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Lipid Profile and 5α-Reductase Inhibition Activity of Proprietary Ultrahigh-Pressure Supercritical Carbon Dioxide and Hexane Saw Palmetto Extracts

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Abstract: Inhibition of 5α-reductase (5αR), which blocks the conversion of testosterone to its active metabolite, dihydrotestosterone, has been shown to impact further prostate enlargement (benign prostatic hyperplasia, or BPH). Clinical trials of standardized lipidosterolic extracts of *Serenoa repens* (LSESr), also known as standardized extracts of saw palmetto, have demonstrated improvement in lower urinary tract symptoms (LUTS) and delayed progression of BPH. The aim of this preclinical study was to compare two standardized LSESr, a proprietary ultrahigh-pressure supercritical carbon dioxide extract of *S. repens* (UHP-sCESr) and the well-established hexanic extract of *S. repens* (HESr), for both 5αR inhibition activity and lipid profiles. UHP-sCESr and HESr had nearly identical inhibition curves and comparable IC_{50} values for 5αR-1 (9.25 ± 0.87 and 9.86 ± 0.11 μg/mL, respectively; $p = 0.43$) and 5αR-2 (7.47 ± 0.07 and 7.72 ± 0.05 μg/mL, respectively; $p = 0.0544$). UHP-sCESr and HESr also had comparable lipid profiles based on similar total fatty acid levels (87.7% and 91.5%, respectively), weight/weight comparisons of individual fatty acids, and individual fatty acid ratios to lauric acid. In addition, UHP-sCESr meets the standard set by the United States Pharmacopeia (USP) monograph for authenticity and purity for a supercritical carbon dioxide ($SCCO_2$) extract of saw palmetto, whereas HESr meets the standard set by the European Medicines Agency (EMA) for a well-established medicinal product. In conclusion, based on enzyme inhibition curves and IC_{50} values, a standardized lipid profile is important to achieve comparable mechanisms of action for lipidosterolic extracts of saw palmetto. UHP-sCESr offers a comparable, standardized LSESr for men with LUTS/BPH in regions where the proprietary HESr is not available.

Keywords: saw palmetto; lipidosterolic extract of *Serenoa repens* (LSESr); hexanic extract of *Serenoa repens* (HESr); ultrahigh-pressure supercritical carbon dioxide extract of *Serenoa repens* (UHP-sCESr); 5α-reductase inhibitors; benign prostatic hyperplasia; lower urinary tract symptoms; complementary therapies; phytotherapy; dietary supplements; watchful waiting

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1. Introduction

Benign prostatic hyperplasia (BPH) is one of the most prevalent chronic conditions in aging men [1] and the most common cause of lower urinary tract symptoms (LUTS) [2]. Although some men with BPH have no symptoms, as many as 50% will develop bothersome LUTS [3]. These symptoms are categorized as either obstructive voiding symptoms (e.g., weak urine flow, intermittency, straining, and incomplete emptying) or bladder storage symptoms (e.g., urinary frequency, urgency, and nocturia) [4]. The incidence and progression of LUTS increase linearly with age [5], with ~80% of men affected by 70 years of age [6]. Both the incidence and prevalence of LUTS are expected to increase as the population ages [5,7], placing a burden on men who experience a health-related quality of life (HRQOL) due to their symptoms [8]. The impact on global healthcare systems has a cost estimate in the billions of dollars per year [6,9]. The HRQOL impact of LUTS/BPH is

on par with that of asthma [10] and is the primary reason men seek physician or urologist guidance on medical care [11].

The pathogenesis of LUTS/BPH is multifactorial and not completely understood. Increased sympathetic nervous system activity, hormonal alterations, and age-related tissue remodeling are known factors [11]. Androgens play an important role in normal prostate growth and development and also influence the development of BPH [12]. Activation of androgen receptors stimulates numerous pathways in prostate cells, including prostate-specific antigen production, cell growth, and cell survival [13]. Approximately 90% of androgens in the prostate are in the form of dihydrotestosterone (DHT), which is known to be a more potent activator of androgen receptors than testosterone [3]. The 5α-reductase (5αR) enzymes, of which there are three isoforms (types 1, 2, and 3), convert testosterone to DHT [14]. All three isoforms are found throughout the body, including in prostate tissue. As men age, these isoforms are overexpressed in prostate tissue, particularly the expression of 5αR-2 in men with BPH [14]. In addition to an increase in prostate tissue mass (e.g., hyperplasia, hypertrophy), an increase in adrenergically driven smooth muscle tone can lead to an impact on urine flow [11]. Finally, chronic inflammation also appears to play an important role in both the development and progression of BPH and LUTS [2,9,11,15].

