Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v35i4.16900

Microbiological activity of caraway (*Carum carvi* L.) essential oil obtained from different origin

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ABSTRACT. Caraway is one of the most important medicinal plant cultivated in Poland. Caraway essential oil performs medium antimicrobial activity, although it inhibits growth of many bacteria and fungi. The aim of the study was to evaluate microbiological activity of caraway oil obtained from fruit originated from different genotypes and investigate the correlation between this activity and essential oil content and its main components content. In the experiments done in 2008 and 2009, essential oil of 20 selected caraway genotypes originated from: European botanical gardens (17), cultivar 'Konczewicki' and our own breeding strains (2) was tested. The Minimal Inhibitory Concentration (MIC) of oil, which inhibited standard bacterial strain was investigated. MIC value was recalculated to antibiotic units (AU). The microbiological activity of caraway oil of the tested objects was significantly different. The strongest activity performed oil of population from genotype Cluj (MIC = 0.16 mg mL^{-1}; AU = 8650), while the weakest activity was determined for oil of population from genotype Krakow (MIC = 1.75 mg mL^{-1}; AU = 582). A significant negative correlation was observed between MIC and carvone content, however positive correlation was observed between MIC and limonene content. Caraway essential oil exhibited medium antimicrobial activity and carvone can be recognized as a one of the active component.

Keywords: caraway, carvone, essential oil, limonene, microbiological activity.

A atividade microbiológica do óleo essencial de alcaravia (*Carum carvi* L.) de várias origens

RESUMO. A alcaravia é uma das plantas medicinais mais importantes cultivadas na Polônia. O óleo essencial da alcaravia tem atividade média antimicrobiana embora ele iniba o crescimento de várias bactérias e fungos. Esse estudo não somente avalia a atividade microbiológica do óleo de alcaravia obtido da fruta oriunda de vários genótipos, mas também investiga a correlação entre essa atividade e o conteúdo do óleo essencial e seus componentes principais. Em experimentos realizados em 2008 e 2009, foi testado o óleo essencial de 20 genótipos selecionados, cultivar Konczewicki, oriundos de jardins botânicos europeus (17) e de nossas cepas (2). Foi analisada a Concentração Mínima Inibitória (CMI), a qual inibiu a cepa bacteriana padrão. O valor de CMI foi recalculado para unidades antibióticas (UA) e registrou-se que a atividade microbiológica do óleo da alcaravia foi diferente. A mais forte atividade produziu óleo oriundo do genótipo Cluj (CMI = 0,16 mg mL^{-1}; AU = 8650), enquanto o mais fraco foi derivado do óleo oriundo do genótipo Krakow (MIC = 1,75 mg mL^{-1}; AU = 582). Uma correlação negativa significante foi registrada entre CMI e o conteúdo carvone. Todavia, a correlação positiva foi registrada entre CMI e o conteúdo limonene. O óleo essencial de alcaravia mostrou uma atividade média antimicrobiana enquanto o carvone pode ser determinado como um dos componente ativos.

Palavras-chave: alcaravia, carvone, óleo essencial, limonene, atividade microbiológica.

Introduction

Caraway (*Carum carvi* L.) is one of the most important medicinal plant cultivated in Poland on the area 8.000 ha (SEIDLER-ŁOŻYKOWSKA; BOCIANOWSKI, 2012). Caraway fruit (*Carvi Fructus*) are used as a component of herbal mixtures recommended as a digestive, carminative and lactogenic. Caraway essential oil (*Carvi Aetheroleum*) obtained from fruit by hydro-distillation is transparent liquid, colorless with pleasant smell and spicy taste (SADOWSKA; OBIDOSKA, 1998). The main constituents of oil are monoterpenes: carvone and limonene which usually make 95% of all oil (KALLIO et al., 1994; RUSZKOWSKA, 1998; SEDLÁKOVÁ et al., 2001). According to Bouwmeester et al. (1995) carvone content determines the quality of caraway fruit.

Kalemba (1999) reports that most of essential oils performs strong antimicrobal activity specially oils (thyme, savory, clove) contained such phenols as: thymol, carvacrol or eugenol. Similar results were obtained by Baratta et al. (1998), who indicated high activity of cinnamon, clove, oregano, savory and thyme oils, while testing 20 different oils (Caraway oil was not tested) by 45 bacteria species from 11 genus (*Clostridium*, *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus*, *Enterococcus*, *Bacillus*, *Xanthomonas*, *Pseudomonas*, *Agrobacterium*, *Erwinia*) and group of eight species of yeasts. Results obtained by Kędzia et al. (1990) showed inhibited effect of sage oil on gram-positive bacteria (*Bacillus*, *Staphylococcus*, *Streptococcus*, *Mycobacterium*) and some fungi (*Cryptococcus*, *Candida*, *Aspergillus*, *Mucor*, *Rhizopus*, *Alternaria*, *Helminthosporium*, *Nigrospora*). All authors pointed out on correlation between antibacterial activity and composition of the tested oils.

