

Comparison of the potency of 10 different brands of *Serenoa repens* extracts

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Abstract. – **Background:** The extract of *Serenoa repens* is the phytopharmaceutical product most often used for the treatment of urological symptoms associated with benign prostatic hyperplasia (BPH). Several extracts are commercially available but extraction processes vary between manufacturers and thus not all these products are equivalent in terms of active ingredient content and composition of preparations.

Aim: As there is a paucity of comparative studies, we compared the activity of different extracts of *Serenoa repens* widely available on the world market.

Materials and Methods: Beltrax Uno®, Permicsaps®, Permixon®, Prostadyn®, Prostagutt®, Prostamen®, ProstaX®, Urocaps® and Urogutt® were assayed for 5- α -reductase activity on 10 day fibroblasts and epithelial cells co-cultures. Human fibroblast growth factor (hFGF)-induced-proliferation inhibition was also assayed.

Results: As to extract activity, differences were observed between the tested extracts, but all were able to inhibit 5- α -reductase types I and II isoenzymes (5 α R-I and 5 α R-II) as well as fibroblast proliferation.

Conclusions: Extract potency differs between products and so does proliferation inhibition potency. Quantitative and qualitative variations in the active ingredient are likely to account for these differences.

Key Words:

Serenoa repens, Benign prostatic hyperplasia, 5 α -reductase, Fibroblasts.

Introduction

About one third of men with benign prostatic hyperplasia (BPH) who choose non-surgical options will resort to phytotherapeutical medication alone or in combination with traditional pharmacotherapy. One widely used phytoceutical is the extract from the drupes of the American Saw palmetto (*Serenoa repens*, but the taxon is often la-

belled *Sabal serrulatum*, actually a trademark¹ and a different genus of the *Arecaceae* family)^{2,3}. However, there are several saw palmetto extracts available and all differ qualitatively because of varying proprietary extraction process and quantitatively because of variations in the active ingredient content and the composition of preparations. Variability between clinical studies using heterogeneous methodologies and the relative paucity of comparative studies also explain why biological medicine cannot substantiate the equivalence of all *Serenoa* preparations. Also because the composition of proprietary preparation may not be exactly known, it is conceivable that clinical efficacy may also vary between brands. We, therefore, carried out a comparative study to assess the different pharmacological activity of various commercial *Serenoa repens* extracts available on a worldwide basis. In particular, we mounted assays designed to compare the ability of extracts to inhibit the two 5 α -reductase-1 (5 α R-I) and 5 α -reductase 2 (5 α R-II) isoenzymes in co-cultured human prostate fibroblasts and epithelial cells as well as the anti-proliferative effect of extracts on fibroblasts.

Materials and Methods

Extracts

The preparations we procured were Beltrax Uno® (Beliarda, Argentina), Permicsaps®, (Bago, Argentina) Permixon® (Pierre-Fabre, France), Prostadyn® (Dr. Dunner, China), Prostagutt® (Schwabe Pharma, Russia), Prostamen® (Ancalmo, Panama), ProstaX® (Interfarma Corporation, Panama), Urocaps® (Division Fitoterapeutica, Mexico) and Urogutt® (Farmasa Schwabe, Thailand). For the sake of comparison, the solid-state active ingredient was extracted by evaporation with hexane. This solid was dissolved in 10 mL

ethanol to yield a concentration of 10 mg/mL. The stock solution obtained was further diluted in appropriate media to provide a working solution of 1 mg/mL.

