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Comparison of different methods for extracting phenolic and flavonoid compounds from Balangu (royleana Lallemantia) seeds

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ABSTRACT

Balangu is a plant from the mint family that, in addition to producing gum from its seeds, has phenolic compounds and antioxidant properties. The aim of this study was to investigate the effect of traditional extraction methods, ultrasound extraction and microwave extraction on the extraction of phenolic and flavonoid compounds from Balangu seeds. The treatments studied included water, acetone and ethanol solvents and different extraction times. The highest amount of phenolic compounds extracted from balangu fruit was obtained when acetone solvent was used for 24 hours. The tests performed included the measurement of phenolic compounds by the Folin-Ciocalteu method, and the measurement of antioxidant activity by the method of reducing power and total antioxidant capacity.The results showed that like the traditional extraction method, the use of acetone solvent in the microwave method in all extraction times, more phenolic compounds were extracted from balangu fruit than other solvents and with increasing the extraction time up to 12 minutes in all solvents used the amount of phenolic compounds increased. . The maximum and minimum free radical scavenging activity of DPPH belonged to acetone solvent extract with 24 hours of extraction and water with 3 hours of extraction, respectively. With increasing ultrasound time, the amount of iron-reducing power in the extracts decreased and the extracts obtained from acetone solvent had more iron reducing power than other solvents at any time. In all three extraction processes, the best solvent, acetone and the best process time for the traditional method, microwave and ultrasound were 24 hours, 12 minutes and 30 minutes, respectively. The highest amount of total phenol related to the process for extracting the extract from Balangu fruit was selected by ultrasound.

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1- Introduction

Balango, a plant with a scientific name *royleana Lallemantia* It belongs to the mint family and has dark seeds and oval-shaped stripes. Among the known species of the genus*Lallemantia* 5 species are native to Iran and very few species have been studied so far. The Iranian names of this plant are "Balango" and "Balango Shirazi". Balango seeds are a good source of carbohydrates (45.25%), crude fiber (30.67%), ash (3.63%), oil (30% oil), protein (25.60%) and a series of compounds with medicinal properties. and it is nutritious. This plant has different species that are also found in abundance in Iran. Immediately after being placed in water, balango seeds absorb it and produce a sticky and tasteless gum. This gum also has many medicinal, industrial and food properties(1) Due to the production of high amounts of mucilage, this seed can be used as a new source of hydrocolloid in food formulation. In addition to oil, the most important composition of balango seeds is the presence of mucilage. It also has the phytochemical composition of tocroferol, which tocroferols are phenolic compounds with antioxidant effect (2).

Traditional (submerged) extraction is one of the classic methods used to extract phenolic compounds from plants, during which phenolic compounds enter the liquid matrix (solvent) based on the diffusion process from the solid matrix (plant sample). This method can be used without the need for complex equipment to extract phenolic compounds from large amounts of raw material, but the efficiency of extracting phenolic compounds in this method is relatively low and this method is time-consuming and requires a large amount of organic solvent, so the methods Modern extraction such as microwave and ultrasound are proposed as suitable and economic alternatives (3).

High-frequency ultrasound waves create contraction and expansion cycles and destroy plant cell walls due to the occurrence of cavitation phenomenon, and in this way help solvent penetration. Also, the dimensions of the plants have been greatly reduced, which increases the solid surface and better penetration of the solvent and increases the productivity. In addition toExtractionThe usual decrease in the concentration of extractive compounds with time is also observed, which is due to the long-term exposure of temperature-sensitive compounds to high temperature. Also, studies show that the type of solvent, the power of ultrasonic waves, and time are important quantities that must be carefully determined to increase efficiency (4).

