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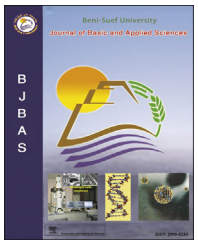

Full Length Article

A comparative in vivo and in vitro evaluation of hair growth potential of extracts and an isolate from petroleum ether extract of *Cuscuta reflexa* Roxb

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ABSTRACT

This study examined the inhibitory effect of Stigmast-5-en-3-O-glucopyranosidetriacetate-5[1]-ol (SGTA), an isolate from petroleum ether extract of *Cuscuta reflexa* and performed comparative study of petroleum ether extract (PTE), ethanolic extract (ETE) and SGTA on hair growth activity in androgenic alopecia rat model. Alopecia induced in albino rats by testosterone administration subcutaneously for 21 days. Finasteride solution was applied topically served as standard. *In vitro* experiment to study the effect of extracts and isolate on activity of 5α-reductase enzyme and comparing with finasteride. *In vivo* experiment showed that rat follicular density and anagen/telogen (A/T) ratio were increased in the PTE, ETE and SGTA treated group when compared to a control group. Skin histological results shown that the PTE, ETE and SGTA treated group had an increase in number and shape of the hair follicles and increase in the follicle anagen/telogen ratio when compared to the finasteride and control group. The result indicated that the ethanolic, petroleum ether extract and isolate of petroleum ether extract of *C. reflexa* found useful in the treatment of androgen-induced alopecia in the experimental animal. In summary, SGTA and extract control the apoptosis of hair cells and retarded the testosterone induce alopecia and therefore be a natural product with much impending for use as a treatment for androgenic alopecia.

1. Introduction

The term androgenetic alopecia frequently used to express the patterned loss of scalp hair in genetically vulnerable men and women. Androgenetic alopecia (AGA) is an androgen-driven condition in genetically prone individuals that affects half of the male population (Otberg et al., 2007). There are various genetic and environmental factors, which are engaged in causing AGA. In androgenetic alopecia miniaturization of

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genetically vulnerable terminal hairs into vellus hairs in the affected region, that is mainly drive by androgen. The key androgen is supposed to be an active metabolite of testosterone called dihydrotestosterone, rather than testosterone itself. The 5α-reductase type-2 isoenzyme is mainly responsible catalyst for the conversion of testosterone into dihydrotestosterone because of this it is main target for treatment of alopecia (Sinclair, 2005).

Presently available therapies for treatment and management of androgenetic alopecia are antiandrogens and biological response modifiers. On the other hand, the low success rate and related adverse effects confines their clinical use (Price, 1999). Nowadays natural products consider in cosmetics and about many kinds of extracts from plant investigated for hair growth activity. Various plants and herbal formulations reported as hair growth promoter as well as used for enhancement of hair quality in traditional Indian system of medicine, but being deficient in sound scientific support and information confines their use (Roy et al., 2005).

C. reflexa Roxb belongs to family Convolvulaceae. It is a parasite, perennial herbs with slender long yellow or golden stems. It is also called as dodder or akashabela or amarabela or swarnalata. It is widely distributed in tropical and temperate regions and common in all over India and Ceylon. It depends on host plants, mostly thorny herbs for growth and nutrition and sometimes entirely covering the bushes and trees (Dorr, 1990; Patel et al., 2012). Conventionally, it is mainly use as a purgative in the management of protracted fever, diaphoretic, and demulcent (Chopra et al., 1992; Kritikar and Basu, 1984). Antisteroidogenic properties of methanolic extract of *C. reflexa* have been reported (Gupta et al., 2003). In our earlier study, we reported the hair growth promotion of the petroleum ether extract of this herb on denuded skin surface of albino (Roy et al., 2006, Pandit et al., 2008). The present study, our investigation was an attempt to compared the efficacy of petroleum ether (PTE), ethanolic extract (ETE) and Stigmast-5-en-3-O-glucopyranosidetriacetate-5[1]-ol (SGTA), an isolate from petroleum ether extract of *C. reflexa* for promoting hair growth in testosterone-induced hair loss and to showed that the ETE and SGTA also inhibits conversion of testosterone to its more potent metabolite, dihydrotestosterone by inhibiting 5α-reductase type 2 enzymes thus inhibiting hair loss as PTE.

2. Materials and methods

2.1. Plant material and authentication

Stems of *C. reflexa* growing on *Bougainvillea spectabilis* and *Jasminum multiforum* were collected in the month of Nov–Dec 2010 from forests surrounding our university campus, Sagar and were authenticated by Dr. P.K Tiwari, Department of Botany, Dr. H.S.Gour University (Herbarium no. Bot/Her/2123). The plant material dried in sunlight and reduced to a coarse powder.

2.2. Extraction

Coarsely powdered stems of *C. reflexa* feed in a soxhlet apparatus and extracted with petroleum ether (60–80 °C) and with ethanol (95%) in separate assemblies to obtain petroleum ether extract and ethanolic extract respectively.

2.3. Chromatographic characterization

C. reflexa is a parasitic plant that draws its nutrients from the host. Phytoconstituents expected to show a discrepancy with the host, and it is accordingly desirable that material collected from the recognized host. For the present study, stems of *C. reflexa* collected from plants growing on the *B. spectabilis* and *Jasminum multiforum*. The Petroleum ether extract and ethanolic extract characterized by thin layer chromatography (TLC) on precoated silica developed in toluene/ethyl acetate (97: 3) and Chloroform: Methanol (9:1) as mobile phase respectively. The plates sprayed with anisaldehyde sulfuric acid reagent and heating at 105 °C for 10 min gave eight and four spot respectively.

2.4. Isolation, purification, and characterization of active compound

The isolation of compound done based on solubility. For isolation, the petroleum ether extract suspended in acetone and shaken strongly to dissolve the extract. The insoluble mass centrifuged at 2000 rpm, collected, and suspended into ethyl acetate later than this it separated into ethyl acetate soluble and insoluble fraction. Then, concentrated the ethyl acetate soluble fraction and it yielded yellowish white solid crystalline material after keeping in a refrigerator for about 12 h. It was further purified by crystallization; the isolated compound SGTA melted at 120 °C and gave an R_f value of 0.83. In solvent system toluene: ethyl acetate (97: 3) it gave a single spot. It gave Lieberman burchard test and positive Salwonski test that confirms the sterols moiety. Based on IR, NMR and mass spectroscopic data it confirms the presence of steroidal molecule (Fig. 1).

2.5. In vivo studies on hair growth

2.5.1. Animals

Male Swiss albino rats (6–8 months age, 130–140 g weight) used. The animals were adapted to conventional laboratory conditions with providing standard food and water. The photoperiod kept at 12 h of light and 12 h of darkness and temperature (25 ± 3 °C). All animal experimentation carried out after approval of the protocol by the Institutional Ethical

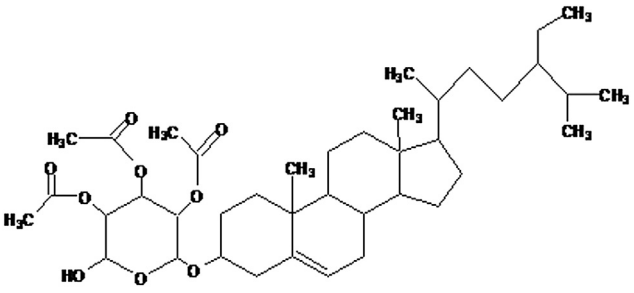

Fig. 1 – Stigmast-5-en-3-O-glucopyranosidetriacetate-5[1]-Ol.