention indices (Kovats indices) to that published in the previous literature [17-20].

2.4 Assessment of Cytotoxic Activity

2.4.1 Mammalian cell lines

Human liver (HepG-2) and colon cancer cells (Caco-2) were purchased from VACSERA, Egypt.

2.4.2 Media of cell line culture

For perpetration of growth medium, Dulbecco Modified Eagle Medium (Bio Wittaker® Lonza, Belgium) was accompanied with inactivated fetal bovine serum (10%), HEPES buffer (10 mM, pH 7.3), glutamine (2 mM) and gentamycin (50 μg/ml).

2.4.3 Evaluation of cytotoxic activity

The hexane extracts were investigated for their cytotoxic effects towards two tumor cell lines; liver (HepG-2) and colon (Caco-2) using MTT assay [21]. After the cells grown on 75 cm^2 tissue culture flasks

reached confluence (usually 24 hr), we prepared the cell suspension of the two tumor cell lines 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 have a medium in place of cell suspension. The incubation of the cells occurred at 37 ^{o}C for 24 hr in a humidified atmosphere of 5% CO_2. When a complete monolayer cell sheet was formed in each well of the plate, the medium was aspirated and replaced with DMEM with 2% fetal bovine serum. Then, the hexane extract was dispensed into 96-well 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 hexane extract were added into a 96-well sterile tissue culture plate using multichannel pipette (Eppendorf, 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 ℃ in humidified atmosphere of 5 % CO_2. At the end of incubation, the plate was examined using the inverted microscope. Photos for cells at different concentrations of hexane extract were taken under the microscope *(*Zeiss West, Germany) using a Nikon camera (Nikon Inc., Japan) for evaluation of observed morphological changes.

2.4.4 Determination of viable cells

The survival cells were detected through stain with 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT), which is reduced by metabolically active cells into formazan with purple color. 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 MTT for 20 min. Then, remove excess stains, rinse the board with deionized water, and then dry. To obtain quantitative date 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 ELISA reader (SunRise TECAN, Inc.®, USA) at 490 nm. The absorbance is proportional to the number of survival cells in the culture plate. The percentage cell viability was calculated using the Microsoft Excel® version 2010. Percentage cell viability was calculated as follows:

$$\%\ \text{Cell viability} = \frac{\text{Mean Abs} - \text{Mean Abs plant extract}}{\text{Mean Abs control}} \times 100$$

Where Abs = absorbance at 490 nm cell quantity.

2.5 Assessment of In Vitro Anti-arthritis Property

Albumin denaturation test was carried out with some modifications [22]. Two folds serial dilutions of sample ranging between 1000-7.81 µg/mL were prepared in 96-well plate. Reaction mixture for each concentration was prepared from 100 µL of test drug and 100 µL of 1% albumin solution. These prepared solutions were stored for 15 min at 28 ℃. Then, the reaction mixtures were kept at 70 ℃ for 10 minutes to induce denaturation. The reaction mixture was cooled, and turbidity was measured using microplate reader at 660 nm. Diclofenac sodium was used as standard drug in the concentration of 1000-7.81 µg/mL and treated similarly as test extracts. Percentage inhibition of denaturation was calculated using control in which no drug was added. Each experiment was done in triplicate and the average was taken. Percentage inhibition of denaturation was calculated by the following equation:

$$\%\ \text{inhibition of denaturation} = (1 - \frac{\text{A2}}{\text{A1}}) \times\ 100$$

Where A_1 = absorbance of the control; and A_2= absorbance of test/standard sample with albumin solution

IC_{50} value is the concentration at which 50% of protein denaturation is inhibited under the assay conditions.

