Quality, safety and activity of an ointment formulated from *Butyrospermum parkii* and *Ricinus communis* oils on rabbits hair growth

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Abstract:
Hair loss is a dermatologic disorder, and the surge for discovering natural products anti hair loss with less side effects is continuous. This study aimed to promote hair growth of a safety ointment on rabbits. The ointment was formulated by a mixture of oils of *Ricinus communis* as active and *Butyrospermum parkii* as an excipient, two species well known for their uses in hair maintenance. Eight healthy, male and female rabbits weighting 1.5-1.8 kg were divided in four groups. The ointment was applied to the rabbits previously shaved on the flank area at the rate of twice treatments daily for 28 days. Hair length was measured each two days while the hair mass was assessed on day 29. In addition, quality of this ointment was evaluated by specific microbiological control, stability by observation of some appearance parameters along the experiment and safety by *in vivo* skin irritation test. PKR ointment with yellowish color obtained had an excellent consistency, was homogeneous, with an interesting odor and pH= 4.1. After 28 days of ointment application, results showed for the male a cumulative growth length round to 20 mm i.e. average daily growth of 0.714 mm/day against 15.5 mm i.e. average daily growth of 0.553 mm/day for female. On Day 29, hair mass also increased in treated groups. The ointment did not show any microbial contamination, it remained stable over time with a good pH and non irritative.
The ointment could be used as potential topical formulation hair grower in replace to synthetic cosmetics.

Keywords: Ointment, PKR, Formulation, Vegetable oils.

Qualité, sécurité et activité d'une pommade formulée à partir des huiles de *Butyrospermum parkii* et *Ricinus communis* sur la croissance des poils chez le lapin.

Résumé :
La chute des cheveux est un trouble dermatologique pour lequel la recherche sur des produits naturels avec des effets supportables est continue. Cette étude vise à promouvoir une pommade de qualité pour la croissance des poils chez les lapins. La pommade a été formulée par un mélange des huiles de *Ricinus communis* comme actif et de *Butyrospermum parkii* comme excipient, deux espèces bien connues pour leurs utilisations dans l'entretien des cheveux. Huit lapins sains, mâles et femelles, pesant entre 1,5 et 1,8 kg ont été répartis en 4 groupes. La pommade a été appliquée deux fois par jour sur les lapins préalablement rasés sur les flancs pendant 28 jours. La longueur des poils a été mesurée tous les deux jours tandis que la masse des poils a été évaluée au 29ème jour. Les tests de contrôle microbiologique, de stabilité et d'innocuité ont été également réalisés. La pommade jaunâtre de pH= 4,1 présentait une excellente consistance, une bonne homogénéité et une agréable odeur. Après 28 jours, les résultats ont montré pour le mâle une longueur de croissance cumulée de 20 mm soit une croissance journalière moyenne de 0,714 mm/jour contre 15,5 mm soit une croissance journalière moyenne de 0,553 mm/jour pour la femelle. Au jour 29, la masse des poils a également augmenté dans les groupes traités. La pommade n'a montré aucune contamination microbienne, elle est restée stable avec un bon pH et était non irritant.
Cette pommade pourrait être utilisée comme potentiel produit capillaire en remplacement des cosmétiques synthétiques.

Key words: Pommade, PKR, Formulation, Huiles végétales.

Introduction

Hair is one of the vital parts of the body considered to be protective and esthetic on the body (Melanie, 2016). Today, many people still suffer from distress condition due to hair loss, thinning, baldness and pre-mature graying among younger generation. Hair loss affects millions of people worldwide in framing their personality and general appearance of an individual. This leads to significant psychosocial manifestation which may cost additional expenses on hair treatments. In the era of hair care technology, synthetic cosmetic products were developed to solve the problem of hair loss. However, there is a greater risk of side effects such as headache, irritation, hypertrichosis and sexual health problems (Semalty *et al.*, 2011). These unsupportable side effects encouraged scientists to research new hair grower products

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which will be safe and gentle to use on hair without the harsh side effects caused by synthetic available hair growers. In traditional system of medicine, many plants such as *Persea americana*, *Aloe vera*, *Peperomia pellucida*, *Zizyphus jujuba,* etc. and herbal formulations are reported for hair growth promotion but lack of sound scientific backing and information limits their use (Ake-Assi, 1984; Rathi *et al*., 2008).

