

# Blueberry Leaves: A Valuable Antimicrobial and Antibiofilm Agent Against Multidrug-Resistant Pathogens

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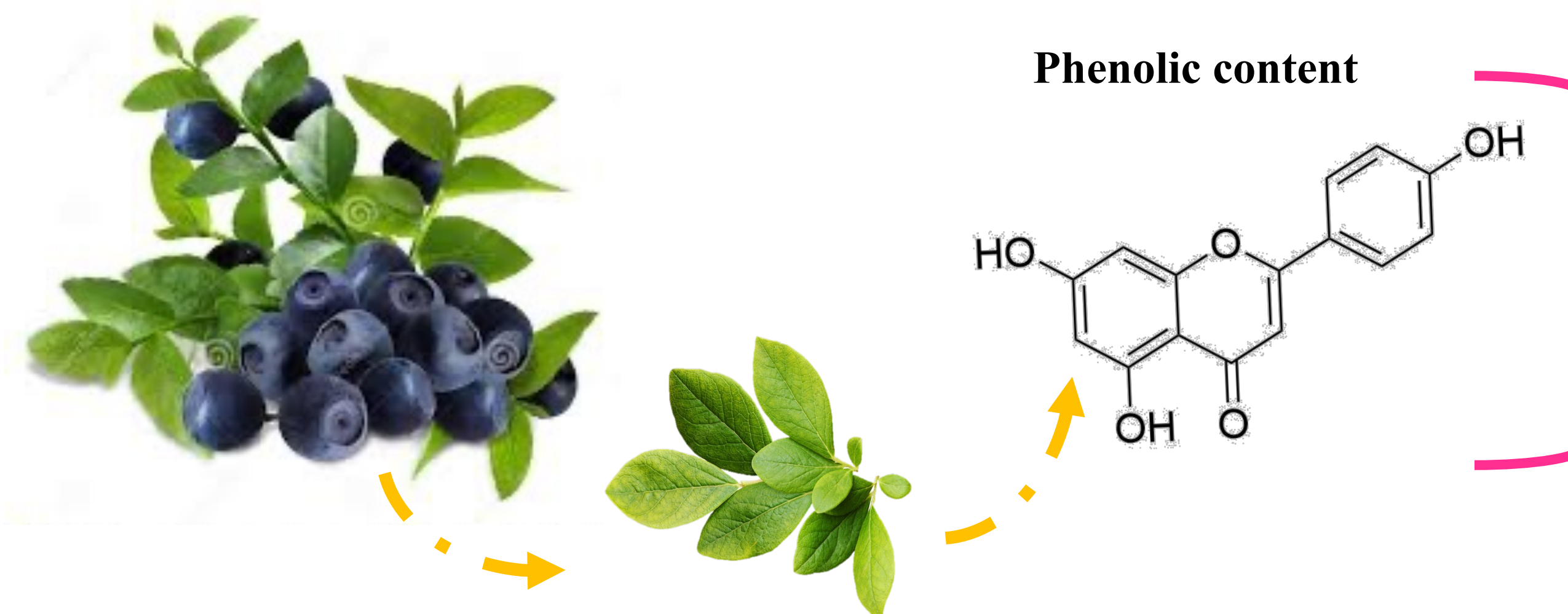
