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Phytochemicals Screening and Antimicrobial Activity of Veronica Agrastis Extract

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P A P E R I N F O

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A B S T R A C T

Phytochemical compounds are of medical importance for many diseases, including inhibitory activity against bacterial and fungal pathogens. In this paper , multiple phytochemicals were isolated from *Veronica agrestis* in Iraq by subjecting the stem and leaves to gas chromatography-mass spectrometry (GC-MS). The study aims to identify the active compounds of the vegetative parts of the species and test their inhibitory activity against pathogens. The analysis revealed the presence of ten biologically active phytochemicals: benzeneacetonitrile, 3-hydroxy; (E)-4-(3-hydroxyprop-1-en-1-yl)-2;methoxyphenol; phytol; 9,12,15,-octadecatetrienoic acid (Z,Z,Z); 13,17Seco-5 pregn-13(18)-n-20-one; stigmasterol; octatriacontyl pentafluoropropionate; 5-(7a-isopropyl-4,5-dimethyl-octahydroindin-4-yl)-3-methyl-penta-2,4-dien-1-ol; cholestan-3-ol; 2-Methylenediamine-,(3β,5α)-. These compounds showed high inhibitory activity against the pathogenic fungus *Candida albicans* and the bacteria *Proteus mirabilis*. Six concentrations of the extract were used (5 ,10, 20 ,40 ,60 ,80)mg/ml showed results (00.00, 0.00, 0.00, 6.00,10.00,16.00) mm diameter of colonies in *Candida albicans* whil the diameter of colonies in *Proteus mirabilis* presented results (0.00,0.00, 10.00, 14.00, 22.00, 30.00) mm respectivly .

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1. INTRODUCTION

The genus *Veronica* is an important plant genera belonging to the Plantaginaceae family (Albach D. C., 2006) , and includes a large number of species that are rich in biologically active phenolic compounds. This genus has attracted the attention of researchers due to its potential uses in various fields, including pharmaceuticals, cosmetics, and natural products used in food preservation (Brčić A., 2024).Studies have shown that some Veronica species, such as *V. rosea*, contain flavonoids with antioxidant activity. These compounds have also been shown to be effective in protecting against sun exposure and reducing heat-induced hemolysis, reflecting their potential role in supporting skin health and reducing muscle tension (Chaira S. et al., 2022).

As for *V. amygdalina*, leaf analysis has revealed a wide range of active plant compounds, such as alkaloids, flavonoids, tannins, saponins, and others, which have demonstrated cholesterol-lowering, antioxidant, and anticancer properties, as well as their effectiveness in lowering blood sugar levels (Great I. et al., 2023).Studies have also been conducted on *V. biloba*, where active compounds such as dichloromethane, ethyl acetate, and n-hexane were extracted. (Hassan A. M. I. R., 2019). These extracts demonstrated remarkable activity against a range of bacteria and fungi, particularly due to the presence of ginkolic acid, known for its antibacterial properties (Hassan A. et al., 2019) Other species of the genus, such as *V. officinalis* (Rajakumar S. et al., 2022) *V. orchidea*, and *V. teucrium,* have also proven effective as antimicrobials, particularly against Gram-positive bacteria such as *Bacillus cereus* and *Listeria spp*. (Nazlić M. et al., 2023) . Some Veronica species also contain compounds such as benzaldehyde, known for its role in fungicidal resistance (Ebrahimzadeh M. & Navaei N., 2024). On the other hand, extracts of *V. montana* have demonstrated activity against *Escherichia coli* and *Staphylococcus aureus*. This activity is attributed to the

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effect of flavonoids and tannins in disrupting bacterial cell membranes (Brčić A., 2024).
Many other species, such as *V. persica*, *V. hederifolia*, *V. polita poir*, and *V. anagallis*-aquatica, have been observed to be rich in phenolic compounds with diverse health effects, including antimicrobial activity (Bačič K., 2024) . Despite this significant research on the various species of the genus, insufficient information is available on the phytochemical composition of *Veronica agrestis*, highlighting the need for a detailed study of this species. Therefore, this study aims to identify the active compounds in the vegetative parts of this species and test their inhibitory activity against certain pathogens, such as bacteria and fungi, particularly the genus *Candida.*

2. MATERIAL AND METHODES

2.1. Study Area and Sample Collection

The samples used in this study were obtained from a number of infected patients, in collaboration with the University of Karbala. The samples were collected under sterile conditions and then transported to the laboratory for isolation. Fungi and bacteria were isolated from the biological samples, and the species under study were identified after obtaining pure cultures using standard microbiological methods.

2.2 Collection and Preparation of Plant Materials

sciences college laboratory, Kerbala

The plant known as "*Veronica agrastis*" was found in several locations throughout Iraq. After that, the plants were cleaned and allowed to dry at room temperature. After absorbing 100 milliliters of ethanol, 10 grams of powdered plants were filtered.

