

Antioxidant and Biological Activities of Fresh and Dried Apricot Extracts Obtained by Cold Soaking and Ultrasonic Extraction

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Abstract

The objective of this study was to evaluate the antioxidant (AA), antibacterial, and antifungal activity of fresh and pre-treated apricot extracts, dried by microwave at different powers (200, 400, and 800 W), extracted by the cold soaking method, and ultrasound-assisted extraction (UAE). Biological activity (bacterial and fungal) was estimated by agar disk diffusion test against four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus* sp.), and two fungal strains (*Candida* spp. and *Geotrichum capitatum*). Methanolic extracts of apricot fruits: fresh, dried processed, and apricot dough extracted by cold soaking showed a higher AA ranging from 34.22 to 96 % than the other extracts extracted by UAE with values ranging from 14.37 to 66.88 %. The results of tested extracts from fruits (*Prunus armeniaca* L.) extracted by both extraction methods showed the highest inhibitory activity against most of the tested bacterial and fungal strains with inhibition zones ranging from 4 to 45 mm. The biological activity (antibacterial and antifungal activity) has been improved using different treatments and microwave powers for drying apricots. In addition, the results of the biological activity of the extracts obtained by UAE are the best compared to cold soaking. However, it was determined that the UAE extraction method of cold soaking and drying of apricot fruits was more appropriate for the food industry due to the obtaining of extracts with good antibacterial and antioxidant potential, and their incorporation in foods would increase their production and uses, thus leading to the development of superior biofunctional foods, and the use of bio-solvent, such as methanol, which is easily available in high purity, highly safe, and completely biodegradable.

Keywords

Antioxidant, antibacterial and antifungal activities, apricot, cold soaking, ultrasonic extraction, strains

1 Introduction

Apricot is the fruit of the common apricot tree, *Prunus armeniaca* L., of the *Rosaceae* family (subfamily *Pomoides*). In 2019, Algerian apricot production amounted to 256.890 t.¹ The fruit of the apricot is distinguished by its high dietary value, and is a valuable raw material for processing.² It is highly appreciated by consumers due to its delicious taste, appealing smell, vivid colours, and numerous nutritional properties which are a result of its rich content of minerals, fibres, sugars and bioactive phytochemicals, including vitamin C, β -carotene, thiamine, ri-

boflavin, niacin, and pantothenic acid, as well as phenols, carotenoids, and tocopherols.³

Recently, research trends are focused on studying healthy foods and natural products due to their potential use as biologically active compounds and their consecutive connection in treating and mitigating diseases.⁴ Natural antioxidants such as phenolic, vitamins, minerals, and carotenoids are contributing substantially to the prevention of oxidation and quenching of free radicals species. These antioxidants are highly effective against oxidative damage related to health disorders, including cardiovascular diseases, neurological syndromes, aging process, cancer, cataract development, weakness in the immune system, and inflammation.^{5,6} The use of antioxidant-rich fruits has been

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described to control the myriad of degenerative diseases that influence human health.^{5,7} These phytochemicals are the main contributors to the antioxidant, antimutagenic, antimicrobial, hepatoprotective, cardioprotective and anti-inflammatory properties of apricots, in addition to their role in taste, colour, and nutritional value.⁸ Pathogenic microorganisms are the main cause of infectious diseases in the world and have been considered a major public health problem worldwide. Plant extracts have been considered as a source of new antimicrobial agents for the future. Phenolic compounds are known to have activity against a wide range of microorganisms. Microbial pathogens in food may cause spoilage and contribute to foodborne disease incidence, while the emergence of multidrug-resistant and disinfectant-resistant bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* has increased rapidly, causing the increase in morbidity and mortality.⁹ Natural products like apricot could be used as effective drugs to treat human diseases, with high efficiency against pathogens and negligible side effects. Fresh and dried fruits are valuable components of our daily diet and are rich sources of antioxidants. For this purpose, we used two efficient methods for the extraction of bioactive compounds (polyphenols and flavonoids), such as cold soaking and ultrasound-assisted extraction (UAE). Cold soaking consists of soaking plant materials in a container sealed with a solvent and leaving them to stand at room temperature for 24 h, with frequent agitation.¹⁰ The UAE was considered to be a simple, efficient extraction method, saving time and reducing chemical consumption.^{11,12}

In our work, the drying efficiency of apricots treated with sucrose, NaCl, and sodium bisulphite solutions dried in microwaves was tested at different powers with AA by scavenging DPPH free radicals, and biological activity tested with four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* sp.), and two fungal strains (*Candida* spp. and *Geotrichum capitatum*), and the same for apricot dough dried in an electric oven at 60 °C. The extraction was based on two methods: cold soaking and UAE. To our knowledge, such work has never been done before.

