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Research article

Screening of hair growth promoting activity of *Punica granatum* L. (pomegranate) leaves extracts and its potential to exhibit antidandruff and anti-lice effect

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ABSTRACT

The intent of the present investigation was to explore the utility of alcoholic and aqueous extract of *Punica granatum* L. as hair growth promoter along with anti-lice and antidandruff activity. A filter paper diffusion approach was employed for screening of the pediculocidal and ovicidal activity. Albino mice, preselected for their telogen phase of hair growth were used during the study. The prepared extracts, Minoxidil and control were applied over shaved skin surface on to the backs of mice to assess telogen to anagen transition. The qualitative and quantitative analysis was performed. The outcome of the studies revealed that *Punica granatum* L. alcoholic and aqueous extracts exhibited prominent anti-lice activity. The transition of telogen to anagen phase of the number of anagen hair follicle was observed in approximately 45, 27 and 51% of animals treated with alcoholic and aqueous extract of *Punica granatum* L., and Minoxidil, respectively, which suggest the hair growth promoting potential of the extract of *Punica granatum* L. Also, 3 % *Punica granatum* L. alcoholic extracts exhibited a potent antidandruff activity against fungal strains tested. Maltol, was identified as a principal phytoconstituent in the alcoholic extract. The findings greatly suggest anti-lice, antidandruff and hair growth promoting potential of the extract of *Punica granatum* L.

1. Introduction

Hair is one of the crucial parts of body which is derived from ectoderm of the skin. It is often considered as one of the protective appendages on the body of human beings and animals (Ebling, 1987). It has a marked effect on overall personality of an individual and appeal of the human body (Stough et al., 2005). Basically, hair is made up of a protein termed as keratin which is produced in the hair follicles in the outer layer of skin. The follicles keep on generating newer hair cells and the older ones are propelled out through the surface of the skin at the rate of about six inches a year. In fact, the hair what we observe on the skin surface is nothing but a string of dead keratin cells (Cash, 2001). On an average an adult head have nearly 100,000 to 150,000 hair, amongst which around 100 or more are normally shed off every day.

Alopecia refers to a dermatological disorder which has been known since years back and is considered to be a curse to mankind. Androgenic alopecia is a genetic condition which is observed in both populations, men as well as women. The classification of the balding scalp in men into types I-VIII proposed by Hamilton is still one of the most extensively used and well-known classification (Venning and Dawber, 1988). In the male pattern baldness, Men generally start experiencing hair thinning and loss of hair as early as in their teen ages or early 20s. It is generally characterized by a receding hairline which is followed by gradual thinning and loss of hair from the crown and frontal scalp. Conversely, women suffering from female pattern baldness usually do not suffer from the problem of thinning and loss of hair till their 40s or later. Generally, females experience an overall hair thinning over the entire scalp, with the most widespread hair loss at the crown (Stough et al., 2005). Studies on

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finding of novel hair growth promoters is the need of time as only two drugs namely Minoxidil (topical) and Finasteride (oral) have been approved by Food and Drug Administration (FDA) and are available in the market for the treatment of alopecia. Moreover, these drugs are not totally devoid of side effect(s) (Kakali et al., 2009).

In the traditional Indian system of medicine, numerous plants, herbs and herbal formulations have been reported for hair growth promotion as well as for improvement of quality of hair. However, owing to lack of sound scientific studies and supportive evidences the entire information finds limited usage (Kirtikar and Basu 2012). Countless, plant extracts and herbs have been traditionally claimed for their effective use in the treatment of hair loss in the oriental medicine. However, there is no report available on hair growth promoting activity of *Punica granatum* L. (*P. granatum*) leaf extract. Therefore, the present study was focused on the scientific investigation of the hair growth promoting potential of *P. granatum* extracts.

P. granatum (Pomegranate) is a member of family *Punicaceae* (now in *Lythraceae*) is a deciduous spreading shrub or small tree only in temperate regions but evergreen in tropical and subtropical condition and has thorns with it and the plant is found all over India. Pomegranate peel is an inedible part obtained during preparation of pomegranate juice and is often discarded. It is a prosperous source of tannins, flavonoids, polyphenols and some anthocyanins namely Delphinidins and Cyanidins (Li et al., 2006). Several studies on the extracts of *P. granatum* and compounds have exhibited high antioxidant (Aviram et al., 2008), anti-inflammatory (Kitchen et al., 2004), anti-carcinogenic (Bell and Hawthorne 2008), and antimicrobial potential (Duraipandiyan et al., 2006). Copious herbal plants and remedies have been documented in Ayurveda and other ancient Indian texts and their preparations have been found to be imperative in the treatment of a range of diseases (Bhutkar et al., 2018; Randive et al. 2016a, b, 2019, 2020, Randive et al. 2019). Based on these traditional uses of the plant we have made an attempt to investigate the extracts of *P. granatum* (alcoholic and aqueous) for its hair growth promoting activity along with antidandruff activity.

Pediculus humanus capitis, referred to as the human head louse is considered as a vital concern in public health-associated problems. There has been an immense arousal in pediculocidal drug resistance towards head louse which has initiated research to explore novel anti-lice agents from medicinal plants (Sunilson et al., 2009). This study was therefore designed to screen the anti-lice activity of *P. granatum* (alcoholic and aqueous) extract by using a filter paper diffusion approach.

2. Materials and methods

2.1. Animals

Healthy Swiss Albino mice of (male and female) weighing 18–25 gm were housed in polypropylene cages maintained under standard conditions in the animal house of RMES's College of Pharmacy, Gulbarga, in accordance with the guidelines laid by the Institutional Animal Ethical Committee (IAEC) committee. The research work was approved by RMES's College of Pharmacy, Gulbarga, Karnataka, INDIA as per guidelines of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India with an protocol approval no. RMES/CPCSEA/IAEC/01/09.

The mice in all groups were morphologically preselected for their hair growth cycle (Datta et al., 2009). The mice were clustered in 4 groups of 4 each from either sex. A 4 cm × 3 cm area of hair from dorsal portion of all mice was shaven with hair trimmer. 0.2 mL of 3 % alcoholic and aqueous plant extracts were applied to the selected mice. A standard group was applied with 2 % Minoxidil (MINTOP) solution in alcohol (Jaybhaye et al., 2011). Vehicle control group received 95% alcohol as a vehicle treatment. The application of MINTOP solution was continued for 1 month and simultaneously during the treatment hair growth pattern was observed (Jung et al., 2010; Seok-Seon et al., 2002).

2.2. Chemicals

MINTOP 2% Solution (Dr. Reddy's) containing Minoxidil was purchased from local market at Kasegaon, Sangli (MS), India.

2.3. Collection and extraction of plants parts

Fresh leaves of *P. granatum* were collected from Bhingewadi, INDIA and taxonomically recognized at the YC College, Karad (Voucher No - YC-22). The collected plant material was dried in hot air oven at 40 °C for 48 h, thereafter it was crudely powdered and properly stored. 500 g powder was defatted with petroleum ether for 65–85 °C in a Soxhlet extractor followed by extraction with alcohol and water (Bhinge et al. 2017). The collected alcoholic and aqueous extract was further concentrated on rotary evaporator and stored in a vacuum dryer until used (Bhinge et al. 2017).