In recent years, the use of microwaves has been valuable in extracting results. This method increases the extraction efficiency in less time and by using less solvent, increasing the amount of extracted compounds and causing less damage to the environment. The better effect of microwave waves can be related to the mechanical effects of internal heating, which is based on the dielectric conductivity and polarizability due to microwave irradiation. As a result, the pressure created inside the cells of the sample leads to the effective and efficient transfer of this energy to the plant material, which is through molecular interaction with the electromagnetic field and the rapid transfer of energy to the solvent and raw plant material. Water absorption of plant materials has increased, and these changes provide an opportunity to improve the extraction performance of target molecules from plant materials.**(5).**

Lianfu et al. (2008) investigated the optimization and comparison of ultrasound/microwave extraction and ultrasonic extraction of lycopene from tomatoes and reported that the optimal

conditions for microwave extraction are 98 W microwave power combined with 40 kHz ultrasonic process, ratio Solvents to tomato paste 10.6:1 (V/W) and extraction time should be 367 seconds(6). Also, Bagharian et al. (2011) investigated the comparison between conventional, microwave and ultrasound methods for extracting pectin from grapefruit. The highest total pectin yield was 27.81% (w/w) for 6 min of extraction at 900 W. In addition, the effects of temperature and time on the quality and quantity of extracted pectin were investigated. The highest yield was for the ultrasound time of 25 minutes (17.92%) at a constant temperature of 70°C, and in general, ultrasound extraction had better results than microwave (7).

Various researches have been conducted on the comparison of different methods of extracting different plant compounds (8-12), however, no study has been conducted on the comparison of different methods of extracting balango. Therefore, the purpose of this study is to extract balango with different methods and to examine the characteristics of the resulting extract.

2-materials and methods

2-1- Materials

To carry out this research, Balingo seeds were procured from the local market. Other chemicals were purchased with laboratory grade and valid.

2-2- Balango extract extraction

Balango seed after preparation, cleaned and waste and foreign materials were removed. Then it was turned into flour by a hand mill and passed through a sieve with a mesh of 0.05 mm.

2-2-1-Traditional method (submerged)

10 grams of Balango powder was mixed with a solvent (water, 80% acetone and 80% ethanol) at a ratio of 10:1 and placed in a shaker incubator at a temperature of 25 degrees Celsius. After the extraction period (3, 6, 9, 12, 18, 21 and 24 hours), the extracts were filtered with Whatman filter paper number one. First, the alcoholic extracts were concentrated in a rotary evaporator until the extraction solvent was completely removed, and then they were dried with a freeze dryer, and the aqueous extracts were dried only with a freeze dryer. (5).

2-2-2- Microwave method

In this method, like the flooding method, 5 grams of balango powder and solvent (water, 80% acetone, and 80% ethanol) were poured into the extraction flask with a ratio of 1:20. At the same time, the contents of the balloon were mixed together with the help of a magnetic stirrer, irradiation was done during different times (2, 4, 6, 8, 10, 12 and 15 minutes) (Samsung,B-12). After the end of irradiation and cooling of the balloon, the resulting extract was filtered with Whatman No. 1 filter paper. First, the alcoholic extracts were concentrated in a rotary evaporator until the extraction solvent was completely removed, and then they were dried with a freeze dryer, and the aqueous extracts were dried only with a freeze dryer. Then, the amount of phenolic compounds in the extract was immediately measured. In this method, the maximum power was 900 watts and the device was connected and disconnected automatically (13, 14).

2-2-3- Ultrasound method

20 grams of powder was mixed with a solvent (water, 80% ethanol, and 80% acetone) at a ratio of 1:6, and then exposed to ultrasonic waves (31 kHz frequency) in an ultrasonic bath for different times (30, 60, and 90 minutes). .

Then, the extracted extract was separated from the plant solids using filter paper. Alcoholic extracts were first concentrated in a rotary evaporator until the extraction solvent was completely removed, and then dried with a freeze dryer, and aqueous extracts were dried only with a freeze dryer. (14).

2-3- Tests of extracted extracts

2-3-1- Measuring the amount of phenolic compounds

. 20 microliters of the extract solution was mixed with 1.160 milliliters of distilled water and 100 microliters of Folin Ciocalto reagent. After 1 to 8 minutes, 300 microliters of sodium carbonate solution (20%) was added to them. After shaking, the test tubes were placed in a water bath with a temperature of 40 degrees Celsius and after 30 minutes, their absorption was read with a spectrophotometer at a wavelength of 760 nm. The total amount of phenolic compounds in the extract was calculated in terms of gallic acid using the equation obtained from the standard curve and the results were expressed in terms of milligrams of gallic acid per gram of dry extract. Experiments were performed in three repetitions and their averages were reported (5).

