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ORIGINAL ARTICLE

Trichosanthes kirilowii Exerts Androgenic Activity via Regulation of PSA and KLK2 in 22Rv1 Prostate Cancer Cells

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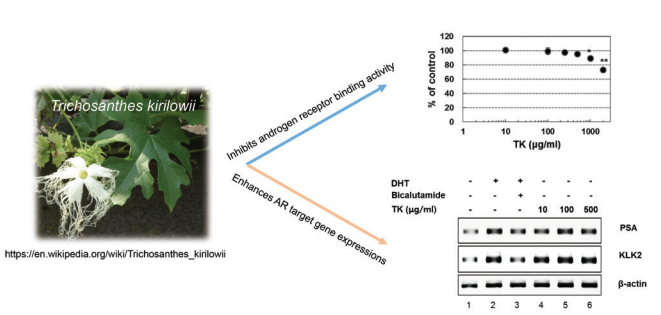
ABSTRACT

Background: The androgen comprises a group of hormones that play roles in male reproductive activity as well as personal characteristics. **Objective:** We investigated the androgenic activity of various herbal medicines in human prostate cancer 22Rv1 cells. **Materials and Methods:** Herbal extracts of *Trichosanthes kirilowii* (TK), *Asarum sieboldii* (AS), *Sanguisorba officinalis* (SO), and *Xanthium strumarium* (XS) were selected to have androgenic effects based on a preliminary *in vitro* screening system. **Results:** TK, AS, SO, and XS enhanced the proliferation of 22Rv1 cells without having cytotoxic effects. All tested herbal extracts increased androgen receptor (AR)-induced transcriptional activity in the absence or presence of dihydrotestosterone (DHT). In an AR-binding assay, TK, but not AS, SO, or XS, produced a significant inhibition of AR binding activity, indicating it has androgenic activity. Additionally, TK treatment positively regulated mRNA expression of the AR-related molecular targets prostate-specific antigen (PSA) and kallikrein 2 (KLK2) compared with untreated control. **Conclusion:** Taken together, TK-enhanced AR-mediated transcriptional activity might be an attractive candidate drug for treating androgen-related diseases.

Key words: Androgen, androgen receptor, transcription, Trichosantheskirilowii, 22Rv1 cells

SUMMARY

- Trichosantheskirilowii (TK), Asarumsieboldii (AS), Sanguisorbaofficinalis (SO), and Xanthium strumarium (XS) enhanced the proliferation of 22Rv1 cells without having cytotoxic effects.
- TK, AS, SO, and XS increased androgen receptor (AR)-induced transcriptional activity.
- TK, but not AS, SO, or XS, produced a significant inhibition against AR-binding activity.
- TK treatment positively regulated mRNA expression of the AR-related molecular targets prostate-specific antigen and kallikrein 2.



Abbreviations used: BPH: benign prostatic hyperplasia; AR: androgen receptor; DHT: dihydrotestosterone; PSA: prostate-specific antigen; TK: Trichosanthes kirilowii; AS: Asarum sieboldii; SO: Sanguisorba officinalis; XS: Xanthium strumarium; ATCC: American Type Culture Collection; FBS: fetal bovine serum; PBS: phosphate-buffered saline; SD: standard deviation; ARE: androgen-responsive element; KLK: kallikrein

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INTRODUCTION

The androgens, also called testoids or male sex hormones, are essential for the health and well-being in men. Excesses or shortages of androgen can lead to various diseases or disease-like syndromes, including prostate cancer, benign prostatic hyperplasia (BPH), the andropause, female hypertrichosis, acne, and androgenetic alopecia. The androgen directly interacts with androgen receptors (ARs) present in many body tissues, such as the reproductive system, central nervous system, cardiovascular system and immune system,[1] and influence transcriptional activation of the AR.[2]

To date, several medications have been developed to regulate androgen secretion in patients with androgen-related diseases. For instance, finasteride[3] and dutasteride[4] are medications used for treating BPH by targeting 5α- reductase, an enzyme involved in steroid metabolism that converts testosterone into dihydrotestosterone (DHT). However, they produce serious side effects for patients, such as decreased libido, and ejaculatory or erectile dysfunction.[3] In this regard, several recent studies have suggested that herbal medicines might be attractive candidates to reduce the deleterious side effects and enhance efficacy in treating androgen-related diseases. For instance, androgenic effects of *Buteasuperba*, a Thai herb used in traditional medicine, have been reported in male and female rats.[5,6] Green tea extract and a specific green

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tea catechin, (-) epigallocatechin–3-gallate can control the production and biological activities of androgens as well as other hormones.[7]

In our preliminary work, we performed *in vitro* screening using over 30 kinds of herbal extracts to detect any androgenic or anti-androgenic effect in human prostate cancer 22Rv1/MMTV cells. The 22Rv1 cells are derived from the human prostatic carcinoma xenograft, CWR22R, and express both the AR and prostate-specific antigen (PSA).[8] Kim *et al.* reported that, compared with LNCaP and PC3/AR+ cells, 22Rv1 is the most appropriate prostate cancer cell line for detecting androgenic actions *in vitro* because of its sensitivity to androgenic drugs.[9] The ethanol extracts from *Trichosanthes kirilowii* (TK), *Asarum sieboldii* (AS), *Sanguisorba officinalis* (SO), and *Xanthium strumarium* (XS) were selected as candidates in primary screening among 30 tested herbs (data not shown), and we further confirmed their activities by additional experiments to identify the best herbal candidates among them.

