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Antibacterial and Antifungal Activity of Ethanol Extracts from *Centaurea montana*, *Centaurea macrocephala* and *Psephellus dealbatus*, Depending on the Phases of Their Phenological Development

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ABSTRACT

Background: Plants are an important source of biologically active compounds that can be used as crop protection agents. When using them, it is important to take into account the period when these biologically active substances accumulate in the plant to the maximum level. This article presents the results of a study on the antibacterial and antifungal activity of ethanol extracts obtained from *Centaurea montana*, *Centaurea macrocephala* and *Psephellus dealbatus* at different stages of their development.

Methods: Plants of *Centaurea montana*, *Psephellus dealbatus* and *Centaurea macrocephala* were grown in the experimental fields of the Tatar Research Institute of Agriculture in the Republic of Tatarstan, Russian Federation. Extracts were prepared from the freshly harvested biomass using single maceration with stirring. In experiments, the minimum inhibitory concentration (MIC) of bacterial and fungal extracts from *C. montana* and *P. dealbatus* and *C. macrocephala* were determined using a double sequential dilution method in a liquid medium. To determine the minimum bactericidal and fungicidal concentrations (MBC and MFC), 10 µl of an inoculum (or a piece of fungal mycelium) was added to agarized nutrient media in Petri dishes using a bacteriological loop, taken from test tubes without visible growth.

Result: Extracts from the buds, flowers, leaves and stems of these plants exhibited the greatest antimicrobial activity during the budding and early flowering stages. Bacterial growth was inhibited at an extract concentration of 625 µg/ml and fungal growth at a concentration of 312 µg/ml. The data obtained demonstrates the potential of using these ethanol extracts as herbal preparations for combating plant pathogens during the budding and early flowering periods.

Key words: Antimicrobial activity, Crop protection, Organic farming, Pesticide, Phytopathogens.

INTRODUCTION

As the world's population continues to grow, so does the demand for food. In order to meet this demand, we need to increase agricultural productivity. However, currently, it is not possible to achieve consistently high yields without the use of fertilizers and other chemical plant protection products (Khakimov *et al*., 2020). As a result, pesticides have become an essential part of modern agriculture (Riedo *et al*., 2023). Pesticides are designed to target specific organisms and should decompose quickly Sánchez-Bayo, (2021). However, in reality, a large percentage of substances used do not reach their intended destination and end up elsewhere in the environment (Kumar *et al*., 2023). During application, most pesticides reach the soil surface and are absorbed and destroyed by soil microorganisms (Copaja and Gatica-Jeria, 2021). Residual amounts of pesticidal preparations (2-8% of the initial application), which are usually considered unstable or moderately resistant, can remain in soils for years after their last use (Riedo *et al*., 2023).

Constant exposure to pesticides in the soil can potentially affect both the diversity and activity of plants, animals and fungi, leading to a deterioration of soil fertility. Biorational drugs are used as an alternative to chemical pesticides to combat diseases in crops. Before the development of synthetic pesticides, plants have been used for thousands of years to protect crops in their natural and

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processed forms. The plant-based ingredients of biopesticides are natural chemicals derived from plants that act as repellents, attractants, anti-feeders and growth inhibitors (Stankovic *et al*., 2020; Ngegba *et al*., 2022; Ahmed *et al*., 2024; Krishnasamy *et al*., 2025).

Herbal preparations are used in organic agriculture due to their safety (Suteu *et al*., 2020; Achraf *et al*., 2023; Saha *et al*., 2024). They are also useful in integrated pest management, as they have a positive impact on environmental conservation and have low toxicity to mammals, as well as a low risk of resistance development in target pests (Daraban *et al*., 2023; Iqbal *et al*., 2024).

Centaurea L. is a genus of herbaceous plants belonging to the family of composite flowers, comprising more than 700 species that grow in steppe, forest, floodplain and dry meadow areas, as well as on sediment, field margins and meadow slopes Bouafia *et al*. (2020). Extracts and essential oils from some *Centaurea* L. species have been shown to possess antitumor, anti-inflammatory and anti-diabetic properties (Shaldaeva *et al*., 2022; Guvensen *et al*., 2019; Kubik *et al*., 2022; Sen, 2023; Fattaheian-Dehkordi *et al*., 2021; Yirtici *et al*., 2023).