Approaches that can be used to manage the care of men with LUTS/BPH depend on symptom severity and HRQOL impact [7,11]. For men with mild-to-moderate symptoms, watchful waiting (i.e., WW, an annual medical evaluation and reassessment of symptom severity but no medical intervention) is generally recommended [7,16,17]. For men with moderate to severe symptoms, bothersome symptoms, or both, medical treatment may be considered [7,16]. When medical treatment is considered, options include medication therapy [16,18], alternative or complementary therapy [17,19], or medication in combination with alternative or complementary therapy [20].

The most commonly used therapeutics for LUTS/BPH are α-adrenergic–receptor antagonists (α-blockers) and 5αR enzyme inhibitors (5αRIs). Each class of medication targets one of the pathways known to be involved in the multifactorial pathophysiology of LUTS [21]. Specifically, α-blockers (e.g., doxazosin, silodosin, tamsulosin) interfere with sympathetic adrenergic-receptor–mediated contraction of the prostatic smooth muscle cells and bladder neck [16]. Of note, α-blockers provide only symptom relief and do not address prostate enlargement [7]. 5αRIs (e.g., finasteride, dutasteride), on the other hand, shrink the prostate and halt further growth by blocking the conversion of testosterone to its active metabolite, DHT [16]. Finasteride has been found to reduce serum DHT levels by 70% to 90% by inhibiting 5αR-2, whereas dutasteride reduces serum DHT levels to nearly 0 by inhibiting both 5αR-1 and 5αR-2 [16]. A large retrospective observational study (n = 7103) found that 38% of patients with LUTS suggestive of BPH were treated with 5αRIs [22]. Both α-blockers and 5αRIs are associated with sexual side effects (e.g., decreased libido, erectile dysfunction, decreased ejaculation, anejaculation, orgasmic dysfunction, and gynecomastia) [16,23].

In many European countries, lipidosterolic extracts of *Serenoa repens* (LSESr), also known as saw palmetto berry oil extracts, are sold as prescription herbal medicines and are frequently prescribed as first-line therapy for patients with mild to moderate LUTS [24]. For example, phytotherapeutic agents represent 20% to 25% of prescriptions for LUTS/BPH in Switzerland, Germany, and France [21]. In a survey of German urologists, half preferred LSESr to medications for the treatment of LUTS/BPH [24]. In a study of 200 physicians in France and Spain, the main drivers for initiating LSESr monotherapy in patients with BPH included patient acceptability, avoidance of sexual side effects, and reduction of inflammation [25].

Of the commercially available LSESr products, the most well studied is the pure hexanic oil extract, that is, the hexanic extract of *S. repens* (HESr) [1,19]. This extract product has been shown in preclinical studies to inhibit the activity of the enzymes 5αR-1 and -2 [26,27]. For more than 40 years (Supplementary Materials), HESr has been shown in clinical studies to be well-tolerated with a low risk of sexual side effects [28]. Because

LSESr have a different mode of action than α-blockers, LSESr may act synergistically with silodosin or tamsulosin to provide better symptom relief than either treatment alone in men with LUTS/BPH [29,30].

Several systematic reviews and meta-analyses have evaluated the effectiveness of LSESr for LUTS/BPH. A Cochrane systematic review published in 2012 concluded that LSESr did not improve LUTS compared with placebo [31]. However, because this review included a number of different LSESr products and doses, the authors noted that their conclusions might not be generalizable to standardized proprietary LSESr products, such as HESr [31]. A 2018 meta-analysis by Fusco et al. reviewed data from studies evaluating urodynamic clinical outcome with several different interventions (e.g., α-blockers [18 studies]; 5αRIs [3 studies]; phosphodiesterase type 5 therapy [1 study]; and LSESr [2 studies]) [32]. Quantitative data synthesis found a significant impact on benign prostate obstruction for α-blockers and 5α-RIs but no conclusive beneficial effect from the LSESr studied [32]; however, only two LSESr studies were considered, and each used a different product and different study design.