In traditional medicine, TK is usually used to treat coughing, fever, pain, inflammation, and discharge of phlegm. A variety of biological actions of TK have been reported against cancers,[10-12] diabetes,[13] immune diseases,[14] and osteoclasogenesis.[15] However, no androgenic effects of these herbs have been reported. We examined effects of TK on the transactivation and binding activity of the AR using 22Rv1 cells, and we report here on its androgenic activity.

MATERIALS AND METHODS

Materials and preparation of 70% ethanol extracts

The four dried herbal medicines, TK, AS, SO, and XS were purchased from HMAX (Jecheon, Korea). The identity of each was confirmed taxonomically by Prof. Je Hyun Lee, Dongguk University, Gyeongju, Korea. Voucher specimens (ST2, ST14, ST24, and ST25) have been deposited at the K-herb Research Center, Korea Institute of Oriental Medicine. The medicines (200.0 g, each) were extracted three times with 70% ethanol (2.0 L) by sonication for 60 min. The extracts were filtered, evaporated to dryness, and freeze-dried using a freeze dryer (PVTFD100R; Ilshin Lab. Ltd., Gyeonggi, Korea). The yields of TK, AS, SO, and XS were 24.5, 8.9, 12.2, and 5.9%, respectively.

Cell culture and cell line establishment

The human prostate cancer cell line 22Rv1 (CRL–2505) and African Green Monkey kidney cells COS–7 (CRL-1651) were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). 22Rv1/MMTV cells were established by the stable transfection of pMMTV-Luc plasmid into the 22Rv1 cells selected with G418, as previously described.[9] The cells were maintained in RPMI1640 medium (Gibco BRL, Carlsbad, CA) containing 10% fetal bovine serum (FBS), penicillin (100 units/mL), 1% L-glutamine and streptomycin (100 μg/mL) under humidified 5% CO_2 in air at 37°C.

Proliferation assay

The 22Rv1/MMTV cells were plated onto 96-well microplates at a density of 2 × 10^4 cells/well. The cells were treated with various concentrations (10, 100, 250 or 500 μg/mL) of herbal extract for 24 h. The 3-(4,5-dimethylthiazol–2-yl)–5-(3-carboxymethoxyphenyl)–2-(4-sulfophenyl)–2H-tetrazolium(inner salt; MTS) solution (Cell Titer 96 non-radioactive cell proliferation assay kit; Promega Corp., Madison, WI) was added to the cells in serum-free medium and incubated under humidified 5% CO_2 in air at 37°C for 90 min. The absorbance was measured at 490 nm using a Molecular Dynamics plate reader (Sunnyvale, CA).

Transactivation assay

For reporter gene assay, 22Rv1/MMTV cells were seeded in 96-well white plates (Thermo Scientific, Rockford, IL, USA), at an initial density of 1 × 10^4 cells per well, with RPMI1640 medium containing 10% dextran-charcoal stripped FBS. After 24h. the cells were treated with various concentrations of the herbal extract for 24h. Luciferase activity was determined using a Steady-Glo Luciferase assay kit (Promega, Madison, WI), and luminescence was measured using an EnSpire Multimode Plate Reader in the luminescence mode. DHT was purchased from Wako Pure Chemicals (Osaka, Japan).

Competitive AR binding assay

The whole cell binding assays were carried out, as previously described.[16] COS–7 cells were transfected with pCMV-hAR using Lipofectamine reagent (Invitrogen, San Diego, CA). Twenty-four hours before the binding reaction, the cells were placed in the serum-free and phenol red-free medium, and then maintained at 37°C for 2 h with 5 nM [^{3}H]5α-DHT in the absence or presence of unlabeled herbal extracts. Nonspecific binding of [^{3}H]5α-DHT was measured by adding a 100-fold molar excess of unlabeled [^{3}H]5α-DHT. The cells were washed twice in phosphate-buffered saline (PBS), collected into a buffer containing 2% sodium dodecyl sulfate (SDS), 10% glycerol and 10 mMTris (pH 6.8), and radioactivity was determined using a scintillation counter.

