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Content determination of total saponins from *Opuntia*

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ABSTRACT

Objective: To establish for content determination of total saponins in *Opuntia*. **Methods:** Total saponins was determined by UV-VIS spectrophotometry after extracts of the sample had been coloured. **Results:** The methods was linear in the range of 0.05~0.30mg (r=0.9987), and the average recovery was 98.47%, RSD was 3.29% (n=5). **Conclusion:** The methods is sensitive, reliable, simple and reproducible for the determination of the contents of saponins in *Opuntia*.

KEYWORDS

Opuntia; Total saponin; Content determination; UV-VIS spectrophotometry.

INTRODUCTION

Opuntia dillenii Haw. is a herbal plant belonging to genus *opuntia* in cactaceae family. The plants of *Opuntia* have a wide distribution in the world. In China, its stems are used as a folk medicine for the treatment of diabetes, gastric ulcer, mastitis, parotitis and several other diseases[1]. More and more present pharmacological studies have revealed that the plants of *Opuntia* had a lot of interesting biological effects such as anti-inflammatory, anti-diabetes[2], antioxidant[3,4]. *Opuntia dillenii* Haw. contains a variety of more complex chemical composition, they are organic acids, sterols, flavonoids[5,6], sugars, fatty acids, alkaloids, polysaccharides[7], and terpenes[8]. Saponin compounds in the Cactus is one of its active ingredients[9], have significant analgesic effect[10].

In this paper, on the bases of previous research of total of *Opuntia* saponins and the facilities of the laboratory, a colorimetric method for quantitative analysis of total of *Opuntia* saponins. Given there are no statutory reference substance saponin constituents of a cactus, we picked oleanic acid to the reference substance, using spectrophotometric method for determination of total saponin content of cactus, so as to lay the foundation for use of in-depth development of cactus.

APPARATUS AND MATERIALS

Appartus

Electronic balance (Shanghai Balance Instrument Factory, FA2104), Centrifugal machine (BECKMAN company), Vacuum rotating evaporation instrument (Shanghai Senco machinery Institute, R-501), Thermostat water bath (Shanghai Senco machinery Institute, R501), ULtrascan2000 UV-vis spectrophotometer (Amersham Pharmacia Biotech), DRAGON Pipette (large Dragon medical equipment (Shanghai) limited), Small vacuum pump, Buchner funnel, filter, dryer, etc.

Reagents and materials

Oleanolic acid (Abbreviation OA, purity >95%, National Institute for the Control of Pharmaceutical and biological Products, lot number: 110709-200304), industrial alcohol; methanol (Tianjin NO.3 Chemical Reagents Factory), perchloric acid (70%~72%) (Shanghai Taopu Chemical Industrial Factory), vanillin (Tianjin Kemiou Chemical Reagent Co., Ltd.), glacial acetic acid (Tianjin NO.3 Chemical Reagents Factory), petroleum ether (60~90°C) (Tianjin NO.3 Chemical Reagents Factory), n-butanol (Tianjin NO.3 Chemical Reagents Factory), Cactus collected from Kunming, deionized water.

METHODS AND RESULTS

Extracting total saponins from *Opuntia*

The dried cactus powder (5.0 g) were placed in flasks of 250 mL, soaked for 12 hours with 100mL 70% aqueous EtOH, extracted for three times at 80 °C in a water bath, 2 hours each. The combined solution was concentrated under reduced pressure to about 5 mL. The residue was and transfered to separatory funnel, and extracted with petroleum ether (60~90°C) for three times (per 10 mL) to give petroleum ether extract. The petroleum ether extract was washed a small amount of water, discarded the ether layer, merged wash water and water phase to give water extract. The water extract was enriched to approximately 5 mL. The concentrated liquid was put in a separatory funnel, using water saturated butanol to extract till colorless, discarded water layer to give n-butanol extract. The n-butanol extract was concentrated in water bath. The residue was dissolved with methanol to transfer 25 mL volumetric flask, constant volume, shake, give 200mg/mL ethanol extract of cacti.

Determination of total saponins content

Preparation of reference solutions

The reference substance oleanic acid（10.0 mg）were placed in a 50 mL volumetric flask, dissolved in methanol, diluted to scale and shaked to give the concentration of 0.20 mg/mL oleanic acid reference substance solution.

Determination of wavelength choice

0.20 mg/mL oleanic acid reference substance solution (0.25mL) and 200mg/mL samples ethanol extract of cacti (0.10mL) were put in tube with plug, waved dry solvent on water bath, joined 0.2 mL new preparation of 5% vanillin-ice acetate solution, dissolved and joined 0.8 mL perchlorate, shaked uniform, heated 15 min at 60°C water bath. Then the tube was removed and cooled with water to suspended reaction. 5 mL glacial acetic acid was joined in the tube, shake uniform, accompanying blank reagents was regarded as comparison, was scaned for 400-700 nm wavelength range. Oleanic acid reference substance at 548 nm had maximum absorption, samples has not maximum absorption at 548 nm, but has strong absorption, so choosed 548 nm as the determination of the wavelength. The measured results were calculated total saponin content as oleanic acid for the baseline.

