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IN VITRO RESEARCH STUDY ON THE ANTIMICROBIAL ACTIVITY OF SEA BUCKTHORN, BLACK CUMIN AND GRAPE SEED ESSENTIAL OILS AGAINST SELECTED FOOD SPOILAGE FUNGI

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Abstract

Natural antimicrobial agents such as essential oils obtained from plants can control the growth of different food spoilage microorganisms, thus prolonging the shelf-life and quality of food products. As essential oils are not toxic and already used as flavoring agents, they can be used as substituents for chemical preservatives used to control microbial growth. This study aimed to evaluate the antimicrobial activity by determining the minimum inhibitory concentrations (MIC) of sea buckthorn, black cumin, and grape seed essential oils over four different mould strains usually occurring in the spoilage of fruit products. The fungi used in the experimental research were Penicillium expansum, Fusarium oxysporum, Botrytis cinerea, and Aspergillus flavus. To establish the minimum inhibitory concentration of the essential oils, the agar disc diffusion technique was used. The results showed that all essential oils inhibited the growth of Penicillium expansum and Aspergillus flavus strains at a minimum volume of essential oil of 30 µL.

Key words*: antimicrobial activity, essential oils, fungi, food spoilage.*

INTRODUCTION

Nowadays, food safety and the extension of food products shelf-life are vital factors that are closely studied and analyzed by both researchers and the food industry. Although modern techniques, such as modified atmosphere packaging (Stan et al., 2021), have been emerging over the past few years, food borne diseases are still a significant concern worldwide (Burt, 2004). Therefore, there is a stringent motivation to develop novel methods and techniques that could reduce or eliminate food borne pathogens in all types of food products (Liu et al., 2009; Tenea, 2021). Food products are in general perishable, and protection against spoilage is required during the critical phases of preparation, packaging, storage, and distribution, where the chance to be contaminated or cross-contaminated is high. As consumers have become more concerned about the food they consume and the processing methods used for conserving it, researchers all over the world are looking to replace the traditional methods used for conservation, which may depreciate the nutritional value and sensorial proprieties of the food products, with novel processing techniques (Wendy et al., 2014). Synthetic preservatives have been used to maintain the shelf-life and nutritional quality of large varieties of agri-food products for decades. The main disadvantage of using artificial preservatives is that they may have a negative outcome on the health of the final consumers. The food industry is trying to replace them with non-synthetic, non-toxic, and natural preservatives. Novel and innovative techniques such as essential oils (EOs) and spice extracts are used to extend the shelf-life of different food products (Reis et al., 2022). Essential oils are natural volatile compounds that possess antimicrobial activity, obtained from the plant's secondary metabolism, the most important ones being monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, esters, ethers, aldehydes, ketones, phenols) (Khodaei et al., 2021). To extract them from plants, steam distillation is the most used technique, but there are several other techniques in use, such as expression, fermentation, or extraction. In recent years there have been a large number of research studies that demonstrate the antimicrobial (Michel et al., 2012; Mangalagiri et al., 2021; Lisboa et al., 2022) and antioxidant (Baydar et al., 2007; Mohamed et al., 2016; Rodrigues et al., 2020) activity of EOs. Because of these proprieties, EOs have been used in the food and pharmaceutical industry. EOs and their active components show promising activities against many food-borne pathogens and spoilage microorganisms (Mangalagiri et al., 2021). When applied into a food matrix, a higher concentration of EOs is needed to exert similar antimicrobial activity as those obtained in *vitro* assays. EOs and their active components are novel and innovative techniques used to extend the shelf-life of food products (Tao et al., 2021). The antimicrobial properties of EOs have been reported in several studies (Ermumcu, 2022). The EOs antimicrobial activity results from the complex interaction between the different compounds (phenols, aldehydes, ketones, alcohols, esters, ethers, or hydrocarbons) found in EOs. In some cases, the bioactivities of EOs are closely related to the activity of the main components of the oils.

