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Research Article

Moringa Oleifera Ethosomes a Potential Hair Growth Activator: Effect on Rats

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ABSTRACT

The hair growth promoting activity of *Moringa oleifera* leaf extract formulated as an ethosome (MOE) had been investigated. The skin irritation test evaluated in terms of erythema and edema. Five groups of five rats each evaluated for the hair growth activity: Groups 1 and 2 treated with minoxidil and placebo ethosome while groups 3, 4 and 5 treated with ethosome containing 1, 2 and 5 % w/v MO leaf extract respectively for 30 days. The effects of MO ethosome on hair length, growth pattern and growth phase characteristics were evaluated. No erythema or oedema appeared on the skin of the rats when MO ethosome was liberally applied. The hair growth activity of MO ethosome showed concentration dependent activity. The hair growth initiation time, hair length and hair growth completion time of MO ethosomes showed concentration dependent activity. The activities of the 5% MO ethosome were comparable to minoxidil solution and remarkably higher than that of the placebo ethosome. The group treated with 5% MO ethosome showed more follicles in the anagen phase than either the minoxidil or the placebo groups after 30 days of treatment. The MO ethosome thus, showed a safe, hair growth promotion activity.

KEYWORDS: *Moringa oleifera*; monoxidil; ethosomes; *Moringa oleifera* leaf extract; hair growth.

Source of support: None

Competing interest / Conflict of interest
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INTRODUCTION

Hair loss popularly known as alopecia is a dermatological disorder with psychosocial implications in patients. It is a common disorder that has been recognized for over 2000 years and has been estimated to affect about 2% of the world's population[1]. Apart from metabolic and hereditary causes alopecia has been observed as a major side effect of anticancer, immunosuppressant and many other drug treatments. The urgent needs to get the hair back in order to avoid the psychosocial effect and to the large extent feeling of incomplete have been of major concern. Many people have tried conventional hair restoration methods such as laser treatments, harsh chemicals, pharmaceutical drugs or even surgery. Often these methods have limited success. In literatures, there are about, 300,000 products claimed to help hair regrowth over the years, but none of them was found to be effective in hair growth promotion except minoxidil and finasteride. Minoxidil is a synthetic cardiovascular agent whose side effect of hair growth promotion has been scientifically proven to help treat alopecia[3,4]. After five years of use, 2 and 3% topical minoxidil, the improvement showed to peak at one year with a slow decline in growth over subsequent years[2]. Finasteride is contraindicated for use in women[5]. However, apart from the cost of these agents, the side effects of minoxidil and finasteride have limited their use[6], therefore, the need to search for a more reliable alternative in term of cost and effectiveness becomes inevitable.

The conventional medical approach of simply using synthetic agents to control and restore hair loss is not only costly, but also inadequate and associated with several health risks, complications and poor compliance[7]. Therefore, there is a pressing need to source for new and affordable drugs. In response to this, the World Health Assembly, in 1989, adopted among its resolutions, key interest in the use of herbal medicines[8]. The use of indigenous herbal medicines is an important strategy towards the attainment of these objectives. This is because it would reduce cases of noncompliance caused by side effects and economical reasons. Many plants such as *Cuscuta reflexa* Roxb, *Prunus dulcis* seeds and herbal formulations of *Hibiscus rosa-sinensis* Linn[9] have shown hair growth promotion activity[10]. Also pure compounds from plants examined for hair growth activity; proanthocynidine from grape seeds (*Vitis vinifera*) and beta-sitosterol in saw palmetto (*Serenoa serrulata*) some of which have shown remarkable effect. In recent years, attention shifted toward a popular plant called *Moringa oleifera*, this plant has gained wide support in its various uses in the health care of many countries[11,12].

Moringa oleifera (Moringaceae) is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands[13]. Almost every part of the tree is considered to have medicinal value or nutritional value[15]. Many Nigerian rural communities referred to it as the miracle tree corroborating the Indian Ayurvedic medicine that believes the leaves treats about 300 diseases. The leaves of *M. Oleifera* is particularly rich in vitamins, amino acids, potassium, calcium, phosphorous, iron, and antioxidants[14,15]. All these constitute components needed for tissue generation as well as a good hair formation.

