Analysis of Oxidation resistance of Flavonoids in Northern Shaanxi Red Jujube

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Abstract. With Shaanxi Ziziphus jujuba var. goutouzao as an experimental material, soxhlet extraction and ultrasonic extraction were adopted to extract flavonoids in Ziziphus jujuba var. goutouzao, and qualitative detection of flavonoids in extracting solution was scanned through color reaction and ultraviolet spectrum and the result showed positive. With rutin as a standard, the determined content of flavonoids in Ziziphus jujuba var. goutouzao was respectively 3.80 mg/g and 4.95 mg/g. The determination of total reducing power of flavonoids in Ziziphus jujuba var. goutouzao and DPPH scavenging determination showed that, in certain concentration range, the total reducing power and DPPH scavenging are directly proportional to the content of flavonoids, explaining that flavonoids in Ziziphus jujuba var. goutouzao has strong antioxidant ability.

Introduction

Red jujube is a Rhamnaceae native to China, which is a fruit of "homology of medicine and food" containing many nutrient substances useful to mankind with very high nutritional value and medicinal value. It is a health food integrating medicine, food, tonifying functions, known as "woody food, good tonic product", with the efficacy of nourishment for vitality and blood enriching. The use of red jujube as medicine has a long history in China. Red jujube has the medicinal value of stomach invigorating and spleen raising, blood enriching and vital energy tonifying, lung moistening to arrest cough, production promoting of body fluid and throat nourishing, sedative and sleeping, liver-qi stagnation soothing, toxicity alleviating, immunity improving, etc. [1]. It is a topic of research for many scholars. Among them, flavonoids have a multi-biological health effect. In-depth study on the nature of flavonoids in red jujube is a gospel of human health, so it is of profound significance for the research and development of northern Shaanxi red jujube.

Flavonoid exists in our daily vegetables, fruits, although flavonoid is not recognized by people, its reaction in vivo is considered to have nutritional function and it has the effects of oxidation resistance, anti-inflammation, anticancer, anti- vascular proliferation and anti-virus. At present, the research on flavone in jujube is focused on the extraction of general flavone or total phenols in jujube and their content determination, Some researchers emphasize the study of the method of flavonoids extraction in all species, and solvent extraction, supercritical fluid extraction, enzymic method, backflow and soxhlet and other methods are adopted[2] for further optimization. And some researchers study different flavonoids in various jujubes. In recent years, people begin to pay attention to personal health care. Meanwhile, the development of natural antioxidants and their clinical application are more and more. Therefore, the development of natural antioxidants is more and more urgent and of far-reaching significance. Red jujube is a characteristic species in northern Shaanxi. If the contained natural antioxidants are developed and utilized, the development of natural health products and natural drugs [3] that can prevent and cure a variety of diseases will certainly produce good social benefits. On the basis of previous studies, with northern Shaanxi red jujube as a research material, oxidation resistance of flavonoids in northern Shaanxi red jujube was studied and analyzed, providing the basis for further study and experiment of flavonoids in northern Shaanxi red jujube.

Materials and methods

Material processing.

Red jujube: Ziziphus jujuba var. goutouzao is produced by Yanchuan Senhai Agricultural Products Distribution Limited Liability Company. Standard Q/YAZX0001S-2011 is implemented. fleshy unmouldy red jujubes are selected, which are denucleated and chopped after rinsing and draining off, baked in oven for 12 h at 80℃, grinded into powder with mortar and sieved through 200 mesh sieve and finally put in beaker for standby application.

Reagents and instruments.

Rutin standard substance: Bohu Biological (Shanghai) Co., Ltd.; DPPH (1,1-diphenyl-3 - nitrophenylhydrazine): Tokyo Kasei Co., Ltd.; sodium nitrite, aluminium nitrate, sodium hydroxide, petroleum ether, ethyl acetate and trichloroacetic acid are all analytic reagents.

Electro- thermostatic blast oven (DHG-9140A); ultrasonic cell disruptor (JY96- II); low speed centrifuge (KDC-40); Soxhlet extractor; rotatory evaporator (RE-52B); electric-heated thermostatic water bath; vacuum drying chamber (DZF-6050B); ultraviolet spectrophotometer UV-2550, etc.

Test method.

