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Bioactive Compounds from *Portulaca oleracea* L. Extract

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Portulaca oleracea L. (purslane) is a plant with various bioactive compounds, such as polyphenols, flavonoids, and polysaccharides. In this study, we aimed to evaluate the antioxidant, antimicrobial, and anticancer properties of purslane extract obtained by Soxhlet extraction with 80 % methanol for 5 h. The extract was characterized by measuring the contents of polyphenols, flavonoids, and polysaccharides, as well as the DPPH free radical scavenging activity. The antimicrobial activity of the extract was tested against six bacterial strains and one fungal strain using the agar well diffusion method. The anticancer activity of the extract was assessed by measuring the cell survival rates of K562 and HepG2 cancer cells treated with different concentrations of the extract. The results showed that the purslane extract had high contents of polyphenols (16.717 ± 0.11 mg GAE/g DW), flavonoids (8.403 ± 0.23 mg RE/g DW), and polysaccharides (23.169 ± 0.27 mg glucose/g DW), and exhibited significant antioxidant activity (IC50 = 1269.019 µg/ml). However, the extract did not show any antimicrobial activity against the tested microorganisms. The extract also demonstrated anticancer activity against both cancer cell lines, with cell survival rates of 72.73 % and 69.79 % for K562 and HepG2 cells, respectively. These findings suggest that purslane extract has potential applications in the food and pharmaceutical industries as a natural source of bioactive compounds.

1. Introduction

Portulaca oleracea L., commonly known as purslane, is a widely distributed succulent plant that belongs to the *Portulacaeae* family. It is native to India and the Middle East, but it can grow in various climatic conditions around the world (Kumar et al., 2021). It is a short-day species that originated from India and the Middle East but can adapt to different environmental conditions (Minh et al., 2019). Purslane has a sour taste and is used as a vegetable in the Mediterranean and some Asian countries, where it is cooked in soups or salads (Melilli M.G. et al., 2018). Purslane contains various bioactive compounds, such as flavonoids, alkaloids, omega-3 fatty acids, vitamins, and minerals (Fukalova et al., 2022). These compounds give purslane medicinal properties, such as cooling, antipyretic, diuretic, anti-parasitic, anti-liver disease, anti-dysentery, etc. Purslane is recognized by the World Health Organization (WHO) as one of the most popular herbs (Zhou et al., 2015).
These abundant bioactive compounds explain the reason why purslane is used as a traditional herbal medicine. Purslane has been used as a traditional herbal medicine in many countries to treat various diseases, such as fever, inflammation, infection, and cancer (Iransh et al., 2017). However, there is still limited scientific evidence to support its therapeutic effects and mechanisms. Previous studies have mainly focused on the chemical composition and antioxidant activity of purslane extracts, but few have investigated their antimicrobial and anticancer activities (Rahman and Sulltana, 2013). Moreover, different extraction methods may affect the yield and quality of bioactive compounds in purslane extracts (Minh et al., 2019).
Therefore, it is important to optimize the extraction conditions and evaluate the biological activities of the extracts obtained from purslane. In this study, we aimed to evaluate the antioxidant, antimicrobial, and anticancer properties of purslane extract obtained by Soxhlet extraction with 80 % methanol for 5 h. This is one of the most commonly used methods for extracting bioactive compounds from plant materials (Haque and Abubakar, 2020).

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We also characterized the extract by measuring the contents of polyphenols, flavonoids, and polysaccharides, as well as the DPPH free radical scavenging activity. We tested the antimicrobial activity of the extract against six bacterial strains and one fungal strain using the agar well diffusion method. We assessed the anticancer activity of the extract by measuring the cell survival rates of K562 and HepG2 cancer cells treated with different concentrations of the extract. The results of this study may provide useful information for the potential applications of purslane extract in the food and pharmaceutical industries as a natural source of bioactive compounds.