In the last decade there has been a lot of pharmacological research on the hair-growth promoting activities of plant extracts using test animals and even man (Adhirajan *et al*., 2001). Herbal cosmetics were very developed in the world market in the goal to stop hair loss and to enhance hair growth. Adding herbs in cosmetics is safer for our skin. They provide numerous essential nutrients such as vitamins, carbohydrates, flavonoids, polyphenols, saponins and phytosterols, which were required to maintain normal functions of the sebaceous gland and promote natural hair growth (Cho *et al*., 2014; Agrawal & Singh, 2017). In this field, there is interesting results of research works; for instance herbal topical formulation containing crude corms extract of pisang kepok (*Musa balbisiana*) significantly increases the hairs length and mass of rabbits in comparison to the normal and negative control (Mashuri *et al*., 2017). Formulation containing extract of *Hibiscus rosa sinensis*, *Calotropis gigantea* and the combination of both plants extracts reported to show better hair-growth acitivitie (Pathan *et al*, 2012). Pumpkin seed oil extracted from *Cucurbita pepo* L. reported to increase mean hair count up to 40% at 24 weeks in men suffer from androgenetic alopecia (Cho *et al*., 2014).

In addition to the above mentioned plants, there are numerous vegetables oils known to be utilized in ivorian folk medicine as hair maintenance and growth, such as *Ricinus communis* L. and *Butyrospermum parkii* (G. Don) Kotschyi (Ake-Assi, 1984; Kamanzi, 2002). These oils were also reported to be used as analgesic, anti-inflammatory, antimicrobial, antioxidant activities (Lewis, 1986; Aslania, 2007; Dumeignil, 2012).

The present work was aimed to prepare and evaluate an ointment for hair growth containing *Ricinus communis* and *Butyrospermum parkii* oils in rabbits.

Materials and Methods

1. Matérial

- Plant material:

Caspsules from *Ricinus communis* L. (Euphorbiaceae) identified by a botanist of our unit were collected from Daloa (Côte d'Ivoire) and shea butter from *Butyrospermum parkii obtained* from Korhogo market (Côte d'Ivoire). Materials were collected from July 2019- to September 2019.

- Animal:

Eight (8) healthy rabbits (*Oryctolagus cuniculus*) aged 5 months old weighting 1.5-1.8 kg were used for the study of efficacy of the ointment. Rabbits were provided by the pet shop of Bioactive Natural Substances Unit of Jean Lorougnon Guede University. Both during the acclimation period (7 days) and throughout the treatment, the animals were individually housed in a room with natural light cycle with the temperature range of 20°C – 25°C, humidity monitored 45%-65% environment and fed with normal diet and water *ad libitum*. All experiments performed on animals followed the standard operation recommended by National Ethic and Research committee (NERC) during a workshop in 2010 (CSRS, 2010).

2. Methods

- Ricinus communis oil extraction:

Oil extraction was performed according to the traditional method described by Oluwole *et al.* (2012). *Ricinus communis* capsules were collected and were dried in a dark ventilated room for 7 days. Then, the seeds were isolated, washed and crushed into a paste. A quantity of 250 g of the paste was boiled in 2 L of water at 100 °C for 2 hours. After this boiling time, oil appeared on the surface of the water, and it was collected in graduated eppendorffs in order to quantify the volume of oil.

- Shea butter oil from Butyrospermum parkii:

A quantity of 150 g shea butter (*Butyrospermum parkii*) was melted completely into oil at 40 °C under a hot plate.