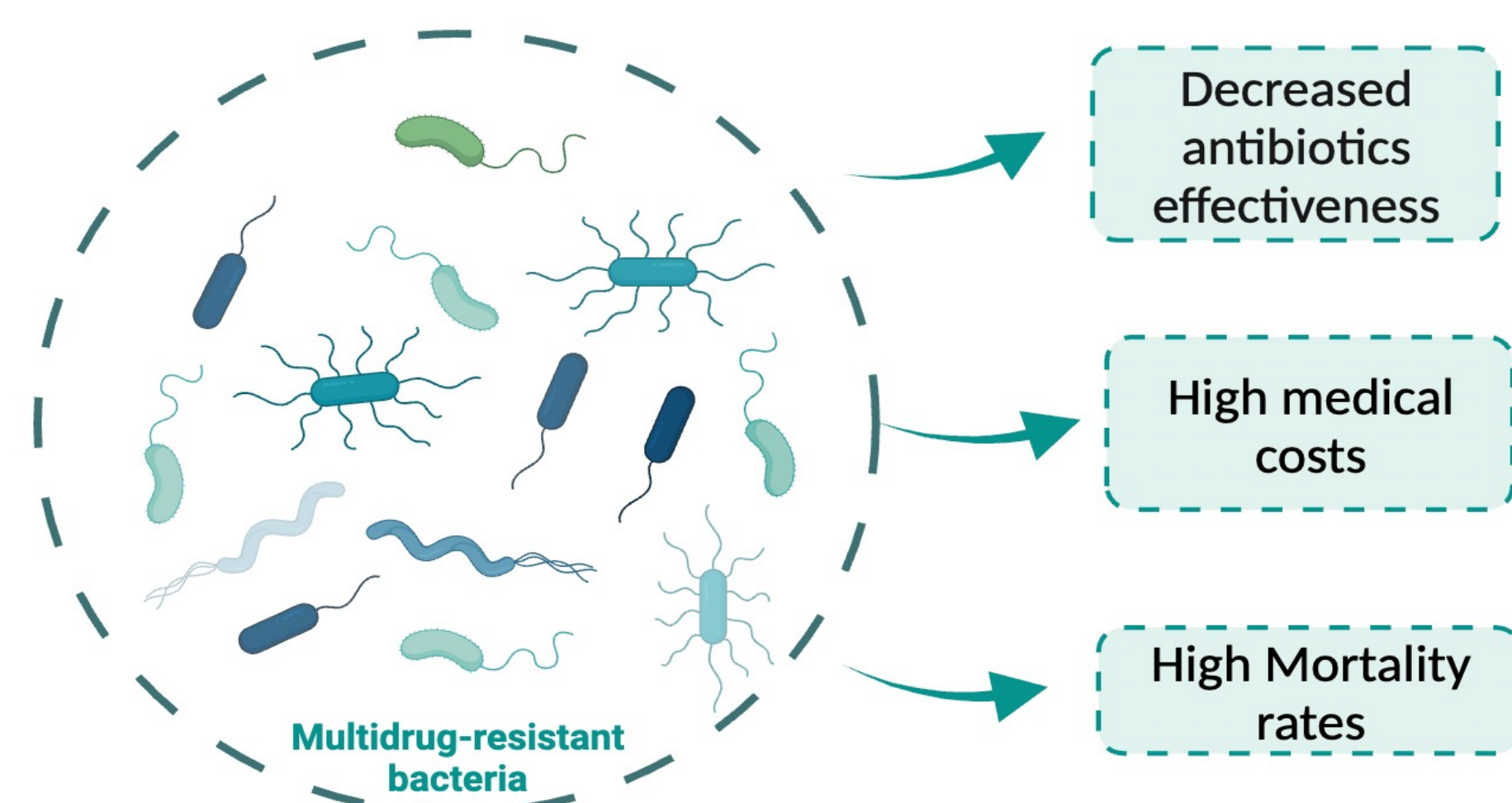
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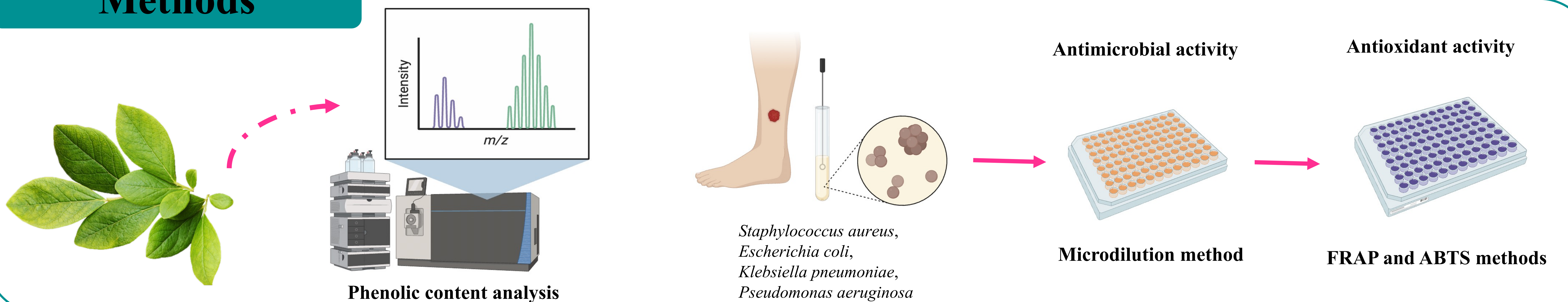
## Introduction



