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Original paper

Antimicrobial activity of camelina oil and hydroalcoholic seed extracts

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Abstract

Camelina sativa is well-known for its polyunsaturated fatty acids content. Beside the lipidic profile, the seeds contain phenolic compounds with antioxidant, antimicrobial and anticarcinogenic activity.

This study aims to assess the antimicrobial activity of some camelina extracts, using the disc diffusion assay. Camelina oil and seed extracts in tree types of hydroalcoholic solvents (ethanol, i-propanol and methanol) were used in this study. All these were evaluated for antimicrobial activity against six different human pathogens: *Bacillus cereus, Salmonella typhimurium, Enterococcus faecalis, Staphylococcus aureus, Candida albicans* and *Candida parapsilosis,* and other five plant pathogens: *Botrytis cinerea, Fusarium oxysporum, Monilinia sp., Penicillium sp.* and *Sclerotium sp. B. subtilis* ATCC 6633 was used as reference, due to its high sensitivity to antimicrobial compounds. Three methods to obtain hydroalcoholic extracts were tested. The 90% propanolic extracts have shown the highest antimicrobial activity against *Salmonella typhimurium, Enterococcus faecalis* and *S. aureus*. A larger spectrum of antimicrobial activity was revealed when the extraction was made under shaking.

Keywords Camelina sativa seed extract, Mădălina variety, antimicrobial activity, disc diffusion assay.

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Introduction

Camelina sativa is an oleaginous plant belonging to *Brasicaceae* family. Its seeds are well-known for their polyunsaturated fatty acids high content. Camelina oil and meal are obtained through mechanical cold pressing. Both of them have fatty acids as major compounds. Beside the remarkable lipid profile, camelina seeds contain a range of compounds accessible only through polar solvent extraction (POPA et al [1], DOBRE et al [2]). Other valuable camelina compounds are phenols. They are usually associated with organic acids, carbohydrates or other phenolic compounds as part of vacuoles or cell walls. Aquaous methanolic solution is frequently used to obtain hydroalcoholic extracts, due to its ability to extract phenols (TERPINC et al [3]). Different extraction protocols have been used by researchers to obtain camelina hydroalcoholic seed extracts. These protocols mention different extraction times and solvents' concentrations, while using shaking or sonication for extraction. When ultrasounds are used, the biologic material and solvent stay in contact for 45 min. On the other hand, shaking is more time consuming, requiring up to 2 days of extraction. The most common extraction reports suggest 70% to 90% alcohol concentration. Considering the final extracts destination, additional treatments can be applied on the seeds to remove lipid compounds (TERPINC et al [3], QUEZADA & CHERIAN [4], GUPTA & KUMAR [5]).

Camelina seeds contain polyphenols as coumarins, flavonoids, hydroxilated derivatives of cinnamic and benzoic acids, lignins, (TERPINC et al [6]), protocathechuic acid, p-hydroxy-benzoic acid, salicylic acid, ellagic acid, sinapine, sinapic acid, phytic acid, flavonol derivatives (quercitin). The phenolic compunds identified in camelina seeds have antioxidant, antimicrobial and anticarcinogenic properties (GUPTA & KUMAR [5], TERPINC et al [6], GUPTA et al [7]).

Glucosinolates represent another group of chemicals found in camelina seeds extracts, with antimicrobial activity (GUPTA et al [7]).

Methanolic and ethanolic extracts are the most frequently mentioned as having antimicrobial activity. GUPTA et al [7] have reported significant and intermediate antimicrobial activity of methanolic and ethanolic extracts on gram-positive and gram-negative bacteria like: *Bacillus pumilus, Bacillus subtilis, Pseudomonas fluorescens, Escherichia coli, Agrobacterium tumefaciens and Acinetobactor junii*. In addition, the following fungal cultures have shown sensibility to both methanolic and ethanolic extracts: *Trichoderma reesei, Phanerochaete chrysosporium, Tilletia indica.*

Considering the previously reported results on antimicrobial activity of camelina seed extracts and their chemical composition, the present paper aims to analyze the antimicrobial spectrum of camelina oil and hydroalcoholic seed extracts, by testing 3 diferent extraction methods, on 13 microbial strains. As microorganisms were used different human and plant pathogens.