Ethanolic extracts of *C. ptosomipappoides*, *C. odyssei*, *C. ptosomipappa*, *C. amonicola* and *C. kurdica* at concentrations of 65 µg/ml and above inhibit the growth of *P. vulgaris*, *B. cereus*, *E. coli*, *A. hydrophila*, *L. monocytogenes* and *S. aureus*, as well as *M. luteus* Guven *et al*. (2008). Extracts of *C. cyanus* L., *C. jacea* L. and *C. scabiosa* L. at concentrations ranging from 60 to 120 µg/ml inhibit the growth of the gram-positive phytopathogens *C. michiganensis* and *A. solani* Sharonova *et al*. (2021). The fungicidal activity of methanolic extracts of six *Centaurea* species collected in Iran has been demonstrated against the growth of *Pythium aphanidermatum mycelium, Phytophthora melonis and Rhizoctonia solani* (Abbasi, 2012). Ethanolic extracts from 12-week-old *Centaurea solstitialis* plants collected from the USA showed activity against *A. helianthi*, *F. arthosporioides*, *F. oxysporum*, *F. solani*, *B. cinerea*, *P. palmivora* and *S. sclerotiorum* (Guermache and Widmer, 2004).

The data presented in the literature indicate changes in the antimicrobial activity of plants from the Centaurea genus, depending on the region where they grow, the stage of their growth and the extraction method used. The aim of this study is to investigate the antimicrobial activity of extracts from different parts of *C. montana*, *P. dealbatus* and *C. macrocephala* plants, in relation to their phenological development phase.

MATERIALS AND METHODS

Plants and extraction

To conduct research in 2022, plants of *Centaurea montana*, *Psephellus dealbatus* and *Centaurea macrocephala* were grown in the experimental fields of the Tatar Research Institute of Agriculture in the Republic of Tatarstan, Russian Federation. The selection of these plants was carried out twice a month from May to September 2022 during the morning hours from 7.00 to 10.00. After harvesting, the plants were washed with distilled water and separated into flowers, leaves and roots. Extracts were prepared from the freshly harvested biomass using single maceration with stirring. The plant material was ground in a laboratory mill (LM 202, Russia) and 150 mL of extractant (70% ethanol solution in water) was added to a 15 g suspension of the ground material. The mixture was continuously stirred for 1.5 hours at extraction temperature 45°C. The selected extraction conditions, based on the results of our own research published in articles Sharonova *et al*. (2021) and Nikitin *et al*. (2023), make it possible to extract biologically active compounds as completely as possible without their thermal degradation. The resulting mixture was filtered using filter paper (Whatman No.1) and the filtrate was then concentrated using a rotary evaporator (LabTexRe 100-Pro). The final extracts were stored in the dark at 4°C.

Strains of microorganisms and nutrient media

The test cultures used in this study were strains of gram-positive bacteria *Clavibacter michiganensis* BKM Ac-1404 (Cm) and gram-negative bacteria *Erwinia carotovora* spp. Carotovora (Ec), as well as the fungi *Alternaria solani* K-100054 (As) and *Rhizoctonia solani* BKM F-895 (Rs), which were obtained from the All-Russian Collection of Microorganisms at the Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences" and the collections of phytopathogenic organisms from the All-Russia Scientific Research Institute for Phytophathology.

Liquid broth with microbial spores was prepared using standard nutrient media, including Potato Extract Glucose broth for *Ec*, *As* and *Rs* and Corynebacterium Selective Agar for *Cm*. Tebucanazole and chloramphenicol (produced by Kazan Pharmaceutical Plant in Russia) as well as diphenoconazole (from Score 250 EC by Syngenta in the USA) were used as reference compounds for the experiments.

Antimicrobial analysis *in vitro*

In experiments, the minimum inhibitory concentration (MIC) of bacterial and fungal extracts from *C. montana* and *P. dealbatus* and *C. macrocephala* were determined using a double sequential dilution method in a liquid medium Sharonova *et al*. (2021).

