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Research Article

Commercially Available Saw Palmetto Products: Quality Control Testing

Antoine Al-Achi,[1] Andrea F. Locklear,[1] and Lewis Fetterman[1,2]

1. **Campbell University College of Pharmacy and Health Sciences, P.O. Box 1090, Buies Creek, North Carolina 27506**
2. **Posthumously**

Abstract

The use of saw palmetto (*Serenoa repens*) berries extract for the management of benign prostatic hyperplasia by men is well documented. Patients have a vast number of commercially available products to choose from. In this study, we performed pharmaceutical quality control tests on five brands of the dietary supplement, available on the market in the greater Raleigh area in North Carolina. The products were evaluated for their fatty acids content (the active principles in saw palmetto), weight variation among their units (capsules), and disintegration time of the capsules in an aqueous environment. The results of these tests showed that products differed greatly in their fatty acids content, showing high variability within the products. Two products failed the disintegration test and one product did not pass weight variation test. A price comparison among the products showed that the least among the products in pharmaceutical quality was the most costly. Pharmacists should be aware of such discrepancies existing among the saw palmetto products they carry in their drug stores.

Key Words: palmetto, quality control, disintegration time.

Introduction

Saw Palmetto (*Serenoa repens*) is currently used as an alternative to conventional medicinal practices for symptomatic treatment of benign prostatic hyperplasia (BPH). It is found in nature in the southern coastal states of Florida and Georgia and also in the Bahamas and Cuba.[1] Currently, saw palmetto is not approved for a therapeutic treatment in the U.S. Saw palmetto has also been marketed to maintain the overall urinary health in men and women. The recommended dose for this indication is 160 mg at breakfast and the evening meal to help decrease the incidence of side effects. The potential side effect of this supplement is GI disturbance.[2] BPH is seen in the older male population and can drastically alter the quality of life. Possible symptoms include pain during urination, feeling of urgency, increased frequency, straining, slow to start a urine stream.[3] Saw Palmetto is a dietary herbal supplement that offers an alternative to drug therapy for some patients. Saw palmetto has been documented in study trials to decrease the symptoms related to BPH and information regarding improvement of symptoms of BPH have sparked a trend of consumer buying of saw palmetto.[4,5]

***Corresponding Author**
E-mail: alachi@campbell.edu
Mob. 910.893.1703

Currently, the Food and Drug Administration (FDA) is responsible for ensuring that nutritional supplements are safe for consumer use. After a product is on the market it is the responsibility of the FDA to prove the supplement is unsafe before removing it from circulation. However, the FDA, due to limited resources, does not routinely analyze contents nutritional supplements before the product is marketed. The FDA does require a label with a list of ingredients for nutritional products.[6] With the limited involvement of the FDA with the dietary supplements, manufacturers should take on the responsibility to produce quality products with little variability in content and physical properties.

The purpose of this study was to investigate a variety of store brand saw palmetto extracts for content uniformity, weight variation, and disintegration properties. In doing this study, the brands as well as bottles of the same brand were investigated for variation. Weight variation and disintegration tests were performed in compliance with USP requirements.

Material and Methods

Material

ChromaDex Saw Palmetto Free Fatty Acid Standard Kit; acetonitrile HPLC grade (EMD lot:45297, Fisher Scientific lot:050579); methanol HPLC grade (Fisher Scientific, lot:010652); hexanes (Fisher Scientific, lot:01168); potassium hydroxide pellets ACS grade (Sigma-Aldrich lot:221473); glacial acetic acid (Fisher Scientific, lot: 884930); sodium acetate trihydrate (Fisher Scientific, lot: 996219); 2-propanol HPLC grade (Fisher Scientific, lot:011200; 032332); 2, 4'-Dibromoacetophenone (Sigma-Aldrich) 98%; cis-Dicyclohexano-18-crown-6, 98% (Sigma-Aldrich). VanKel Disintegration apparatus with wire basket, Model 35-1000(VanKel Cary, NC) and Precision-All stainless steel water bath Model 181 (Precision Scientific Inc, Chicago, IL) were used in the disintegration testing. Saw palmetto brand products (labeled A, B, C, D, and E) were purchased from local stores in North Carolina. These standardized products were labeled to contain 160 mg of saw palmetto extract per capsule (not less than 85% of the extract was fatty acids) (Table 1).

Methods

Preparation of Methanolic KOH solution: The solution was prepared using 0.557g KOH, added to 400 mL methanol and stirred with magnetic stirrer until all KOH was dissolved. The approximate concentration was 0.028 N.