RNA isolation and reverse transcription polymerase chain reaction

Total RNA was isolated from 22Rv1 cells using TRIzol reagent (Life Technologies, Inc., Grand Island, NY), and cDNA was synthesized according to the manufacturer's instructions. In brief, total RNA was reverse-transcribed using MMLV-reverse transcriptase (Promega Corp., Maison, MI) and oligodT primers. Gene expression was investigated as previously described.[17]

Quantitative analysis of 70% ethanol extract of TK

The analysis was performed using a Shimadzu Prominence LC–20A system (Shimadzu, Kyoto, Japan) consisting of a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a photodiode array (PDA) detector, was employed. The data processor used LCsolution software (version 1.24; Shimadzu, Kyoto, Japan). The 3,29-dibenzoyl-rarounitriol was separated on a Phenomenex Gemini C_{18} column (250 × 4.6 mm; particle size 5 μm, Phenomenex, Torrance, CA) and maintained at 40°C. The mobile phases for chromatographic separation were carried out using isocratic elution (A : B = 5 : 95) of solvent A (distilled water) and solvent B (acetonitrile) for 30 min. The analysis was performed at a flow-rate of 1.0 mL/min using a detection wavelength of 230 nm. The injection volume was 10 μL.

Statistical analysis

All results were expressed as the means ± standard deviation (SD). Any significance of differences between groups was determined by an analysis of variance (ANOVA) and Student's *t*-test.

RESULTS AND DISCUSSION

Here we report the *in vitro* androgenic activity of TK. We screened over 30 herbal extracts in an initial study using human prostate cancer 22Rv1/MMTV cells (data not shown). The 22Rv1 cells are derived from the human prostatic carcinoma xenograft, CWR22R, and express both the AR and PSA.[8] Kim *et al.* reported that, compared with LNCaP and PC3/AR+ cells, 22Rv1 is the most appropriate prostate cancer cell line for detecting androgenic actions *in vitro* because of its sensitivity

to androgenic drugs.[9] The ethanol extracts from TK, AS, SO, and XS revealed androgenic effects among 30 tested herbs, and we further confirmed their activities by additional experiments.

Effects of four herbal extracts on AR-mediated transcription in 22Rv1/MMLV cells

The MTS assays were conducted to evaluate the effects of TK, AS, SO, and XS on the proliferation of 22Rv1/MMLV cells. As shown in Figure 1, all tested herbal extracts increased cell proliferation in a dose-dependent manner with no cytotoxicity. The herbal extracts increased the cell growth rate by 1.3 to 1.9-fold. Next, to assess the androgenic activity of the herbal extracts, we conducted luciferase assays for AR-mediated transactivation. The cells were treated with various concentrations of each herbal extract (10, 100, 250, or 500 μg/ml) for 24 h. As shown in Figure 2, all herbal extracts increased AR-mediated transcriptional activity compared with untreated control. We also examined whether each herbal extract could influence DHT-activated transcription [Figure 3]. The cells were concurrently stimulated with various concentrations of each herbal extract and DHT (10 nM) for 24 h. Treatment with the XS extract increased transcriptional activity in a dose-dependent manner. In contrast, treatment with TK and AS increased the activity at ≤ 250 μg/ml, but decreased it at 500μg/ml. Treatment with SO increased the activity at ≤ 100 μg/ml, but decreased it at ≥ 250μg/ml.

Effects of four herbal extracts on AR binding affinity in COS–7 cells

Next, we investigated the AR-binding affinities of the herbal extracts using recombinant human AR transiently expressed in monkey kidney COS–7 cells. The cells were transiently transfected with pCMV-hAR plasmid and incubated with the labeled androgen [^{3}H]5α-DHT in the absence or presence of each unlabeled herbal extract. The treatment with TK extract significantly reduced androgen binding to the AR in a dose-dependent manner [Figure 4A]. Although treatment with SO also revealed competitive inhibition of androgen binding, there was no statistical significance between untreated control and SO treatment [Figure 4C]. The treatment with AS and XS extracts had no significant effect on AR binding ([Figure 4B and D], respectively). Thus, our results

