

INHIBITION OF TYPE I 5 α -REDUCTASE BY MEDICINAL PLANT EXTRACTSPatil Vijaya¹, Samuel Grace², Mirapurkar Shubhangi², R. Krishna Mohan², Dasgupta Debjani^{1*}¹Department of Biochemistry, The Institute of Science, Mumbai - 400032, India²Board of Radiation and Isotope Technology, Navi Mumbai-400705, India

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ABSTRACT

Type I 5 α -reductase has been implicated in skin disorders such as acne, hirsutism and male pattern baldness and its inhibition offers a potential treatment for these disorders. The aim of this study was to investigate the inhibition of type I 5 α -reductase activity by extracts from Indian medicinal plants. Plant extracts were screened and selected based on their ability to inhibit *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Since type I 5 α -reductase metabolises testosterone to Δ^4 -androstene-3, 17-dione, the activity of enzyme was determined using RIA for testosterone and Δ^4 -androstene-3, 17-dione. It was found that methanolic extract of *Embelia ribes* was a potent inhibitor of type I 5 α -reductase (IC₅₀:100 μ g/mL). Extracts of *Vitex negundo*, *Terminalia chebula*, and *Terminalia bellerica* which also inhibited type I 5 α -reductase (IC₅₀: 200-390 μ g /mL). Therefore herbal formulation of these plant extracts may be used in the treatment of skin disorders involving type I 5 α -reductase.

KEYWORDS: Type I 5 α -reductase; acne; MBC; medicinal plants

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INTRODUCTION

5 α -reductase is a NADPH dependent enzyme that catalyzes the conversion of substrates with a 3-oxo- Δ^4 ,⁵ structure in the steroid A ring into 5 α - reduced compounds. Two isoforms of the enzyme are known; type I with an optimal pH of 6-9 and type II with an optimal pH of 5.5. The type I isoform occurs in non genital skin, scalp, sebaceous gland, liver and brain while type II isoform is present in the prostate, genital skin and seminal vesicles¹⁻². Type I 5 α -reductase is responsible for hirsutism³, acne and male pattern baldness whereas hyperactivity of type II enzyme leads to several androgen dependent disorders such as polycystic ovary syndrome⁴ and benign prostatic hyperplasia⁵. Localization of type I 5 α -reductase in the sebaceous glands of skin makes it a potential target for treatment of androgen dependent skin disorders.

The sebaceous glands of acne patients have increased levels of type I 5 α -reductase which converts testosterone to 5 α -dihydrotestosterone. This metabolite then accumulates and stimulates the secretion of sebum which provides a better environment for growth of *P. acnes*⁶. Therefore, the inhibition of type I 5 α -reductase would significantly control sebum production and reduce the

growth of *P. acnes*. Here the source of type I 5 α -reductase was PC-3 cell line. In PC-3 cell line, the major product of conversion of testosterone by type I 5 α -reductase is Δ^4 -androstene-3, 17-dione (4-androstenedione) and minor products are 5 α -Adione, dihydrotestosterone, androsterone and its epimer epiandrosterone⁷⁻⁸.

Specific type I 5 α -reductase inhibitors from natural sources include catechin, epigallocatechin -3-gallate⁹ from green tea and theaflavin -3'-gallate¹⁰ from black tea. Synthetic 5 α -reductase inhibitors are azasteroids and derivatives such as finasteride MK 906 (specific for type II), dutasteride GG745 (dual inhibitor)¹¹, turosteride, aryl substituted 6-azasteroid, pregnadiene-diones, pregnatriene-diones, steroidal carboxylic acids, non steroidal inhibitors such as 1H-benzoquinolizin-3-one derivative¹² and oxime inhibitors¹³. These synthetic compounds have side effects such as teratogenicity, impotence, decreased libido, ejaculation disorders and breast tenderness¹⁴⁻¹⁷. Hence it is important to identify compounds from natural sources which eliminate these side effects and provide safe treatment for acne.

Here, we have investigated the efficacy of *Embelia ribes*, *Vitex negundo*, *Terminalia chebula* and *Terminalia bellerica* as potential inhibitors of type I 5 α -reductase, *P.*

acnes and *Staphylococcus epidermidis*. In this investigation extracts from *Embelia ribes* was found to be highly potent inhibitor of type I 5 α -reductase. *Vitex negundo*, *Terminalia chebula* and *Terminalia bellerica* were also significant inhibitors of type I 5 α -reductase.