- ✓ Antimicrobial effect;
- ✓ Anti-inflammatory activity;
- ✓ Antioxidant properties

Blueberry leaves have been extensively studied for their rich composition of bioactive compounds, and numerous publications have delved into identifying and characterizing these valuable components [1,2]. Specifically, anthocyanins found in blueberries have garnered attention for their diverse biological activities, among which their antimicrobial properties have been identified.

## Methods



## Results

Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region ( $\lambda_{max}$ ), mass spectral data, identification and quantification

Peak	$\lambda_{max}$ (nm)	[M-H] m/z	MS <sup>2</sup> (m/z)	Identification	Concentration
1	324	353	191(100),179(55),135(10)	3-O-Caffeoylquinic acid <sup>1</sup>	5.38±0.05 <sup>b</sup>
2	322	707	467(23),353(100),191(15)	Caffeoylquinic acid dimer <sup>1</sup>	4.02±0.05 <sup>c</sup>
3	310	341	179(85), 149(54), 135(100)	Caffeic acid hexoside <sup>2</sup>	1.76±0.02 <sup>d</sup>
4	322	707	467(23),353(100),191(15)	Caffeoylquinic acid dimer <sup>1</sup>	40.7±0.4 <sup>a</sup>
5	323	353	191(100),179(8),161(2),135(3)	5-O-Caffeoylquinic acid <sup>1</sup>	3.7±0.1 <sup>a</sup>
6	283	863	711(28), 573(13), 451(15), 411(18), 289(6)	Procyanidin trimer <sup>3</sup>	5.8±0.1 <sup>b</sup>
7	282	863	711(25), 573(18), 451(13), 411(31), 289(10), 285(8)	Procyanidin trimer <sup>3</sup>	7.7±0.1 <sup>d</sup>
8	282	1153	865(30), 577(17),575(12),561(7), 289(13)	Procyanidin tetramer <sup>3</sup>	0.734±0.00 <sup>b</sup>
9	281	1153	865(37), 577(15),575(11),561(5), 289(10)	Procyanidin tetramer <sup>3</sup>	5.93±0.03 <sup>b</sup>
10	345	609	301(100)	Quercetin-3-O-rutinoside <sup>4</sup>	2.64±0.02 <sup>b</sup>
11	327	463	301(100)	Quercetin-3-O-glucoside <sup>4</sup>	2.67±0.01 <sup>b</sup>
12	319	515	353(100), 191(11),179(8)	3,5-O-Dicaffeoylquinic acid <sup>1</sup>	0.72±0.04 <sup>d</sup>
13	340	593	285(100)	Luteolin di-6,8-C-hexoside <sup>5</sup>	4.87±0.05 <sup>b</sup>
14	334	447	285(100)	Luteolin 6-C-glucoside <sup>5</sup>	3.5±0.1 <sup>b</sup>
				TPA	56.3±0.1 <sup>b</sup>
				TP	20.1±0.1 <sup>c</sup>
				TOF	13.7±0.2 <sup>b</sup>
				TPC	90.1±0.2 <sup>c</sup>

TPA-Total phenolic acids, TP-Total procyanidin, TOF-Total other flavonoids, TPC-Total phenolic compounds; calibration curves used: 1- chlorogenic acid ( $y = 16882x - 161172$ ;  $R^2 = 0.9999$ ; LOD = 0.20  $\mu\text{g/mL}$ ; LOQ = 0.68  $\mu\text{g/mL}$ ), 2- caffeic acid ( $y = 38834x + 406309$ ;  $R^2 = 0.9984$ ; LOD = 0.78  $\mu\text{g/mL}$ ; LOQ = 0.97  $\mu\text{g/mL}$ ), 3- catechin ( $y = 84956x - 23200$ ;  $R^2 = 0.9999$ ; LOD = 0.17  $\mu\text{g/mL}$ ; LOQ = 0.68  $\mu\text{g/mL}$ ), 4- quercetin-3-O-glucoside ( $y = 34845x - 166173$ ;  $R^2 = 0.9996$ ; LOD = 0.21  $\mu\text{g/mL}$ ; LOQ = 0.71  $\mu\text{g/mL}$ ), 5- Apigenin-7-O-glucoside ( $y = 10683x - 45794$ ;  $R^2 = 0.999$ ; LOD = 0.10  $\mu\text{g/mL}$ ; LOQ = 0.53  $\mu\text{g/mL}$ ). nd- not detected. Different letters in the same row show significant difference between means of the same compounds in different extraction methods. Different letters in each row mean statistically significant differences with a significance of 0.05.

