Journal of Medicinal Plants Research Vol. 5(7), pp. 1265-1271, 4 April, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Screening of steroid 5α-reductase inhibitory activity and total phenolic content of Thai plants

Thapana Kumar[1], Chaiyavat Chaiyasut[1]*, Wandee Rungseevijitprapa[2] and Maitree Suttajit[3]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Thailand.
[2]Department of Pharmaceutical Science and Technology, Faculty of Pharmacy, Ubon Ratchathani University, Thailand.
[3]School of Medical Science, Naresuan University at Phayao, Thailand.

Accepted 22 September, 2010

Steroid 5α-reductase is the enzyme responsible for changing androgen testosterone into the more potent androgen dihydrotestosterone (DHT). Overexpression of DHT can cause many disorders including androgenic alopecia and benign prostatic hypertrophy (BPH). The aim of this study is to determine which plants possess 5α-reductase inhibitory activity, and to evaluate the correlation between 5α-reductase inhibitory activity and total phenolic content of these plants. Ten kinds of Thai plants were collected from local areas and extracted with 95% ethanol. The yields of ethanolic extracts of these plants ranged from 2.22 to 16.05%, dry weight. In the present study the ability of the extracts to inhibit 5α-reductase enzyme has, for the first time, been calculated as finasteride equivalent 5α-reductase activity (FEA) value (mg finasteride per 1 g extract). FEA values are easier to understand and to compare their activity. FEA values of the extracts ranged from 5.56 to 17.59 mg finasteride per 1 g extract. The highest FEA value was found in *Ocimum basilicum* L. The red strain of *Oryza sativa* L. was the second most potent 5α-reductase inhibitor, with FEA value of 16.72. Total phenolic content of the extracts ranged from 32.00 to 370.85 mg gallic acid equivalent per 1 g extract. There was no correlation between 5α-reductase inhibitory activity and total phenolic content. Phytochemicals other than phenolic compounds may play an important role in enzyme inhibition. As the usual dosage regimen of finasteride for treating androgen-related disorders is 1 to 5 mg/d, regular intake of these fresh plants or their extracts may be beneficial in health promotion, prevention or treatment effect.

Key words: Androgenic alopecia, benign prostatic hyperplasia, dihydrotestosterone, steroid 5α-reductase, testosterone, Thai plants, total phenolic content.

INTRODUCTION

Steroid 5α-reductase (5aR, EC 1.3.99.5; Δ^4-3-oxo-steroid 5α-oxidoreductase) is a microsomal enzyme that catalyzes the NADPH-dependent reduction of $\Delta^{4,5}$ double bond of a variety of 3-oxo-Δ^4steroids such as testosterone, progesterone and corticosterone. The important role of 5aR is to metabolize testosterone into a more potent androgen, dihydrotestosterone (DHT), which can bind firmly to androgen receptors with higher affinity and slower dissociation rate than testosterone. In humans, DHT is necessary for normal male growth; but high expression of DHT can cause many diseases such as acne, hirsutism, androgenic alopecia, benign prostatic hyperplasia (BPH), and prostate cancer (Bruchovsky et al., 1968; Liu et al., 2006; McGuire et al., 1960). In all animals, including humans, two different 5aR isozymes

*Corresponding author. E-mail: chaiyavat@gmail.com. Tel: +6653944340. Fax: +6653894163.

have been characterized. They are 5α- reductase type 1 (5aR1) and 5α-reductase type 2 (5aR2). In humans, the two isozymes share less than 50% sequence identity. Moreover, the two isozymes differ in their biochemical properties and specific organ distribution. 5aR1 has a broad basic optimum pH and a lower affinity for testosterone (K_m > 1μm), but higher capacity (high V_{max}); whereas 5aR2 prefers a slightly acidic pH and has higher affinity for testosterone (K_m < 10nm), but lower capacity (low V_{max}). 5aR1 can be found in the brain, liver, non-genital skin, and in the dermal papilla of hair follicles, while 5aR2 can be found only in androgen-dependent tissues such as the prostate, epididymis, and seminal vesicles (Eicheler et al., 1998; Iehlé et al., 1999; Liu et al., 2008b).