MATERIALS AND METHODS

Reagents and chemicals

Brain heart infusion agar, brain heart infusion broth, tryptone soya broth, Ham's F-12 K medium, fetal bovine serum (FBS), L-glutamine, antibiotic and antimycotic solution were purchased from Hi Media Pvt Ltd (India). Solvents, testosterone and dexamethasone were obtained from Sigma-Aldrich (St. Louis, USA). The positive control, dutasteride was procured from Pfizer India. Radioimmunoassay kit for testosterone (RIAK-16) and Active androstenedione RIA (DSL 3800) were purchased from Board of Radiation and Isotope technology, India and Beckman and Coulter, Czech Republic respectively.

Cell line

Prostate cancer cell line (PC-3) was procured from National Centre for Cell Science NCCS, Pune. It was maintained in Ham's F-12K medium supplemented with 10% FBS, antibiotic antimycotic solution (10000 U penicillin/L, 10 mg streptomycin/L, 25 μ g amphotericin/L), L-glutamine (2 mM) and sodium bicarbonate (2.2 %).

Plant collection and authentication

The medicinal plants were collected from the western suburbs of Mumbai, identified and authenticated by Dr. Vinayak Naik. The plant specimens were deposited in the herbarium of Zandu Pharmaceuticals Works Ltd, Mumbai, India. The ethnomedicinal uses of plant specimens and voucher specimen numbers are as listed in **Table 1**.

Collection of the culture. The cultures of *Propionibacterium acnes* (MTCC 1951) and *Staphylococcus epidermidis* (MTCC 3615) were obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India.

Preparation and phytochemical investigation of methanolic extracts

The plant parts were air-dried at room temperature (30 °C) and powdered using a mechanical mixer. The plants were extracted with methanol (15 gm in 150 mL solvent) using Soxhlet apparatus and were evaporated to dryness *in vacuo*. The phytoconstituents were examined qualitatively by reported methods¹⁸⁻¹⁹.

Determination of minimum bactericidal concentration (MBC)

The MBC was detected by broth dilution method against *P. acnes* and *S. epidermidis*. The plant extracts were diluted in brain heart infusion broth supplemented with

0.03 % sodium thioglycollate for *P. acnes* and tryptone soya broth for *S. epidermidis*. The cell density of *P. acnes* was adjusted to approximately 3×10^8 CFU/mL for inoculation using 0.5 McFarland standard (OD 0.132) at 530 nm. The standardised *P. acnes* and *S. epidermidis* were added to serial dilutions of each plant extract giving an initial count of approximately 10^6 CFU/mL of culture in triplicates. Incubation was carried out under anaerobic condition at 37°C for 48 h for *P. acnes* and under aerobic conditions at 37°C for 24 h for *S. epidermidis*. To determine the MBC, a loopful of broth was collected from those tubes which did not show any growth and inoculated on brain heart infusion agar. MBC was reported as the concentration at which no visible growth was observed²⁰. Erythromycin (1 mg/mL) and benzoyl peroxide (4 mg/mL) were used as positive controls.

Determination of 5 α -reductase inhibition

PC-3 cell line was seeded in 24 well plates (2×10^5 cells/well) with complete medium, at 37°C and pH 7 (optimum pH for type I 5 α -reductase) and serum depleted for next 24 h. Testosterone (1.25 μ M) and increasing concentrations of plant extracts in the range of 125-1000 μ g/mL were added to the medium. The reactions were terminated at various time intervals (0, 1, 2, 3, 4, 5 h and 24 h). Dutasteride (2mM) was used as control². Uptake of testosterone by PC-3 cell line was studied using a Radioimmunoassay kit (RIAK-16). Ham F12-K cell culture medium was used as a matrix instead of serum and inter and intra assay precision was carried out for the same. The residual testosterone was counted in a gamma counter (Beckman and Coulter) calibrated for ¹²⁵I. The concentration of testosterone in the aspirated cell culture medium was determined from calibration curve generated from testosterone standards provided with the kit. 4-Androstenedione was extracted from aspirated medium using diethyl ether after refrigerating at -15°C for 1 h. The ether layer was separated, evaporated and analyzed for the presence of 4-androstenedione. Active androstenedione RIA (DSL 3800) kit from Beckman and Coulter was used to measure its concentration²¹.

