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Antimicrobial Potential of Extracts from Agroindustrial Residues of Maule Region, Chile †

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Abstract: Bacterial and fungal infections, among which urinary tract infections (UTI) and vulvovaginal candidiasis (VVC) stand out, have a high prevalence among young women. Strategies for their treatment generate the appearance of microorganisms resistant to commonly used antibiotics and antifungals. In this work, the antimicrobial potential of ethyl acetate and methanol extracts from agroindustrial waste from the Maule region, Chile (Chilean papaya, blueberry, and grape) was evaluated against the pathogens that cause UTIs and VVC. The extracts were chemically characterized by analysis of total phenolics and the antioxidant capacity was determined by $DPPH^{\cdot}$ and $ABTS^{+}$ radical scavenging assays. Extracts from agroindustrial residues showed a total phenolic content ranging from 4.91 to 17.22 g GAE/100 g of extract. The antioxidant capacity of the extracts varied between 21.6 and 101.2 µg/mL. According to the results, the antimicrobial activity of the extracts was classified as moderate to good. The results suggest that the proposed agroindustrial waste extracts could potentially be used as functional ingredients to treat CVV and UTIs, providing added value to the residues.

Keywords: urinary tract infections; vulvovaginal candidiasis; antimicrobial; agroindustrial waste; chilean papaya; blueberry; grapes

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1. Introduction

Bacterial and fungal infections are currently highly prevalent worldwide, and there is a marked increase in their frequency and severity. These include urinary tract infections (UTIs), which affect approximately 150 million people worldwide annually and are extremely common among young, healthy women, resulting in considerable morbidity and healthcare costs [1,2]. Vulvovaginal candidiasis (VVC) is another highly recurrent infection affecting millions of women each year and has been considered a major public health problem [3].

A UTI is considered to be the invasion of pathogens into the urinary tract. Common symptoms include burning with urination, frequent urge to urinate, and significant pain. Pathogens causing UTIs include Gram-positive and Gram-negative bacteria and fungi [4]. The main causative agent of complicated and uncomplicated UTIs is *Escherichia coli*, which causes 80–90% of UTIs [5,6]. In uncomplicated UTIs, it is followed by *Klebsiella pneumoniae*,

Staphylococcus saprophyticus, *Enterococcus faecalis*, group B *Streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida spp*. Likewise, in complicated UTIs, after *E. coli*, there are *Enterococcus spp*., *K. pneumoniae*, *Candida spp*., *S. aureus*, *P. mirabilis*, and *P. aeruginosa* [4].

Vulvovaginal candidiasis (VVC) is a commonly diagnosed vaginitis caused by Candida species, with *Candida albicans* being the most common etiology [7]. Symptoms are nonspecific and include vaginal discharge, irritation, burning, among others. VVC is associated with considerable direct and indirect economic costs and increased susceptibility to human immunodeficiency virus (HIV) infection [8]. Risk factors for VVC include reproductive age, pregnancy, hormone replacement therapy, antibiotic use, immunosuppression, uncontrolled diabetes, oral contraceptive pills, and frequent sexual intercourse [9]. When VVC is not treated, many complications have been reported as a consequence, such as pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, spontaneous abortion, and menstrual disorders. Therefore, prevention, early diagnosis, and timely treatment of VVC, especially among risk groups, are essential to avoid complications [10].

The treatment regimen for UTI includes initial empirical therapy, which should be continued according to the characteristics and severity of the patient's condition, clinical syndrome, and definitive treatment based on urine culture and antibiogram results. The problem with this treatment strategy is the emergence of bacteria resistant to commonly used antibiotics and antifungals [11]. Conventional treatments for vulvovaginal candidiasis (VVC) are based on antifungal agents, and orally administered probiotics. However, persistent Candida strains are less susceptible to these antifungal agents, leading to treatment failure and recurrence of the condition.

