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Research Article

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Optimal Extraction of Flavonoids from the *Moringa olifera* Seeds Extract and Study of its Antioxidant Activity

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ABSTRACT

Through this research, the optimal extraction conditions and characterization of flavonoids from the aqueous extract of the seeds of the Moringa olifera is studied. The effect of the extraction parameters such as PH, time of extraction, temperature and material ratio are studied to optimize the extraction conditions. Furthermore the total antioxidant capacity (TAC) of the optimum extract was studied using FRAP method and compared to the other extracts with different polarity.

Keywords: Extraction parameters; Emerson reaction; Flavonoids; Antioxidant activity

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INTRODUCTION

Moringa olifera from the family of *Moringaceae* is an edible plant cultivated in different countries of Middle East such as Iran, Iraq and Pakistan. The plant is one of the best green sources of vitamins, minerals, and potent anti-oxidants such as flavonoid compounds (Figure 1). In fact the different parts of the plant are used as herbal drugs to relieve many human deficiencies and sicknesses such as vitamins and minerals deficiencies, cardiovascular defect and promoting normal blood-glucose levels for its pharmaceutical properties such as anit-flammatory, antioxidant and antimicrobial effects. It also improves eyesight, mental alertness and bone strength, [1-3]. The seeds of *Moringa olifera* use in food making after boiling to remove its bitter test and prepare the dried nuts and other food beverages. Furthermore, the application of different parts of this shrub in folk medicine is common among the local people for remedy of leprosy, rheumatism, stomach ulcer and antidote, [4,5]. Recently the plants containing antioxidant phytochemicals were attracted the interest of many researchers in which some reports concerning the biosynthesis of nanostructures using green methods especially plant sources emphasized on the antioxidant content of the plants extract as bioreducers and stabilizing agents, [6-12]. In this study the optimized extraction conditions of flavonoids from the seeds of *Moringa olifera* is investigated using colorimetric Emerson reaction. Also, the antioxidant activity of the optimized plant extract is compared to the other extracts with different polarities using FRAP method.

EXPERIMENTAL SECTION

All reagents and materials used in this process are from Aldrich. All solutions were prepared with water that was doubly distilled. Glass apparatus and vessels were cleaned by soaking in 10% HNO_3 followed by rinses with distilled water.

Plant Material

The seeds of *Moringa olifera* were collected in July 2014 at Nikshahr region at Sistan and Baluchestan province from Iran. Voucher specimen was deposited at the herbarium of department of biology at university of Sistan and Baluchestan.

Figure 1: Image of the *Moringa olifera* tree

Flavonoid Percent of the *Moringa olifera* Extract

The effect of single extraction parameters such as temperature, time, pH and material ratio [volume of extracting agent (ml) / the weight of the dried powder (g)] for the optimal extraction of flavonoid were studied through the emerson reaction as a systematic study of the colored reaction of phenolics with 4-amino antipyrine and an oxidizing agent, [13] as following: 20 g dried powder of the plant seeds extracted with aqueous solvent then concentrated and demonstrate to 25 ml flask and mixed to ethanol 90% to extend to volume (solvent A). 5 ml of solvent A added to the flask (100 ml) and mixed to 40 ml ethanol 90% then reached to volume with distilled water (solvent B). 5 ml of solvent B, 45 ml distilled water, 0.5 ml NH_3(aq) 3.5% and 1 ml aminoantipyrin 2% respectively mixed together under reflux condition at 70°C. 4 ml from K3 [$Fe(CN)_3$] 2% was added to the mixture and kept for 5 min. finally the obtained mixture was extracted using 25 ml $CHCl_3$ (solvent C). The absorbance of solvent C read at 455 nm then the percent of flavonoids was measured as following:

Percent of Flavonoids = [E×V1×V2] / [E1%1cm × b× y1× y2]

Where E is the absorbent of solvent C; b, is the weight of the dried sample powder(g), E1%1 cm, is the absorbance of 1% solvent of standard Morin in 1 cm cell, V1 and V2 are dilution factors or the volume of the flask containing solvent A and final dilution for solvent C, respectively.

Study of the Single Extraction Parameters on the Percent of Flavonoids

The effect of temperature:

The result of Figure 2 showed the percent of flavonoids at different temperatures under the same conditions. The percent of flavonoids increased gradually with rising the temperature to 80°C and upper than 80°C it decreased. These changes is for increasing the molecular kinetics which increased the rate of diffusion from cell to extracting agent in higher temperature than 80°C but after that the percent was reduced for thermal oxidizing and decomposing processes.