The study used 24-hour bacterial cultures. The final inoculum contained 10^2 KE/mL for bacteria and 1.1-1.5 × 10^2 KOE/mL for fungi. The bacterial concentration was determined using a DEN-1B densitometer (Biosan, Latvia). Control tubes containing only the nutrient medium were also used. Fungi for experiments were grown from mycelium on a solid loop medium for 7-14 days. Discs of mycelium with a diameter of 5 mm were cut with a sterile steel tube, at a distance of 8-10 millimeters from the edge of an actively growing colony and placed in a test tube containing a nutrient medium and an extract of the concentration being studied.

To determine the minimum bactericidal and fungicidal concentrations (MBC and MFC), 10 µl of an inoculum (or a piece of fungal mycelium) was added to agarized nutrient media in Petri dishes using a bacteriological loop, taken from test tubes without visible growth.

The results of bacterial growth were recorded daily for 5 days at 30°C for *Cm* and at 25°C for *Ec*. Fungi were incubated in a thermostat at 26°C for 7 days to determine the growth of microorganisms visually Sharonova *et al*. (2021). All analyses were performed in triplicat.

RESULTS AND DISCUSSION

Gram-positive bacterium *Cm* isolated from corn and gram-negative bacterium *Ec*, a pathogen of potato and tomato blackleg, were used to test antibacterial activity. To assess antifungal activity, *As*, the causative agent of plant alternariasis in the Solanaceae family and *Rs*, a causative agent of wheat root rot disease, were used as strains. Table 1 presents the values of minimum inhibitory fungicidal and bactericidal concentrations of flower and stem extracts of plants collected between May and August 2023. Extracts were obtained from plants at different stages of their phenological development, starting with the appearance of leaves and ending with fruit dropping. These stages include budding and flowering. Chloramphenicol is an antibiotic that inhibits protein synthesis in bacterial cells by disrupting peptidyl transferase. It is used as a shading agent to control bacteria. Tebuconazole is a systemic fungicide with a wide range of activity. It has protective, curative and eradicating properties. It quickly penetrates the plant and is evenly distributed throughout it (Leite *et al*., 2024).

Bacteriostatic and fungistatic concentrations of ethanol extracts from the stems and leaves of the plants studied were found to start at 312 µg/ml. All extracts, during the initial stages of the appearance of inflorescences, showed high activity against the gram-positive bacterium Cm and remained active until the stage of budding and the beginning of flowering. However, during the period of full flowering and further plant growth, extracts from flowers and stems of all species under study were found to be less active. The MIC of the extracts was above 2500 µg/ml.

In relation to gram-negative bacteria *Ec*, extracts *C. montanà* and *P. dealbatus* were active during the emergence of inflorescences and budding and extract *C. macrocephala* was active during budding and the onset of flowering. The data are presented in Table 2.

Extracts of stems and leaves from *C. montanà* and *P. dealbatus* plants, collected during the period when inflorescences begin to appear before full flowering, showed high antifungal activity against *As*. The extracts were most active at the beginning of the flowering period. The MIC started at a concentration of 312 µg/ml of extract.

In relation to *Rs*, flower extracts were also active during the early stages of flowering. *C. montana* flower extract showed the maximum inhibition of growth. The lowest concentration that inhibited the development of the Rt test culture was 312 µg/mL.

Extracts of the stems and leaves of *C. macrocephala* were not active against the fungi studied. Extracts of plant buds showed high antibacterial and antifungal activity against all test cultures. They started to inhibit the growth of bacteria at a concentration of 625 µg/ml. Extracts of *C. montana* buds with a MIC of 1250 µg/ml showed the least bacteriostatic activity. The antibacterial activity of flower extracts at the beginning of flowering was similar to that of bud extracts. As the plant developed further, the antimicrobial activity of flowers decreased.

Extracts of flowers collected at the beginning of flowering in all studied plants, at a concentration of 625 µg/ml, began to inhibit the growth of Cm, but already at the stage of full flowering, the minimum inhibitory concentration (MIC) increased to 2500 µg/mL. With regard to gram-negative bacteria, all flower extracts were inactive.