2 Material and methods

2.1 Preparation of apricot extracts

The variety of apricots selected in our study was widespread (local variety; rosé de Manaa from the region of Batna, Algeria). The fruits were randomly selected from several clusters at different heights and orientations, harvested at full maturity (July), and stored in cold storage at 4 °C.

In this work, the efficiency of the domestic microwave drying process of apricot was tested by AA by scavenging DPPH free radicals, and the biological activity was tested with four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* sp.), and two fungal strains (*Candida* spp. and *Geotrichum capitatum*), and the same for apricot dough dried in an electric oven at 60 °C and compared to the raw material (apricot without drying). For this purpose, three treatments were

carried out with the following solutions, sucrose (60° Brix), NaCl (6 %), and sodium bisulphite (6 %), before the drying. Then, the drying process was started at powers of 200, 400, and 800 W; the drying process was to be stopped at a residual moisture content of 5 %. In addition, the drying time was set according to the time prediction model obtained by the artificial neural network (ANN) (200, 400, and 800 W for 540, 440, and 320 s, respectively), according to a previous study¹. For this purpose, microwave power (W), total apricot weight (g), moisture content (%), dry matter content (%), and moisture ratio (MR) were calculated and fed into the ANN model to predict drying time. Finally, the extraction of polyphenols and flavonoids was carried out for each treatment and each power according to two techniques: cold soaking and UAE, it should be noted that the present work is a continuation of a previous work already published in 2021.¹

2.1.1 Cold soaking

Four grams of fresh apricot and dry products were ground and homogenised, then macerated in 400 ml of methanol for 24 h under continuous stirring in a shaker. After filtration through 0.45 µm filter paper, the filtrates were concentrated in a vacuum rotary evaporator at 80 rpm rotation and 35 °C. After concentrating, the residue was recovered with 10 ml of pure methanol, and stored at –18 °C.

2.1.2 Ultrasound-assisted extraction (UAE)

UAE was performed using an ultrasonic bath (Soniclean 220 V, 250 W, and 50 kHz, Soniclean Pty Ltd., Thebarton, Australia). Four grams of fresh apricots were considered, and the processed products were crushed and homogenised followed by maceration in 400 ml of methanol for 30 min in an ultrasonic bath at 45 °C. Filtration was then carried out before concentration in a vacuum rotary evaporator. Recovery of the residue was performed with 10 ml of pure methanol; it was then stored at –18 °C.

2.2 Evaluation of the antioxidant activity of fresh apricot and dried products by the effect of the free radical DPPH

Free radical scavenging activity was measured employing the stable free radical DPPH (C₁₈H₁₂N₅O₆). The reduction of this radical is accompanied by its change from the characteristic purple colour of the DPPH solution to yellow.^{13,14} This radical is an oxidant that can be reduced by the antioxidant (AH) according to the following reaction:¹⁵



Antioxidant activity was evaluated using the trapping method with modifications, where 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as a relatively stable free radical. An amount of 50 µl of the sample (fresh apricot extract and the processed products) was added to 1.95 ml of DPPH methanolic solution (6 · 10⁻⁵ M) and vortexed. The reduction of this radical is accompanied by its change from

the characteristic purple colour of the DPPH solution to yellow.¹⁶ The mixture was left in the dark for 30 min, and the absorbance was measured at 517 nm with the corresponding blank. The AA is expressed in % and determined by applying the following equation:

$$\% \text{ AA} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \cdot 100 \quad (2)$$

where % AA is antioxidant activity (percentage of anti-free radical activity), Abs sample is absorbance of the sample after 30 min, and Abs control is absorbance of the negative control after.^{17–20}

2.3 Evaluation of the antibacterial and antifungal activity of *Prunus armeniaca* L.

The antimicrobial activity of the extracts was determined by the agar diffusion method standardised by the National Committee for Clinical Laboratory Standards.^{21,22}

The following bacterial and fungal strains were tested: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* sp., *Candida* spp. and *Geotrichum capitatum* were used to test the effect of the extracts: fresh apricot, dry samples.