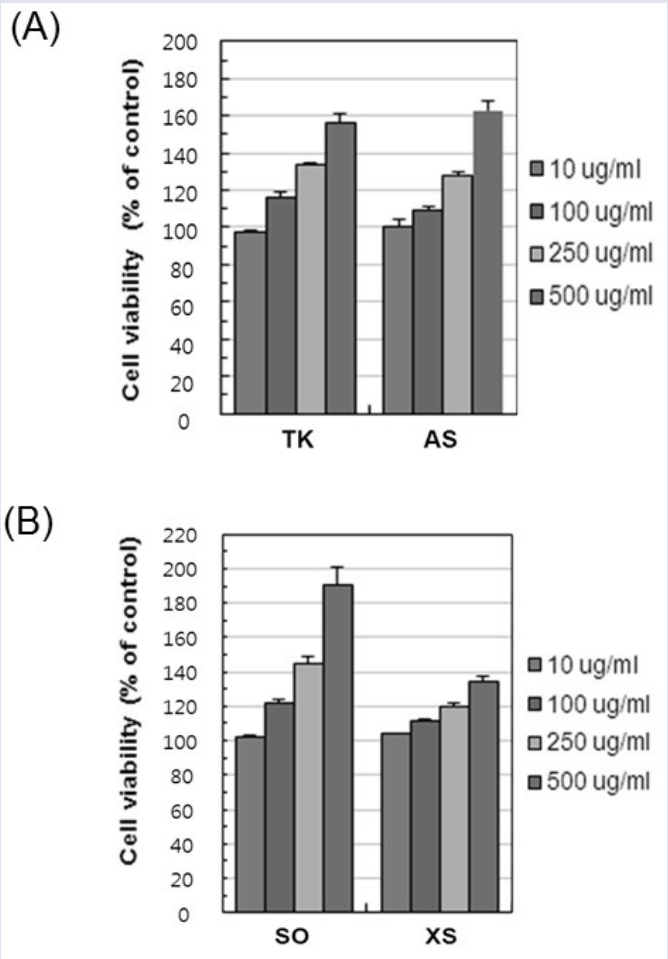

Figure 1: Effects of herbal extracts on the proliferation of 22Rv1/MMLV cells. (A) *Trichosanthes kirilowii* (TK) and *Asarum sieboldii* (AS). (B) *Sanguisorba officinalis* (SO) and *Xanthium strumarium* (XS). Cells were treated with various concentrations (10, 100, 250, or 500 μg/mL) of herbal extracts for 24 h. Changes in cell proliferation induced by each herb were determined by MTS assays. The bar graphs represent the means ± SD from three independent experiments.

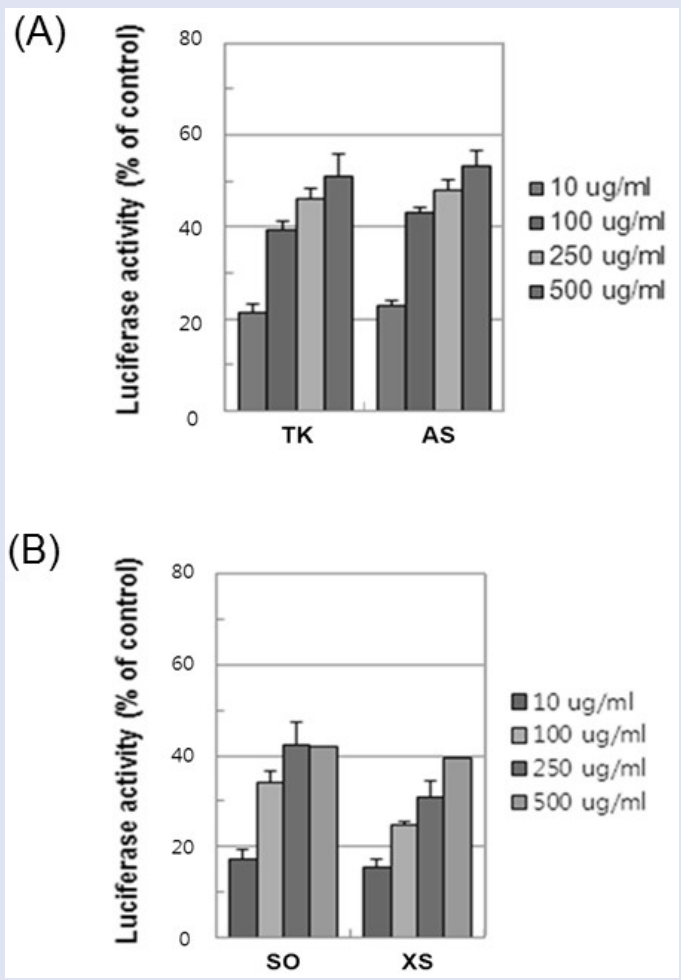

Figure 2: Androgenic effects of herbal extracts in 22Rv1/MMLV cells. (A) *Trichosanthes kirilowii* (TK) and *Asarum sieboldii* (AS). (B) *Sanguisorba officinalis* (SO) and *Xanthium strumarium* (XS). Cells were transiently transfected with a pRL-TK vector and incubated for 24 h with various concentrations (10, 100, 250 or 500 μg/mL) of each herbal extract. The cell lysates were prepared for luciferase activity assay as described in the methodology above. Transfection efficiency for luciferase assay was normalized to the Renilla luciferase activity. DHT (10 nM) was used as a positive control. The bar graphs represent the means ± SD from three independent experiments.