Co-culture Cell Model

Human prostate tissue was obtained after informed consent from men undergoing transurethral resection of the prostate for BPH. Primary fibroblast and epithelial cultures were established as previously described⁴⁻⁶. Briefly, fragments 1 to 3 mm in diameter were digested with 150 U/mL collagenase and 150 U/mL hyaluronidase for 18 to 20 hours at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 5 percent foetal bovine serum, 50 IU/mL penicillin, 50 µg/mL streptomycin and 2 mM L-glutamine. Cells were isolated on a Ficoll gradient and the presence of epithelial cells and/or fibroblasts was confirmed by phase-contrast microscopy and immunocytochemical staining. AE1/AE3 epithelial cell markers (Dako A/S, Milan Italy) and TE-7 fibroblast markers (Chemicon Europe, Ltd Hampshire, UK) were used. This *in-vitro* co-culture model of fibroblasts and epithelial cells expresses many of the phenotypical characteristics of the prostate, including both 5αR-I and 5αR-II⁴. From the primary cultured cells, fibroblast and epithelial cell co-cultures were prepared (as described in reference⁷) by fitting the cell culture plates with a microporous membrane to separate the two cell populations while maintaining cell communication by means of diffusible factors. Co-cultures were maintained in a mixed medium of DMEM and RPMI-1640 (1:1, v/v) containing 10% foetal calf serum (FCS) at 37°C in 5% CO₂.

5α-reductase Activity Assay

As per protocol, to compare the efficacy of the various *Serenoa* extracts in the inhibition of 5αR-I and 5αR-II, prostate fibroblasts and epithelial cells were equally mixed and assayed as described at 10 day co-culture for 5α-reductase activity, as in references 7 and 8. In brief, cells were harvested by trypsinization, centrifuged and the pellet re-suspended in RPMI-1640 supplemented with 10% FCS and counted. Cells were re-suspended in 4 mmol/mL sodium phosphate buffer either at pH 7.5 (5αR-I) or pH 5.5 (5αR-II).

Cell suspensions were added to glass tubes containing [³H]testosterone ([1,2,6,7-³H]testosterone, specific activity 10⁵ Ci/mmol, 8 × 10⁶ dpm/dish, 20 mmol/L; 1 µCi, from Amersham

International, Cardiff, UK), as substrate, a NADPH-regenerating system and different concentrations of each extract. The tubes were next incubated at 37°C for 30 minutes in a shaking water bath. The reaction was stopped by immersion into liquid nitrogen.

Samples were evaporated to dryness in a vacuum oven at 40°C and the residues re-suspended in ethanol. 5αR-I and 5αR-II isoenzyme activities were determined by measuring the conversion of [³H]-testosterone to dihydrotestosterone (DHT) – as described elsewhere^{7,8}.

Results of isoenzyme activity are expressed as percent control. The conversion of 1 µM [³H]-testosterone in the absence of inhibitors is defined as 100 percent activity (0.8-1.5 nmol DHT/10⁶ cell/min for 5αR-I; 30-35 nmol DHT/10⁶ cell/min for 5αR-II). Dose-effect response curves were analysed using a sigmoid maximum effect (E_{max}) model with a variable slope (GraphPad Software, Inc. La Jolla, CA, USA). Inhibitory potency was assessed by determining the half maximal inhibitory concentration (IC₅₀).

Proliferation Assay

In this experiments was evaluated the potency of various extract to inhibit the proliferative effect of human Fibroblast Growth Factor (hFGF basic) that induces a considerable increase in proliferation of human prostatic fibroblasts.

Fibroblasts (20 000 cells per well) were plated on 96-well cell culture plates and cultured in the presence or absence of basic FGF (0.5 ng/mL). One, 10 and 30 ng/mL of each *Serenoa* extract were added after 24 hours. Extracts had been initially solubilised in dimethyl sulfoxide (DMSO) and further diluted in minimum essential medium. Appropriate controls to which equivalent dilutions of DMSO were added showed no interference with cell proliferation. The relative increase in cellular proliferative activity against a bovine serum albumin (BSA)-treated negative control sample was calculated using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion in an MTT cell proliferation assay (Sigma Chemical Co., St Louis, MO, USA). Cultures were stopped after 72 hours and each assay was run in triplicate.

Statistical Analysis

Non-linear regression curves were compared by ANOVA tested by Bonferroni's adjustment in post-hoc comparisons.