A 2018 meta-analysis by Vela-Navarrete et al., however, only considered studies that used the same LSESr product, specifically HESr, at a standard dosage of 320 mg/day as monotherapy [28]. The authors found that HESr improved peak urinary flow rate and decreased nocturia, with relief of LUTS similar to that of tamsulosin and short-term 5αRI regimens. Of the LSESr available in Europe, only the standardized HESr formulation has been designated as a "well-established" medicinal product by the European Medicines Agency (EMA) [33,34]. The standardized HESr formulation is also the only LSESr included in recommendations from the recently updated treatment guidelines for male LUTS by the European Association of Urology [35]. However, the standardized HESr product is not available in some countries, including the United States, where saw palmetto products are regulated as herbal supplements and can vary widely in dose, lipid profile, and even authenticity [36].

A global review of the past 40 years of clinical research on use of LSESr for LUTS/BPH emphasized the importance of using a LSESr with a standardized profile at the clinically effective dose (320 mg/day) to achieve a clinical benefit in symptoms [18,20]. In addition, an international panel of urologists recently developed seven consensus statements based on their evaluation of more than 50 original clinical research studies on LSESr and also noted the need for the saw palmetto extract product to meet a recognized standard to be effective [37].

The objective of this study was to investigate the link between an established mechanism of action of LSESr (i.e., the ability to inhibit 5αR enzyme activity) and the possible importance of the standardized lipid profile. The primary aim of this preclinical study was to compare the 5αR-1 and -2 enzyme inhibition activities of two standardized LSESr products, a proprietary, standardized, high-quality, ultrahigh-pressure supercritical carbon dioxide ($SCCO_2$) extract of *S. repens* (UHP-sCESr) and a well-established, standardized HESr [28]. In addition, comprehensive lipid profiles for both the standardized UHP-sCESr and HESr products were determined and compared.

2. Materials and Methods

2.1. 5αR Enzyme Inhibition Assays

Cell-free 5αR enzyme inhibition assays were performed by VivaCell Biotechnology (Denzlingen, Germany), using methods similar to those previously described by Pais 2010 [38] and Pais et al., 2016 [24]. Separate assays were performed to determine the inhibition of either 5αR isoenzyme 1 or 2.

2.1.1. Sample Preparation for 5αR Inhibition Assays

Two commercial saw palmetto lipidosterolic extract products were tested: USPlus® lot 170710 (a proprietary UHP-sCESr produced by Valensa International, Eustis, FL, USA) and Permixon® lot G06743 (a proprietary HESr produced by Pierre Fabre Medicament,

Castres, France, July 2018 retail purchase, Troistorrents, Switzerland, expiry date July 2020 [note: experiments were performed in 2019, before the expiration date]). Permixon herbal supplement capsules contain approximately 36% saw palmetto oil in a polyethylene glycol (PEG) matrix (molecular weight: 10,000 g/mol). Therefore, the commercial USPlus extract was formulated with PEG to mimic the Permixon product for proper comparison in the enzyme inhibition assays. The PEGylated USPlus lot 170710 (170710-PEG) had a waxy, homogenous appearance, similar to the appearance of the content of Permixon capsules G06743.

The saw palmetto extract in the Permixon capsules and the PEGylated USPlus samples were liberated from the PEG using the following protocol:

- For each variable, the equivalent of three capsules' fill weight was transferred to three reaction tubes, respectively, yielding six reaction vessels
- 600 µL of hexane was added to each reaction tube
- Each sample was mixed using a vortex mixer and sonicated until the solids dissolved
- The samples were then centrifuged for 5 min at 4800× *g*
- The hexane phase of each sample was then transferred to a fresh tube
- The procedure was repeated twice
- The hexane phases for each sample of each were then combined
- The hexane solvent for each sample was then evaporated using a speedvac
- The three samples were pooled using acetone (0.5%) and diluted to a 10 mg/mL stock solution, stored at room temperature

2.1.2. Cell Cultures and Preparation of Cell Homogenates

Human embryonic kidney cells (HEK293) (RRID: Addgene_126445) stably transfected to express either 5αR isoenzyme 1 or 2 (HEK-1 or HEK-2, respectively) [39] were kindly provided by Professor R. W. Hartmann, Pharmaceutical and Medicinal Chemistry, University of the Saarland. The HEK293 cells were cultured in Dulbecco's Modified Eagle Medium (pH 7.4) with 10% fetal calf serum, penicillin/streptomycin (100 U/mL and 100 µg/mL), and 0.5 mg/mL of geneticin in a humidified 5% CO_2 atmosphere at 37 °C.

