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ANETHUM SOWA ESSENTIAL OIL AN ALTERNATIVE IN THE TREATMENT OF VARIOUS BACTERIAL INFECTIONS - IN VITRO STUDY

ULEIUL ESENȚIAL DE *ANETHUM SOWA* O ALTERNATIVĂ ÎN TRATAMENTUL INFECȚIILOR BACTERIENE – STUDIU IN VITRO

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ABSTRACT | REZUMAT

A major objective of the latest research in the biomedical field is to find natural alternatives to antibiotics to reduce the occurrence of multidrug-resistance bacterial strains. The essential oil from the genus *Anethum*, according to data from the literature, is an important candidate. Still, its ability to inhibit bacterial growth is influenced by a number of factors. Starting from these aspects, this paper characterises an essential oil of *Anethum sowa*, produced and marketed in Romania, regarding antioxidant capacity, chemical composition and antimicrobial activity. The results demonstrate an increased antioxidant capacity, with positive RSA values from a concentration of 1.25µL/mL. Through GC-MS, 21 compounds were identified, 4 being the majority, respectively D-limonene (41.491%), carvone (23.074%), apiol (16.218 %), D-dihydrocarvone (8.228%) and trans-dihydrocarvone (6.586%). The antimicrobial activity, highlighted by microdilution methods, is evident at the lowest concentration tested (1.25 µL/mL) of essential oil against Gram-positive bacteria, while inhibition of all strains of Gram-negative bacteria needs a higher concentration (5 µL/mL). The MIC values for *Candida spp.* are 1.25 µL/mL for *C. parapsilopsis* and 5 µL/mL for *C. albicans*.

Keywords: Anethi essential oil, antioxidant activity, antimicrobial activity, horses

Un obiectiv major al ultimelor cercetări în domeniul biomedical îl constituie găsirea unor alternative naturale la antibiotice pentru reducerea apariției tulpinilor bacteriene cu antibiorezistență crescută. Uleiul esențial din genul *Anethum*, conform datelor din literatura de specialitate pare a fi un candidat important, însă capacitatea lui de inhibare a creșterii bacteriene este influențată de o serie de factori. Pornind de la aceste aspecte, prezenta lucrare caracterizează uleiul esențial de *Anethum sowa*, produs și comercializat pe teritoriul României, din punct de vedere al capacității antioxidante, a compoziției chimice și a activității antimicrobiene. Rezultatele obținute demonstrează o capacitate antioxidantă crescută, cu valori pozitive ale RSA încă de la concentrația de 1,25µL /mL. Prin GC-MS s-au identificat 21 de compuși, 4 fiind majoritari, D-limonen (41,491%), carvonă (23,074%), apiol (16.218%), D-dihidrocarvonă (8,228%) și trans-dihidrocarvonă (6,586%). Activitatea antimicrobiană, evidențiată prin metoda microdiluțiilor, este evidentă la cea mai mică concentrație (1,25 µL/mL) testată a uleiului esențial împotriva bacteriilor Gram pozitive, în timp ce inhibarea tuturor tulpinilor de bacterii Gram-negative necesită o concentrație mai mare (5 µL/mL). Valorile MIC pentru *Candida spp.* sunt de 1,25 µL/mL pentru *C. parapsilosus* și 5 µL/mL *C. albicans*.

Cuvinte cheie: Anethi ulei esențial, activitate antioxidantă, activitate antimicrobiană haloterapie, cai

During the last decades, numerous researchers from the biomedical field have been based on finding natural products as an alternative method of prevention and treatment of numerous pathologies. Medicinal and aromatic plants, being rich reservoirs of bioactive molecules, are able to promote health and be used as drugs (1, 3, 17). Essential oils of different plants are studied to be used to replace synthetic antioxidants and antimicrobial agents in the food and pharmaceutical industries, as well as in phytotherapy.

The importance of their use as antimicrobial agents derives precisely from the emergence of multidrug-resistant bacterial strains in most antibiotics used in medical practice (19).

