Cancer Investigation, 24:588–592, 2006
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.1080/07357900600894815

informa
healthcare

ORIGINAL ARTICLE

Tocopherols and Saponins Derived from *Argania spinosa* Exert, an Antiproliferative Effect on Human Prostate Cancer

A. Drissi, Ph.D.,[1] H. Bennani, Ph.D.,[1] F. Giton, M.Sc.,[2] Z. Charrouf, Ph.D.,[3] J. Fiet, M.D.,[2] and **A. Adlouni, Ph.D.[1]**

Laboratoire de Recherche sur les Lipoprotéines, Faculté des Sciences Ben M'sik, Casablanca, Morocco.[1]
Equipe de Recherche Emi INSERM 03-37, (Professeur Dominique Chopin), Centre de Recherches Chirurgicales, CHU Henri Mondor, Faculté de Médecine, Créteil, France.[2]
Département de Chimie, Faculté des Sciences, Université Mohammed V, Rabat, Morocco.[3]

ABSTRACT

The aim of our study is to evaluate the antiproliferative effect of tocopherols obtained from alimentary virgin argan oil extracted from the endemic argan tree of Morocco and of saponins extracted from argan press cake on three human prostatic cell lines (DU145, LNCaP, and PC3). The results were compared to 2-methoxyestradiol as antiproliferative drug candidates. Cytotoxicity and antiproliferative effects were investigated after cells' treatment with tocopherols and saponins compared to 2-Methoxyoestradiol as the positive control. Tocopherols and saponins extracted from argan tree and 2-methoxyestradiol exhibit a dose-response cytotoxic effect and an antiproliferative action on the tested cell lines. The best antiproliferative effect of tocopherols is obtained with DU145 and LNCaP cell lines (28 μg/ml and 32 μg/ml, respectively, as GI_{50}). The saponins fraction displayed the best antiproliferative effect on the PC3 cell line with 18 μg/ml as GI_{50}. Our results confirm the antiproliferative effect of 2-methoxyestradiol and show for the first time the antiproliferative effect of tocopherols and saponins extracted from the argan tree on hormone-dependent and hormone-independent prostate cancer cell lines. These data suggest that argan oil is of potential interest in developing new strategies for prostate cancer prevention.

INTRODUCTION

Prostate cancer (PC) is among the most frequent metastatic (1) malignancies contributing to cancer deaths in males (1, 2). The exact causes of PC are not known. In addition, the tumor development of the human prostate gland is critically dependent on the deregulated balance between various different growth factor regulators (3, 4). It strongly is suggested, however, that differences in the incidences of clinical prostate cancer and the passage from an indolent tumor to a clinically detected tumor are not due to genetic variations but to environmental and dietary factors. For example, although the incidence of PC is similar in diverse geographical areas (5), PC mortality rates are 4–5 times higher in Northern European countries and the United States than in Japan. Compared with the rates observed in Japan, the incidence of PC in Japanese men who migrated to the United States increased 4–9 times within one generation (6).

Dietary modifications for PC prevention continue to gain attention following demonstrations that various dietary nutrients/supplements such as fatty acids (7, 8) and antioxidants (9–12) are related to a reduced risk of developing PC. In this respect, several antioxidant plant compounds such as green tea, olive oil, and wine have been well individualized. Of all these nutrients, those having the most beneficial influence on PC prevention are lycopene, polyphenols, vitamin E and selenium (13–16).

We are grateful to Laboratoire Officiel d'Analyses et de Recherches Chimiques (Casablanca, Morocco) for its excellent technical assistance. Also, we are indebted to Arganoil Company (Casablanca, Morocco) for its financial support.
Keywords: Argan tree, Virgin argan oil, Tocopherols, Saponins, 2-Methoxyestradiol, Prostate cancer, Antiproliferative effect.
Correspondence to:
A. Adlouni
Laboratoire de Recherche sur les Lipoprotéines
Faculté des Sciences Ben Msik
B.P. 7955, Sidi Othman
Casablanca
Morocco
email: adlounia@yahoo.fr