2.4. Microorganism used

The test organisms used in this study were *Candida albicans (C. albicans)*, *Aspergillus niger (A. niger)* and *Penicillin notatum (P. notatum)*. The culture was collected from Yashawantrao Chavan College of Science, Saidapur, Karad (MS) INDIA - 415110.

2.5. Qualitative analysis of P. granatum (alcoholic and aqueous) extract

Qualitative test was executed for the identification of phenolic compounds, flavonoids, alkaloids, anthraquinone glycosides, cardiac glycosides, saponin glycosides, flavonoids, carbohydrates, tannins and other phytoconstituents like organic and inorganic matter. It was carried out as per methods described by Siddiqui and Ali (1997), Sithara et al. (2016) (Sithara et al., 2016; Siddiqui and Ali 1997).

2.6. Pediculocidal and ovicidal effects

As per the procedure mentioned by Sunilson et al. (2009), *Pediculus humanus capitis* (head lice) were collected from the girls at the age between 10-15 years by combing their scalp using a new comb for screening of the anti-lice activity (Sunilson et al., 2009). Nits, nymphs, and adults of the *Pediculus humanus capitis* was carefully clustered in a steel container. The samples of *P. granatum* alcoholic and aqueous extract were tested for pediculocidal activity by employing filter paper diffusion method (Picollo et al., 2000). *P. granatum* alcoholic and aqueous extract were dissolved in distilled water to yield three concentrations of each extract (3, 5 and 10 %). Subsequently, the test organisms were clustered in 7 groups, and each group contained 5 human lice in a proportion of 2:3 (Nymphs/adults). The selected lice were placed in the Petri dish containing filter paper. With the aid of micropipette, a 500 μL of selected concentrations of *P. granatum* alcoholic and aqueous extract samples were poured over lice to get thin layer up to 4 cm^2 (Carpinella et al., 2007). Group I which was treated with distilled water served as a Control. Whereas, Group II, III, and IV were treated with 500 μL of 3 %, 5% and 10% *P. granatum* aqueous extract dissolved in distilled water, respectively. Group V, VI and VII received 500 μL of 3%, 5%, and 10% of *P. granatum* alcoholic extract respectively, and Group V, VI and VII received 500 μL of 3%, 5%, and 10% of benzyl benzoate 25% (w/v) (RidPed) as a Standard respectively. Thereafter, petri dishes were kept in dark room maintained at a temperature of 27 ± 1 °C with relative humidity 70 ± 5 %. After completion of 1 h treatment, all the petri dishes were added with 500 μL of distilled water sample and placed in a dark room as per the aforementioned condition. After 18 h the movements of lice were observed under the dissecting microscope, whereas, the movement less lice was considered as dead lice (Meinking et al., 1986).

Five oval eggs (unbroken operculum) with brown color were placed in each Petri dish containing Whatmann filer paper (6 cm diameter in size). 500 μL of samples, control, *P. granatum* alcoholic and aqueous

Table 1. Effects of *P. granatum* alcoholic and aqueous extracts against *Pediculus humanus apitis*.

Treated Sample	Against *capitis* (nymphs and adults)	Against nits	
	Mortality in Average (%) (Mean ± S.D.)	Emergence (%) (Mean ± S.D.)	
		After 6th Day	After 14th Day
Distilled water	8.27 ± 1.3868	83.05 ± 1.6575	92.44 ± 0.9098
3 % *P. granatum* alcoholic extract	20.76 ± 0.7809	45.95 ± 1.6757	32.60 ± 1.6849
5 % *P. granatum* alcoholic extract	46.06 ± 1.1278	28.06 ± 1.0908	22.89 ± 1.8787
10 % *P. granatum* alcoholic extract	65.50 ± 1.4380	15.65 ± 2.0910	10.33 ± 1.8966
3 % *P. granatum* aqueous extract	5.34 ± 1.6575	67.98 ± 1.6756	80.56 ± 1.9909
5 % *P. granatum* aqueous extract	14.65 ± 1.6565	52.45 ± 2.0901	43.76 ± 1.9898
10 % *P. granatum* aqueous extract	20.78 ± 2.0091	43.65 ± 1.7868	35.67 ± 1.6750
3 % benzyl benzoate 25% (w/v) (RidPed)	39.04 ± 1.9091	4.62 ± 2.0910	0.00 ± 0.0000
5 % benzyl benzoate 25% (w/v) (RidPed)	60.04 ± 1.9980	0.00 ± 0.0000	0.00 ± 0.0000
10 % benzyl benzoate 25% (w/v) (RidPed)	100.00 ± 0.0000	0.00 ± 0.0000	0.00 ± 0.0000

extract in concentration 3, 5, and 10 % were dropped as per the aforementioned procedure. Subsequently, all the petri dishes were kept in a dark chamber maintaining the condition mentioned above for continuous 14 days with proper maintenance of the moisture by addition of 100 μL of distilled water after an interval of 48 h. Finally, hatching of all placed eggs was confirmed under a dissecting microscope, and % of emergence, i.e., partially hatched nits, was noted in triplicate (Sunilson et al., 2009).

2.7. Anti-dandruff activity

Saboraud's agar plate was streaked with selected fungi (*C. albicans*, *A. niger* and *P. notatum*) as per standard protocol (Palla et al., 2018). 100 μl dose of 3 % *P. granatum* aqueous and alcoholic extracts solutions and standard drug Fluconazole in concentration 0.5 % was loaded per bore respectively in aseptic condition with help of sterile micropipette. Plates were kept at room temperature for 30 min and then incubated at 37 °C for 24 h (Palla et al., 2018). The diameter of the zones of inhibition was measured with the help of scale in mm (Chavan et al., 2020b; Bhinge et al., 2020).

2.8. Skin irritation test

Shaven dorsal area of the mice was cleaned using surgical spirit and thereafter alcoholic and aqueous extracts (3%) were applied on skin over 1 sq cm area and the mice were observed for 48 h. The studies of primary skin irritation test revealed no abnormal effect(s) on the shaved area of the mice (Chavan et al., 2020a).

2.9. In-vitro and in-vivo hair growth activity

Two parameters namely, hair growth initiation (minimum period in days to begin detectable hair growth) and hair growth completion time (minimum period in days taken to fully cover the shaved skin area with new hair) were considered for confirmation of hair growth activity (Roy et al., 2008; Savali et al., 2011). Hair growth initiation and completion period were counted for *P. granatum* aqueous and alcoholic extract, standard drug treated mice group and compared with control vehicle treated group after beginning the treatment.

Hair was plucked randomly from the earlier denude area of all mice on 20th, 25th and 30th days after beginning the treatment. The actual length of 20 hair was measured and average was taken for further calculation (Jung et al., 2010; Adhirajan et al., 2003). The outcomes were represented as the mean length ± S.D. of twenty hair.

The mice from *P. granatum* extract (aqueous and alcoholic), 2 % Minoxidil and vehicle treated group were euthanicated on the 30 day of drug treatment. Also, the excised skin was fixed on frame consisting of a metal coin with a radius of 0.9cm. After weighing skin with hair and without hair, difference in weight was calculated as a hair weight (Dascalu, 2013; Zhanga et al., 2016).

