ABSTRACT

Hair related problems are common dermatologic disorders and more than 50% world populations irrespective of gender, race, region or culture are affected. Treatment of hair related problems such as seborrheic dermatitis, telogen effluvium, anagen effluvium, male and female pattern hair loss, head lice infection and dandruff are the major concerns among the cosmetologists and dermatologists. Among the hair related disorders, alopecia is the most common form of abnormal hair loss, which affects a large population of men and women as well as children and young adults. About 30-48% patients are reported to be affected before the age of 20 and around 70% cases occur between 10-25 years of age. Alopecia is characterized by rapid non-scarring hair loss that range from single to multiple confluent patches. Apart from the metabolic and hereditary causes, alopecia has also been observed as a major side effect of anticancer, immunosuppressant and many others drug treatments. In addition to this, abnormal hair loss may also be induced in the body by severe illnesses, injuries, infections, surgery, crash diets, psychological stresses, pregnancy, thyroid disorders, iron deficiency, anemia or drugs.

The present investigation was undertaken to isolated useful components from the traditionally known medicinal plants and to determine their effect in hair follicle regeneration in the case of alopecia. The plants were selected on the basis of their availability, potentiality and use in different hair ailments. Two medicinal plants *Eclipta alba* Hassk and *Aloe barbadensis* Miller were selected and their morpho-phenological characters were studied. Isolation of genomic DNA was

standardized using a modified CTAB method from the plants. Genome size was determined following the standard protocol.

Compounds Ea 1 and Ea 2 was isolated and purified from the methanolic and ethylacetate extracts of *Eclipta alba* by performing TLC, column chromatography (CC) and HPLC. The spectral data from IR, MS, proton and carbon NMR suggested the compound Ea 1 to be a saponin with the probable structur 6-(2'-β-D-glucopyranoslyoxy-4'-hydroxy-6'-methyl)-phenyl-4-methoxy-2-pyrone, possessing molecular weight 620.5 and in the case of the compound Ea 2, probable structure was found to be 3-(5-amino-2,3-dimethylcyclohexyl)-4-hydroxy-1,6-dimethylpiperidin-2-one with a molecular weight 268.22. Compound Av 3 and Av 4 isolated and purified from the ethylacetate extract of *Aloe barbadensis* using TLC, column chromatography (CC) and HPLC, identified to be pyrone. The probable structure of the compounds was determined using IR, MS, proton and carbon NMR. The structure of compound Av 3 was determined to be 1-(3-ethoxy-6-hydroxy-4-methoxy-2, 5-dimethylphenyl) ethanone, with molecular weight 238.12; and in the case of compound Av 4, the probable structure was found to be methyl 2-(7-hydroxy-4-methoxy-5-methyl-2H-chromen-2-ylidene) acetate, with molecular weight 262.26.

The antimicrobial activity as assessed by agar well diffusion method of the compounds against five bacterial species such as *Bacillus subtilis* (MTCC 619), *Klebsiella pneumoniae* (MTCC 109), *Escheichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 737) and *Pseudomonas aeruginosa*; also two fungal strains *Candida albicans* (3017) and *Fusarium oxysporium* (MTCC 284)

exhibited the compound Ea 1 to have the largest zone of inhibition (24.0 ± 0.14 mm) against *B. subtilis* (MTCC 619) followed by Av 4 (23.0 ± 0.29 mm). Compound Ea 1 and Av 4 also exhibited strong antifungal activity against both the fungal strains as compared to the compounds Ea 2 and Av 3. The radical scavenging activity of all four phyto-compounds was biochemically studied using DPPH scavenging assay. Both the compound Ea 1 and Av 4 exhibited good scavenging property as compared to gallic acid and quercetin. In the case of IC_{50}, the lowest value was 8.64±0.79 mg exhibited by Ea 1 and maximum was 82.06±0.12 mg exhibited by Av 3. Cytotoxicity of the phyto-compounds was studied in murine macrophage cell line (RAW 264.7) which showed cell proliferation in comparison to standard Kanamycin proving their non-toxic characters. The high LD_{50} values of the isolated phyto-compounds supported the same.

Wistar albino rat was used in the present investigation as the animal model to induce alopecia. Warfarin was used as alopecia inducing chemical as it is known to cause alopecia as a side effect of medications. Minoxidil 2% solution was used as the standard drug to compare the hair regeneration ability of the isolated phyto-compounds. Before the application of four phyto-compounds on the animal model, their acute dermal irritation study was conducted on rabbits according to the OECD guidelines 404. The study revealed that all four compounds were non-irritant as compared to the positive control, 0.8% formaldehyde solution after 72 h of exposure. All four phyto-compounds were applied topically on alopecia affected area of wistar rats for 15 days. Among the

four phyto-compounds, only two Ea 1 and Av 4 exhibited hair follicle regeneration and growth stimulation activities. These two phyto-compounds stimulated the hair follicles in a shorter time period as compared to minoxidil. Same effect was observed in the case of completion of hair growth. The average length and weight of hair in the case of the positive control and the phyto-compounds treated animals showed promising result. Histological study of the control and the treated animal skin tissue confirmed distinct change in follicular activity. Hematological and biochemical effects due to the topical application of the phyto-compound no significant changes as against the control.

In the present investigation, out of four phyto-compounds, two compounds Ea 1 and Av 4 showed strong hair follicle regeneration and growth promotion as compared to the minoxidil treatment. The study suggested the use of these two phyto-compounds in the cosmetics and drugs to be used against hair fall and alopecia. Also these two compounds may possibly be used as alternative medications to minoxidil.