Cells were harvested, freed from culture medium by centrifugation (500× *g*), and resuspended in a homogenate buffer consisting of 50 mM Tris HCl (pH 7.4), 300 mM sucrose, 0.1 mM ethylenediamine tetra-acetic acid (EDTA), 10 mM dithiothreitol (DTT), and 100 µM phenylmethanesulfonyl fluoride (PMSF). The cells were then solubilized by freezing at −196 °C and thawing on ice. The thawed cells were incubated at 4 °C with 1 mg/mL DNAse in 50 mM magnesium chloride and incubated with vigorous shaking for 30 min. The obtained cell homogenate centrifuged at 20,200× *g* in a refrigerated centrifuge for 50 min at 4 °C. The pellet was resuspended in homogenate buffer and was centrifuged again at 20,200× *g* for 30 min at 4 °C. This procedure was repeated twice. The cell homogenate pellet was detached from the tube bottom with homogenate buffer and resuspended using an Ultra-Turrax homogenizer at the highest speed. Protein concentrations in the resulting cell homogenates were quantified. The fractionated cell suspension was aliquoted and stored at −80 °C.

2.1.3. Preparation of Saw Palmetto Samples and 5αR Enzyme Assays

The saw palmetto samples were diluted using acetone (0.5%), and the positive controls (dutasteride and finasteride, used to show the assays were working properly) were diluted using DMSO (1%). Serial dilutions of the saw palmetto extract samples (50, 10, 5, 1, 0.1, and 0.01 µg/mL) were prepared in duplicate. Serial dilutions of positive controls were also prepared in duplicate. The 5αR-1 assays included finasteride concentrations of 1250 nM and 250 nM and dutasteride concentrations of 50, 10, 5, 1, 0.1, and 0.01 µg/mL. The 5αR-2 assays included finasteride concentrations of 4 nM and 1 nM.

The measurement of 5αR enzyme activity was based on the level of conversion of the substrate androstenedione to the 5α-reduced product 5α-androstanedione in the presence of the test compound compared to levels produced in the presence of the positive control. Cell

homogenates were added to test sample dilutions, positive controls, or untreated controls in Tris HCl-EDTA assay buffer to yield a final volume of 250 µL containing 0.24 mM NADPH, 250 nM androstenedione, and 10 µg cell homogenates. The reaction vessels were then incubated at 37 °C for 15 min, following previously published methodology [24,38]. Enzyme activity was stopped by adding an equal volume (250 µL) of acetone containing the internal standard griseofulvin (0.1 µM). After shaking at 800 rpm for 10 min at room temperature, samples were centrifuged for 5 min at 4800× *g*. The supernatants were then transferred into fresh microcentrifuge tubes. An injection volume of 20 µL for each sample was then subjected to liquid chromatography/mass spectrometry, as described in Pais 2010 [38], to determine levels of androstenedione and 5α-androstanedione.

2.1.4. Data Analysis

5αR inhibition rates were calculated using the data analysis methods previously described [24,38]. For quantification, the ISTD (internal standard) method was applied for correction for the loss of analyte during sample preparation or sample inlet. Results were displayed as a peak area ratio (the area of the analyte peak divided by the area of the internal standard peak). Conversion rates were calculated according to the following formula: conversion (%) = (area ratio androstenedione × 100)/(area ratio androstenedione + area ratio 5α-androstanedione). Inhibition rates, expressed as percent inhibition values relative to untreated controls, were calculated from the mean conversion rates with and without inhibitor. The half-maximal inhibitory concentration (IC_{50}) values were calculated by linear interpolation of the concentrations (conc) of test compounds and the corresponding percentage of inhibition (inh) that brackets 50%: IC_{50} = (50% − low inh %)/(high inh % − low inh %) × (high conc − low conc) + low conc. The in vitro assay results were acceptable if they met a threshold of ≥60% inhibition of 5αR by the positive control. The inhibition curves for each experiment were analyzed using a one-way analysis of variance and the IC_{50} data were compared using Bonferroni's multiple comparisons tests with $\alpha = 0.05$.

2.2. Lipid Profile Assays

For lipid profile analysis, the non-PEGylated USPlus (lot 170710 manufactured by Valensa International, Eustis, FL, USA) and Permixon (lot G06743 manufactured by Pierre Fabre Medicament, Castres, France) were used. The total fatty acid levels of the saw palmetto products were determined in duplicate using the method described in the United States Pharmacopeia (USP) monograph for saw palmetto extract.