The growing problem of antimicrobial resistance and side effects of antibiotics is a major health concern worldwide. Due to the limited choice of effective drugs and the high resistance rates, there is a need to explore new strategies and possible alternatives to reduce the rate of treatment failure in uropathogenic bacteria. Given the challenges associated with current treatments, natural extracts, which are multi-component drugs with the potential to bind to multiple targets through various mechanisms of action, are considered as alternative therapies that may reduce the likelihood of microorganisms developing resistance.

The Maule Region, known for its agriculture and food industry, including a significant wine industry, generates large amounts of agricultural waste, including grape pomace and fruit residues. Despite being recognized as sources of valuable compounds, these residues are mostly used as animal feed or discarded, causing environmental pollution.

Previous studies suggest that products derived from cranberries, such as juice and fresh or dehydrated fruit, can reduce the incidence of bacteriuria and urinary infections, particularly in healthy women. Other studies have also demonstrated the antimicrobial of grape pomace, and Chilean papaya extracts [12–14]. However, further research is needed to fully understand their effects on the pathogens that cause VVC and UTIs. In this work, the antimicrobial potential of ethyl acetate and methanol extracts from agroindustrial waste from the Maule region, Chile (Chilean papaya, blueberry, and grape) was evaluated against the pathogens that cause UTIs and VVC.

2. Materials and Methods

2.1. Sample Collection and Extraction Procedure

The Chilean papaya residues (mucilage and seeds) were obtained from the local artisan production of preserves in the town of La Pesca, Iloca, Maule Region, Chile, in March of 2022. Blueberry pomace was obtained from Agrozzi, Maule Region, Chile, in December of 2023. Grape pomace was provided by Santa Carolina winery in the Maule Region, Chile, in march of 2024. The samples were transported to the laboratory and kept at −6 °C until processing.

Chilean papaya residues (mucilage and seeds), blueberry pomace or grape pomace were subjected to extraction following the procedures described by Razali et al., 2012 and Gopčević et al., 2019 [15,16] with modifications. Plant material (1000 g) was homogenized in a blender and extracted three times with petroleum ether (3 × 1 L each) at room temperature in the dark for 1 day and then sonicated for 10 min using a Branson ultrasonic cleaner bath, model 1510, 115v, 1.9 L with a mechanical timer (10 min with continuous hold) and heater switch, 47 kHz to obtain the low polarity metabolites. The de-fatted residue was extracted three times with ethyl acetate, using the same extraction procedure (3 × 1 L each, at room temperature in the dark for one day and then sonicated for 10 min using a Branson ultrasonic cleaner bath). The remaining residue was re-extracted with methanol three times (3 × 1 L each, at room temperature in the dark for one day and then sonicated for 10 min using a Branson ultrasonic cleaner bath) to obtain the high polarity extract. Extracts were then filtered, combined and concentrated under reduced pressure below 40 °C in a rotary evaporator Heidolph 517-61000-00-0 (Germany), frozen at −20 °C and subsequently lyophilized in a Biobase-BK-FD10P lyophilizer (HES, Chile). The freeze-dried extracts were stored at 4 °C for further analysis.

2.2. Chemical Characterization of Extracts

Total polyphenols and total catechins were measured on an Y15 automatic analyzer (Biosystems, Barcelona, Spain), using the kits COD 12815 and COD 12834 respectively, acquired from BioSystems (Santiago, Chile). Total polyphenols method (kit 12815) is based on the reaction of polyphenols in the samples with the Folin-Ciocalteu's reagent in basic media, generating a colored complex quantified spectrophotometrically at 750 nm. Determination of total catechins by means of the kit 12834 is based on the reaction of catechins (mainly monomeric fraction of flavan-3-ols) with the chromogen p-(dimethylamine)-cinnamaldehyde (p-DAC) in ethanol/acid medium, generating a colored complex quantified spectrophotometrically at 620 nm. Measurements were performed in triplicate, and results were reported in mg/L of gallic acid, and catechin as mean ± SD.