The samples had the following treatments:

- Sample 1: Apricot was subjected to treatment with sucrose and dried at 800 W.
- Sample 2: Apricot was subjected to treatment with NaCl and dried at 800 W.
- Sample 3: Apricot was subjected to treatment with sodium bisulphite and dried at 800 W.
- Sample 4: Apricot was subjected to treatment with sucrose and dried at 400 W.
- Sample 5: Apricot was subjected to treatment with NaCl and dried at 400 W.
- Sample 6: Apricot was subjected to treatment with sodium bisulphite and dried at 400 W.
- Sample 7: Apricot was subjected to treatment with sucrose and dried at 200 W.
- Sample 8: Apricot was subjected to treatment with NaCl and dried at 200 W.
- Sample 9: Apricot was treated with sodium bisulphite and dried at 200 W, and extracted from the apricot dough dried in an electric oven at 60 °C.

The fresh and dried extracts of *Prunus armeniaca* L. were dissolved in methanol to a final concentration of 1 g/100 ml.^{23,24}

Bacterial and fungal strains were spread on nutrient and malt agar, respectively, incubated at 37 °C for 24 h, and 28 °C for 3–5 days to optimise their growth for bacteria and fungi, respectively. After growth, a suspension was prepared with diluted distilled water and adjusted to a concentration of 0.5 McFarland (10⁶ CFU/ml).

Bacterial suspensions were spread on the surface of Mueller-Hinton agar using a swab to ensure uniform inocula-

tion. Inoculation was performed three times while rotating the plate approximately 60 after each application. The discs impregnated with 50 µl of extracts were deposited with forceps on the surface of the inoculated agar. Two antibiotics (cefotaxime for gram-negative bacteria and gentamicin for gram-positive bacteria) were used as the positive, and the negative control was a methanol-impregnated disc. After 3 min of diffusion at laboratory temperature, the Petri dishes were incubated at 37 °C for 18 to 24 h, and the diameters of the inhibition zones were measured and discussed. The experiment was performed in duplicate.^{25,26}

The antifungal activity of the extracts was determined by the agar diffusion method. The stock cultures were grown on a suitable agar (Sabouraud with chloramphenicol and actidione), in order to inhibit the growth of the contaminating bacterial flora and to reach the stationary phase of growth. The discs impregnated with 50 µl of extracts were deposited with forceps on the surface of the Sabouraud agar. The fungi were incubated for 48 h at 30 °C. Antifungal activity was determined by measuring the diameter of the zone of inhibition.

Antimicrobial and antifungal activity was expressed by the diameter of the zone of inhibition of the strains against the extract, and interpreted according to Celikel & Kavas and Kumaran & Joel Karunakaran (resistant: diameter less than 8 mm; sensitive: diameter between 9 and 14 mm; very sensitive: diameter between 15 and 19 mm; extremely sensitive: diameter greater than 20 mm).^{27,28}

2.4 Statistical analysis

Statistical analysis of results was performed using XLStat 2014 software. An equal mean assumption was tested by analysis of variance (ANOVA). The mean was significantly different by comparison with Duncan's test ($p \leq 0.05$).

3 Results and discussion

3.1 Antioxidant activity

The results of the effect of drying on the DPPH radical activity of the elaborated dried products extracted by two extraction techniques: cold soaking and UAE, are presented in Table 1.

According to the results in Table 1, fresh apricot extracts have a considerable AA of 61.77 % by the cold soaking method. This AA value found was higher than that given by Incedayi et al.²⁹ and Noui³⁰, who reported AA values of 46.52 and 28.97 %, respectively, and in agreement with those given by Wani et al.³¹, who reported an AA range between 30.2 and 71.2 % for the apricot variety Rakausilk, grown in India. The highest AA was observed for apricots treated with the sodium bisulphite solution dried at 800 and 400 W, products treated with sucrose solution dried at 200 W, and those treated with NaCl solution dried at 400 and 800 W (96.00, 91.11, 75.40, 71.89, and 64.66 %, respectively), with significant differences.