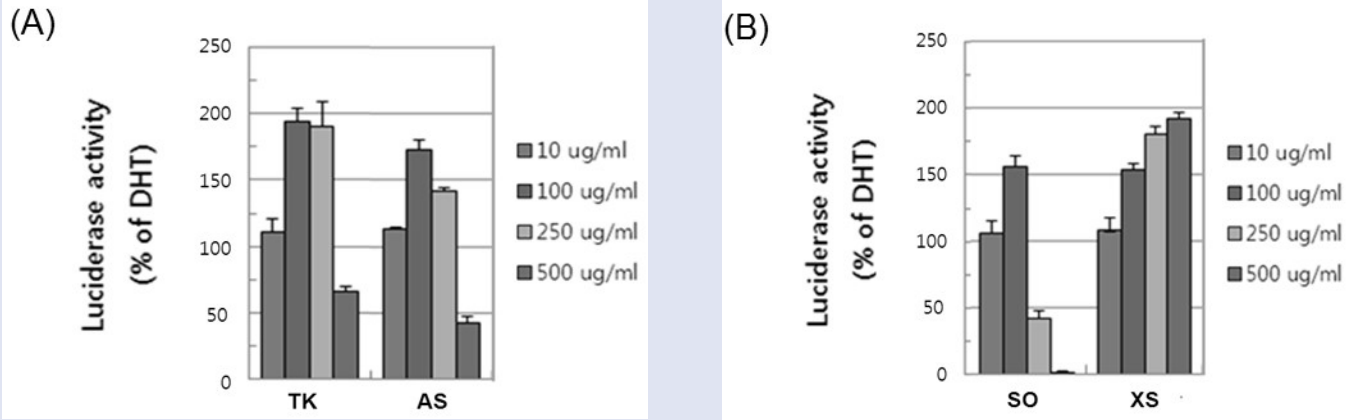

Figure 3: Effects of herbal extracts on DHT-activated androgenic activity of herbal extracts in 22Rv1/MMLV cells. (A) *Trichosanthes kirilowii* (TK) and *Asarum sieboldii* (AS). (B) *Sanguisorba officinalis* (SO) and *Xanthium strumarium* (XS). Cells were transiently transfected with apRL-TK vector and incubated for 24 h with various concentrations (10, 100 or 500 µg/mL) of each herbal extract in the presence of DHT (1 nM). The cell lysates were prepared for luciferase activity assay as described in the methodology above. Transfection efficiency for luciferase assay was normalized to the Renilla luciferase activity. The bar graphs represent the means ± SD from three independent experiments.