In treating DHT-related disorders, many synthetic 5aR inhibitors have been studied. For example, finasteride (MK-906, Proscar™) has been a drug of choice to treat BPH (Robinson et al., 2003). However, finasteride has a number of unfavorable side effects including impotence (erectile dysfunction), abnormal ejaculation, decreased ejaculatory volume, abnormal sexual function, gynecomastia, testicular pain, and myalgia (Lacy et al., 2008). To avoid these side effects, natural products may be used instead. In recent years, many researchers have found that some phytochemical classes possess an anti-5α-reductase activity. For example, aliphatic polyunsaturated fatty acids such as γ-linoleic acid can inhibit 5aR enzymes (Liang et al., 1992). Phenolic compounds such as tannin, isoflavones and chalcones have also been found to be effective in inhibiting 5aR enzymes *in vitro* (Hiipakka et al.; 2001, Liu et al., 2008a; Shimizu et al., 2000). Moreover, some triterpenoids isolated from *Ganoderma lucidum* can also inhibit 5aR (Liu et al., 2006).

Some plants with reported 5aR inhibitory activity are *Serenoa repens* (saw palmetto) fruit (Niederprûm et al., 1994), *Myrica rubra* (red bayberry) bark (Matsuda et al., 2001a), *Boehmeria nipononivea* (Shimizu et al., 2000a), *Artocarpus incisus* (Thai breadfruit) leaf (Shimizu et al., 2000b), *Alpinia officinarum* (lesser galangal) rhizome (Kim et al., 2003), *Lygodium japonicum* (Japanese climbing fern) spore (Matsuda et al., 2002), *Pleurotus ostreatus* (oyster mushroom) fruiting body, and *Lentinula edodes* (shiitake) fruiting body (Fujita et al., 2005).

Thailand is located in Southeast Asia and has thousands of varieties of plants, one of which might prove useful as a medicinal supplement to treat androgen-related disorders. Ten kinds of plants were randomly selected for screening tests. This study proposed to screen the 5α-reductase inhibitory activity of certain Thai plants, in order to find new sources of potential agents against several symptoms caused by excess 5α-reductase activity, and to determine the relationship between phenolic content and 5α-reductase inhibitory activity.

MATERIALS AND METHODS

Plant materials

Ten kinds of plants were purchased from local markets in Chiang Mai, Thailand. They were then identified by comparison with the herbarium specimens at the Faculty of Pharmacy, Chiang Mai University.

Animals

Six-week-old male Sprague-Dawley (SD) rats were obtained from the National Laboratory Animal Center, Bangkok, Thailand, and housed under a 12 h light/dark cycle with free access to food and water. This study was approved by the Animal Research Ethical Committee of the Faculty of Pharmacy, Ubon Ratchathani University, Ubon Ratchathani, Thailand.

Reagents

Dithiothreitol, sucrose, testosterone, finasteride and NADPH were purchased from Sigma (St. Louis, MO). Methanol, dichloromethane and absolute ethanol were purchased from Fisher Chemical (Fair Lawn, NJ). Other chemical compounds were purchased from Wako Pure Chemical Industry (Osaka, Japan).

Extraction of plants

Each plant was ground and dried in a hot air oven at 40°C for 48 h, and then extracted by maceration in 95% ethanol for 3 d. Each marc extract was re-macerated in 95% ethanol for another 3 d. The ethanol phase was evaporated to dryness under controlled pressure by using a rotary evaporator (Eyela, Tokyo, Japan).

Preparation of rat microsomes

Rat microsomal suspensions were prepared by following the method reported by Liu et al. (2006), with some modifications. Three male SD rats were sacrificed. The livers were removed and rinsed with cold normal saline solution. Specimens were then minced with scissors and homogenized in a solution composed of 0.32 M sucrose and 1 mM dithiothreitol in 0.02 M phosphate buffer (pH6.5). The homogenate was then centrifuged twice at 4500 x *g*, 0°C for 30 min each time. All of the supernatants were collected. The resulting supernatants containing microsomal particles were tested for soluble protein by the Lowry method (Lowry et al., 1951) and kept at -50°C until use.