3. Results

3.1 Investigation of Gas Chromatography/Mass Spectrometry Analysis

The hexane extract of *S. polygamus* leaves (HSPL)

was analyzed by GC/MS, and 55 (86.65%) compounds were identified, including aliphatic hydrocarbons (21.51%), triterpenoids (20.71%), fatty acid methyl esters (FAMEs) (17.36%) and acyclic diterpenes (6.26%), as illustrated in Table 1. Further, betulin (6.54%) is the predominant component, followed by phytol acetate (5.27%), lupeol (3.96%), palmitic acid methyl ester (3.58%), heptacosane (3.25%) and palmitaldehyde (2.72%). On other side, 17 components were characterized in the hexane extract of *S. terebinthifolius* leaves (HSTL), (92.21%): triterpenoids (42.15%), FAMEs (21.16%), acyclic diterpene (14.70%), sesquiterpenes (8.19%) and fatty acids (3.28%). The HSTL was predominately characterized by phytol (14.70%), lupeol (13.33%), linolenic acid methyl ester (11.17%), palmitic acid methyl ester (7.74%), 28-oxours-12-en-3-yl acetate (7.44%), *β*-amyrin (6.73%), lupeol acetate (5.23%) and *α*-amyrin (5.14%).

Table 1 The phytoconstituents of hexane extracts of *S. polygamus* and *S. terebinthifolius* leaves.

No.	Compound	RI		Molecular formula	Composition (%)		Identification method
		Calculated	Reported		HSPL	HSTL	
	n-Undecane	1107	1100	$C_{11}H_{24}$	0.94	-	
	3,7-Dimethyldecane	1117	1125	$C_{12}H_{26}$	0.94	-	RI, MS
	1-Cyclohexylpentane	1124	1130	$C_{11}H_{22}$	0.94	-	RI, MS
	Hexylcyclopentane	1129	1134	$C_{11}H_{22}$	1.19	-	RI, MS
	4-Methylundecane	1148	1147	$C_{12}H_{26}$	1.09	-	RI, MS
	n-Dodecane	1198	1199	$C_{12}H_{26}$	-	0.83	RI, MS
	2,4-Dimethylundecane	1201	1208	$C_{13}H_{28}$	1.72	-	RI, MS
	β-Methyltetraline	1216	1218	$C_{11}H_{14}$	1.94	-	RI, MS
	1-Cyclohexylhexane	1227	1233	$C_{12}H_{24}$	1.14	-	RI, MS
	6-Methyldodecane	1246	1253	$C_{13}H_{28}$	1.81	-	RI, MS
	2-Butyl-1-octanol	1267	1277	$C_{16}H_{34}O$	1.99	-	RI, MS
	n-Octylcyclohexane	1332	1356	$C_{14}H_{28}$	1.67	-	RI, MS
	2,6-Dimethyldecalin	1368	1370	$C_{12}H_{22}$	0.99	-	RI, MS
	Farnesane	1373	1376	$C_{15}H_{32}$	0.95	-	RI, MS
	Butylated hydroxytoluene	1519	1519	$C_{15}H_{24}O$	-	1.90	RI, MS
	1-Butylhexyl benzene	1526	1535	$C_{16}H_{26}$	1.10	-	RI, MS
	Citronellyl butyrate	1530	1529	$C_{14}H_{26}O_2$	0.98	-	RI, MS
	Spathulenol	1587	1576	$C_{15}H_{24}O$	-	2.97	RI, MS
	α-Eudesmol	1658	1652	$C_{15}H_{26}O$	-	2.63	RI, MS
	1,6-Methanonaphthalene,decahydro-1,4,8a-trimethyl-9-methylene	1666	1669	$C_{15}H_{24}$	-	2.59	RI, MS
	Myristic acid	1717	1720	$C_{14}H_{28}O_2$	-	3.28	RI, MS
	Palmitaldehyde	1828	1830	$C_{16}H_{32}O$	2.72	-	RI, MS
	Palmitic acid methyl ester	1927	1927	$C_{17}H_{34}O_2$	3.58	7.73	RI, MS
	Palmitic acid	1947	1946	$C_{16}H_{32}O_2$	1.17	-	RI, MS
	α-Eicosene	1979	1980	$C_{35}H_{70}$	1.02	-	RI, MS
	Dodecanoic acid-2-hexen-1-yl ester	1980	1986	$C_{18}H_{34}O_2$	0.90	-	RI, MS
	Methyl isoheptadecanoate	2006	1996	$C_{18}H_{36}O_2$	0.95	-	RI, MS
	Palmitic acid trimethylsilyl ester	2026	2041	$C_{19}H_{40}O_2Si$	1.00	-	RI, MS
	Octadecanal	2069	2037	$C_{18}H_{36}O$	0.95	-	RI, MS
	9,12-Octadecenoic acid methyl ester	2080	2075	$C_{19}H_{34}O_2$	1.33	-	RI, MS
	Methyl (8*E*,11*E*)-8,11-octadecadienoate	2097	2093	$C_{19}H_{34}O_2$	-	0.93	RI, MS
	Linolenic acid methyl ester	2109	2108	$C_{19}H_{32}O_2$	1.49	11.17	RI, MS
	Methyl stearate	2111	2112	$C_{19}H_{38}O_2$	1.25	-	RI, MS
	Phytol	2120	2119	$C_{20}H_{40}O$	0.94	14.70	RI, MS
	Linoleic acid ethyl ester	2162	2167	$C_{18}H_{34}O_2$	0.99	-	RI, MS