2.2.1. Sample Preparation

For each preparation, 100 mg of sample was combined in a pressure-proof vial with 3 mL of 5% sulfuric acid in methanol. Vials were then heated for 2 h at 100 °C and shaken every 15 to 20 min. After cooling to room temperature, 5 mL of a hexane-based nonadecane internal standard solution and 10 mL of a saturated sodium chloride aqueous solution were added. The mixture was shaken for 1 min, and the layers were allowed to separate. From the upper hexane layer, 1 mL was transferred into a gas chromatography (GC) autosampler vial.

2.2.2. Analytical Testing/Sample Analysis

Prepared test samples and methyl ester standard solutions for the individual fatty acids (USP; Rockville, MD, USA) were analyzed in duplicate with GC (PerkinElmer Clarus GC instrumentation with a flame ionization detector; a fused silica capillary column 0.25 × 30 m; 0.25 µm film of USP phase G16 coating). The column was held isothermally for 1 min at 80 °C, then increased at a rate of 20 °C/min to 220 °C, held for 4 min, followed by a 20 °C/min increase to 240 °C for 2 min. Injector temperature was 300 °C, and 1 µL was injected using the split mode (60:1) with helium carrier gas at 2.0 mL/min. The concentration of each fatty acid was calculated from the nonadecane peak response

ratio of each methyl ester in the standard solution relative to the response ratios in the sample solution.

For the HESr product, the fatty acid concentration was interpreted following the USP Monograph for capsules containing saw palmetto extract to account for the weight of inactive material (or inert components) in the capsule fill. Thus, the saw palmetto extract fatty acid concentration in the capsule was determined by multiplying the measured sample fatty acid concentration by the ratio of: (average fill weight of 20 capsules/labeled amount of saw palmetto extract per capsule).

2.2.3. Data Analysis

The percentage of each fatty acid was calculated from the peak response ratio of each methyl ester in the standard solution relative to the sample stock solution. The USP has established a monograph for *S. repens* (saw palmetto) extract that establishes the profile for authenticity, potency, and purity. For authenticity identification, the ratios of the concentration of lauric acid to the concentration of the nine other individual fatty acids were calculated and compared to the USP monograph profile [40].

3. Results

3.1. Inhibition of 5αR Enzyme Activity

UHP-sCESr was found to be a potent, dose-dependent inhibitor of both 5αR-1 and 5αR-2, equivalent to the 5αR inhibition activity of HESr.

3.1.1. Inhibition of 5αR-1

UHP-sCESr and HESr had nearly identical 5αR inhibition curves and comparable IC_{50} values for 5αR-1 (9.25 ± 0.87 and 9.86 ± 0.11 µg/mL, respectively; $p = 0.43$) (Figure 1).

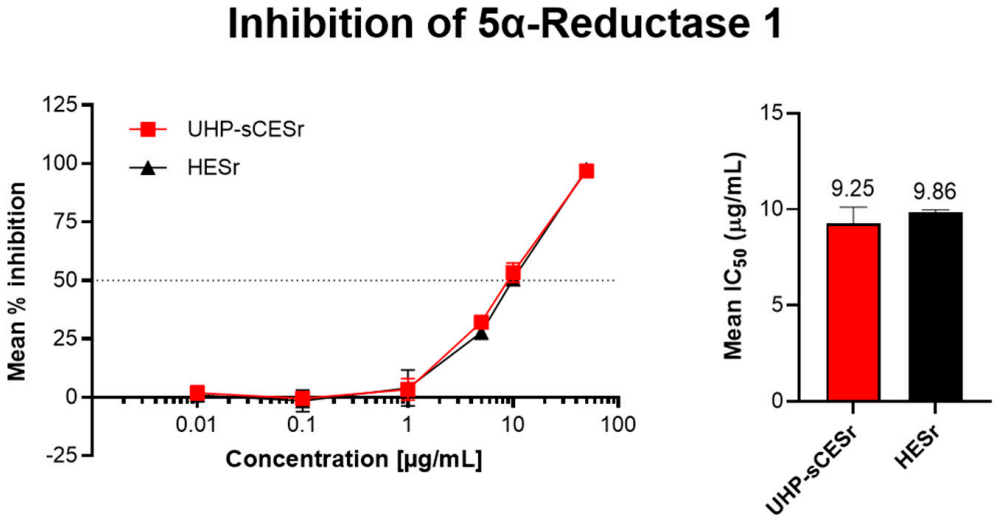

Figure 1. Inhibition of 5αR-1 by an ultrahigh-pressure supercritical carbon dioxide extract of *Serenoa repens* (UHP-sCESr; lot 170710-PEG) and hexanic extract of *S. repens* (HESr; lot G06743). For the mean IC_{50} values, error bars represent the standard error.