Materials and Methods

1. Materials

Camelina sativa seeds, Mădălina variety, were used in this study. The seeds were collected from plants grown at Moara Domnească Teaching Farm, in 2017, from a pesticide and fertilizer free cropping system. After harvest, mechanical automatized seeds processing was carried out using an ICS equipment from National Institute of Research–Development for Machines and Installations Designed to Agriculture and Food Industry-INMA Bucharest.

Camelina oil was obtained through mechanical cold pressing at the same Institute.

2. Hydroalcoholic seed extracts preparation

Three extraction methods, named Method 1, 2 and 3, were performed to obtain the hydroalcoholic extracts. Seeds were in contact with the solvents for different timeframes. Method 1 and Method 2 were adapted following KUMAR et al [8] method, while for the 3rd one, QUEZADA & CHERIAN [4] protocol was used as reference.

Camelina seeds used for extraction were surface disinfected in 70% ethanol for 5 minutes, than rinsed 3 times with ultrapure water. Seeds were then dried at 50-60°C, for 2 hours.

To obtain camelina seed extracts through Method 1, grounded seeds were suspended in 90% methanol, ethanol, i-propanol solvents in 1:4 w/v ratio. The mixture was incubated at room temperature, 125 rpm under orbital shaking, for 48 h. The mixture was then centrifuged for 30 minutes at 4000 × g. The supernatant was concentrated in a rotary evaporator and used for antimicrobial activity assessments.

For the extraction Method 2, the solvents used had 90% concentration. All following steps were maintained. However, the clear supernatants were not concentrated, but used as they resulted.

For extraction Method 3, camelina seeds were ground to fine powder using a blender. Grounded seeds were suspended in 70% methanol, ethanol, or i-propanol solvents, in 1:10 w/v ratio. Samples were sonicated for 45 minutes at room temperature and centrifuged for 30 minutes at 4000 × g. The resulted supernatant was used for antimicrobial activity determination.

3. Antimicrobial activity evaluation

Antimicrobial activity was evaluated *in vitro* using agar disc diffusion assay. A total of 13 microorganisms were used in this study (Table 1). All provided from the Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine Bucharest.

Table 1. Microbial strains tested to determine the antimicrobial activity of hydroalcoholic extracts

Chategory	Microbial strain	Abbreviation	Characteristics
Bacteria	*Bacillus subtilis* ATCC 6633	B.s. 6633	Reference strain sensitive to antimicrobial compounds
	Bacillus cereus	B.c.	Human pathogenic bacteria
	Salmonella typhimurium	S.t.	
	Enterococcus faecalis	E.f.	
	Staphylococcus aureus ATCC 6536	S.a. 6536	
	Staphylococcus aureus ATCC 43300	S.a. 43300	
Yeasts	*Candida albicans*	C.a.	Human pathogenic yeasts
	Candida parapsilosis	C.p.	
Fungi	*Penicillium* sp.	P.	Plant pathogenic fungi
	Fusarium oxysporum	F.o.	
	Botrytis cinerea	B. cin.	
	Monilinia sp.	M.	
	Sclerotium sp.	Scl.	

Appropriate culture media were used for each microbial category, namely: LB broth for bacteria, YPG broth for yeasts and PDA for fungi.

The antagonistic activity against phytopatogenic fungi was performed in a similar manner. However, the fungal inoculum consisted in 5 mm mycelia plugs placed in the center of the agar plates. Equidistant, at 2 cm from the fungal inoculum sterile paper discs were placed on the growth media, and inoculated with 7 µl of camelina oil or hydroalcoholic seed extracts. Several controls were also included in the tests. Each alcoholic solvent was subjected for analysis. As negative control, plates inoculated with each fungi were used to evaluate the growth rate of the pathogen in normal *in vitro* conditions, without the presence of any potential inhibitor. Plates were incubated at 28±2°C, for 3 to 5 days.

