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***In vivo* hair growth activity of *Prunus dulcis* seeds in rats**

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Abstract

Seeds of *Prunus dulcis* were traditionally known for its hair growth activity. The study was aimed to investigate the efficacy of various extracts of *P. dulcis* as a potential hair growth promoter. Petroleum ether, methanol, chloroform and water extracts of *P. dulcis* seeds incorporated in oleaginous ointment base were applied topically on shaved denuded skin of albino rats and screened for hair growth activity. Petroleum ether extract showed consistent and significant increase in the length of hair ($p < 0.001$) and also showed a good percentage of hair follicles in the anagen phase after histological studies. The total number of days taken to complete hair growth for petroleum ether was 24 where as for methanol, water and chloroform extracts were 28, 29 and 30 respectively. From this study it can be concluded that the seed extracts of *P. dulcis* exhibits a significant potency in promoting hair growth.

Keywords: *Prunus dulcis*; hair growth; cosmeceutics; ointments; minoxidil.

Introduction

In mammals, hair plays a vital role in thermal insulation and also for social and sexual communication, both visually and as a means for dispersing scents secreted by skin glands. Humans are relatively hairless compared to other mammals and human hair has no known significance for survival of species. However, it remains an important cosmetic asset (Andrew, 1993). Hair loss is one of the dermatological disorders to human race which is common throughout the world and is of great concern for decades (Olsen, 1995). Many factors such as metabolism, hormones, heredity and side effects of antineoplastic and immunosuppressant drugs, have been negatively affecting the healthy growth of hair. The usage of synthetic drugs, minoxidil and finasteride approved by FDA (Goodman and Gilman, 1996; Libecco and Bergfeld, 2004) have abbreviated due to their side effects. After 5 years of minoxidil usage the improvement has been shown to peak for one year with a slow decline in regrowth over subsequent years. These crises lead to the search for natural products from plant origin possessing potential hair growth activity. The folklore claim of medicine in Malaysia acclaims the hair growth promotion of medicinal plants belonging to various families, but lack of scientific literatures limited the use of these plants among community. *Prunus dulcis* (Mill.) D.A.Webb belongs to the family Rosaceae is a

tree of 10 m height, fruits are oblong drupe of 30–60 mm length and its seeds are flat, long-ovoid with a brown seed coat (James, 1983). Seeds of *P. dulcis* has been traditionally claimed to possess hair growth promoting activity and the oil from the seeds were used by ethnic tribes for premature hair fall (Phondke, 1992). These seeds are a good source for proteins (arginine, histidine, lysine, phenylalanine, leucine, valine, tryptophane, methionine, cystine) and vitamins (thiamine, riboflavin, niacin, ascorbic acid, biotin, folic acid) and tocopherols. The fatty acid contents of the oil are myristic, palmitic, oleic, and linoleic acids (Chakre, 1992). In the present study, the hair growth initiation and promotion of various extracts such as methanol, chloroform, petroleum ether and water of *Prunus dulcis* seeds were investigated.

Materials and Methods

Collection and extraction of plant material

Seeds of *P. dulcis* were purchased from the local market in the month of February 2008, Alam Jaya, Selangor, Malaysia. It was identified and authenticated by Dr. J. Anbu Jeba Sunilson, Pharmacognosist, Masterskill University College of Health Sciences, Selangor, Malaysia. A specimen sample (MUCHPH/03/F(2)/20008) was preserved in the herbarium, School of Pharmacy, Masterskill University College of

Health Sciences, Malaysia for future reference. All the seeds were then dried in an oven at 50°C for 2 hours and later size reduced to coarse powder in an electric mill (Chiheb *et al.*, 2009). The powder (1000 g) was successively extracted with petroleum ether, chloroform, methanol and water by Soxhlet extraction (Bose *et al.*, 2007). These extracts were concentrated using rotary vacuum evaporator and kept in dessicator until further investigation.

Preparation of ointment

The petroleum ether, chloroform, methanol and water extracts of *P. dulcis* were incorporated into ointment base in concentration of 5% (w/w). All the ointments were made following the procedure given in Indian Pharmacopoeia, 1996. The extracts were then incorporated in the prepared oleaginous base.