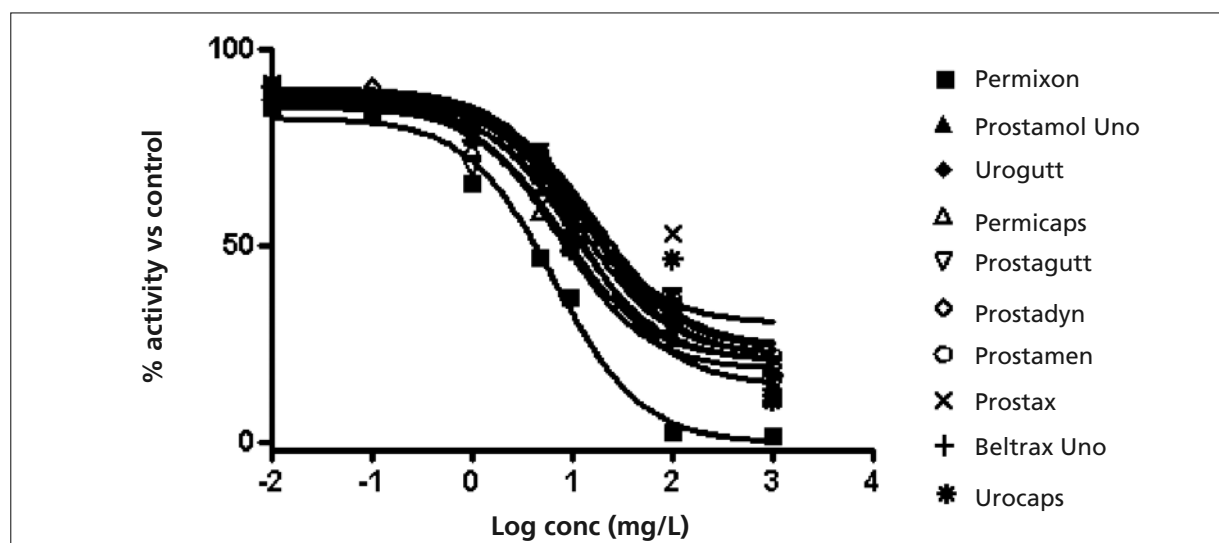


Figure 1. Inhibition of 5 α -reductase type I. Enzyme activity is expressed as a percent of the control. (The conversion of 1 μ M of testosterone in absence of inhibitors is defined of 100% activity).

Results

5 α -Reductase Activity Assays

All *Serenoa* extracts inhibit 5 α -reductase isoenzymes in co-cultures of prostate epithelial cells as well as fibroblasts but their inhibitory activity (IC_{50}) differs widely.

Figure 1 plots the activity (versus control) of the extracts on 5 α R-I concentrations and Table I summarises the dose-response curve for agonist assay (IC_{50}) of the various brands of *Serenoa* extracts (with unadjusted comparisons).

Permixon[®] (ic_{50} = 6.717) shows the highest efficacy on 5 α -reductase type I and is statistically different compared to the others extracts. However, there is a different weight in statistical differences.

Permixon[®] is statistically superior versus Permicsaps[®] (ic_{50} = 8.063) and Prostagutt[®] (ic_{50} = 7.737) with $p < 0.05$; versus Prostamol uno[®] (ic_{50} = 10.24) and Urogutt[®] (ic_{50} = 13.51) with $p < 0.01$; versus Prostadyn[®] (ic_{50} = 13.86), Prostamen[®] (ic_{50} = 15.30), Prostax[®] (ic_{50} = 10.71), Beltrax uno[®] (ic_{50} = 18.58) and Urocaps[®] (ic_{50} = 12.16) with $p < 0.001$.