2. Material and method

2.1 Experiment apparatus

The experiments were conducted in a biotech laboratory at the Faculty of Chemical Engineering, Ho Chi Minh City University of Technology. Ethanol, methanol, gallic acid, Folin-Ciocalteu reagent and trypan blue were obtained from Merk Chemical Co., Inc. The extract was evaporated to dryness using a 110-DAVS rotary evaporator. The following equipment was used for the microbial culture and assay: a horizontal shaker (IKA®HS260 basis), a biological safety cabinet (Esco LHG-6AG-F8), an incubator (Memmert), and an OD spectrophotometer (Chromtech CT-2200).

2.2 Materials and microbial strains

Portulaca oleracea L. (purslane) was collected from a field in Khanh Tan village, Nhon Hai commune, Ninh Hai district, Ninh Thuan province, Vietnam. The plant material was washed with tap water, cut into small pieces (approximately 1 cm), and oven-dried at 60 °C for 24 h (Gallo et al., 2007). The dried plant material was ground into a fine powder and stored in airtight containers at 4 °C until further use. The following bacterial and fungal strains were purchased from the American Type Culture Collection (ATCC): *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 43300, *Salmonella typhimurium* ATCC 14028, and *Candida albicans* ATCC 10231.

2.3 Extraction conditions of purslane

The powder of *Portulaca oleracea* L. (purslane) was subjected to extraction with three solvents: distilled water, ethanol, and methanol, to obtain the bioactive compounds: polyphenols, flavonoids, and polysaccharides. Two extraction methods were employed: lixiviation and Soxhlet, at three temperatures (ambient, 64 °C, and 78 °C), and two durations (5 h and 24 h) (Table 1). The extract was filtered using Whatman No.1 filter paper (ϕ 110 mm), and the solvent was evaporated to yield the residue, which was stored at 4 °C. The total polyphenols, polysaccharides, and flavonoids in the purslane extract were quantified by standard methods.

Table 1: Extract conditions of Portulaca Oleracea L. M_W, M_E, and M_M represented water, ethanol, and methanol lixiviation. S_E and S_M represented ethanol and methanol Soxhlet extraction

Sample	Method	Solvent	T (°C)	T (h)
M_W	Lixiviation	H_2O	RT	24
M_E	Lixiviation	Ethanol 70 %	RT	24
M_M	Lixiviation	Methanol 80 %	RT	24
S_E	Soxhlet	Ethanol 70 %	78	5
S_M	Soxhlet	Methanol 80 %	64	5

2.3.1 Polyphenol quantification

The total polyphenols in the purslane extract were quantified by the Folin-Ciocalteu (F-C) method, using gallic acid as a standard (Singleton et al., 1999). A mixture of 0.5 mL of extract and 2.5 mL of F-C reagent was incubated for 5 min, then 2.5 mL of Na_2CO_3 solution was added. After 2 h of dark incubation, the absorbance was measured at 760 nm.

2.3.2 Flavonoid quantification

The flavonoid content in the extract was quantified by the aluminum complexation method, using rutin as a standard (Pekal A. et al, 2014). A mixture of 0.5 mL of extract and 2 mL of H_20 was treated with 0.15 mL of 5 % $NaNO_2$ and incubated for 6 min. Then, 0.15 mL of 10 % $AlCl_3$ was added, followed by 1 mL of 1M NaOH. The absorbance was measured at 510 nm.

2.3.3 Polysaccharide quantification

The total carbohydrate content in the extract was determined by the phenol-sulfuric acid method, using glucose as a standard (Nielsen, 2017). A mixture of 1 mL of extract and 1 mL of 5 % phenol was treated with 5 mL of concentrated H_2SO_4. The mixture was heated at 100 °C for 5 min, cooled, and the absorbance was measured at 490 nm.

2.4 Antioxidant activity assay

The DPPH radical scavenging activity of the purslane extracts was evaluated by the method of Blois (1958). The extracts (1 mL) were mixed with 1 mL of 0.2 mM DPPH solution in methanol and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH scavenging was calculated in the Eq(1):

$$S\% = \frac{A_C - A_S}{A_C} \times 100\% \quad (1)$$

where A_c is the absorbance of the control (without extract) and A_s is the absorbance of the extract. The IC_{50} value, which represents the concentration of extract required to scavenge 50 % of DPPH radicals, was determined by interpolation from the dose-response curve. Vitamin C at concentrations ranging from 2 to 10 µg/mL was used as a positive control.

