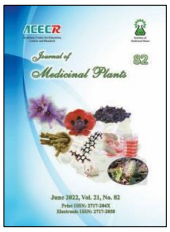
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Research Article

Capparis spinosa L. tablet: from traditional to modern dosage form

Somayeh Esmaeili[1], Behnaz Keramatian[2], Haniyeh Kashafroodi[2], Rasool Choopani[3], Homa Hajimehdipoor[1,*]

[1] *Traditional Medicine and Materia Medica Research Center and Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2] *Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[3] *Traditional Medicine and Materia Medica Research Center and Department of Traditional Medicine, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Background: *Capparis spinosa* is used in Persian medicine for treatment of various diseases. In order to easier use, better patient's acceptance and more stability, preparing a suitable dosage form is necessary. **Objective:** The aim of the study was preparing tablet form from caper fruits and quality assessment of the product. **Methods:** The fruit extraction was performed using ethanol 80% and maceration technique and the extract was dried by freeze dryer. Eight formulations were made using lactose, avicel PH-102, SiO_2 and magnesium stearate. The best formulation was failed during stability tests; therefore, the extract was dried by spray drying method along with maltodextrin and SiO_2 as excipients. Eight formulations were prepared using lactose, avicel PH-102, croscarmellose sodium, PVP K30 and magnesium stearate and the best one was selected. Physicochemical and microbial assessments were performed on the selected formulation and stability tests were done in 40°C and 75% humidity as well as 30°C. **Results:** Caper tablets with freeze dried extract were deformed and their color changed but tablets with spray dried extract were stable in 30°C. They were oblong, green-blue, biconvex, scored tablets with 20.3×9.9×6.7 mm dimensions. Weight, hardness, disintegration time, rutin assay and dissolution were 1115 mg ± 10%, 18.33 ± 1.52 kp, 15±3.5 min, 0.58 ± 0.02 mg/tab and 93.03 ± 3.61 % in 60 min, respectively. **Conclusion:** Caper tablets are good candidate for production in industrial scale after *in vivo* and clinical studies. Moreover stability assessment of the tablets should be performed in suitable packaging in long term study.

Abbreviations: C. spinosa, *Capparis spinosa*; PVP K30, Polyvinylpyrrolidone K30; CCS, Croscarmellose Sodium; SiO_2, Colloidal Silicon Dioxide; MgSt, Magnesium Stearate; ITM, Iranian traditional medicine; WHO, World Health Organization

*Corresponding author: hajimehd@sbmu.ac.ir

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1. Introduction

According to World Health Organization (WHO), 80 % of the world's people relies on traditional medicine for prevention and treatment of diseases [1]. Increasing use and popularity of traditional medicine in both developing and industrialized countries, demonstrate global market for traditional medicine [2]. Medicinal plants are one of the choices in traditional medicine due to their availability and the possibility of fewer side effects [3]. It is estimated that the international market for herbal medicines is over $60 billion in year and it increases gradually [2]. *Capparis spinosa* L. (Caper) is one of the medicinal herbs with diverse effects. It is native to Mediterranean region and widely distributed from Morroco to Crimea, Armernia and Iran [4]. Some countries such as Turkey, Spain, Greece and Italy widely produce caper [5]. Different parts of the plant including fruits and roots have been used since ancient times due to its beneficial properties on human diseases such as liver and kidney diseases, paralysis, diabetes, splenomegaly, mental disorders [6], rheumatoid arthritis, gout [7], and hemorrhoids [8]. Caper pickle is also widely used as a food-medicine in many parts of the world [9]. The pickle is mentioned in traditional medicine texts as an eliminator of phlegm and soda [10]. Lots of studies have been conducted on the biological effects of this plant and it has been shown that the plant has several properties especially hepatoprotective and anti-diabetic effects [11, 12]. This plant also has strong antioxidant activities [13, 14]. Since caper has many beneficial health effects, the chemical and bioactive components of the species have been extensively investigated. The plant has a wide range of bioactive compounds such as alkaloids, flavonoids, streroids, terpenoids and tocopherols [5, 15-20]. It is rich in flavonoids; therefore, many studies have identified and quantified flavonoids in the plant [5]. Rutin is one of the most important flavonoids in caper [21]. This compound is a powerful antioxidant agent which strengthens capillaries and inhibit platelet clump formation in the blood vessels [22]. In addition, rutin reduces low density lipoprotein (LDL) and improves CVD risk biomarkers [23].