Extracts of *C. montana* and *P. dealbatus* buds had lower MIC values against fungi compared to bacteria. Inhibition of the growth of *As* and *Rs* test cultures began at an extract concentration of 312 µg/ml. Fungicidal concentrations were greater than 1250 µg/ml. The antifungal activity of flower extracts was lower than that of bud extracts. At a concentration of 625 µg/ml, extracts from *C. montana* and *P. dealbatus* inhibited the growth of *As*.

Flower extracts were not active against *Rs*. Only the extract from *C. montana* flowers, collected at the beginning of flowering, showed an MIC value of 625 µg/ml. In addition to extracts from leaves, stems and flowers, we also analyzed the antimicrobial activity of roots, obtained at the stage of fruit shedding and the completion of plant regeneration signs. The data are presented in Table 3.

The data from the study showed that the plants under investigation have antimicrobial activity against test cultures of phytopathogenic bacteria and fungi. The ability of extracts from *C. montana*, *P. dealbatus* and *C. macrocephala* to inhibit the growth of microorganisms depends on both the stage of plant development and the parts used for extraction.

Álvarez-Martínez *et al*. (2021) developed a scale for measuring the antimicrobial activity of plant extracts. According to this scale, plant extracts with an MIC of more than 2500 µg/ml should be considered inactive. Extracts with an MIC between 500 and 2500 µg/ml are considered moderately active. Extracts with MICs between 100 and 500 µg/ml are significantly active. And extracts with MICs less than 100 µg/ml are very active (Alvarez-Martinez *et al*., 2021).

During the leaf emergence stage, extracts from the leaves and stems of all plants studied exhibit moderate activity against bacterial strains, which persists for extracts of *C. montana* until the flowering stage and for *C. macrocephala* and *P. dealbatus* until the full flowering stage. Moderate antibacterial activity, similar to that of leaf and stem extracts, is also shown by bud and flower extracts during their emergence phase. As plants develop further, the antibacterial activity of all *Centaurea* L. parts decreases. During the full flowering and end of flowering stages, the

Table 1: Antimicrobial activity of stems and leaves.

Plants	Date of collection of plants	Phenological stages	*Cm*		*Ec*		*As*		*Rs*	
			MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MFC, µg/ml	MIC, µg/ml	MFC, µg/ml
C. macrocephala	11.05.23	Germination	625±28	1250±56	1250±44	1250±44	1250±62	2500±124	1250±102	2500±204
	5.06.23	Budbloom	625±62	1250±124	1250±52	1250±52	1250±48	2500±96	1250±56	2500±102
	19.06.23	The appearance of a bud	625±56	625±56	625±42	2500±168	1250±44	2500±88	2500±160	>2500
	5.07.23	The beginning of blooming	1250±48	2500±96	625±40	2500±160	1250±36	2500±72	2500±214	>2500
	17.07.23	Full bloom	2500±130	2500±130	1250±58	2500±116	2500±172	2500±172	2500±180	>2500
	31.07.23	The end of flowering	2500±188	>2500	2500±154	>2500	>2500	>2500	2500ñ	>2500
	15.08.23	Shedding of flower seeds	2500±154	>2500	1250±62	2500±124	>2500	>2500	>2500	>2500
P. dealbatus	11.05.23	Germination	625±62	1250±124	625±32	1250±64	625±38	1250±76	1250±62	2500±124
	5.06.23	Budbloom	625±46	1250±92	625±32	1250±64	625±32	1250±64	1250±48	2500±96
	19.06.23	The appearance of a bud	625±60	625±60	1250±68	2500±136	312±16	1250±64	625±28	1250±56
	5.07.23	The beginning of blooming	2500±140	2500±140	1250±52	2500±104	625±42	2500±168	2500±194	2500±194
	17.07.23	Full bloom	2500±208	>2500	>2500	>2500	>2500	>2500	>2500	>2500
	31.07.23	The end of flowering	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
	15.08.23	Shedding of flower seeds	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
C. montana	11.05.23	Germination	625±40	1250±80	625±62	1250±124	625±32	1250±64	1250±72	> 1250
	5.06.23	Budbloom	625±56	625±56	625±40	625±40	625±43	> 1250	625±44	1250±88
	19.06.23	The appearance of a bud	2500±160	2500±160	>2500	>2500	312±12	625±24	312±20	625±40
	5.07.23	The beginning of blooming	2500±148	2500±148	625±56	>2500	625±52	1250±104	1250±60	2500±120
	17.07.23	Full bloom	2500±152	2500±152	1250±82	2500±164	2500±208	2500±208	2500±218	>2500
	31.07.23	The end of flowering	2500±134	2500±134	2500±172	2500±172	2500±160	2500±160	2500±144	2500±144
	15.08.23	Shedding of flower seeds	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500