Measurement of steroid 5α-reductase inhibitory activity

5α-reductase assay was performed according to the method of Matsuda et al. (2001b) with some modifications. The 3.0 ml reaction solutions each contained 0.2 ml of various plant extracts in 50% ethanol solution, 1.0 ml of 0.02 mM phosphate buffer (pH 6.5), 0.3 ml of freshly prepared 500 ppm testosterone solution in 50% ethanol solution, and 1.0 ml of microsomal suspension. Reactions were then initiated by the addition of 0.5 ml of 0.77mg/ml NADPH in phosphate buffer; samples were then incubated at 37 °C for 30 min. The reactions were then stopped by adding 5.0 ml dichloromethane, followed by adding 0.5 ml of 100ppm propyl *p*-hydroxybenzoate in 50% ethanol (as an internal standard for

Table 1. Plants used and their percentage yield of extraction.

Scientific name	Family	Part used	% Yield of the ethanolic extract
Centella asiatica (L.) Urb.	Apiaceae	Leaf	10.26
Terminalia chebula Retz.	Combretaceae	Fruit	11.50
Terminalia bellirica (Geartn.) Roxb.	Combretaceae	Fruit	16.05
Oryza sativa L.	Poaceae	Grain	2.22
Garcinia mangostana L.	Guttiferae	Peel	14.78
Ocimum basilicum L.	Lamiaceae	Whole plant	2.74
Piper nigrum Wall.	Piperaceae	Fruit	13.60
Citrus reticulata Blanco	Rutaceae	Peel	14.06
Houttuynia cordata Thunb.	Saururaceae	Whole plant	2.59
Curcuma longa L.	Zingiberaceae	Rhizome	7.84

HPLC). Samples were shaken for 60 s, and then centrifuged at 400 x *g* for 10 min. The water phase was frozen at -50°C. Four ml of organic phase was decanted and evaporated to dryness. The residue was redissolved in 5.0 ml methanol. An aliquot of 10.0 µl was analyzed for remaining testosterone content using high pressure liquid chromatography (HPLC). Samples were injected into an analytical Hypersil®-ODS column (Thermo Scientific, USA) 250 x 4.6 mm i.d. with 5µm internal particle size, using testosterone (>98% pure) as a standard. The mobile phase used was 65% methanol with a flow rate of 1 ml/min and detected by UV absorbance at 242 nm. The temperature of the column was controlled at 40°C.

To determine inhibitory activity, two special reactions must be completed: firstly, a complete reaction (rxn) containing 0.2% of 50%ethanol instead of the extract; secondly, an enzyme blank (ctrl) that receives 5.0 ml dichloromethane before the addition of NADPH, so that the conversion of testosterone into DHT does not occur. The % inhibition was calculated using peak area ratio (r) of testosterone/internal standard following the equation:

$$\% \text{ inhibition} = [(r_{sample} - r_{rxn})/(r_{ctrl} - r_{rxn})] \times 100$$

Finasteride, a well-known 5α-reductase inhibitor, was used as a standard enzyme inhibitor. The IC_{50} of finasteride was calculated. From the remaining testosterone content in each sample, finasteride equivalent anti-5α-reductase activity of each extract was calculated and recorded in terms of finasteride equivalent 5α-reductase inhibition activity (FEA) as a unit of mg finasteride equivalent per 1 g extract.

Determination of phenolic content

Total phenolic content (TPC) was determined using Folin-Ciocalteu reagents with gallic acid as a standard, following the method of Stoilova (2007) with some modification. Briefly, 0.2 ml of diluted plant extracts was added to 1.0 ml of 0.2 N Folin-Ciocalteu phenol reagent in a test tube and kept for 5 min. After that, 3.0 ml of 7.5% sodium carbonate solution was then added. Reactions were kept in a dark place for 2 h, and then read for UV absorbance at 750 nm. Gallic acid was used as a standard. TPC of each sample was expressed as mg gallic acid equivalent (GAE) per 1 g extract.

Statistics

All samples were analyzed in triplicate. All values were expressed as mean ± SD. To compare several groups, analysis of variance was used. Significant differences between means were determined by Duncan's multiple range tests. Pearson's correlation coefficient was used to predict the relationship between 5α-reductase inhibitory activity and TPC. A probability value of $p < 0.05$ was adopted as the criteria for the significant differences.

