Antimicrobial Properties, Cytotoxic Effects and Fatty Acids Composition of Vegetable Oils from Purslane, Linseed, Luffa and Pumpkin Seeds

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INTRODUCTION

- Vegetable oils are fundamental components in numerous diets, including the Mediterranean diet, offering essential nutrients and important bioactive compounds that promote health.
- Nowadays, the food industry is striving to discover new natural compounds to act as non-synthetic antimicrobial agents across the entire food supply chain, aiming to satisfy consumer preferences for healthier, chemical-free food options.

METHODOLOGY

- Oil samples were obtained from seeds of purslane (*Portulaca olearacea* L.), pumpkin (*Cucurbita maxima* L. cv. Nychaki), luffa (*Luffa aegyptica* Mill.) and linseed (*Linum usitatissimum* L.).
- Purslane and pumpkin oils were obtained from seeds harvested from plants cultivated at the experimental farm of the University of the Thessaly in the growing period of spring–autumn 2022. Pumpkin seeds were sown directly in soil in single rows on 27 July 2022, and fruit were harvested on 7 December 2022.
- Luffa and linseed oils were obtained from local retail shops and from Giachanas—Cold Pressed Seed Oils S.A. (Evros, Greece).

RESULTS AND DISCUSSION

Table 1. Fatty acids composition (%) of the studied vegetable oils (mean ± SD).

Fatty acids	Linseed oil 1	Linseed oil 2	Linseed oil 3	Luffa oil	Purslane oil	Pumpkin oil
C14:0	0.042±0.001e	0.043±0.001e	0.054±0.001c	0.095±0.002b	0.049±0.001c	0.155±0.003a
C15:0	-	-	-	-	0.028±0.001	-
C16:0	4.61±0.04e	4.32±0.04f	5.36±0.01d	13.77±0.07c	14.1±0.1b	14.72±0.05a
C16:1	0.068±0.004c	0.044±0.001d	0.086±0.004b	0.153±0.005a	0.086±0.001b	0.15±0.01a
C17:0	0.057±0.004e	0.055±0.004e	0.072±0.002c	0.166±0.004a	0.105±0.003c	0.113±0.002b
C18:0	2.85±0.01d	3.10±0.01c	3.06±0.01c	6.99±0.02a	3.046±0.006c	6.24±0.01b
C18:1n9c	7.09±0.01c	6.43±0.01e	6.95±0.01d	15.14±0.03b	5.24±0.02f	21.76±0.01a
C18:2n6c	12.87±0.01f	14.58±0.01e	17.98±0.02d	61.86±0.01a	34.10±0.05c	55.25±0.05b
C18:3n3	71.90±0.05a	70.93±0.01b	65.62±0.04c	0.94±0.02f	41.25±0.08e	0.323±0.006g
C20:0	0.095±0.002e	0.102±0.001e	0.153±0.001d	0.312±0.003c	0.60 ± 0.01a	0.401±0.005b
C20:1	0.080±0.002c	0.081±0.001c	0.124±0.002a	0.057±0.001d	0.102±0.001b	0.119±0.008a
C22:0	0.081±0.001e	0.088±0.005d	0.133±0.004c	0.092±0.006d	0.25±0.01a	0.144±0.002b
C22:2	-	-	-	-	0.49±0.05a	0.35±0.03b
C23:0	0.186±0.004e	0.164±0.002f	0.222±0.005d	0.251±0.001c	0.51±0.01a	0.292±0.003b
C24:0	0.085±0.005b	0.061±0.001c	0.183±0.002a	0.180±0.001a	-	-
SFA	8.01±0.04e	7.94±0.02f	9.24±0.01d	21.86±0.06b	18.7±0.1c	22.06±0.06a
MUFA	7.24±0.01c	6.56±0.01d	7.16±0.01d	15.35±0.03b	5.43±0.02e	22.03±0.02a
PUFA	84.76±0.04b	85.51±0.01a	83.60±0.02c	62.79±0.04e	75.83±0.08d	55.92±0.08f
PUFA/SFA	10.58±0.02b	10.77±0.01a	9.05±0.01c	2.87±0.05e	4.06±0.04d	2.55±0.07f
n6/n3	0.18±0.03e	0.21±0.01e	0.27±0.03d	65.81±0.02b	0.83±0.06c	166.57±0.03a

*C14:0 myristic acid; C15:0 pentadecanoic acid; C16:0 palmitic acid; C16:1 palmitoleic acid; C17:0 heptadecanoic acid; C18:0 stearic acid, C18:1n9c oleic acid, C18:2n6c linoleic acid, C18:3n3 α-linolenic acid, C20:0 arachidic acid; C20:1 eicosenoic acid; C22:0 behenic acid; C22:2 docosadienoic acid; C23:0 tricosylic acid; C24:0 lignoceric acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6/n3: ratio of omega-6/omega-3 fatty acids; - : not detected. In each row, different letters mean statistical differences among samples.