Virgin argan oil is extracted from the pit of argan tree, *Argania spinosa*, which is endemic to southwestern Morocco. We evaluated the effect of tocopherols obtained from virgin argan oil and saponins extracted from the argan press cake, on proliferation in three human prostatic cell lines (PC3, DU145, and LNCaP) as a possible means of diet-associated prostate cancer prevention. This oil is rich in unsaturated fatty acids (45 percent oleic and 37 percent linoleic) and minor compounds (tocopherols, polyphenols, sterols, carotenoids, xanthophyls, and squalene). The unsaponifiable fraction is very abundant in these compounds, which have powerful antioxidant effects as proved on isolated LDL from human plasma (17). These interesting findings, with regard to the prevention of LDL oxidation due to the rich minor compounds, provoked our interest in investigating virgin argan oil's antiproliferative effect on PC. The results are compared to 2-methoxyestradiol ($2ME_2$) as antiproliferative drug candidate (18).

MATERIALS AND METHODS

Cell lines

Three human prostatic cell lines (PC3, DU145, and LNCaP) had grown as monolayers in RPMI 1640 medium (Invitrogen, Cergy Pontoise, France) supplemented with 5 percent fetal bovine serum (FBS, Invitrogen) and 2 mM L-glutamine (Invitrogen), at 37°C with 5 percent CO_2 and 95 percent humidity. The human prostate cancer cell lines LNCaP, DU145, and PC3 were purchased from the American Type Culture Collection.

Argan compounds extraction

The virgin argan oil used in this study was extracted by a cold pressing process (19); its chemical composition is listed in Table 1 (20). The fruit provided is from the city of Essaouira in southwestern Morocco. The argan oil used was in its rough state, without any preliminary processing. In order to investigate the antiproliferative effect of this oil, we analyzed the unsaponifiable fraction that represents one percent of the total mass of the oil, from which we extracted the tocopherols. The tocopherols were separately obtained after extraction of the unsaponifiable fraction of argan oil, which was obtained using the hexane extraction method. Using the HPLC technique, the purified fraction of tocopherols was reconstituted in chloroform (20). While the saponins were isolated from the MeOH extract of the argan press cake (21).

Table 1. Composition of virgin argan oil (20)

Fatty acid	%
C16:0	13.4
C18:0	5.1
C18:1 n-9	44.8
C18:2 n-6	35.7
C18:3 n-3	0.1
Sterols	mg/100 g oil
Schottenol	142
Spinasterol	115
Stigmasta-8,22-dien-3β-ol	9
Others	29
Tocopherols	mg/kg oil
α	35
β	122
γ	480
Phenolic compounds	μg/kg oil
Vanilic acid	67
Syringic acid	37
Ferulic acid	3147
Tyrosol	12

Steroid

2-Methoxyestradiol ($2ME_2$) was provided by Steraloids Laboratory (New Hampshire, USA). A stock solution was prepared in DMSO (Sigma, Saint-Quentin Fallavier, France) and stored at −20°C. $2ME_2$ is synthesized in vivo by hydroxylation at the 2-position of estradiol and subsequent O-methylation by catechol-O-methyltransferase (COMT) (22, 23).

Methyl Thiazolyl Tetrazolium (MTT) assay

PC3, DU145, and LNCaP cell lines were seeded on 96-well plates at density of 10,000 cells/well in a total volume of 100 μl. Cells were incubated for 24 hours prior to addition of saponins, tocopherols and $2ME_2$. The different media were changed 24 hours after. Saponins, tocopherols, and $2ME_2$ were added at 7 different concentrations between 1 and 100 μg/ml. The amounts of DMSO used to dissolve tocopherols and saponins were the same as in the control plate. The maximum final concentration of DMSO was 1 μl per ml of culture medium (0.1% v/v).

Living cells were counted at 48 hours using the MTT method (24). Cells were incubated for 2 hours at 37°C with 1 mg/ml MTT (Sigma). MTT was then discarded and replaced by isopropanol. Optical density (OD) was measured on an EL800 universal microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at 550 nm. IC_{50} was defined as the concentration entailing a 50 percent reduction of OD in comparison with the controls.