2.3. Antioxidant Activity Measurements

The chilean papaya residues, grape pomace and blueberry pomace extracts were evaluated for DPPH• free radical scavenging capacity, according to the method previously described by Brand-Williams [17] and modified by Polo-Cuadrado [18]. All measurements were performed in triplicate. Ascorbic acid was used as the reference compound with an EC_{50} value of 1.5 μg/mL in methanol. The extracts were also evaluated for the radical scavenging capacity of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) according to [19] and modified by Polo-Cuadrado [18]. All measurements were performed in triplicate. Ascorbic acid was used as the reference compound with an EC_{50} value of 28 μg/mL in methanol.

2.4. Antimicrobial Activity

The antimicrobial activity of extracts was evaluated against *E. coli* (*ATCC® 25922*), *Acinetobacter baumannii (ATCC® 19606)*, *Pseudomonas aeruginosa (ATCC® 27853)*, *Staphylococcus aureus (ATCC® 43300)*, *Pseudomonas aeruginosa (ATCC® 9027)*, *Staphylococcus aureus (ATCC® 29213)*, *Enterococcus faecalis (ATCC® 51299)*, *C. albicans* (*ATCC® 10231*), and *C. krusei (ATCC® 14243)*, using the conventional plate diffusion method and determination of the minimum inhibitory concentration through the doubling dilution in 96-well microliter plates using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) [20]. The extracts were dissolved to produce 2 mg/mL in Mueller-Hinton media as a stock solutions. Stock solutions of extracts were diluted to give serial 2-fold dilutions, resulting in concentrations ranging from 1000 to 1.95 μg/mL.

3. Results and Discussion

3.1. Chemical Caracterization of Extracts from Agroindustrial Residues

The chemical characterization of ethyl acetate and methanol extracts from Chilean papaya residue, blueberry pomace, and grape pomace with respect to total phenolic content and total catechins are shown in Table 1. The total phenolic content of extracts was similar in all of them, except for methanolic extract from blueberry pomace, which showed the highest amount. In the case of total catechins, the extracts from Chilean papaya and methanol extract from blueberry pomace showed similar content that was lower in grape pomace extracts and not detected in grape pomace ethyl acetate extract.

Table 1. Chemical characterization of extracts from Chilean papaya residue, blueberry pomace, and grape pomace.

	Ethyl Acetate Extracts		**Methanol Extracts**	
	Total Phenolics (g GAE/100 g of Extract)	**Total Catechins (g CE/100 g of Extract)**	**Total Phenolics (g GAE/100 g of Extract)**	**Total Catechins (g CE/100 g of Extract)**
CPR	6.24 ± 0.34	3.79 ± 0.22	4.90 ± 0.70	2.80 ± 0.56
BP	5.74 ± 0.27	0.27 ± 0.09	17.22 ± 1.03	2.35 ± 0.26
GP	4.91 ± 0.66	BQL	6.67 ± 0.58	0.97 ± 0.06

CPR: Chilean papaya residues; BP: Blueberry pomace; GP: Grape pomace; BQL: Below quantification limit.

3.2. Antioxidant Activity

The antioxidant capacity ethyl acetate and methanol extracts from Chilean papaya residues, blueberry pomace and grape pomace was assessed using different assays, including $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, and expressed as the amount of sample that can scavenge the radical by 50% (EC_{50} in μg/mL) (Table 2).

Table 2. EC_{50} values of Chilean papaya residue, blueberry pomace and grape pomace extracts in scavenging $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals.

	Ethyl Acetate Extracts (EC_{50} in μg/mL)		**Methanol Extracts (EC_{50} in μg/mL)**	
	$DPPH^{\bullet}$	**$ABTS^{\bullet+}$**	**$DPPH^{\bullet}$**	**$ABTS^{\bullet+}$**
CPR	55.99 ± 3.55	29.77 ± 1.25	94.80 ± 2.69	56.74 ± 1.96
BP	101.21 ± 7.38	72.02 ± 4.91	21.60 ± 0.99	35.08 ± 2.68
GP	47.35 ± 4.05	33.46 ± 0,93	33.67 ± 0.95	24.12 ± 2.55

CPR: Chilean papaya residues; BP: Blueberry pomace; GP: Grape pomace.