RESULTS

The plants and the parts used in this research are shown in Table 1. The plants were randomly selected from a variety of families. All of these plants are easily acquired and widely used in Thailand. The % yields of their ethanolic extracts ranged from 2.22 to 16.05%. *Terminalia bellirica* (Geartn.) Roxb. had the highest % yield of extraction at 16.05%, followed by *Garcinia mangostana* L. (14.78%) and *Citrus reticulata* Blanco (14.06%), while the lowest yields were found in the red strain of *Oryza sativa* L. (2.22%), *Houttuynia cordata* Thunb. (2.59%) and *Ocimum basilicum* L. (2.74%).

Rat microsomal suspensions appeared opaque pinkish in color, and contained 4.71 mg/ml soluble protein as assessed by the Lowry method. With given HPLC conditions, propyl *p*-hydroxybenzoate (an internal standard) and testosterone gave retention times of around 5 and 8 min, respectively. HPLC chromatograms of the complete reaction, enzyme blank, 0.5µM finasteride, and *O. basilicum* are shown in Figures 1A, B, 2A, and B, respectively.

5α-reductase inhibitory activity of finasteride was calculated as IC_{50} of 0.39 µM. The correlation between inhibitory activity (as % inhibition) and concentration of finasteride was expressed as:

$$y = 166.78x - 15.285 \ (R^2 = 0.999)$$

with y representing % inhibition and x the concentration of finasteride. Based on the given equation, FEA values of the extracts were calculated and expressed in Table 2. The 5aR inhibitory activity of each extract can be