The genus *Anethum*,from the *Apiaceae* family, contains four species known as aromatic and spice herbs, native to the Middle East and the Sahara in northern Africa but currently spread throughout the world. The medicinal importance of these plants is attributed to numerous phytoconstituents with various pharmacological attributes such as antioxidant, antimicrobial, anti-inflammatory, anti-Alzheimer, and neuromodulatory, antitumoral, antidiabetic, antiparasitic and insecticidal activities (8, 13, 14). The essential oils of plants

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from the Anethum genus, due to their increased content of polyphenols, are proposed as natural antibiotics for infections caused by both Gram-positive and negative bacteria, as well as fungal infections. Antimicrobial activity has been demonstrated against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginous*, *V. cholarae*, *A. parasiticus*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. albicans* (5, 6, 9, 16, 21). However, this activity is influenced by the amount of polyphenols, which varies depending on the botanical origin, the geographical origin, the meteorological conditions where the plant is cultivated, and last but not least, the process of obtaining the essential oil.

The purpose of this study was to characterise an essential oil of *Anethum sowa*, produced in Romania, through the prism of antioxidant capacity, chemical composition, and antimicrobial activity.

METHODS AND MATERIALS

The essential oil of Anethi (*Anethum sowa*), produced and marketed by Oleya, Romania, was tested. The oil was characterised by antioxidant activity, chemical composition, and antimicrobial activity.

Antioxidant Capacity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay

The antioxidant capacity of the Anethi essential oil was determined according to Cocan et al. (2021) with minor modifications (4). First, the alcoholic extract was prepared by adding 1 ml of essential oil to 10 ml of 70% ethanol (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The alcoholic extract was mixed using an ultrasonic water 136 bath (FALC Instruments, Treviglio, Italy) for 30 minutes at room temperature. After 30 minutes of stirring, the extracts were filtered through Whatman filters fitted with a 0.45 µm nylon membrane of 30 mm diameter (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and stored at 2-4°C until analysis. The positive control used consisted of butylated hydroxytoluene (BHT) (200 ppm concentration) and the negative control of 70% ethanol.

To highlight the antioxidant capacity of the essential oil, 1 mL of alcoholic in 2.5 mL of DPPH alcohol solution (0.3 mM) (Sigma-Aldrich, Taufkirchen, Germany) was added. The mixture was shaken to homogenise and then incubated for 30 minutes, at room temperature in the dark. At the end of the incubation period, the absorbance of the samples was read using a UV-VIS spectrophotometer (Specord 205; Analytik Jena AG, Jena, Germany), at a wavelength of 518 nm. For each concentration of the essential oil, three determinations were performed, and the results were reported as a mean.

The control sample consisted of a mixture of distilled water and DPPH solution in the same volume and concentration as the ones used for essential oil.

The antioxidant activity was calculated as a percentage of Radical Scavenging Activity, according to the formula:

$$RSA\ (\%) = (A_{control} - A_{sample}) / A_{control} * 100$$

where: RSA - Radical Scavenging Activity

$A_{control}$ - the absorbance value of control sample

A_{sample} - the absorbance values of extract sample

Gas chromatography-mass spectroscopy (GC/MS)

The chemical composition of the Anethi essential oil was analysed by GC-MS using a Shimadzu QP 2010 Plus apparatus (Columbia, SC, USA) equipped with an AT WAX 30 m 0.32 mm 1 m capillary column. The carrier gas was helium with a the discharge rate of 1 mL/min. The temperatures of the injector and ion source were 250°C and 220°C, respectively. For compound separation, a temperature gradient was utilised with an initial oven temperature of 40°C maintained for 1 minute, followed by an increase to 210°C at a rate of 5°C/min and a subsequent 5-minute hold at this temperature. The sample injection volume was 1 µL of a 2% BEO hexane solution, and a split ratio of 1:50 was utilised. The volatile components of the essential oil evaluated were identified using the NIST 5 Wiley 275 library database. The match of detected compounds to the database was a minimum of 90%. The results were presented as percentages of total compounds. LRI was calculated using Normal alkane RI for the same polar column.

