



Development of a NADES-SPE Extraction Approach for Olive Pomace: Selectivity and Antibacterial Evaluation

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Abstract:

Olive pomace (OP), a major by-product of olive oil production, is rich in valuable bioactive compounds with antioxidant and antimicrobial potential. This study developed a NADES-SPE approach for recovering OP phenolics. C18 cartridge with acidified methanol achieved the best purification efficiency and antioxidant activity, but with also high recovery changing methanol to ethanol. The extract showed strong antibacterial activity, with MIC values of 12.25, 50.00, and 100.00 mg/mL for *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively.

• Introduction

Recovering bioactive compounds from agrifood by-products such as OP promotes a circular economy due to their antioxidant and antimicrobial potential, with promising applications in pharmaceutical and biotechnological fields. Conventional organic solvents show limited efficiency and toxicity issues. Natural deep eutectic solvents (NADES) provide a greener alternative but are difficult to remove since they cannot be evaporated like organic solvents. This study explores NADES extraction combined with solid-phase extraction (SPE) for phenolic recovery and evaluates the extract's minimal inhibitory concentration (MIC).

• Results and discussions

Table 1. Influence of solvents on phenolic recovery and antioxidant activity from NADES-based OP extracts.

SPE	TPC (mg GAE/100g FW)	TFC (mg CE/100g FW)	FRAP (μmol FSE/100g FW)	DPPH•-SA (mg TE/100g FW)
50% Et./H ₂ O (no SPE)	1035.91±4.67 ^b	599.46±5.44 ^c	7483.53±195.51 ^c	660.45±40.06 ^{bc}
NADES (no SPE)	1368.35±0.00^a	740.09±6.27^a	10543.48±406.46^a	822.04±15.75^a
C18*	770.14±24.07 ^e	447.34±6.27 ^e	6253.32±351.3 ^e	587.49±41.36 ^c
C18†	409.33±44.32 ^d	394.11±8.30 ^f	4736.77±31.85 ^f	447.21±13.09 ^d
C18‡	1106.23±3.08 ^b	649.16±3.14 ^b	8493.54±232.56 ^b	737.74±23.06 ^{ab}
C18§	1015.62±24.24 ^c	582.62±22.62 ^c	7908.26±64.73 ^{bc}	710.1±28.02 ^b
C18¥	948.07±8.15^c	569.32±27.34^c	7890.96±67.37^{bc}	660.65±13.66^{bc}

Table 2. Influence of solvents on individual phenolic recovery from NADES-based OP extracts.

SPE	Individual Phenolics compounds (mg/100g FW)							
	Hydroxytyrosol	Tyrosol	Caffeic acid	Verbascoside	Luteolin-7-O-glucoside	Apigenin-7-O-glucoside	Luteolin	Apigenin
50% Et./H ₂ O (no SPE)	84.53±0.81 ^d	78.29±2.09 ^a	12.73±0.61 ^a	35.82±1.38 ^d	15.10±0.23 ^a	10.29±0.12 ^a	75.78±0.92 ^a	N/D
C18*	137.11±4.75 ^c	31.53±0.29 ^d	7.80±0.46 ^c	51.95±1.74 ^c	7.49±0.09 ^e	4.49±0.12 ^d	12.67±0.40 ^c	N/D
C18†	68.2±3.43 ^d	19.25±0.29 ^e	4.72±0.30 ^d	27.62±0.50 ^f	4.30±0.07 ^f	2.37±0.10 ^f	8.85±0.39 ^f	N/D
C18‡	190.17±6.36^a	40.47±1.08^b	10.32±0.56^b	74.40±2.11^a	11.01±0.35^c	6.53±0.46^{bc}	29.96±0.48^b	N/D
C18§	164.77±12.97 ^b	40.13±0.55 ^b	9.76±0.57 ^b	66.46±4.30 ^b	9.88±0.12 ^d	7.04±0.09 ^b	30.77±2.32 ^b	0.03±0.00 ^a
C18¥	148.62±6.85^{bc}	36.26±1.16^c	9.12±0.61^{bc}	58.28±2.27^c	11.96±0.25^b	5.90±0.30^c	30.11±0.22^b	N/D