3.1.2. Inhibition of 5αR-2

UHP-sCESr also had similar inhibition curves and IC_{50} values as HESr for 5αR-2 (7.47 ± 0.07 and 7.72 ± 0.05 µg/mL, respectively; $p = 0.0544$) (Figure 2).

3.2. Analysis of Lipid Profiles

The composition of UHP-sCESr was found to be comparable to that of HESr based on similarities in total fatty acid content (HESr [lot G06743], 91.5%; UHP-sCESr [lot 170710], 87.9%) and equivalent weight/weight comparisons of individual fatty acids (Figure 3). The

HESr and UHP-sCESr lipid profiles from those individual lots were then compared with the typical lipid profile of UHP-sCESr (UHP-sCESr-T) determined using averages from GC data gathered over a consecutive 3-year period (n = 81) and the typical lipid profile of HESr (HESr-T) averaged from five different lots of commercial product (lot G06743 and four lots obtained subsequently: G06959, G06963, G06968, and G06970). The typical total fatty acid content for UHP-sCESr during the consecutive 3-year period (UHP-sCESr-T; 90.1%) and the typical total fatty acid content for HESr from the five lots tested (HESr-T; 89.8%) were comparable, as were the weight/weight comparisons of individual fatty acids for the two LSESr products (Figure 3).

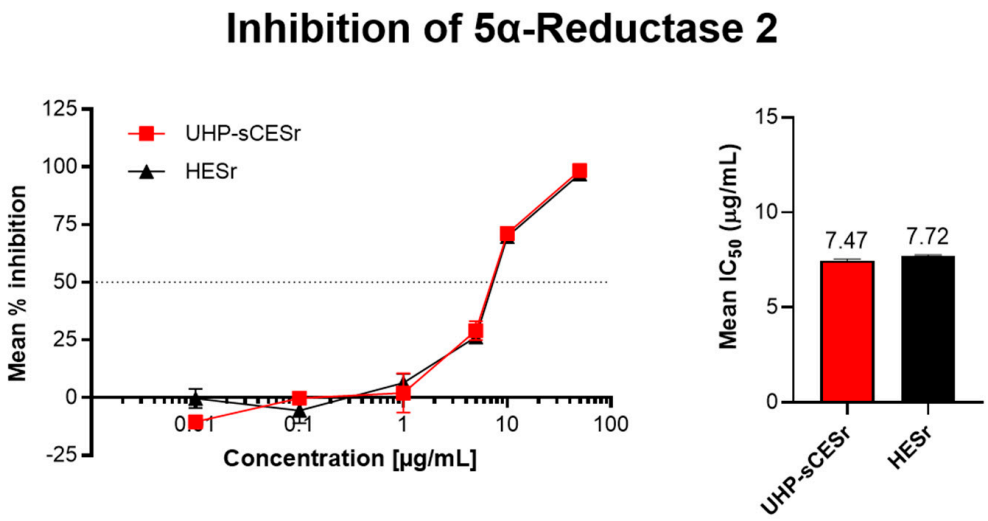

Figure 2. Inhibition of 5αR-2 by an ultrahigh-pressure supercritical carbon dioxide extract of *Serenoa repens* (UHP-sCESr; lot 170710-PEG) and hexanic extract of *S. repens* (HESr; lot G06743). For the mean IC_{50} values, error bars represent the standard error.