According to the results, the methanol extract from blueberry pomace was the most active sample scavenging the $DPPH^{\bullet}$ radical, followed by methanol and ethyl acetate extracts from grape pomace respectively. The most active samples scavenging the $ABTS^{\bullet+}$ radical were the methanolic extracts from grape pomace, and blueberry pomace and ethyl acetate extracts from grape pomace and Chilean papaya residues. In both assays, the antioxidant capacity is concentration-dependent and could be attributable to the polyphenolic components present in each extracts.

3.3. Antimicrobial Activity

In the disk diffusion technique, the ethyl acetate extract from grape pomace and methanol extract from Chilean papaya inhibited moderately *E. coli* and *Acinetobacter baumannii* growth. Both extracts from grape pomace and Chilean papaya methanol extracts, showed the ability to inhibit moderately *Pseudomonas aeruginosa* growth. All extracts moderately inhibited the growth of *Staphylococcus aureus*. The plates of *C. albicans* and *C. krusei*

showed inhibition zone with methanol extracts from grape pomace and blueberry pomace.

The minimal inhibitory concentration (MIC) was determined against nine pathogens and results are shown in Table 3. Although in the assay to determine the minimum inhibitory concentration performed in 96-well plates, all the extracts showed inhibition at different percentage levels, not all the extracts inhibited 50% of the growth of the pathogens at the concentrations tested (1.95–1000 μg/mL), being classified as moderate antimicrobials. Grape pomace and blueberry pomace were the most active samples. According to the results, the antimicrobial activity of these residues was classified as good against *A. baumannii*, *P. aeruginosa*, and *S. aureus*, while the methanol extract from grape pomace was the only sample that showed an IC_{50} lower than 1000 against *C. spp*.

Table 3. Minimal inhibitory concentration (MIC) of agroindustrial residue extracts on nine pathogens.

	PEA	PM	BEA	BM	GEA	GM
A. baumannii	>1000	>1000	719.7 ± 77.9	>1000	>1000	>1000
P. aeruginosa (ATCC 27853)	>1000	>1000	>1000	>1000	611.3 ± 18.5	916.2 ± 15.8
S. aureus (ATCC 43300)	>1000	>1000	627.3 ± 63.4	>1000	>1000	470.4 ± 88.3
P. aeruginosa (ATCC 9027)	>1000	>1000	678.4 ± 27.5	>1000	>1000	>1000
S. aureus (ATCC 29213)	>1000	>1000	501.8 ± 27.6	>1000	>1000	>1000
C. albicans	>1000	>1000	>1000	>1000	>1000	335.6 ± 11.7
C. krusei	>1000	>1000	>1000	>1000	>1000	729.8 ± 59.0
E. coli	>1000	>1000	>1000	>1000	>1000	>1000
E. faecalis	>1000	>1000	>1000	>1000	>1000	>1000

PEA: Ethyl acetate extract from Chilean papaya; PM: Methanol extract from chilean papaya; BEA: Ethyl acetate extract from blueberry pomace; BM: Methanol extract from blueberry pomace; GEA: Ethyl acetate extract from grape pomace; GM: Methanol extract from grape pomace.

4. Conclusions

This study showed the antimicrobial potential of Chilean papaya residues, blueberry pomace, and grape pomace against pathogens that cause UTIs and VVC. The antimicrobial activity of methanol extracts from grape pomace and blueberry pomace was classified as good against *A. baumannii*, *P. aeruginosa*, and *S. aureus. The* methanol extract from grape pomace was the only sample that showed an IC_{50} lower than 1000 against *C. spp*.

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Conflicts of Interest:

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