Using method of Adhirajan et al. (2003), with slight modification, the histological studies were performed. After the mice were daily treated with *P. granatum* extract (aqueous and alcoholic), standard drug and vehicle for 30th days, the mice from each group were euthanicated on the 31st day. The dorsal skin biopsies at the same position of different groups were taken from shaved area and prepared their slides (Chavan et al., 2020b). From the prepared slides, the number of hair follicles/mm (Sawada et al., 1987) and % ratio of the anagen and telogen of hair follicles was calculated. Also the skin thickness from epidermis to panniculus carnosus was determined (Johnson and Ebling, 1964) using UTHSCSA image tool 300 (Chavan et al., 2020a). Nikon optical microscope was used for the measurement of length of hair follicles (Zhanga et al., 2016).

2.10. Gas chromatography-mass spectrometry (GC-MS) analysis of alcoholic P. granatum extract

GCMS-QP2010 Ultra technology (Shimadzu, Kyoto, Japan) having DB-WAX column (60m, 0.25 mm × 0.25 mm) (Agilent Technologies, Santa Clara, CA, USA) was used for the assessment of crude *P. granatum* alcoholic extract. Mass spectra were acquired in the mass range of m/z 45e850. Compounds were identified by matching their retention time and mass spectra with those from Shivaji University, Kolhapur (MS) 416004, using an automated library search, and percentage of composition was calculated by peak area (Yuan and Yuk, 2018).

Table 2. Antidandruff activity of 3 % *P. granatum* aqueous and alcoholic extracts.

Bacterial strains	Inhibition Zone in mm			
	Control (DMSO)	3 % *P. granatum* aqueous extract (Mean ± SD)	3 % *P. granatum* alcoholic extract (Mean ± SD)	Fluconazole (Mean ± SD)
A. niger	-	12.55 ± 1.2545	14.35 ± 1.5486	16.35 ± 2.0584
C. albicans	-	10.25 ± 2.0214	13.45 ± 1.6547	15.85 ± 1.5460
P. notatum	-	11.55 ± 1.5640	14.25 ± 1.8564	16.45 ± .1.5487

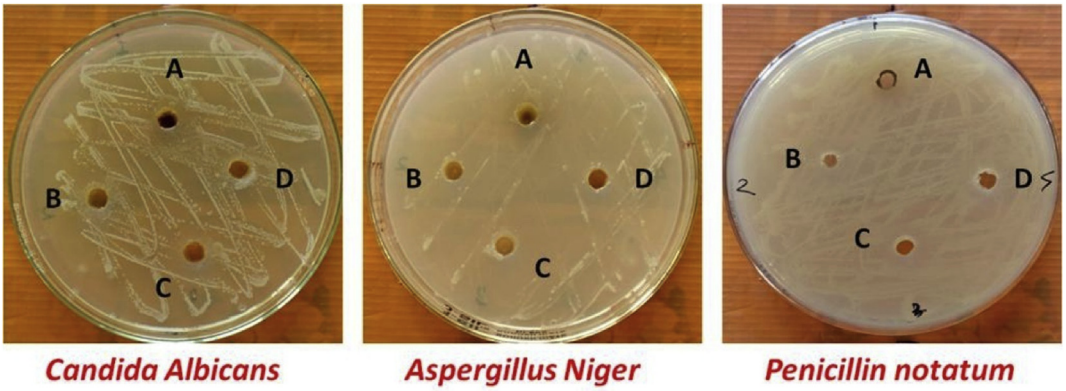

Figure 1. Antidandruff activity of A) *P. grantum* alcoholic extract B) *P. grantum* aqueous extract C) Fluconazole D) Control against *Candida Albicans*, *Aspergillus Niger* and *Penicillin notatum.*

2.11. Statistical analysis

The values mentioned in the tables and texts indicate the mean ± standard error mean (SEM) of the respective groups. Statistical investigation was executed with Student's t-test. A value of $P < 0.0001$, 0.05, 0.01, or 0.001 was considered statistically significant (Chavan et al., 2020a).

3. Result

3.1. Qualitative phytochemical analysis of P. granatum extracts

Qualitative phytochemical analysis of alcoholic extract exhibited the presence of alkaloids, anthraquinone glycosides, cardiac glycosides, saponin glycosides, flavonoids, carbohydrates, tannins. Whereas, the aqueous extract was found to possess alkaloids, cardiac glycosides, saponin glycosides, flavonoids and tannins.

3.2. Pediculocidal and ovicidal effects

The observations were recorded for the Pediculocidal effects of Control, *P. granatum* alcoholic and aqueous extract (3, 5 and 10 %) and the Standard (3, 5, and 10 %). The average mortality for control (vehicle) treated against nymphs and adults lice was noted to be 8.27 ± 1.3868. At the highest concentration of 10 % *P. granatum* alcoholic and aqueous extract the average mortality against the lice (nymphs and adults) was observed to be 65.50 ± 1.4380 and 20.78 ± 2.0091 respectively. 10 % standard treated lice group exhibited 100.00 ± 0.0000 average mortality. The results have been presented in Table 1, which exhibits the mean values ± SEM. For.

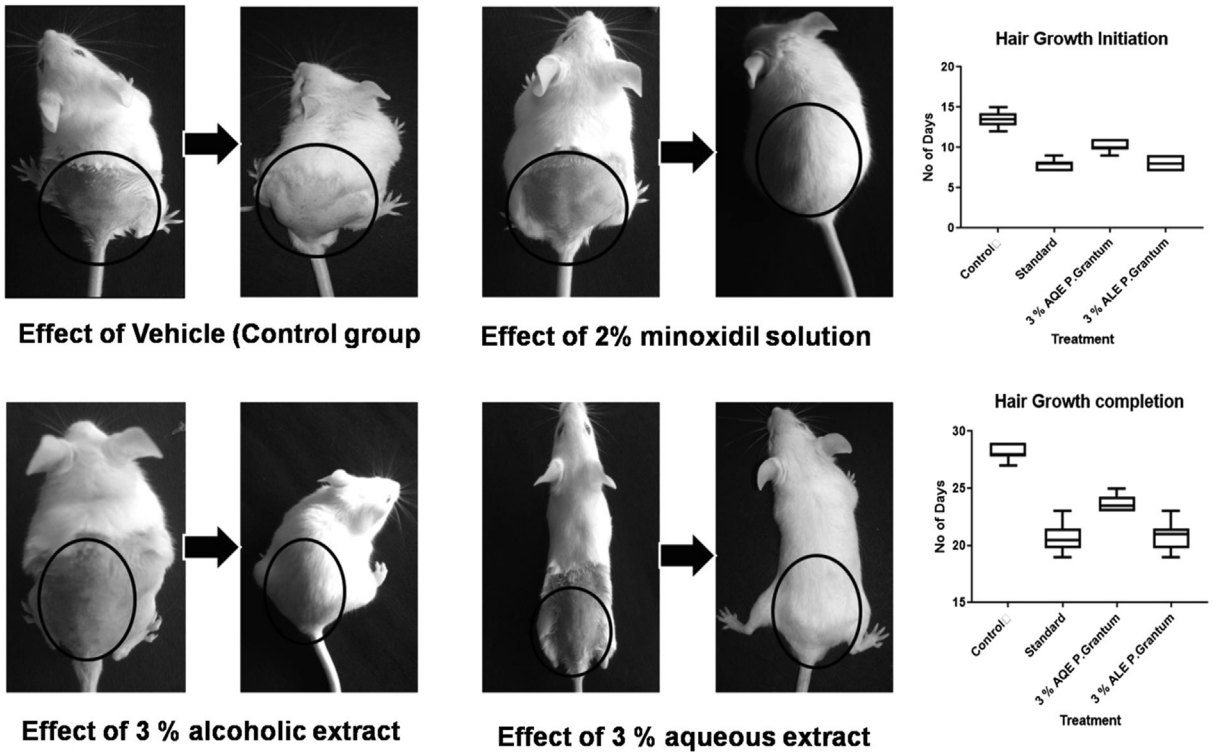

Figure 2. Effects of topical *P. grantum* aqueous and alcoholic extract on hair regeneration in mice. The back skin was topically applied with *P. grantum* aqueous extract (3 %), *P. grantum* alcoholic extract (3 %), 1% minoxidil or vehicle. Photographs were taken on 0^{th} and, 30^{th} days after applying extract or vehicle on the shaved dorsal skin.

