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Research paper

Influence of solvents on polyphenol content and antioxidant activity of fig leaf extracts obtained by maceration and ultrasonic extraction

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Abstract

In this paper, the polyphenol content and antioxidant activity of fig leaf extracts were analyzed. Extraction was performed with methanol, ethanol, acetone and their aqueous mixtures (50:50 v/v). Extractions were performed with stirring at 300 rpm on a vibromix and ultrasonic bath for 15 minutes. The polyphenol content was determined spectrophotometrically using the Folin-Ciocalteu test. Antioxidant capacity was tested using the FRAP and DPPH methods. The results showed a significant effect of extraction of bioactive components using an organic solvent:water mixture in relation to the organic solvent itself. Ultrasonic extraction proved to be a more efficient technique compared to mixing at 300 rpm.

Keywords: *Fig Leaf; Polyphenols; FRAP; DPPH Inhibition.*

1. Introduction

Ficus carica (common fig) is a tree native to southwest Asia and the eastern Mediterranean and is among the first plants cultivated by humans (Kucukerdonmez et al., 2021). F. carica L. is an important member of the genus Ficus. The fig is an important harvest worldwide for its dry and fresh consumption (Mawa et al., 2013). Figs are rich in carbohydrates, minerals, and bioactive compounds, among other properties. In addition, some compounds can be found in the leaves (Garza-Alonso et al., 2019). Medicinal plants belonging to the genus Ficus have been reported as better remedies for metabolic disorders (Mopuri et al., 2018). F. carica has been reported to have numerous bioactive compounds such as arabinose, β-amyrins, β- carotenes, glycosides, β-sitosterol and xanthotoxol (Kannur and Khandelwal, 2014). Organic acids profile of fig leaves is composed by six organic acids: oxalic, citric, malic, quinic, shikimic, and fumaric acids (Oliveira et al., 2009). F. carica fruit phytochemical such as 6-O-acyl-β-D-glucosyl-β-sitosterol was reported as a potential cytotoxic agent and the latex of fig demonstrated the inhibition of cancer cell proliferation (Lazreg et al., 2011; Rubnov et al., 2001). Fig leaf extract has been found to have good antioxidant, anti-inflammatory and hepatoprotective activity (Mopuri et al., 2018).
In this paper, the influence of extraction technique and different organic solvents and their aqueous mixtures on antioxidant capacity and polyphenol content is analyzed. This research can help in a better understanding of the extraction conditions for the extraction of bioactive components from this plant.

Fig. 1: Fig Leaf.

2. Material and methods

Dried fig leaves were bought in one of the Tuzla markets. The leaves were ground to a powder using an electric grinder and subjected to extraction in this form. Ultrapure water, prepared with a TKA Smart2Pure device, was used for the extraction process. Methanol, glacial acetic acid, hydrochloric acid, sodium carbonate were purchased from Merck (Darmstadt, Germany). 2,2`-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Iron(II) sulphate heptahydrate and iron(III) chloride hexahydrate were purchased from Honeywell (Charlotte, North Carolina, USA). Folin & Ciocalteu's reagent was purchased from Semikem (Bosnia and Herzegovina).

2.1. Preparation of extracts

1 gram of powdered fig leaves was transferred to a flat-bottomed flask and poured with 50 mL of organic solvent or organic solvent: water mixture (50:50 *v/v*). Maceration was performed with stirring at 300 rpm on a Libra 40 vibromix. Ultrasonic extraction was performed in an Elmasonic S ultrasonic bath. Extraction was performed for 15 minutes in the case of both extraction techniques. After the extraction, the mixture was filtered through a blue strip of filter paper and the obtained extracts were immediately analyzed.

2.2. Determination of total phenolic content (TPC)

Total phenolic compounds presented in the extracts were quantified spectrophotometrically using the Folin-Ciocalteu test following the protocol (Singleton et al., 1999), with some modifications. 200 μL of extracts was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420 μL of 10% sodium carbonate was added. The absorbance of the resulting blue-coloured solution was measured at 765 nm after incubation at room temperature for 1 hour. Quantitative measurements were performed, based on a standard calibration curve of gallic acid ($y = 0{,}0042x + 0{,}0076$, $R^2 = 0{,}9998$). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gram of fig leaves.

2.3. Ferric-reducing antioxidant power (FRAP) assay

The reducing powers of the extracts that reflected their antioxidant activity were determined following the protocol (Benzie and Strain, 1999). 3 mL of prepared FRAP reagent is mixed with 100 μL of extracts. Absorbance at 593 nm is recorded after a 30 min incubation at 37 °C. The FRAP value was calculated from the calibration curve of iron (II) sulfate heptahydrate ($y = 0{,}001x + 0{,}0698$; $R^2 = 0{,}9997$) and expressed in mol per gram of fig leaves.

2.4. DPPH radical scavenging activity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method (Horozić et al., 2019). 100 μL of the diluted extract was mixed with 1900 μL of methanol and 500 μL of 0.5 mM DPPH radical solution. The absorbance was measured at 517 nm with methanol as a blank sample. 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used as a control sample. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$[(Ac - As) / Ac] \times 100$

Where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the DPPH solution.

3. Results and discussion

Tables 1 and 2 show the results of polyphenol content and antioxidant activity of the prepared extracts *in vitro*. The highest polyphenol content in the case of both extraction techniques has the water-ethanol extract, followed by the water-acetone and water-methanol extract. The weakest effect in the extraction of bioactive components was shown by acetone, whose extract showed significantly the lowest polyphenol content and at the same time the weakest antioxidant activity.