Table 1 to be continued

Ethyl 9,12,15-octadecatrienoate	2171	2175	$C_{21}H_{44}O$	1.00	-	RI, MS
Palmitic acid butyl ester	2179	2188	$C_{20}H_{40}O_2$	1.09	-	RI, MS
Phytol acetate	2214	2218	$C_{22}H_{42}O_2$	5.32	-	RI, MS
Arachidic acid methyl ester	2336	2333	$C_{21}H_{42}O_2$	0.93	1.33	RI, MS
4,8,12,16-Tetramethylheptadecan-4-olide	2340	2364	$C_{21}H_{40}O_2$	1.08	-	RI, MS
1-Heneicosanol	2368	2365	$C_{21}H_{44}O$	1.08	-	RI, MS
1-Tetracosene	2387	2396	$C_{24}H_{48}$	0.91	-	RI, MS
Hexadecyl octyl ether	2453	2459	$C_{24}H_{50}O$	0.98	-	RI, MS
Cyclogallipharaol	2489	2499	$C_{21}H_{36}O$	2.57	-	RI, MS
Methyl docosanoate	2528	2531	$C_{23}H_{46}O_2$	0.94	-	RI, MS
3*β*-Hydroxy-5α-androstan-17-one	2508	2504	$C_{19}H_{30}O_2$	0.91	-	RI, MS
Heptacosane	2697	2700	$C_{15}H_{24}$	3.28	-	RI, MS
Lignoceric acid methyl ester	2720	2725	$C_{25}H_{50}O_2$	0.93	-	RI, MS
Squalene	2813	2819	$C_{30}H_{50}$	1.98	-	RI, MS
α-Tocospiro A	2855	2860	$C_{29}H_{50}O_4$	1.80	-	RI, MS
α-Tocospiro B	2876	2881	$C_{29}H_{50}O_4$	2.42	-	RI, MS
6a,14a-Methanopicene, perhydro-1,2,4a, 6b, 9,9,12a-heptamethyl-10-hydroxy-	2858	2863	$C_{30}H_{50}O$	0.96	-	RI, MS
2-Methyloctacosane	3029	2864	$C_{29}H_{60}$	0.94	-	RI, MS
1-Octacosanol	3067	3074	$C_{28}H_{58}O$	0.96	-	RI, MS
β-Sitosterol	3187	3197	$C_{29}H_{50}O$	1.49	-	RI, MS
Olean-12-en-3-one	3322	3327	$C_{30}H_{48}O$	1.11	-	RI, MS
β-Amyrin	3335	3337	$C_{30}H_{50}O$	1.12	6.73	RI, MS
α-Amyrin	3380	3376	$C_{30}H_{50}O$	-	5.13	RI, MS
Lup-20(29)-en-3-one	3389	3384	$C_{30}H_{48}O$	1.23	4.29	RI, MS
lupeol	3440	3442	$C_{30}H_{50}O$	3.96	13.33	RI, MS
Lupeol acetate	3509	3516	$C_{32}H_{52}O_2$	0.94	5.23	RI, MS
Betulin	3512	3518	$C_{30}H_{50}O_2$	6.40	-	RI, MS
Betulinaldehyde	3620	3628	$C_{30}H_{48}O_2$	2.59	-	RI, MS
28-Oxours-12-en-3-yl acetate	3722	3727	$C_{32}H_{50}O_3$	-	7.44	RI, MS
Aliphatic hydrocarbons				21.51	0.83	
Triterpenoids				20.76	42.15	
FAMEs				17.36	21.16	
Acyclic diterpenes				6.26	14.70	
Sesquiterpenes				-	8.19	
Fatty alcohols and aldehydes				7.70	-	
Fatty acids				1.17	3.28	
Others				11.89	1.90	
Total identified				86.65	92.21	

