

Determination of Anti-Dandruff and Hair Growth Promoting Activity of combined coconut oil extract of Marking Nut

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Abstract:

Dandruff, a most common dermatological disorder, is an unpleasant, chronic and pruritic scalp condition which may leads to hair loss also. This study attempted to know the activity of an herbal formulation against a dandruff causing organism *Malassezia furfur* which was cultured and identified from human dandruff sample and also performed the hair growth activity. Marking nuts were extracted with coconut oil and its anti-malassezial activity was evaluated based on diffusion dependent assays. Extract showed effective results comparable to a standard drug. In vivo hair growth stimulating activity for the extract was tested on denuded dorsal skin of 5-week old mice against the controls and the standard drug minoxidil. The parameters used to evaluate hair growth were hair growth completion time, hair length, hair weight, and hair follicle length and hair density. The present results provides insights on the use of Marking nuts extract for anti-malassezial activity and hair rejuvenation in traditional practices.

Keywords: Marking nut, and Anti-dandruff, hair promoting activity.

1. Introduction

Dandruff is a common problem for the most of the people in the world. Dandruff sufferers usually complain because the scalp becomes more oily, hair becomes brittle and falls out [1]. There are various possible causes, including seborrheic dermatitis, allergic reactions, psoriasis, and eczema. Seborrheic dermatitis is the condition mainly caused by the reaction of the fungi *Malassezia* on the scalp [2]. Poor hygiene is not a condition, other factors like person's age, stress levels, medical conditions, and also hair products may cause dandruff. It may be more visible if a person does not wash or brush their hair frequently.

Alopecia is a Universal problem, it affects both genders. The drug of plant origin is necessary to replace the synthetic one to overcome their adverse effects. India is a repository of medicinal plants. Many of the herbs are using for preparation of various cosmetics but there were limit in use because of the lack of sound scientific backing and information [3]

Complications

Complications can sometimes result from treatment with the other drugs. Dandruff is treated by many antifungal drugs are available. The synthetic active substances include ketoconazole, zinc

pyrithione, selenium sulfide and salicylic acid, where many of them are used to control the abundance of fungi in scalp [4]. But problems like development of fungal resistance to antibiotics, reduced efficiency and increased toxicity of synthetic drugs, are now creating the need for an alternative way of treatment. By the way, ethno botanical research gained more interest and phytoactive constituents of plants are now some effective approach to deal antimicrobial resistance. Minoxidil is a vasodilator drug used for the treatment of alopecia. To replace the synthetic one the drug of plant origin is necessary.

2. Material and Methods

2.1 Isolation and Identification of Dandruff causing Fungi

2.1.1 Sample Collection and Culture

By partitioning with a sterile comb and scrapping using a sterile blunt scalpel, flakes shedding from scalp of a volunteer were collected. The specimen was then inoculated to Potato Dextrose Broth and observed for growth by incubating at 25°C for 48 hours. Later it was stored at 2-8°C for further use. The fungi broth culture was introduced to a Potato Dextrose Agar (PDA) plate, incubated at 25°C for 48 hours to observe colony characters. PDA plates were prepared by adding 10% tartaric acid to maintain the pH at 3.5, to inhibit bacterial growth.

2.2 Preparation of Plant extract

2.2.1. Collection of plant materials

Dried nuts of *Semecarpus Anacardium* Linn., were collected from the local market at Hyderabad, Telangana, India. The fruits were authenticated by department of Botany Osmania University, Hyderabad.

2.2.2 Sample selection

The dried nuts were mixed thoroughly and sample was selected randomly

2.2.3 Purification of Plant material

The selected nuts were taken and cleaned by using cotton cloth. Seeds contains corrosive property so which were soaked in milk for overnight, then after removed from milk and dried under sun light. Completely dried nut were used for extraction.

2.2.4 Extraction of plant material

Dried seeds were grounded and taken in a beaker and boiled with coconut oil for 30 mins. Then the extract was filtered, the supernatant was used to check the activity.

2.2.5 Preliminary Phytochemical Analysis

For the Preliminary Phytochemical screening [5].the extract was tested for carbohydrates, alkaloids, glycosides, sterols, phenolic compounds, tannins, Flavonoids, Saponins, proteins and amino acids by using standard procedure [6].

2.3 Determination of anti-dandruff activity

Anti-dandruff activity was tested by disc diffusion and agar well diffusion methods; the diffusion dependent activity of the extract was evaluated based on Zone of Inhibition (ZOI).