Cm - Clavibacter michiganensis, Ec - Erwinia carotovora, As - Alternaria solani, Rs -Rhizoctonia solani.

Table 2: Antimicrobial activity of inflorescences.

Plants	Date of collection of plants	Phenological stages	*Cm*		*Ec*		*As*		*Rs*	
			MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml
C. macrocephala	05.06.23	The appearance of a bud	625 ±32	1250±64	625±42	625±42	2500±166	2500±166	2500±206	2500±206
	19.06.23	The beginning of blooming	625±36	1250±72	1250±82	>2500	1250±58	2500±116	2500±180	2500±180
	5.07.23	Full bloom	2500±202	>2500	1250±94	2500±188	2500±162	2500±162	2500±220	>2500
P. dealbatus	05.06.23	The appearance of a bud	625±36	625±36	625±46	1250±92	312±22	1250 ± 88	312 ±22	1250 ± 88
	19.06.23	The beginning of blooming	625±42	625±42	1250±60	2500±120	625±36	2500 ± 144	1250±52	2500±104
	5.07.23	Full bloom	2500±190	>2500	1250±48	2500±96	1250±93	>2500	2500±134	2500±134
C. montana	05.06.23	The appearance of a bud	625±30	1250±60	1250±66	2500±132	312±28	1250 ± 112	312±28	1250± 112
	19.06.23	The beginning of blooming	625±46	625±46	1250±82	>2500	625±46	1250±46	625±30	1250±60
	5.07.23	Full bloom	2500 ±218	2500±218	1250±64	>2500	1250±72	2500±144	2500±176	2500±176

Cm - Clavibacter michiganensis, Ec - Erwinia carotovora, As - Alternaria solani, Rs -Rhizoctonia solani.

Table 3: Antimicrobial activity of the roots.

Plants	Date of collection of plants	Phenological stages	*Cm*		*Ec*		*As*		*Rs*	
			MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml
C. macrocephala	15.08.23	Shedding of flower seeds	2500±204	>2500	1250±78	2500±156	>2500	>2500	1250±66	>2500
	4.09.23	There are no signs of plant growth.	2500±156	>2500	>2500	>2500	1250±84	>2500	1250±62	>2500
P. dealbatus	15.08.23	Shedding of flower seeds	2500±170	>2500	1250±92	1250±92	>2500	>2500	1250±90	>2500
	4.09.23	There are no signs of plant growth.	2500±218	>2500	>2500	>2500	1250±80	>2500	1250±88	>2500
C. montana	15.08.23	Shedding of flower seeds	>2500	>2500	1250±72	2500±144	>2500	>2500	1250±104	>2500
	4.09.23	There are no signs of plant growth.	2500±210	>2500	>2500	>2500	1250±92	>2500	2500±216	>2500

bacteriostatic activity of stem, leaf and flower extracts is already in a range between moderate and inactive activity. Bactericidal concentrations of the extracts are slightly different from bacteriostatic concentrations and are 2 times higher or equal to MIC values.

The test strains of fungi were more sensitive to the action of bacteria. We identified the significant antifungal activity of extracts from *P. dealbatus* and *C. montana* at the budding, flowering and leaf fall stages. During the budding stage, extracts from the buds showed significantly antifungal activity, while leaves and stems had moderate activity. In the flowering stage, the flowers showed decreased antifungal activity to moderate levels, while the stems and leaves showed increased activity to strong levels. In the final leaf fall stage, all *Centaurea* L. extracts showed decreased activity to moderate levels and then became inactive. Extracts from *C. macrocephala* have been moderately active and inactive throughout the entire period of their study.