- Ointment formulation:

The ointment was formulated with *Ricinus communis* vegetable oil (castor oil) as an active ingredient and shea butter oil as an excipient. For the formulation, castor oil was incorporated into shea butter oil in order to obtain an homogeneous phase. The homogeneous mixture obtained was distributed directly into 150 mL plastic container. Then, these containers were left

to stand at a temperature below 25 °C until their solidification.

Formulation PKR ointment: X% $_{\text{shea butter oil}}$ + Y% $_{\text{castor oil}}$. The parameters X and Y represented the proportions by volume of each vegetable oil.

- Evaluation of PKR ointment formulation:

The physical appearance was visually checked for the colour, odour, homogeneity and consistency as per the methods mentioned earlier in evaluation for Herbal hair formulations (Adhirajan *et al*., 2001; Rathi *et al*., 2008). The pH was detected using pH meter (HANNA HI 8010, USA).

- Microbiological control of PKR ointment:

Microbial control was carried out in the goal to examinate safety of the ointment PKR. Microbiological analysis was achieved according to method based on normalized process from 3 samples of the ointment (FAD, 2014, Jwel *et al*., 2014).

To enumerate total viable bacteria (TVB) and fungal count, ten grams of the ointment were homogeneously mixed with 90 mL of buffer peptone water (BPW), and serial dilutions were prepared up to 10^{-2} following the standard protocols. An aliquot of 0.1 mL of each suspension from the dilution 10^{-2} was spread onto nutrient agar (NA) plate to enumerate the total bacteria and on Sabouraud + Chloroamphenicol (SC) plate for the estimation of fungal load. Then, the NA plate and SC plates were incubated at 37 °C for 24 hours and at 25 °C for 72 hours, respectively (Jwel *et al*., 2014).

To enumerate specific pathogens, 0.1 mL from the dilution of 10-2 of the sample was spread onto Violet Red Bile Lactose Agar (VRBL), Rapid E. coli 2, Baird Parker and BEA Bile-Esculine-Azide (BEA) for the enumeration of total coliform, *Escherichia coli*, *Staphylococcus spp*., and *Streptococcus spp.* consecutively. All the plates were incubated at 37 °C for 24 hours (Jwel *et al*., 2014).

Number of microorganisms present in a sample of the ointment was obtained by the formula described by AFNOR (2001). The number of germs per mL or g of sample was calculated for each germ, then compared to the normative reference of the microbiological criteria for herbal medicinal products from FAD (2014).

- Primary skin irritation assay of PKR ointment in rabbits:

A primary skin irritation test was conducted on rabbits to determine the potential for PKR ointment to produce an irritation after a single topical application. Two rabbits, male and non-pregnant female *Oryctolagus cuniculus* weighing between 1.5 kg and 1.8 kg, were allowed free access to water and food. Two areas of flanks (left and right) measuring 25 cm^2 each one of rabbits were shaved. Five-tenths of a gram of the ointment was applied at the right side and the excipient (Shea butter) as negative control at the left side on each rabbit (OECD, 2017; Nor zafirah *et al*., 2020). Four test areas (Two on the right side and two on the left side) were marked for the test, then animals were acclimatized at least 5 days before the tests. Each rabbit was housed in an individual cage in a temperature-controlled (20–25°C) and humidity-monitored (45–65 %) environment. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. No pre-existing skin irritations were observed. After application, the pad and entire trunk of each animal were then secured with semi-occlusive micropore tape to avoid dislocation of the pad and rabbits were returned to their designated cages. After 30-60 min, 24 h and 72 h of exposure to ointment, the pads were removed and individual dose sites were scored according to Draize with scoring system for dermal reactions (Nair *et al*., 2010; Nor Zafirah *et al*., 2020).