2.5 Antimicrobial activity assay

The antimicrobial activity of the purslane extracts was assessed by the agar well diffusion method (Nadzir et al., 2017). Mueller Hilton Agar (MHA) plates were inoculated with test microorganisms (100 µl of 10^6 CFU/mL) and wells of 6-8 mm diameter were made on the agar surface. The wells were filled with 50 µl of the extracts, while the negative control was DMSO and the positive control was gentamicin (500 µg/mL). The plates were incubated at 37 °C for 16-20 h and the zones of inhibition were measured.

2.6 Anticancer activity assay

The anticancer activity of the purslane extracts was determined by the MTT assay (Amarala et al., 2018). The cells were thawed from liquid N_2 and centrifuged to remove DMSO. The cells were resuspended in RPMI medium and seeded in a 24-well plate at a density of 10^5 cells/mL (1 mL/well). After 24 h of incubation at 37 °C, 1 µL of the extracts was added to each well. The negative control had no extract or solvent. After 72 h of incubation, the cells were stained with trypan blue and counted using a hemocytometer under a microscope. The cell viability was calculated the Eq(2):

% cell viability = (number of viable cells/number of total cells) x 100 (2)

2.7 Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean ± SD. One-way ANOVA was used for statistical analysis and $p < 0.05$ was considered significant (R, v4.3.1).

3. Result and discussion

3.1 Extraction conditions of purslane

The powder of *Portulaca oleracea* L. (purslane) was extracted with three solvents (methanol, ethanol, and distilled water) using two methods (Soxhlet and lixiviation) as shown in Table 1. The color of the extracts varied depending on the solvent used (Figure 1). This was due to the different polarity of the solvents and the compounds extracted. For instance, water, as a highly polar solvent, would extract mainly polar compounds. The total content of polyphenols, flavonoids, and polysaccharides in the extracts was determined by standard methods.

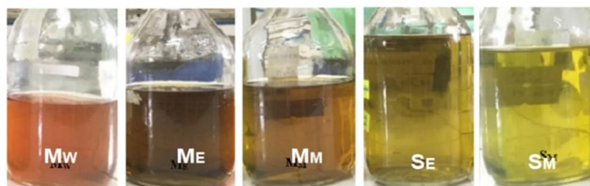

Figure 1: Purslane extracts in different solvents and extraction methods. The extracts were obtained by water lixiviation (MW), ethanol lixiviation (M_E), methanol lixiviation (M_M), ethanol Soxhlet extraction (S_E), and methanol

Soxhlet extraction (S_M). The colors of the extracts are shown as follows: red-orange (M_W), dark brown (M_E), light brown (M_M), and yellowish-green (S_E and S_M).

3.2 Total polyphenol content, flavonoids contents, and polysaccharides contents

The total polyphenol content of the purslane extracts obtained with different solvents and methods was shown in Figure 2a. The extract with 80 % methanol had the highest polyphenol content among all the extracts, while the extract with water had the lowest. The Soxhlet method with 70 % ethanol (S_E) and 80 % methanol (S_M) also yielded higher polyphenol content than the lixiviation method. The S_M extract had the highest polyphenol content of 13.605 ± 0.88 mg GAE/g DW, while the M_W extract had the lowest of 6.584 ± 0.66 mg GAE/g DW (Figure 2a). There was no significant difference in polyphenol content among the M_E, M_M, and S_E extracts. These results are consistent with previous studies that showed higher polyphenol content in purslane extracts with methanol or ethanol than with water (Gatea et al., 2017). Methanol is considered to be the most suitable solvent for extracting polyphenols from plant tissues because it can prevent the oxidation of polyphenols by inhibiting the activity of polyphenol oxidase and it is more volatile than water (Uddin et al., 2012).