Currently, to the best of our knowledge, there is no suitable pharmaceutical formulation or scientific data published on *M. oleifera* hair growth or belonging to the extract formulated in the form of ethosomes. In this study, a vital point on extract formulation and evaluation is reported. The aim of this study was to develop an ethosomes formulation of aqueous leaf extract of *M. oleifera* for the management of alopecia in rat model.

MATERIALS AND METHODS

Sterile water produced in the department of microbiology and biotechnology of NIPRD and used within 24 h of production, phosphate buffer solution, cholesterol, ethanol and methanol (Sigma–Aldrich Chemie, Germany), phospholipon 90® H (Nattermannallee, Germany) chloroform (BDH, UK), minoxidil (Regaine, UK), Veet® (Reckitt Benckiser, UK).

COLLECTION OF THE PLANT

The leaves of *M. oleifera* collected from NIPRD botanical garden in the month of April- May, 2012. The plant material has been identified by a botanist, Mallam Muazam Wudil, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja, Nigeria.

PREPARATION OF EXTRACT

M. olifera leaves washed with distilled water and shade dried for 3 days was then finely powdered

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using electrical blender. About 300 g of powder was subjected to soxhlet extraction with distilled water (3.0 liters) for about 72 h. The extract filtered using a fine muslin cloth of 100 µm mesh size, concentrated in vacuum under reduced pressure using a rotary flash evaporator (Sigma – Aldrich, USA) and dried in a desiccator over fused calcium chloride for 48 h. The solid extract (25 % yields) packed into a close tight container and used for further studies.

Animals

Male Wistar albino rats weighing about 190-200 g acclimatized to the experimental room at a temperature (26±2 °C) and relative humidity (60-65%), and light, (approximately12/12 h light-dark cycle). Three rats placed per cage and fed on standard food pellets and given water ad libitum. The animal study was carried out with prior approval of the animal Ethical Committee of NIPRD Abuja Nigeria. The principle of Good Laboratory Practice and animal handling (National Institutes of Health guide for the care and use of laboratory animals; Publication No. 85-23, revised 1985) followed.

PREPARATION OF *M. OLEIFERA* EXTRACT ETHOSOME GEL

The solvent evaporation[16,17] adopted with slight modification for preparation of the ethosomes. Three batches of the ethosomes containing 1, 2 and 5 % w/v of *M. oleifera* leaf extract (MO), respectively, formulated. Phospholipon 90® H (2.5 g) and cholesterol (0.1 g) mixed and dissolved in a mixture of 7.5 ml chloroform and 2.5 ml methanol in a round-bottomed flask. The solution evaporated over a water bath at 40 °C. The flask containing thin film of the lipid mixture was kept overnight (about 12 h) to allow for complete evaporation of any remaining traces of the organic solvents. The dry film then hydrated with a mixture of the MO dispersion in PBS (pH 6.5), (previously warmed to 40 °C), and ethanol, added in a fine spray, and shaken vigorously in a closed vessel. While still mixing, 10 ml of polyethylene glycol added and the volume made up to 100 ml with PBS. Vigorous mixing continued in a blender (MX-J220P, Panasonic, China) at 13,000 revolutions per min for further 5 min. A 0.75 % w/v sodium alginate dispersion in distilled water used to prepare gel of the ethosome suspension which was respectively packaged into a screw-capped plastic container and stored in the refrigerator (4-8 °C) until further use.

SKIN IRRITATION TEST

Three rats were chosen and caged individually with food and water given 24 h as previously described prior to the test. A 4 cm^2 area of the dorsal portion of the rats shaved with an electric hair clipper (Wahl, USA) and wiped with methylated spirit. Approximately, 1.0 ml quantity of the formulation was applied over the site. The test sites observed every 6 h for erythema and edema for a total duration of 48 h after application of the formulations in accordance to the earlier researcher[18].