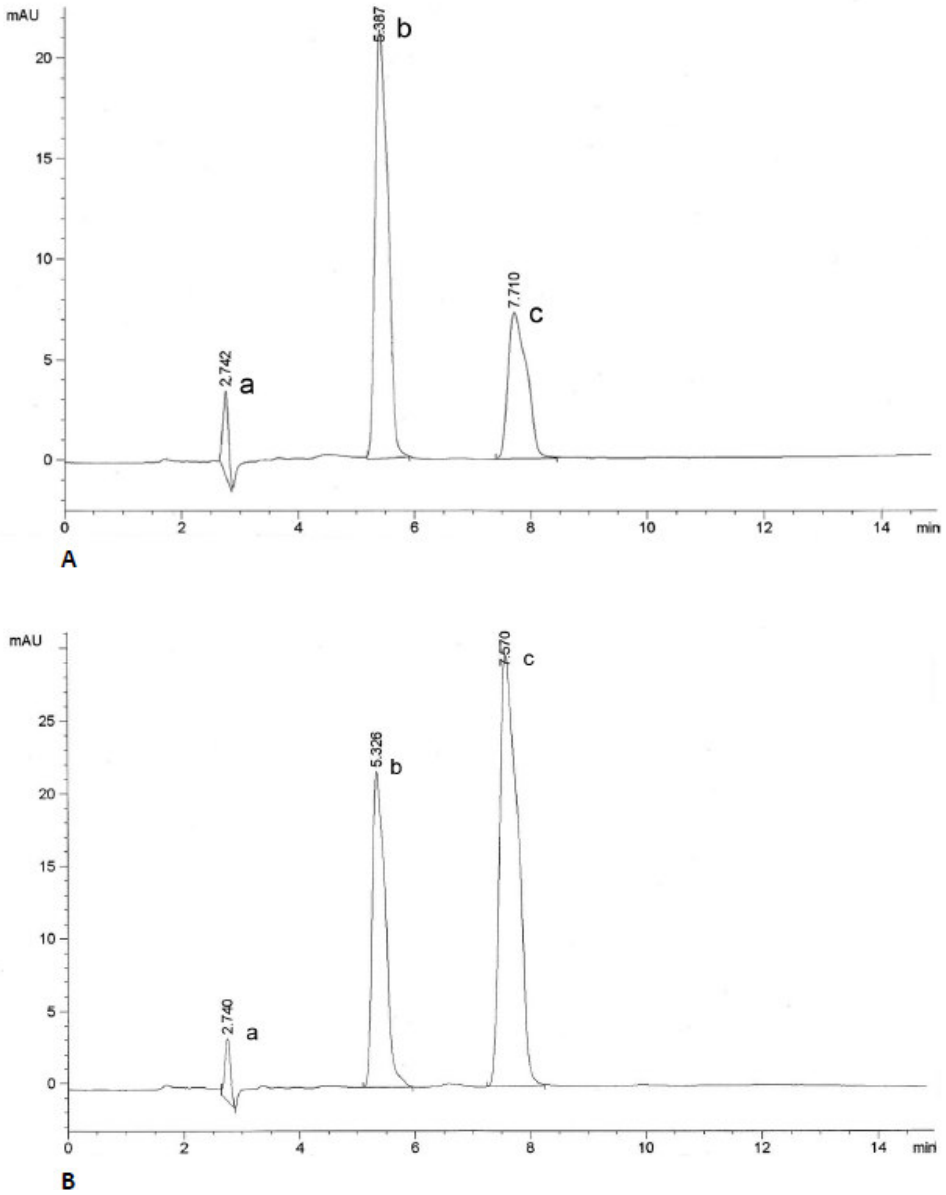

Figure 1. HPLC chromatogram of: (A) complete reaction control and (B) enzyme blank. a, b and c represent dithiotreitol, propyl *p*-hydroxybenzoate, and testosterone, respectively.

arranged from higher to lower, as follows: *O. basilicum*, *O. sativa*, *H. cordata*, *Curcuma longa* L., *Centella asiatica* (L.) Urb., *Terminalia chebula* Retz., *G. mangostana*, *T.bellirica*, *Piper nigrum* Wall., and *C. reticulata*, respectively. FEA values of the extracts ranged from 17.59 to 5.56 mg finasteride equivalent per 1 g extract. The best inhibitory activity was achieved by *O. basilicum* extract, and the lowest inhibitory activity was found in *Citrus reticulata* extract. There were no significant differences in 5aR inhibitory activity in *C. longa* and *C. asiatica*, or in *G. mangostana*, *T. bellirica*, and *P. nigrum*.

TPC of each extract (Table 3) ranged from 32.00 to 370.85 mg GAE per 1 g extract. They can be arranged from higher to lower as follows: *T. bellirica*, *T. chebula*, *C. longa*, *G. mangostana*, *O. sativa*, *C. reticulata*, *H. cordata*, *P. nigrum*, *O. basilicum*, and *C. asiatica*, respectively. *T. bellirica* had the highest TPC, followed by *T. chebula* (286.04 mg GAE per 1 g extract). *C. asiatica* had the lowest TPC. There were no significant differences in TPC among *C. longa* and *G. mangostana*, or among *O. sativa*, *C. reticulata*, *H. cordata*, *P. nigrum*, and *O. basilicum*.