Caraway essential oil performs medium antimicrobial activity. Hence it inhibits growth of many bacteria and fungi: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Mycobacterium tuberculosis* (SADOWSKA; OBIDOSKA, 1998; TOXOPEUS; BOUWMEESTER, 1992). Youself and Tawil (1980) described inhibited effect of caraway oil in concentration MIC = 190 μg mL^{-1} on yeast and mould growth. The investigation done by Bailer et al. (2001) and Frank et al. (2002) showed that carvone, the main compound of caraway oil, inhibited growth of some bacteria and fungi and can be used as a natural sprout suppressant during potato storage. Also research done by Hartmans et al. (1995) showed high activity of caraway oil against fungi occurred during potato storage: *Fusarium sulphureum*, *Phoma exigua* var. foveata, *Helminthosporium solani*. Similar effect of antifungal activity of caraway oil against Cladosporium cladosporioides, Fulvia fulvium, Alternaria alternata, Phoma macdonaldii, Phomopsis helianthi in concentration 2.5 mg mL^{-1} was observed by Simic et al. (2008). Caraway oil antimicrobial activity is used in medicine, food preservation, but also in cosmetic industry (DACHLER, 1998).

The aim of the study was to evaluate antibacterial activity of Caraway essential oil obtained from fruit originated from different genotypes and investigate the correlation between this activity and essential oil content and its main components (carvone and limonene).

Material and methods

In the experiments done in 2008 and 2009, essential oil of 20 selected caraway genotypes originated from: European botanical gardens (Bayreuth, Berlin, Bonn, Cluj, Göttingen, Jena, Krakow, Nantes, Poznan, Prague, Reykjawik, Riga, Salzburg, Ulm, Warsaw, Wroclaw, Zurich), cultivar 'Konczewicki' and our own breeding strains (9/1, 9/10) was tested. Two Caraway collections were established in 2007 and 2008 in Institute of Natural Fibres and Medicinal Plants in Poznan (Poland). In April each year seeds of the selected accessions were sown in greenhouse. Plants with 5-8 leaves were planted in the field at the beginning of May, 25-140 plants per accession. Mature fruit were collected from 10 plants of each accession by hand, separately from each plant in June 2008 and 2009. After harvest fruit were dried in the well-ventilated, shadow place.

The content of essential oil was estimated after hydro-distillation of Caraway fruits (10 g) of each accession in two replication using Dering's apparatus following the methods recommended by Polish Pharmacopoeia VIII (2008). The hexane solution of the oil (1:10) was analyzed with gas chromatography using Perkin Elmer Clarus 500 system in the following conditions: chromatographic column Elite 1 (30 m x 0.32 mm x 0.25 μm), volume of each injected sample = 1 μL, injector temperature = 200°C, carrier gas – helenium, flow = 1 mL $min.^{-1}$, FID detector temperature = 220°C. Time of components retention of the tested solution: carvone = 45.17 min., limonene = 15.69 min.

Antibacterial activity of caraway oil was analyzed by standard dilution in liquid media according to Cavanagh (1963). Hundred mg of essential oil was dissolved in 1 mL dimethylosulfoxide (DMSO). The range of oil dilution from 1 to 20 mg mL^{-1} was prepared in media Mueller-Hinton Broth of Merck. Then to the media was added 0.1 mL of 18 hours liquid culture of standard strain (*Staphylococcus aureus* ATCC 6538 P) diluted 1:10.000 in the same medium (number of inoculum contained 10^4-10^5 bacterial cells in 1 mL). Incubation of the tested samples was conducted in 37°C for 18 hours. The MIC (Minimal Inhibitory Concentration) was defined as the lowest concentration of the oil completely inhibiting the growth of standard strain. These value was calculated on antibiotic units (AU), based on that value of MIC is equivalent of 1AU. The results were referenced to 1 g of oil.

