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Chemical Composition and Microbiological Activity of Some Plants Belonging to the Fabaceae Family

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Introduction

Despite the availability of several antibiotics and antimycotics, the treatment of patients, especially the immunocompromised ones, is still limited because of low drug potency. The emergence of resistant strains and diseases due to certain free radicals, mainly oxygen reactive species also makes treatment challenging.

Based on the reports received through the European Antimicrobial Resistance Surveillance Network (EARS-Net), the WHO reports alarming growth rates of pathogenic microorganisms that have developed multiple resistances to common drugs.

Unfortunately, the pharmaceutical industry does not have the required rate of production of synthetic, allopathic drugs to cover the development of these multi-resistant organisms. This study is intended to obtain untested, natural compounds existing in the spontaneous flora, which have antimicrobial activity.

Our research investigates the chemical composition, antimicrobial, and phytochemical activity of four extracts obtained from the Fabaceae family flowers, which grow wild in Western Romania.

The four selected plants were: Melilotus officinalis (MO) (Melilot, Sweet clover); Coronilla varia (CV) (Scorpion vetch); Ononis spinosa (OS) (Spiny restharrow), and Robinia pseudoacacia (RP) (Black locust).

The *Fabaceae*, or *Leguminosae*, is one of the three largest flowering plants, exceeded only by the *Compositae* and *Orchidaceae*, with an estimated ~ 750 genera. From an economic point of view, the *Fabaceae* family is second only to Gramineae, including many economically and medicinally important flowering plants. The main criteria for their selection was their abundance in the wild flora in Western Romania, with all four plants being native or naturalized in our country but need further research.

In view of this, the purpose of this study was to determine which of the selected plants have antimicrobial activity and to identify the chemical components responsible for these properties.

The experimental part involved: i) obtaining MO, CV, OS and RP hydroalcoholic extracts; ii) analysing the total polyphenols content and polyphenolic profile of MO, CV, OS and RP extracts using the LC methodology; iii) testing the in vitro antimicrobial effect of extracts.

To the best of our knowledge, the local wild plant species selected for this study are being screened for the first time regarding their chemical composition and their antimicrobial effects linked to their specific composition.

Materials and Methods

Plant material

The aerial parts of the investigated medicinal plant species (MO, CV, OS and RP) were collected during the flowering period from the wild flora located on the outskirts of Timisoara, Romania (45°47'00.1"N 21°12'37.2"E). From each species, ~500 g of fresh material was used. Additionally, voucher specimens were botanically identified and deposited in a temperature-controlled herbarium (22–25 °C and 30–40% relative humidity) Vouchers Specimen Number Herbarium - Botany Department, M.O - VSNH.BUASTM-BD56, C.V - VSNH.BUASTM-BD57, 0.S - VSNH.BUASTM-BD58, R.P - VSNH.BUASTM-BD59).

Preparation of extracts

The plant material was air dried at 25 °C and ground to a fine powder using a grinder (GM 2000; Grindomix; Retsch Technology GMbH, Haan, Germany). The powdered material (2 g) was extracted with 20 mL 60% ethanol (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 30 min at room temperature using an ultrasonic water bath (FALC Instruments, Treviglio, Italy). Extracts were then filtered using Whatman membrane filters nylon 0.45 µm with 30 mm diameter (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and stored at 2–4 °C for subsequent chemical and antimicrobial analyses.

Determination of total polyphenols content by Folin-Ciocalteu assay

The total phenolics content was determined according to Folin-Ciocalteu modified method. The samples were incubated for 30 min at 50°C in INB500 thermostat, Memmert GmbH, Schwabach, Germany) after read absorbance at 750 nm using a UV-VIS spectrophotometer (Specord 205; Analytik Jena AG, Jena, Germany). The calibration curve was obtained using gallic acid (concentration range: 2.5-250 µg/mL). The results were expressed in mg GAE per g of dry matter (d.m.). All determinations were performed in triplicate.

Determination of individual polyphenols by LC analysis

LC analysis was performed using a Shimadzu chromatograph (Shimadzu 2010 EV, Kyoto, Japan) equipped with SPD-10A UV detector, EC 150/2 NUCLEODUR C18 Gravity SB 150 x 2 mm x 5 µm column (Macherey-Nagel GmbH & Co. KG, Germany). The calibration curves were performed in the range of 20-50 µg/mL. The calibration curves were produced in the range of 1–10 µg/mL. The results were expressed in mg·g−1 d.m. The experiments were performed in triplicate.