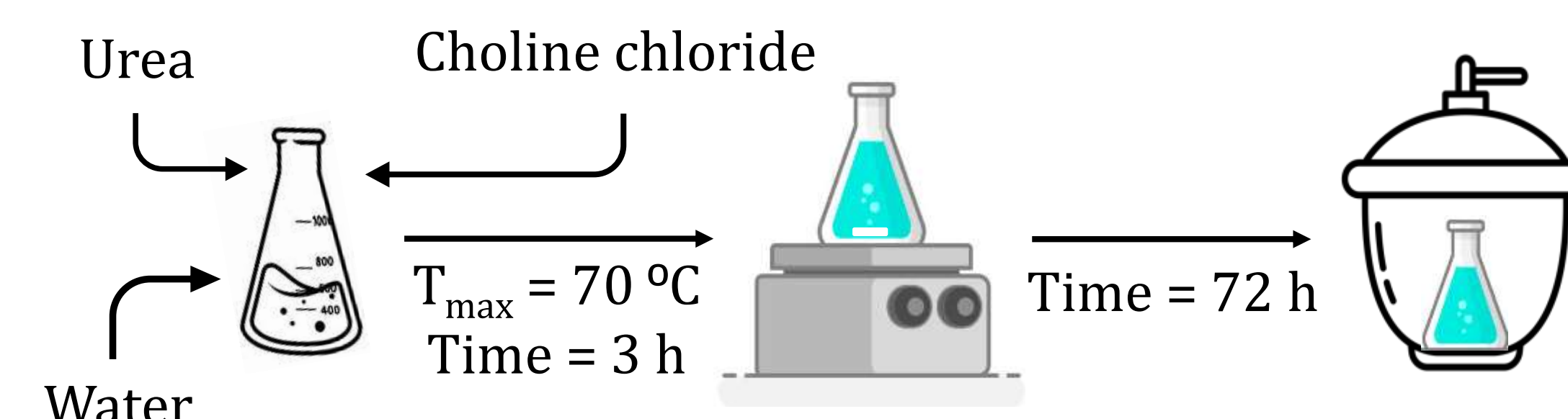
Results are expressed as mean ± standard deviation (n=3). Within each column, different letters represent significant differences between samples at $p < 0.05$. *conditioning and desorption with methanol; †conditioning and desorption with ethanol; ‡conditioning with methanol and desorption with methanol + 2% acetic acid; §conditioning with methanol and desorption with ethanol + 2% acetic acid; ¥conditioning with ethanol and desorption with ethanol + 2% acetic acid; FW = fresh weight; C18 = Bakerbond C18; Et = ethanol; TPC = total phenolics content; TFC = total flavonoids content; FRAP = ferric reducing antioxidant power; DPPH•-SA = 2-diphenyl-1-picrylhydrazyl radical scavenging activity; GAE = gallic acid equivalents; CE = catechin equivalents; FSE = ferrous sulfate equivalents; TE = trolox equivalents; N/D = non-detected.