- Shelf life control of the ointment PKR:

Six samples of formulated ointment were stored in different temperature conditions (room temperature 25 °C, 30 °C, 45°C), opened, handled and hermetically closed in the goal to follow their degradation for 28 days. Every week, the samples were observed for drug decomposition by physical analysis of odour, colour, consistency and homogeneity according methods describes by Adhirajan *et al*., 2001. However, the sample hermetically closed was observed only D28.

- Evaluation of PKR ointment activity on hair growth in rabbits:

Test was carried out on 4 males and 4 females of rabbits. Animals were divided into 4 groups: 2 test groups including Test Male (Group A: 2 males) and Test Female (Group B: 2 females) then 2 control groups including Male control (Group C: 2 males) and Female control (Group D: 2 females). Twenty-four hours (24 h) before the ointment application, the right flank of rabbits were shaved by electric pet clipper on an area of 25 cm^2, on where the ointment PKR was applied without irritating the skin. Immediately, the site was occluded by placing sterile gauze flats. A quantity of 0.5 g PKR ointment was administered

topically on the animal shaved skin of the test groups, twice a day (morning and afternoon), for 28 days according to the method described by Mohammad *et al*. (2017). Every 2 days, from each treated area was taken randomly 10 hairs, then the length of each hair was measured using caliper and the mean of hair length was calculated (Mashuri *et al*., 2017). On day 29, all hairs in the treated area and control were taken and weighed using a digital microbalance and expressed in milligram. From data collected, curves of the cumulative rabbit's hair growth were made. All these experiments were carried out within the research unit on Natural Bioactive Substances according to the guidelines of the National Ethics and Research Committee (NERC) of Côte d'Ivoire (CSRS, 2010).

- Statistical analysis:

The data were described as ***mean*** ± Standard Error (SE). One-way ANOVA and Least Significant Difference (LSD) test were used to determine the statistical significance ($p < 0.05$) of the differences between values of various experimental and control groups.

Results and Discussion

- Extraction yield and ointment formulation:

Extraction yield of castor oil from the method described above was evaluated at 8.45% for a volume of 24 mL from 250 g of castor seeds. This method would therefore not be suitable for a good extraction of castor oil. Indeed, according to Dumeignil (2012), castor seeds contain 50 to 70 % oil. In addition, Guergour (2011) obtained an extraction yield of 30% with the extraction process using the Soxhlet apparatus. The low yield of 8.45 % obtained with the boiling method would due to the very high viscosity of castor oil, making it to be the densest vegetable oil (Dumeignil, 2012).

- Evaluation of PKR ointment formulation:

Formulation adopted for PKR ointment was as follows: ***X% shea butter oil + Y% castor oil***, where X and Y are quantities of each oil expressed in percentage. In the goal to request later a patent for this formulation, values X and Y were not indicated.

Physical appearance for this formulation were collected in Table I. In terms of physical analysis, PKR ointment exhibited good macroscopic qualities. It was homogeneous with a pleasant odor of shea butter. These data suggested that the proportions X and Y retained for the formulation of the ointment were well chosen.

Table I: Description of appearance of PKR ointment quality.

Parameters	PKR ointment
Color	Yellowish
Odour	Pleasant
Consistency	Excellent
Homogeneity	Good

In addition, shea butter is the preferred excipient for formulating ointments because it releases the active compounds better (Semde, 2003; Toé, 2004). Considering parameters obtained, PKR ointment showed significant physical parameters evaluation as cited by Adhirajan *et al.* (2001). The subjective properties such as consistency and homogeneity were good. The pH value of the ointment was evaluated at 4.1 at room temperature (Fig 1). This suggested that the ointment was compatible to cosmetic use because it's closer to skin pH which is 4.5 (Semdé, 2003; Toé, 2004). Therefore, PKR ointment could not interfere with the physiology of skin. After formulation, pH of PKR ointment was also closer to the components. This would mean that oils mixture didn't increase or decrease significantly pH. This showed that there wasn't additional compound produced from this mixture and the original components still stable.