Figure 2 reports the 5 α R-II activities of *Serenoa* extracts and Table II their IC_{50} concentrations. Also against this enzyme, the inhibitory activity of Permixon[®] yields a lowest IC_{50} with a value of 3.994 and outperforms Urocaps[®] in this ability by five orders of magnitude, Beltrax Uno[®], Prostamol Uno[®] and Urogutt[®] by four, Prostadyn[®] by almost four, Prostamen[®] by three and Permi-

caps[®], Prostagutt[®] and ProstaX[®] by two. The pairwise comparisons with Permixon[®] reach less than 0.001 significance levels in all cases except Urogutt[®] ($p < 0.05$), Permicsaps[®] ($p < 0.05$), and Prostagutt[®] ($p < 0.01$), though without losing statistical superiority ($0.05 > p < 0.01$).

Proliferation Assay

Figure 3 shows the effect of three-day *in-vitro* treatment with the various extracts on proliferation of fibroblasts against pooled controls and on hFGF induced proliferation *vs.* a negative control (with bovine serum albumin). None of the extracts tested were able to influence the

Table I. EC_{50} of various extracts of *Serenoa repens* on 5 α reductase I compared to Permixon.

Sigmoidal dose-response	EC_{50}	p value vs permixon
Permixon [®]	6.717	
Prostamol Uno [®]	10.24	$p < 0.01$
Urogutt [®]	13.51	$p < 0.01$
Permicsaps [®]	8.063	$p < 0.05$
Prostagutt [®]	7.737	$p > 0.05$
Prostadyn [®]	13.86	$p < 0.001$
Prostamen [®]	15.30	$p < 0.001$
Prostax [®]	10.71	$p < 0.001$
Beltrax Uno [®]	18.58	$p < 0.001$
Urocaps [®]	12.16	$p < 0.001$

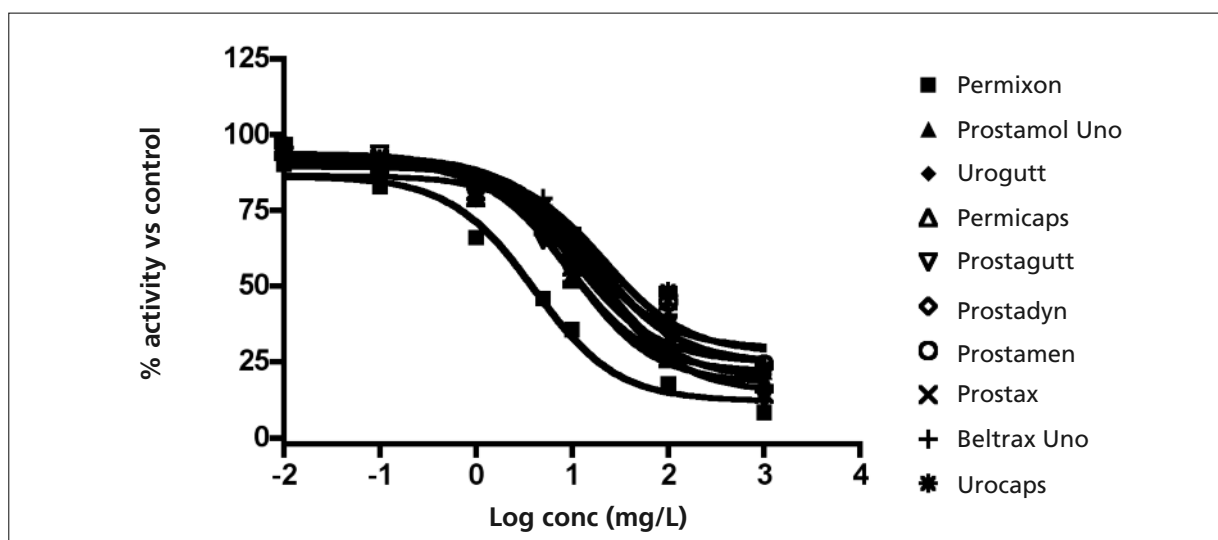


Figure 2. Inhibition of 5 α -reductase type II (for details see Figure 1).

Table II. EC₅₀ of various extracts of *Serenoa repens* on 5-reductase II compared to Permixon.