Antimicrobial activity

The extracts were tested against: *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. flexneri* (ATCC 12022), *S. typhimurium* (ATCC 14028), *H. influenzae* type B (ATCC 10211), *C. albicans* (ATCC 10231) and *C. parapsilopsis* (ATCC 22019).

The microorganisms used in this study were obtained from the culture collection of the Laboratory of Microbiology in the Interdisciplinary Research Platform within Banat's "King Michael I of Romania" University of Agricultural Science and Veterinary Medicine Timisoara.

A 10-3 dilution of the fresh culture was used to perform the assay, an inoculum equivalent to a 0.5 McFarland standard. The suspensions were tested using a 96 microdilution well plate. Using a Calibra digital 852 multichannel pipette, 100 µL of microbial suspension was placed in each well. The extracts were used directly, placing either 25, 50 or 100 µL in each well. The plates were covered and left 24 hours at 37 °C. After 24 hours 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 MIC is defined as the lowest compound concentration that yields no visible microorganism growth. The method of MIC determination based on the microbial mass loss by measurement of OD by spectrophotometry according to ISO 20776-1:2019 was described in our previous research [75]. To interpret the results, two indicators were calculated BGR and BIR by using the following formulas:

$$BGR = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%)\ (1)$$

$$BIR = 100 - BGR\ (\%)\ (2)$$

where:

OD sample—optical density at 540 nm as mean value of triplicate readings for extracts and standards in the presence of the selected bacteria;
OD negative control—optical density at 540 nm as mean value of triplicate readings for the selected bacteria in BHI.
Concerning the standards tested, the method used was identical with the one used for the extract analysis. The quantities tested were calculated as the minimum and maximum amount of standard contained in the extracts within the same amount of extract tested, therefore, resulting 50 and 500 mg·g-1.

$$MGR = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%)\ (1)$$

$$MIR = 100 - MGR\ (\%)\ (2)$$

where:
OD sample—optical density at 540 nm as mean value of triplicate readings for extracts and standards in presence of the selected fungy;
OD negative control—optical density at 540 nm as mean value of triplicate readings for the selected fungy in BHI.

Results

Chemical composition of extracts

Figure 1 presents the total polyphenols content (TPC) expressed as mg gallic acid equivalent GAE/ g sample