HAIR GROWTH ACTIVITY TEST

The rats divided into 4 groups of five rats each. A 4 cm^2 area of the dorsal portion of all the rats shaved and wiped with surgical spirit. Hair remover (Veet®) also applied over the shaved area to assure the complete removal of any trace of hair from the denuded area. Groups 1 and 2 treated with minoxidil (positive control) and ethosome containing no extract (negative control), respectively. Groups 3 and 4 treated with ethosome formulation containing 2 and 5 % of the extract, respectively. The treatment continued for 30 days.

QUALITATIVE HAIR GROWTH STUDY

For each of the groups the hair growth pattern assessed for the entire period of treatment and by observing the hair growth initiation (time taken to initiate hair growth on denuded skin region) and completion time (time taken to completely cover the denuded skin region with new hair according to the earlier reported worked[19].

QUANTITATIVE HAIR GROWTH STUDY

The hair length determined by plucking hair randomly from the shaved area of the mice on the 10^{th}, 20^{th} and the 30^{th} day of the beginning of treatment. The lengths of ten hairs measured using a hand lens and an electronic a vernier caliper (Mitutoyo, Japan), and average length of hair strand determined, the results expressed as mean length ± Standard error of mean (S.E.M) of ten hair strands[19,20].

HISTOLOGICAL STUDY

One rat from each group was euthanized on day thirty of treatment. Skin biopsies taken from the shaved area and specimens preserved in 10% formalin. The specimens embedded in paraffin wax and the blocks prepared for microtomy. After fixation, vertical sections of the skin sectioned into uniform thickness of 10 µm. The sections were

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stained with hematoxylin and eosin and the number of hair follicles per mm area of skin[21] and ratio of hair follicles in different phases of growth: anagen (active growth phase) and telogen (resting phase) determined using the microscope fitted with an ocular micrometer facility.

STATISTICAL ANALYSIS

All the values were expressed as mean ± S.E.M. (n=5) in each group. Statistical analysis performed using student t-test. A value of *P<0.05* considered statistically significant.

RESULTS

PHYSICAL EVALUATION OF *M. OLEIFERA* EXTRACT ETHOSOME GEL

The MO ethosome is a light green gel with slight leafy odor and smooth texture, when touched.

SKIN IRRITATION TEST

No erythema or oedema formed on the hair denuded skin of the rats when MO ethosomes applied liberally.

EFFECT ON HAIR GROWTH INITIATION AND COMPLETION

The hair growth initiation and completion pattern of the formulations are presented in Table 1. Observed that growth was initiated in the control on the ninth day, the 5%, 2% and 1% MO ethosome, hair growth initiation were on the fourth, fifth and seventh day respectively while it recorded on the seventh day for the standard. The hair growth completion time was 19±1.2 days, 19±0.20 days and 11.4±0.40 for the1%, 2 % and 5% MO ethosmes respectively while 25.4±0.25 days and 19±0.20 days observed for the placebo ethosome and minoxidil solution respectively.

Table 1. Effect of *Moringa oleifera* leaf extract ethosomes on hair growth initiation and completion time.

	Hair growth (Days) [mean ± SEM]	
Treatment(Topical)	Initiation time	Completion time
Placebo ethosome	5.0±0.20	25.4±0.25
2% Minoxidil	6.6±0.25	19±0.20
1% MO ethosome	5.0±0.20	14±0.20
2% MO ethosome	4.6±0.25	11.4±0.40
5% MO ethosome	3.4±0.25	7.8± 0.20

Significant difference at P>0.05

EFFECT ON HAIR LENGTH

The effect of *M. oleifera* leaf extract ethosomes on hair length of rats presented on Figure 1. At the end of the 30 days period of application on the hair denuded skin of the rats, the MO leaf extract ethosome showed a concentration dependent hair growth activity. The effect on hair length was of the order: 2 % minoxidil (standard) > 5 % > 2 % > 1 % MO ethosomes > placebo, with significant differences (*P < 0.05*) between the standard and 1 %, 2 % MO ethosomes. However, there was no significant difference between the effects of the standard and the 5 % MO ethosome.