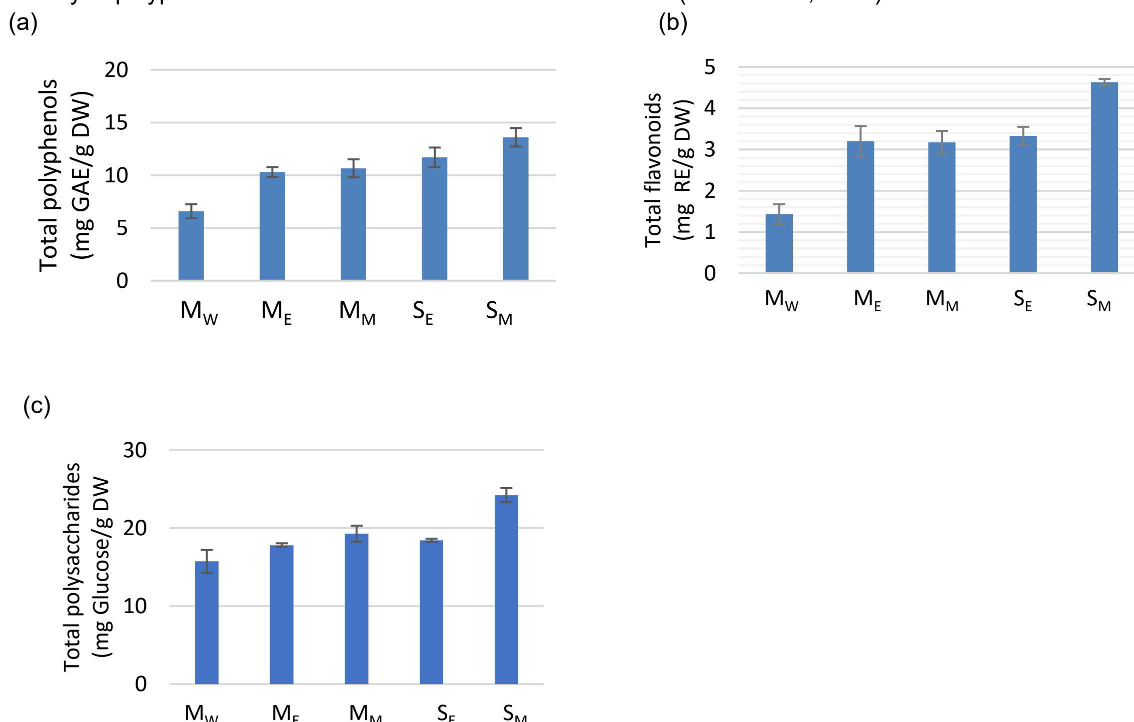

Figure 2: The total (a) polyphenol content,(b) flavonoid content and (c) polysaccharide content of purslane extracts obtained by different methods and solvents

Figure 2b showed the flavonoid content of the purslane extracts with different solvents and methods. The Soxhlet method with ethanol or methanol gave more flavonoids than the lixiviation method. The S_M extract had the most flavonoids (4.63 ± 0.08 mg RE/g DW). This differed from a previous study with lower flavonoids (2.96 mg RE/g DW) in a purslane extract with ethanol (Gatea et al., 2017). This could be due to the variation in the purslane plants.

Figure 2c showed the polysaccharide content of the purslane extracts with different solvents and methods. The Soxhlet method with 80 % methanol gave the highest content of 24.23 ± 0.9 mg Glucose/g DW (S_M). This method also yielded the most polyphenols, flavonoids, and polysaccharides among all the extracts. The heat and methanol probably helped to extract more compounds from the plant cells.

3.2 Antioxidant activity of purslane extract

Purslane is a rich source of bioactive compounds, such as flavonoids (kaempferol, myricetin, luteolin, apigenin, quercetin, genistein, and genistin), polyphenols, polysaccharides, omega-3 fatty acids, and ascorbic acid. These compounds have antioxidant properties that can scavenge free radicals and may prevent cardiovascular

diseases and cancer. The S_M purslane extract showed the highest antioxidant activity among the extracts tested, with an IC_{50} value of 1,269.02 µg/mL for DPPH radical scavenging (Figure 3).