• Conclusions

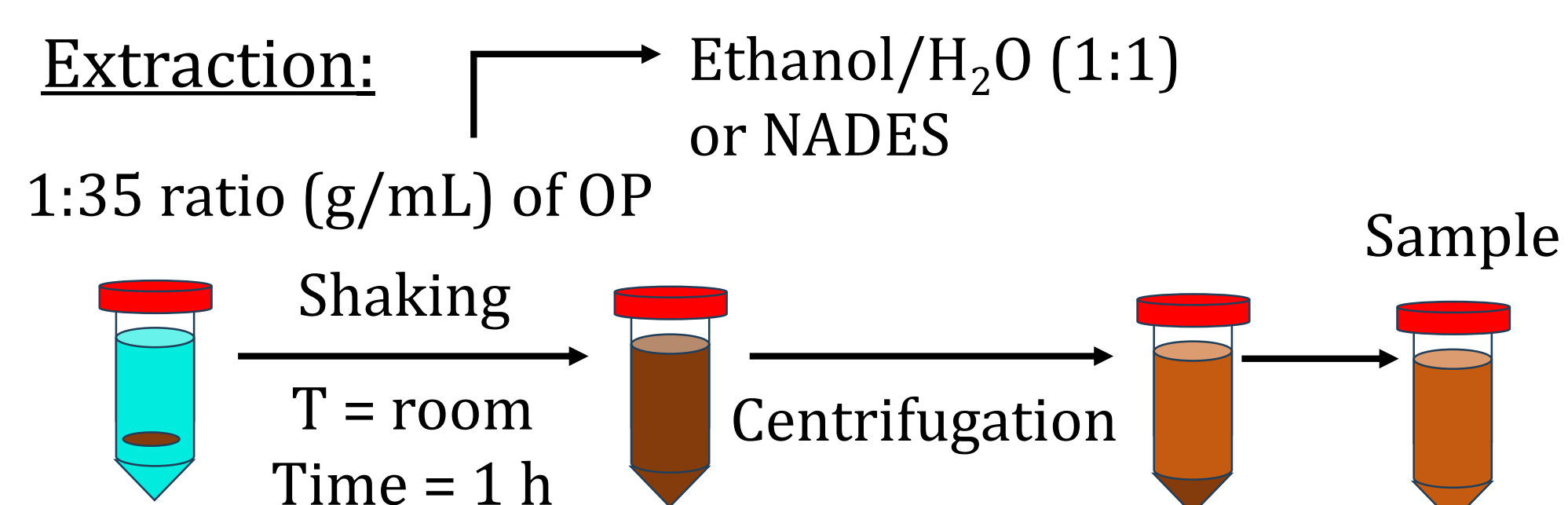
It is concluded that ethanol with 2% acetic acid efficiently recovered phenolics from OP, producing antioxidant and antibacterial extracts, notably against *S. aureus*, and proving a greener alternative to methanol for bioactive extract production.

• Material and method

NADES preparation:



Extraction:



SPE purification:

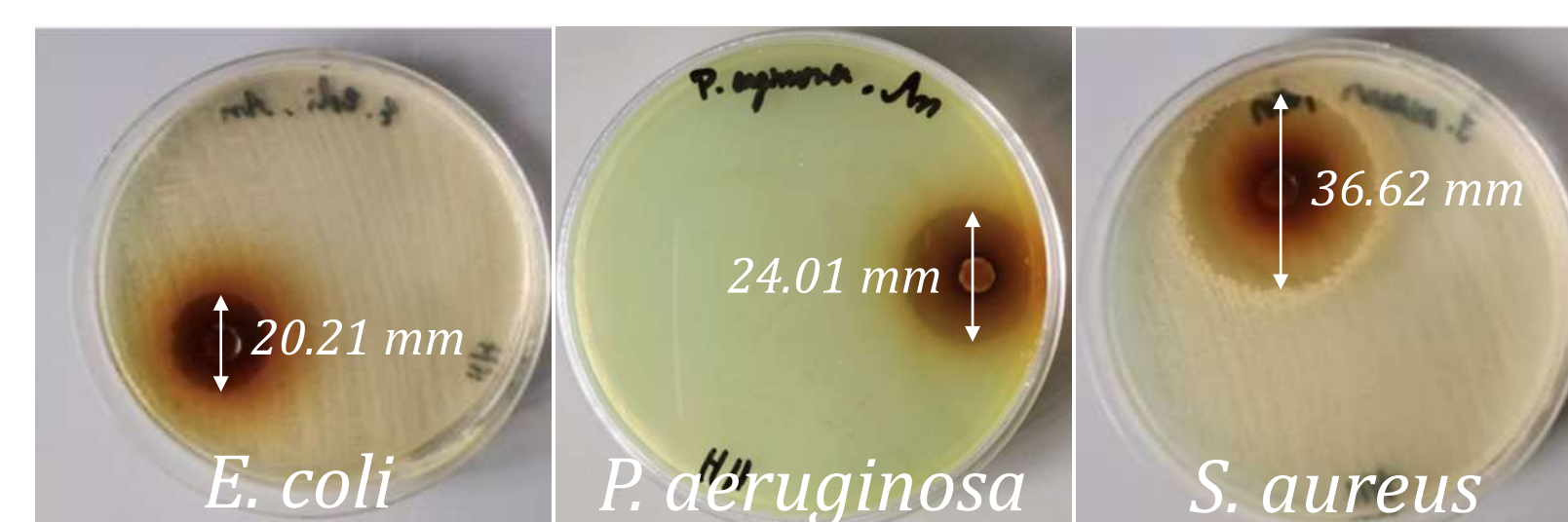
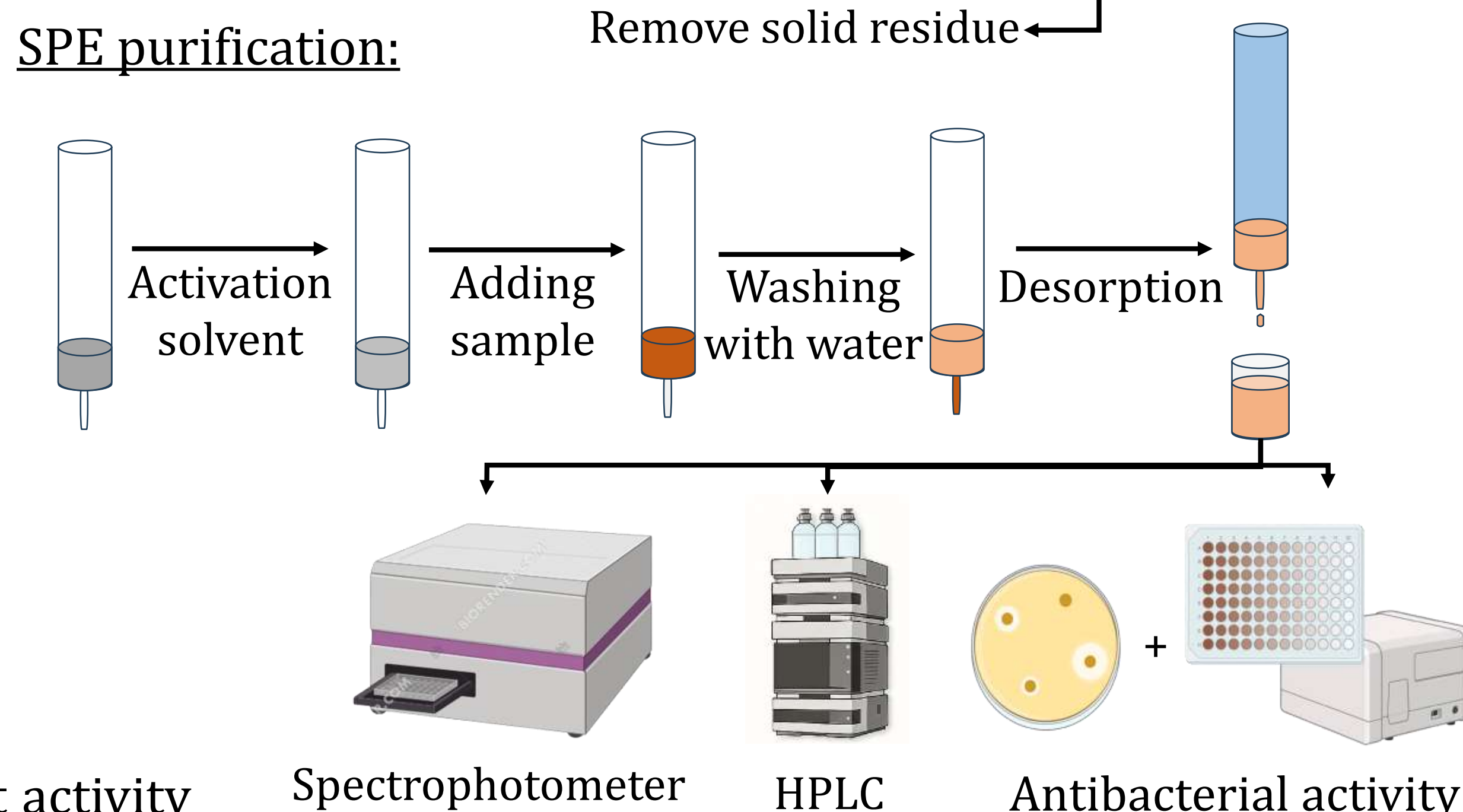
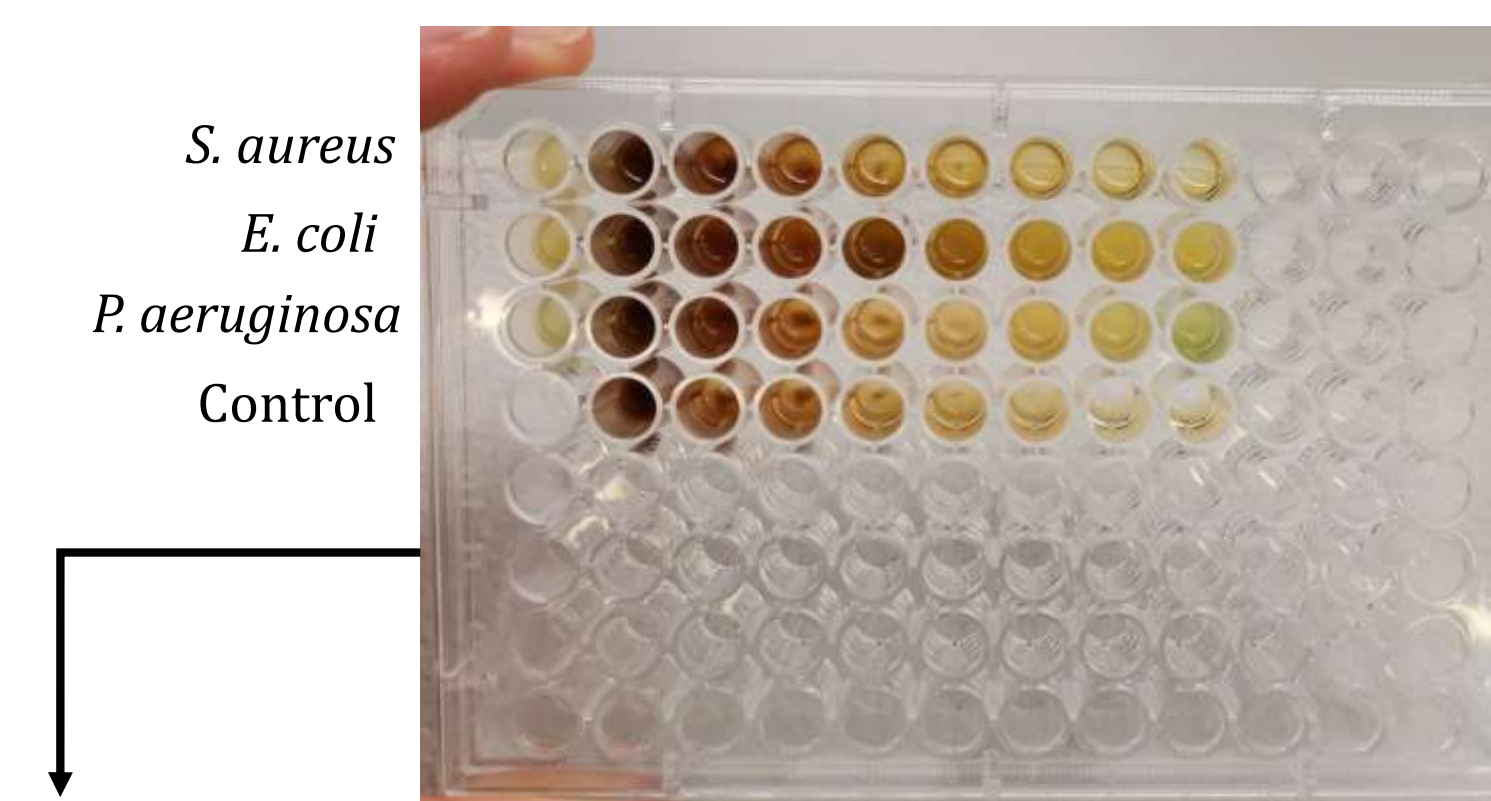


Figure 1. Antibacterial activity of purified OP extracts evaluated by agar well diffusion on Mueller–Hinton agar.



MIC values for *E. coli* (100.00 mg/mL), *P. aeruginosa* (50.00 mg/mL), *S. aureus* (12.25 mg/mL).

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