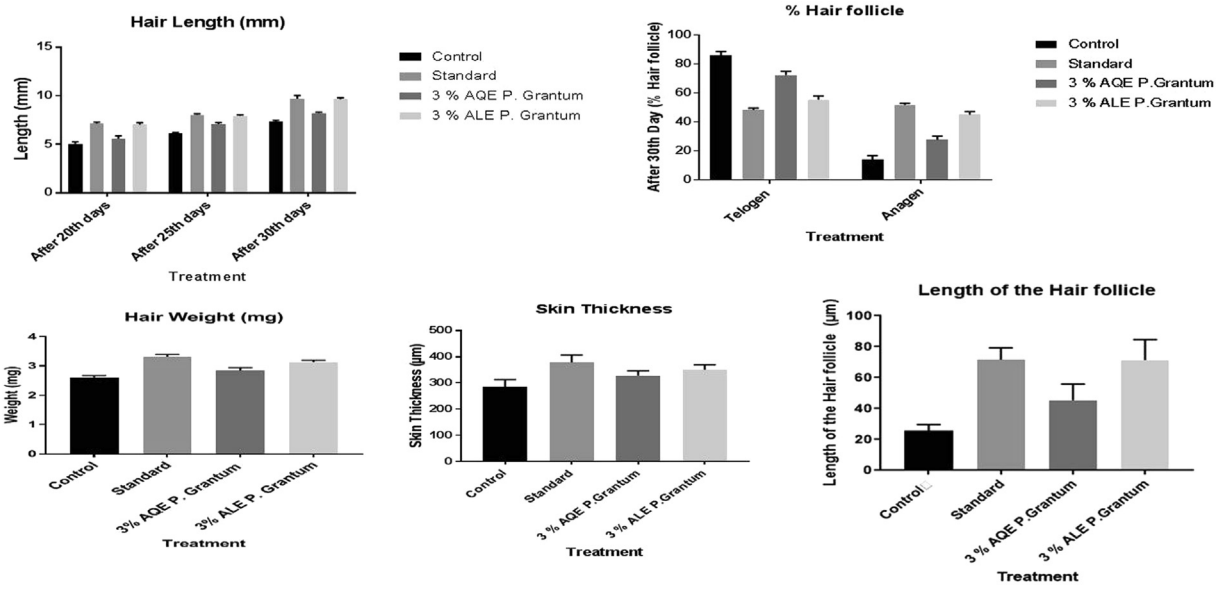

Figure 3. Effect of *P. grantum* aqueous and alcoholic extracts on hair weight (mg), hair length parameter after 20th, 25th and 30th days, % hair follicle (Telogen and Anagen), skin thickness and length of the hair follicle.

Ovicidal effect of Control, *P. granatum* alcoholic and aqueous extract (3, 5 and 10 %) and the Standard (3, 5, and 10%), it was noted to be 83.05 ± 1.6575 and 92.44 ± 0.9098 % emergence for control (vehicle) treated against selected nits after 6th and 14th day of treatment. At the highest concentration of 10 %, the *P. granatum* alcoholic extract exhibited 15.65 ± 2.0910 and 10.33 ± 1.8966 % emergence against the treated nits after 6th and 14th days of treatment respectively. Whereas, no emergence was observed in Standard (10 %) treated nits group after 6th and 14th days treatment. The results are presented in Table 1, which indicate the mean values ± SEM. Based upon the observed outcomes of Pediculocidal and Ovicidal effects of *P. granatum* leaves alcoholic extract posses comparable results as that of used standard.

3.3. Anti-dandruff activity

Anti-dandruff activity of 3 % *P. granatum* aqueous and alcoholic extracts against *C. Albicans*, *A. Niger* and *P. notatum* as models for fungi were investigated. The zone of inhibition of 3 % *P. granatum* aqueous, 3 % *P. granatum* alcoholic extract and Fluconazole were found to be 12.55 ± 1.2545, 14.35 ± 1.5486 and 16.35 ± 2.0584 respectively, against *A. niger*. In case of *C. albicans* zone of inhibition was noted to be 10.25 ± 2.0214, 13.45 ± 1.6547 and 15.85 ± 1.5460 for 3 % *P. granatum* aqueous, 3% alcoholic extract and standard, respectively. While, the zone of inhibition was found to be 11.55 ± 1.5640, 14.25 ± 1.8564 and 16.45 ± .1.5487 for 3 % *P. granatum* aqueous, 3 % *P. granatum* alcoholic extract and Fluconazole respectively, against the *P. notatum*. 3 % *P. granatum* aqueous and alcoholic extracts has been used as a comparable antidandruff drug by antidandruff drugs like Fluconazole shown in Table 2 and Figure 1. Three % *P. granatum* alcoholic extracts exhibited a potent antidandruff activity against fungal strains tested. Three % *P. granatum* alcoholic extracts showed almost same activity like fluconazole (0.5 %) against selected pathogens. The results were found to be statistically significant ($p < 0.05$). Anti-dandruff effect of *P. granatum* leaves alcoholic extract was observed to be satisfactory and comparable as that of standard used in the study.

3.4. In-vitro and in-vivo studies for hair growth activity

It was observed that the hair growth was initiated from the shaved area on 13th to 14th days in control, whereas for the standard and alcoholic extract treated animals the growth was initiated on 6th to 7th day of treatment. For the group treated with the aqueous extract, hair growth was observed to be initiated on 10th to 11th day of treatment. The hair growth on the shaved area was observed to be completed on 28th to 29th days in control, whereas, in the standard and alcoholic extract treated animals the growth was completed on 20th to 21th day of treatment Figure 2. In the aqueous extract treated animals the hair growth was observed to be completed on 23th to 24th day of treatment. As shown in the result in Figure 2, the whole denuded area of vehicle control, standard, aqueous extract and alcoholic extract treated mice was observed to be covered.

The length of hair for the group treated with aqueous extract of *P. granatum* was found to be 5.59, 7.06 and 8.22 mm after 20th, 25th and 30th days respectively. Whereas for the group treated with alcoholic extract of *P. granatum* the hair length was observed to be 7.03, 7.93 and 9.62 mm after 20th, 25th and 30th days respectively. Comparable results were observed for hair length for the group treated with standard drug Minoxidil. In case of vehicle treated control group the hair length was found to be 5.06, 6.10 and 7.30 mm after 20th, 25th and 30th days respectively as compared to other groups, as depicted in Figure 3.