2-3-2- Evaluation of DPPH free radical inhibition activity

For this purpose, solutions of extracts in solvent were prepared. One milliliter of DPPH solution (with a concentration of 1 mM) was added to 3 milliliters of the extract and the resulting mixture was vigorously stirred. The test tubes were placed in a dark place for 30 minutes. After this time, the absorbance was read at a wavelength of 517 nm. Finally, the percentage of inhibition of DPPH radicals by the extract was calculated with the following formula (1).

Percentage of free radical inhibition = $(A_c - A_s)/A_c \times 100$ (Relation 1-3)

In this regard, A_c and A_s They are control absorption and sample absorption respectively.

2-3-3- Evaluation of the antioxidant activity of the extracts by the reducing power method

powerThe ability of the extracts to reduce trivalent iron was determined by the method of Arabshahi and Arouj (2007). In this method, solutions of powdered extracts were prepared in extraction solvents. 1 ml of the extract solution was mixed with 2.5 ml of phosphate buffer (pH = 6.6 and M = 0.2) and 2.5 ml of potassium ferricyanide (10 g/l) and kept for half an hour in a water bath with temperature^{O}C was placed 50. Then 2.5 milliliters of 10% trichloroacetic acid (weight: volume) was added to the samples and centrifuged for 10 minutes at a speed of 1650 rpm. From the supernatant solution after centrifugation, 2.5 ml was carefully removed and after adding 2.5 ml of distilled water and 0.5 ml of iron (III) chloride (1 g/l), the absorbance of the samples was read at a wavelength of 700 nm. . The high absorption rate indicates the high regenerating power of the extracts**.(15).**

2-3-4- Evaluation of the antioxidant activity of the extracts by the method of total antioxidant capacity

0.1 milliliter of the extract solution was mixed with 1 milliliter of the reagent (0.6 M sulfuric acid, 28 M sodium phosphate and 4 M Ammonium molybdate) in a test tube and kept in a water bath at a temperature of 90 degrees for 1.5 hours. Celsius was placed. After cooling, the absorbance of the samples was read at a wavelength of 695 nm. The method of preparing the reagent is that after preparing the 0.6 M sulfuric acid solution, the

calculated amounts of sodium phosphate and ammonium molybdate were dissolved separately in sulfuric acid to reach the desired concentration in one liter of the reagent. Then, these two solutions were combined and the resulting mixture was brought to a volume of one liter with the remaining sulfuric acid. (16).

2-4- Statistical analysis

In this research, a factorial experiment was used in the form of a completely randomized block design. The comparison of the experimental treatments was also done using the least mean square test. After conducting the experiment in the form of research method and data collection, data analysis was done in the form of experimental designs and using SAS software. Graphs were also drawn using Excel software.

1- Results and discussion

3-1- Effect of solvent type and extraction time on the amount of phenolic compounds in different extraction methods

The results showed that the use of acetone solvent in all extraction times, more phenolic compounds were extracted from balango fruit and with the increase of extraction time in all solvents used, the amount of phenolic compounds increased (Table 1). The highest amount of phenolic compounds extracted from balango fruit was obtained It was found that acetone solvent and 24 hours of extraction were used. The results showed that, like the traditional extraction method, the use of acetone solvent in the microwave method also extracted more phenolic compounds from balango fruit than other solvents at all extraction times, and with increasing the extraction time up to 12 minutes in all the used solvents, the amount of phenolic compounds increased. It was found (Table 2) and increasing the process time (more than 12 minutes) had no effect on the amount of phenolic compounds in the total extract, and in the extraction of phenolic compounds with the help of microwaves from balango fruit, the most efficient solvents were acetone, ethanol and water respectively. The results shown in Table 3 showed that the amount of phenolic compounds in all three solvents used decreased with the increase of extraction time in the extraction process with the help of ultrasound. On the other hand, it was found that at any fixed time of the extraction process, the use of acetone solvent led to the extraction of more phenolic compounds of the extract.