The antimicrobial activity against bacteria and yeast was evaluated by measuring the clear inhibitory halo surrounding the paper discs. Inhibitory zones were compared with the appropriated solvent controls. Antibiotic inhibition halo was also taken into account.

For fungi two biometric measurements were possible: the clear inhibition zone, where fungi were not able to colonize the agar due to the inhibitory activity of the samples, and the mycelia radius, which was compared with the fungal radius from the pure cultures, as described by URSAN et al [9].

Results and Discussion

Some of the camelina seed extracts, and also the camelina oil, showed antimicrobial activity against the microorganisms tested. The antagonistic activity varied with the type of the sample, but mostly from one microbial strain to another.

Bacillus subtillis ATCC 6633 has been slightly inhibited by i-propanol extract obtained through Method 2 (P2) and by the camelina oil (UC) (Figures 1 a, b).

Against *Bacillus cereus*, the only samples having antagonistic activity were ethanol extract obtained through Method 2 (E2) and camelina oil (UC). However, they presented a low inhibitory activity against *B. cereus* (Figure 1 c).

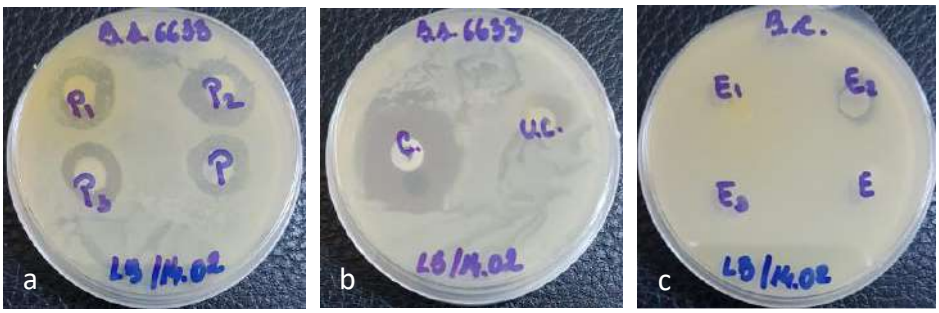

Figure 1. Antimicrobial activity of camelina oil and hydroalcoholic seed extracts against *Bacillus subtilis* ATCC6633 (**a, b**) and *Bacillus cereus* (**c**).

As depicted in Figure 2a, the P1 extract showed a high antimicrobial activity against *Salmonella typhmurium*. Additionally P2, E3 and UC also lead to inhibition zones against this pathogen (Figures 2 a, b and c), but were considered to be not as good as the P1 extract.

Against *Enterococcus faecalis* there has been noticed a very good inhibitory activity of P1 extract (Figure 2 d). The inhibition zone was comparable in size with the inhibition zone induced by kanamycin control.

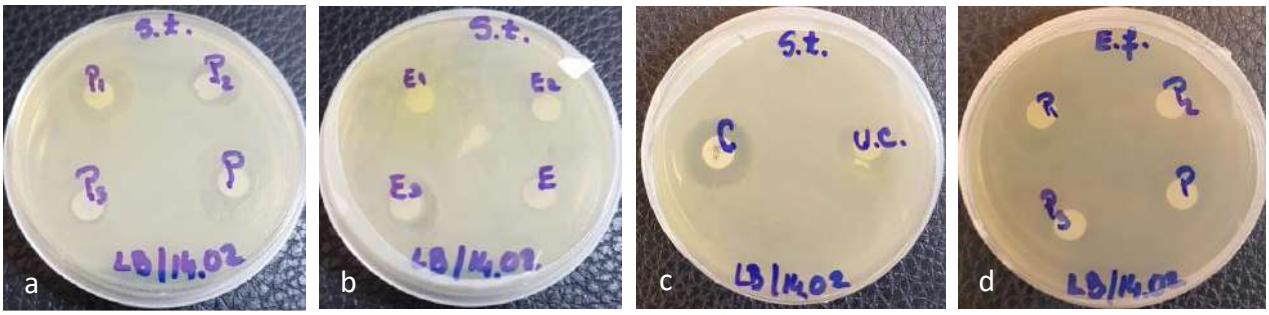

Figure 2. Antimicrobial activity of camelina oil and hydroalcoholic seed extracts against *Salmonella typhmurium* (**a, b, c**) and *Enterococcus faecalis* (**d**).