The one-way analysis of variance was carried out to determinate the effect of origin on the variability of MIC, AU, essential oil content, carvone content and limonene content. Means values and coefficients of variation were calculated for each trait. The least significant differences (LSDs) for each trait were calculated and, on this basis, homogeneous groups for the analyzed traits were determined. Procedure FCORRELATION in GENSTAT Release 10.1 (2007) was used for correlation analyses. Associations among traits were studied with the help of a scatterplot matrix (CLEVELAND, 1994; KOZAK et al., 2010).

Results and discussion

Analysis of variance indicated that the main effect of plant origin was significant for all traits in the study, except for limonene content. Mean values and coefficient of variation for each origin and each trait were shown in the Table 1. Based on the minimal inhibitory concentration of the selected caraway genotypes three homogeneous groups were distinguished (Table 1). Similarly, three homogeneous groups were distinguished based on differences in antibiotic units (Table 1).

The microbiological activity of caraway oil of the tested objects was significantly different. The strongest activity performed oil of population from Cluj (MIC = 0.2 mg mL^{-1}; AU = 8650); in that same group can be also classified oil of population from Bonn (MIC = 0.3 mg mL^{-1}; AU = 4350) and from Jena (MIC = 0.38 mg mL^{-1}; AU = 3000). The weakest activity was determined for oil of population from Krakow (MIC = 1.8 mg mL^{-1}; AU = 582). Also low activity performed oils of population from Wroclaw (MIC = 1.6 mg mL^{-1}; AU = 875) and Warszawa (MIC = 1.5 mg mL^{-1}; AU = 750). The oils of cultivar 'Konczewicki' and breeding strains showed medium activity (cv. 'Konczewicki' MIC = 0.8 mg mL^{-1}; AU = 1350, strain 9/1 MIC = 0.8 mg mL^{-1}; AU = 1350, strain 9/10 MIC = 0.9 mg mL^{-1}; AU = 1175 and there was no significant differences among them. The lowest coefficient of variation (0%) for MIC and AU was found for population from Nantes, Prague, cv. Konczewicki and strain 9/1, whereas the highest coefficient of variation (84.9 %) was revealed for population from Göttingen.

The content of essential oil in caraway fruit was significantly different and varied from 3.4% (Krakow) to 5.2% (Cluj and Göttingen). Fruit of cultivar 'Konczewicki' and breeding strains contained higher amounts of oil (cv. 'Konczewicki' - 4.5%, strain 9/1 - 4.8%, strain 9/10 - 4.9%). The analysis of caraway genotypes oil content distinguished six homogeneous groups (Table 1). The coefficient of variation for oil content ranged from 0% (Reykjawik) to 24.3% (Warsaw). The content of carvone in oil was also significantly different and oscillated from 53% (Krakow) to 68% (Bayreuth). Oil of six population (Bayreuth, Cluj, Poznan, Prague, Reykjawik, Warsaw) contained more then 60% of carvone, while oils obtained from breeding materials contained lower amounts of carvone (cv. 'Konczewicki'- 57%, strain 9/1 - 59%, strain 9/10 - 56%). Two homogeneous groups were distinguished based on differences in content of carvone in oil (Table 1). The coefficient of variation for carvone content ranged from 1.17% (Ulm) to 25.0% (Zurich). The content of limonene was not significantly different and varied from 28% (Poznan, Prague) to 40% (Bonn, Jena, Ulm). The coefficient of variation for limonene content ranged from 4.0% (Cluj) to 41.6% (Zurich).

Table 1. Mean values, coefficient of variation (cv) and homogeneous groups for caraway antimicrobial activity and essential oil, carvone and limonene content