RESULTS AND DISCUSSION

Current treatment for acne is based on the use of combination therapy using antibiotics and benzoyl peroxide²². However, the increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogens have necessitated search for other alternative medicines²³. The present study involved screening of plant extracts for inhibition of type I 5 α -reductase which plays a significant role in the control of acne. Acne lesions are predominantly colonised by *P. acnes* and *S. epidermidis*, methanolic extracts of medicinal plants

have been screened here for their antimicrobial activity against these organisms²⁴. *E. ribes* and *T. chebula* had strong inhibitory effects against *P. acnes* as shown in **Table 2**. Methanolic extracts of *T. bellerica* and *V. negundo* also showed significant antimicrobial activity against *P. acnes* and *S. epidermidis*. The MBC values for the plant extracts against *P. acnes* were found to be in the range 0.1 mg/mL to 0.4 mg/mL. It was also found that inhibition potencies against *P. acnes* of methanolic extracts of *E. ribes* and *T. chebula* (MBC: 0.1mg/mL) were comparable with erythromycin (MBC: 0.08 mg/mL). These extracts were potent inhibitors as compared to benzoyl peroxide (MBC: 0.6mg/mL). The MBC values for the plant extracts against *S. epidermidis* were found to be in the range 1 mg/mL to 1.9 mg/mL. The phytochemical investigation revealed the presence of tannins, alkaloids, glycosides, terpenoids, amino acids, sugars, quinones and saponins as shown in **Table 2**²⁷.

Anti acne activity of these plants was further confirmed by type I 5 α -reductase inhibition at pH 7. The ability of type I 5 α -reductase to metabolize testosterone to 4-Androstenedione is very high as compared to 17-keto metabolites such as 5 α -adione, androsterone and epiandrosterone⁶. The RIA assays were used to evaluate the metabolism of testosterone in PC-3 cells by detecting concentration of 4-androstenedione and testosterone. Concentrations of testosterone and 4-androstenedione were evaluated by RIA, since the sensitivity of this method is higher than classical spectrophotometric or spectrofluorimetric techniques². Higher residual levels of testosterone and lower 4-androstenedione production indicated significant inhibition of 5 α -reductase in presence of plant extracts.

Fig. 1 and **Fig. 2** indicate the residual levels of testosterone in presence of plant extracts at a concentration of 125 and 500 μ g/mL respectively over a period of 5 h. Complete uptake of testosterone (1.25 μ M) occurred within 5 h as no residual testosterone was observed in absence of plant extracts. High residual levels of testosterone were observed in presence of all the plant extracts at concentration of 500 μ g /mL indicating inhibition of 5 α -reductase.

Extract of *E. ribes* showed effective inhibition of 5 α -reductase resulting in higher residual levels (40 %) of testosterone at 125 μ g /mL and 56 % at 500 μ g /mL. However, *T. chebula*, *T. bellerica* and *V. negundo* showed lower inhibition resulting in lower levels of residual testosterone (13-26 % at 125 μ g /mL and 17-40 % at 500 μ g /mL). Dutasteride exhibited an inhibition of 5 α -reductase similar to that of *E. ribes* at concentration of 2 mM (1056 μ g /mL). Thus of all the plants

investigated here, extract of *E. ribes* was found to be the most potent inhibitor of type I 5 α -reductase.

The inhibition of 4-androstenedione production in presence of varying concentration of plant extracts after 24 h is shown in **Fig. 3**. All the plant extracts inhibited the production of 4-androstenedione significantly (76-87 %) at concentration of 500 and 1000 μ g /mL. *E. ribes* inhibited the production of 4-Androstenedione by 57 % at a concentration of 125 μ g/mL whereas it exhibited 87 % inhibition at a concentration of 1000 μ g/mL. *E. ribes* showed significant reduction (87 %) in the production of 4-Androstenedione at (1000 μ g /mL) which indicated significant inhibition of 5 α -reductase. Dutasteride showed 98 % inhibition of 4-Androstenedione production at concentration of 2 mM (1056 μ g /mL). The above results indicated that extract of *E. ribes* was the most potent inhibitor of 5 α -reductase with IC₅₀ value of 100 μ g /mL as against other plant extracts which showed IC₅₀ values in the range of 260-360 μ g /mL as shown in **Table 2**.