Table 1 – Effect of drying on the values of antiradical activity of dried products elaborated by cold soaking extraction method and UAE (%)

Product	Cold soaking	UAE
Apricot	61.770 ± 0.009 ^{bc}	14.370 ± 0.014 ^f
Sample 1	63.440 ± 0.020 ^{bc}	52.510 ± 0.037 ^b
Sample 2	57.290 ± 0.011 ^c	42.780 ± 0.073 ^c
Sample 3	75.400 ± 0.000 ^b	26.630 ± 0.044 ^e
Sample 4	64.660 ± 0.007 ^{bc}	54.090 ± 0.102 ^b
Sample 5	71.890 ± 0.053 ^b	32.040 ± 0.079 ^d
Sample 6	34.220 ± 0.001 ^e	45.160 ± 0.003 ^c
Sample 7	91.110 ± 0.007 ^{ab}	56.960 ± 0.019 ^b
Sample 8	96.000 ± 0.002 ^a	22.700 ± 0.078 ^e
Sample 9	53.660 ± 0.019 ^c	66.880 ± 0.014 ^a
Apricot dough	43.880 ± 0.065 ^d	21.310 ± 0.045 ^e

Values marked with different letters differ significantly ($p < 5\%$; Duncan's test).

The increase or decrease in AA after drying is mainly attributed to the change in phenolic composition. The same finding was reported by Pradeep and Guha, who reported that the increase in AA after heat treatment is highly correlated with the polyphenolic content.³²

The studies of Dragovic-Uzelac et al., which sought to highlight the correlation between polyphenol content and AA in fruit and vegetable extracts, showed that phenolic compounds contribute directly to the antioxidant capacity.³³

According to Table 1, the results of AA revealed that fresh apricot extracts extracted by UAE method possessed an antiradical activity of 14.37 %, which was much lower than that found by the cold soaking method. The increase in antioxidant activity after drying was mainly attributed to the change in phenolic composition. The highest antioxidant activity was observed for apricots treated with sodium bisulphite solution dried at 200 and 800 W, NaCl solution dried at 200 W, and sucrose solution dried at 800 W (66.88, 56.96, 54.09, and 52.51 %, respectively), with a significant difference. The lowest values were recorded in apricot dough, dried apricot treated with sodium bisulphite solution dried at 400 W, and that treated with sucrose solution dried at 200 W (respectively 21.13, 22.70, and 26.63 %, with a non-significant difference). The cold soaking method was considered the best method for extraction of fresh and dried apricots compared to the UAE method, because it allowed the extraction of a large quantity of polyphenols and consequently of very important AA values.

3.2 Antimicrobial and antifungal activity

The antimicrobial and antifungal activities of fresh, dried apricot, and apricot dough (*Prunus armeniaca* L.) extracts were estimated by the agar disk diffusion test against four

bacterial and two fungal strains. The antimicrobial and antifungal activities of the studied extracts were demonstrated by measuring the diameter of the inhibition zone around the discs.

The inhibition zones of the extracts obtained by the cold soaking method and the ultrasound-assisted extraction obtained by the agar diffusion method show that antibacterial and antifungal activities of fresh and dried apricots depend on the targeted bacteria and fungus. The results are summarised in Figs. 1 and 2.

Most of the methanol extracts from fresh and dried apricots showed biological activity (antibacterial and antifungal activity) against clinical isolates of microorganisms of six species, with zones of inhibition ranging from 4 to 45 mm (Figs. 1 and 2). The results confirmed that apricots, whether fresh or dried, are potentially rich sources of antimicrobial agents. Yiğit et al.³⁴ found that apricot kernels have antimicrobial activity against the strains of microorganisms studied (*Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Candida glabrata*, *Candida albicans*, and *Candida parapsilosis*) with zones of inhibition ranging from 8 to 15 mm. In contrast, apricot dough extracts and sample

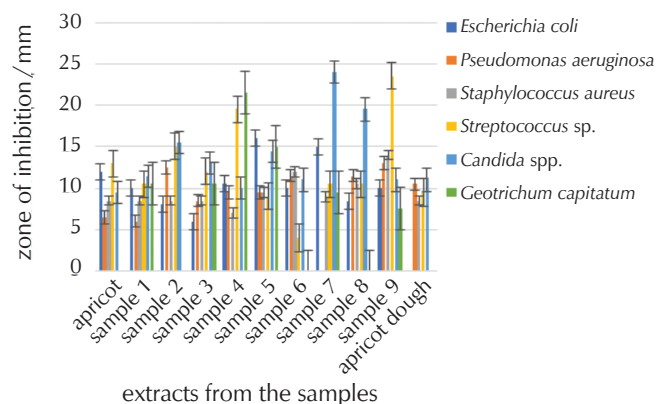


Fig. 1 – Diameter of the antibacterial inhibition zone of methanolic extracts of fresh apricot and dried products prepared by cold soaking method