Tritiated thymidine incorporation (3HdThd)

PC3, DU145, and LNCaP cell lines were cultured on 24-well plates at a density of 20,000 cells/ well in a total volume of 1 ml. After plating in 24-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) and 24-hour incubation, cells were treated with saponins, tocopherols, and $2ME_2$ for 24 hours at different concentrations between 1 and 100 μg/ml. A 2 hour pulse of 2 μCi/ml ^{3}[H] thymidine was then applied to cells. Thereafter, cells were incubated for 10 minutes at 4°C in 10 percent trichloroacetic acid (TCA), washed 3 times with phosphate buffered saline (PBS) pH 7.4 and lyzed in 200 μl of 0.2 M NaOH/1% SDS solution. Radioactivity incorporated in the cells was measured by scintillation counting for one minute on Beckman LS 6000SC scintillation counter (Beckman Coulter, Roissy, France). The GI_{50} was defined as the concentration inhibiting growth by 50 percent compared to the controls.

Table 2. Relative cytotoxicity of compounds extracted from argan and 2-methoxyestradiol, ($2ME_2$ used as the control), in 3 human cancer cell lines (measured after 48 hours)

	IC_{50}, μg/ml		
Cell lines	Tocopherols	Saponins	$2ME_2$
DU145	24.89 ± 3.05	35.08 ± 2.39	20.11 ± 1.84
LNCaP	20.04 ± 1.32	50.34 ± 4.19	$15.63 \pm .32$
PC3	59.54 ± 4.12	24.24 ± 2.84	6.16 ± 1.17

IC_{50}, the concentration that inhibits growth of cell by 50 percent versus that of control cells. Data are presented as the mean $\pm$ SD of 3 separated experiments on each cell line.

Statistics

Statistical analysis was performed using stat view software (SAS Institute, Inc., Cary, NC, USA). The results are reported as mean $\pm$ SD.

RESULTS

Relative cytotoxicity of saponins, tocopherols, and $2ME_2$

The relative cytotoxic activity of saponins, tocopherols, and $2ME_2$ on human tumor cells was measured by means of a MTT assay in human cancer cells including the DU145, LNCaP, and PC3 lines. Table 2 summarizes the mean IC_{50} values for saponins, tocopherols, and $2ME_2$. The data indicated that tocopherols, saponins, and $2ME_2$ all effectively inhibited the growth of the 3 human tumor cells after 48 hours. For the DU145 and LNCaP cell lines, the most cytotoxic effect was obtained with the tocopherol fraction at 24.89 μg/ml and 20.04 μg/ml, respectively, as IC_{50}. The saponins fraction showed the most cytotoxic effect on the PC3 cell line with 24.2 μg/ml as IC_{50}.

Saponins, tocopherols, and $2ME_2$ inhibit cell proliferation

Saponins, tocopherols, and $2ME_2$ showed a dose-dependant antiproliferative effect on DU145, LNCaP, and PC3 cell lines (Figure 1). The calculated GI_{50} at 24 hours on DU145, LNCaP, and PC3 cell lines were, respectively, 28, 32, 48 μg/ml for tocopherols, 26, 30, 18 μg/ml for saponins, and 12, 11, 6 μg/ml for $2ME_2$. Proliferation in the 3 cell lines was more inhibited by $2ME_2$ than tocopherols and saponins.