Preparation standard curve

Oleanic acid reference substance solution was be measured accurately 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 mL were put in 10 mL 7 tube with plug respectively. Methanol was volatilized at water bath, again joined 0.2 mL new

preparation of 5% vanillin-ice acetate solution and 0.8 mL perchlorate, shaked uniform, heated 15 min at 60 °C water bath, removed, cooled by water to room temperature, again joined glacial acetic acid 5 mL, shake uniform, accompanying blank reagents was regarded as comparison, measured absorbency by the ULtrascan2000 UV-vis spectrophotometer at 548 nm, then drawed standard curve to give regression equations: A=6.425 C-0.0016, r=0.9987 (n=7). The results showed that the linear relationship is right in the range 0.05~0.30 mg/mL.

Methods of study

Stability test

Oleanic acid solution and sample solution were drawed 0.25 mL respectively. Absorbance values were measured under the same conditions depending on the method of preparation of standard curve, accompanying blank reagents was regarded as comparison, measured absorbency at 548 nm, each 10 min determination once absorbency, total 100 minutes, the results are shown in TABLE 1. The results indicated that the generated of colored materials were stability which the color reagents and oleanic acid and samples ethanol extraction solution reacted in 100 minutes. Operation quickly could be used as quantitative analysis, and reliable degree is high.

TABLE 1 : The results of stability experimebnt

Color Time(min)	0	10	20	30	40	50	60
Standard sample Absorbency	0.303	0.302	0.299	0.309	0.307	0.302	0.300
Sample Absorbency	0.320	0.320	0.318	0.314	0.319	0.320	0.318

Precision test

Oleanic acid solution and sample solution were drawed precision 5 pieces, each 0.25 mL. Absorbance values were measured under the same conditions depending on the method of preparation of standard curve. The results are shown in TABLE 2, precision RSD<5%, indicating good instrument precision.

TABLE 2 : The result of precision experiment

NO	Sample Volume (mL)	Absorbency	RSD (%)	NO	Sample Volume (mL)	Absorbency	RSD (%)
1	0.25	0.304		1	0.25	0.330	
2	0.25	0.302		2	0.25	0.320	
3	0.25	0.298	2.09	3	0.25	0.305	2.82
4	0.25	0.296		4	0.25	0.316	
5	0.25	0.288		5	0.25	0.318	

Reproducibility test

5 same medicine powder(5.0 g) were extracted by ethanol according with 2.1 to give cactus sample solution. Accurately imbibed 0.25 mL sample solution, absorbance was determinated in the light of the standard curve, the results are shown in TABLE 3, reproducibility RSD=4.06% (n=5).

TABLE 3 : The result of repetition experiment

NO	Sample Volume(mL)	Absorbency	Average	RSD(%)
1	0.25	0.330		
2	0.25	0.326		
3	0.25	0.320	0.326	4.06
4	0.25	0.344		
5	0.25	0.308		

Recovery test

Precision drawing on a known concentration of the sample solution 5 parts, each part was 100 μL, precise joined 50,100,150,200,250 μL of standard solution respectively. Then, absorbance values were measured by standard curve method at 548 nm, results are shown in TABLE 4. The results indicated that the average recovery rate reach to 98.47%, RSD=3.29% (n=5).

TABLE 4 : The results of recovery rate experiment

No	Total quantity of saponins in sample(μg)	Added quantity(μg)	Analysis result (μg)	Recovery rate(%)	Average (%)	RSD(%)
1	21.0	10.0	31.25	102.50		
2	21.0	20.0	39.83	94.15		
3	21.0	30.0	50.36	97.87	98.47	3.29
4	21.0	40.0	59.86	97.15		
5	21.0	50.0	71.35	100.7		

Sample size determination

5.0 g Cactus powder was extracted by ethanol to give sample solution according to 2.1. Absorption was mersured by standard curve method, the contents of total quantity of saponins in sampLe was calculated, the results are shown in TABLE 5.

TABLE 5 : The contents of total quantity of saponinsin sample

No	Total quantity of saponins in sample (mg/g)	RSD(%)
1	0.254	
2	0.265	
3	0.250	3.06
4	0.251	
5	0.244	

RESULT AND DISCUSSION

Determination of total saponins had spectrophotometry and HPLC method, due to instrument is expensive in the method of HPLC, measurement methods is complex. Spectrophotometric is easy, operation is simple, reproducible and suitable for field determination and routine analysis.

Currently, because there is no statutory reference substance about saponin constituents of a cactus, so the structure and selection of Oleanic acid triterpene saponin from cactus near could be as reference substances. The total saponin content in cacti was measured using Spectrophotometric method. While test results had a little difference, their principle of showed colour was consistency, therefore, the method is still a better choice, in the case of lack of standards provisional.

Extracts used in the test, after degreasing by petroleum ether and excepting small molecules of sugar by n-butanol extraction, the reduced the interference of impurities. Because of vanillin-glacial acetic acid reagents is more sensitive with the color reaction of saponins from *Opuntia.* Chromogenic reagent is preparated prior to the use. But experimental results is affected by temperature and time, thus rendering time and temperature should be strictly controlled. Before joining the glacial acetic acid and determination of absorbency, test tubes must be with water cooling to keep the timely termination of reaction and determination in a timely manner. Before the heating components to show color, tubes must be shake, or experimental error is more. Test tubes must be fully dry, otherwise results are disturbing.

Determinated of total saponins in the samples, the color is stability within 30~60min, linear range is 0.05~0.30mg (r=0.9987), RSD of precision test is 2.09%, an average recovery rate is 98.47%, which is indicatd that these photometric precision, reproducibility, stability is better, and the saponins is without larger losses in extract purification, you can ensure that the final determination of the validity of the results.

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