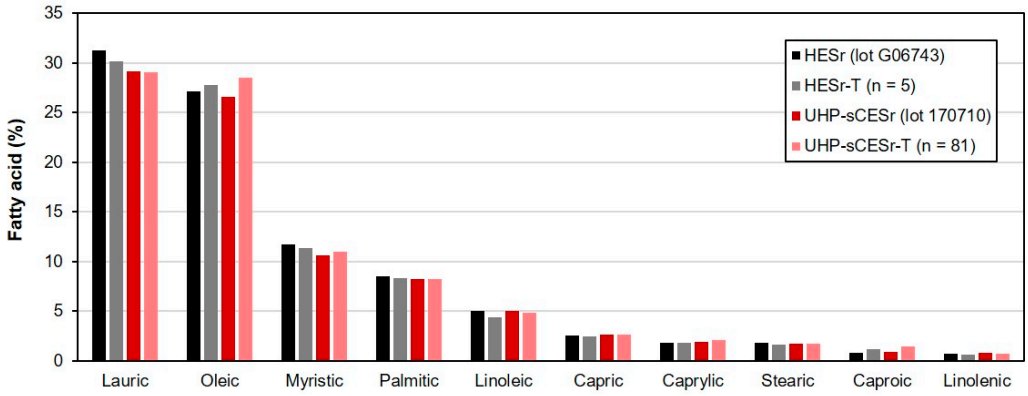

Figure 3. Individual fatty acid content (*w*/*w*) for the hexanic extract of *Serenoa repens* (HESr; lot G06743), HESr typical data (HESr-T; n = 5), an ultrahigh-pressure supercritical carbon dioxide extract of *S. repens* (UHP-sCESr; lot 170710), and UHP-sCESr typical data (UHP-sCESr-T; n = 81).

The lauric acid ratios (lauric acid %: individual fatty acid %) for each fatty acid were also similar for HESr (lot G06743), HESr-T (n = 5), UHP-sCESr (lot 170710), and UHP-sCESr-T (n = 81) (Table 1).

Of note, UHP-sCESr (lot 170710) and UHP-sCESr-T (n = 81) typical data were also within the specifications for total fatty acid content and lauric acid ratios for each individual fatty acid as defined in the USP monograph for $SCCO_2$ [40].

Table 1. Lauric acid ratios for individual fatty acids in HESr, HESr-T, UHP-sCESr, and UHP-sCESr-T.

	Ratio of Lauric Acid (%) to Individual Fatty Acids (%)			
Fatty Acid	**HESr (lot G06743)**	**HESr-T (n = 5)**	**UHP-sCESr (lot 170710)**	**UHP-sCESr-T (n = 81)**
Oleic	1.2	1.1	1.1	1.0
Myristic	2.7	2.7	2.8	2.6
Palmitic	3.7	3.6	3.6	3.5
Linoleic	6.2	6.9	5.9	6.1
Capric	12.3	12.5	11.4	11.3
Caprylic	17.6	16.5	15.4	14.4
Stearic	17.7	18.2	17.4	17.5
Caproic	37.8	27.7	31.4	23.4
Linolenic	42.5	50.8	34.8	43.4

HESr, hexanic extract of *S. repens*; HESr-T, HESr typical data; %, percent; UHP-sCESr, ultrahigh-pressure supercritical carbon dioxide extract of *S. repens*; UHP-sCESr-T, UHP-sCESr typical data.

4. Discussion

Standardized LSESr have been found to influence prostate and urinary tract health by several important mechanisms of action. Specifically, LSESr inhibits 5αR-1 and 5αR-2. Inhibition of 5αR, which blocks the conversion of testosterone to its more active metabolite, DHT, has been shown to delay further prostate growth in men with LUTS/BPH [7]. LSESr have also been found to inhibit cyclooxygenase and lipoxygenase, induce apoptosis of prostate epithelial cells, exhibit antiestrogenic activity, and exhibit spasmolytic effects via calcium channels blockade and β-adrenergic antagonism [19].

Scaglione et al. reported the 5αR inhibition activity for seven different LSESr in one study [26] and 10 different LSESr in another [27]. The hexanic extract product used in these experiments and analyzed for chemical composition was used in both papers authored by Scaglione and colleagues. The EC_{50} values from these earlier publications showed that HESr is a significantly more potent inhibitor of 5αR-1 and 5αR-2 than the other commercial LSESr tested [27]. However, in the present study, UHP-sCESr exhibited nearly identical inhibition curves and comparable IC_{50} values to HESr in experiments with both 5αR isoforms.

In a comprehensive 3-part global review published in 2021, US urologist Dr. Stephen Strum reviewed the past four decades of clinical literature on saw palmetto and identified 58 high-quality clinical trials that form the evaluable body of evidence for LSESr monotherapy in patients with LUTS/BPH [18]. These evaluable clinical trials were ≥2 months in duration, had data for ≥20 patients at end of the study, and assessed the clinically established dose (320 mg/day) of a standardized LSESr product as monotherapy. More than half of the studies were non-English-language publications, many of which had never been considered in previous English-language meta-analyses or reviews on the clinical evidence of standardized LSESr products. Dr. Strum's comprehensive review concluded that monotherapy with a standardized LSESr at a dose of 320 mg/day in men with LUTS/BPH was associated with a rapid onset of response (as early as 4 weeks), a durable response, and delayed progression of symptoms [18]. Dr. Strum noted that the proprietary hexanic extract product was the most widely studied standardized LSESr product represented in the global literature, and further noted that inhibition of 5α-reductase was one of the mechanisms by which LSESr exerted an effect on the prostate and addressed LUTS [18]. Within the global body of literature, 46 clinical trials have evaluated HESr monotherapy in nearly 4900 patients for durations of up to 5 years (see Supplementary Materials).