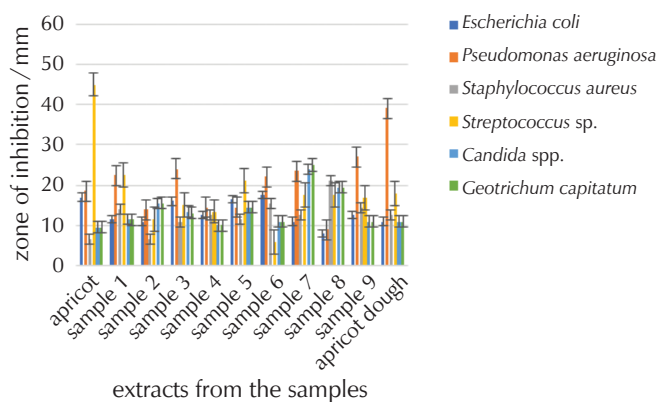


Fig. 2 – Diameter of the antibacterial inhibition zone of methanolic extracts of fresh apricot and dried products prepared by UAE method

7 extracted by cold soaking showed resistance to bacterial strains of *Escherichia coli* and *Pseudomonas aeruginosa* strains, respectively. Extracts of fresh apricots, samples 2, 6, 8, and apricot dough extracted by cold soaking were resistant to the fungal strain *Geotrichum capitatum* (no zone of inhibition produced around the discs).

The lack of antibacterial and antifungal activity of these exceptional extracts towards these strains could be explained by many factors that are related either to the nature of fruit processing or to the wrong choice of techniques, conditions, and tools of manipulation. The poor choice of the adopted method for performing antibacterial activity may be explained by the observation of bacterial resistance. Indeed, *Jorgensen and Turnidge* found that the well diffusion method was more suitable and reproducible for the evaluation of the antibacterial activity of organic extracts than the agar medium diffusion method.³⁵

However, the absence of activity of these extracts towards these strains *in vitro* cannot condition their antibacterial activities *in vivo*. Some fruit metabolites may be active without being metabolised, and in this case, their *in vitro* and *in vivo* activity would be detectable. However, some metabolites are only active after their biochemical reaction; they will be inactive *in vitro* while they are active *in vivo*.³⁶

The results also showed that the activity of the extracts against sensitive bacteria and fungi increased in the extracts of dried apricots treated with sucrose solution, NaCl, and sodium bisulphite. This behaviour can be explained by the effectiveness of our treatment and the extraction methods used.

On the other hand, the fresh apricot extract extracted by UAE showed the highest bacterial activity against *Streptococcus* sp. at the concentration of 1 g/100 ml with an inhibition diameter of 45 mm. However, apricot dough extract and extracts 9 and 3 showed high activity against *Pseudomonas aeruginosa* (inhibition zone of 39, 27, and 24 mm respectively, at a concentration of 1 g/100ml), followed by extract 8 against *Staphylococcus aureus* (inhibition zone of 21 mm, at a concentration of 1 g/100 ml) and finally, the extracts apricot, 6, 5, and 3 against *Escherichia coli* (zone of inhibition of 17, 17, 16.5, and 16 mm respectively, at a concentration of 1 g/100 ml). Concerning fungal activity, extracts 7 and 8 showed high activity against *Candida* spp. (inhibition zone of 24 and 19.5 mm respectively, with a concentration of 1 g/100 ml), and finally, extract 8 had activity against *Geotrichum capitatum* (inhibition zone of 19.5 mm, with a concentration of 1 g/100 ml).

For the cold soaking method, we found that extracts 9 and 4 showed strong bacterial activity against *Streptococcus* sp. (inhibition zone of 23.5 and 19.5 mm respectively, at a concentration of 1 g/100 ml), followed by extracts 5 and 6 that showed bacterial activity against *Escherichia coli* (inhibition zone of 16 and 15 mm respectively, with a concentration of 1 g/100 ml), extract 14 showed activity against *Staphylococcus aureus* (inhibition zone of 14 mm, with a concentration of 1 g/100 ml), and finally, extracts 9 and 2 showed activity against *Pseudomonas aeruginosa* (inhibition zone of 13 and 12.5 mm respectively, with a concentration of 1 g/100 ml). Concerning fungal activity,

extracts 7 and 8 showed strong activity against *Candida* spp. (inhibition zone of 24 and 19.5 mm respectively, with a concentration of 1 g/100 ml), and extracts 4 and 6 against *Geotrichum capitatum* (inhibition zone of 21.5 and 15 mm respectively, with a concentration of 1 g/100 ml).