Hair weight was determined after 30th days. For the group treated with 3 % *P. granatum* aqueous and alcoholic extracts, the weight of hair was found to be 2.8466 ± 0.0548 and 3.1366 ± 0.0328 respectively. Whereas for the group treated with 1 % Minoxidil solution, weight of the hair was observed to be 3.3166 ± 0.0440. In case of vehicle treated control group less hair weight was observed as compared to other groups used in the study (Figure 3).

As shown in Figures 3 and 4, the skin biopsies exhibited marked difference in the different cyclic phases (anagen and telogen) of hair follicles in treated and control mice. On the last day of treatment the transition of telogen to anagen phase of the number of % hair follicle was

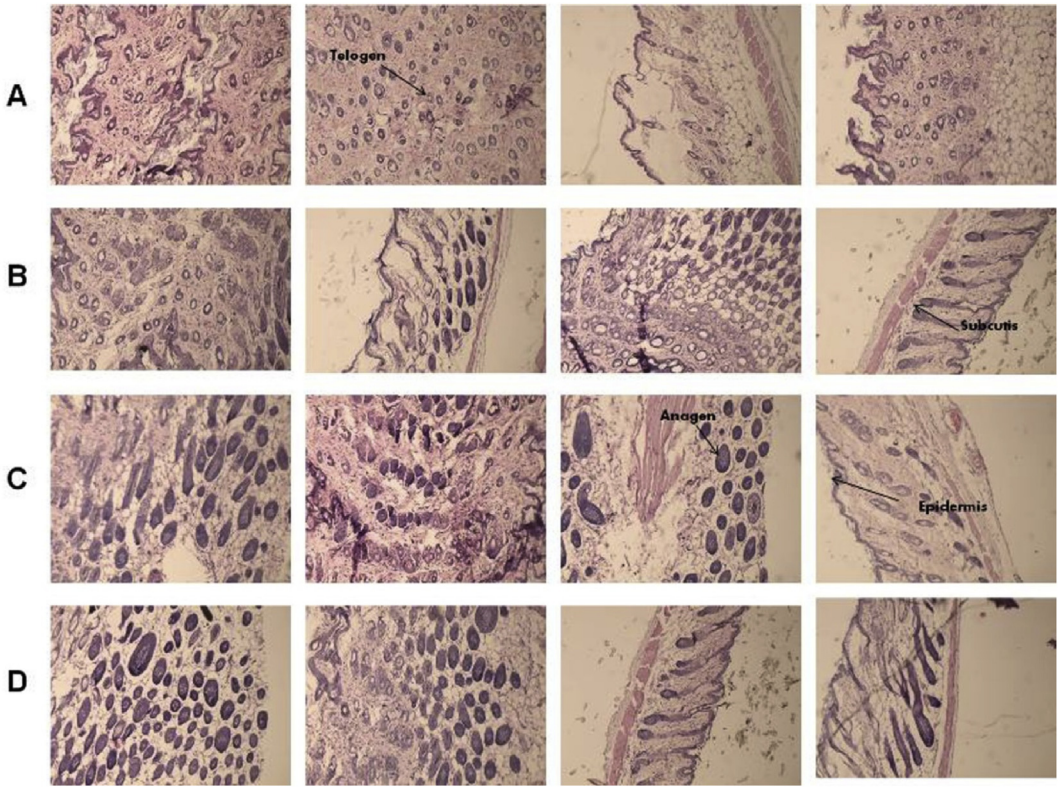

Figure 4. Histopathological studies, the number of hair follicles counted in subcutis A. Effect of vehicle (Control group); B. Effect of 2% minoxidil solution; C. Effect of 3 % *P. grantum* aqueous extract; D. Effect of 3 % *P. grantum* alcoholic extract.

Table 3. Effect of *P. granatum* aqueous and alcoholic extract on quantitative hair growth.

Treatment (Topical) animal groups	After 30th Day (% Hair follicle)		
	Telogen	Anagen	Ratio
Control Vehicle treated	86.00 ± 1.5275	14.00 ± 1.5275	6.1428
Minoxidil 1 % alcoholic solution treated	48.33 ± 0.6666****	51.66 ± 0.6666****	0.9354
P. granatum aqueous extract (3 %) treated	72.33 ± 1.4529****	27.66 ± 1.4529****	2.6144
P. granatum alcoholic extract (3 %) treated	55.33 ± 1.4529****	44.66 ± 1.4529****	1.2296

The results are shown as the mean values ± SEM. ****P < 0.0001, when compared to respective control values by Student's t-test.

observed in approximately 45 % animal treated with alcoholic extract of 3% *P. granatum* whereas 27 % of the animals treated with aqueous extract of *P. granatum* also showed transition from telogen phase to anagen phase characterized by hair follicles. The animals treated with 1 % Minoxidil showed 51 % anagen hair follicle induction. The animals treated with vehicle showed very less anagen induction. The results are depicted in Table 3.

Skin thickness was determined using UTHSCSA image tool 300 after 30th days (Chavan et al., 2020a)., for the group treated with 3 % *P. granatum* aqueous and alcoholic extracts, the thickness of skin was found to be 328.33 μm and 351.00 μm respectively. Whereas for the group treated with 1 % Minoxidil solution, thickness of the skin was observed to be 378.33 μm (Table 4). In case of vehicle treated control group less skin thickness was observed as compared to other groups (Figure 3).

Treatment with 3 % alcoholic extract in mice exerted a more noteworthy effect on the hair follicle length than those of the 3 % aqueous extract, control and 1% Minoxidil groups (Figure 3). The groups treated with extracts (aqueous and alcoholic) attained an average length of 45.33 and 71.34 μm in mice, respectively, whereas hair follicle length in the standard and vehicle treated group was observed to be 71.66 and 25.65 μm respectively. Certainly, by comparing the data, we could find that alcoholic extract had selective promoting activity in the mice. Amongst the studied extract the alcoholic extract was observed to be more

Table 4. Effect of *P. granatum* aqueous and alcoholic extract on skin thickness and length of the hair follicle after 30th Day.

Treatment (Topical) animal groups	Skin Thickness (μm ±mean)	Length of the Hair follicle (μm ±mean)
Control Vehicle treated	285.66 ± 15.5599	25.65 ± 2.3333
Minoxidil 1 % alcoholic solution treated	378.33 ± 16.4147**	71.66 ± 4.4095***
P. granatum aqueous extract (3 %) treated	328.33 ± 10.1379ns	45.33 ± 6.0644ns
P. granatum alcoholic extract (3 %) treated	351.00 ± 10.6926*	71.34 ± 7.6883***

The results are shown as the mean values ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns – not significant when compared to respective control values by Student's t-test.

prominent to exhibit *in vitro* and *in vivo* hair growth promoting potential and the said activity was almost comparable as that of Minoxidil (standard).

3.5. GC-MS analysis of extract

Nowadays, the study of the organic compounds from plants and their activity has been increased (Patel et al., 2017). The combination of a greatest separation technique (GC) with the most excellent identification technique (MS) made GC-MS an ideal approach for qualitative estimation for volatile and semi-volatile bioactive compound (Grover and Patni 2013). In the present investigation total fourteen bioactive chemical constituents were identified in the plant extract with important chemical properties. The most abundant components found in the plant extract were Maltol (36.23 % RT at 8.213 min). The results are showed in Table 5 and Figure 5.