Table 1: Results of Analysis of Polyphenol Content, Reduction Ability and Inhibition of DPPH Radicals for Extracts Obtained by Ultrasonic Extraction

Extract	TPC [mg GAE/g]	FRAP [μmol/g]	IC_{50} value [mg/mL]
Methanol	3.45	26.9	2.08
Ethanol	2.48	13.7	3.51
Acetone	1.62	13.9	9.07
Methanol:Water	9.47	99.9	0.49
Ethanol:Water	12.8	129.8	0.39
Acetone:Water	10.9	120.8	0.41

Table 2: Results of Analysis of Polyphenol Content, Reduction Ability and Inhibition of DPPH Radicals for Extracts Obtained by Maceration

Extract	TPC [mg GAE/g]	FRAP [μmol/g]	IC_{50} value [mg/mL]
Methanol	2.13	26.3	2.58
Ethanol	1.34	13.5	3.82
Acetone	1.04	12.9	10.0
Methanol:Water	8.58	90.1	0.68
Ethanol:Water	9.01	120.0	0.51
Acetone:Water	8.86	119.1	0.59

The order of extraction efficiency of the bioactive components decreases in the following order: water-ethanol mixture > water-acetone mixture > water-methanol mixture > methanol > ethanol > acetone. It was found that when extracting bioactive components from dry plant material, a better effect is achieved by a higher proportion of the aqueous phase in the organic (Bimakr et al., 2011). Water increases the diffusion process, thus facilitating the extraction of phenolic compounds from plant tissue (Altiok et al., 2008).

Abdel-Aziz et al. (2020) examined fig leaf extracts. Water was used as the extraction solvent. The aqueous extract was shown to be more effective in neutralizing DPPH radicals, with an IC_{50} value of 30 µg/mL. The reason for better results can be explained by the different geographical origin of fig leaves, the method of extraction, etc.
Comparison of the content of isolated polyphenols and antioxidant activity using two extraction techniques is shown in Figures 2 and 3. Higher efficiency in isolating biologically active components was recorded using ultrasonic extraction. This can be explained by the effect of cavitation on the cell material, more precisely the cell wall, which allows greater penetration of the solvent into the material and increases the mass transfer. Due to the rupture of the cell walls, there is a direct contact with the contents of the cell, which accelerates the extraction and increases its efficiency.

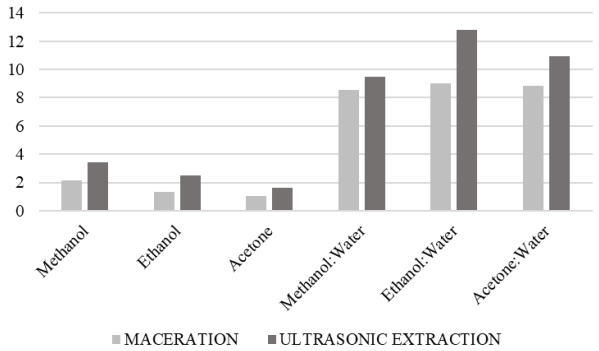

Fig. 2: Comparison of Polyphenol Contents Obtained by Maceration and Ultrasonic Extraction.

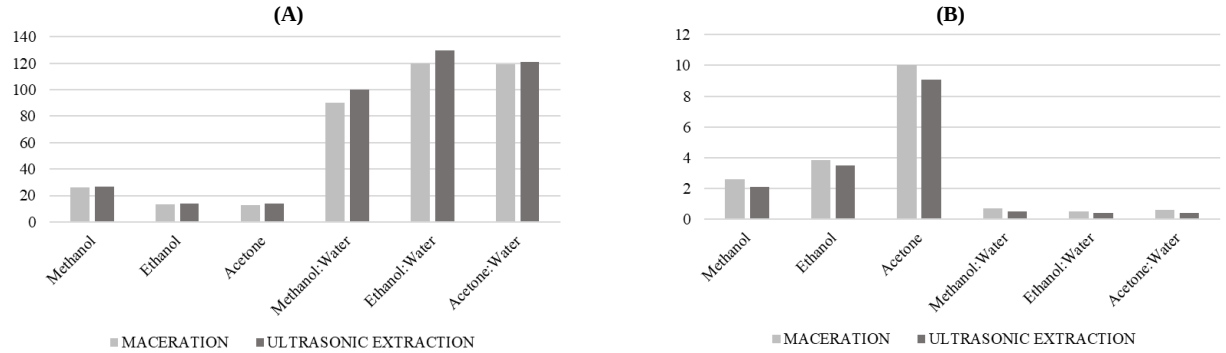

Fig. 3: Comparison of (A) Reduction Ability and (B) Inhibition of DPPH Radicals for Fig Leaf Extracts Obtained by Maceration And Ultrasonic Extraction

4. Conclusion

Based on the performed analysis, it is concluded that the extracts obtained by mixing organic solvents with water have a higher polyphenol content and higher antioxidant activity compared to the extracts obtained with the organic solvents themselves. The best results in the extraction of bioactive components were shown by the mixture of ethanol:water, in the case of both extraction techniques. Acetone showed the weakest extraction potential. By comparing extraction techniques, ultrasonic extraction proved to be more efficient.

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