Table 1 - The effect of solvent type and extraction time in the traditional method on phenolic compounds (ppm) of the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	21.43±0.001^{cG}	24.853±0.009^{aG}	22.193±0.008^{bG}
6	21.406±0.003^{cF}	25.573±0.007^{of}	22.673±0.006^{bF}
9	21.737±0.007^{This}	25.930±0.005^{aE}	23.126±0.004^{bE}
12	22.323±0.002^{cD}	26.323±0.005^{aD}	23.560±0.003^{bD}
18	22.683±0.005^{cC}	26.773±0.02^{BC}	24±0.007^{bC}
21	23.020±0.006^{cB}	27.310±0.004^{aB}	24.716±0.006^{bB}
24	23.88±0.005^{that}	27.947±0.003^{aA}	25.320±0.004^{not}

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 2 - The effect of solvent type and extraction time in the microwave extraction method on phenolic compounds (ppm) of the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	24.680±0.002^{cF}	28.110±0.07^{of}	26.323±0.03^{bF}
6	25.027±0.002^{This}	28.543±0.06^{aE}	26.723±0.04^{bE}
9	25.437±0.05^{cD}	28.960±0.004^{aD}	27.217±0.04^{bD}
12	25.960±0.02^{cC}	29.420±0.04^{BC}	27.627±0.02^{bC}
18	26.320±0.03^{cB}	29.857±0.03^{aB}	28.070±0.05^{bB}
21	26.933±0.05^{that}	30.256±0.02^{aA}	28.443±0.06^{not}
24	26.917±0.03^{that}	30.243±0.02^{aA}	28.433±0.05^{not}

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 3 - The effect of solvent type and extraction time in the ultrasound extraction method on phenolic compounds (ppm) of the extract

Extraction time (minutes)	Solvents		
	Water	Acetone	Ethanol
30	30.553±0.007^{that}	32.483±0.002^{aA}	31.223±0.06^{not}
60	30.523±0.006^{cB}	32.453±0.007^{aB}	31.015±0.05^{bB}
90	29.740±0.05^{cC}	31.943±0.06^{BC}	30.653±0.04^{bC}

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Traditional methods of extracting natural plant compounds have disadvantages such as the loss of volatile compounds, low yield, long extraction time, destruction of unsaturated compounds and the remaining toxic solvent (17). Also, since the compounds of the extract are very sensitive to heat, as a result of direct heat in traditional methods, chemical changes take place in them, and the long extraction time in these methods causes the polymerization of the extract. The basis of microwave extraction is absorption of microwave energy by polar molecules of chemical compounds (18). At higher times of microwave radiation, the extraction rate of phenolic compounds may decrease due to thermal degradation (19). The choice of solvent for microwave extraction depends on the solubility of the target compound, the interaction between the solvent and the plant material, and factors such as constant and dielectric loss. has it. Using this method to extract phenolic compounds reduces the use of solvent, increases extraction efficiency, increases the purity of the final product and reduces energy consumption compared to other methods. The extraction temperature in this method is a direct function of time and power (watts). And it has the photo ratio with the mass of the solvent sample and heat capacity (20).

The reason for the increase in the amount of extraction of phenolic compounds with increasing ultrasonic time can be attributed to

the phenomenon of cavitation, which is actually due to the propagation of sound waves in the solid-liquid phase, contraction and expansion cycles are created in the environment, which causes the formation of bubbles that continue to grow. And finally they disintegrate. This action causes solid and liquid particles to oscillate and they speed up under ultrasonic action. As a result, solutes quickly diffuse from the solid phase to the solvent. In addition, other effects such as emulsification, diffusion and tissue damage also help to increase the extraction of desired components from raw materials. At higher times, the amount of extraction may decrease due to the occurrence of oxidation (due to exposure to ultrasound waves) (21). The use of ultrasonic waves increased the mass transfer between the solvent and the plant tissue, reduced dependence on the solvent, extraction at a lower temperature, extraction at a high speed and higher extraction efficiency. According to the results, the amount of phenolic compounds extracted with ultrasonic waves is more than other methods used. Is. In line with these results, Zhang et al. (2017) (22) inA research aimed at extracting blueberry extract using ultrasound and microwaves reported that ultrasound is more effective in extracting phenolic compounds. Ravi et al. (2018) also reported that the amount of total phenol in the extract extracted from tomato pomace with ultrasound waves is higher than the amount of total phenol in the extract extracted with microwave waves (23).