Black cumin (*Nigella sativa* L.) seeds essential oils have been used in medicinal applications for a long time because of their anti-inflammatory (Woo et al., 2012) and antimicrobial properties (Chaieb et al., 2011). Over 100 different chemical constituents, including all the essential fatty acids (linolenic and linoleic acid), terpenoids, aliphatic alcohols and unsaturated hydroxy ketones) are found in black cumin seeds (Ramadan & Moersel, 2002a). Other beneficial compounds found in the black cumin oils are carvone, an unsaturated ketone, terpene or d-limonene, a-pinene, and p-cymene (Ramadan, 2007). Yasni et al. (2009) studied the antimicrobial activity of black cumin (*Nigella sativa* L.) extracts on the growth of several pathogenic bacteria (*Salmonella typhimurium*, *Bacillus cereus,* and *Staphylococcus aureus*). The results showed that black cumin oil managed to inhibit the development of the pathogenic bacteria at different concentrations. Black cumin oil was incorporated into edible films with chitosan and alginate as matrixes, and the antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was assessed. The results showed that the films containing black cumin oil inhibited the growth of the bacteria in chicken meat samples (Takma & Korel, 2019).

Grapeseed oil is a by-product obtained from the winemaking process, which has great importance in the food industry and the medical sector, due to its therapeutic proprieties (Rosa da Mata, 2022; Maier et al., 2009). Grape seeds contain large amounts of vitamin E and proteins (Lachman et al., 2015). The oil obtained from processing the grape seeds has a significant amount of unsaturated fatty acids (oleic and linoleic acids) (Baydar & Akkurt, 2001; Lachman et al., 2015) and several bioactive components, such as tocopherols, phytosterols, tocotrienols, flavonoids, and phenolic acids (Bail et al., 2008). In a study realized by Mohamed et al. (2016), it analysed the antioxidant activity and bioactive compounds in different grape seed oils samples extracted by supercritical CO_2 and organic solvent. The results showed that this extraction technique represents an efficient green solvent for high-quality oil extraction. Rodrigues et al. (2020) conducted an experiment to assess the antimicrobial activity of a chitosan and gelatine film incorporated with phenolic extracts of grape seed and jaboticaba peel. The antimicrobial test showed that all samples inhibited microorganism growth, extending the shelf-life of the food sample analyzed.

Sea-buckthorn leaves (*Hippophae rhamnoides* L.) are a good source of biologically active substances and nutrients and a rich source of phenolic compounds, fatty acids, and vitamins A, C, E. Some of the most important compounds found in sea-buckthorn are carotenoids, sterols, flavonoids, lipids, tannins, and ascorbic acid, which possess biological activities (Jaroszewska & Biel, 2017). Sea-buckthorn oil is known to have the antioxidant capacity (Tkacz et al., 2020) and antimicrobial activity (Upadhyay et al., 2011), thus making it a valuable component for the agri-food industry. Jeong et al. (2010) studied the antimicrobial and antioxidant activities of sea-buckthorn extracts. The results showed that the EOs extracted from roots and stems had antimicrobial activity compared to other antimicrobial agents.

MATERIALS AND METHODS

Materials

In the present study were used three types of essential oils from Sea-buckthorn (*Hippophae rhamnoides* L.), Black cumin (*Nigella sativa* L.) and Grape seeds (*Vitis vinifera* L.), purchased from Dacia Plant and Hofigal. The four fungi strains used in the experiments (*P. expansum*, *F. oxysporum*, *B. cinerea* and *A. flavus*) were obtained from the collection of the Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The fungal strains were inoculated in Potato Dextrose Agar (PDA) medium and incubated at 25°C for a period of seven days.