- Fatty acids profile varied among the studied oils. In particular, α-linolenic acid (C18:3n3) was the most abundant fatty acid in the case of linseed oils (71.90%, 70.93%, and 65.62% for linseed oil 1, 2, and 3, respectively), while the second most abundant compound was linoleic acid (C18:2n6c) followed by oleic (C18:1n9c), palmitic (C16:0), and stearic (C18:0) acids.
- Luffa seed oil included mostly linoleic acid (61.86%) followed by oleic acid and palmitic detected in similar amounts (15.14% and 13.77%, respectively), and stearic acid which was found in lesser amounts (6.99%).
- Purslane seed oil was also a rich source of α-linolenic acid (41.25%) and linoleic acid (34.10%), followed by palmitic, oleic, and stearic acids (14.1%, 5.2%, and 3.0%, respectively).
- Pumpkin oil showed similarities with luffa oil and contained slightly less linoleic and more oleic acid (55.25% and 21.76%, respectively), while palmitic and oleic acids were found in amounts similar luffa oil.

Table 2. Cytotoxicity and antitumor activity of the studied vegetable oils (GI_{50} values μg/mL).

Seed oil	Cytotoxicity to non-tumor cell lines	Cytotoxicity to tumor cell lines			
	PLP2 (porcine liver primary culture)	HeLa (cervical carcinoma)	HepG2 (hepatocellular carcinoma)	MCF-7 (breast carcinoma)	NCI-H460 (non-small cell lung cancer)
Linseed oil 1	301±23a	291±27b	> 400	> 400	369±33a
Linseed oil 2	> 400	> 400	> 400	> 400	> 400
Linseed oil 3	> 400	> 400	> 400	> 400	> 400
Luffa oil	215±17c	189±17c	> 400	> 400	136±12b
Purslane oil	>400	307±12a	>400	>400	>400
Pumpkin oil	259±21b	270±25b	> 400	> 400	> 400

*GI50 values correspond to the sample concentration responsible for 50% inhibition of growth in a primary culture of liver cells-PLP2 or in human tumor cell lines or. GI50 values for Ellipticine (positive control): 3 μg/mL (PLP2), 1.0 μg/mL (MCF-7), 1.0 μg/mL (NCI-H460), 2.0 μg/mL (HeLa) and 1.0 μg/mL (HepG2). In each row, different letters mean statistical differences among samples.

- None of the tested oils were effective against all the tested tumor cell lines, while linseed oil 1, luffa oil, and pumpkin oil showed a slight toxicity against the non-tumor porcine liver primary culture (PLP2) cell line.
- All the tested oils (except for linseed oil 1 and 2) showed efficacy against cervical carcinoma (HeLa) cell lines, especially luffa oil which recorded the lowest GI50 values (215 μg/mL), followed by pumpkin oil, linseed oil 1, and purslane oil in decreasing order of effectiveness. Similarly, luffa oil was the most efficient against non-small cell lung cancer (NCI-H460) cell line, followed by linseed oil 1, whereas none of the tested oils were effective against hepatocellular carcinoma (HepG2) and breast carcinoma (MCF-7) cell lines.

Table 3. Antibacterial activity of the studied seed oils (MIC and MBC mg/mL).

Seed oil	*Staphylococcus aureus* (ATCC 11632)		*Bacillus cereus* (food isolate)		*Micrococcus flavus* (ATCC 10240)		*Enterobacter cloacae* (ATCC 35030)		*Salmonella Typhimurium* (ATCC 13311)		*Escherichia coli* (ATCC 25922)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Linseed oil 1	2.00	4.00	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00
Linseed oil 2	4.00	8.00	1.00	2.00	1.00	2.00	0.50	1.00	2.00	4.00	2.00	4.00
Linseed oil 3	2.00	4.00	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00	0.50	1.00
Luffa oil	2.00	4.00	1.00	2.00	1.00	2.00	0.50	1.00	1.00	2.00	2.00	4.00
Purslane oil	2.00	4.00	2.00	4.00	2.00	4.00	2.00	4.00	2.00	4.00	1.00	2.00
Cucurbit oil	2.00	4.00	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00	0.50	1.00
E211	4.00	4.00	0.50	0.50	1.00	2.00	2.00	4.00	1.00	2.00	1.00	2.00
E224	1.00	1.00	2.00	4.00	1.00	2.00	0.50	0.50	1.00	1.00	0.50	1.00

*MIC: minimum inhibition concentration; MBC: minimum bactericidal concentration; E211: sodium benzoate; E224: potassium metabisulphite.