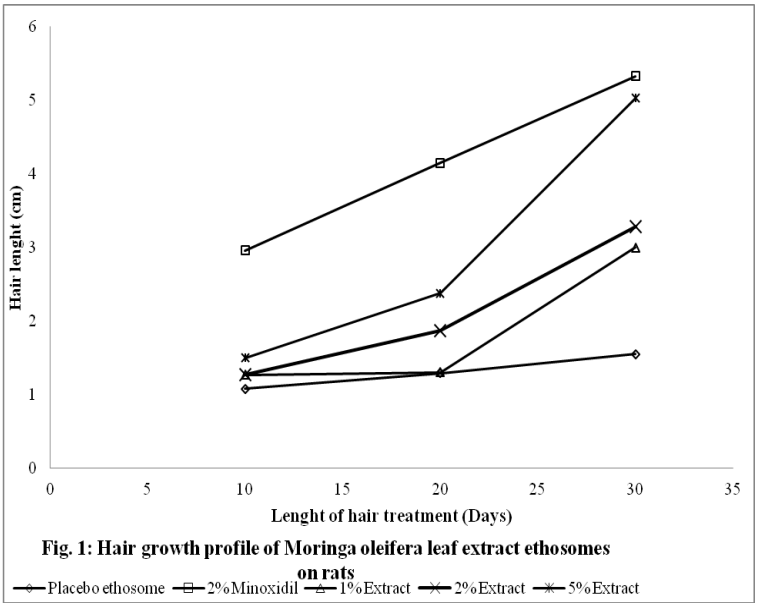

Fig. 1: Hair growth profile of Moringa oleifera leaf extract ethosomes on rats

EFFECT ON HAIR GROWTH PHASES

The effect of the MO ethosome on the different phases of hair growth as related to the active growth phase (anagen) and resting phase (telogen) presented in Fig. 2 and 3. Fig. 2 shows the relative concentrations of the hair follicles at the different hair growth phase while Figure 3 shows the images of the different stages of the hair follicles of the rats treated with different formulations. The group treated with 5% of the extract showed 60% of follicles in the anagen phase, the minoxidil group showed 57% of follicles in the anagen phase, while the 1% and 2% MO ethosome groups showed 51% and 55% respectively, the placebo group was 48% in the anagen phase respectively after the 30 days period of treatment.

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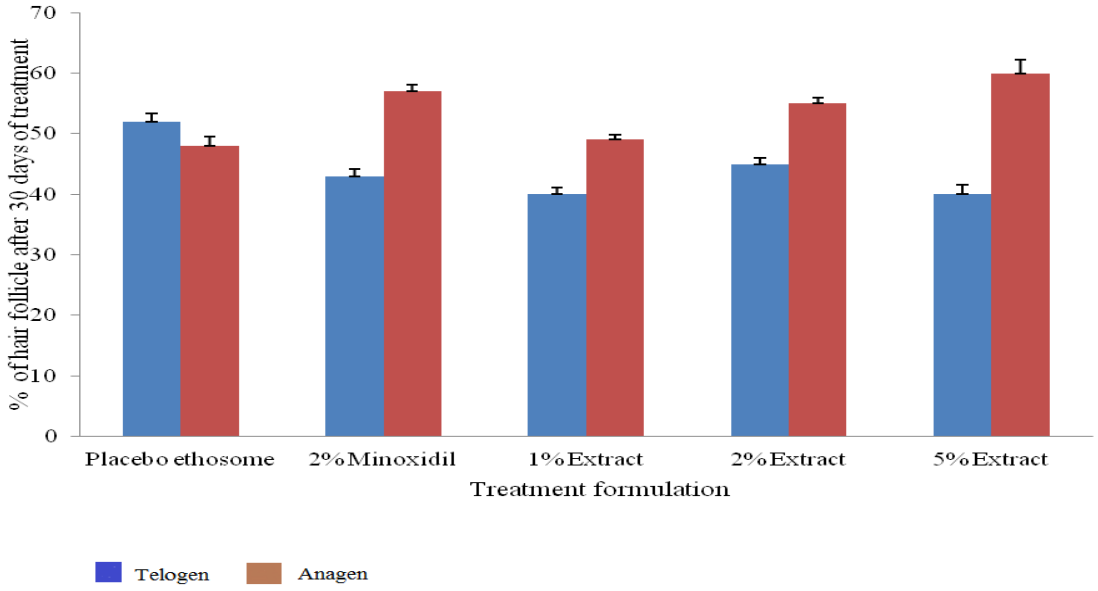