Traits	MIC mg mL^{-1}		AU		Essential oil content [%]		Carvone content [%]		Limonene content [%]	
Origin	mean	cv	mean	cv	mean	cv	mean	cv	mean	cv
Bayreuth	0.5bc	70.71	2675bc	70.05	3.83cdef	2.77	68a	10.40	30.5a	20.87
Berlin	0.8abc	47.14	1500bc	47.14	4.6abcde	9.22	60.5ab	12.86	35a	20.20
Bonn	0.3c	76.15	4350b	76.40	4.4abcdef	6.43	56ab	10.10	40a	10.61
Cluj	0.2c	76.15	8650a	76.02	5.2a	5.44	61ab	4.64	35a	4.04
Göttingen	1.3abc	84.85	1250bc	84.85	5.2a	13.60	57.5ab	13.53	36.5a	25.18
Jena	0.4c	47.14	3000bc	47.14	4.55abcde	10.88	56.5ab	11.26	40.5a	15.71
Konczewicki	0.8abc	0.00	1350bc	0.00	4.5abcde	15.71	57ab	4.96	38.5a	12.86
Krakow	1.8a	20.20	582c	20.03	3.4f	12.48	53.5b	9.25	43a	6.58
Nantes	0.8abc	0.00	1350bc	0.00	4.6abcde	12.30	55ab	7.71	39.5a	12.53
Poznan	1.0abc	70.71	1325bc	72.04	4.05bcdef	1.75	66.5ab	5.32	28.5a	17.37
Prague	1.0abc	0.00	1000bc	0.00	3.75def	16.97	65ab	4.35	28.5a	27.29
Reykjawik	0.9abc	20.20	1175bc	21.06	4.2abcdef	0.00	61ab	18.55	33.5a	31.66
Riga	1.3abc	28.28	850bc	24.96	3.6ef	11.79	58ab	14.63	36.5a	25.18
Salzburg	1.1abc	47.14	1000bc	49.50	4.4abcdef	12.86	60.5ab	12.86	36.5a	17.44
Ulm	0.6bc	28.28	1675bc	27.44	3.85cdef	5.51	60.5ab	1.17	40a	14.14
Warsaw	1.5ab	47.14	750bc	47.14	3.93bcdef	24.32	61ab	6.96	36.5a	9.69
Wroclaw	1.6ab	76.15	875bc	76.77	4.8abcd	20.62	55.5ab	16.56	39.5a	23.27
Zurich	1.0abc	70.71	1325bc	72.04	4.2abcdef	3.37	59.5ab	24.96	34a	41.59
strain 9/1	0.8abc	0.00	1350bc	0.00	4.85abc	1.46	59ab	7.19	37.5a	13.20
strain 9/10	0.9abc	20.20	1175bc	21.06	4.95ab	7.14	56.5ab	3.75	39.5a	5.37
$LSD_{0.05}$	1.06		3744		1.05		14.22		14.55	

In table means followed by the same letters are not significantly different.

A significant negative correlation was observed between MIC and AU (r = -0.58), MIC and carvone content (r = -0.45), carvone content and limonene content (r = -0.93) (Table 2, Figure 1). However, positive correlation was observed between MIC and limonene content (r = 0.37) (Table 2). Some of relationships are not linear (Figure 1 and Figure 2). The function defining dependence between AU and MIC were found. This function is given by AU = $1002.7MIC^{-1.001}$ and explains in 99.97 % of total variation (Figure 2).

Table 2. Correlation of microbiological activity of essential oil and content of the oil, carvone and limonene

Traits	MIC	AU	Essential oil ontent	Carvone content	Limonene content
MIC	1				
AU	-0.58***	1			
Essential oil content	0.004	0.22	1		
Carvone content	-0.45**	0.23	-0.27	1	
Limonene content	0.37*	-0.16	0.20	-0.93***	1

*p < 0.05, **p < 0.01, ***p < 0.001.