Streptococcus sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida* spp. and *Geotrichum capitatum* strains are more sensitive to extracts of samples: fresh apricot, apricot dough, 7, 7 and 8, respectively extracted by UAE with inhibition zones of 48, 39, 25, 24, and 21 mm, respectively. Similarly, it has been reported previously that *Candida* spp., *Streptococcus* sp. and *Geotrichum capitatum* strains are more sensitive to extracts of samples 7, 9, and 4 extracted, respectively, by cold soaking with inhibition zones of 24, 23.5, and 2.5 mm, respectively. By comparing the two extraction methods, we found that the UAE method showed moderate bacterial and fungal activity compared to the cold soaking method. In addition, these results indicate that fresh and dried apricot fruits could not only serve as a rich source of food, but also be of importance in ethno-botanical studies because of their high antioxidant and antimicrobial activities. It can be concluded from this study that the potential use of dried fruit extracts as effective antimicrobial and antifungal agents should be further evaluated. Due to their activity against bacteria and fungi, these extracts could have broad-spectrum antimicrobial properties.

4 Conclusion

In the present work, *Prunus armeniaca* L. showed various antioxidant and biological (antibacterial and antifungal) activities desirable for human health. Particularly, the AA of both fresh and processed dried fruits is quite high due to their high polyphenolic content. Hence, the consumption of apricots has been suggested as having health benefits for humans. The results clearly show that the methanolic extracts of fresh and processed dried apricots extracted by UAE and cold-soaking were found to be significantly active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* sp., *Candida* spp. and *Geotrichum capitatum*, with different zones of inhibition ranging from 4 to 45 mm, which explains the effectiveness of our treatment and the extraction methods used. These dried fruits exhibited high antioxidant and biological properties, and should thus be recommended for incorporation in foods firstly by increasing their agricultural production leading to the formulation of superior biofunctional foods, and secondly, their consumption should be encouraged. This research is of great importance to advance research on the characterisation and isolation of active molecules contained in this fruit, and thus their exploitation by the pharmaceutical and agri-food industry.

ACKNOWLEDGEMENTS

This study was supported by the research laboratory LET-PPÉM (Laboratory for the Improvement of Phytosanitary Protection Techniques in Mountain Ecosystems), and LA-

PAPEZA (Laboratory for the Improvement of Agricultural Productions and Protection of Ecosystems in Dry Areas) of the University Batna 1, Algeria.

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SAŽETAK

Antioksidativna i biološka aktivnost svježih i suhih ekstrakata marelica dobivenih hladnim namakanjem i ekstrakcijom potpomognutom ultrazvukom

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Cilj ovog istraživanja bio je procijeniti antioksidativno, antibakterijsko i antifungalno djelovanje ekstrakata svježih i obrađenih marelica (*Prunus armeniaca* L.), sušenih u mikrovalnoj pećnici pri različitim snagama (200, 400 i 800 W), dobivenih metodom hladnog namakanja i ekstrakcijom potpomognutom ultrazvukom. Biološka aktivnost (antibakterijska i antifungalna) procijenjena je difuzijskim testom na agar disku koristeći četiri bakterijska soja (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* i *Streptococcus* sp.) i dva gljivična soja (*Candida* spp. i *Geotrichum capitatum*). Metanolni ekstrakti svježih i sušenih plodova te tijesta marelice dobiveni hladnim namakanjem pokazali su antioksidativno djelovanje u rasponu od 34,22 do 96 %, što je više od ekstrakata dobivenih ekstrakcijom potpomognutom ultrazvukom s vrijednostima u rasponu od 14,37 do 66,88 %. Ekstrakata iz plodova marelice dobiveni objema ekstrakcijskim metodama pokazali su najveću inhibitornu aktivnost prema većini ispitivanih sojeva bakterija i gljiva sa zonama inhibicije u rasponu od 4 do 45 mm. Biološka aktivnost (antibakterijska i antifungalna) poboljšana je primjenom različitih intenziteta sušenja marelica. Osim toga, rezultati biološke aktivnosti ekstrakata dobivenih ekstrakcijom potpomognutom ultrazvukom bolji su u usporedbi s hladnim namakanjem.

Ključne riječi

Antioksidans, antibakterijska i antifungalna aktivnost, marelica, hladno namakanje, ekstrakcija potpomognuta ultrazvukom, sojevi

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Izvorni znanstveni rad
Prispjelo 4. rujna 2022.
Prihvaćeno 23. prosinca 2022.