Study on Extraction Method and Optimization of Total Flavonoids kelp

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Abstract: In this study, seaweed kelp as the raw material, the use of ultrasound-assisted method of extraction process of total flavonoids kelp discussed. Discussion on the ethanol concentration, extraction temperature, time and other factors affecting the extraction of total flavonoids ultrasound, by orthogonal experiment The optimum conditions were: extraction temperature 65 ˚C, extraction time 4h, ethanol concentration of 40%. The results showed that ultrasonic strengthening of total flavonoids extracted kelp is feasible, ultrasound has to save time, extraction rate, saving energy and environmental advantages.

Introduction

With increasing environmental pollution and human longevity, cardiovascular disease, cancer, diabetes, senile dementia disease growing threat to human health, AIDS, Marburg virus, Ebola hemorrhagic fever New diseases are emerging, only viral disease in the world each year on average two or three new species. Human urgent need to find new effects of drugs to treat these diseases. Species of marine life than the rich land, allegedly of more than 500,000 kinds. We can expect to develop from marine organisms and their metabolites in biological origin different from the land, with specific, innovative, diverse new substances chemical structure. Currently, only less than 3,000 abroad have reported compounds in marine species, they found that nearly 5 both terrestrial organisms are not seen or very different natural products.

Seaweed is an important part of living marine resources. On the taxonomy, algae belong low implicit flower plants, divided into four categories a cyanobacteria, green algae, red algae and brown algae, also includes diatoms, dinoflagellates, golden algae and other microalgae. Growth in the world's oceans is estimated that there are more than 15,000 species of algae. Algae are the original producers of organic matter in the ocean and inorganic natural enrichment (including chlorine, Australia, iodine and other halogen), which is at the bottom of the pyramid of a devouring predator status in the marine ecosystem, algae and epiphytic They were born where there are complex microbial antagonistic, symbiotic relationship, so algae can often be the synthesis of certain secondary metabolites having cytotoxic activity, antibacterial to protect themselves. The phenomenon of chemical ecology enlightenment researchers on the biological activity of natural ingredients seaweed-depth study. Numerous reports indicate that a lot of seaweed ingredients have good anti-tumor, anti-viral, anti-fungal, anti-bacterial, immune enhancement or suppression, anti-oxidation, prevention and treatment of cardiovascular disease brain, inhibiting the biological activity of certain target enzymes.

In this paper, on the kelp total flavonoids were discussed, discussed ethanol concentration, extraction temperature, time and other factors affecting ultrasonic extraction of total flavonoids by orthogonal experimental analysis optimum conditions, with a view to large-scale industrial applications provide a reference.

Materials and Methods

Test Materials

Kelp: origin of Hainan Province, China; Ethanol, petroleum ether, chloroform, ethyl acetate, n-butanol, gallic acid, sodium nitrite, aluminum nitrate, sodium hydroxide, sodium carbonate and other analytical reagent

Experimental Instrument

Swing-speed grinder: Wenling Lin Machinery Co.; AY120 electronic analytical balance: Shimadzu Corporation; KH-400KDB High Power CNC ultrasonic cleaner: Ultrasonic Instrument Co., Ltd. Kunshan Wo Chong; SHZ-D (Ⅲ) circulating water pumps: Gongyi City to Hua instrument Co., Ltd.; RE-52AA rotary evaporator: Haiya Rong Biochemical instrument Factory.

Experimental Methods

Sample Preparation

The kelp after the use of clean water and dried, and pulverized with a pulverizer spare.

Orthogonal Test

Factors affecting extract yields have extraction time, extraction temperature, extract ethanol concentration. In order to determine these three integrated factors on kelp yield of flavonoids and polyphenols design L9 (33) orthogonal test ultrasonic extraction and determination. 10g each experiment were taken to a solid-liquid kelp experimental operation 1:10(see Tab.1).

Tab.1 Factor and orthogonal experiment

Level A Time / b B temperature / ˚C ethanol concentration /% D Power / W

Level	A Time/h	B Temperature/˚C	C Ethanol concentration/%	D Power/W
1	2	45	40	300
2	3	55	60	300
3	4	65	80	300

Determination of total flavonoids

Traditional Chinese Medicine method for the determination of total flavonoids have UV spectrophotometry, colorimetry and $AlCl_3$ aluminum nitrate chromogenic colorimetric assay after a variety of methods, etc., but the content of kelp Total Flavonoids multi aluminum nitrate colorimetric assay, because this method has high accuracy, good stability, simple operation, low equipment requirements, color sensitivity and other advantages.