2.3.1 Disc diffusion method

Hot air sterilized Whatman Filter paper discs of 6 mm diameter was loaded with extract of various diluted concentrations (50mg/ml, 100mg/ml and 150mg/ml) respectively. Ketoconazole (30µl) was also

loaded to disc and used as test standard. Standardized fungal test suspension was inoculated and uniformly spread on a PDA plate by a cotton swab. Using sterile forceps, the discs containing standard and extract of varied concentrations were laid over the surface of the agar plate; incubated at 25°C for 48 hours and observed.

2.3.2 Agar well diffusion Method

About 20-25 ml of potato dextrose agar medium for each Petriplate cooled to 45 °C and was added to pre sterilized plates (150 mm in size). After this, Old cultures of fungal strain was spreaded over the agar plates. Petri plates were allowed to dry. About four wells in each plate of 10mm diameter was punched in agar surface with the help of sterilized cork borer for sphere for placing the extracts into the well. Standard and, extract of various diluted concentrations (50mg/ml, 100mg/ml and 150mg/ml) were loaded into the wells and incubated. The plates were kept for incubation at 30°C for about 48 to 72 hours. Zone of inhibition was clearly visible after 72 hours of incubation and the diameter of the zone was measured.



Fig. 1: Growth of *Malassezia furfur* against *M.furfur*.



Fig. 2: Antidandruff activity of semecarpus against *M.furfur*.



Fig. 3: Antidandruff activity of Ketoconazole against *M.furfur*.

2.4 *In-vivo* Determination of Hair Growth Promoting activity:

2.4.1 Experimental animals.

Experimental studies of Marking nuts extract was studied by using Thirty animals in 5 randomized groups The selected Five-week-old male C57BL/6 mice were allowed to adapt to their new environment for one week, with food and water provided under room temperature, 50 ± 5% relative humidity and 12 hrs of a light/dark cycle before the experiment was begun. The dorsal area (2 cm × 4 cm) of the 6-week-old mice was shaved with an animal clipper. After removing of hair the mice all of the hair follicles were synchronized in the telogen stage. All

animals were randomized into 4 groups based on different topical applications: coconut oil (CO), Marking nuts extract (ME) minoxidil (MXD), and Marking nut and coconut oil combined extract (MECO). Once a day, 6 days a week, for 4 weeks (100 μ l) of each compound was topically applied to the shaved dorsal area of the mice.

2.4.2 Quantitative hair growth study

The method reported was followed for the quantitative evaluation of treatment. Two mice from each group were euthanized after 10 days, 20 days, and 30 days of treatment: Skin biopsies were taken from shaved area, and the specimen was preserved in 10% formalin. Tissues were placed in paraffin wax and stained with hematoxylin and eosin. Sections from all the groups were evaluate for the number of hair follicles per mm area of skin and percentage ratio of hair follicles in different cyclic phases, such as anagen (growth phase), catagen, and telogen (resting phase), was determined microscopically [7].



Figure 4: Hair growth-promoting effects of the extract.(1) control consisting of coconut oil only, (2) consisting of Marking nut extract only, (3) experiment consisting of coconut oil extract of marking nut (4) experiment consisting of standard dug Minoxidil after 21 days treatment.

2.4.3 Hair length

With the help of electric clipper hair was plucked randomly from the depilated area and measured the hair length by with the help vernier caliper Or Scale and calculated the mean of hair length [8].

2.4.4. Hair density

From the desired depilated area, hair was trimmed and the hair was count manually [9].

2.4.5 Determination of Hair Weight.

After 21 days of treatments Hair weight measurements were done. Mice were killed by physical dislocation and a from the previously shaved skin region a one square centimeter portion was cut. Skin weight with and without hair was determined, the differences in weight was recorded as the net weight of the new regrown hair.

3. Results and Discussion

3.1 Preliminary Phytochemical Observation

Preliminary Phytochemical screening, the extract was tested for carbohydrates, alkaloids, glycosides, sterols, phenolic compounds, tannins, flavonoids, Saponins and amino acids using standard procedure [10].

Table.1: Preliminary Phytochemical Observation

S.No	Phytochemical Analysis	Observation
	Alkaloids	-
	Carbohydrates	+
	Steroids	+
	Phenols	+
	Tannins	-
	Flavonoids	-
	Glycosides	-
	Saponins	-
	Terpenes	+

Key (+) =Presence, (-) =Absence

3.2 Qualitative Studies on Anti-dandruff activity

Table.2: Dose response and MIC of the extract.