Medicinal plants should be converted to dosage forms in order to easier patient's acceptance and controlled deliver of active substances [24-26]. Due to widespread use of caper in traditional and folk medicine [27], in this study, tablets of the plant fruits were prepared and physicochemical and microbial characteristics of the dosage form were assessed. Moreover, rutin has been considered as a marker for quality assessment of the tablet and stability evaluation.

2. Materials and Methods

2.1. Plant materials

Capparis spinosa fruits were collected from Polur, Tehran province, Iran and identified in Traditional Medicine and Materia Medica Research center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran (No. HMS-552).

2.2. Chemicals

Silicon dioxide (SiO_2) was purchased from Evonik (Germany). Avicel PH-102 was from FM (Ireland). Lactose was prepared from Armor Co. (France). Magnesium stearate was prepared from Sun Here Co. (China). Croscarmellose sodium and PVP K30 were purchased from Hiranya cellulose product Co. (India) and Merck Co. (Germany), respectively. Rutin standard material was prepared from sigma Co. (Germany). HPLC grade methanol was from J.T.Baker Co, (USA). Hydrochloric acid and formic acid were from

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Merck Co. (Germany). Ethanol 96 % was purchased from Taghtir Khorasan Co. (Iran).

2.3. Instrumentation

Electric furnace was from Tebazma Co., Iran. Tablet press machine, friability tester, disintegrator, dissolution tester and granulator were from Noavaran Co., Iran. Hardness tester was from Electropharmed Azin Gostar, Iran. HPLC experiment was performed using a Shimadzu system equipped with a vacuum degasser, quaternary solvent mixing, manual-injector and a Shimadzu UV/V detector. Lab solution software was applied for instrument control, data collection and data processing.

2.4. Physicochemical analysis of caper

Quality control tests were performed on caper fruit including foreign matter, total ash, acid insoluble ash, loss on drying, alcohol and water soluble extractives [28].

2.5. Extraction and formulation

Powdered caper fruits were extracted with ethanol 80 % by maceration method for 3 days and the plant: solvent ratio 1:10. After every 24 hours, the mixture was filtered and a new solvent was added to the powder. The filtered extract was dried by rotary evaporator at 40°C and freeze dryer. The final dry extract was converted to viscose form after leaving the freeze dryer.

Daily dose of caper fruit in traditional medicine is about 8 g. Relative to the extraction yield (25 %), average dosage of the extract was considered 2 g/day and tablets containing 500mg of the extract were prepared for using 4 tablets a day.

According to the oily and sticky form of the extract, avisel PH-102, lactose, SiO_2 and magnesium stearate excipients were used for the formulation. Different concentrations of the excipients were mixed with the extract and passed through sieve to obtain granules (Table 1). The granules were dried and the tablets were pressed with a single punch tablet press machine (9×20 mm dimensions).

The F8 tablets was packaged in polyethylene bottles with silica packs as humidity adsorbent (30 tablet in each bottle) and undergone accelerated stability conditions (40 ºC and 75 % humidity) as well as 30 °C. After three months, it was found that the tablets absorbed moisture, changed color, and softened; therefore, it was decided to dry caper extract by spray drying method. This extract was prepared using ethanol 80 % as solvent (plant: solvent 1:10) by maceration method for 24 hours. Maltodextrin (27 %) and SiO_2 (3 %) were used for drying and obtaining powdered extract. Eight formulations were prepared according to Table 2.

2.6. Quality control of caper tablets

Quality assessment of the tablets was performed including appearance, dimensions, weight variation, hardness, disintegration time, marker assay (rutin), dissolution profile and microbial levels [28].

2.6.1. Assay of rutin in caper tablets

Rutin as the main flavonoid in the caper was selected as tablet marker [21] and evaluated in the tablet by HPLC method.

2.6.1.1. Standard preparation

A solution with concentration of 60 µg/ml was made from rutin standard material with methanol as solvent.

2.6.1.2. Sample preparation

Twenty tablets were powdered and 500 mg of the powder was added to a volumetric flask (5 ml) and extracted with methanol by using sonication for 30 min. Then the flask adjusted to 5 ml with methanol. The solution was

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centrifuged and filtered through a 0.45 µm filter and injected to HPLC.