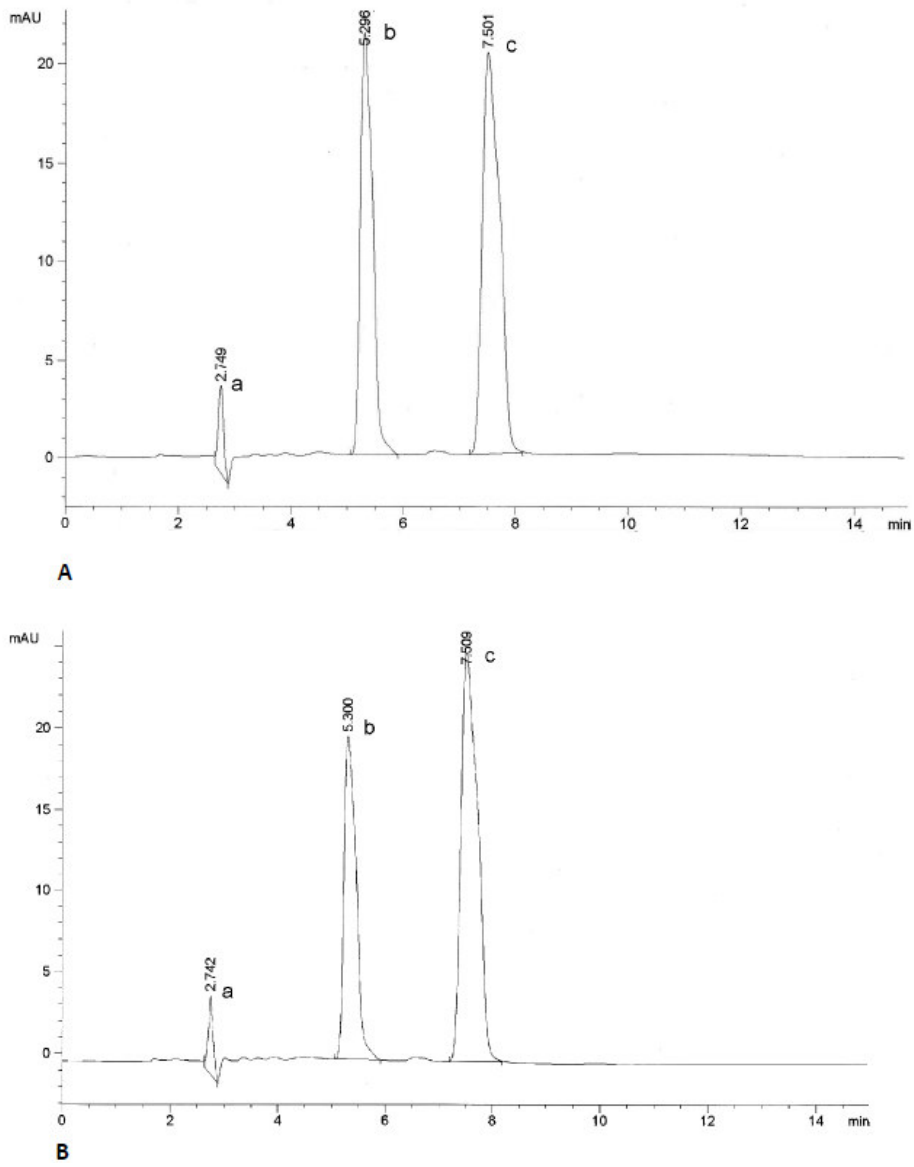

Figure 2. HPLC chromatogram of: (A) 0.5 µM finasteride and (B) *Ocimum basilicum*. a, b, and c represent dithiotreitol, propyl *p*-hydroxybenzoate, and testosterone, respectively.

DISCUSSION

Ten kinds of plants from different families were randomly selected. The parts of the plants used in this experiment are common used by Thai people for cooking, and also by traditional practitioners for medicinal treatment. After the processes of extraction, it was found that the crude extracts of all plants seemed to have a sticky, semi-solid appearance.

Rat microsomal suspensions consisted of a 5α-reductase enzyme and other enzymes that may be able to metabolize the substrate testosterone. Therefore, a control reaction was necessary to minimize errors. Two control reactions were evaluated. Firstly, the completed reaction control was one in which 5aR had full activity and could metabolize testosterone into DHT. Secondly, the enzyme blank was a reaction in which 5aR had none of the activity which is acquired by denaturing the enzyme; this helps in determining the total amounts of testosterone in the reactions studied. When the reactions were complete, the reacted tubes were further treated as described, and injected into a HPLC system. Generally, the determination 5α-reductase inhibitory activity is performed by radioimmunoassay (RIA); but the RIA

Table 2. Activity of plant extract on inhibition of 5α-reductase enzyme.

Plants	Finasteride equivalent 5α-reductase inhibition activity (mg finasteride/ 1 g crude extract)[1]
O. basilicum L.	17.59 ± 1.00a
O. sativa L.	16.72 ± 0.95b
H. cordata Thunb.	15.37 ± 1.50c
C. longa L.	13.83 ± 1.03d
C. asiatica (L.) Urb.	13.73 ± 1.05d
T. chebula Retz.	12.74 ± 0.84e
G. mangostana L.	11.62 ± 1.18f
T. bellirica (Geartn.) Roxb.	11.58 ± 0.84f
P. nigrum Wall.	11.18 ± 0.81f
C. reticulata Blanco	5.56 ± 1.12g

[1]-value in table expressed as mean ± SD of triplicate experiments. Means in column with different letters are significantly different ($p<0.05$).

Table 3. Total phenolic content of extracts.