The effect of pH:

Figure 3 showed the percent of extracted flavonoids obtained by pH changes from strong acid to strong alkaline media. As it is shown, the maximum result achieved in neutralized media at pH 7 whereas the percent of flavonoids strongly decreased with shifting the pH toward the alkaline media probably for structural decomposition and conversion.

The effect of extraction time:

Figure 4 shows an ascending rate of flavonoid extraction with prolonging extraction time. This may increase the rate of diffusion from plant cell to the solvent medium with the rise of time.

The effect of the dilution:

The factor was studied per V/W (W, the weight of the dried seeds (g), V, is the volume of the extracting

medium (ml)). The percent of flavonoids strongly increased to the V: W ratio of 20 then decreased strongly. This is to increase of the diffusion from the plant cells to the solvent media but in dilutions further than 20 the effect of this parameter is negligible because of hyper concentration of the solution and losing the diffusion rate (Figure 5).

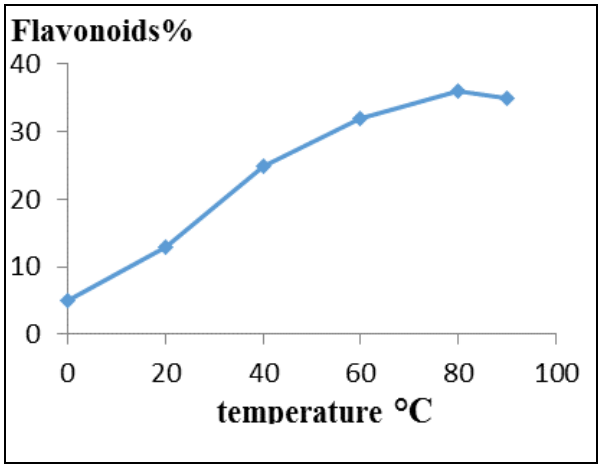

Figure 2: The effect of temperature on the percent of flavonoids

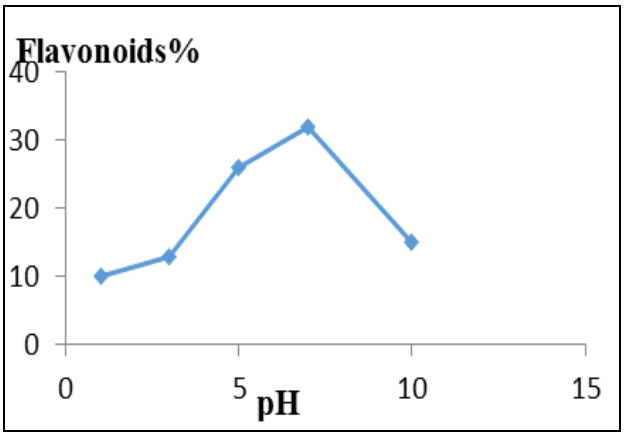

Figure 3: The effect of pH on the percent of flavonoids

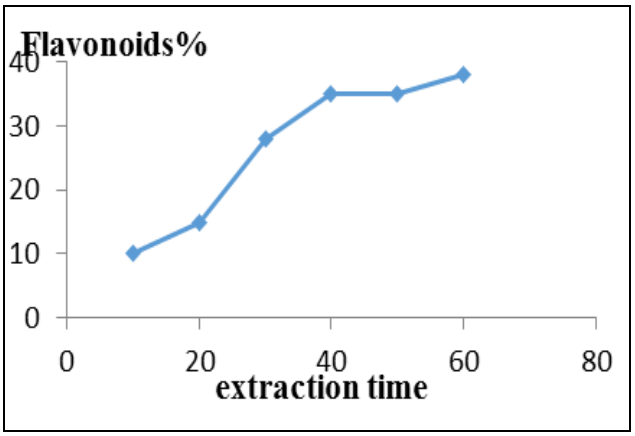

Figure 4: Flavonoid extractin with prolonging the time of extraction

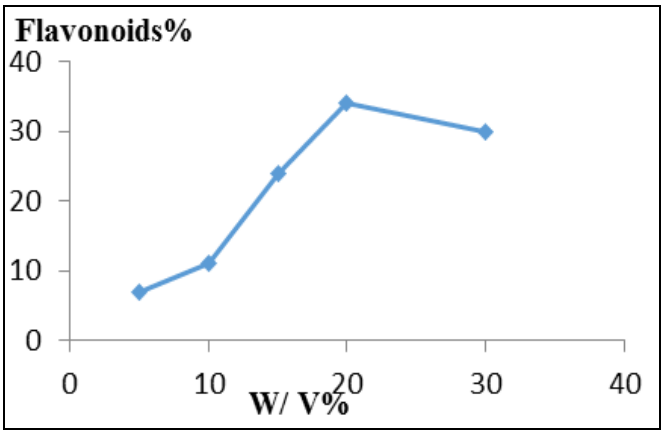

Figure 5: The effect of the dilution on the percent of flavonoids

Antioxidant Activity

All the solvent and chemicals used are of Merck grade. Through this study the FRAP assay was applied to study the antioxidant activity0 This method is based on the colored reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) at 593 nm by a reductant at low pH, [14]. Briefly, the FRAP assay was studied with no modification as following: The stock solutions included 300mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_20$ solution. The fresh working solution was prepared freshly by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37ºC before using. Aqueous solution of known Fe (II) concentration as standard solution was used for calibration (in a range of 0.1 to 1 mM). Assay: Blank FRAP reagent, Sample: 1.5 ml FRAP reagent and 50 µl sample solution. The reaction was monitored up to 4 min at 593 nm, at 37ºC. The Fe (II) standard solution was tested in a parallel process. Calibrations were made by a calibration curve. The standard curve was linear between 1 to 10 mmol also additional dilution was needed if the FRAP value was over the linear range of the standard curve, furthermore the results are expressed in mmol Fe^{+2}/ mg sample.