Selection of red jujube → stoving→flavonoids extraction → preliminary identification of flavonoids → content determination of flavonoids →flavonoids oxidation resistance experiment → conclusion drawing

1) Extraction of flavonoids from red jujube

Accurately weight and select 2 copies of 10g dry red jujube powders, and extract flavonoids from northern Shaanxi red jujube by means of Soxhlet and ultrasonic cell break [4-5].

a. Soxhlet extraction

Wrap 10.034g red jujube samples weighted in filter paper, and put into Soxhlet extractor, add 150 ml 80% ethyl alcohol into distilling flask, and distill in liquid-to-solid ratio 1:15 at 90℃ thermostat water bath. Refluxed in a water bath for 6h till getting colourless reflux, dilute the extracting solution, use petroleum ether, ethyl acetate to remove impurities, which is then soluble in 80% ethanol solution after concentrating and transferred into 25ml volumetric flask for constant volume with serial number I to be measured.

b. Ultrasonic cell break

Put10.015g red jujube weighted into beaker, add 100 ml 80% ethyl alcohol, liquid-to-solid ratio 1:10, preheat it in thermostat water bath at 20℃ for 10 min, put centrifugal tube as red jujube solution, treat it for 20 min in ultrasonic cell breaker under the condition of power 400 w. After being treated by ultrasonic cell breaker, treat the solution with low speed centrifuge, take the supernatant for 10 min at 4200 r/min, dilute resultant solution, use petroleum ether, ethyl acetate to remove impurities, which is then soluble in 80% ethanol solution after concentrating and transferred into 25ml volumetric flask for constant volume with serial number II to be measured.

2) Preliminary identification of extractive from northern Shaanxi red jujube

Respectively Extract a small quantity of flavonoids from extracting solution from northern Shaanxi red jujube in volumetric flask I and volumetric flask II for qualitative test, and qualitatively analyze extracting solution through ferric trichloride's color change test, K3Fe(CN)6＋FeCl3 color change test, hydrochloric acid - magnesium powder test by means of ultraviolet spectroscopy.

a. Color reaction

Ferric chloride reaction test: take a small amount of extracting solution from northern Shaanxi red jujube in test tube I1 and test tube II1, and respectively add 2ml 0.02g/ml ferric chloride solution and the color changes from faint yellow to blue green. K3Fe(CN)6＋FeCl3 color change test: take a small amount of extracting solution from northern Shaanxi red jujube in test tube I2 and test tube II2, and respectively add 2 ml 0.02 g/ml ferric chloride solution and then add 2 ml 0.02 g/ml K3Fe(CN)6 and the color changes from faint yellow to dark blue green. hydrochloric acid - magnesium powder test reaction: take a small amount of extracting solution from northern Shaanxi red jujube in test tube I3 and test tube II3, add a little magnesium powder and shake them up, and then drop in several drops of concentrated hydrochloric acid and shock it, and observe the change and a small amount colorless

bubbles generate, and then put test tube I3 and test tube II3 in boiling water bath for heating, and observe the change after 3min finding the bubble aggravated. Record the experiment phenomenon timely.

By synthesizing the two kinds of experimental phenomena and reaction results, it showed that the extractive from northern Shaanxi red jujube contains flavonoids.

b. UV-visible absorption spectrum and UV scanning

Dilute the extracting solution from rutin standard substance and northern Shaanxi red jujube with 80% ethyl alcohol. Scan it within the range of 200 nm—800 nm with ultraviolet spectrophotometer, and further confirm the existence of flavonoids in extractive from northern Shaanxi red jujube.