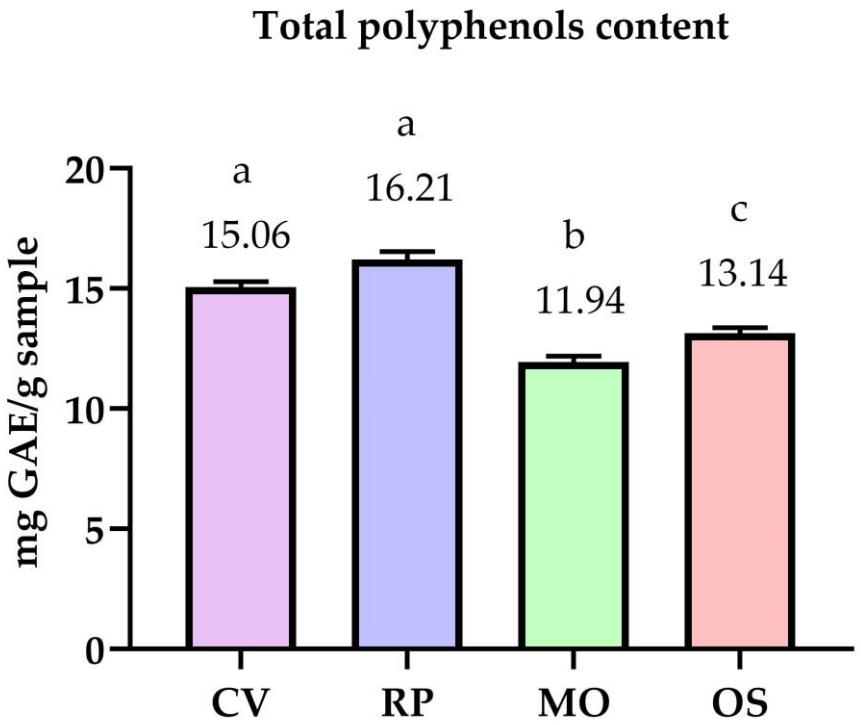


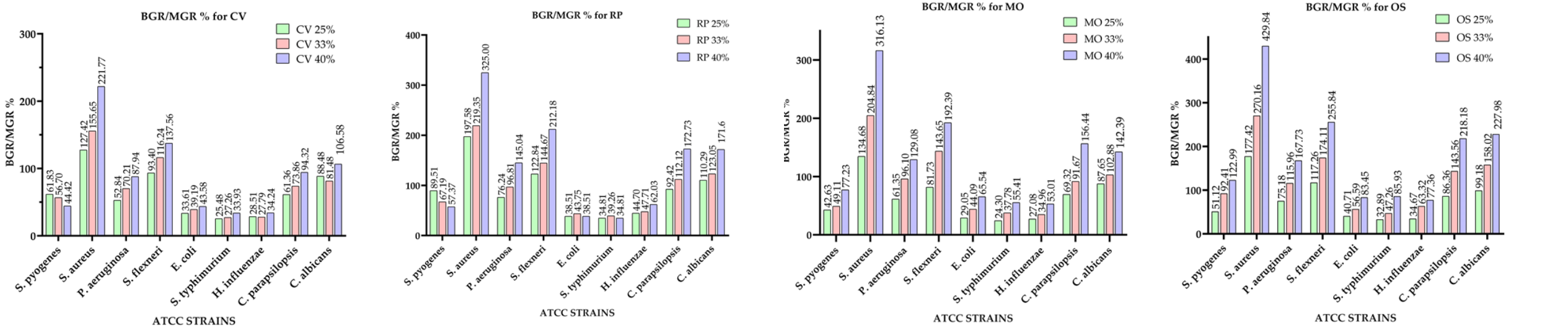
Table 1. The individual profile of polyphenols detected using LC method (mg·g-1)

Compound	Retention time (min)	RP	MO	CV	OS
Gallic acid	4.8	0.693±0.011^{a}	nd	0.249±0.006^{b}	0.007±0.0001^{c}
Protocatechuic acid	10.8	0.701±0.012^{a}	0.696±0.007^{a}	0.155±0.006^{b}	0.011±0.0003^{c}
Caffeic acid	21.9	0.567±0.008^{a}	2.441±0.03^{b}	0.594±0.008^{a}	0.668±0.013^{c}
Epicatechin	22.7	17.002±0.181^{a}	65.879±0.424^{b}	2.219±0.025^{c}	nd
Coumaric acid	24.4	0.179±0.004^{a}	0.999±0.014^{b}	0.104±0.002^{c}	0.043±0.001^{d}
Ferulic Acid	24.7	nd	nd	nd	0.073±2.76
Rutin	25.7	35.257±2.84^{a}	7.865±0.71^{b}	2.779±44.42^{c}	2.156±60.88^{d}
Rosmarinic acid	28.8	4.430±0.43^{a}	0.640±21.12^{b}	2.051±38.98^{c}	4.391±115.79^{a}
Resveratrol	31.9	2.176±12.73^{a}	1.518±27.54^{b}	1.256±29.50^{c}	1.107±21.24^{d}
Quercetin	32.1	1.786±14.14^{a}	nd	0.536±5.50^{b}	2.838±50.54^{c}
Kaempferol	34.9	0.669±7.07^{a}	1.114±199.46^{b}	1.878±37.9^{c}	4.861±54.29^{d}

The four investigated Fabaceae species revealed distinct chemical patterns (Table 1). In the MO alcoholic extract, the main components were: epicatechin (65.879 mg·g-1), kaempferol (1.114 mg·g-1), rutin (7.865 mg·g-1) and caffeic acid (2.441 mg·g-1). Smaller quantities of protocatechuic acid (0.696 mg·g-1), coumaric acid (0.999 mg·g-1), resveratrol (1.518 mg·g-1) and rosmarinic acid (0.64 mg·g-1) were also detected.

Our data showed that in case of the the RP alcoholic extract the main polyphenolic components were rutin (35.257 mg·g-1), epicatechin (17 mg·g-1), rosmarinic acid (4.439 mg·g-1) and resveratrol (2.175 mg·g-1). Other compounds found in minority were: gallic acid (0.693 mg·g-1), quercetin (1.786 mg·g-1), protocatechuic acid (0.701 mg·g-1), caffeic acid (0.567 mg·g-1) and kaempferol (0.669 mg·g-1).

The CV extract presented as primary polyphenolic components the following: rutin (2.779 mg·g-1), epicatechin (2.219 mg·g-1), rosmarinic acid (2.051 mg·g-1), kaempferol (1.878 mg·g-1), gallic acid (0.249 mg·g-1) and resveratrol (1.256 mg·g-1) and in the OS extract: kaempferol (4.861 mg·g-1), rosmarinic acid (0.043 mg·g-1), quercetin (2.838 mg·g-1) and rutin (2.156 mg·g-1).