Animals

Wistar albino rats of either sex, weighing 200–250 g, were obtained from Masterskill laboratory animal house, Selangor, Malaysia. All the animals were housed in cages at a room temperature of 25 ± 3°C with 12h dark/12h light cycles. The rats were fed with rat pellets and water *ad libitum*. The experiments were approved by the Institutional Animal Ethical Committee of Masterskill University College of Health Sciences, Malaysia.

Primary skin irritation test

Hairs from the dorsal side of the rats were removed using hair clippers and electrical shavers. A commercially available hair remover (Veet) was applied for complete removal of hair from 1 sq cm area (Roy *et al.*, 2007). The shaved area was cleaned with surgical spirit and the animals did not show any toxic effects when petroleum ether, chloroform, methanol and water extracts were applied in a concentration of up to 10% for 48 hours post application. Hence the prepared extracts were considered safe for topical application (Uno and Kurata, 1993).

Treatment for hair growth activity in vivo

Thirty albino rats were divided into six groups of five animals in each group. Hairs from a 3 cm² area at the dorsal portion of all the rats were sheared using electric shavers and applied with marketed hair remover to completely remove

hair. Group 1 served as a control was applied with simple ointment. Group 2 – 5 were topically applied with ointments of various extracts such as petroleum ether, chloroform, methanol and water respectively. Group 6 was applied with 1ml of 2% minoxidil over the shaved area. All the ointments and standard drug were applied once in a day. The treatment was continued for 30 days and hair growth pattern was observed and tabulated (Mithal and Shah, 2000; Tortora, 1996). Skin biopsies were taken on the 10th, 20th, and 30th day for follicular observation.

Hair length determination

Hair was plucked randomly using sterile forceps from the shaved dorsal area of rats on 15th, 20th, 25th, and 30th days of treatment. Hair length was measured and the results were recorded as mean length ± SEM of 25 hairs.

Histological studies

One rat from each group was euthanicated after 10, 20 and 30 days of treatment. Skin biopsies were obtained from the shaved portion and preserved in 10% formalin. Sections of tissues were implanted in paraffin wax and sectioned into a thickness of 10 µm. The sectioned tissues were stained with haematoxylin and eosin and the follicular phases of hairs were examined under microscope with an ocular micrometer (Sawada *et al.*, 1987).

Statistics

The data for hair growth length of albino rats were expressed as mean ± SEM. The values obtained for the above parameters were compared with control group using Student's t-test. The values of p<0.01, p<0.05 and p<0.001 were considered to indicate a significant difference between the groups.

Results

Growth of hair from the shaved dorsal area was monitored and the number of days taken for the initiation of growth was recorded in table 1. In comparison to the standard, the time taken to complete the hair growth was found to be 24 days for petroleum ether ointment followed by methanol (28 days), water (29 days) and chloroform (30 days).

Table 1: Qualitative observation of hair growth.

Treatment group	Dose (%)	Number of days taken to initiate hair growth	Number of days taken to complete hair growth
Control	-	8	31
Petroleum ether extract	5%	7	24
Methanol extract	5%	7	28
Chloroform extract	5%	9	30
Water extract	5%	8	29
Standard (minoxidil)	2%	6	22

The increase in the length of hair from the denuded area at the end of 2nd week and the further observations in length on the due course of the treatment were tabulated as table 2. On comparing with the control, all the extract treated groups and the standard were fully covered with

hair on the 4th week of the treatment. At the end of treatment, maximum length for hair was observed in the group treated with petroleum ether extract. The growth of hair was sparse for chloroform and water extracts and there was no substantial difference in the texture of hair.

Table 2: Effect of various extracts of *Prunus dulcis* on hair length of albino rats.

Treatment group	Dose (%)	Length of hair (mm)			
		Day 15	Day 20	Day 25	Day 30
Control	-	4.6 ± 0.5	7.8 ± 1.3	11.4 ± 0.8	14.8 ± 0.6
Petroleum ether extract	5%	7.3 ± 0.8**	10.4 ± 1.1***	16.3 ± 1.4***	16.4 ± 0.4***
Methanol extract	5%	5.8 ± 0.9	8.2 ± 1.3*	12.7 ± 1.5*	15.5 ± 0.8
Chloroform extract	5%	6.1 ± 0.8*	7.9 ± 1.6	11.2 ± 0.9	15.1 ± 0.7
Water extract	5%	5.1 ± 0.4	8.0 ± 0.5	12.8 ± 0.6*	16.0 ± 0.6**
Standard (minoxidil)	2%	8.2 ± 0.6***	11.2 ± 1.3***	16.7 ± 1.6***	16.9 ± 0.5***

* p<0.01, ** p<0.05, *** p<0.001. Compared to control group by student's t-test (n = 25 hairs).