Antimicrobial activity

To test the antimicrobial activity of Anethi essential oil, bacterial and fungal reference strains (ATCC) obtained from the culture collection of the Microbiology Laboratory of the Interdisciplinary Research Platform within the University of Life Sciences "King Mihai I of Romania" in Banat, Timisoara, were taken into study. The tested reference strains were: *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Shigella flexneri* (ATCC 12022), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae type B* (ATCC 10211), *Clostridium perfringens* (ATTC 13124), *Candida albicans* (ATCC 10231), and *Candida parapsilopsis* (ATCC 22019). The concentrations of Anethi essential oil tested were 1.25 µL/100 µL, 2.5 µL/100 µL, 5µL/100 µL, 10 µL/100 µL, 20µL/100 µL.

Bacterial culture. All the bacterial reference strains were revived by overnight growth in brain heart infusion (BHI) broth (Oxoid, CM1135) at 37°C and, subsequently, passed on BHI Agar (Oxoid, CM1136) for

24h at 37°C. The antimicrobial activity of the essential oil was tested by the microdilution method, according to Hulea et al. (2022), with minor modifications (7). A dilution of 10^{-3} from each fresh bacterial culture was prepared, equivalent to an optical density (OD) of 0.5 McFarland standard (1.5×10^8 UFC × mL), using BHI broth. The suspensions were tested by spotting 100 µL of microbial suspension in each well of the 96 microdilutions well plate, using a Calibra digital 852 multichannel pipette. The tested concentrations of Anethi essential oil were added over microbial suspensions. Then, the plates were covered and left for 24h at 37°C. After the incubation period, the OD was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Triplicate tests were performed for all samples. The negative control was considered a suspension of bacterial strain into BHI.

Fungal Culture. The antifungal activity of Anethi essential oil was tested by microdilution assay, according to Obiștioiu et al. (2021). Briefly, from the ATCC fungal strains revived by growth in brain heart infusion (BHI) broth (Oxoid, CM1135) at 37°C for 48 h, a 10^{-2} dilution was performed, an inoculum equivalent to a 0.5 Mc Farland standard (10). The diluted cultures (100 µL) were pipetted in each well of the 96 microdilutions well plate, over which different concentrations of essential oil were tested. The plates were covered and incubated for 48 h at 37°C. After 48 h, the OD was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Triplicate tests were performed for all samples. The negative control consisted of fungal suspension strains growing in BHI. The antibacterial activity is reported as BIR% (bacterial inhibition rate), a rate that is calculated by the formula:

BGR (bacterial growth rate) %=
OD samples / OD control *100

BIR%= 100 – BGR (%) (3)

where:

OD sample—optical density at 540 nm as the mean value of triplicate readings for EOs in the presence of the selected bacteria;

OD control—optical density at 540 nm as the mean value of triplicate readings for the selected bacteria in BHI.

For the antifungal activity, it was used MGR% (mycelial growth rate) and MIR% (mycelial inhibition rate), by using the same formula.

RESULTS AND DISCUSSIONS

The antioxidant capacity expressed by the R.S.A. of *Anethi sowa* essential oil tested is presented in Fig. 1. Starting with the concentration of 1.25 µL/mL, the RSA values are positive, respectively 15.33%, reaching the concentration of 20 µl/mL at 33.08%, with a value of IC_{50} of 30.15%. Similarly, Saleh et al. (2016), demonstrated that the methanol, ethyl acetate, and chloroform extracts of *Anethum sowa* had high antioxidant activity with IC_{50} values between 13.08 and 36.42 µg/mL (14). On the other hand, the same author clearly demonstrated that the essential oil of the same plant has an IC_{50} of 3.07 mg/mL (15). The results obtained by GC-MS are presented in Table 1 and Fig.2.