- Primary skin irritation of PKR ointment in rabbits:

Under the experimental conditions, PKR ointment was not irritating (Table II). This result showed this ointment was safety for skin. This skin tolerance in rabbits would indicate that PKR ointment was not irritating to the skin; this could be explained by the fact that shea butter (excipient) was not aggressive for the skin, as was castor oil (Alsania et al., 2007; Toe, 2007).

- PKR ointment safety:

The microbiological analysis carried out on the samples of the PKR ointment did not show any presence of the targeted germs, namely yeasts and molds, total coliform, *Escherichia coli*, *Staphylococcus spp*., and *Streptococcus*.

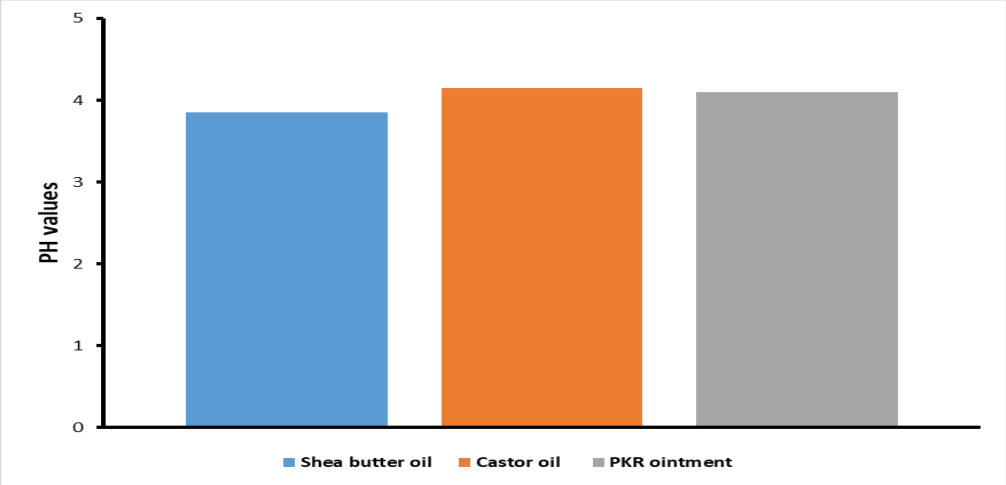

Fig. 1: pH values of components and PKR ointment.

Table II: Skin irritation scores in rabbits after exposure to Negative control and PKR ointment.

Rabbits	Negative Control				PKR Ointment			
	Erythema		Edema		Erythema		Edema	
	24 h	72h	24 h	72h	24h	72h	24h	72h
Male	0	0	0	0	0	0	0	0
Female	0	0	0	0	0	0	0	0

This result suggested the formulation of the ointment was made in Good Manufacturing Pactices (GMP) by compliance with the hygiene measures adopted. The acidity of PKR ointment could limit the microbial proliferation, because the growth of most microorganisms was slowed down when the pH is below 4.2 (FAD, 2014).

- ***PKR ointment shelf life:***

Beyond a temperature above 30°C, PKR ointment became unstable and began to melt. The observations carried out on different days on PKR, left open, handled and hermetically closed showed that the color, the odor, the consistency and homogeneity remained unchanged Table III. The physical parameters evaluated indicated that preparation was physically stable at temperatures inferior to 30 °C.

- ***Efficacy of PKR ointment on hair growth on rabbits:***

The choice of *Ricinus communis* and *Butyrospermum parkii* species was justified by their use in some local communities for their different needs for well-being, food and aesthetics. Indeed, the butter from the seeds of *B. parkii* is known in West Africa particularly for its many benefits in food, cosmetics and therapy (Lewis, 1986; Aslania, 2007, Dumeignil, 2012). As for the oil of R. communis, it enters in the preparation of several cosmetic proposals and is widely used locally to reinforce the shine of the hair (Maroyi *et al*., 2007; Olowule *et al*., 2012).