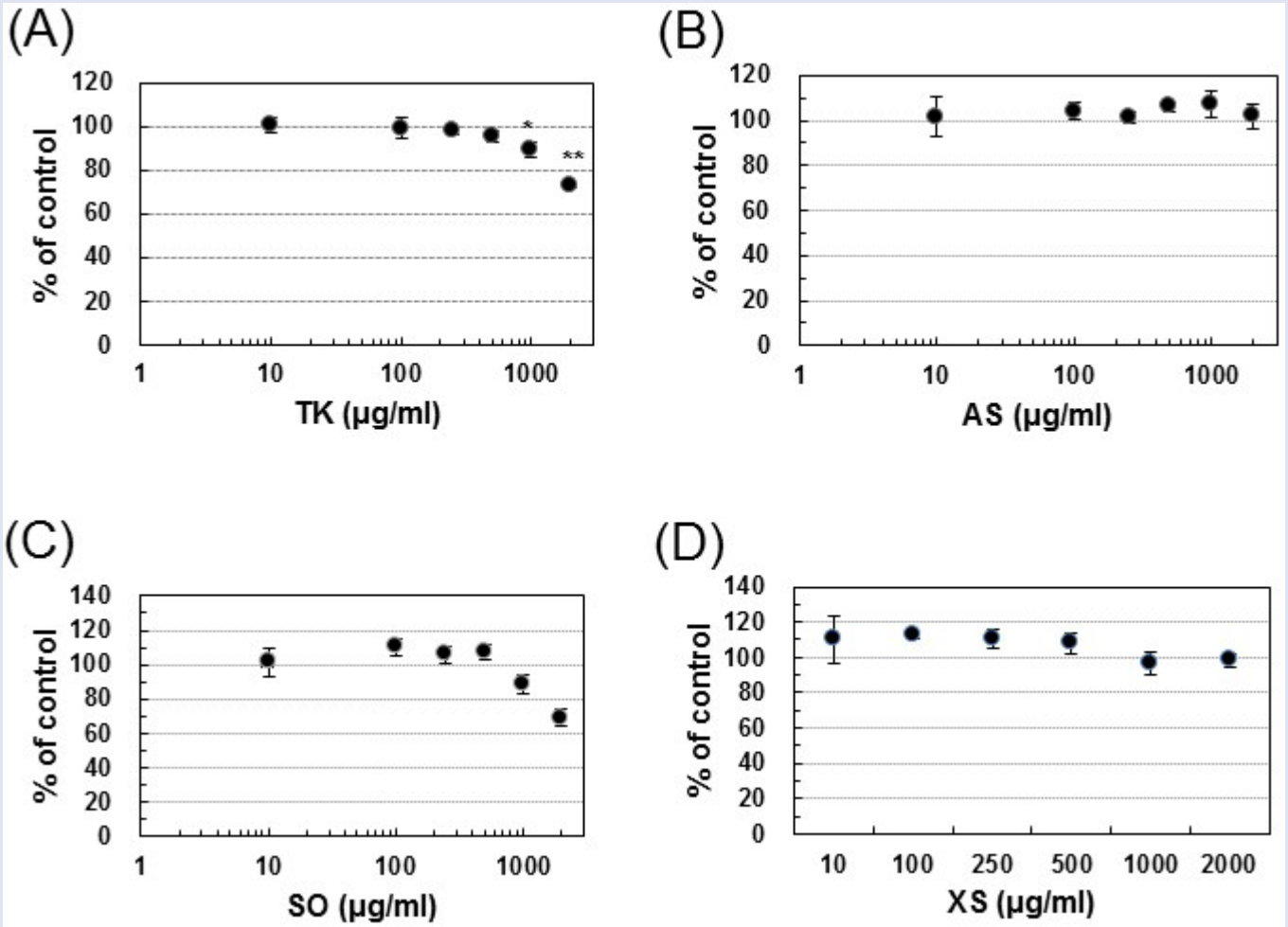

Figure 4: Effects of herbal extracts on [^{3}H]5α-DHT binding to the AR in COS-7 cells. (A) *Trichosanthes kirilowii* (TK), (B) *Asarum sieboldii* (AS), (C) *Sanguisorba officinalis* (SO) and (D) *Xanthium strumarium* (XS). AR-binding inhibition was determined in COS-7 cells transiently transfected with pCMV-AR as described in the methodology above. Results are expressed as the percentage of binding relative to [^{3}H]5α-DHT alone and are shown for unlabeled herbal extracts. Data are expressed as the means ± SD from three independent experiments.$^*p < 0.05$ and $^{**}p < 0.01$ vs. untreated control.

imply that TK is the most effective androgenic agent among these four tested herbal extracts.

Effects of TK on mRNA expressions of PSA and KLK2 in 22Rv1/MMLV cells

Several biomarkers have been used to evaluate the molecular mechanisms of androgenic or anti-androgenic drugs.[18] As mentioned above, androgens interact with the AR, and the complex subsequently translocates into the nucleus and binds to specific androgen-responsive elements (AREs) on the target gene promoters.[19] PSA, also known as kallikrein 3 (KLK3), and KLK2 are well known target molecules of androgen receptor for expressing AR.[20,21] Therefore, we performed RT-PCR to analyze whether TK could change the mRNA expression of PSA and KLK2. DHT was used as a positive control. As shown in Figure 5, DHT clearly enhanced both PSA and KLK2 expression levels (lane 2) and these were inhibited by co-treatment of anti-androgen bicalutamide (lane 3). The TK treatment increased PSA and KLK2 expression levels in a dose-dependent manner compared with untreated control (lanes 4–6).

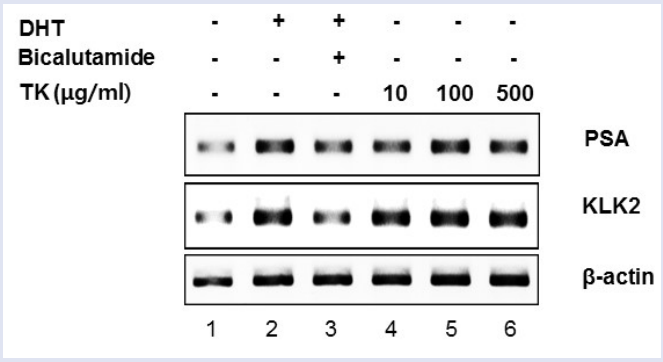

Figure 5: Effects of the *Trichosanthes kirilowii* extract on the mRNA expression of androgen-related genes in 22Rv1/MMLV cells. Cells were treated with various concentrations (10, 100 or 500 μg/mL) of TK extract for 24 h, or with DHT (1 nM) in the absence or presence of bicalutamide (20 μM). The mRNA expressions of PSA and KLK2 were determined by RT-PCR as described in the methodology above.