Sigmoidal dose-response	EC ₅₀	<i>p</i> value vs permixon
Permixon®	3.994	
Prostamol Uno®	17.37	< 0.001
Urogutt®	19.51	< 0.05
Permicaps®	9.888	< 0.05
Prostagutt®	8.237	< 0.01
Prostadyn®	15.52	< 0.001
Prostamen®	13.49	< 0.001
Prostax®	10.41	< 0.001
Beltrax Uno®	17.61	< 0.001
Urocaps®	20.96	< 0.001

basal proliferation of prostate fibroblasts within the 72-hour range of the experiments. While the addition of hFGF increases the proliferation of fibroblasts by about 87%, there is no difference with the negative control-treated culture (Figure 3). All extracts inhibit the proliferation induced by hFGF but Permixon® supplementation highly and significantly correlates ($r^2 = 0.85$; $p < 0.05$) with proliferation control, in contrast to all other extracts (Figure 4). The adjusted post-hoc comparisons show highly significant ($p < 0.01$) proliferation differences with Prostadyn®, Urocaps® and Urogutt® and statistical significance is maintained in comparisons with other extracts (Table III).

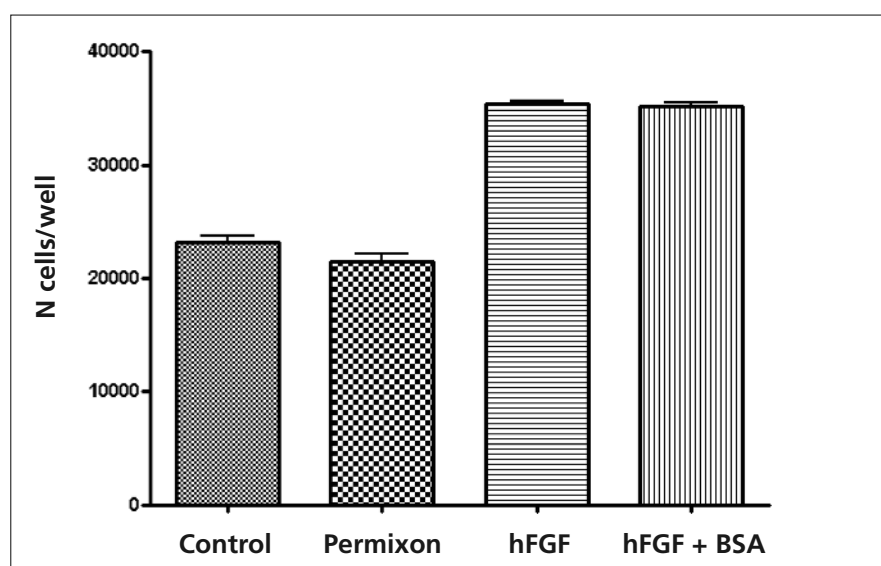


Figure 3. Fibroblasts proliferation in presence of Permixon, hFGF and hFGF + BSA.

Table III. Statistical analysis of proliferation.

Bonferroni's multiple comparison test	<i>t</i>	<i>p</i> value
Permixon vs Prostamol Uno	4.872	< 0.01
Permixon vs Urogutt	4.854	< 0.01
Permixon vs Permicaps	4.038	< 0.05
Permixon vs Prostagutt	3.974	< 0.05
Permixon vs Prostadyn	5.285	< 0.01
Permixon vs Prostamen	4.504	< 0.05
Permixon vs Prostax	4.321	< 0.05
Permixon vs Beltrax Uno	4.398	< 0.05
Permixon vs Urocaps	4.639	< 0.01