Figure 2.Effect of Moringa olifera ethosomes on the hair follice development.

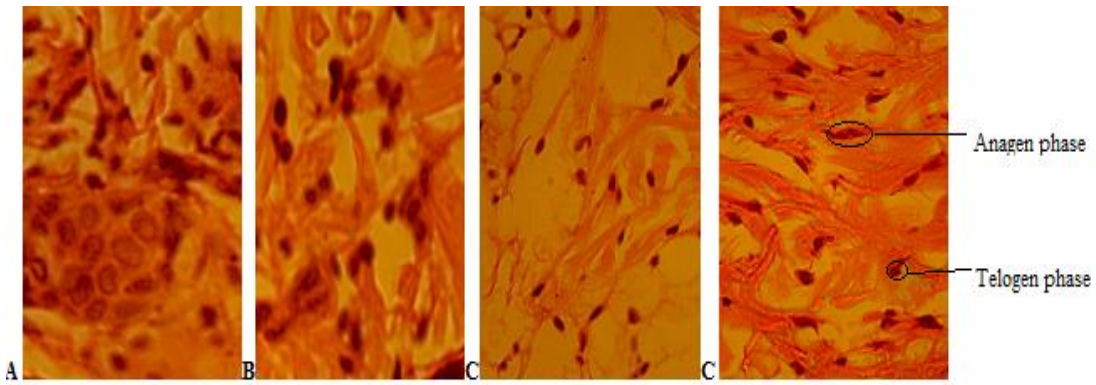

Figure 3: Hair follicle of rats treated with placebo ethosome, minoxidil and different concentrations of MO ethosome in the different phases of development.

A=Placebo ethosome; B= 1%MO ethosome; C=Minoxidil solution; D=5% MO ethosome.

DISCUSSION

Ethosome is an advanced drug delivery system that promote cellular communication and particle transportation by enhancing interaction between the lipid molecules and the polar groups, and also reducing the rigidity of the lipids in the stratum corneum thereby increasing their fluidity thus, resulting in high skin penetration and enhanced compound delivery to deep skin strata[23,24],stratum corneum[25] as well as ensure stability of bioactives. MO leaf water extract was formulated as ethosome gel so as to optimize the delivery of the extract to the stratum corneum by facilitating the deep penetration of the bioactive components of the MO leaf extract to the hair follicle also to ensure stability of the active component on storage and application.

Erythema is characterized by the reddening of the skin due to hyperemia of the capillaries in the lower layers of the skin which occurs when capillaries dilate due to inflammation while edema result when there is a build-up of fluid resulting in the affected tissue to become swollen. All these are toxic reactions that may occur when the skin

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comes into contact with toxic substances[26]. The absence of erythema or oedema when the MO ethosomes was applied on the hair denuded skin of the rats indicates the absence irritation when the formulation is applied to the skin. The absence of toxicity further collaborated by the wide margin safety as shown by the LD50 which is greater than 5000 mg/kg po and the absence of toxicity observed when MO leaf extract is formulated as an ethosome[27]. Topical minoxidil and oral finasteride are the primary drugs approved for the treatment of hair loss however; their use is limited by their side effects and contraindications. Local irritation such as dermatitis, dry skin, scaling and erythema has been observed with topical application of minoxidil[22] a side effect that is not observed with MO ethosome. Though MO is used as a remedy for different diseases it also used as a vegetable in many Nigerian communities therefore occasions of toxicity is not expected. Thus, the MO ethosome is considered safe to use for the purpose of hair growth enhancement.