CONCLUSION

The present study is an attempt to find novel type I 5 α -reductase inhibitors. Earlier investigations of type I 5 α -reductase inhibitors include use of plant extracts such as *Saw palmetto*, *Pygeum africanum*, *Artocarpus incisus*, *Thuja orientalis*,²⁵ *Laminaria saccharina*, *Salvia officinalis*, *Thymus officinalis*, *Arnica Montana*, *Mentha piperita* and *Rosamarinus officinalis*²⁶. These experimental results indicated that methanolic extract of *E. ribes* was a potent inhibitor of type I 5 α -reductase and had antimicrobial activity against *P. acnes* and *S. epidermidis* may be sufficient to control acne vulgaris. This study revealed that extracts of *Embelia ribes*, *Vitex negundo*, *Terminalia chebula* and *Terminalia bellerica* could be used in herbal formulations as an alternative treatment for acne.

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Table1: Ethnomedicinal plant species investigated

Botanical name (Voucher specimen number)	Family	Local name	Organ Tested	Ethnomedicinal uses
<i>Vitex negundo</i> Linn ZPS- 1209	Lamiaceae	Nirgundi	Leaves	Astringent, febrifuge, sedative, removes foetid discharges and worms from ulcers, bactericidal, antitumor, insecticidal, fumigant, skin disorders, and antidote for snake bites.
<i>Embelia ribes</i> Burm.f. ZPS-1638	Myrsinaceae	Vavding	Seeds	Anthelmintic, skin diseases, tumors, ascites, bronchitis, jaundice, mental disorders, wound healing, leprosy.
<i>Terminalia chebula</i> Retz ZPS-1154	Combretaceae	Harada	Fruit	Hyperlipidemia, fever, cough, skin diseases, diarrhoea, constipation, indigestion, topically as a mouthwash and gargle, vaginitis.
<i>Terminalia bellerica</i> Gaertn.Roxb ZPS-0386	Combretaceae	Behada	Fruit	Hyperlipidemia, indigestion, diarrhoea, constipation, sore throat, hepatoprotectant, respiratory tract infections, cough, sore eyes.

Table2: MBC values of plant extracts against *P. acnes* and *S. epidermidis* and reductase inhibitory activity²⁷.

Plants	Phytochemicals identified ¹	MBC mg/mL		5 α -reductase inhibition IC ₅₀ μ g /mL
		<i>P. acnes</i>	<i>S. epidermidis</i>	
<i>V. negundo</i>	SA, AL, TA, GL, AA, CBH, TE	0.4	1.9	260
<i>E. ribes</i>	AL, TA, AA, CBH, QU, TE	0.1	1.0	100
<i>T. chebula</i>	SA, AL, TA, GL, AA, CBH, TE	0.1	1.0	310
<i>T. bellerica</i>	SA, AL, TA, GL, CBH, TE	0.2	1.3	360
Erythromycin ²	NA	0.08	0.9	NA
Benzoyl peroxide ²	NA	0.6	1.2	NA
Methanol ³	NA	-	-	NA

¹AL: alkaloids, AA: amino acids, GL: glycosides, QU: quinones, TA: tannins, TE: terpenoids, SA: saponins, CBH : carbohydrate, NA: Not Applicable.
 (-): no inhibition of growth at the concentrations tested