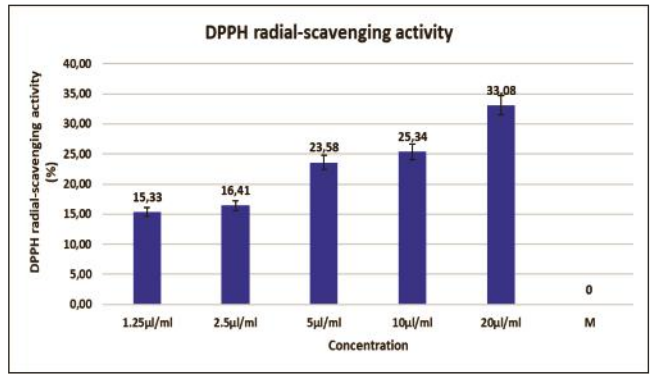

Fig. 1. Antioxidant capacity of *Anethum sowa* essential oil in different concentrations

Table 1

The composition of Anethi essential oil obtained by GC-MS

Crt. no.	Compound Name	R.Time	Conc.
1.	alpha-Pinene	11.650	0.387 %
2.	3-Carene	14.460	0.083 %
3.	Sabinene	14.764	0.184 %
4.	beta-Myrcene	15.402	0.803 %
5.	alpha-Phellandrene	15.613	0.336 %
6.	D-Limonene	16.239	41.491 %
7.	beta-Phellandrene	17.319	0.191 %
8.	p-Cimene	20.043	0.890 %
9.	p-Isopropenyl toluene	25.354	0.162 %
10.	Limonene oxide	26.703	0.231 %
11.	Linalool	27.677	0.269 %
12.	Decanal	27.800	0.094 %
13.	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	28.815	0.131 %
14.	p-Mentha-1(7),8(10)-dien-9-ol	29.433	0.056 %
15.	D-Dihydrocarvone	33.475	8.228 %
16.	trans-Dihydrocarvone	34.101	6.586 %
17.	(+)-Carvotanacetone	34.959	0.269 %
18.	Carvone	36.183	23.074 %
19.	Myristicin P2380	46.449	0.267 %
20.	Lemicin	46.560	0.050 %
21.	Apiol	47.408	16.218 %

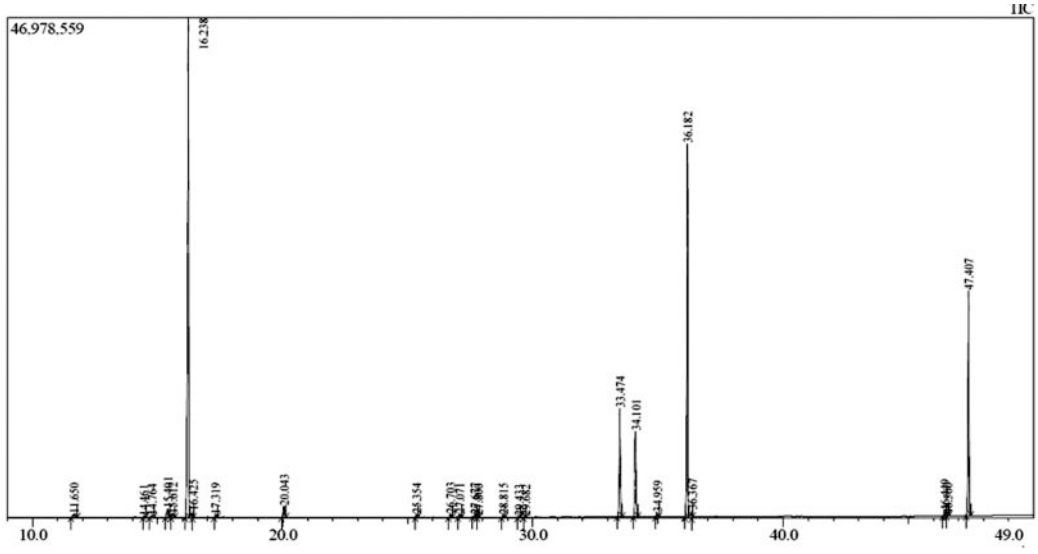

Fig. 2. Chromatogram of *Anethum sowa* essential oil

The major compounds of the *Anethum sowa* essential oil detected by GC-MC were D-limonene (41.491%), 18 carvone (23.074%), apiol (16.218%), D-dihydrocarvone (8.228%) and trans-dihydrocarvone (6.586%). Other compounds were detected with values under 1%.