Slight antimicrobial activity was determined against *Staphylococcus aureus* ATCC 6536 when testing E2 and P1 extracts (Figures 3 a, b).

S. aureus ATCC 43300 was slightly inhibited by M1, M3, P2, P3 and camelina oil, while P1 showed a medium antimicrobial activity compared with the other extracts (Figures 3 c, d). As *S. aureus* ATCC 43300 is a MRSA strain, it cannot be inhibited by kanamycin antibiotic.

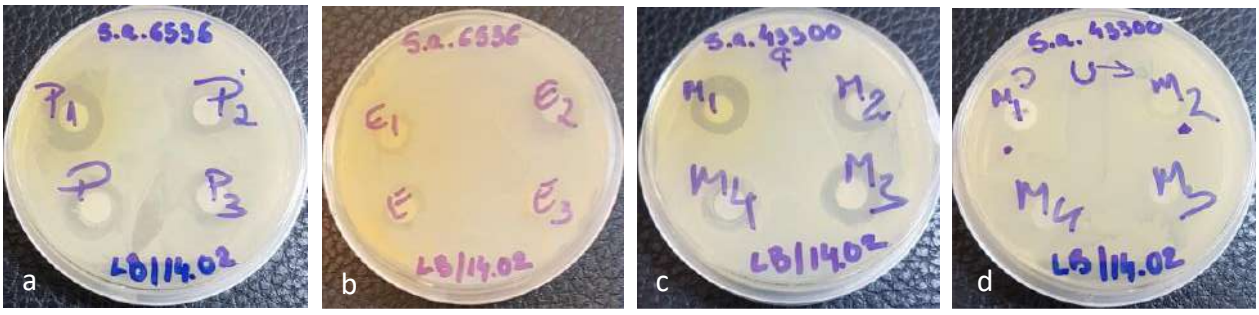

Figure 3. Antimicrobial activity of camelina oil and hydroalcoholic seed extracts against *Staphylococcus aureus* ATCC 6536 (**a, b**) and *S. aureus* ATCC 43300 (**c, d**).

Best results against *Candida albicans* were obtained when using P2 extract. P3 and E3 extracts have also presented antimicrobial activity against this pathogen (Figures 4 a, b).

The only camelina extract inhibiting *Candida parapsilosis* was P2, which showed a slight antimicrobial activity.

Regarding the antifungal activity against the analyzed plant pathogens the camelina oil was the only one showing fungal inhibitory activity, with slight antagonism, only against *Botrytis cinerea* (Figure 4 c). The antifungal efficacy of the camelina oil was evaluated to 27.3% taking into account the fungal growth in the control plate.

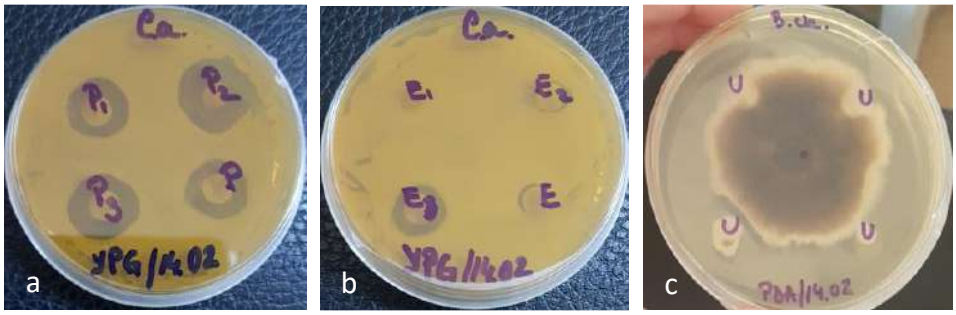

Figure 4. Antimicrobial activity of camelina oil and hydroalcoholic seed extracts against *Candida albicans* (**a, b**), and *Botritis cinerea* (**c**).