Table 3: Hair growth activity of different extracts of *Prunus dulcis* on anagen/telogen phases.

Treatment group	Dose (%)	Percentage of hair follicles					
		Day 10		Day 20		Day 30	
		Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Control	-	36	64	46	54	54	46
Petroleum ether extract	5%	43	57	60	40	72	28
Methanol extract	5%	40	60	54	46	63	37
Chloroform extract	5%	39	61	49	51	62	43
Water extract	5%	41	59	54	46	60	40
Standard (minoxidil)	2%	48	52	62	38	71	29

There were 5 to 7 hair follicles counted per mm of the skin for both extract and control treated groups and on histological observation, there was no difference in the number of hair follicles post treatment. The difference in the various cyclic phases of the hair follicles such as anagen and telogen phases for the treated and control groups were examined and the findings were recorded in table 3. On the 10th day of the

Discussion

The need for the identification of novel hair growth promoter is of immense need as there are only two drugs such as minoxidil (topical) and finasteride (oral) which were approved by FDA for the treatment of alopecia (Kakali *et al.*, 2009). Minoxidil, a potassium channel opener proved to be effective in 54% of the treated subjects in a human randomized control trial by Upjohn Company. The treated subjects exhibited significant adverse dermatological reactions such as pruritis, dryness, scaling, local irritation and dermatitis (De Villez and Richard, 1990). In a pattern of baldness shown in male population otherwise termed as androgenic alopecia, finasteride which is a 5 alpha reductase inhibitor showed to be effective in increasing the growth of hair. Merck research laboratories reported that 48% of the hair growth was observed in finasteride treated subjects in one year clinical trial study. This drug was showed to be well tolerated in patients and a few has been withdrawn from the trial because of sexual disorders due to the treatment (McClellan and Markham, 1999). The American Hair loss association reported that loss of hair has been emotionally distressing and disturbing and makes the affected people vulnerable (Washington post, 2006). These factors lead to the search for novel drugs that revitalize the hair growth pattern and appearance with less adverse effects. So screening of medicinal plants for its potential as a hair growth promoter was brought into limelight. In this study the denuded dorsal area of albino rats when treated with various extracts of *Prunus dulcis* revealed that petroleum ether extract possessed greater potential in promoting the hair growth and induced the anagen phase in telogen (resting) phase of hair follicles. This pattern of growth may be due to the premature shifting of hair follicles from the telogen to anagen phase (Philpot *et al.*, 1992). Uno and Kurata (1993) reported that the conversion of short hairs to long terminal hair, follicular size enlargement and prolonged anagen phase is due to

treatment, nearly 45 – 50 % of the follicles were in anagen phase. At the end of the treatment, petroleum ether extract of *P. dulcis* exhibited with maximum number of hair follicles at the anagen phase. The methanol, chloroform and water extract also showed a higher percentage of anagen phase follicles than the control but lesser than the petroleum ether extract.

increased rate of proliferation of cells. The growth of hair was dense in petroleum ether extract treated group which explains the increased induction of follicles in anagen phase. The transformation of hair follicles from telogen phase to anagen phase in treated groups may be due to the epithelial cell proliferation near the base of the follicles that induce vasodilatation of blood vessels in the scalp as reported by Savin and Atton (1993). All the other extracts also showed considerable hair growth promoting activity which was well comparable with that of standard drug minoxidil. The results obtained validate the folklore claim of the plant being used for the treatment of jaundice.

Conclusion

From this study, we conclude that the hair growth activity of petroleum ether extract of *Prunus dulcis* exhibited more substantial effect compared to methanol, chloroform and water extracts. However, the exact mechanism of *Prunus dulcis* has been unknown and hence identification and isolation of active constituents from the extracts may laminate new directions for treatment of alopecia. Further research is needed for structural elucidation and identifying the mechanism of action responsible for using *Prunus dulcis* as an apparent hair growth promoter.

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