2.6.1.3. HPLC condition

The column was a C_{18} (Shim-pack VP-ODS); 250×4.6 mm, 5µm. The mobile phase was a combination of methanol: formic acid 1 % with the conditions mentioned in Table 3. Oven temperature was ambient. The flow rate was 1 ml/min. Injection volume for all samples and standard solutions was 20 µl. The wavelength was set at 257 nm.

Table 1. Different formulations of caper tablet containing freeze dried extract

No.	Percentage				
	Caper extract	SiO_2	Avisel PH-102	Lactose	MgSt
F1	60	3	-	37	-
F2	60	3	17	20	-
F3	60	3	37	-	-
F4	60	5	35	-	-
F5	60	10	30	-	-
F6	50	5	45	-	-
F7*	50	8	41	-	1
F8*	50	10	39	-	1

*Only F7 & F8 were used for pressing. The tablet weight was 1000 mg. SiO_2: silicon dioxide; MgSt: Magnesium stearate

Table 2. Different formulations of caper tablet containing spray dried extract

No.	Ingredient (mg)						
	Caper extract	Lactose	PVP K30	Avisel PH-102	CCS	MgSt	Tablet weight (mg)
F1	715*	150	-	-	40	10	915
F2	715	150	15	-	40	10	930
F3	715	150	30	-	40	10	945
F4	715	150	30	50	40	10	995
F5	715	150	30	70	40	10	1015
F6	715	200	50	70	40	10	1085
F7	715	200	50	90	40	10	1105
F8	715	200	50	90	50	10	1115

*500mg extract and 215mg maltodextrin and SiO_2, CCS: croscarmellose sodium, MgSt: magnesium stearate
The tablets were film-coated by using green-blue color.

Table 3. Gradient program for analysis of rutin in caper tablet

Time (min)	Methanol	Formic acid 1 %
0	40	60
15	75	25
20	75	25
30	98	2
34	98	2
35	40	60

2.6.2. Dissolution test of caper tablet

Dissolution test was performed on six tablets. The dissolution apparatus type II (paddle) at 75 rpm, 900 ml distilled water as medium at 37 ºC was used and the samples were analyzed after 15, 30, 45 and 60 min. The percentage of released rutin was determined using 5 ml filtered samples. A rutin solution with concentration of 0.96 µg /ml was used as standard.

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3. Results

3.1. Quality control of caper fruits

The results of physicochemical characteristics of caper fruits have been shown in Table 4. There are no pharmacopoeia monograph for *C. spinosa*.

3.2. Caper tablet formulation

As shown in Table 1, eight different formulations were prepared by using caper freeze dried extract and different excipients. Due to the oily and sticky form of the extract, formulations of F1-F6 produced no suitable granules for pressing. All granules were sticky or absorbed moisture over the time. First, lactose and low percentage of SiO_2 (3 %) were used, and then avisel PH-102 changed with lactose. Finally, because of the sticky granules, the percentage of SiO_2 increased. F7 and F8 created appropriate granules for pressing, but tablet no. 7 stuck to the punch and die, so the last one (F8) was the choice. After three months in stability tests, it was found that the tablets absorbed moisture, changed color, and softened; therefore, this formulation was failed and spray dried extract was used. As shown in Table 2, eight formulations were prepared. At first, lactose was used for the formulation, but the prepared tablet showed no suitable press-ability, so avisel PH-102 was added to the formula in different amounts (Table 2). In the last formulation, to reduce disintegration time of the tablets, the percentage of croscarmellose sodium was increased. The properties of the F1-F8 tablets have been shown in Table 5. Among different formulations, the last one showed suitable compressibility, hardness and disintegration time and was chosen as the best one.

Table 4. Quality control results of caper fruits

Test	%
Foreign matter	0.0
Total ash	6.41 ± 0.22
Acid insoluble ash	0.52 ± 0.042
Loss on drying	6.33 ± 0.37
Water soluble extractives	16.99 ± 0.53
Alcohol soluble extractives	32.5 ± 0.53

Table 5. Physical characteristics of different formulations prepared from spray dried extract

No.	Physical characteristics
F1	Unsuitable compressibility
F2	Unsuitable compressibility
F3	High disintegration time
F4	Suitable disintegration time, Low compressibility
F5	Suitable disintegration time, Low compressibility
F6	Suitable compressibility, High disintegration time
F7	Suitable compressibility, High disintegration time
F8	Suitable compressibility and Disintegration time

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3.3. Quality control of caper tablet

Chromatograms of rutin standard solution and caper tablets have been shown in Fig. 1. Rutin peak was in 9.1 min and its concentration in tablets was found 0.58 mg/tab.