Few specialised studies describe the chemical composition of *Anethi sowa* essential oil, most focusing on the description of the essential oil of *Anethum graveolens*. However, the chemical composition of the essential oil of the two chemotypes European dill (*Anethum graveolens*) and Indian dill (*Anethum sowa*) are differentiated, especially for the monoterpenes contained. *Anethum sowa* contains high quantities of apiol, whereas *Anethum graveolens* is rich in carvone (9, 13, 16). Regarding the concentration of apiol contained in *Anethum sowa* essential oils, this varies from one tested essential oil to another, which underlines that geographical area and climate may influence the chemical composition. Thus, Saleh et al. (2017) highlighted apiol as the main component in a concentration of 81.99 % (15), while Vats et al. (2012) reported concentrations of 26.15% (20). In contrast, the results obtained in the present study demonstrate a content of 16,218 % of apiol, but not as a major component, dominating D-limonene, followed by carvone. However, Sumitra et al. (2012) provided similar results to those obtained in the present study, showing that *Aethum sowa* essential oil is a rich source for isolation of limonene (18).

The antimicrobial activity of the essential oil tested is presented as BIR% in Figures 3–5. Fig. 3 presents the antibacterial activity against Gram-positive bacteria. As it is observed, starting with the first concentration of essential oil tested, the values of BIR% for each strain were positive, which proves that the MIC for *S. aureus*, *S. pyogenes*, *L. monocytogenes*, *Cl. Perfringens*, and *B. cereus* is 1.25 µL/mL.

Fig. 3 presents the antibacterial activity against Gram-negative bacteria. Starting with the first con-

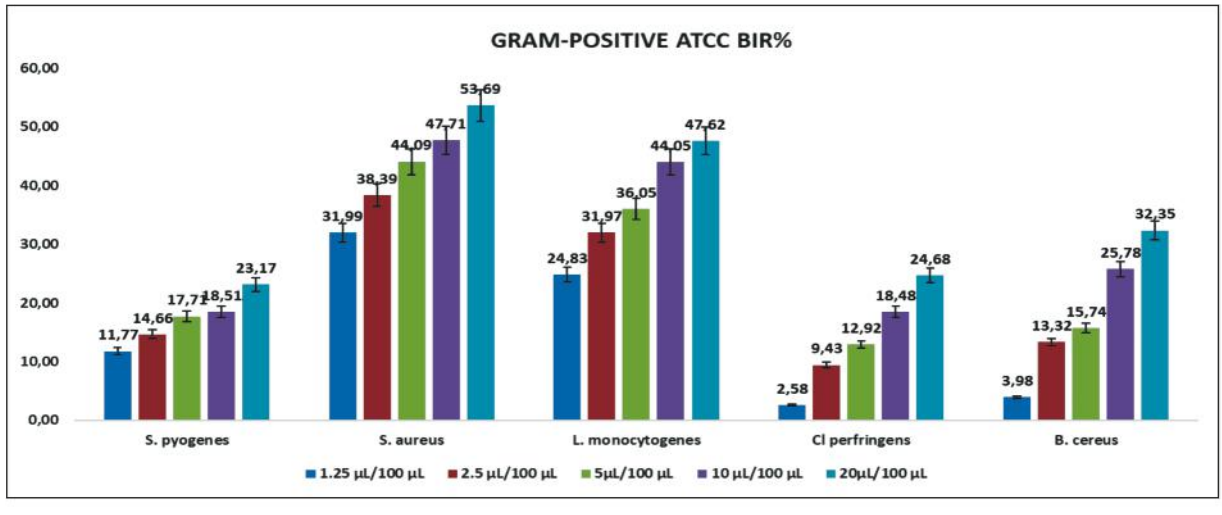

Fig. 3. The bacterial inhibition rate for *A. sowa* essential oil against Gram-positive bacteria