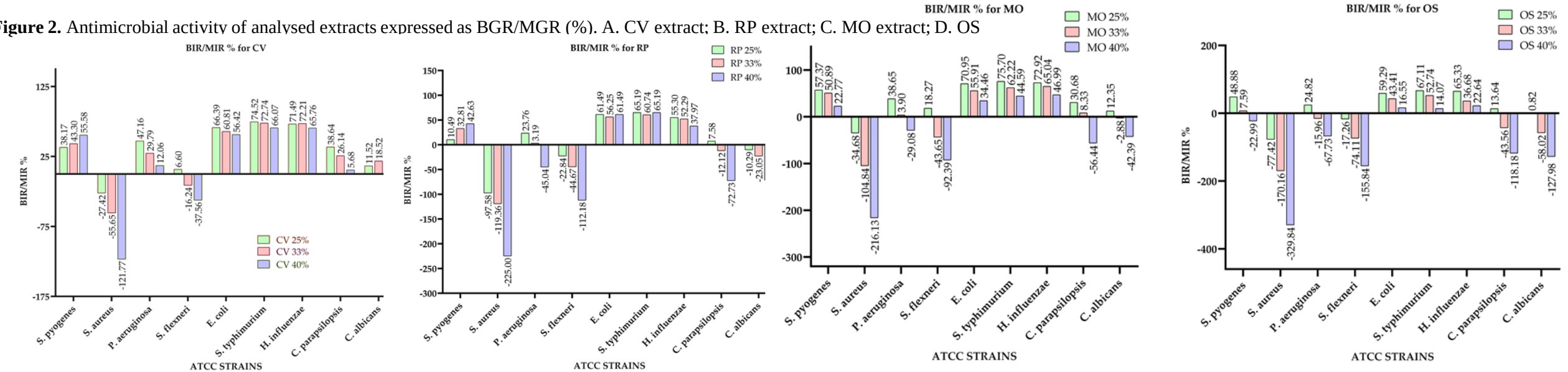
A. Antimicrobial activity (expressed as BGR/MGR%) of CV extract

B. Antimicrobial activity (expressed as BGR/MGR%)of RP extract

C. Antimicrobial activity (expressed as BGR/MGR%) of MO extract

D. Antimicrobial activity (expressed as BGR/MGR%) of OS extract

Figure 2. Antimicrobial activity of analysed extracts expressed as BGR/MGR (%). A. CV extract; B. RP extract; C. MO extract; D. OS



A. Antimicrobial activity (expressed as BIR/MIR%) of CV extract

B. Antimicrobial activity (expressed as BIR/MIR%) of RP extract

Antimicrobial activity (expressed as BIR/MIR%) of MO extract

D. Antimicrobial activity (expressed as BIR/MIR%) of OS extract

The MIC (µL/100 mL) for plant extracts CV, RP, MO and OS

	S. pyogenes	S. aureus	P. aeruginosa	S. flexneri	E. coli	S. typhimurium	H. influenzae	C. parapsilopsis	C. albicans
CV	25	25	25	25	25	25	25	25	25
CV	33	33	33	33	33	33	33	33	33
CV	40	40	40	40	40	40	40	40	40
RP	25	25	25	25	25	25	25	25	25
RP	33	33	33	33	33	33	33	33	33
RP	40	40	40	40	40	40	40	40	40
MO	25	25	25	25	25	25	25	25	25
MO	33	33	33	33	33	33	33	33	33
MO	40	40	40	40	40	40	40	40	40
OS	25	25	25	25	25	25	25	25	25
OS	33	33	33	33	33	33	33	33	33
OS	40	40	40	40	40	40	40	40	40

The samples that had no inhibition effect, causing a mass growth of the strain are marked in dark grey colour. The light gray colour represents the samples in which the MIC was found, but subsequent concentrations showed a potentiating effect, therefore the effect decreased together with the concentration. The white colour highlights the samples in which the MIC was determined and the effect was maintained together with an increase in concentration.

Conclusion

The study conducted on the antimicrobial potential of plant extracts belonging to the Fabaceae family pointed out that they can be taken into consideration as promising antimicrobial agents and open a new pathway for further research in order to find new complementary antibiotics against Gram-positive and / or negative bacteria and antifungal agents.

Thank you for your attention!