Discussion

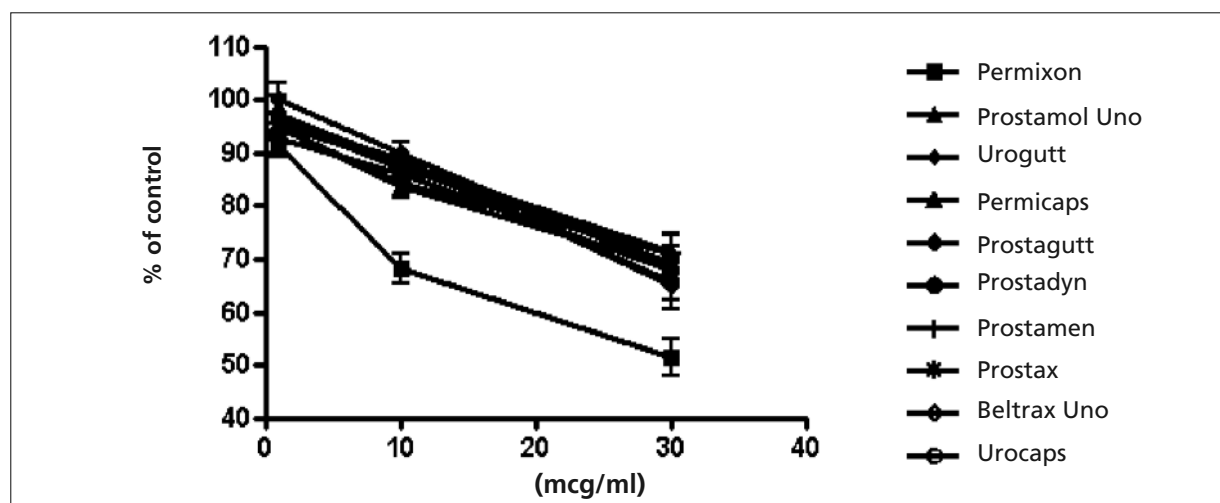
In this study we demonstrated that various extract of *Serenoa repens* purchased in various countries show different intrinsic activity *in vitro*, even if all show activity.

In 2008, our group studied different brands of *Serenoa repens* extracts, purchased in Italy, for their comparative potency and the present study falls in line with our then conclusion that each preparation should be individually tested for bioactivity beyond clinical efficacy⁹ Earlier still, a pioneering study by Habib and Wyllie¹⁰ had drawn attention to the importance of variations among brands resulting in a discrepancy between actual and stated dose of *Serenoa repens* across the spectrum of commercially available preparations, due to different product formulations, plant sources, extraction process and content in the active ingredient and bioactive adju-

vants¹⁰. In this context, the present study completes the earlier investigation by focusing on the potency of 10 lipidosterolic extracts of *Serenoa repens* on the inhibition of 5 α R-I and 5 α R-II isoenzymes and on the inhibition of the proliferation of human prostatic fibroblasts after the induction with hFGF. We have demonstrated that the brands assayed are significantly different. The possible explanation of this to *t* variance in effective concentrations may be attribute to the different composition in the active components¹¹.

One limitation of this study is in its nature. Our work has been performed to assess comparative potency but not directly address clinical efficacy or safety. All products analyzed are recommended at 300-320 mg per day in spite of their different potency. Theoretically, we do not know if the clinical efficacy of the tested extracts is different because the exact dose of the *Serenoa* extract has not been established. However, many trials extensively reviewed¹², using mainly Permixon®, have demonstrated mild to moderate improvement in urinary symptoms and in flow measures in men with Benign Prostatic Hyperplasia. Compared to finasteride¹³ and tamsulosin¹⁴, Permixon® has produced similar improvements in urinary symptoms and flow measure, with fewer Adverse Drug Reactions.

Further comparative studies are needed to assess the bioequivalence of other bioactive compounds (e.g., *Pygeum africanum* or *Urtica dioica*)¹⁵ which may affect the effectiveness of *Serenoa repens* in mixed-type preparations¹⁶.