Compounds are arranged according to their elution. RI: Kovats retention index on DB-5 column. HSPL: hexane of Schinus polygamus leaves extract, HSTL: hexane of Schinus terebinthifolius leaves. RI, identification based on comparison of reported Kovats retention indices. MS, identification based on mass spectral data and fragmentation profile.

3.2 MTT Assay and Cytotoxic Activity

The cytotoxic potency of both extracts was investigated towards two cancer cell lines (HepG2 & Caco-2) using MTT assay with concentrations ranging from 19.53 to 625 μg/ml (Figure 1). The HSPL and HSTL extracts showed moderate cytotoxic activities against HepG2, with IC_{50} of 102.65 and 65.55 μg/mL, respectively. While the Caco-2 cell line presented IC_{50} values of 217.78 and 83.55 μg/mL, respectively. The HSTL extract showed more remarkable cytotoxic effects than HSPL extract against both cell lines. The

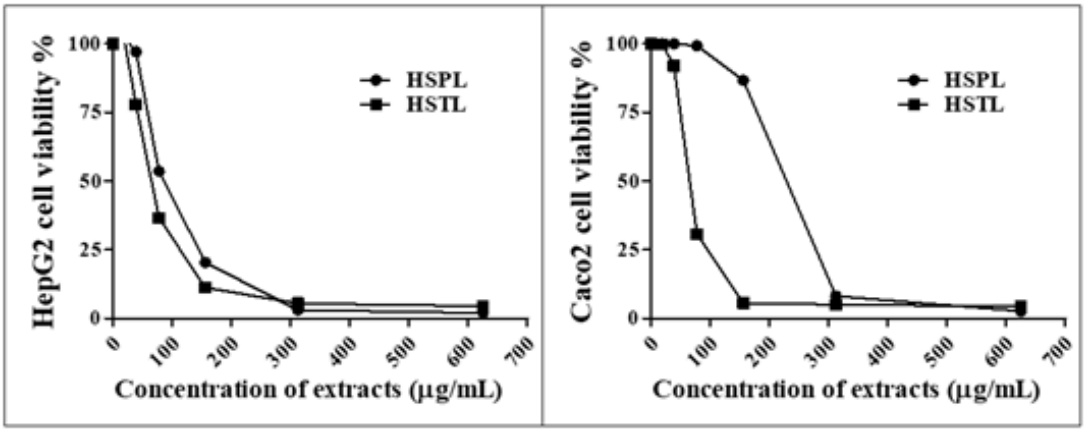

Fig. 1 The effect of extracts on the cell viability of HepG2 and Caco-2 Cells.

cytotoxic effects observed for both extracts are ascribable to synergism between the active predominant compounds found in the plant extract. The synergism between these compounds isdependent on the concentration or predominance of compounds, their structure and interactions between them to exert cytotoxic activity [23, 24].

In consistent with our results, the triterpenoids, the predominant class in the HSTL extract, were reported to display antineoplastic and antiproliferative properties upon testing against several cancer cell lines [25, 26]. In our study, the identified triterpenoids are structurally composed from lupine, cycloartane, friedelane, ursane and oleanane groups [27]. Mechanistically, the triterpenes could directly arrest the tumor proliferation, cell cycle progression via modulation of apoptotic pathway [28]. Upon correlation with the phytoconstituents, phytol which is the major compound of HSTL extract, exerted antitumor activity in hepatocellular carcinoma SMMC7721 cell line by different signaling cascades related tocaspase3 activation, down expression of death receptor3 (DR3) and upregulation of FADD mRNA [29]. It could selectively inhibit topoisomerase II [30]. Furthermore, Caco-2 cells provoked lower sensitivity to both extracts than hepatic carcinoma cell line (HepG2); this is attributed to p-glycoprotein transporter over expression which actively pumps any lipid-soluble substance out the cells [31].