2.3. Plant Extract preperation

The dried stem and leaves were pulverized and extracted in a Soxhlet. The polar compounds were extracted with 1.5 liters of eythanol, then filtered and evaporated. After that, the compounds were weighed, and their yield percentage, expressed as a percentage of their dry weight, was calculated. The yield of the dark green extract from eythanol was 10 grams (0.095%), the plant extracts were placed in the °C. (Wahid A. Z. & Jafar F. N., 2005) dark and refrigerated at a temperature of 4

2-4-Testing the Effect of the Alcoholic Extract of Veronica agrestis on the Growth of Fungi and Bacteria

The effectiveness of the alcoholic extract of V. agrestis against pathogenic strains of fungi and bacteria (Proteus spp. and Candida spp.) was evaluated using the well diffusion method on Mueller-Hinton agar

PROCEDURE

1. Preparation of Inoculum:
   - Inoculate a single colony of Proteus and Candidain sterile broth and incubate overnight at 37°C (for Proteus) and 30°C (for Candida).
   - Adjust the inoculum to match a 0.5 McFarland standard.
2. Inoculating the Plates:
   - Pour Mueller-Hinton agar into sterile Petri dishes and allow it to solidify.
   - Use a sterile swab to evenly spread the inoculum across the surface of the agar plates.
3. Creating Wells:
   - Once the agar is inoculated and dried, use a sterile cork borer or well-maker to create wells in the agar plates.
4. Adding Plant Extracts and Controls:
   - Using a micropipette, add 100 µL of each concentration of the plant extract (80, 60, 40, 20, 10, and 5) mg/ml into separate wells.
   - Add 100 µL of ciprofloxacin into one well as the positive control and 100 µL of distilled water into another well as the negative control.
5. Incubation:
   - Incubate the plates at the appropriate temperature (37°C for Proteus and 30°C for Candida) for 24-48 hours.
6. Measuring Inhibition Zones
   - After incubation, measure the diameter of the inhibition zones around each well using a ruler or caliper.
   - Record the results for each concentration of the plant extract and the controls.

2- Using gas chromatography and mass spectrometry (GC/MS) to identify the chemical components of the extract, GC-MS analysis was used to chemically identify the compounds of *V. agrastis*. At Al-Zahraa Center for Medical and Pharmaceutical Sciences Research, the sample was prepared according to the method (Abu-Serag N. A. et al., 2019) (Al-Zahraa Center for Medical and Pharmaceutical Research Sciences, n.d.)

2.5. Statistical Analysis

Data were analyzed using STATGRAPHICS Centurion XV (version 20.1.16). ANOVA and LSD tests were employed at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1 These results are consistent with (Ertas A. et al., 2014), when they studied the activity of the family species against the obtained pathogens. It was found that the ethanolic extract of V. agrestis parts inhibited the fungi causing candidiasis and other skin diseases and that some of the secondary metabolites had antifungal properties. Six concentrations of the extract (5, 10, 20, 40, 60, 80) g/ml were used. The colony diameter of Candidia albicans showed results (0.00, 0.00, 0.00, 6.00, 10.00, 16.00) mm, respectively. Therefore, the results are presented in Table (1) figure (1) . The alcoholic extract showed high antibacterial activity against Proteus mirabilis, which causes urinary tract infections. Six concentrations of the extract (5, 10, 20, 40, 60, 80) g/ml were used. The colony diameter of Proteus mirabilis showed results of (0.00, 0.00, 10.00, 14.00, 22.00, 30.00) mm, respectively. Therefore, the results are shown in Table (2) and figure (2). These results are consistent with the results of Salehi (Salehi B. et al., 2019), when they studied the activity of the family species against some secondary metabolites that act as antimicrobials and they found that.

Table 1. Antifungal activity of ethanol extracts from *V.agrastis* against *Candida albicans*

Table (1)	Mean of Inhibition zone (mm)						
Comparison with distilled water (0.00) mg/ml	Comparison with Nystatin 10mg	Concentration (5mg/ml)	Concentration (10mg/ml)	Concentration (20mg/ml)	Concentration (40mg/ml)	Concentration (60mg/ml)	Concentration (80mg/ml)
0.00	8.00	0.00	0.00	0.00	6.00	10.00	16.00

Table 2. Antibacteral activity of ethanol extracts from *V.agrastis* against *P. mirabilis*

Comparison with distilled water (0.00) mg/ml	Comparison with Ciproflox acin 10mg	Concentration (5mg/ml)	Concentration (10mg/ml)	Concentration (20mg/ml)	Concentration (40mg/ml)	Concentration (60mg/ml)	Concentration (80mg/ml)
0.00	8.00	0.00	0.00	10.00	14.00	22.00	30.00

When reviewing the results of the current study on the chemical compounds of Veronica, it is noted that the percentage of compounds with fungal growth inhibitory properties is high, such as steroids, sticasterol, lanosterol, organofluoro ester compounds, unsaturated fats, in addition to phenols and diterpenes, all of which play a role in the inhibitory action against Candida (Alqurashi A. S. et al., 2022).

Figure 1. *Candida albicans* growth grown in varying concentrations of *V. agrestis* ethanol extract.