Recently, an international panel of urologists reviewed the 58 high-quality clinical trials identified in the Strum literature review and determined that "the current evidence links the fatty acid fingerprint of LSESr to increased effectiveness in LUTS". In particular, the

experts identified the proprietary HESr as a standardized extract with a composition that meets USP standards and consistently demonstrates the ability to address LUTS. The panel concluded that any extract with a similar fingerprint to that found in HESr and aligned with the USP monograph standards is likely to be clinically effective [37]. While no comparative LSESr clinical trials have been published, the important role of a standardized lipid profile in clinical effectiveness would appear to be important [20]. While the proprietary HESr is an herbal medicine product available mostly in Europe, this product is not commercially available in North America [37]. Habib and Wyllie [41] noted the tremendous variability in the composition of marketed saw palmetto supplement products. This is specifically true in the United States, where supplement products for LUTS/BPH vary widely in lipid profile and potency and may not even be authentic [36]. Our study indicates that the proprietary UHP-sCESr is comparable to the proprietary HESr in both 5αR inhibitory activity and lipid profile. UHP-sCESr is available in North America (and elsewhere) [37], providing a standardized LSESr for men where the proprietary HESr is not available. In addition, these preclinical data could support future clinical investigations of the UHP-sCESr in men with LUTS/BPH.

Standardized lipid extracts of *Serenoa* have been reported to be composed of ~90% free and esterified fatty acids, 6.8% glycerides, and 2.3% unsaponifiable matter (including approximately 0.34% phytosterols) [12]. The therapeutic effect of LSESr is attributed to the entire phytocomplex rather than a single component [12]. Lipid profile testing can confirm the presence of the "bioactive fingerprint" of a high-quality LSESr product. Established standards for authenticity, potency, and purity are included in the USP monograph, which has established defined ratios of key fatty acids compared with lauric acid for different extraction methods (hexane, $SCCO_2$, and ethanol).

Several factors influence the ultimate composition and quality of LSESr. The maturity of the saw palmetto berry determines the fatty acid profile and content of total and free fatty acids [42], similar to the related berry from *Roystonea regia* or the royal palm [43]. The method of extraction and the extraction solvent used can also influence the lipid profile [12]. Unlike other manufacturers that may use ethanol or lower pressure and temperature parameters for $SCCO_2$ extraction, the proprietary ultrahigh-pressure $SCCO_2$ process used to produce the standardized UHP-sCESr tested in this study yields a chemical profile and enzyme inhibition activity comparable to that obtained with hexane extraction but does not require organic solvents.

Analytical testing of the lipid profile of the proprietary UHP-sCESr product found it to be comparable to the HESr product in terms of total fatty acid content, weight/weight comparisons of individual fatty acids, and ratios of individual fatty acids to lauric acid. In addition, the typical lipid profiles of UHP-sCESr, averaged from 81 production lots collected consecutively over 3 years, and the typical lipid profiles of HESr, averaged from testing five different commercial lots of product, were comparable to the UHP-sCESr product used in these experiments and to lots produced over a period of time using the proprietary extraction process.

5. Conclusions

A high-quality, standardized LSESr produced using a proprietary ultrahigh-pressure $SCCO_2$ process was shown to contain a lipid profile, including total fatty acids, individual fatty acids, and lauric acid ratios, nearly identical to that of a proprietary hexanic LSESr designated as a well-established medicinal product by the EMA [33,34]. The lipid profile of UHP-sCESr also met the specifications defined by the USP monograph for $SCCO_2$ extracts. Extracts with lipid profiles similar to HESr that also meet USP monograph standards are more likely to be clinically effective [37]. Finally, the proprietary UHP-sCESr was shown to be a strong inhibitor of both 5αR-1 and 5αR-2 enzymes, with 5αR inhibition activity equivalent to that of HESr based on nearly identical inhibition curves and comparable IC_{50} values for both the 5αR-1 and 5αR-2 isoforms. A LSESr such as UHP-sCESr with a

standardized lipid profile that provides the same magnitude of 5αR enzyme inhibition as the proprietary HESr may be effective for men with LUTS/BPH.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/uro3010005/s1, HSESr Monotherapy Clinical Trials (in chronological order), including references [44–91].

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