2-3- The effect of solvent type and extraction time on DPPH free radical scavenging activity in different methods

The results showed that the maximum and minimum activity of inhibiting DPPH free radicals belonged to the extract obtained from acetone solvent with 24 hours of extraction and water with 3 hours of extraction, respectively. Also, with the increase of extraction time, the amount of DPPH free radical inhibition activity increased and the extracts obtained from acetone solvent had more DPPH free radical inhibition activity than the other two solvents at all times (Table 4).

The results showed that in all the solvents used in this study, the DPPH free radical inhibition activity of the extracts increased with the increase in the extraction time of the extract with microwave (up to 12 minutes), and the DPPH free radical inhibition activity of the extract extracted with acetone solvent was more than the extracts obtained from the solvent. was ethanol as well as water (Table 5).

According to the results, in extraction with ultrasonic process, the extracts obtained from acetone solvent at any time had more DPPH free radical inhibition activity than other solvents, followed by the extracts obtained from water solvent. It was also found that by increasing the extraction time in the extraction process with the help of ultrasound, the DPPH free radical inhibition activity decreased in all three solvents used (Table 6).

DPPH radical trapping model is widely used to evaluate free radical trapping ability in different samples. This compound is a free radical with a central nitrogen atom that becomes stable by reviving and producing a molecule that changes color from purple to yellow. This radical absorbs at 515 to 528 nm. The DPPH radical scavenging test evaluates the antioxidant activity of water-soluble phenols, so greater ability to scavenge the DPPH radical indicates more hydrophilic molecules, as a result, they have more antioxidant activity. In addition to the number of hydroxyl groups in the structure of an antioxidant molecule, the position of hydroxyl groups, the presence of other functional groups such as double bonds and the combination of hydroxyl and ketone groups play an important role in the antioxidant activity of compounds. The antioxidant activity of natural extracts is related to their ability to give hydrogen, as a result, their free radical inhibitory activity indicates that the extracts have primary antioxidants. Mass transfer, reducing the particle size of the material, increasing the contact with the solvent and improving the diffusion of the solvent in the material. In this context, Chuyen et al. (2018), in their report on the antioxidant property of the extract extracted from the skin of prickly pear, considered ultrasound waves to be a high efficiency method in extracting antioxidant compounds compared to microwave waves (24). In the present research, changing the extraction method had a significant effect on the DPPH radical trapping power. In some previous studies, a good linear relationship between antioxidant activity and total phenol has been reported in many plants (25), and in this study, the extract with a higher amount of phenol showed a higher antioxidant activity.

Table 4 - The effect of solvent type and extraction time in the traditional method on DPPH the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	021 cG /0±09/48	018 aG /0±75/53	078 bG /0±13/50
6	024 cF /0±06/49	032 of /0±37/54	016 bF /0±79/51
9	016 This /0±86/49	062 aE /0±06/55	019 bE /0±92/52
12	013 cD /0±33/50	019 aD /0±87/55	021 bD /0±45/53
18	020 cC /0±99/50	025 BC /0±41/56	026 bC /0±61/54
21	017 cB /0±63/51	049 aB /0±00/57	023 bB /0±73/55
24	074 that /0±17/52	033 aA /0±49/59	046 not /0±06/57

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 5 - The effect of solvent type and extraction time in the microwave extraction method DPPH of the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	002 cF /0±267/57	007 of /0±283/59	003 bF /0±236/58
6	002 This /0±730/57	006 aE /0±856/60	004 bE /0±853/58
9	005 cD /0±117/58	004 aD /0±367/61	005 bD /0±119/59
12	002/0±543/58cC	004 BC /0±637/62	002 bC /0±340/60
18	003 cB /0±210/59	003 aB /0±857/65	005 bB /0±650/62
21	005 that /0±670/59	002 aA /0±720/69	006 not /0±123/64
24	003 that /0±667/59	002 aA /0±719/69	005 not /0±120/64

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 6 - The effect of solvent type and extraction time in the ultrasound extraction method on DPPH of the extract