Although genetics play an important role in hair growth however, certain overwhelming factors such as hormone and nutrition essentially influence hair generation and regeneration. The vitamins and minerals most often linked to hair growth are vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin E, copper, iron, zinc, folic acid and biotin. There are three basic ways that vitamins and minerals may influence hair follicles. These nutrients may act directly on the hair stimulating growth activity or they may act indirectly by influencing the production of the hormone that controls hair follicles development. Apart from the direct action on hair growth some of these nutrients act indirectly by influencing the levels of other vitamin and minerals that have a direct effect.

The formulated MO leaf extract as shown by this study has effective hair growth activity comparable to minoxidil. Indeed minoxidil and the MO ethosomes showed favorable differences in the onset of hair growth initiation as well as the follicles in the growing phase. Faster hair growth initiation of MO ethosomes compared to minoxidil is a successful indicator of the hair growth promoting activity of MO leaf extract ethosome. Though the exact mechanism by which minoxidil stimulates hair growth is not known, however one of the postulations is the direct stimulation of the hair follicle cells in the telogen phase to pass into the anagen (active) phase. Apart from carbohydrates and proteins MO contains over twenty vitamins and mineral in functional quantities and usable form. Many of these nutrients are linked directly and indirectly to hair growth. Thus, the mechanism of the hair growth activity of MO ethosome is related to the various mechanisms that are related to these bioactive constituents. The presence of high content of ascorbic acid, tocopherol, flavonoids and other phenolic compounds which has established antioxidant activities due to their effective free radical scavenging activities also contribute indirectly by mopping-up free radicals that compromise the integrity of cell membranes in the hair follicles, thus inhibiting their potential anti hair growth factors as with iron, which promote the flow of blood to the follicle[28]. Also the ability of the formulation to effectively deliver the bioactive components of the MO extract to the stratum cornium constitutes also another contributory factor.

To produce new hairs, existing follicles undergo cycles of growth (anagen), regression (catagen) and rest (telogen). The duration of anagen determines the length of the hair and is dependent upon continued proliferation and differentiation of matrix cells at the follicle base. During each anagen phase, the follicles produce an entire hair shaft from tip to root; during catagen and telogen, follicles reset and prepare their stem cells so that they can receive the signal to start the next growth phase and make the new hair shaft. During hair growth cycle a transition from telogen to anagen occurs when one or two quiescent stem cells at the base of the telogen follicle are activated to produce a new hair shaft[27,29]. Rats treated with 5% MO ethosomes showed more hair follicles in the anagen phase compared to minixoidil which showed more follicles in the telogen phase. The anagen is an important phase in hair growth as this is the regenerative phase, characterized by the continuous cell division, which pushes older cells upwards and forms the hair shaft. As these hair cells reach a third of the way up a follicle, they start to die and harden. The telogen phase corresponds to the period of rest. During this phase, the follicle stays shortened, and the outer root sheath remains attached to the papilla forming the root germ. As telogen ends, the root germ starts growing downwards to form a new hair bulb around the dermal papilla and allows the next hair growth cycle to begin[30].

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CONCLUSION

M. oleifera leaf extract formulated as an ethosome showed an effective hair growth activity favorably comparable to minoxidil. The absence of irritation, faster initiation and high rate of hair growth are favorable activities that show its effectiveness as a hair growth promoter. This may be because the extracts incorporated into ethosome gels which are known to have an affinity for the keratin horny layer of the skin thereby improving better absorption and penetration. More so, the various constituents of the herbal extracts such as minerals and amino acids may be the cause for the significant hair growth activity. The formulation not only show remarkable activity, but are also devoid of potential side effects compared to synthetic drugs like minoxidil. This thus, portends another great value of MO as a cosmetic product for hair growth promoter. Further study proposes for use in human subjects and pilot scale production.

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