Colorimetric establish a standard reference curve aluminum nitrate: Precision weighing 10.0mg rutin, with 60% ethanol volume to 25mL flask, shake, that give a concentration of 0.4mg / mL rutin standard solution. Imbibe 0,0.4,0.8,1.2,1.6,2.0mL rutin standard solution, add 10mL volumetric flask, were added to 60% ethanol solution 2.0,1.6,1.2,0.8,0.4,0mL, and then add 5% sodium nitrate solution 0.5mL, shake, place 6min, followed by addition of 10% aluminum nitrate solution 0.5mL, after 6min place, adding 4% sodium hydroxide solution 4.0 mL, 60% ethanol was added to volume, shake, place 15min measured absorbance (A) at 510nm Department, draw the standard curve. Rutin standard concentration of c as abscissa, rutin standard solution to determine the absorbance value A ordinate, prepare a standard curve (Fig.1). By regression statistics, was the standard curve equation: A = 11.825 c-0.0007, R^2=0.9990. Showed that the concentration of rutin standard within 0~0. 08mg/mL range and the absorbance values good linear relationship. To learn of the prepared test sample solution 1.0mL, determination of total flavonoids as described above.

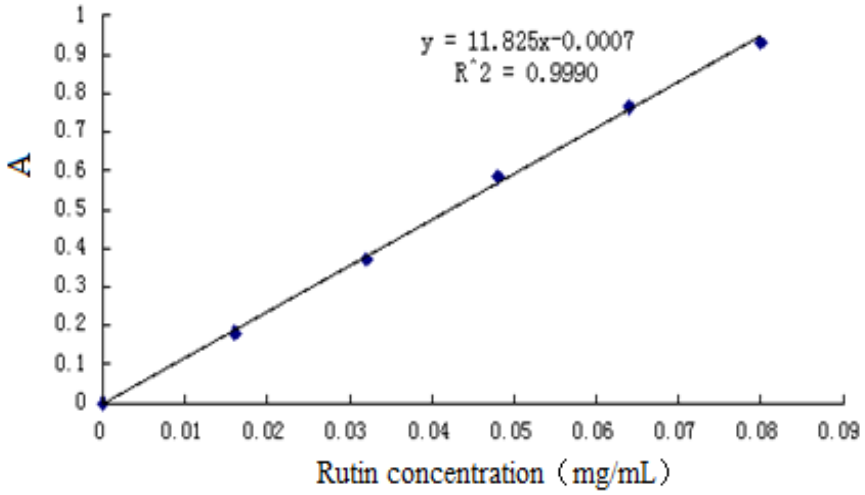

Fig.1 Standard curve

Results and analysis

Orthogonal test results and analysis

Tab.1 orthogonal to optimize the extraction conditions, based on the study, experimental factor table and experimental results of univariate analysis (Tab.2).

Tab.2 Orthogonal Level Analysis

No.	Factor				Total Flavonoids /mg
	A	B	C	D	
1	1	1	1	1	10.3
2	1	2	2	2	11.6
3	1	3	3	3	11.7
4	2	1	2	3	9.5
5	2	2	3	1	10.3
6	2	3	1	2	14.9
7	3	1	3	2	9.8
8	3	2	1	3	14.0
9	3	3	2	1	13.9
k1	33.6	29.6	39.2		
k2	34.7	35.9	35.9		
k3	38.7	40.5	31.8		
R	1.7	3.6	2.5		

From the analysis results in Tab.2, the maximum temperature of the poor extraction factor, followed by ethanol concentration, extraction time then. That extraction temperature in these three factors influence the maximum polyphenol extraction, extraction time with minimal impact. In this experiment, parameter optimization based on optimization of process parameters for each factor A3B3C1, namely optimum conditions were: extraction temperature 65 ˚C, extraction time 4h, ethanol concentration of 40%.

Tab.3 Variance Table

Variance sources	square	degrees of freedom	mean square	F value	P value
A	0.007	2	0.003	4.586	0.179
B	0.044	2	0.022	31.002	0.031
C	0.021	2	0.010	14.487	0.065
Error blank column	0.001	2	0.001		

Results from Tab.3 that the variance in the ethanol concentration, extraction time and temperature of these three factors, the extraction temperature was significant. Comprehensive Tables 2 and 3 are two optimization process, and consider saving materials and energy, in order to obtain a higher extraction capacity, optimum conditions were: extraction temperature 65˚C, extraction time 4h, ethanol concentration of 40% .

Conclusions

In this study, ultrasound strengthen seaweed extract total flavonoids of ethanol concentration, extraction temperature, extraction time on the ultrasonic extraction of total flavonoids influence, the use of orthogonal design its optimum conditions, the experimental results obtained optimum : extraction temperature 65 ˚C, extraction time 4h, ethanol concentration of 40%, ultrasonic power is 300W. This method has to save time, extraction rate, saving energy and environmental advantages.

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