Plants	Total Phenolic content (mg GAE/ 1 g extract)[1]
T. bellirica (Geartn.) Roxb.	370.85 ± 26.80a
T. chebula Retz.	286.04 ± 3.37b
C. longa L.	218.26 ± 14.90c
G. mangostana L.	205.90 ± 6.05c
O. sativa L.	75.48 ± 6.04d
C. reticulata Blanco	75.53 ± 5.46d
H. cordata Thunb.	67.67 ± 3.43d
P. nigrum Wall.	60.75 ± 3.26d
O. basilicum L.	63.12 ± 0.57d
C. asiatica (L.) Urb.	32.00 ± 0.91e

[1]-value in table expressed as mean ± SD of triplicate experiments. Means in column with different letters are significantly different ($p<0.05$).

method has many limitations, such as the dangerous from radioactive compounds and requiring complex equipment. The HPLC method was developed by Matsuda et al. (2001) to replace the RIA method. This method for determination of 5α-reductase inhibition activity is comparable to RIA and GC-MS. In our experiment, the IC_{50} of finasteride was 0.39 μM, which is comparable to the previous report of 0.34 μM (Park et al., 2003) which was assessed by the RIA method. According to HPLC conditions in this experiment, it was found that propyl *p*-hydroxybenzoate and testosterone have a good resolution and selectivity. Finasteride was used as a standard enzyme inhibitor in the 5α-reductase inhibition experiment because it is a well-known drug of choice to treat DHT-related disorders. In the process of comparing the activities of samples, we formulated a new term – finasteride equivalent 5α-reductase inhibitory activity, or FEA value – based on the inhibitory activity of selected plants at selected concentrations, converted into a finasteride equivalent in units of mg finasteride equivalent per g extract. FEA value is proportionally related to 5α-reductase inhibition activity. The higher the FEA value, the higher the 5α-reductase inhibition activity.

All of the plant extracts used in this report had a different ability to inhibit 5α-reductase enzyme. Among the extracts, *O. basilicum* or basil, the most potent 5aR inhibitor, contained volatile compounds in a class of terpenoids and aliphatic alcohols (Politeo et al., 2007). These compounds may be responsible for the highest FEA value. The red strain of *O. sativa*, or red rice, was used in this experiment. It contains a high level of anthocyanin, which was classified as one of the phenolic compounds, reported to be 5aR inhibitors (Hiipakka, 2001). The lowest activity of 5α-reductase inhibition was

found in *C. reticulata*, or tangerine, with the FEA value of 5.56. This was surprising because tangerine peel contains several flavonoids which have been reported to be 5aR inhibitors (Hiipakka et al.; 2001).

To determine whether phenolic compounds in the plants studied were the main active phytochemicals involved in enzyme inhibition, TPC of these plant extracts were determined. The results showed that each plant extract had different TPC. Unfortunately, there was no correlation between 5α-reductase inhibitory activity and TPC; Pearson's correlation coefficient was -0.169, p = 0.373. This suggested that phytochemicals other than phenolic compounds may play an important role in enzyme inhibition. As seen in *O. basilicum*, the plant with the highest FEA value has lower TPC.

The usual doses of finasteride to treat alopecia and benign prostatic hypertrophy (BPH) are 1 and 5 mg/d, respectively. From FEA values of each plant, it may be assumed that regular intake of these fresh plants or their extracts may be beneficial in preventing and treating symptoms related to excess 5aR activity. Further investigations of other bioactive phytochemical classes, which may play a role in enzyme inhibition and the *in vivo* activity of these extracts, will be conducted.

ACKNOWLEDGMENTS

The author would like to thank the Office of Higher Education Commission, Thailand for supporting by grant fund under the program Strategic Scholarships for Frontier Research Network for the Join Ph.D. Program Thai Doctoral degree for this research. This research was also supported by Office of the National Research Council of Thailand and Faculty of Pharmacy, Ubon Ratchathanee University.

REFERENCES

Bruchovsky N, Wilson JD (1968). The conversion of testosterone to 5-alpha-androstan-17-beta-ol-3-one by rat prostate *in vivo* and *in vitro*. J. Biol. Chem., 243: 2012-2021.

Eicheler W, Happle R, Hoffmann R (1998). 5α-reductase activity in the human hair follicle concentrates in the dermal papilla. Arch. Dermatol. Res., 290: 126-132.

Fujita R, Liu J, Shimizu K, Konishi F, Noda K, Kumamoto S (2005). Anti-androgenic activities of *Ganoderma lucidum*. J. Ethno., 102: 107-112.