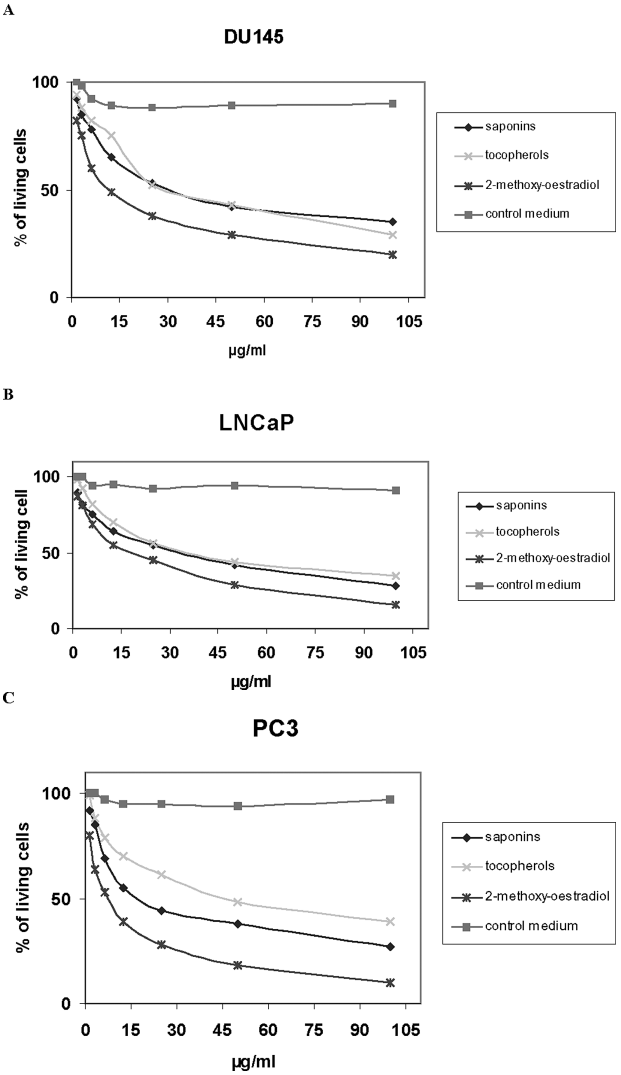

Figure 1. Effect of argan compounds on cell proliferation compared to $2ME_2$ as positive control. After 24 hour incubation, cell proliferation of (A) DU145, (B) LNCaP, and (C) PC3 cell lines was assessed by ^{3}[H] thymidine incorporation. The percentage of living cells was determined in comparison with control medium which contains corresponding concentrations of DMSO.

DISCUSSION

Several studies have focused on the antioxidant effects of various dietary substances on the prevention of prostate cancer. Natural antioxidants in vegetable foods such as tea, olive oil, and wine are believed to be responsible for the reduced risk of PC observed in association with these foods (9, 11, 15). Evidence supporting this hypothesis is based on epidemiological observations, animal case-studies, and cell culture experiments.

Argan tree compounds also can be an important dietary source of antioxidants (20). Argan oil—a product from the fruit of the argan tree produced and consumed largely in southwestern Morocco—is known for its cosmetic, pharmaceutical, and nutritional virtues. It has been demonstrated that compounds such as tocopherols, sterols, and polyphenols from argan oil have an antioxidative effect (17, 25), while saponins from argan cake press have antiinflammatory properties (26), suggesting a possible role in inhibiting carcinogenesis. To our knowledge, no study of the effects of argan-derived saponins or tocopherols on prostate cancer has been reported yet.

In the present study, we investigated the antiproliferative effect of tocopherols obtained from virgin argan oil and saponins extracted from argan press cake compared with $2ME_2$ as an

antiproliferative drug candidate. We have studied the compounds' viability and proliferation in two hormone-independent (DU145 and PC3) and one hormone-dependent (LNCaP) human prostate cell lines. Our results indicate that tocopherols and saponins display cytotoxic activity and exert an inhibitory effect on the proliferation of the three prostatic cell lines. The effect is not the same for the three cell lines; LNCaP was clearly the most sensitive of the three. These results are significant because tocopherols' role as a candidate molecule for the prevention of prostate cancer have been well documented (27, 28).

Venkateswaran et al. (28) demonstrated that physiological concentrations of vitamin E induced cell cycle arrest mediated by up-regulation of the P27 cell cycle regulatory protein. This observation provides a theoretical basis for the putative chemopreventive effect of vitamin E. Also, such clinical trials as the SELECT study (29, 30), the second large-scale study of chemoprevention for prostate cancer which included more than 32,000 men, has suggested that both selenium and vitamin E have potential efficiency in prostate cancer prevention. Considering γ-tocopherols in comparison with all vegetable oils, argan oil seems to be a unique γ-tocopherols-rich oil. Thus, it would be possible that γ-tocopherols extracted from argan oil could have more benefit in chemopreventive action against cancer of the prostate. Taken together, our data are promising for the future use of argan oil in patients developing prostate cancer. The use of γ-tocopherols should be considered for study in future prevention trials.