4. Discussion

Owing to the unhygienic conditions the infestation of head lice occurs and in public health-associated problem, *Pediculus humanus* capitis, which is commonly referred to as human head louse, infestation is considered to be of vital concern. Due to increased instances of resistance of the head louse to the synthetic drugs, presently the researchers have focused on exploring either new substitutes to the synthetic compounds or phytoconstituents obtained from various plant sources. Our study aimed at screening of the anti-lice activity of *P. granatum* alcoholic and aqueous extract by using a filter paper diffusion approach. The results exhibited potential Pediculocidal and Ovicidal effects of *P. granatum* alcoholic extract.

Anti-dandruff activity of *P. granatum* extracts was evaluated against the *A. Niger, C. Albicans and P. notatum.* As per the research attempted by Anitha et al. (2015), the presence of principal fungal species namely *Candida albicans*, *Aspergillus niger*, *Cryptococcus spp* and *Penicillium spp* species which may be responsible for formation of dandruff were recovered and identified from the scalp of human volunteers (Anitha et al., 2015). Hence, the aforesaid fungal species were particularly selected and used in our study for evaluation of antidandruff potential of *P. granatum* extract.

Very few drugs have been recommended for the treatment of alopecia, amongst which is Minoxidil which is known to stimulate telogen bulb and convert them to larger anagen follicles than those in previous cycle (Adhirajan et al., 2003; Chavan et al., 2020a).

Literature survey revealed that there has been no reports available relating to the utilization of *P. granatum* extract for the management of hair disorder. Our studies on experimental animals evidenced that, the topical application of 3 % *P. granatum* alcoholic and aqueous extract solution significantly reduced the time required for hair-growth initiation and completion as compared to control group. Results of hair length determination studies indicated that the topical application of *P. granatum* alcoholic extract (3 %) almost comparable in hair length determination when compared to Minoxidil treated group. Thus, these observations confirmed the effectiveness of 3 % *P. granatum* alcoholic and aqueous extract (3 %) in promoting hair growth. It was observed that the mice groups treated with *P. granatum* (alcoholic and aqueous) 3 % extract solution groups exhibited significant hair growth as compared to vehicle treated mice. Moreover, this group experienced less number of the hair follicles in the anagen phase, and we could easily visualize hair follicles in the telogen phase. Specifically, in the group treated with alcoholic extract, majority of the hair follicles were noted in the anagen phase. Thus, the effect of *P. granatum* alcoholic extract on the hair growth activity was notably high as compared to the vehicle control mice cluster. Even for Minoxidil, it was stated that they readily stimulate telogen bulb and convert them to bigger anagen follicles than those in the previous cycle (Adhirajan et al., 2003). Also, Minoxidil also helps to stimulate the vasodilation of scalp blood vessels and the proliferation of epithelial cells near the base of hair follicles (Savin and Atton 1993). Our findings revealed that, the hair follicles got transformed from telogen to anagen phase in all the groups, and to a greater extent in the alcoholic extract treated group.

In order to reveal the hair growth promotion effect of *P. granatum* leaves alcoholic extract, GC/MS study was undertaken to investigate the possible phytoconstituent(s) responsible for the said effect. Eleven probable compounds were observed after analyzing the data of GC/MS of alcoholic extract of *P. granatum* leaves. Amongst the eleven compounds Maltol was observed to be the major phyto-constituent. James et al. have also proved that Maltol acts as an *in-vivo* skin lightener and exhibits an *ex-vivo* melanogenesis reducing activity along with antioxidant potential, skin tone and skin quality improving activities (Cheetham et al., 2017). Melanogenesis is a biosynthetic pathway for the formation of melanin in human skin (Te-Sheng, 2012). Melanin is type of pigment cell especially made up of called melanocytes (https://www.loc.gov/everyday-mysteries/item/why-does-hair-turn-gray/). In active hair follicles, melanocytes typically occur in the wall of the pilary canal (infundibulum) and in the pigmented part of the bulb, close to the upper part of the dermal papilla (Jean-Paul and Giuseppe, 1993). During the development of murine anagen hair follicle, the two melanogenic proteins, tyrosinase and Gp75, are regulated in a time-restricted frame (Burchill et al., 1991). Melanocytes position themselves at the openings on the skin's surface through which hair grows (follicles) (https://www.loc.gov/everyday-mysteries/item/why-does-hair-turn-gray/). On the basis of aforesaid facts it has been concluded that the principal compound namely Maltol along with other phytoconstituent(s) may be responsible to exhibit hair growth promoting activity. Also, third major phytoconstituent in the extract as per GC/MS report was 1,2,3 Benzotriazole, which has been known to possess strong anti-fungal activity as reported earlier by Sudhir et al. (2013) (Sudhir et al., 2013). In this research, the authors have tried to explore the hair growth promoting activity of Pomogranate which was

Table 5. Major volatile compounds from *P. granatum* extract by GC-MS.

Sr. no.	Compound	Retention time	Molecular weight	Molecular formula	Concentration (%)
1.	Maltol	8.213	126.11 g mol^{-1}	$C_6H_6O_3$	36.23
2.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	8.968	144.12 g mol^{-1}	$C_6H_8O_4$	3.75
3.	5-Hydroxymethylfurfural	11.039	126.11 g mol^{-1}	$C_6H_6O_3$	23.80
4.	1,2,3-Benzenetriol	14.065	126.11 g mol^{-1}	$C_6H_6O_3$	19.70
5.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	22.778	652.90 g mol^{-1}	$C_{38}H_{68}O_8$	6.09
6.	Hexadecanoic acid, ethyl ester	23.103	284.48 g mol^{-1}	$C_{18}H_{36}O_2$	0.79
7.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	26.062	278.40 g mol^{-1}	$C_{18}H_{30}O_2$	5.46
8.	Ethyl 9,12,15-octadecatrienoate	26.166	306.48 g mol^{-1}	$C_{20}H_{34}O_2$	1.98
9.	Palmitoyl chloride	30.087	274.87 g mol^{-1}	$CH_3(CH_2)_{14}COCl$	0.27
10.	hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	30.391	330.50 g mol^{-1}	$C_{19}H_{38}O_4$	0.47
11.	Squalene	33.900	410.70 g mol^{-1}	$C_{30}H_{50}$	0.84

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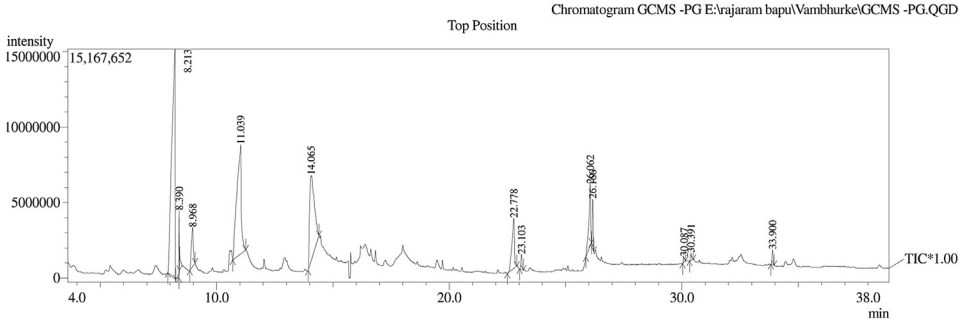
Sample Information