- All the oils exhibited high bactericidal and growth inhibitory effects against *S. aureus* and *M. flavus* with MIC and MBC similar to E211 and E224 (positive controls), respectively (except for linseed oil 2 and purslane oil, which showed the lowest activity in the case of *S. aureus* and *M. flavus*, respectively).
- All the tested oils were similarly or more effective than E224 against *B. cereus*, while E211 was the most effective.
- Linseed oil 2 and luffa oil recorded MIC values similar to E224 against *E. cloacae*, while the rest of the tested oils (with the exception of purslane oil) were more effective than the other positive control (E211).
- Regarding *S. typhimurium*, the tested oils (except for linseed oil 2 and purslane oil) were similarly effective to positive controls, apart from E224 which recorded the lowest MBC values. Finally, linseed oil 3 and pumpkin oil showed higher effectiveness against E. coli than the rest of the oils and similar to E224.

Table 4. Antifungal activity of the studied seed oils (MIC and MFC mg/mL).

Seed oil	*Aspergillus fumigatus* (ATCC 9197)		*Aspergillus versicolor* (ATCC 11730)		*Aspergillus niger* (ATCC 6275)		*Penicillium funiculosum* (ATCC 36839)		*Penicillium verrucosum* var. *cyclopium* (food isolate)		*Trichoderma viride* (IAM 5061)	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Linseed oil 1	0.50	1.00	1.00	2.00	2.00	4.00	2.00	4.00	1.00	2.00	1.00	2.00
Linseed oil 2	2.00	4.00	1.00	2.00	1.00	2.00	0.5	1.00	0.5	1.00	0.5	1.00
Linseed oil 3	1.00	2.00	2.00	4.00	0.50	1.00	2.00	4.00	2.00	4.00	2.00	4.00
Luffa oil	0.50	1.00	0.50	1.00	0.50	1.00	0.50	1.00	0.50	1.00	0.50	1.00
Purslane oil	4.00	8.00	2.00	4.00	2.00	4.00	2.00	4.00	2.00	4.00	2.00	4.00
Cucurbit oil	1.00	2.00	0.50	1.00	0.50	1.00	0.50	1.00	0.50	1.00	1.00	2.00
E211	1.00	2.00	2.00	2.00	1.00	2.00	1.00	2.00	2.00	4.00	1.00	2.00
E224	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	1.00	1.00	0.50	0.50

*MIC: minimum inhibition concentration; MFC: minimum fungicidal concentration; E211: sodium benzoate; E224: potassium metabisulphite.

- Linseed oil 1 and 3 were more effective against *A. fumigatus* compared not only to the rest of the tested oils, but also to the used controls.
- Similarly, luffa and pumpkin oils were the most effective against *A. versicolor*, *A. niger*, *P. funiculosum*, and *P. verrucosum* var. *cyclopium*, without differences from linseed oil 3 in the case of *A. niger*, and linseed oil 2 in the case of *P. funiculosum* and *P. verrucosum* var. cyclopium. Finally, linseed oil 2 and luffa oil were the most effective against *T. viride* with MIC values equal to E224.
- Purslane seed oil and linseed oil 3 (except for the case of *A. niger*) had the least overall effectiveness against the tested fungi, since in most cases they recorded the highest MIC and MFC values.

CONCLUSIONS

- Based on the results of our study, the tested less-conventional seed oils showed promising nutritional value regarding their fatty acids profile, with linseed and purslane seed oils having high amounts of health beneficial α-linolenic acid.
- On the other hand, pumpkin and luffa oil were the most abundant in linoleic acid, which is also associated with beneficial health effects.
- This could be supported by the *in vitro* cytotoxic activity of luffa oil against cervical carcinoma and non-small cell lung cancer cell lines.
- The tested oils also showed a varied effectiveness against several Gram+ and Gram- bacteria, especially linseed oil 3 and pumpkin oil, which showed the significant activity against most of the tested bacteria (especially against *Enterobacter cloacae* and *Escherichia coli*), On the other hand, the antifungal activities were more profound, and the studied oils were more effective than the positive controls for most of the tested fungi, especially luffa and pumpkin oil, which showed the best overall performance.
- Therefore, the studied oils could be used as a source of compounds with antimicrobial potential but could also been exploited for their high nutritional value and cytotoxic effects as a functional ingredient in food products, thus increasing the added value of the corresponding crops.

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