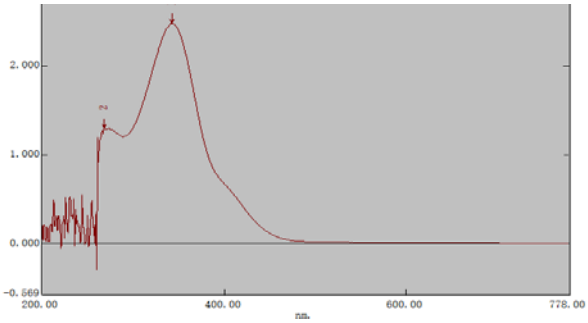

Fig. 1 UV scanning image of rutin standard substance

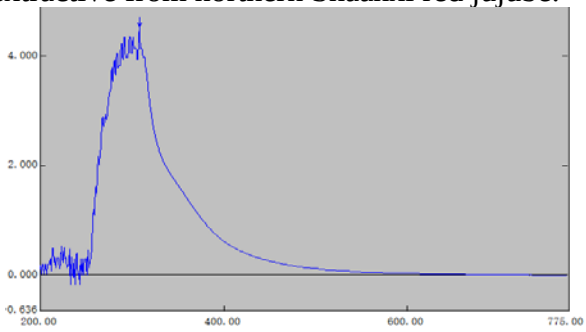

Fig. 2 UV scanning image of extractive from northern Shaanxi red jujube

It can be seen from Fig. 1 and Fig. 2, there are 2 absorption bands in ultraviolet absorption spectrum of rutin standard substance, respectively at 298.5 nm and 363.5 nm, and there is 1 absorption band at 299.5 nm of ultraviolet absorption spectrum of extractive from northern Shaanxi red jujube. Furthermore, same absorption bands exist in 240—280 nm in extractive from rutin standard substance and northern Shaanxi red jujube. So, it can be preliminarily decided that there are flavonoids in extracting solution from northern Shaanxi red jujube.

3) Content determination of flavonoids in northern Shaanxi red jujube

a. Preparation of rutin control solution

Weight 10.013 mg dry rutin control product powder, and dissolve it with 95% ethyl alcohol, put rutin solution in 100 ml volumetric flask, and dilute with water with 95% ethyl alcohol to scale mark.

b. Determination of maximum absorption peak of rutin control solution

Adopt NaNO2-Al(NO3)3NaOH for complexcolorating, accurately take 4 ml 0.1 mg/ml control solution in test tube, and then add 0.4 ml 5% sodium nitrite solution and shake it up before quiescence. After 6 min of reaction, add 0.4 ml 10% aluminum nitrate solution and shake it up before quiescence. After 6 min of reaction, add 4 ml 4% sodium hydroxide solution and shake it up before quiescence. After 15 min of reaction, scan it under ultraviolet spectrophotometer through complexcolorating, and the complex has got its maximum absorption peak at 508 nm in visible region. In addition, when the linear relation is good, measure its absorbance at 508 nm.

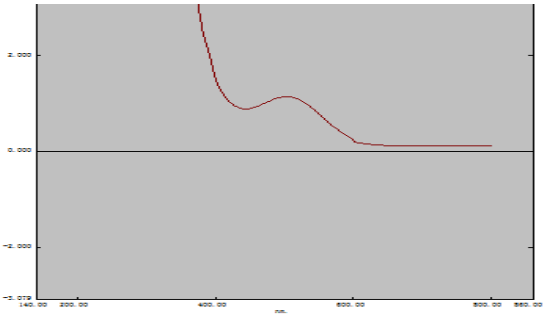

Fig. 3 Ultraviolet absorption spectrum of rutin control product

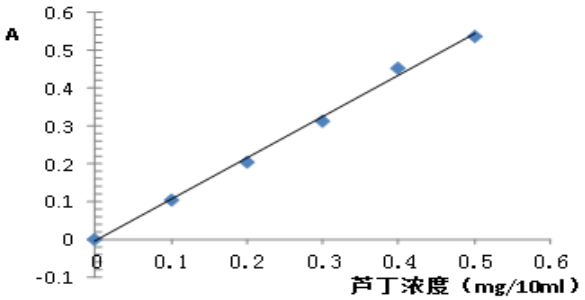

Fig. 4 Standard curve of rutin control solution

c.Drawing of standard curve of rutin control solution

Respectively take 0, 2.5, 5.0, 7.5, 10.0, 12.5 ml rutin solution in 25 ml volumetric flask, and separately add 0.4 ml 5% sodium nitrite solution and shake it up before quiescence. After 6 min of reation, separately add 0.4 ml 10% aluminum nitrate solution and shake it up before quiescence. After 6 min of reation, respectively add 4 ml 4% sodium hydroxide solution and shake it up before quiescence. Dilute it with 95% ethyl alcohol. After 15 min of reation, respectively measure the absorbance A1, A2, A3, A4, A5, A6 under ultraviolet spectrophotometer at maximum absorption peak wavelength 508 nm and get rutin standard curve. The data of absorbance of rutin control solution measured in different concentrations is shown in Table 1 below, and the plotted standard curve is shown in Fig. 4.