Only a few studies have showed an antiproliferative effect of saponins on human prostate cancer, notably from ginseng (31, 32). As reported in the literature, 7 major saponins have been identified in argan press cake, which is rare in vegetable oils (33). Saponins isolated from argan press cake are specific and chemically different from those extracted from ginseng and soy. In our study, the saponins isolated from the press cake exhibited cytotoxic activity and inhibited proliferation of LNCaP, DU145, and PC3 cell lines. Our observations that saponins' antiproliferative effect is dose dependant and that PC3 was the most sensitive cell line are similar to those of Kim et al. (31) and Liu et al. (32), who reported the antiproliferative activity of ginseng saponins in the LNCaP and PC3 cell lines. They suggested that ginseng saponins activate the expression of P21 and P27 as cyclin kinase inhibitors. Also, these saponins blocked LNCaP cells at G1 phase and subsequently inhibited cell growth. Since the chemical structure of argan saponins is different from that of other saponins, it is expected that argan saponins may act with a different mechanism. Thus, the argan saponins could offer a new opportunities for cancer prevention. We believe it would be of use to investigate all argan saponins separately in order to elucidate the role of each in prostate cancer prevention.

Before drawing final conclusions on the mechanisms of the anticancer effects of tocopherols and saponins derived from the argan tree, further investigations concerning the cell proliferation and apoptosis would be necessary and of interest.

Our study suggests for the first time that argan tree compounds could play a role in developing new strategies for the prevention and treatment of prostate cancer. Argan oil and argan press cake constitute additional products that reduce prostate cancer risk factors and delay the onset of prostate carcinogenesis.

REFERENCES

1. Lalani, E.N.; Stubbs, A.; Stamp, G.W. Prostate cancer; the interface between pathology and basic scientific research. Semin. Cancer Biol. **1997**, *8* (1), 53–59.
2. Greenlee, R.T.; Murray, T.; Bolden, S.; Wingo, P.A. Cancer statistics, 2000. Cancer J. Clin. **2000**, *50* (1), 7–33.
3. Untergasser, G.; Rumpold, H.; Hermann, M.; Dirnhofer, S.; Jilg, G.; Berger, P. Proliferative disorders of the aging human prostate: involvement of protein hormones and their receptors. Exp. Gerontol. **1999**, *34* (2), 275–287.
4. Feldman, B.J.; Feldman, D. The development of androgen-independent prostate cancer. Nat. Rev. Cancer **2001**, *1*, 34–45.
5. Yatani, R.; Chigusa, I.; Akazaki, K.; Stemmermann, G.N.; Welsh, R.A.; Correa, P. Geographic pathology of latent prostatic carcinoma. Int. J. Cancer **1982**, *29*, 611–616.
6. Shimizu, H.; Ross, R.K.; Bernstein, L.; Yatani, R.; Henderson, B.E.; Mack, T.M. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br. J. Cancer **1991**, *63*, 963–966.
7. Connolly, J.M.; Coleman, M.; Rose, D.P. Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice. Nutr. Cancer **1997**, *29* (2), 114–199.
8. Giovannucci, E.; Rimm, E.B.; Colditz, G.A.; Stampfer, M.J.; Ascherio, A.; et al. A prospective study of dietary fat and risk of prostate cancer. J. Natl. Cancer **1993**, *85* (19), 1571–1579.
9. Paschka, A.G.; Butler, R.; Young, C.Y. Induction of apoptosis in prostate cancer cell lines by the green tea component, (-) epigallocatechin-3-gallate. Cancer Lett. **1998**, *130*, 1–7.
10. Yoshizawa, K.; Willett, W.C.; Morris, S.J.; Stampfer, M.J.; Spiegelman, D.; et al. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J. Natl. Cancer Inst. **1998**, *90* (16), 1219–1224.
11. Norrish, A.E.; Skeaff, C.M.; Arribas, G.L.; Sharpe, S.J.; Jackson, R.T. Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study. Br. J. Cancer **1999**, *81* (7), 1238–1242.
12. Ni, J.; Chen, M.; Zhang, Y.; Li, R.; Huang, J.; Yeh, S. Vitamin E succinate inhibits human prostate cancer cell growth via modulating cell cycle regulatory machinery. Biochem. Biophys. Res. Commun. **2003**, *300* (2), 357–63.
13. Giovannucci, E.; Ascherio, A.; Rimm, E.B.; Stampfer, M.J.; Colditz, G.A.; Willett, W.C. Intake of carotenoids and retinal in relation to prostate cancer. J. Natl. Cancer Inst. **1995**, *87* (23), 1767–1776.
14. Kim, L.; Rao, A.V.; Rao, L.G. Effect of lycopene on prostate LNCaP cancer cells in culture. J. Med. Food **2002**, *5* (4), 181–187.
15. Kampa, M.; Hatzoglou, A.; Notas, G.; Damianaki, A.; Bakogeorgou, E.; et al. Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines. Nutr. Cancer **2000**, *37*, 223–233.
16. Zang, Y., Ni, J.; Messing, E.M.; Chang, E.; Yang, C.R.; Yeh, S. Vitamin E succinate inhibits the function of androgen receptor and the expression of prostate-specific antigen in prostate cancer cells. Proc. Natl. Acad. Sci. **2002**, *99* (11), 7408–7413.
17. Drissi, A.; Girona, J.; Cherki, M.; Godas, G.; Derouiche, A.; et al. Evidence of hypolipemiant and antioxidant properties of argan oil derived from the argan tree (Argania spinosa). Clin. Nutr. **2004**, *23* (5), 1159–1166.