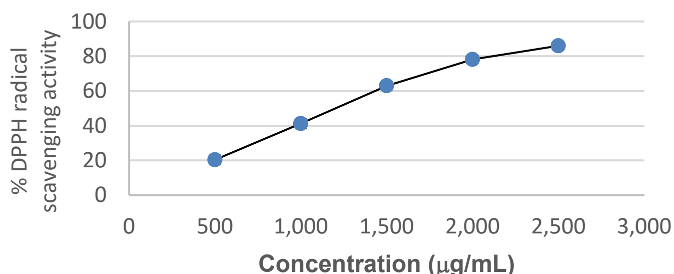

Figure 3: DPPH free radical scavenging activity of purslane extract

3.3 Antimicrobial activity of perslane extract

The purslane extract had no antimicrobial activity against six bacteria and one fungus, as shown by the agar well diffusion method (Figure 4a and 4b). This result contradicts previous studies that reported antibacterial activity of purslane extract against Gram-negative bacteria, such as *E. coli* and *P. aeruginosa*, and Gram-positive bacteria, such as *S. aureus* and *E. faecalis* (Saad et al., 2023), as well as antifungal activity against *C. albicans* (Oraibi et al., 2017). The difference could be attributed to the variation in the extraction methods, solvents, concentrations, and sources of the purslane plant material that were used in different studies.

(a) (b)

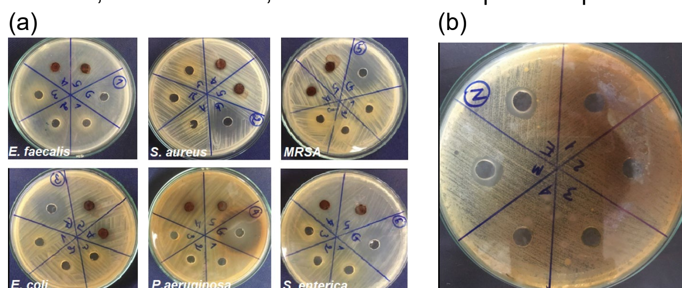

Figure 4: Antimicrobial activity of purslane extract by agar well diffusion method. (a) Zones of inhibition of bacterial growth by purslane extract (1: M_W; 2: S_M; 3: S_E; 4: Ethanol; 5: Methanol; G: Gentamicin 0.5 mg/mL). (b) Zones of inhibition of fungal growth by purslane extract (1: M_W; 2: S_M ; 3: S_E; 4: M; E: Ethanol; M: Methanol).

3.4 Anticancer activity of purslane extract

The MTT assay showed the purslane extract's cytotoxic effect on HepG2 and K562 cells, with survival rates of 69.79 % and 72.73 %, respectively (Table 2). This agreed with Al-Sheddi et al. (2015), but not with Zhao et al. (2017). The extract also altered CDK1 and P53 genes in pancreatic cancer cells, suggesting its therapeutic potential (Alipour et al., 2021).

Table 2: The survival rate of cancer cells treated by SM extract (100 µg/mL)

Cells/control	The percentage of viable cells (%)
Blood cancer cells K562	72.73
Liver cancer cells HepG2	69.79
DMSO	100
Methanol 80 %	100

4. Conclusion

This study used different solvents and extraction methods to obtain polyphenols, flavonoids, and polysaccharides from purslane and evaluated their biological activities. The extract exhibited antioxidant and moderate cytotoxic activities against HepG2 (hepatocellular carcinoma) and K562 (chronic myeloid leukemia) cells, with survival rates of 69.79 % and 72.73 %, respectively, but no antimicrobial activity. Further studies are needed to isolate and identify the bioactive compounds in the purslane extract and to elucidate their pharmacological mechanisms.

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