3.3 Assessment of In vitro Anti-arthritic Activity

Both extracts provided a moderate protective effect against protein denaturation with IC_{50} = 80.12 & 63.09 µg/mL for HSPL and HSTL, respectively, compared to diclofenac sodium (IC_{50} = 15.12 µg/mL) as standard anti-inflammatory drug (Table 2). Strikingly, most of the major compounds of both extracts have been reported for anti-inflammatory and antiarthritic properties. Among these compounds; betulin showed high affinity to glucocorticoid receptor and activated its expression and biosynthesis resulted in a potent anti-inflammatory activity [32]. Also, it was found to suppress phospholipase A2 activity [33]. Consistent with our results, the oral administration of phytol to arthritis-prone rats significantly restored antioxidant and abolished the occurrence of arthritis [34]. In addition, it was found that the preventive effects of phytol were mediated through indirect effect on arthritogenic T cells [35]. It also elicited a significant prophylactic property in combination with vaccine against *Staphylococcus aureus* to avoid the progression of rheumatoid arthritis in mice [36]. Also, it significantly attenuated the inflammatory reaction through modulation of neutrophil production, IL-1β, TNF-α release and reactive oxygen species during carrageenan-induced acute inflammation [37]. Strikingly, lupeol as major common compound, displayed anti-inflammatory properties in arthritic mice; this was associated with

Table 2 The protein denaturation test of both extracts.

Concentration of extract (µg)	% Inhibition of albumin denaturation		
	HSPL extract	HSTL extract	Diclofenac sodium
1000	76.32 ± 1.60	79.35 ± 0.96	89.35 ± 0.58
500	71.65 ± 0.58	73.24 ± 2.10	84.12 ± 1.2
250	63.48 ± 1.20	68.18 ± 0.58	76.52 ± 0.63
125	59.37 ± 0.63	61.35 ± 1.30	70.14 ± 0.58
62.5	46.32 ± 1.30	49.89 ± 0.58	68.28 ± 0.63
31.25	31.08 ± 2.10	38.15 ± 0.92	59.14 ± 1.20
15.63	22.87 ± 2.50	25.34 ± 1.50	51.21 ± 0.58
7.81	9.38 ± 1.20	12.34 ± 0.63	31.12 ± 1.20
IC_{50}	80.12	63.09	15.12

All determinations were done in triplicates and values are expressed as the mean ± SE.

the immune system modulation and stabilization of inflammation mediators [38]. Consistent with another study, lupeol regulated the phagocytic ability of immune cells, such as macrophages, T-lymphocytes, and CD4+ T cell-mediated cytokines in arthritic rats [39]. Further, Latha and co-workers exerted a negative impact on lysosomal enzymes and glycoproteins, and their functions to reduce collagen in arthritic animals [40]. Concerning to palmitic acid methyl ester as major common compound in the two extracts, the topical application was effective in reducing inflammation mediated by PGE2, TNF-α, IL-6, NF-κB expression and neutrophil infiltration [41].

4. Conclusions

The current study is the first report to analyze the phytochemical constituents of hexane extracts of *Schinus* species through GC-MS analysis. Consequently, results of the GC-MS profile can be utilized as pharmacognostical tool for the identification and authentication of these plants. Also, the current study provided the first report for their cytotoxic and anti-arthritic properties which opens avenues for pharmaceutical researchers to develop anti-arthritic natural agent. However, further quantifiable *in vivo* research is now recommended to categorize the specific mechanism responsible for the cytotoxic and anti-arthritic pathways.

Conflicts of Interest

The authors declare no conflicts of interest.

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