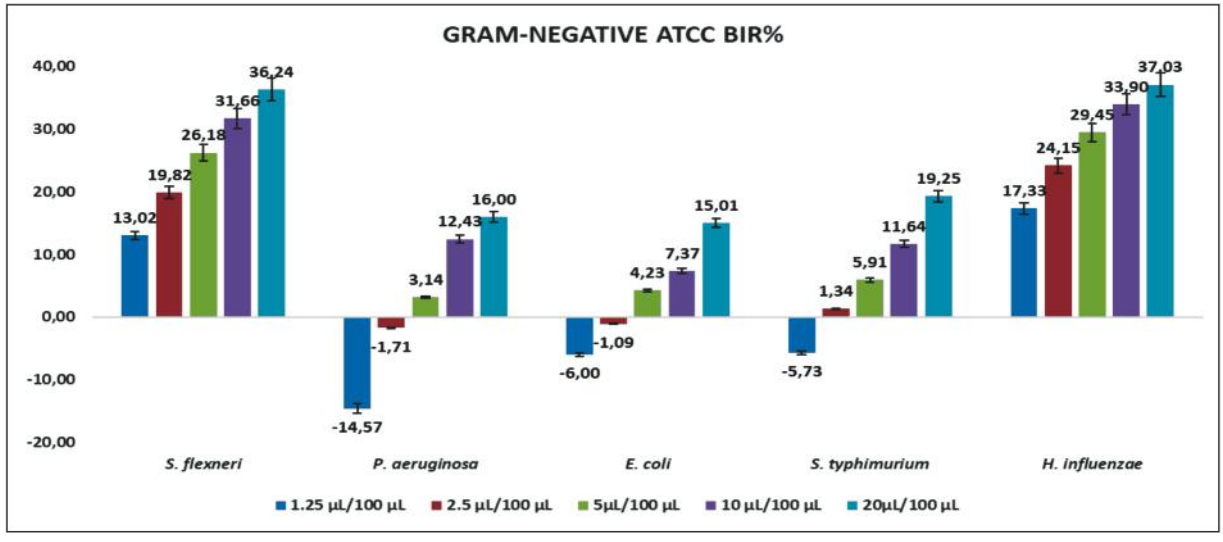

Fig. 4. The bacterial inhibition rate for *A. sowa* essential oil against Gram-negative bacteria

centration tested, respectively 1.25 µL/mL, the values of BIR% were negative for *P. aeruginosa* (-14.5%), *E. coli* (-6.00%), and *S. typimurium* (-5.73%) and positive for *S. flexneri* (13.02%) and *H. influenzae* (17.33 %). At a concentration of 2.50 µL/mL, the values of BIR% remain negative for *P. aeruginosa* (-1.71%) and *E. coli* (-1.09%), but positive for *S. typhimurium* (1.34 %). For all the tested strains, the essential oil of *A. sowa* shows antibacterial activity starting at a concentration of 5 µL/mL. The MIC values are 1.25 µL/mL for *S. flexneri* and *H. influenzae*, 2.5 µL/mL for *S. typhimurium* and 5 µL/ mL for *P. aeruginosa* and *E. coli.*

The antimicelial activity of *Anethum sowa* tested essential oil against strains of *Candida spp*. is presented in Fig. 5. The BIR% values are positive for *C. parapsilosus* (1.81%–23.80%) starting with a concentration of 1.25 µL/mL of essential oil but negative for *C. albicans*. For *C. albicans*, BIR% becomes positive at a concentration of 5 µL/mL (0.34%).