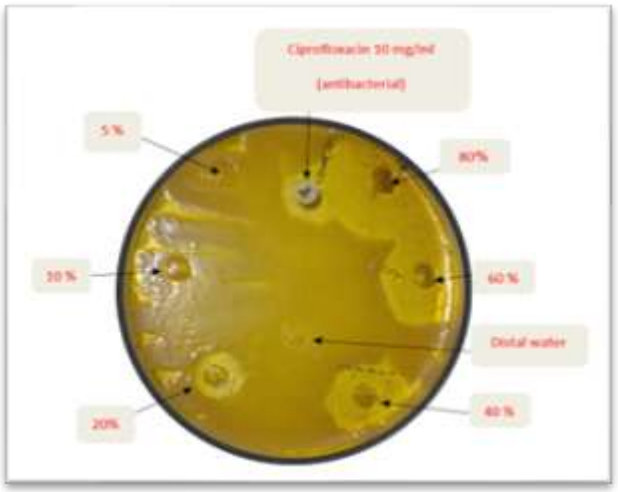

Figure 2. Growth of Proteus mirabilis cultured in varying concentrations of *V. agrestis* ethanol extract

3.1. Gas chromatography-mass spectrometry (GC-MS)

The ethanolic extract of the vegetative parts of Veronica agrestis was analyzed using gas chromatography-mass spectrometry (GC-MS). Ten major chemical compounds were detected, as shown in Table (3).

Table 3. Major phytochemical compounds in ethanolic extract *V. agrastis.*

No.	R. t. (min)	Area	%Total	M. wt	Prob %	Name
1	18.347	1.005e+8	2.036	133	68.8 %	Benzeneacetonitrile, 3-hydroxy
2	18.788	1.544e+8	3.129	133	68.7 %	Benzeneacetonitrile, 3-hydroxy
3	24.536	7.685e+7	1.557	180	51.0 %	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol
4	31.788	1.850e+8	3.749	296	64.6 %	Phytol
6	41.327	1.752e+7	0.355	400	15.3 %	Cholestan-3-ol, 2-methylene-, (3β,5α)-
7	43.501	3.723e+8	7.543	302	8.12 %	13,17-Seco-5α-pregn-13(18)-en-20-one
8	44.628	1.179e+9	23.887	412	57.1 %	Stigmasterol
9	45.998	7.685e+7	1.557	696	5.06 %	Octatriacontyl pentafluoropropionate
10	48.737	3.119e+7	0.632	288	12.8 %	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien- 1-ol

Table 4. Classification and Biological Relevance of Phytochemicals Isolated from the ethanolic extract *V. agrastis.*

No.	Compound Name	Compound Type	
1	Benzeneacetonitrile, 3-hydroxy	Aromatic nitrile	Antimicrobial, antioxidant
2	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (Coniferyl alcohol)	Phenylpropanoid	Antioxidant, anti-inflammatory, anticancer
3	Phytol	Diterpene alcohol	Antimicrobial, antioxidant, anticancer,
4	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (α-Linolenic acid)	Omega-3 fatty acid	Anti-inflammatory, antioxidant
5	Cholestan-3-ol, 2-methylene-, (3β,5α)-	Plant sterol	Cholesterol-lowering, anti-inflammatory, anticancer
6	13,17-Seco-5α-pregn-13(18)-en-20-one	Steroid derivative	Hormonal activity, anti-inflammatory effect
7	Stigmasterol	Plant sterol	Antioxidant, anti-inflammatory, cholesterol-lowering
8	Octatriacontyl pentafluoropropionate	Long-chain ester	antibacterial
9	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-1-ol	Unsaturated cyclic terpenoid	Antifungal, antibacterial, antioxidant

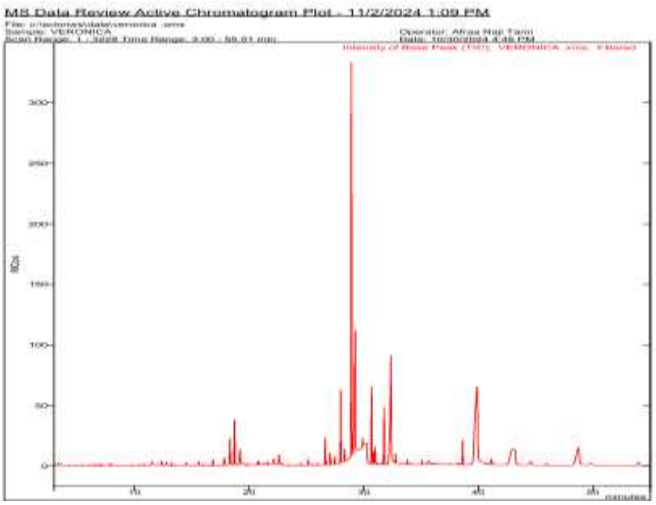

Figure 3. GC-MS chromatogram of ethanolic extract the leaves and stem for *V. agrestis*

4. CONCLUSION

The ethanolic aroma of *V. agrestis* leaves and stems exhibits potent antifungal activity against the fungus Candidia albicans and the bacterium Protus mirabilis. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the extracts contained a variety of bioactive plant compounds, including aromatic compounds, phenols, steroids, and terpenes, which likely contribute to their antimicrobial properties. This suggests *V. agrestis* as a natural source of antifungal and antibacterial properties.

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