Extraction time (minutes)	Solvents		
	Water	Acetone	Ethanol
30	007 cA /0±170/61	002 aA /0±167/71	006 not /0±693/67
60	006 cB /0±137/59	007 aB /0±143/67	005 bB /0±573/65
90	005 cC /0±673/57	006 BC /0±740/63	004 bC /0±843/63

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

3-3- The effect of solvent type and extraction time on iron reduction power in different extraction methods

The extracts obtained from the acetone solvent at the same time of the process had more iron reducing power than the other two solvents, and on the other hand, it was found that the iron reducing power of the extracts increased with the increase in the extraction time. The results showed that the maximum reducing power of the extracts that were extracted by microwave method belonged to the extract obtained from the acetone solvent after 12 minutes of the extraction process and the lowest one belonged to the sample obtained from the water solvent with 2 minutes of the extraction process. On the other hand, it was found that by increasing the time of the microwave extraction process up to 12 minutes, the reductive power of the extracts increased and after this period, when water and acetone solvents were used, this feature decreased (Table 7). The comparison of the average data of the iron reducing power of extracts extracted with the help of ultrasound, which was carried out by Duncan's test method, showed that with the increase of ultrasound time, the amount of iron reducing power in the extracts decreased, and the extracts obtained from acetone solvent had iron reducing power at any time. were more than other solvents.

Arkoub-Djermoune et al. (2019) by investigating the effect of different solvents (acetone, ethanol, methanol and water) on the phenolic content and iron reduction power of eggplant skin extract, stated that there is a direct relationship between the amount of total phenol in the extract and its reduction power(26) . An extract may have a high amount of phenolic compounds but low antioxidant power, this could be because the use of different solvents leads to the extraction of different phenolic compounds and the antioxidant activity of different compounds according to their chemical structure. Is different. In addition, during the extraction process, other compounds (ascorbic acid, protein and tocopherol) that have high solubility in water or other solvents are also extracted.

Table 7 - The effect of solvent type and extraction time in the traditional method on ferric reducing antioxidant power assay in the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	002 ^{cG} /0±221/0	002 ^{aG} /0±482/0	001 ^{bG} /0±303/0
6	004 ^{cF} /0±253/0	003 ^{of} /0±511/0	003 ^{bF} /0±322/0
9	001 ^{This} /0±282/0	003 ^{aE} /0±551/0	001 ^{bE} /0±351/0
12	004 ^{cD} /0±312/0	002/0±603/0^{aD}	003 ^{bD} /0±371/0
18	003 ^{cC} /0±363/0	004 ^{BC} /0±641/0	002 ^{bC} /0±411/0
21	001 ^{cB} /0±381/0	003 ^{aB} /0±662/0	003 ^{bB} /0±452/0
24	002 ^{that} /0±412/0	001 ^{aA} /0±701/0	002 ^{not} /0±501/0

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 8 - The effect of solvent type and extraction time in the microwave extraction method ferric reducing antioxidant power assay in the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	003 cG /0±620/0	002 of /0±821/0	001 bF /0±731/0
6	001 cF /0±663/0	003 aE /0±850/0	002 bE /0±753/0
9	004 This /0±692/0	006 aD /0±871/0	003 bD /0±770/0
12	003 cD /0±720/0	005 BC /0±917/0	003 bC /0±812/0
18	002 cC /0±751/0	004 BC /0±920/0	004 bB /0±842/0
21	003 that /0±792/0	003 aA /0±972/0	004 not /0±872/0
24	002 cB /0±782/0	001 aB /0±963/0	004 not /0±871/0

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 9 - The effect of solvent type and extraction time in the ultrasound extraction method on ferric reducing antioxidant power assay of the extract

Extraction time (minutes)	Solvents		
	Water	Acetone	Ethanol
30	007 that /0±952/0	002 aA /0±213/1	006 not /0±120/1
60	006 cB /0±932/0	007 aB /0±001/1	005 bB /0±976/0
90	005 cC /0±821/0	006 BC /0±913/0	004 bC /0±888/0

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

4-3- The effect of solvent type and extraction time on total antioxidant power in different extraction methods

The extracts obtained from acetone solvent at all times had higher total antioxidant power than the other two solvents, and on the other hand, it was found that the total antioxidant power of the extracts increased with the increase of extraction time. The analysis of the data obtained from the total antioxidant power of microwave extracts showed that the type of solvent, extraction time, and the interaction between the type of solvent and the extraction time had a significant effect on the antioxidant power of the total extracts ($p<0.01$).