HPLC analysis of TK

Several groups have reported analyses of the chemical constituents of TK.[22-26] We quantified the major compound of TK, 3,29-dibenzoyl-rarounitriol, using HPLC-PDA detection. The retention time of 3,29-dibenzoyl-rarounitriol was 15.19 min [Figure 6]. Reproducibility was assessed by repeatedly measuring retention times and peak areas for six independently prepared samples of the analyte. Reproducibility of the reference standard (RSD) was <0.6% for peak response and <0.2% for retention time (data not shown). The line equation and correlation coefficient (r^2) of calibration curves for 3,29-dibenzoyl-rarounitriol were $y = 19814.27x + 2740.33$ and 1.0000, respectively. The limits of detection and quantification were 0.14 μg/mL and 0.45 μg/mL, respectively. The content of 3,29-dibenzoyl-rarounitriol identified in the TK extract was 3.84 ± 0.01 mg/g.

Sun *et al.* have isolated nine compounds from this herb and identified them as 5-ethoxymethyl–1-carboxyl propyl-^{1}H-pyrrole–2-carbaldehyde, 5-hydroxymethyl–2-furfural, chrysoeriol, 4'-hydroxyscutellarin, vanillic acid, α-spinasterol, β-$_D$-glucopyranosyl-a-spinasterol, stigmast–7-en-3β-ol, and adenosine.[22] Among them, two compounds showed biochemical actions on androgen-related diseases. Kim *et al.* reported that chrysoeriol had anti-prostate cancer activity through suppression of the activation of signal transducer and activator of transcription 3 (STAT3) activation.[23] Our group has also reported that treatment with α-spinasterol attenuated BPH in a rat model.[24] Furthermore, it is of interest that TK has the inhibitory effects on breast cancer cells,[12] implying that this herb might have wider biological effects on endocrine diseases although no data have been reported on its anti-prostate cancer effects. Taken together, these studies suggest positive aspects of TK as an androgen regulator.

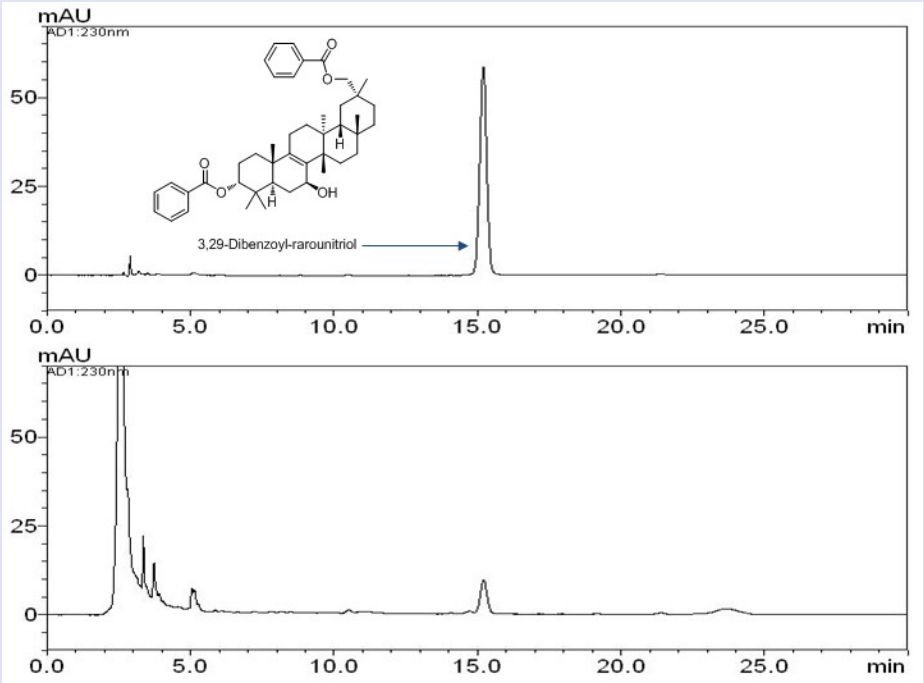

Figure 6: HPLC chromatograms of the standard (A) and 70% ethanol extract of *Trichosanthes kirilowii* (B) with detection at 230 nm.