RESULTS AND DISCUSSION

Optimum Extraction of the *Moringa olifera* Extract

After studying the optimum extraction parameters to obtain the maximum percent of flavonoids in the plant extract, 20 g of the plant seeds powder dissolved in 100 mL distillated water at 80°C for 60 min while stirring at neutralized pH. The obtained extract then was then filtered and kept in refrigerator for more application.

Antioxidant Activity and FRAP Method

The FRAP (Ferric Reduction Antioxidant Power) method is a standard method to evaluate the antioxidant activity using investigation of reducing ability of Fe^{+3} to Fe^{+2} by antioxidant plant extract. In this study the antioxidant property of the optimized plant extract was investigated to evaluate the relation of the obtained maximum flavonoid percent and its antioxidant ability and also its comparison to the antioxidant activity of different extracts of the same plant with various polarity. During this study, we found that the antioxidant property of the optimized extract is significantly more than all other types of extracts (Figure 6). The results clearly revealed the presence of a high amount of flavonoid content as strong antioxidants while applying the optimum condition to the extraction process and its effect on the antioxidant ability of the optimized extract against the other types of extracts.

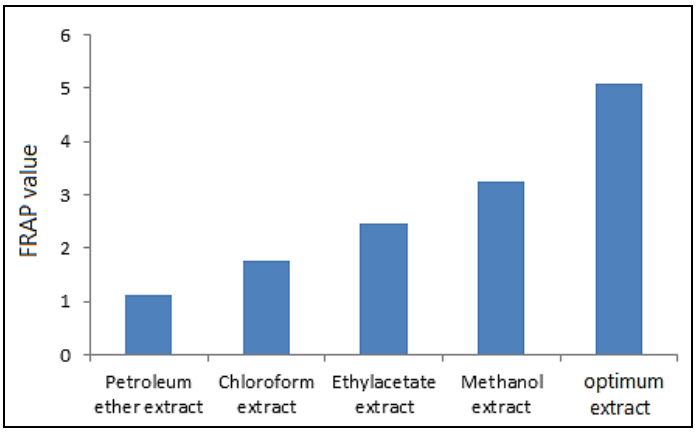

Figure 6: The antioxidant activity of different extracts

CONCLUSION

A highly antioxidant extract was obtained from the seeds of *Moringa olifera* using the obtained optimum extraction parameters as 20 g plant seed powder mixed to 100 mL distillated water at 80°C for 60 min at pH 7. The optimal extract showed strong antioxidant activity as demonstrated with FRAP method compared to the other types of crude extracts with different polarities in which it probably referred to the high concentration of antioxidant flavonoids of the optimum extract.

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