Table 1 Absorbance of rutin control solution

Rutin concentration	0	0.01	0.02	0.03	0.04	0.05
A	0	0.1010	0.2050	0.3134	0.453	0.538

The standard curve equation is: Y=1.1013x-0.0069, R^2=0.997, and it has a good linear relationship within the range of 0-0.06 mg/ml.

d.Content determination of flavonoids in northern Shaanxi red jujube

Conduct complex chromogenic reaction to two kinds of extractives from northern Shaanxi red jujube by means of NaNO2-Al(NO3)3-NaOH, measure the absorbance of the sample at 508 nm, draw the curve through the data obtained, and calculate the concentration of extractives obtained from northern Shaanxi red jujube by the two methods.

4) Experiment of oxidation resistance of flavonoids in northern Shaanxi red jujube

a.Determination of DPPH· scavenging ratio

Weight and take extracting solution of flavonoids from northern Shaanxi red jujube and dilute it to a certain concentration. Further wait for the gradient dilution of concentration of diluted resin solution to get 20 μg/ml, 40 μg /ml, 60 μg /ml, 80 μg /ml and100 μg/ml samples. Respectively take 2.0 ml sample solution into small test tubes and number them test tube 1, test tube 2, test tube 3, test tube 4 and test tube 5. Accurately measure and take 2×0.0001mol/L 2.0 ml DPPH· solution to each small test tube. After 25 min of reaction, respectively take flavonoids extracted from northern Shaanxi red jujube samples as blank zero setting to measure its absorbance Ai at 364 nm under ultraviolet spectrophotometer. Then accurately weight and take 2×0.0001mol/L 2.0 ml DPPH· solution to test tube no. 6, and use the 2.0 ml DPPH· solution weighted to prepare 80% ethyl alcohol of flavonoids in northern Shaanxi red jujube and add it to test tube 6. After fully shaking and mixing, measure the absorbance Ac of mixed liquor. Then, accurately weight and take 2.0 ml flavonoids solution from northern Shaanxi red jujube to the test tube no. 7, and add 2.0 ml 80% ethyl alcohol weighted and taken acutually to the test tube 7. After fully shaking and mixing, measure the absorbance Aj of mixed liquor and take the mean value by three times of parallel determination. According to the equation, calculate the DPPH· suppression ratio (%) of flavonoids in northern Shaanxi red jujube. DPPH· scavenging ratio (%)=[1-(Ai-Aj)/Ac] x 100%.

b.Determination of total reducing power of flavonoids in northern Shaanxi red jujube

Measure and take flavonoids solution from northern Shaanxi red jujube and dilute it to a certain concentration. Further wait for the gradient dilution of concentration of diluted resin solution to get 2 μg/ml, 4 μg /ml, 6 μg /ml, 8 μg /ml and 10 μg/ml samples. Respectively measure and take 1ml 2 μg/ml, 4 μg/ml, 6 μg/ml, 8 μg/ml and 10 μg/ml samples at different concentration gradients to small test tubes, and number them as test tube 1, test tube 2, test tube 3, test tube 4 and test tube 5 respectively. Then, accurately measure and take 2 ml 0.01 g/mlK3Fe(CN)6 and 2 ml 0.2mol/ml phosphate buffered solution to various test tubes for fully shaking and mixing, and put them in water bath at 50℃. After 30 min of reaction, take out the tubes and observe them after shaking. Then add 3.0 ml trichloroacetic acid and put them into centrifugal machine after shaking. After 15 min of

centrifugation, take 5.0 ml upper solution from test tube 1, test tube 2, test tube 3, test tube 4 and test tube 5 and put them into the numbered test tube 11, test tube 22, test tube 33, test tube 44 and test tube 55, and separately add 5.0 ml distilled water and shake it up. And add 1.0 ml 1 mg/ml FeCl3 to each tube. After shaking and mixing, add the solution to be tested into the cuvette respectively, measure their absorbance at 700 nm under spectrophotometer, and record A1, A2, A3, A4 and A5 timely.

Results and analysis

Content determination of flavonoids in northern Shaanxi red jujube.

Calculate the content of flavonoids in northern Shaanxi red jujube extreated by different methods according to results of curves based on two kinds of extractives from northern Shaanxi red jujube and rutin control products, and further calculate the concentration of flavonoids in northern Shaanxi red jujube. And the calculation result is shown in following table.

Table 2 Contents of flavonoids in northern Shaanxi red jujube

Extraction method	Soxhlet extraction	Ultrasonic extraction
Content	3.80 mg/g	4.95 mg/g

Experiment of oxidation resistance of flavonoids in northern Shaanxi red jujube.