At all times, the extracts obtained from acetone solvent had more total antioxidant power than the other two solvents, followed by the extracts extracted with ethanol solvent, and on the other hand, it was shown that up to 12 minutes of the microwave extraction process, the amount of total antioxidant power of the extracts increased and after this time there was no significant change at the level of 5% on the antioxidant power of all the extracts. The extracts obtained from acetone solvent had higher total antioxidant power than the other two solvents at any time of the process, and the total antioxidant power of the extracts decreased with the increase of the ultrasound process time..

The results reported from previous studies also consider the ultrasound extraction method to be an efficient method for extracting antioxidant compounds compared to other extraction methods (27). Li et al.

(2005) investigated the antioxidant activity of five jujube species and reported that the difference in antioxidant activity is due to the fact that antioxidant activity is not only limited to phenolic compounds, but also other compounds such as ascorbic acid and tocopherols, as well as their synergistic effects. It leads to the emergence of antioxidant activity (8).

Table 10 - The effect of solvent type and extraction time in the traditional method on total antioxidant power in the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	001 cG /0±097/0	001 aG /0±140/0	001 bG /0±131/0
6	002 cF /0±109/0	002 of /0±161/0	002 bF /0±149/0
9	003 This /0±119/0	002 aE /0±180/0	003 bE /0±160/0
12	003 cD /0±139/0	001 aD /0±191/0	002 bD /0±171/0
18	002 cC /0±150/0	003 BC /0±2083/0	003 bC /0±181/0
21	002 cB /0±169/0	002 aB /0±230/0	002 bB /0±197/0
24	001 that /0±187/0	003 aA /0±252/0	003 not /0±219/0

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 11 - The effect of solvent type and extraction time in the microwave extraction total antioxidant power in the extract

Extraction time (hours)	Solvents		
	Water	Acetone	Ethanol
3	002 cF /0±257/0	003 of /0±390/0	002 bF /0±311/0
6	002 This /0±285/0	001 aE /0±414/0	003 bE /0±342/0
9	001 cD /0±303/0	002 aD /0±448/0	002 bD /0±362/0
12	002 cC /0±322/0	004 BC /0±473/0	001 bC /0±393/0
18	003 cB /0±352/0	001 aB /0±493/0	002 bB /0±422/0
21	002 that /0±380/0	002 aA /0±512/0	003 not /0±451/0
24	001 that /0±380/0	002 aA /0±512/0	002 not /0±451/0

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

Table 12 - The effect of solvent type and extraction time in the ultrasound extraction method on total antioxidant power of the extract

Extraction time (minutes)	Solvents		
	Water	Acetone	Ethanol
30	003 that /0±550/0	003 aA /0±651/0	003 not /0±604/0

60	001 ^{cB} /0±531/0	002 ^{aB} /0±631/0	002 ^{bB} /0±589/0
90	002 ^{cC} /0±502/0	001 ^{BC} /0±601/0	002 ^{bC} /0±561/0

The same lowercase and uppercase letters indicate insignificance at the 5% level in each row and column, respectively

3-5- Balango fruit extract extraction

The results showed that in all three extraction processes used (traditional method, microwave, and ultrasound), the best solvent was acetone, and on the other hand, the best process time for the traditional method, microwave, and ultrasound was 24 hours, 12 minutes, and 30 minutes, respectively. The comparison of the data obtained from the optimal points of these three processes is given in Table 13, and these findings showed that in all the characteristics measured, the extract obtained from the ultrasonic process had the highest value, in other words, the best process for extracting the extract from balango fruit was the ultrasonic process. .