The results of dissolution test have been shown in Table 6. It is obvious that more than 90 % of rutin has been released in 60 min which is in agreement with USP [28].

The tablets containing spray dried extract were unstable in accelerated stability condition but they showed good stability at 30°C. Caper tablets showed suitable disintegration time (≤ 30 min). In addition, more than 75 % of rutin was released after 60 min [28]. Physicochemical characteristics of the tablets have been shown in Table 7. Microbial tests of the tablets were in agreement with pharmacopoeia [28].

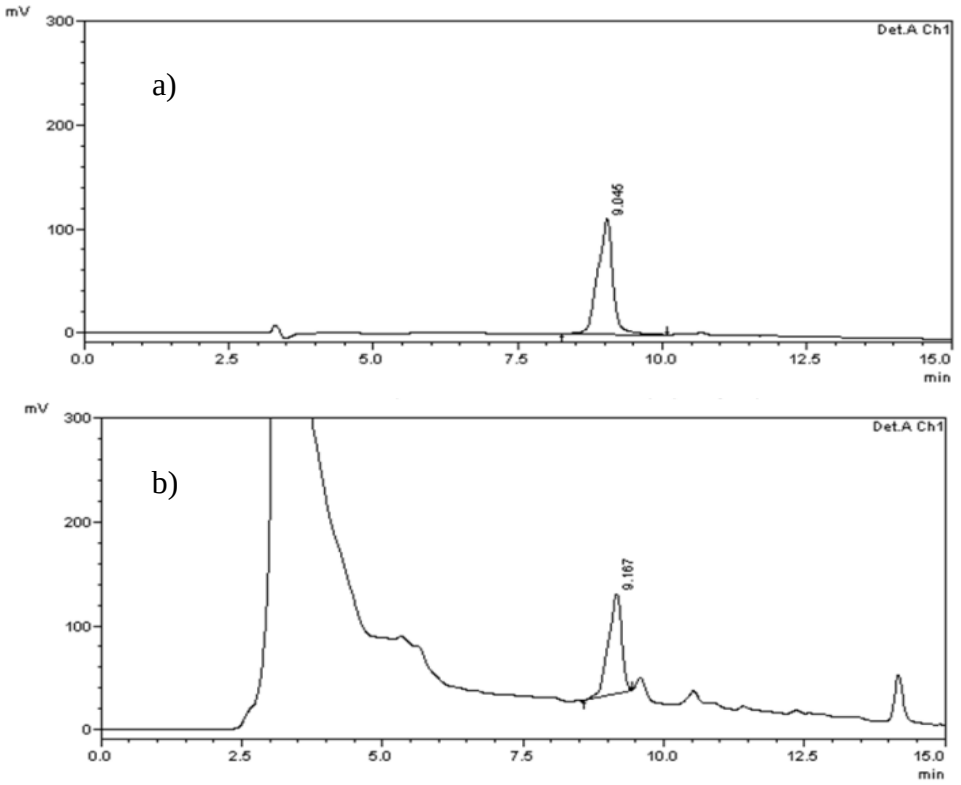


Fig. 1. HPLC chromatograms a) rutin, b) caper tablet

Table 6. Percentage of released rutin from caper tablet

No.	Released Rutin %			
	15 min	**30 min**	**45 min**	**60 min**
1	46.65	63.99	76.35	91.36
2	38.53	65.40	84.55	90.15
3	40.06	74.95	80.89	88.75
4	34.12	55.11	71.36	93.50
5	41.72	64.86	71.84	97.64
6	33.51	57.44	78.89	96.80
Average ± SD	39.10 ± 4.92	63.62 ± 6.98	77.31 ± 5.18	93.03 ± 3.61

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Table 7. Physicochemical characteristics of caper tablets during six months in 30 °C