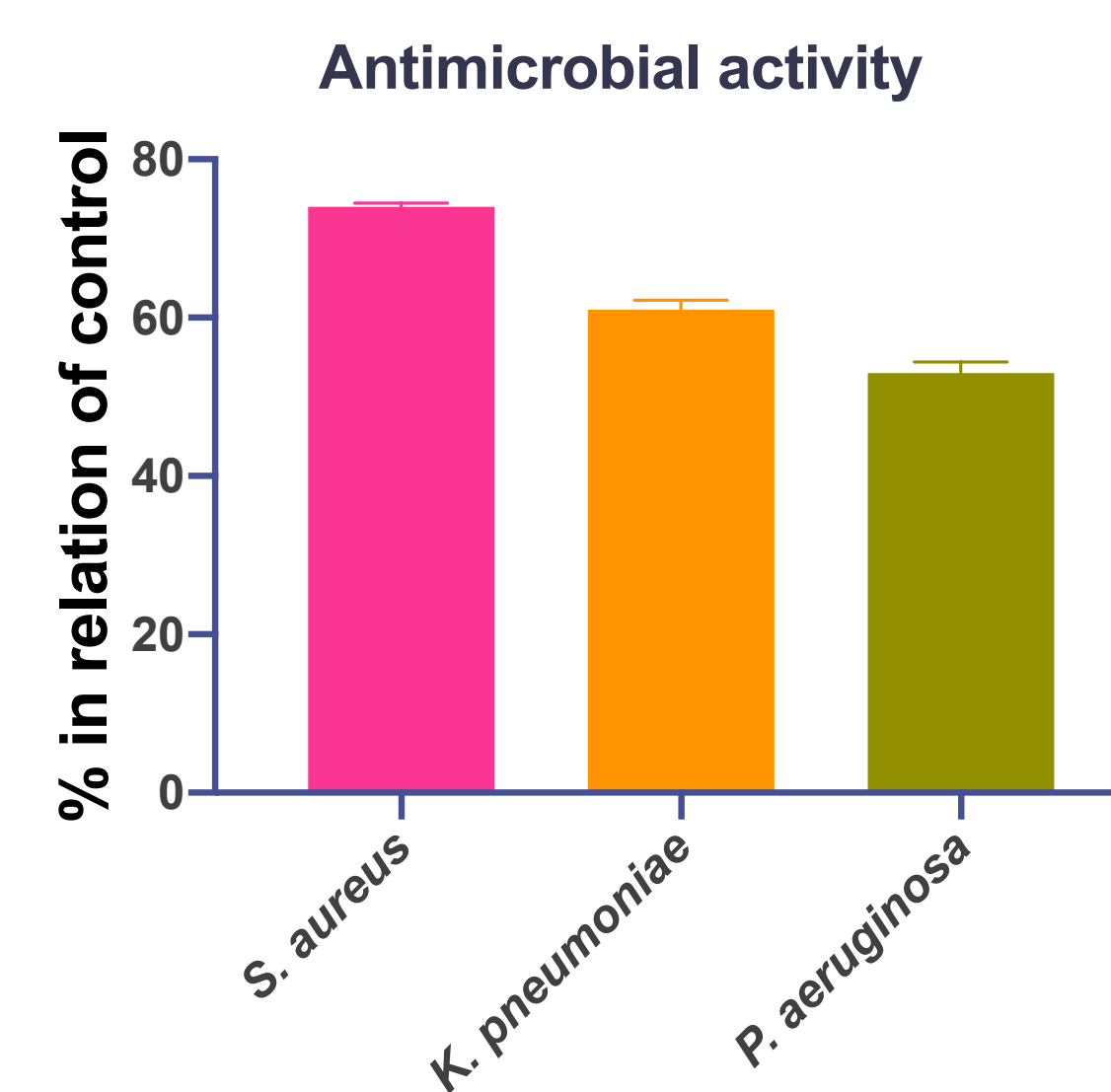


Figure 1. Antimicrobial activity of extracts at 0.5 mg/mL. The results are presented as % of growth inhibition in relation to control. Mean values  $\pm$  SD for three independent experiments are illustrated.