Hiipakka RA, Zhang HZ, Dai W, Dai Q, Liao S (2001). Structure-activity relationships for inhibition of human 5α-reductase by polyphenols. Biochem. Pharmacol., 63: 1165-1176.

Iehlé C, Radvanyi F, Medina SGD, Ouafik LH, Chopin D, Raynuad JP, Martin PM (1999). Differences in steroid 5α-reductase iso-enzymes expression between normal and pathological human prostate tissue. J. Steroid Biochem. Mol. Biol., 68: 189-195.

Kim YU, Son HK, Son HK, Ahn MJ, Lee SS, Lee SK (2003). Inhibition of 5alpha-reductase activity by diarylheptanoids from *Alpinia officinarum*. Planta Med., 69(1): 72-74.

Lacy CF, Armstrong LL, Goldman MP, Lance LL (2008). Drug Information Handbook with international trade names index. 17th ed. US. Lexi Comp Inc., pp. 652-653.

Liang T, Liao S (1992). Inhibition of steroid 5-alpha-reductase by specific aliphatic unsaturated fatty acids. Biochem. J., 285: 557-562.

Liu J, Kurashiki K, Shimizu K, Kondo R (2006). Structure-activity relationship for inhibition of 5α-reductase by triterpenoids isolated from *Ganoderma lucidum*. Bioorg. Med. chem., 14: 8654-8660.

Liu J, Ando R, Shimizu K, Hashida K, Makino R, Ohara S, Kondo R (2008) Steroid 5α-reductase inhibitory activity of condensed tannins from woody plants. J. Wood Sci., 54: 68-75.

Liu S, Yamauchi H (2008). Different patterns of 5α-reductase expression, cellular distribution, and testosterone metabolism in human follicular dermal papilla cells. Biochem. Biophys. Res. Commun., 368: 858-864.

Lowry OH, Rosbrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin Phenol reagent. J. Biol. Chem., 193: 265-275.

Matsuda H, Yamazaki M, Matsuo K, Asanuma Y, Kubo M (2001a). Anti-androgenic activity of Myricae cortex- isolation of active constituents from bark of *Myrica rubra*. Biol. Pharm. Bull., 24(3): 259-263.

Matsuda H, Sato N, Yamazaki M, Naruto S, Kubo M (2001b). Testosterone 5α-reductase Inhibitory active constituents from *Anemarrhenae Rhizoma*. Biol. Pharm. Bull., 24(5): 586-587.

Matsuda H, Yamazaki M, Naruto S, Asanuma Y, Kubo M (2002). Anti-androgenic and hair growth promoting activities of Lygodii spora (spore of *Lygodium japonicum*) I. Active constituents inhibiting testosterone 5α-reductase. Biol. Pharm. Bull., 25(5): 622-626.

McGuire JS, Hollis VW, Tomkin GM (1960). Some characteristics of the microsomal steroid reductases of a rat liver. J. Biol. Chem., 235: 112-117.

Niederprûm HJ, Schweikert HU, Zânker KS (1994). Testosterone 5-reductase inhibition by free fatty acids from *Sabal serrulata* fruits. Phytomed., 1: 127-133 .

Park W, Lee C, Lee B, Chang I (2003). The extract of *Thujae occidentalis* semen inhibited 5α-reductase and androchrogenetic alopecia of B6CBAF1/j hybrid mouse. J. Dermatol. Sci., 31: 91-98.

Politeo O, Jukic M, Milos M (2007). Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. Food Chem., 101: 379-385.

Robinson AJ, DeLucca I, Drummond S, Boswell GA (2003). Steroidal nitrone inhibitor of 5α-reductase. Tetrahedron Lett., 44: 4801-4804.

Shimizu K, Kondo R, Sakai K, Shoyama Y, Sato H, Ueno T (2000a). Steroid 5α-reductase inhibitory activity and hair regrowth effects of an extract from *Boehmeria nipononivea*. Biosci. Biotechnol. Biochem., 64(4): 875-877.

Shimizu K, Kondo R, Sakai K, Baubarn S, Dilokkunanunt U (2000b). A gernylated chalcone with 5α-reductase inhibitory properties from *Atrocarpus incises*. Phytochemistry, 54: 737-739.

Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem., 102: 764-770.