Preparation of Dicyclohexano-18-crown-6 solution (0.025 M): The solution was prepared with 1.017g added to 250 mL bottle and added approximately 135 mL of acetonitrile and sonicated until all solids was dissolved.[7]

Preparation of Dibromoacetophenone 0.2M solution: The solution was prepared by adding 5.56 g of dibromoacetophenone solid to a 200-mL bottle and added 100mL of acetonitrile and sonicated until all solids dissolved.[7]

Derivitization procedure: Sample solutions were prepared by weighing approximately 1000 mg of extract from each bottle (the content of three to four capsules) into a 100-mL volumetric flask in triplicate. Fifty milliliters of 2-propanol were added to each flask and agitated until all extract dissolved. The sample solutions were diluted to volume with 2-propanol and mixed thoroughly. Ten milliliters were transferred into a 50-mL volumetric flask and diluted to volume with 2-propanol.[7] Two milliliters of each sample solution, 2-propanol (blank), and standard solutions (0.02 mg/mL to 0.50 mg/mL in 2-propanol) were transferred into 10mL flasks. Methanolic KOH, 0.02 M Dicyclohexano-18-crown-6 and 0.2 M Dibromoacetophenone were added in the amount of 2.5 mL to each of the 10-mL flasks. Each flask was diluted to volume with acetonitrile and mixed. Five milliliters of each solution were transferred into separate 5-mL reaction vials, capped and heated in a dry block heater for 1 hour at approximately 80°C. The solutions were cooled to room temperature prior to analysis.[7]

The HPLC method: The components for the HPLC analysis were Hewlett Packard 1090 HPLC with diode array UV detector and Phenomenex HPLC column, 3µm, 4.6 x 150mm. The gradient system was set to change linearly from 100% acetonitrile to 100% acetonitrile:water (80:20) over a 30-minute run and 100% acetonitrile was used to re-equilibrate the system for 10 minutes after each run. Acetonitrile was used as the needle wash solvent. A flow rate of 1.5 mL/minute and injection volume of 20 µL were set. The acetonitrile:water (80:20) mixture was equilibrated to room temperature, was loaded into HPLC tank, and was degassed with Helium gas. The organic mobile phase, acetonitrile was loaded into an additional HPLC tank and degassed with Helium gas. The mobile phase was degassed with Helium for approximately 5 minutes and the column was conditioned for 30 minutes prior to first injection. The standard curves for all the fatty acids were linear within the concentration range of 0.02 mg/mL to 0.50 mg/mL (R^2 values for all the acids ranged between 0.9974 to 0.9978).[7]

Weight variation test: Twenty intact capsules were weighed individually, cut open with scissors and contents removed by washing the capsule with hexanes. The empty capsules were air dried, reweighed and weight recorded. The requirements were met if each capsule weight was within the limits of 90% and 110% of the average weight.[8]

Disintegration test: A 0.05M acetate buffer solution was prepared by dissolving 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL solution with an approximate pH of 4.5. The solution was maintained at 37 ± 2°C in a stainless steel water bath. The capsules were observed and time recorded when all six capsules had disintegrated.[8]

Cost comparison: The cost per capsule was calculated for each brand based on the total number of capsule per bottle and its retail price.

Results and discussion

The content uniformity test showed that for the various brands there was a great variability in the overall average amount of fatty acids (Table 2). Brand (E) had two- and-half time higher than that of the labeled amount (329.44 mg vs. 136 mg) while (B) and (D) brands had about 77% of labeled amount (105 mg vs. 136 mg) (136 mg equals to 85% of the extract weight of 160 mg). Variations were found between brands with respect to all fatty acids ($p < 0.001$), except for stearic acid ($p = 0.2157$) (Table 2). The relative standard deviation percent (%RSD) for the content uniformity test, expressed as range, with respect to the seven fatty acids was the highest for product (B) (%RSD = 145.76% - 823.08%) and the lowest for capsules obtained from product (E) (%RSD = 11.60% - 119.74%) ($p = 0.0077$). Overall, capsules from the five brands varied the most with respect to capric acid content (%RSD = 18.58% - 823.08%) and the least with respect to linoleic acid content (%RSD = 7.81% - 102.74%) (Table 2). In comparison, the maximum observed %RSD for all the fatty acids during the standard curves development was less than 3% (n = 10). Therefore, the high-observed variability among the capsules with respect to their fatty acids content cannot be attributed to chromatographic variability, but rather to actual variability existing among and within the brands.

Differences in weight of each capsule observed in this study may be due to the presence of other ingredients (Table 1). In brand (A), there were notable differences in the appearance but the listed ingredients were the same; the brand (A) capsules that had a darker appearance were found to expire at an earlier date. With respect to weight variation test, all brands showed variation within the acceptable limits (90% to 110% of average weight), except for bottle 2 of brand (C) where the capsules failed the test (Table 3)

Table 1: Description of saw palmetto brands.