1) Determination of DPPH • scavenging ratio

DPPH. is a kind of stable nitrogen-centered free radical. If experimental material can remove it, this means the material has the function of reducing hydroxyl radical, peroxy radical concentration and breaking the lipid peroxidation chain reaction. See Fig. 5 as shwon below for cleanup effect of DPPH. in flavonoids extreated from northern Shaanxi red jujube by means of Soxhlet and ultrasonic, in which concentration of flavonoids is taken as x axis and scavenging ratio as y-axis.

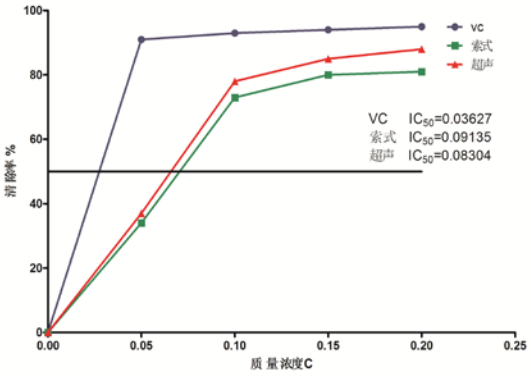

Fig. 5 DPPH. scavenging effect

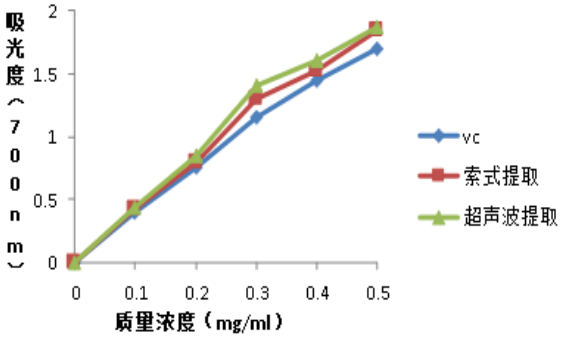

Fig. 6 Total reducing power of flavonoids in northern Shaanxi red jujube

It can be seen form Fig. 5 that flavonoids have relatively strong scavenging ratio to DPPH. at lower concentrations, and the scavenging ratio to DPPH. changes to be steady at higher concentrations. And the intensity sequence of scavenging DPPH· effect is as follows: Vc(IC50=0.03627)> flavonoids(IC50=0.08304) extracted by ultrasonic> flavonoids(IC50=0.09135) extracted by Soxhlet. scavenging DPPH· effect for extractives by Soxhlet and extractives by ultrasonic at the same concentration is lower than that for Vc. The main reason is that the content of antioxidants is low. Furthermore, in addition to flavonoids, extractive also contains other antioxidant substances, such as Vc, polysaccharide, etc. [6] . The contained flavonoids may be the important functional components of it. However, it does not exclude other antioxidant components playing a synergistic effect, which remains to be further studied.

2) Determination of total reducing power of flavonoids in northern Shaanxi red jujube

In this experiment, the total reducing power of flavonoids in northern Shaanxi red jujube is measured, with the concentration of flavonoids as x axis and absorbance as y axis, as shown in Fig. 6 above.

It can be seen from Fig. 6 that, the absorbance and the concentration of flavonoids in northern Shaanxi red jujube are directly proportion, which indicates that the reducing power of flavonoid enhances along with the increase of its concentration, It is shown that such component in northern

Shaanxi red jujube is relatively strong antioxidant, in which the total reducing power sequence is as follows: Vc> flavonoids extracted from northern Shaanxi red jujube by ultrasonic > flavonoids extracted from northern Shaanxi red jujube by Soxhlet, the difference of reducing capacity among various substances is not big. The reducing capacity of extractive obtained by Soxhlet and extractive by ultrasonic is lower than that of Vc at the same concentration. Its main reason is that the content of antioxidants is low. In addition to flavonoids, various extractives contain other antioxidants, such as Vc, polysaccharide, etc., the contained flavonoids may be the important functional components of it. However, it does not exclude other antioxidant components playing a synergistic effect, which remains to be further studied.

Conclusion

In this paper, flavonoids were extracted from northern Shaanxi red jujube by means of Soxhlet and ultrasonic, and the oxidation resistance was analyzed. Results showed that the total reducing power of flavonoids and scavenging DPPH· effect are better, and it has excellent antioxidant effect and is a better in vitro antioxidant and dose-effect relationship exists.

In this experiment, in vitro test was conducted to flavonoids in northern Shaanxi red jujube, but animal experiment wasn't carried out, flavonoids' animal experiment remains to be further studied.

Acknowledgements

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