The results obtained indicate the possibility of using *Centaurea* L. extracts, such as *C. montana* and *P. dealbatus*, as botanical preparations for combating diseases caused by phytopathogenic bacteria and fungi. These extracts have shown promising antifungal activity, especially when extracted from the upper parts of *P. dealbatus* plants harvested in the budding or early flowering phase. However, the antifungal activity of the extracts from *C. macrocephala* has been found to be only moderately active. This is because the accumulation of compounds with strong antifungal activity that can dissolve in 70% ethanol does not occur in *C. macrocephala*. This makes this plant less promising for use as a natural remedy against fungal pathogens. Due to the susceptibility of agricultural crops to fungal diseases, it is recommended to use *C. montana* or *P. dealbatus* extracts as a more effective option for controlling these diseases.

The data obtained complements the knowledge about the antimicrobial activity of *Centaurea* L. extracts. It has been established that extracts from *C. montana* leaves and their derivative fractions inhibit the growth of cholera vibrios, salmonella typhi, *A. baumanni*, *S. dysenteriae*, *B. anthrax*, *M. lacunata*, while not being active against fungi such as *P. chrysogenum*, *C. albicans, A. fumigatus* (Ahmad *et al*., 2015). Methanol, ethanol, n-hexane and ethyl acetate extracts of *C. iberica* successfully suppress the growth of *S. aureus*, *E. coli, K. pneumonia*, *C. albicans*, *M. racemosus A. niger* (Bibi *et al*., 2023). Our data is comparable to the values of antibacterial and antifungal activities of extracts from related species such as *C. bingoelensis, C. sivasica, C. amaena* Boiss. and *Balansa u C. aksoyi* Hamzaoglu and Budak., *C. hypoleuca* (Uysal *et al*., 2021; Yırtıcı *et al*., 2021; Albayrak *et al*., 2017; Ozcan *et al*., 2019).

Changes in the antimicrobial activity of plant extracts during the growing season are dependent on the compounds present in the plant composition. Preliminary phytochemical analysis of plant leaves has revealed a rich source of fatty acids, flavonoids, alkaloids and glycosides (Ahmad *et al*., 2015). Glucosyl-3-cyanidin and diglycosyl-3-5- cyanidin have been identified in the flowers of certain plants (Kamanzi and Raynaud, 1977). Flavonoid glycosides, based on apigenin, luteolin and chrysoeriol, have also been identified (Gonnet, 1992). Additionally, citellarein-6,7-dimethyl ether, scutellarein-6,7,4'-trimethyl ether and various methyl derivatives of 6-hydroxy-luteolin have been found (Wollenweber, 1991). It has been determined that flavonoids and sesquiterpene lactones, particularly germacranolides, eudesmanolides, elemanolides and guaianolides, contribute to the biological activity of Centaurea species, including antimicrobial properties (Sokovic, 2017). During the growth process, the quantitative composition of these compounds in plants can vary significantly. Seasonal fluctuations in the fatty acid composition, tocopherol content and phenolic acid content have been observed. The plant growth stage of *Centaurea* sp., for example, is characterized by a high content of phenolic acids (Bouafia *et al*., 2020; Bouafia *et al*., 2023).

CONCLUSION

Studies of the antimicrobial activity of ethanol extracts from C. *montana*, P. *dealbatus* and C. *macrocephala* plants growing in the Republic of Tatarstan during the active vegetation period have shown their significantly active to inhibit the growth and development of phytopathogenic microorganisms. The minimum inhibitory concentrations for bacteria were 625 µg/ml and for fungi, 312 µg/ml. Extracts obtained from these plants in the budding and early flowering stages have a promising potential for use in botanical preparations for controlling phytopathogens, as they are environmentally friendly and effective alternatives to conventional pesticides. Nevertheless, further research and development are needed to optimize production methods and establish standards for safe and high-quality use of plant extracts in agricultural applications. This is a significant contribution to the field of research into alternative pesticides and it opens up the possibility for further research and development of cornflower extract-based products in the fight against plant disease.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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