Test	Time		
	0	**3rd month**	**6th month**
Appearance	Blue-green oval biconvex, scored tablet	Blue-green oval biconvex, scored tablet	Blue-green oval biconvex, scored tablet
Length (mm)	20.35 ± 0.03	20.32 ± 0.03	19.83 ± 0.04
Diameter (mm)	9.92 ± 0.03	9.94 ± 0.02	9.92 ± 0.02
Thickness (mm)	6.72 ± 0.04	6.93 ± 0.04	6.66 ± 0.04
Weight variation (mg)	1163.0-1205.3	1128.1-1181.7	1163.0-1175.5
Hardness (kp)	18.33 ± 1.52	20.33 ± 1.52	20.33 ± 2.08
Disintegration time (min)	15:00 ± 3.5	26:10 ± 7.2	13:12 ± 2.0
Assay of Rutin (mg/tab)	0.58 ± 0.02	0.57 ± 0.05	0.54 ± 0.03
Dissolution (%)	93.03 ± 3.61	91.12 ± 4.60	87.74 ± 2.39

4. Discussion

Capparis spinosa L. is one of the most important plants in phytotherapy and traditional medicine of many countries [27]. Different parts of the plant are used medicinally in various diseases especially as anti-diabetic, anti-lipidemic and hepatoprotective agent [29]. In Iranian traditional medicine (ITM) (Persian medicine), the plant is used particularly in liver and spleen disturbances [30].

Because of prevalence of diabetes, hepatic failures and dislipidemia and the tendency of many people to natural and traditional medicines, access to the effective natural drugs is very important. Due to the nature of mentioned diseases which usually require long-term treatments, the plants should be converted to suitable dosage forms to easier use and controlled drug delivery which increases the effectiveness of the drug and reduces possible side effects [31]. It should be noticed that herbal drugs, similar to chemicals, need stability tests to maintain their effectiveness and control the side effects of the drug. Despite lots of investigations on caper, a few dosage forms were prepared from the plant. In a study conducted by Hosseini et al. in 2013, *C. spinosa* extract was prepared as a capsule and its effectiveness in type 2 diabetes was investigated in a clinical trial [32]. In other researches, plant extract has been used with other species as a dosage form. Accordingly, the purpose of this study was to prepare tablets from caper fruit extract. Caper contains many compounds, especially oily compounds, which makes the ethanol extract sticky and oily form which couldn't be dried by freeze dryer; therefore, one of the most problems in the formulation of caper tablet was the conversion of the viscous extract to granules with appropriate flowability and compressability which could be pressed and formed tablet with right disintegration time. For solving the problem, SiO_2 was the choice excipient. However, despite the use of different amounts of SiO_2 along with lactose and avisel PH-102 in the tablet formulation, finally the tablets were failed during

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stability tests due to humidity absorption. The use of a spray dried extract prepared by maltodextrin 27 % and SiO_2 3 % was the last choice. The tablets were prepared by wet granulation method using excipients PVP K30, lactose, avisel PH-102, croscarmellose sodium and magnesium stearate. The tablets were oval form, blue-green color with an average weight of 1115 mg, disintegration time of 15 min and hardness of 18.3 kp. The concentration of rutin as a marker compound was found 0.58 mg/tablet. More than 90 % rutin was released in 60 min. However, due to high moisture absorption of caper extract, the tablets absorbed moisture in accelerated stability studies, so they became completely soft, the coating cracked and the color of the tablet core completely changed; therefore, stability test was performed at 30 °C for 6 months. It was found that the tablets were stable in 30 °C during six months. Many natural products fail in accelerated stability tests due to complex matrix and the interactions between chemical compounds at 40° C and 75% humidity in accelerated stability conditions. In this case, according to the ICH instruction [33], to determine the expiration date, the results obtained from long-term stability studies are used. In order to determine the stability of the *C. spinosa* tablets, appropriate packaging of the tablet is required (which was not practical in this study), moreover, the tablet should be kept in suitable final package in long-term stability condition. It should be noticed that *in vivo* and clinical efficacy and toxicity studies should be performed before caper tablets production in industrial scale.

5. Conclusion

The prepared caper film coated tablet containing spray dried extract, lactose, avicel PH-102, croscarmellose sodium, PVP K30 and magnesium stearate showed acceptable physicochemical and microbial characteristics. It was stable in 30 °C for 6 months. Suitable packaging is necessary and the tables should be undergone safety and efficacy tests before industrial production.