For this study, the animal model was rabbits because they are phylogenetically closer to humans and this avoids carrying out these tests on humans for ethical considerations (Kamanzi, 2002).

Prominent hair growth initiation response was observed on 10th day (D10) after applying PKR ointment to Test male group (A) and Test Female Group B in comparison to control groups (C and D). Fig. 2 showed comparative effect on hair growth response in rabbits for a 28-day period with the different groups. Group B (Test male) had the longest mean hair growth after 28 days which is 20 mm. The group B (Test female) attained 15.50 mm mean hair length. In Fig 2, on D28, hair length mean on rabbits control male (Group C) was 11 mm against 8 mm for hair length mean on control female (Group D). This demonstrated that naturally hair growed fatser on male rabbits than female rabbits.

Table III: Ointment PKR shelf life.

Days	Macroscopic parameters	PKR		
		Open	Handled	Hermetically Closed
D0	Colour	Yellowish	Yellowish	–
	Odour	Pleasant	Pleasant	
	Consistency	Excellent	Excellent	
	Homogeneity	Good	Good	
D7	Colour	Yellowish	Yellowish	–
	Odour	Pleasant	Pleasant	
	Consistency	Excellent	Excellent	
	Homogeneity	Good	Good	
D14	Colour	Yellowish	Yellowish	–
	Odour	Pleasant	Pleasant	
	Consistency	Excellent	Excellent	
	Homogeneity	Good	Good	
D28	Colour	Yellowish	Yellowish	Yellowish
	Odour	Pleasant	Pleasant	Pleasant
	Consistency	Excellent	Excellent	Excellent
	Homogeneity	Good	Good	Good