Sample	Concentration(mg/ml)	Extract-Disc Diffusion	Agar Well Diffusion
Extract	50	13.66±0.47	15.33±1.24
Extract	100	20.83±0.62	21.16±0.62
Extract	150	29.83±0.23	29.33±0.47
Standard	30	31.16 ± 1.02	29.16 ± 0.84

The combined extract exhibited a potential activity against the fungi *M. furfur*. With the standard the ZOI was compared and represented in table 2. The ZOI showed by extract on disc diffusion was 13.66±0.47, 20.83±0.62 and 29.83±0.23 mm at 50, 100 and 150 mg/ml concentrations, respectively. The extract on agar well diffusion exerted a ZOI of 15.33± 1.24, 21.16±0.62 and 29.33±0.47 mm at 50, 100 and 150 mg/ml concentrations respectively. ZOI of 31.16±1.02 and 29.16±0.84 mm exhibited the standard solution at 30 µl concentration. Despite differences in the phytochemical constituents the combined extract in both disc and agar well diffusion methods the ZOI was similarly expressed. With increased concentration, the ZOI range increased and it was comparable to that of the standard used. Few patients using ketoconazole suffer dryness and itching. Such problems can be avoided by herbal alternatives procedure [11].

3.2.1 Qualitative Studies on Hair Growth

For qualitative hair rejuvenation studies parameters used were minimum time taken before visible hair growth on the shaven skin and the minimum time taken to completely grow new hair on the denude skin.

3.2.2 Determination of Hair Length.

Randomly selected 20 hairs were pulled and measured the length and the result noted as the mean length \pm standard deviation (SD) of 20 hairs. At time intervals of 6 14 and 21 days of treatment Length measurements were done.

Table of Results

Table 3: Effect of different hair formulation on hair length in mm of mice in hair growth activity

S.NO	Animal Groups	Formulation type	Length of hair in mm(mean \pm SD)
1	Group 1	Vehicle Coconut oil(CO)	2.10 \pm 0.087
2	Group 2	Minoxidil 2% solution(MXD)	5.09 \pm 0.348
3	Group 3	Marking nuts extract(ME)	3.88 \pm 0.155
4	Group 4	Marking nut and coconut oil combined extract (MECO).	4.15 \pm 0.354

Table 4: Effect on hair density of different hair formulation

S.NO	Groups	Drug Formulation	Hair density (mean \pm SD)
1	Group 1	Vehicle Coconut oil(CO)	1195 \pm 37.25
2	Group 2	Minoxidil 2% solution(MXD)	2529 \pm 35.69
3	Group 3	Marking nuts extract(ME)	1676 \pm 26.59
4	Group 4	Marking nut and coconut oil combined extract (MECO).	2657 \pm 39.85

Table 5: Effect of different hair formulation on hair weight in mg of mice in hair growth activity

S.NO	Groups	Drug Formulation	Hair density (mean \pm SD)
1	Group 1	Vehicle Coconut oil(CO)	36.61 \pm 1.30 mg,
2	Group 2	Minoxidil 2% solution(MXD)	51.81 \pm 1.36 mg
3	Group 3	Marking nuts extract(ME)	45.81 \pm 1.36mg
4	Group 4	Marking nut and coconut oil combined extract (MECO).	53.34 \pm 1.72 mg

3.2.3 Hair length

Table 1, In Group-1, Group-2, Group-3 and Group-4, represents hair growth activity, the hair length of all groups shown were found to be 2.24, 5.55, 3.93 mm and 3.89 mm, respectively [12].

3.2.4 Hair density

Table 2. In Group-1, Group-2, Group-3 and Group-4, represents hair density, it was found to be 1195, 2529, 1676 and 2657, per cm², respectively

3.2.5 Determination of Hair Weight.

Mice were treated with the CO, MXD, ME and MECO extracts had hair weight of 36.61 ± 1.30 mg, 51.81 ± 1.36 mg, 45.81 ± 1.36 mg and 53.34 ± 1.72 mg respectively.

3.2.6 Toxicity Studies.

Although marking nuts seeds are a common relish, investigations to determine the toxicity on mice were done before the experiment commenced [13].

CONCLUSION

Crude herbal drugs have been included in traditional medicine and household remedies for a long time. This study revealed the effectiveness of Marking nuts seeds and coconut oil combined extract for the management of dandruff and Hair growth promoting activity this kind would help to combat problems like antimicrobial resistance and other irritant properties of drugs. In vitro evaluation proved its appreciable diffusion dependent Zone of Inhibition against *M. furfur* and In-vivo activity determines the good hair promoting activity.

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