Author contributions

S. E., H. H. and R. C. designed and supervised the project. H. K. involved in tablet formulation. B. K. performed quality control tests.

Conflict of Interest

The authors declare that there is no conflict of interest.

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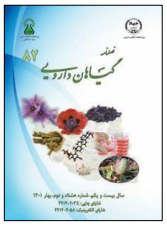
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مقاله تحقیقاتی

قرص کبر: از شکل دارویی سنتی تا نوین

سمیه اسماعیلی[۱]، بهناز کرامتیان[۲]، حانیه کشفرودی[۲]، رسول چوپانی[۳]، هما حاجی مهدی پور[۱،*]

[۱] *مرکز تحقیقات طب سنتی و مفردات پزشکی و گروه داروسازی سنتی، دانشکده طب سنتی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران*

[۲] *مرکز تحقیقات طب سنتی و مفردات پزشکی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران*

[۳] *مرکز تحقیقات طب سنتی و مفردات پزشکی و گروه طب سنتی، دانشکده طب سنتی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران*

اطلاعات مقاله

گل‌واژگان:

کبر

قرص

فرمولاسیون

پایداری

طب سنتی ایرانی

طب ایرانی

چکیده

مقدمه: کبر از گیاهان پرمصرف در طب ایرانی در درمان بسیاری از بیماری‌ها می‌باشد. جهت مصرف راحت‌تر گیاه و پذیرش بهتر توسط بیماران و افزایش پایداری باید گیاه به شکل دارویی مناسب فرموله شود. **هدف:** هدف از این تحقیق، فرمولاسیون قرص از میوه کبر و کنترل کیفیت فرآورده بوده است. **روش بررسی:** عصاره گیری میوه با روش ماسراسیون و اتانول ۸۰ درصد صورت گرفت. عصاره با خشک‌کن انجمادی خشک شد. هشت فرمولاسیون با لاکتوز، آویسل، سیلیکون دی‌اکسید و منیزیوم استئارات تهیه شدند و فرمولاسیون منتخب وارد آزمون‌های پایداری شد ولی به علت ناپایداری، از خشک‌کن پاششی، مالتودکسترین و سیلیکون دی‌اکسید برای خشک کردن استفاده شد. هشت فرمولاسیون دیگر با لاکتوز، آویسل، کراس کارملوز سدیم، PVPK30 و منیزیوم استئارات تهیه شدند و بهترین فرمولاسیون انتخاب و کنترل کیفیت آن انجام شد. پایداری در شرایط تسریع شده و نیز دمای ۳۰ درجه به مدت شش ماه بررسی شد. **نتایج:** قرص‌های حاصله در شرایط پایداری تسریع شده ناپایدار ولی در ۳۰ درجه پایدار بودند. قرص‌ها سبزآبی، دارای ابعاد ۶/۷ × ۹/۹ × ۲۰/۳ میلی‌متر، وزن ۱۰٪ ± ۱۱۱۵ میلی‌گرم، سختی ۱/۵۲ ± ۱۸/۳۳ kp، زمان باز شدن ۳/۵ ± ۱۵ دقیقه بودند. مقدار روتین ۰/۰۲ ± ۰/۵۸ میلی‌گرم در هر قرص بوده و ۳/۶۱ ± ۹۳/۰۳ درصد از این ماده بعد از یکساعت از قرص آزاد می‌شد. **نتیجه‌گیری:** قرص حاصل از عصاره خشک شده با خشک‌کن پاششی، پس از آزمون‌های حیوانی و کارآزمایی‌های بالینی کاندید مناسبی برای تولید صنعتی بوده ولی انجام آزمون پایداری طولانی مدت در بسته‌بندی نهایی مناسب الزامی است.

مخففها: C. spinosa ،Capparis spinosa؛ PVP K30، پلی وینیل پیرولیدون K30؛ CCS، کراس کارملوز سدیم؛ SiO_2، کلوئیدال سیلیکون دی اکساید؛ WHO، سازمان جهانی بهداشت؛ ITM، طب سنتی ایران؛ MgSt، منیزیوم استئارات

* نویسنده مسؤول: hajimehd@sbmu.ac.ir

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