Brand	Other Labeled Ingredients	Extract	Standardized
A	Pure olive oil; Gelatin; Glycerin	160 mg	85-95% Fatty Acids and Biologically Active Sterols 136-152 mg
B	Olive oil, Gelatin (Softgel), Glycerin, Water	160 mg (100-200 mg)	85%-95% of Fatty Acids and Active Sterols
C	Gelatin, Beeswax, Soybean oil mixture, Glycerin Lecithin, Caramel color, Titanium dioxide color	160 mg	85%-95% of Fatty Acids and Active Sterols
D	Pure olive oil, Gelatin, Glycerin	160 mg	85%-95% of Fatty Acids and Active Sterols
E	Olive oil, Gelatin, Glycerin	160 mg	85%-95% of Fatty Acids and Active Sterols

Table 5. Price comparison of the various brands included in this study.

Brand	Price ($)	Bottle Count	Price/capsule
A	9.99	100	$0.10
B	11.99	60	$0.20
C	16.99	120	$0.14
D	6.36	100	$0.06
E	9.99	60	$0.17

Table 2. Average amount of fatty acids (mg) per capsule

Content Uniformity-mg acid (mean ± s.d.)		Brand					
Fatty Acid		**A**	**B**	**C**	**D**	**E**	***p*-value**
Capric		2.01±2.00	0.65±5.35	5.01±1.47	0.78±3.35	6.35±1.18	<0.0001
Lauric		25.26±19.26	34.39±52.58	68.30±5.78	31.59±35.65	95.62±11.55	<0.0001
Linoleic		26.5±17.92	9.20±13.41	36.64±2.86	9.14±9.39	28.15±3.45	<0.0001
Linolenic		4.05±3.00	1.71±2.97	6.81±1.37	1.84±2.08	5.86±0.68	<0.0001
Myristic		6.34±4.78	12.13±18.43	10.74±1.56	12.28±13.76	37.58±4.50	<0.0001
Oleic		102.69±77.22	36.45±54.90	73.74±6.52	37.59±40.79	119.46±14.52	<0.0001
Palmitic		16.26±12.35	9.22±14.23	26.97±14.43	9.53±10.55	29.95±3.63	<0.0001
Stearic		6.08±4.66	1.77±2.87	10.19±19.47	2.56±2.12	6.47±0.83	0.2157
Total mg acid present		189.19	105.52	238.4	105.31	329.44	

Table 3. The weight variation test (20 capsules per bottle)

	Mean ± s.d. (n = 20)	90% of average weight	110% of average weight	Result
A 1	0.266 ± 0.002	0.239	0.292	Pass
A 2	0.265 ± 0.004	0.239	0.292	Pass
B 1	0.315 ± 0.003	0.283	0.346	Pass
B 2	0.317 ± 0.003	0.285	0.348	Pass
C 1	0.399 ± 0.006	0.359	0.439	Pass
C 2	0.401 ± 0.017	0.361	0.442	Fail*
D 1	0.265 ± 0.004	0.238	0.291	Pass
D 2	0.268 ± 0.004	0.241	0.295	Pass
E 1	0.290 ± 0.007	0.261	0.319	Pass
E 2	0.293 ± 0.007	0.264	0.323	Pass

* One capsule out of twenty exceeded the maximum of 110% of average weight.

Table 4. Disintegration test for Saw Palmetto capsules.

Disintegration		
Brand*	# Disintegrated**	Result
A 1	6	Pass
A 2	0	Failed
B 1	0	Failed
B 2	0	Failed
C 1	6	Pass
C 2	6	Pass
D 1	6	Pass
D 2	6	Pass
E 1	6	Pass
E 2	6	Pass

*Numbers 1 and 2 following the product's name are for the bottle nu

**The number of capsules disintegrated within 45 minutes in an acet

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Discussion

In order for any clinical trial to be of value in discovering the potential effectiveness or safety of therapeutic agents, the formulation being used in the trial must be well-defined. Unfortunately, many of the published clinical trials on herbal supplements, including those for saw palmetto, fail to report and/or to document the actual content of the formulation used. This lack of reporting the quality control data could overshadow otherwise well-designed studies.[9] Thus, it is of utmost importance that quality control profile for nutritional supplements be identified. In addition, most of the consumers are under the belief that preparations on the market can be interchangeable, which may not necessary be the case. Studies done on multiple dietary supplements (echinacea, ginseng, kava kava, saw palmetto, and St. John's wort) showed a wide variability among the products available on the market to consumers.[10]