CONCLUSION

Our data demonstrate that the traditional herbal medicine TK exerts androgenic activity via regulation of AR-related genes encoding PSA and KLK2 *in vitro*. Further studies will be required to verify effects of TK on regulating the activities of androgens and the AR using experimental animal models targeting specific androgen-related diseases.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev 2002;23:175-200.
2. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev 2004;25:276-308.
3. Gormley GJ, Stoner E, Bruskewitz RC, Imperato-McGinley J, Walsh PC, McConnell JD. *et al.* The effect of finasteride in men with benign prostatic hyperplasia. The Finasteride Study Group. N Engl J Med 1992;327:1185-91.
4. Roehrborn CG, Boyle P, Nickel JC, Hoefner K, Andriole G. Efficacy and safety of a dual inhibitor of 5-alpha-reductase types 1 and 2 (dutasteride) in men with benign prostatic hyperplasia. Urology 2002;60:434-41.
5. Cherdshewasart W, Nimsakul N. Clinical trial of Butea superba, an alternative herbal treatment for erectile dysfunction. Asian J Androl 2003;5:243-46.
6. Malaivijitnond S, Ketsuwan AN, Watanabe G, Taya K, Cherdshewasart W. Androgenic activity of the Thai traditional male potency herb, Butea superba Roxb., in female rats. J Ethnopharmacol 2009;121:123-29.
7. Liao S, The medicinal action of androgens and green tea epigallocatechin gallate. Hong Kong Med J 2001;7:369-74.
8. Tepper CG, Boucher DL, Ryan PE, Ma AH, Xia L, Lee LF. Characterization of a novel androgen receptor mutation in a relapsed CWR22 prostate cancer xenograft and cell line. Cancer Res. 2002;62:6606-14.
9. Kim HJ, Park YI, Dong MS. Comparison of prostate cancer cell lines for androgen receptor-mediated reporter gene assays. Toxicol *In Vitro* 2006;20:1159-67.
10. Mondal A. A novel extraction of trichosanthin from Trichosanthes kirilowii roots using three-phase partitioning and its *in vitro* anticancer activity. Pharm Biol 2014;52:677-80.
11. Takahashi N, Yoshida Y, Sugiura T, Matsuno K, Fujino A, Yamashita U. Cucurbitacin D isolated from Trichosanthes kirilowii induces apoptosis in human hepatocellular carcinoma cells *in vitro*. Int Immunopharmacol 2009;9:508-13.
12. Kim SR, Seo HS, Choi HS, Cho SG, Kim YK, Hong EH. Trichosanthes kirilowii Ethanol Extract and Cucurbitacin D Inhibit Cell Growth and Induce Apoptosis through Inhibition of STAT3 Activity in Breast Cancer Cells. Evid Based Complement Alternat Med. 2013 2013;975350.
13. Adiga S, Bairy KL, Meharban A, Punita IS. Hypoglycemic effect of aqueous extract of Trichosanthes dioica in normal and diabetic rats. Int J Diabetes Dev Ctries 2010;30:38-42.
14. Gong Q, Deng D, Ding J, Wang C, Bian Z, Ye Z. Trichosanthin, an extract of Trichosanthes kirilowii, effectively prevents acute rejection of major histocompatibility complex-mismatched mouse skin allograft. Transplant Proc 2008;40:3714-18.
15. Youn YN, Lim E, Lee N, Kim YS, Koo MS, Choi SY. Screening of Korean medicinal plants for possible osteoclastogenesis effects *in vitro*. Genes Nutr 2008;2:375-80.
16. Lambright C, Ostby J, Bobseine K, Wilson V, Hotchkiss AK, Mann PC. Cellular and molecular mechanisms of action of linuron: an antiandrogenic herbicide that produces reproductive malformations in male rats. Toxicol Sci 2000;56:389-99.
17. Kim HJ, Park YI, Dong MS. Effects of 2,4-D and DCP on the DHT-induced androgenic action in human prostate cancer cells. Toxicol Sci 2005;88:52-59.
18. Dong Y, Zhang H, Gao AC, Marshall JR, Ip C. Androgen receptor signaling intensity is a key factor in determining the sensitivity of prostate cancer cells to selenium inhibition of growth and cancer-specific biomarkers. Mol Cancer Ther 2005;4:1047-55.
19. Koivisto P, Kolmer M, Visakorpi T, Kallioniemi OP. Androgen receptor gene and hormonal therapy failure of prostate cancer. Am J Pathol 1998;152:1-9.
20. Haese A, Becker C, Noldus J, Graefen M, Huland E, Huland H. Human glandular kallikrein 2: a potential serum marker for predicting the organ confined versus non-organ confined growth of prostate cancer. J Urol 2000;163:1491-97.
21. Recker F, Kwiatkowski MK, Piironen T, Pettersson K, Huber A, Lummen G. Human glandular kallikrein as a tool to improve discrimination of poorly differentiated and non-organ-confined prostate cancer compared with prostate-specific antigen. Urology 2000;55:481-85.
22. Sun XY, Wu HH, Fu AZ, Zhang P. Chemical constituents of Trichosanthes kirilowii Maxim. Yao Xue Xue Bao2012;47:922-25.
23. Kim C, Kim MC, Kim SM, Nam D, Choi SH, Kim SH. Chrysanthemum indicum L. extract induces apoptosis through suppression of constitutive STAT3 activation in human prostate cancer DU145 cells. Phytother Res 2013;27:30-38.
24. Lee MY, Shin IS, Kyoung H, Seo CS, Son JK, Shin HK. Alpha-Spinasterol from Melandrium firmum attenuates benign prostatic hyperplasia in a rat model. Mol Med Rep 2014;9:2362-66.