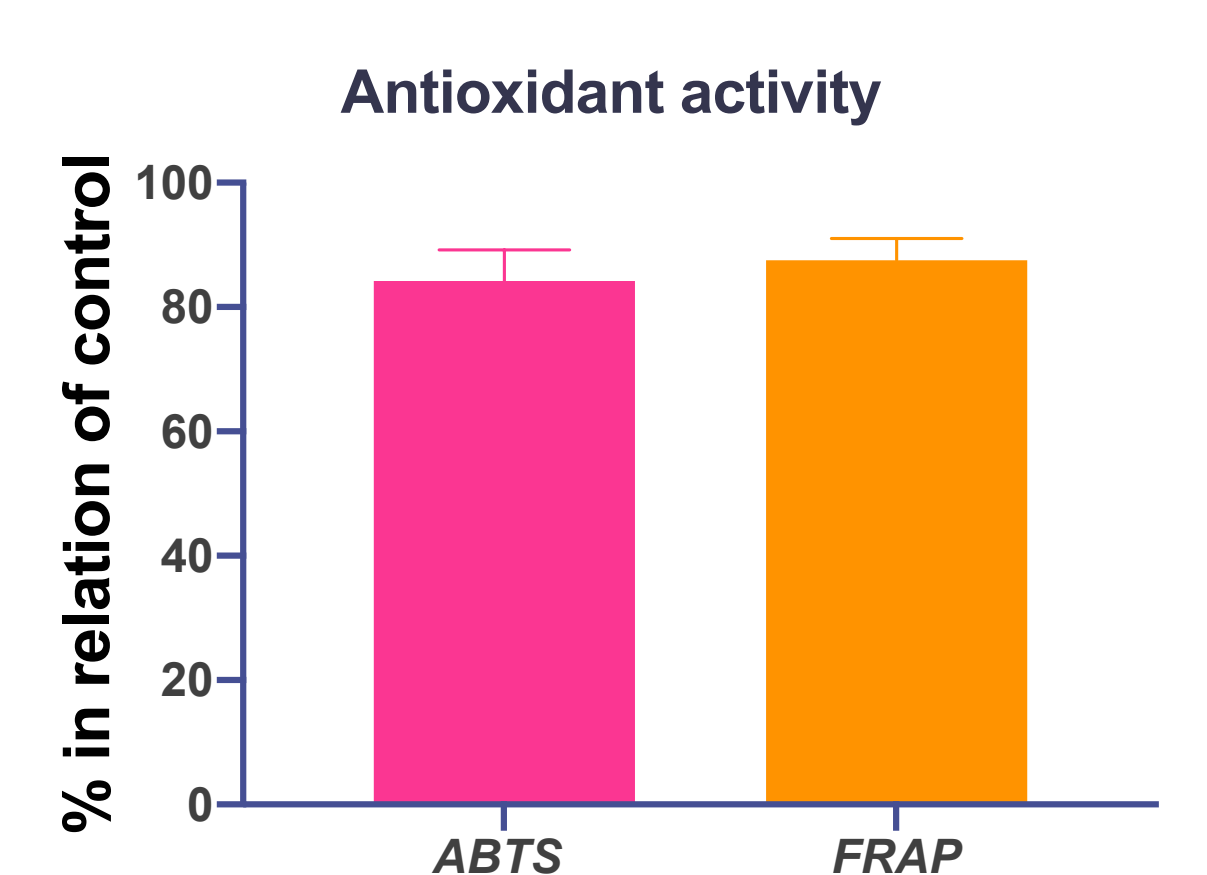


Figure 2. % of antioxidant activity in relation to control. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Ferric reducing antioxidant power (FRAP)

## Conclusion

- ✓ These findings highlight the promising potential of **blueberry leaves** as an avenue to counter **multidrug-resistant bacteria**.
- ✓ Blueberry leaves' **antioxidant and antimicrobial** properties signify their prospective application in tackling **antibiotic-resistant bacterial infections**.
- ✓ This study sheds light on their potential significance in the field of **medical interventions** and **pharmaceutical advancements**.

### References

- [1]. Wang, L.-J., et al. Composition of phenolic compounds and antioxidant activity in the leaves of blueberry cultivars. *J Funct Foods* 2015, 16: p. 295-304.
- [2]. Yang, G., et al. Blueberry leaf extracts incorporated chitosan coatings for preserving postharvest quality of fresh blueberries. *Postharvest Biol Technol* 2014, 92: p. 46-53.
- [3]. Xiaoyong, S. and C. Luming. Phenolic constituents, antimicrobial and antioxidant properties of blueberry leaves (V5). *J Food Nutr Res* 2014, 2(12): p. 973-9.

### Acknowledgments

This work was founded by FCT – Fundação para a Ciência e a Tecnologia and by Fundação BPI La Caixa, within call POCI-01-0145-FEDER-031309 and project titled "AquaVita - Água Termal Como Fonte de Vida e Saúde" and "AquaValor - Centro de Valorização e Transferência de Tecnologia da Água" (NORTE-01-0246-FEDER-000053), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). The authors would like to thank the project UIDB/04033/2020 (CITAB-Center for the Research and Technology of Agro-Environmental and Biological Sciences, National Funds through the Portuguese funding agency).