Approximately 80% of the free fatty acids in saw palmetto extract are lauric, linoleic, myristic, and oleic acids, with lauric and oleic acids constituting the major portion.[11] The content of these acids in brands (A), (B), (C), (D), and (E) was 85.00%, 87.35%, 79.45%, 86.03%, and 85.24%, respectively. The individual fatty acids were shown to have an inhibitory effect in vitro on 5-alpha-reductase, the enzyme responsible for converting testosterone to dihydrotestosterone (DHT). This enzyme is present in two isoforms (types 1 and 2). Although both isoforms are present in prostate tissues, type 2 is more prevalent in normal tissues, whereas type 1 increases significantly to higher levels in prostate cancer tissues. In addition, the level of type 1 enzymes was highly correlated with the severity of cancer.[12] Type 1 and 2 isoenzymes are also found in the human follicular dermal papilla cells (scalp hair and beard), with type 1 being present in much higher level than type 2.[13] Experimentation in cell culture was shown that this inhibition of both types of the enzyme might be important in the reduction of the growth and progression of prostatic cancer cells.[14] However, others have caution against using type 2 inhibitors after the onset of prostate cancer, suggesting that the inhibitors may increase the severity of the disease.[15] They indicated that a slower-catalytic form of the type 2 enzyme in its irreversible conversion of testosterone to dihydoxytestosterone might be associated with a greater severity for prostate cancer. In other words, the higher the level of testosterone remaining (the lower the DHT level), the higher the severity of prostate cancer.[15] Therefore, although type 2 enzyme inhibitors may be beneficial in reducing the risk for prostate cancer, when given at the onset of prostate cancer, they may increase the severity of the disease. Type 2 enzyme is inhibited by the drug finasteride, while dutasteride inhibits both types (both drugs are used in the treatment of BPH).[16] And both finasteride and dutasteride have been shown to decrease the risk for prostate cancer when taken in their recommended doses.[17,18] In vitro studies have shown that linolenic and oleic acids inhibited primarily type 1 enzyme and to a lesser degree type 2, lauric acid inhibited the activity of both types, and myristic acid was active on type 2 enzyme. Palmitic and stearic acids were found to be inactive on either one of the enzyme types.[11] In vivo work in rats given the herbal extract orally showed that oleic acid component in the extract produced the highest accumulation in prostate tissues.[19] As shown in Table 2, oleic acid was the main component in all the brands tested. Collectively, the results from clinical studies have shown favorable effects of saw palmetto extract on BPH symptoms, with limited mild side effects (GI discomfort).[15,20-23]

An overall comparison of the five brands tested in this study, the (E) product appears to be the best in term of quality; its capsules passed the weight variation test and the disintegration test, and it had the lowest variability among all five brands with respect to their fatty acids content. (However, their content of fatty acids was over two-and-half times that of the labeled amount.) On the other hand, product (B) capsules contained a lower average of total fatty acids than that stated on the label (77% of the labeled claims), both of its bottles failed the disintegration test, and it had the highest variability with regard to its capsules' content of fatty acids. Similar to brand (E) product, product (B) passed the weight variation test, however, its capsules were pricier. The closest brand to its labeled claims with respect to the total average fatty acids was that of the (A) brand. Although product (A) capsules passed the weight variation test, the capsules in bottle 2 did not disintegrate within the test time. The brand (C) bottle 2 failed the weight variation test exceeding the upper limit dictated by the USP. However, similar to capsules from brand (E), it had significantly greater average total fatty acids content than the labeled amount. Brand (D) capsules had about 77% of the total labeled amount in fatty acids (similar to product (B)), however unlike product (B) capsules, its capsules passed the disintegration test and its capsules' cost was the least out of all the five brands tested.

In an Internet search for saw palmetto, consumers can retrieve over three million information sites that will allow them to purchase this herbal supplement or get more information regarding its uses. Consumers who have an interest in herbal supplements will purchase this product where available when deciding to use alternative treatments. Questions that may be asked by a consumer regarding the variation between brands may not be answered because this supplement is not subjected to quality control testing. Not all of the saw palmetto products are the same regarding their fatty acid content, weight, or disintegration properties. Consumers will purchase this product based on their comfort level with herbal supplements, the price, and their exposure to evidence that saw palmetto may improve symptomatic

BPH just as well as conventional treatment.[5] And as was found in this study, higher price for the product does not guarantee purchasing better quality formulation. Pharmacists and other clinicians should be aware of these quality control issues when consulting with their patients concerning saw palmetto extract.

Conclusion

Various brands of saw palmetto were found to have significant differences after testing for content uniformity, weight variation, and disintegration properties. Bottles 1 and 2 from brand (B) and bottle 2 from product (A) were determined to be less than optimal selections due to failed disintegration testing. The (E) brand had optimal formulations when evaluating content uniformity for consistency with the label claim. Overall, product (E) met the requirements for disintegration and weight variation and was the least variable in capsule content. Capsules from product (B) demonstrated the least favorable profile for pharmaceutical quality. Pharmacists should be aware of the pharmaceutical quality differences that might exist among the various brands of saw palmetto capsules available on the market, and consult their patients accordingly.

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