The same results were obtained by other authors, who demonstrated that *Anethum sowa* essential oil is effectiveness against *E. faecalis*, *P. aeruginosa*, *S. enteritis*, S. typhimurium, *B. subtilis*, *B. cereus*, *Klebsiella pneumoniae*, *Streptococcus mutants,* and *S. aureus* (2, 11, 12, 15). However, the values for MIC highlighted by other researchers are different from the ones obtained by this study. Saleh et al. (2017) demonstrated that the MIC for *E. faecalis*, *P. aeruginosa*, *S. enteritis*, and *A. aceti* was 62.5 µg/mL, while the MIC values for *E. coli* varied between 62.5-250 µg/mL. The MIC value was also increased for *S. typhimurium* to 250 µg/mL. Also, for some Gram-positive bacteria, such as *B. subtilis*, *B. cereus*, and *S. aureus*, the MIC value is increased to 125 µg/mL (15). All these MIC values are lower compared to

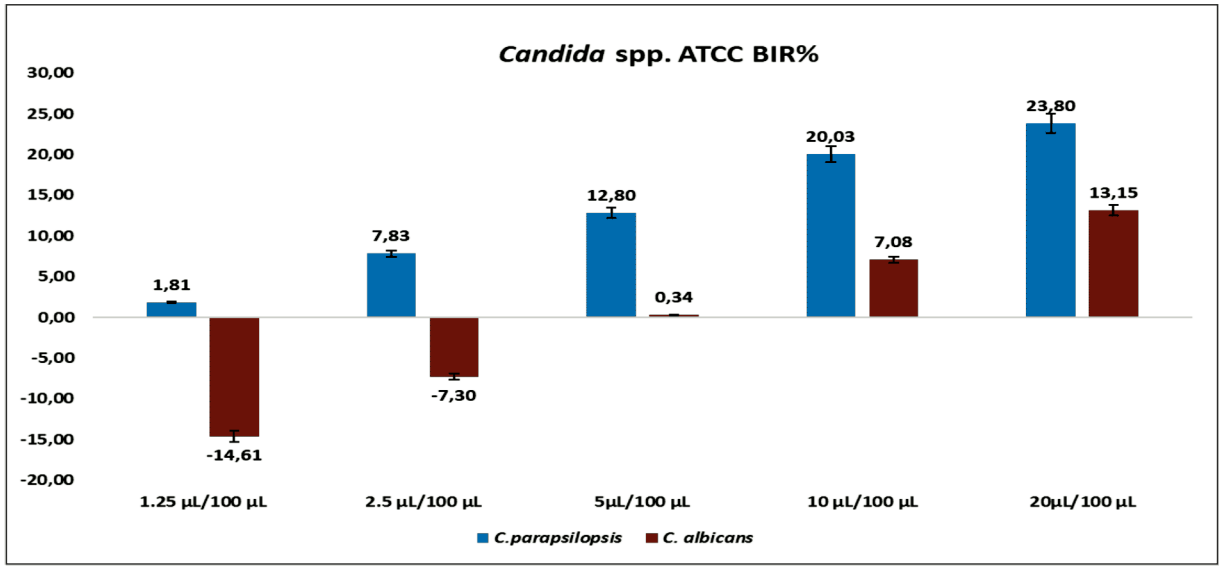

Fig. 5. The bacterial inhibition rate for *A. sowa* essential oil against *Candida spp.* strains

those obtained in our research. These differences regarding the MIC value can be justified by different chemical compositions of the essential oil due to the method of obtaining it and the growing conditions of the plant.

CONCLUSIONS

Anethum sowa essential oil is important natural antioxidant source with high concentrations of D-limonene, carvone, and apiol. The antioxidant capacity is highlighted by an RSA% of 15.33% at a concentration of 1.25 µL /mL. Besides its antioxidant capacity, the essential oil of *Anethum sowa* is a potential natural antimicrobial candidate. The MIC value for Gram-positive strains is 1.25 µL/mL, and for Gram-negative strains, it varies between 1.25 and 5 µL/mL depending on the bacterial species. The anti-mycelial effectiveness is evident at 1.25 µL/mL for *C. parapsilosus* and only at 5 µL/mL for *C. albicans*.

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