²Positive control

³Negative control

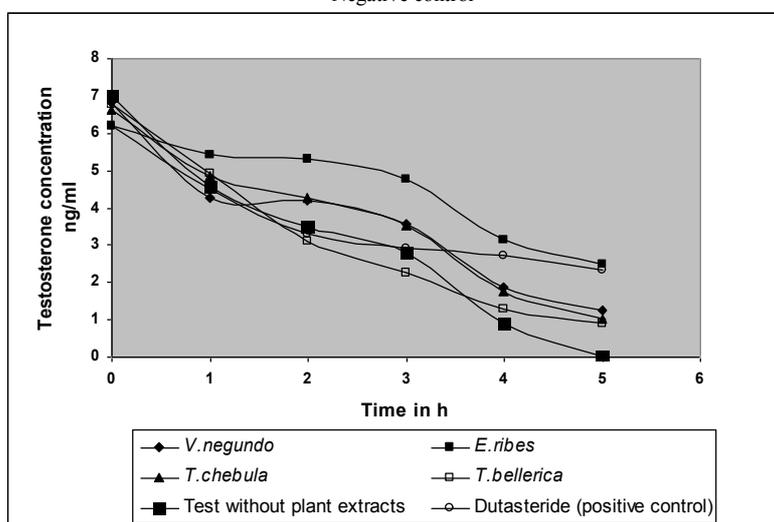


Figure 1: Residual levels of testosterone in presence of plant extracts of *Vitex negundo*, *Embelia ribes*, *Terminalia chebula*, *Terminalia bellerica* (125 μ g/mL), standard (1.25 μ M Testosterone; without inhibitor) and dutasteride - positive control (2mM) after 5 h.

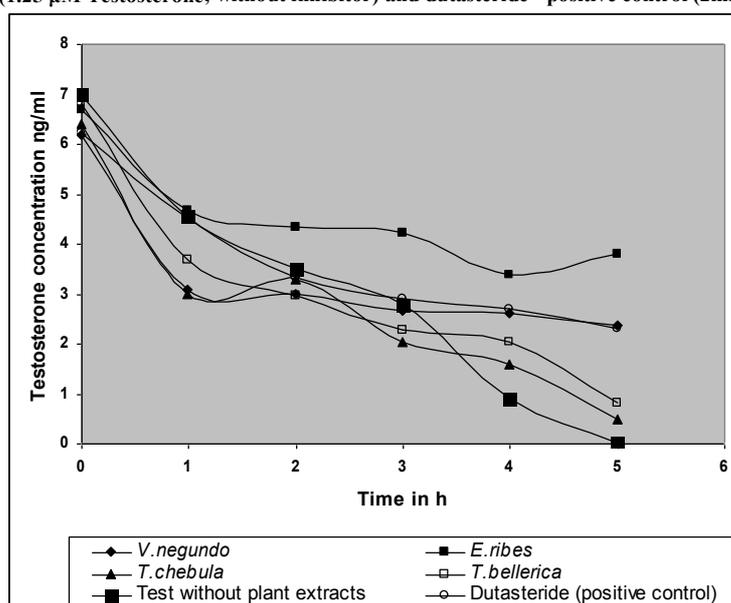


Figure 2: Residual levels of testosterone in presence of plant extracts of *Vitex negundo*, *Embelia ribes*, *Terminalia chebula*, *Terminalia bellerica* (500 μ g/mL), standard (1.25 μ M Testosterone without inhibitor) and dutasteride - positive control (2mM) after 5 h.

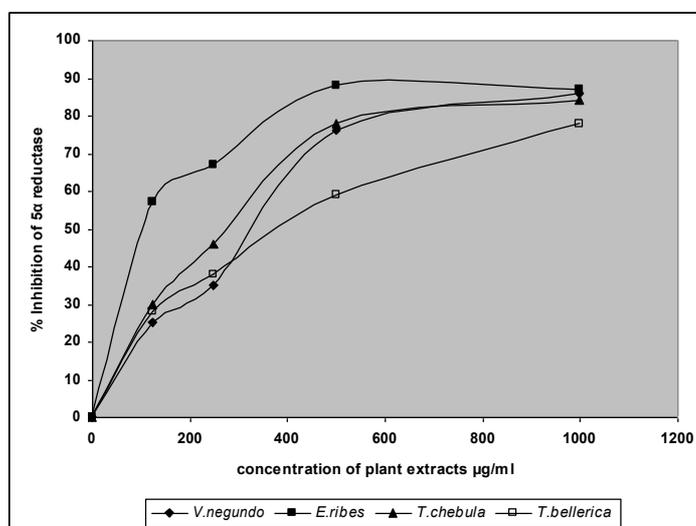


Figure 3: Effect of plant extracts *Vitex negundo*, *Embelia ribes*, *Terminalia chebula* and *Terminalia bellerica* on 4-androstenedione production after 24 h.

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