Analyzed by	ANIL MOHITE:
Analyzed	: 08-11-2016 00:46:32
Sample Type	: Unknown
Level #	: 1
Sample Name	:
Sample ID	: GCMS -PG
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 1
Injection Volume	: 1.00
Data File	: E:\rajaram bapu\Vambhurke\GCMS -PG.QGD
Org Data File	: E:\rajaram bapu\Vambhurke\GCMS -PG.QGD
Method File	: E:\Dr.Ghane SG-II.qgm
Org Method File	: E:\Dr.Ghane SG-II.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\PFTBA_NORMAL_CID OFF_FLM 1_29112018_01.qgt
Modified by	: Admin
Modified	: 22-07-2019 15:19:37



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	8.213	7.905	8.250	148070159	36.23	Maltol
2	8.390	8.380	8.400	2517907	0.62	
3	8.968	8.845	9.075	15340533	3.75	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
4	11.039	10.690	11.250	97295459	23.80	5-Hydroxymethylfurfural
5	14.065	13.940	14.405	80527219	19.70	1,2,3-Benzenetriol
6	22.778	22.495	22.905	24899886	6.09	l-(+)-Ascorbic acid 2,6-dihexadecanoate
7	23.103	23.020	23.190	3234109	0.79	Hexadecanoic acid, ethyl ester
8	26.062	25.870	26.115	22300098	5.46	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
9	26.166	26.115	26.215	8091722	1.98	Ethyl 9,12,15-octadecatrienoate
10	30.087	30.030	30.150	1097066	0.27	Palmitoyl chloride
11	30.391	30.330	30.485	1917818	0.47	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
12	33.900	33.825	33.965	3440019	0.84	Squalene
				408731995	100.00	

E:¥rajaram bapu¥Vambhurke¥GCMS −PG.QGD 1 / 14

Figure 5. GC-MS graph of *P. grantum* alcoholic extract.

not focused in earlier literature. Also, there is another scope to explore more research on phytochemical isolation along with their mode of action which was beyond the purview of our study. The results and findings of the present investigation will be helpful for further researchers.

5. Conclusion

In conclusion, the effect of *P. granatum* leaves alcoholic extract on qualitative and quantitative hair growth was found to be more significant as compared to aqueous extract and control group treated animals. Also its good antidandruff activity supported to as a hair growth promoter. The quantitative effect of *P. granatum* leaves extract definitely promotes hair growth by inducing hair follicles telogen to anagen phase. Animals treated with aqueous and ethanolic extract of *P. granatum* showed better efficacy as compared to control and standard group. The percentage of anagen induction with alcoholic extract of *P. granatum* and Minoxidil were almost comparable. On the basis of similarities observed between the Minoxidil and the *P. granatum* studies, it is expected that *P. granatum* will have similar hair growth activity as shown by Minoxidil. Further research is needed for structural elucidation and identifying the mechanism of action of *P. granatum* as a potential hair growth promoter. The said effect may be attributed to the presence of Maltol and other phytoconstituents as confirmed with GC-MS. Also, it can be concluded that *P. granatum*

alcoholic extract exhibited prominent pediculocidal and ovicidal effects i.e anti-lice effect.

Declarations

Author contribution statement

Somnath D. Bhinge: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mangesh A. Bhutkar; Dheeraj S. Randive; Ganesh H. Wadkar; Sachin S. Todkar; Anil S. Savali; Hariprassanna R. Chittapurkar: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Adhirajan, N., Ravikumar, T., Shanmugasundaram, N., Babu, M., 2003. In vivo and in vitro evaluation of hair growth potential of Hibiscus rosa-sinesis Linn. J. Ethnopharmacol. 88, 235–239.

Anitha, M., Hemapriya, J., Monica Roselin, E., Monisha, D.M., Swathy, S.R., 2015. Fungal infections in dandruff afflicted scalps on medical students. Int. J. Curr. Res. 7 (12), 23712–23716.

Aviram, M., Volkova, N., Coleman, R., Dreher, M., Reddy, M.K., Daneel, F., Mira, R., 2008. Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: studies in vivo in atherosclerotic apolipoprotein E-deficient mice and in vitro in cultured macrophages and lipoproteins. J. Agric. Food Chem. 56, 1148–1157.

Bell, C., Hawthorne, S., 2008. Ellagic acid, pomegranate and prostate cancer-a mini review. J. Pharm. Pharmacol. 60, 139–144.

Bhinge, SD, Bhutkar, MA, Randive, DS, Wadkar, GH, Todkar, SS, 2017. Development and evaluation of antimicrobial polyherbal gel. Ann. Pharm. Fr. 75, 349–358.

Bhinge, S., Bhutkar, M., Randive, D., Wadkar, G., Todkar, S., 2020. Investigation of the synergistic effects of the extract of neem with conventional antibiotic against gram positive and gram negative microorganism. Int. J. Infect. Dis. 101 (1), 16.

Bhutkar, M.A., Bhinge, S.D., Randive, D.S., Wadkar, G.H., Todkar, S.S., 2018. *In vitro* hypoglycemic effects of caesalpinia bonducella and myristica fragrans seed extracts. Indian Drugs 55 (2), 57–62.

Burchill, S.A., Redfern, C.P.F., Thody, A.J., 1991. Expression of mRNA recognized by two pigment-cell-specific cDNA clones and tyrosinase synthesis during the hair growth cycle. Br. J. Dermatol. 122, 279–280.

Carpinella, M.C., Miranda, M., Almiro@n, W.R., Ferrayoli, C.G., Almeida, F.L., Palacios, S.M., 2007. In vitro pediculicidal and ovicidal activity of an extract and oil from fruits of Melia azedarach L. J. Am. Acad. Dermatol. 56, 250–256.

Cash, T.F., 2001. The psychology of hair loss and its implication for patient care. Clin. Dermatol. 19, 161–166.

Chavan, R.R., Bhinge, S.D., Bhutkar, M.A., Randive, D.S., Wadkar, G.H., Todkar, S.S., 2020a. Green synthesis of silver and iron nanoparticles using fresh plant extracts of blumea eriantha dc: evaluation of its hair growth promoting activity and partial characterization. J. Cosmet. Dermatol. 20 (4), 1283–1297.

Chavan, R.R., Bhinge, S.D., Bhutkar, M.A., Randive, D.S., Wadkar, G.H., Todkar, S.S., Urade, M.N., 2020b. Characterization, antioxidant, antimicrobial and cytotoxic activities of green synthesized silver and iron nanoparticles using alcoholic Blumea eriantha DC plant extract. Mat. Today Commun. 24, 101320.

Cheetham, P.S.J., Langwallner, C., Langwallner, M., Tan, W.J.A., 2017. A Cosmetic Composition and the Use Thereof for Regulating Skin Quality. International Publication. Number - WO 2017/127025 Al, Publication Date - 27 July 2017.

Dascalu, A., 2013. Inventor; Medidermis Ltd (IL), assignee. In: Agent for Inducing Hair Growth Containing Extracts of Saw palmetto and Swertia. US No. 8372383 B2, 12 Feb 2013.

Datta, K., Singh, A.T., Mukherjee, A., Bhat, B., Ramesh, B., Burman, A.C., 2009. *Eclipta alba* extract with potential for hair growth promoting activity. J. Ethnopharmacol. 124 (3), 450–456.

Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S., 2006. Antimicrobial activity of some ethnomedical plants used by Paliyar tribe from Tamil Nadu, India. BMC Compl. Alternative Med. 6, 1–7.

Ebling, F.J., 1987. The biology of hair. Dermatol. Clin. 5, 467–481.

Grover, N., Patni, V., 2013. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of Woodfordia fruticosa (L) Kurz. Leaves. Int. J. Pharm. Pharmaceut. Sci. 5, 291–295.

https://www.loc.gov/everyday-mysteries/item/why-does-hair-turn-gray/accessed on 22/03/2020 at 5.30pm.

Jaybhaye, D., Varma, S., Gagne, N., Bonde, V., Gite, A., Bhosle, D., 2011. Effect of Tectona grandis Linn. seeds on hair growth activity of albino mice. Int. J. Ayurveda Res. 1, 211–215.

Jean-Paul, O., Giuseppe, P., 1993. Hair melanins and hair color: ultrastructural and biochemical aspects. J. Invest. Dermatol. 101, 82S–89S.

Johnson, E., Ebling, F.J., 1964. The effect of plucking hairs during different phases of the follicular cycle. J. Embryol. Exp. Morphol. 12, 465–474.

Jung, I.Y., Sharif, M., Al-Reza, C.K., 2010. Hair growth promoting effect of Zizyphus jujuba essential oil. Food Chem. Toxicol. 48, 1350–1354.

Kakali, D., Anu, T.S., Ashok, M., Beena, B., Ramesh, B., Anand, C.B., 2009. Eclipta alba extract with potential for hair growth promoting activity. J. Ethnopharmacol. 124, 450–456.

Kirtikar, K.R., Basu, B.D., 2012. Indian Medicinal Plants, second ed. Orient enterprises, Dehradun (Uttaranchal), India.

Kitchen, D.B., Decomez, H., Furr, J.R., Bajorath, J., 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat. Rev. Drug Discov. 3, 935–949.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S., 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. 96, 254–260.

Meinking, T.L., Taplin, D., Kalter, D.C., Eberle, M.W., 1986. Comparative efficacy of treatments for pediculosis capitis infestations. Arch. Dermatol. 122, 267–271.

Patel, J., Reddy, V., Kumar, G.S., Satyasai, D., Bajari, B., 2017. Gas chromatography and mass spectroscopy analysis of bioactive components on the leaf extract of Terminalia coriacea: a potential folklore medicinal plant. Int. J. Green Pharm. 11 (1), S140.

Picollo, M.L., Vassena, C.V., Mougabure Cueto, G.A., Vernetti, M., Zerba, E.N., 2000. Resistance to insecticides and effect of synergists on permethrin toxicity in Pediculus capitis (Anoplura: pediculidae) from Buenos Aires. J. Med. Entomol. 37, 721–725.

Palla, M.S., Guntuku, G.S., Muthyala, M.K.K., Pingali, S., Sahu, P.K., 2018. Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil. Beni-Suef Univ. J. Basic Appl. Sci. 7 (2), 250–256.

Randive, D.S., Shejawal, K.P., Bhinge, S.D., Bhutkar, M.A., Patil, P.D., Jadhav, N.R., Patil, S.B., 2020. Green synthesis of gold nanoparticles of isolated citrus bioflavonoid from orange: characterization and in vitro cytotoxicity against colon cancer cell lines COLO 320DM and HT29. Indian Drugs 57 (8), 61–69.

Randive, D.S., Sayyad, S.F., Bhinge, S.D., Bhutkar, M.A., 2016a. Preparation of arjunāriṣṭa using microbes isolated from woodfordia fruticosa flowers (Dhayati). Ancient Sci. Life 36, 42–47.

Randive, D.S., Bhinge, S.D., Bhutkar, M.A., Wadkar, G.H., Sayyad, S.F., 2016b. Comparative Standardization of marketed formulations of fermented biomedicine – arjunaristha. Indones. J. Pharm. 27 (4), 220–225.

Randive, D.S., Bhutkar, M.A., Bhinge, S.D., Shejwal, K.P., Patil, P.D., Mane, S.A., 2019. Hypoglycemic effects of Lagenaria siceraria, Cynodon dactylon and Stevia rebaudiana extracts. J. Herbmed. Pharmacol. 8 (1), 51–55.

Roy, R.K., Thakur, M., Dixit, V.K., 2008. Hair growth promoting activity of *Eclipta alba* in male albino rats. Arch. Dermatol. Res. 300, 357–364.

Savali, A., Bhinge, S., Chittapurkar, H., 2011. Evaluation of hair growth promoting activity of Musa Paradisiaca unripe fruit extract. J. Nat. Pharm. 2, 120–124.

Savin, R.C., Atton, A.V., 1993. Minoxidil: update on its clinical role. Dermatol. Clin. 11, 55–64.

Sawada, M., Terada, N., Taniguchi, H., Tateishi, R., Mori, Y., 1987. Cyclosporin Astimulates hair growth in nude mice. Lab. Invest. 56, 684–686.

Seok-Seon, R., Chang, D.K., Min-Ho, L., Seong-Lok, H., Moon-Jeong, R., Yeo-Kyeong, Y., 2002. The hair growth promoting effect of Sophora flavescens extract and its molecular regulation. J. Dermatol. Sci. 30, 43–49.

Siddiqui, A., Ali, M., 1997. Practical Pharmaceutical Chemistry. CBS Publishers and distributors, New Delhi, pp. 126–131.

Sithara, N.V., Komathi, S., Rajalakshmi, S., Queen, J., Bharathi, D., 2016. Phytochemical analysis of Andrographis paniculata using different solvents. Eur. J. Biotechnol. Biosci. 4, 28–30.

Stough, D., Stenn, K., Haber, R., Parsley, W.M., Vogel, J.E., 2005. Psychological effect, pathophysiology and management of androgenetic alopecia in men. Mayo Clin. Proc. 80, 1316–1322.

Sudhir, M.S., Nadh, R.V., Radhika, S., 2013. Antifungal activities of novel 1,2,3-benzotriazole derivatives synthesized by ultrasonic and solvent-free conditions. Drug Invent. Today 5 (2), 126–132.

Sunilson, A.J.S.J., Radhamani, S., Gopinath, R., Anandarajagopal, K., Vimala, A.G.K.A., Husain, H.A., 2009. In vitro screening of anti-lice activity of pongamia pinnata leaves. Kor. J. Parasitol. 47 (4), 377–380.

Te-Sheng, C., 2012. Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity. Materials 5 (9), 1661–1685.

Venning, V.A., Dawber, M.A., 1988. Patterned androgenic alopecia in women. J. Am. Acad. Dermatol. 18, 1073–1077.

Yuan, W., Yuk, H.-G., 2018. Antimicrobial efficacy of *Syzygium antisepticum* plant extract against Staphylococcus aureus and methicillin-resistant *S. aureus* and its application potential with cooked chicken. Food Microbiol. 72, 176–184.

Zhanga, Y., Hanb, L., Chena, S., Guand, J., Qu, F.Z., Zhao, Y.Q., 2016. Hair growth promoting activity of cedrol isolated from the leaves of Platycladus orientalis. Biomed. Pharmacother. 83, 641–647.