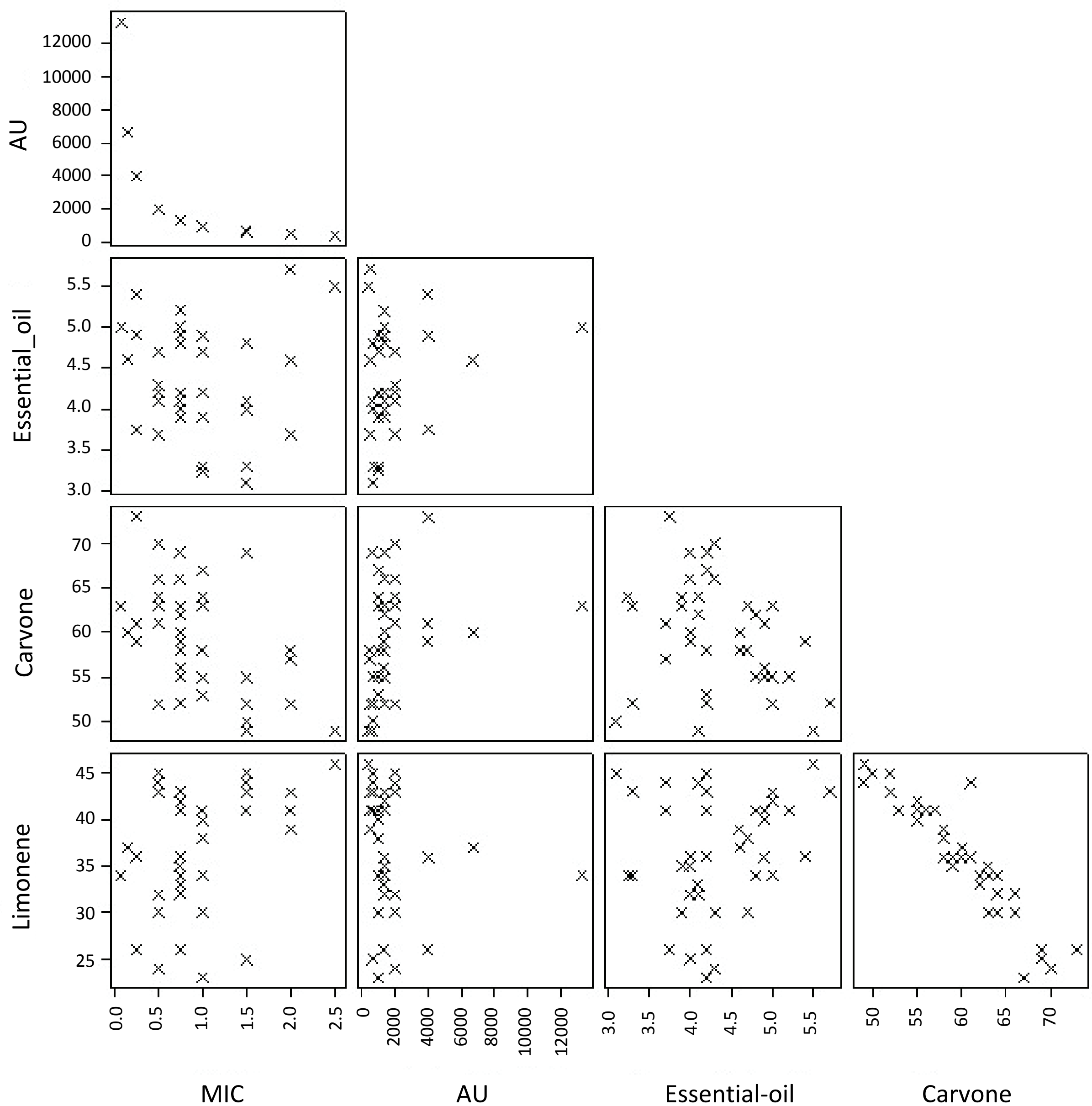

Figure 1. Scatterplot matrix for the caraway characters studied (MIC – Minimal Inhibitory Concentration, AU – antibiotic units).

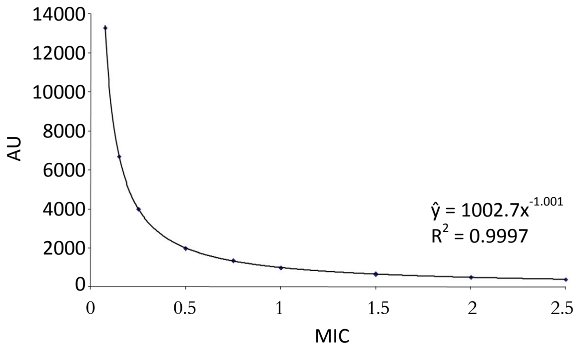

Figure 2. Relationship between Minimal Inhibitory Concentration (MIC) and antibiotic units (AU).

Caraway oil represents lower microbiological activity, which was confirmed by Toxopeus and Bouwmeester (1992) and Sadowska and Obidoska (1998), Dachler (1998). Research done by Hołderna-Kędzia et al. (2006) revealed that the essential oils from tea tree, manuka and sandalwood had strong effect on pathogenic gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*), gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and pathogenic fungi: yeast-like fungi (*Candida albicans*, *Candida krusei*, *Geotrichum candidum*), mould fungi (*Aspergillus flavus*), dermatophytes (*Microsporum gypseum*) in the concentration from 0.1 to 10.0 mg mL^{-1}. Singh et al. (2002) obtained the high activity of essential oil from *Carum copticum* fruit against bacteria gram-positive and gram-negative, although the main compound of this oil was thymol (35-60%). While Simic et al. (2008) reported that MIC of caraway oil inhibited fungi growth was 2.5 mg mL^{-1}, however they did not analyzed the obtained results in comparison to oil composition.

Conclusion

Caraway essential oil exhibited medium antimicrobial activity and carvone can be recognized as a one of the active component.

Acknowledgements

The research was financially supported by Polish Ministry of Science and Higher Education, found no. N310 029 32/1557.

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Received on April 22, 2012.
Accepted on July 14, 2012.