**Figure 4.** Inhibition of fibroblasts proliferation induced by hFGF of the different extracts of *Serenoa repens*.

Acknowledgements

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References

- 1) <http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?103108>.
- 2) <http://www.biomedicine.com/itemDetail.cfm?id=98>
- 3) ZONA S. The genera of Palmae (Arecaceae) in the southeastern United States. *Harvard Pap Bot* 1997; 2: 71-107.
- 4) BAYNE CW, DONNELLY F, CHAPMAN K, BOLLINA P, BUCK C, HABIB F. A novel coculture model for benign prostatic hyperplasia expressing both isoforms of 5alpha-reductase. *J Clin Endocrinol Metab* 1998; 83: 206-213.
- 5) TSUGAYA M, HABIB FK, CHISHOLM GD, ROSS M, TOZAWA K, HAYASHI Y, KOHRI K, TANAKA S. Testosterone metabolism in primary cultures of epithelial cells and stroma from benign prostatic hyperplasia. *Urol Res* 1996; 24: 265-271.
- 6) HABIB FK. Regulation of prostate growth in culture with the pollen extract cernitin T-60 and the impact of the drug on the EGF tissue profiles. In: Vahlensick W, Rutishauser G, eds. *Benign Prostate Diseases*, pp. 120-128. Thieme Verlag, Stuttgart & New York, 1992.
- 7) BAYNE CW, DONNELLY F, ROSS M, HABIB FK. *Serenoa repens* (Permixon®): a 5alpha-reductase types I and II inhibitor – new evidence in a co-culture model of BPH. *Prostate* 1999; 40: 232-241.
- 8) SMITH CM, BALLARD SA, WORMAN N, BUETTNER R, MASTERS JR. 5Alpha-reductase expression by prostate cancer cell lines and benign prostatic hyperplasia *in vitro*. *J Clin Endocrinol Metab* 1996; 81: 1361-1366.
- 9) SCAGLIONE F, LUCINI V, PANNACCI M, CARONNO A, LEONE C. Comparison of the potency of different brands of *Serenoa repens* extract on 5alpha-reductase types I and II in prostatic co-cultured epithelial and fibroblast cells. *Pharmacol* 2008; 82: 270-275.
- 10) HABIB FK, WYLLIE MG. Not all brands are created equal: a comparison of selected components of different brands of *Serenoa repens* extract. *Prostate Cancer Prostatic Dis* 2004; 7: 195-200.
- 11) RAYNAUD JP, COUSSE H, MARTIN PM. Inhibition of type 1 and type 2 5 alpha-reductase activity by free fatty acids, active ingredients of Permixon®. *J Steroid Biochem Mol Biol* 2002; 82: 233-239.
- 12) WILT T, ISHANI A, MAC DONALD R. *Serenoa repens* for benign prostatic hyperplasia. *Cochrane Database Syst Rev* 2002; (3): CD001423.
- 13) CARRARO JC, RAYNAUD JP, KOCH G, CHISHOLM GD, DI SILVERIO F, TEILLAC P, DA SILVA FC, CAUQUIL J, CHOPIN DK, HAMDY FC, HANUS M, HAURI D, KALINTERIS A, MARENCAK J, PÉRIER A, PERRIN P. Comparison of phytotherapy (Permixon) with finasteride in the treatment of benign prostate hyperplasia: a randomized international study of 1098 patients. *Prostate* 1996; 29: 231-240.
- 14) DEBRUYNE F, KOCH G, BOYLE P, DA SILVA FC, GILLENWATER JG, HAMDY FC, PERRIN P, TEILLAC P, VELA-NAVARRETE R, RAYNAUD JP. Comparison of a phytotherapeutic agent (Permixon) with an alpha-blocker (tamsulosin) in the treatment of benign prostatic hyperplasia: a 1-year randomized international study. *Eur Urol* 2002; 41: 497-507.
- 15) LOEW D, KASZKIN M. Approaching the problem of bioequivalence of herbal medicinal products. *Phytother Res* 2002; 16: 705-711.
- 16) WILT T, ISHANI A. Pygeum africanum for benign prostatic hyperplasia. *Cochrane Database System Rev* 2002; (1): CD001044.