Methods

The disc diffusion test method was used to investigate the antifungal activity of the three essential oils. PDA culture medium was sterilized at 121°C for 20 minutes and after cooling it was poured into the Petri dishes. After solidification, a quantity of 100 µL suspension (10^6 UFC/mL) of each fungal strain were spread in each plate with the Drigalsky wand. The inoculated Petri Dishes were left to rest for 30 minutes to incorporate the microorganism into it. Four discs of Whatman paper (Φ = 6 mm) were placed on the prepared medium, on which the different quantities of essential oil (10 µL, 20 µL, 30 µL, 40 µL, 50 µL and 60 µL) were added. The control samples consisted of Petri Dishes inoculated with the selected fungi strain, but without any essential oil on the Whatman disks. For each sample 2 repetitions were performed. The dishes were sealed with parafilm to prevent the evaporation of essential oils and incubated for 7 days at 25°C. Evaluation of antimicrobial activity was performed by measuring the diameter of the inhibition zone (including the diameter of the Whatman disc). There are many and different definitions in the literature for the minimum inhibitory concentration (MIC) (Hammer et al., 1999, Demo et al., 2005, Mihai et al., 2015, Vasile et al., 2017), and as a result of the review of these, and in relation to the specific condition in our study, the MIC was defined as the minimum amount of essential oil for which the inhibition halo diameter was at a minimum of 1 cm.

Statistical analysis

For each sample, three repetitions were performed. The obtained data was statistically analysed by using Microsoft Excel 2016. In all tests, it was considered the significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

The antifungal activity of sea-buckthorn, black cumin and grape seeds was determined against four fungi strains with high occurrence in fresh fruits (*P. expansum*, *F. oxysporum*, *B. cinerea* and *A. flavus*). The evaluation of the antifungal activity was performed by measuring the diameter of the inhibition zone and establishing the minimal inhibitory concentration. The results showed that the diameter of the inhibition zone and antifungal capacity are dependent of the quantity of EO used in the experiments. In order to establish the minimum amount of essential oils necessary to inhibit the development of the tested fungi strains, a quantitative screening was performed with 10 µL to 60 µL of each antimicrobial agent used in these experiments.

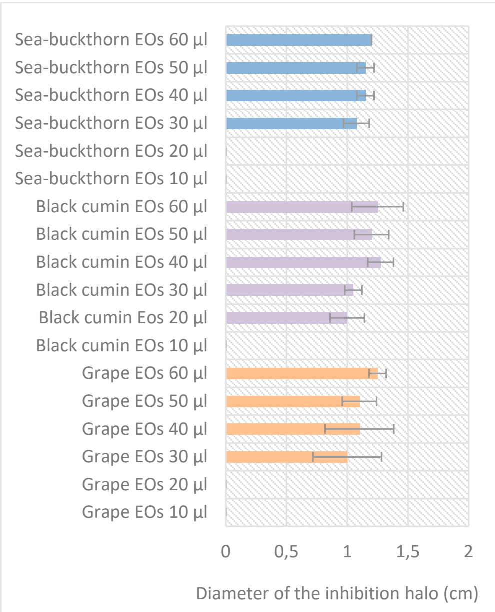

Figure 1. Graphical representation of the inhibition zone (diameter of the inhibition halo) of the essential oils tested on *Aspergillus flavus*

The results showed that in the case of *A. flavus* strain (Figure 1), grape seed and sea-buckthorn EOs presented antifungal activity from 30 µL, the diameter of the inhibition halo being around 1 cm. Black cumin essential oil presented antifungal activity at 20 µL, the diameter of the inhibition halo being 1 cm. The antifungal activity of the three EOs tested was almost the same when the dose was increased from 30 µL to 60 µL, thus the diameter of the inhibition halo for all three EOs ranged from 1 cm at 30 µL to 1.25 at 60 µL.

The tested EOs presented similar antifungal activity results in the case of *P. expansum* strain (Figure 2) as in *A. flavus* strain. Grape seed and sea-buckthorn EOs presented antifungal activity from 30 µL, the diameter of the inhibition halo being around 1 cm. In the case of black cumin essential oil, an inhibitory effect could be seen at 20 µL, the diameter of the inhibition halo being 1 cm.