Table 13 - Comparison of extracts obtained from optimal conditions of different extraction methods

Characteristics	Extraction methods		
	Traditional	Microwave	Ultrasound
Phenolic compound	003 ^{c} /0±947/27	002 ^{b} /0±256/30	002 ^{a} /0±483/32
DPPH	033 ^{c} /0±49/59	002 ^{b} /0±720/69	002 ^{a} /0±167/71
Fe reducing power	001 ^{c} /0±701/0	003 ^{b} /0±972/0	002 ^{a} /0±213/1
Total antioxidant poewer	003 ^{c} /0±252/0	002 ^{b} /0±512/0	003 ^{a} /0±651/0

The supply of new methods of extracting biologically active compounds from plants has increased, especially in the pharmaceutical industry. The reason for this issue is the need for an ideal extraction method that can obtain the largest amount of biologically active compounds in the shortest possible time at the lowest price (28). In the reports, many researchers were able to show that the ultrasonic method significantly increases the extraction efficiency. Reducing the time and reducing the amount of solvent used. In a study conducted by Ebrahimzadeh et al., the effects of different extraction methods on the total phenolic and flavonoid content of the plant.*willow A bottle* was investigated. Dry leaves and salicaria flowers. L was extracted by different methods (including maceration and ultrasound). The extracts showed good antioxidant activity in all tests. This research showed that in this plant, the total phenolic content in the ultrasonic extract was more than the maceration extract, but the total flavonoid content in the maceration extract was more than the ultrasonic extract. Ultrasonic extract in DPPH, nitric oxide and H. free radical trapping tests$_2O_2$ Better than maceration extract has been reported (29).

4 - Conclusion

The results showed that in all three extraction processes used (traditional method, microwave and ultrasound), the best solvent was acetone, and on the other hand, the best process time for the traditional method, microwave and ultrasound was 24 hours, 12 minutes and 30 minutes, respectively. In all the characteristics measured, the extract obtained from the ultrasonic process had the highest value, in other words, the best process for extracting the extract from balango fruit is the ultrasonic process.

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مقاله علمی_پژوهشی

مقایسه روش‌های مختلف استخراج ترکیب‌های فنولی و فالونوئیدی از دانه بالنگو

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چکیده

بالنگو گیاهی از خانواده نعناعیان بوده که علاوه بر تولید صمغ از دانه های آن، دارای ترکیبات فنولی و خواص آنتی اکسیدانی می باشد. هدف از این تحقیق بررسی تأثیر روشهای استخراج سنتی، استخراج به کمک فراصوت و استخراج به کمک مایکروویو بر میزان استخراج ترکیب‌های فنولی و فلاونوئیدی از دانه بالنگو بود. متغیرهای این پژوهش شامل نوع حلال (آب، استون و اتانول)، زمان استخراج (۳، ۶، ۹، ۱۲، ۱۸، ۲۱ و ۲۴ ساعت) و نوع تیمار (سنتی یا غرقابی،فراصوت و مایکروویو) بودند. آزمونهای انجام شده شامل اندازه گیری ترکیبات فنولی به روش فولین سیوکالتو، اندازه گیری فعالیت آنتی اکسیدانی به روش قدرت احیاکنندگی و ظرفیت آنتی اکسیدانی کل بود. نتایج نشان داد که همانند روش استخراج سنتی، استفاده از حلال استون در روش مایکروویو نیز در تمامی زمان‌های استخراج، ترکیبات فنولی بیشتری از میوه بالنگو نسبت به حلال‌های دیگر استخراج شد و با افزایش زمان استخراج تا ۱۲ دقیقه در تمامی حلال‌های مورداستفاده میزان ترکیبات فنولی افزایش یافت. بیشینه و کمینه فعالیت مهار رادیکال‌های آزاد DPPH به‌ترتیب به عصاره حاصل از حلال استون با ۲۴ ساعت استخراج و آب با ۳ ساعت استخراج تعلق داشت. با افزایش زمان فراصوت میزان قدرت احیاکنندگی آهن در عصاره‌ها کاهش یافت و عصاره‌های حاصل از حلال استون در هر زمان دارای قدرت احیاکنندگی آهن بیشتری نسبت به حلال‌های دیگر بودند. در هر سه فرایند استخراج مورداستفاده بهترین حلال، استون و بهترین زمان فرایند برای روش سنتی، مایکروویو و فراصوت به ترتیب برابر با ۲۴ ساعت، ۱۲ دقیقه و ۳۰ دقیقه بود. بیشترین میزان فنول کل مربوط به فرایند برای استخراج عصاره از میوه بالنگو، فرایند فراصوت انتخاب شد.