No activity was noticed against *Penicillium* sp., *Fusarium oxysporum*, *Monilinia* sp. and *Sclerotium* sp. phytopatogens when using camelina seed extracts or camelina oil.

For each tested microorganism there have been mentioned only the extracts and/or camelina oil that revealed antimicrobial activity. To all other extracts, the analyzed microbial strains shown tolerace or resistance.

The antimicrobial activity assessement results are outlined in Tabel 2. Four levels of inhibitory activity were atributed, as follows: no antimicrobial activity (-), low antimicrobial activity (+), medium antimicrobial activity (++) and high antimicrobial activity (+++), based on the comparison with control variants, of all tested cultures.

Table 2. Antimicrobial activity of camelina oil and hydroalcoholic seed extracts

Camelina extracts / **Microbial strains**	**M1**	**M2**	**M3**	**E1**	**E2**	**E3**	**P1**	**P2**	**P3**	**UC**
Bacillus subtilis ATCC 6633	-	+	-	-	-	-	+	+	+	+
Bacillus cereus	-	-	-	-	+	-	-	+	-	+
Salmonella typhimurium	-	-	-	-	-	++	+++	++	-	++
Enterococcus faecalis	-	-	-	-	-	-	+++	-	-	-
Staphylococcus aureus ATCC 6536	-	-	-	-	+	-	+	-	-	-
Staphylococcus aureus ATCC 43300	+	-	+	-	-	+	++	+	+	+
Candida albicans	-	-	-	-	+	+	-	+	+	-
Candida parapsilosis	-	-	-	-	-	-	-	+	-	-
Penicillium sp.	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporum	-	-	-	-	-	-	-	-	-	-
Botrytis cinerea	-	-	-	-	-	-	-	-	-	-
Monilinia sp.	-	-	-	-	-	-	-	-	-	-
Sclerotium sp.	-	-	-	-	-	-	-	-	-	-

Legend: ”- ” no antimicrobial activity, ”+ ” low antimicrobial activity, ”++” medium antimicrobial activity, ”+++” high antimicrobial activity comparing with control variants

As shown in Table 2, i-propanol extracts have a wider antimicrobial spectrum, being able to inhibit the growth of 8 microbial strains out of 13 tested. The ethanol extracts inhibited 5 of the tested strains, and camelina oil inhibited 4 strains. Reduced antimicrobial spectrum was seen on the methanol extracts, were only 2 strains were inhibited.

Correlating the antimicrobial spectrum with the inhibition activity, among i–propanol extracts, best results in terms of number of inhibited strains were obtained with P2 extract, and higher antimicrobial efficacy when using P1 extract and camelina oil.

The methanolic extracts have not shown anti-microbial activity, except of M2 extract against *B. subtilis* ATCC 6633, and M1 and M3 extracts against *S. aureus* ATCC 43300. As methicillin resistant strains are a high problem in medicine, these extraction methods from camelina seeds are a valuable result, although methanol traces should be eliminated from the final product.

Pathogenic yeasts were more resistant, being slightly inhibited only by the ethanolic and propanolic extracts obtained through Methods 2 and 3.

Conclusions

In conclusion, the propanolic extracts obtained through Method 1 have shown the best results in terms of antimicrobial activity against *Salmonella typhimurium* and *Enterococcus faecali*s.

The tested camelina oil, i-propanolic and ethanolic hydroalcoholic extracts have had medium and high antimicrobial activity.

The optimum extraction solvents, considering the antagonistic spectrum range were ethanol and i-propanol. Using camelina oil and various hydroalcoholic seed extracts the microbial growth of several pathogenic strains was inhibited: *Bacillus cereus, Salmonella typhimurium, Enterococcus faecalis, Staphylococcus aureus* ATCC 6536 and ATCC 43300*, Candida albicans, Candida parapsilosis* and *Botrytis cinerea*. Inhibiting human and animal pathogens by camelina oil and hydroalcoholic seed extracts show a good perspective of continuing studies in this field.

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