18. Tinley, L.L.; Leal, R.M.; Deborah, A.; James, W.C.; Lynne, R.W.; et al. Novel 2-methoxyestradiol analogues with antitumor activity. Cander Res. **2003**, *63*, 1538–1549.

19. Boukhobza, M.; Pichon-Prun, N. L'arganier ressource économique et médicinale pour le Maroc. Phytothérapie **1988**, 27, 21–26.

20. Khallouki, F.; Younos, C.; Soulimani, R.; Oster, T.; Charrouf, Z.; et al. Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects. Eur. J. Cancer Prev. **2003**, *12* (1), 67–75.

21. Alaoui, A.; Charrouf, Z.; Soufiaoui, M.; Carbone, V.; Malorni, A.; et al. Triterpenoid saponins from the shells of Argania spinosa seeds. J. Agric. Food Chem. **2002**, *50* (16), 4600–4603.

22. Martucci, C.P. Metabolic fate of catechol estrogens. In *Catechol Estrogens*, Merriam, G.R., Lipsett, M.B., Eds. (Raven Press, New York, 1983), 115–122.

23. Ball, P.; Haupt, M.; Knuppen, R. Biogenesis and metabolism of catechol estrogens in vitro. In *Catechol Estrogens*, Merriam, G.R., Lipsett, M.B., Eds. (Raven Press, New York, 1983), 91–104.

24. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **1983**, *65* (1–2), 55–63.

25. Cherki, M.; Derouiche, A.; Drissi, A.; Bamou, Y.; El Messal, M.; et al. Consumption of argan oil protects against atherosclerosis process by promoving paraoxonase activities and antioxidant status: interventional study in healthy Moroccan men. Nutr. Metab. Card. Dis. **2005**, *15* (5), 352–360.

26. Alaoui, K.; Lagorce, J.F.; Cherrah, Y.; Hassar, M.; Amarouch, H.; Roquebert, J. Activité analgésique et antiinflammatoire des saponines d'Argania spinosa. Ann. Pharmaceutiques Françaises **1998**, *56*, 220–228.

27. Zhang, M.; Altuwaijri, S.; Yeh, S. RRR-alpha-tocopheryl succinate inhibits human prostate cancer cell invasiveness. Oncogene **2004**, *23* (17), 3080–3088.

28. Venkateswaran, V.; Fleshner, N.E.; Klotz, L.H. Modulation of cell proliferation and cell cycle regulators by vitamin E in human prostate carcinoma cell lines. J. Urol. **2002**, *168* (4), 1578–1582.

29. Klein, E.A. Selenium and vitamin E cancer prevention trial. Ann. NY Acad. Sci. **2004**, *41* (1031), 234.

30. Klein, E.A.; Thompson, I.M.; Lippman, S.M.; Goodman, P.J.; Albanes, D.; et al. SELECT: the selenium and vitamin E cancer prevention trial. Urol. Oncol. **2003**, *21* (1), 59–65.

31. Kim, H.S.; Lee, E.H.; Ko, S.R.; Choi, K.J.; Park, J.H.; Im, D.S. Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. Arch. Pharm. Res. **2004**, *27* (4), 429–435.

32. Liu, W.K.; Xu, S.X.; Che, C.T. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. Life Sci. **2000**, *67* (11), 1297–1306.

33. Charrouf, Z.; Wieruzeski, J.M.; Fkih-Tétouani, S.; Leroy, Y.; Charrouf, M.; Fourne, B. Triterpenoid saponin from Argania spinosa, Phytochemistry **1992**, *31*, 207–209.