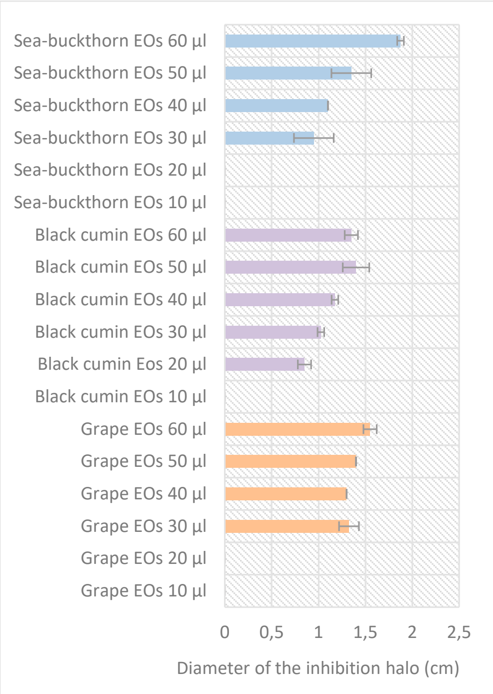

Figure 2. Graphical representation of the inhibition zone (diameter of the inhibition halo) of the essential oils tested on *Penicillium expansum*

The best results regarding the inhibitory effect were obtained at a concentration of 60 µL for the sea-buckthorn essential oil, the diameter of inhibition halo for *P. expansum* being 1.85 cm. At quantities ranging between 40 µL to 60 µL, black cumin and grape seed EOs presented similar inhibitory proprieties, the diameter of the inhibition halo measuring between 1.25 cm at 40 µL to 1.5 cm at 60 µL.

In the case of *F. oxysporum* and *Botrytis cinerea*, sea-buckthorn, black cumin and grape seeds EOs did not show antifungal activity, at any of the tested concentrations.

The minimum inhibitory concentration of sea-buckthorn, black cumin and grape seeds EOs were determined in order to assess their antimicrobial activity. Black cumin essential oil demonstrated lower MIC (20 µL) for both *A. flavus* and *P. expansum* compared with grape seed and sea-buckthorn EOs (Table 1). Grape seed and sea-buckthorn EOs had a MIC of 30 µl for *A. flavus* and *P. expansum*. For *F. oxysporum* and *B. cinerea* all three EOs tested did not show any antimicrobial activity even at concentrations of 60 µL.

Table 1. Minimum inhibitory concentration (MIC) of essential oils tested on *Aspergillus flavus*, *Penicillium expansum*, *Fusarium oxysporum* and *Botrytis cinerea*

Fungal strain	**Grape seed EO**	**Black cumin EO**	**Sea-buckthorn EO**
	MIC (µL)		
Aspergillus flavus	30	20	30
Penicillium expansum	30	20	30
Fusarium oxysporum	NA	NA	NA
Botrytis cinerea	NA	NA	NA

NA - no activity

CONCLUSIONS

In the present study, the antifungal activity of three EOs (sea-buckthorn, black cumin and grape seeds) was determined against four fungi strains with high occurrence in fresh fruits (*P. expansum*, *F. oxysporum*, *B. cinerea* and *A. flavus*). The results showed that all three EOs inhibited the growth of *A. flavus* and *P. expansum* strains at concentrations of 30 µL, the diameter of the inhibition halo being 1 cm. As for *F. oxysporum* and *B. cinerea* strains, none of the EOs tested had any growth inhibitory effect. Sea-buckthorn EO presented the best inhibitory effect over the *A. flavus* and *P. expansum* strains at 60 µL, the diameter of inhibition halo being at 1.85 cm. It can be concluded that the three EOs tested can inhibit the growth of *A. flavus* and *P. expansum* strain. It can be concluded that the three essential oils tested, sea-buckthorn, black cumin and grape seed, presented antimicrobial activity over the four fungi strains tested at concentrations over 30 µL. In order to establish the level of essential oil needed to inhibit the fungal growth in food matrix, further researches need to be made.

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