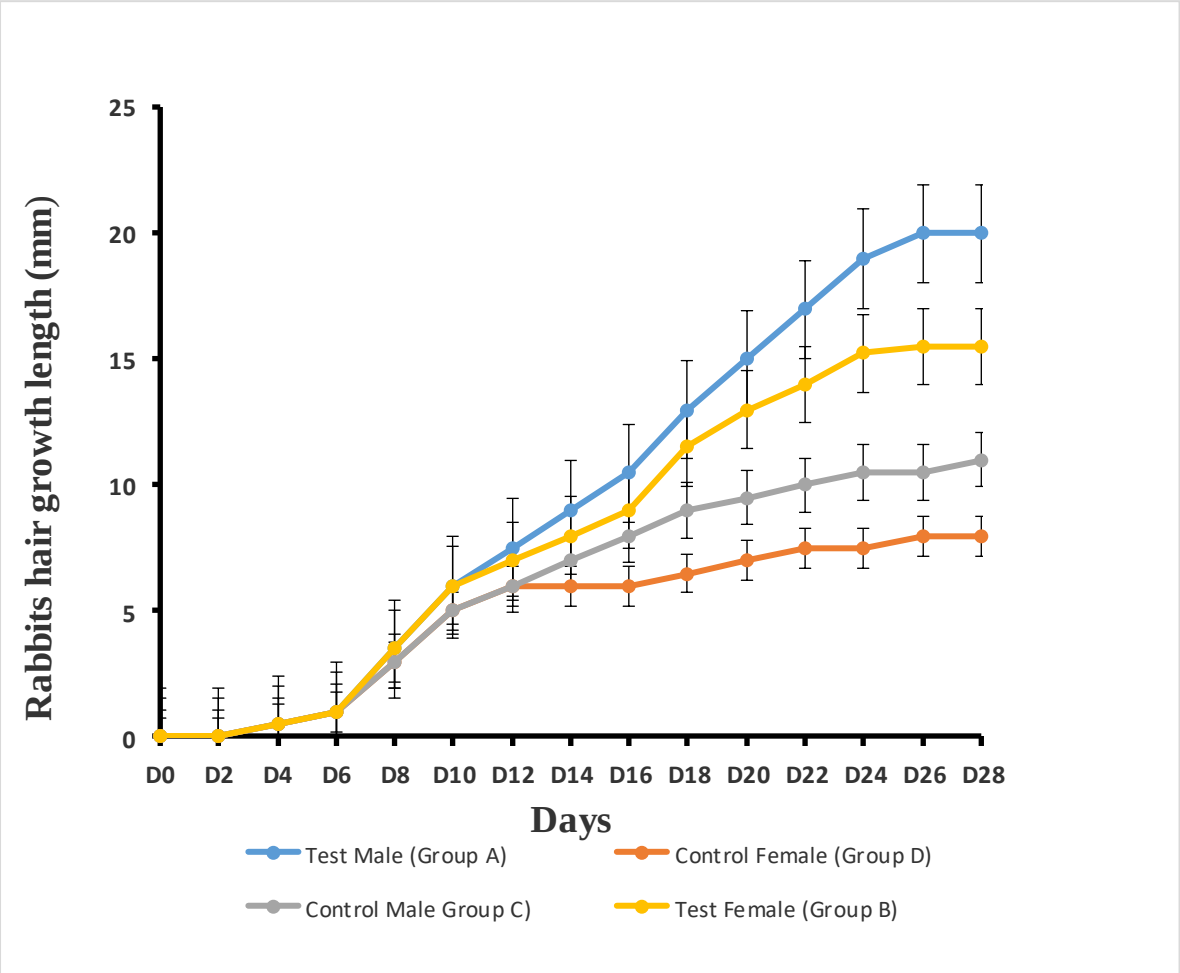

Fig. 2: Effect of PKR ointment on a cumulative rabbit's hair growth length during 28 days experiment.

Tables IV and V present descriptive and analytical data of the effect of PKR ointment treatment on rabbit hairs daily growth and mass after 28 days of application. The one-way ANOVA results in F= 7.286 for Table IV and F= 105.25 for Table V at P< 0.05. LSD test on the mean values between groups for the data in these tables suggest that PKR ointment significantly increase the animal hairs length and mass in comparison to the control.

Hair growth rate in rabbits male treated with ointment PKR (Group A) is highest with 0.714±0.009 mm/day during the 28th day against 0.553±0.006 mm/day for rabbits female treated (Table IV).

Table IV: Daily hair growth length in rabbits.

Groups	Daily growth (mm/day)		Mean±SE (mm/day)
	1	2	
Group A Test Male	0.725	0.703	0.714±0.009[a]
Group B Test Female	0.512	0.594	0.553±0.006[b]
Group C Control Male	0.402	0.382	0.392±0.025[c]
Group D Control Female	0.298	0.272	0.285±0.015[d]

Mean±SE values followed by the different superscript are differ at a =0.05. Group A (Test Male) and Group B (Test Female) are shaved skin treated with PKR ointment; whereas Group C (Control Male) and Group D (Control female) are the shaved skin without treating with PKR ointment.

Table V: Rabbits hair mass in shaved skin area on Day 29.

Groups	Hair mass of rabbits (mg)		Mean±SE
	1	2	
Group A Test Male	43.7	45.5	44.25±0.022a
Group B Test Female	41.9	41.6	41.75±0.051b
Group C Control Male	39.8	39.5	39.65±0.015c
Group D Control Female	40.5	40.3	40.40±0.003d

Mean±SE values followed by the different superscript are differ at a =0.05. Group A (Test Male) and Group B (Test Female) are shaved skin treated with PKR ointment; whereas Group C (Control Male) and Group D (Control female) are the shaved skin without treating with PKR ointment.

This result suggested that PKR ointment increased hair growth rate nearly twice times per day in comparison to male control (0.392±0.025 mm/day) and female control (0.285±0.015 mm/day).On the Day 29, the weight of newly grown hairs in all the treated groups were measured and compared with that of the control group. Also, It was found that hair mass was higher for the male treated group A (44.25±0.022 mg) than female treated group B (41.75±0.051 mg) as shown in the Table V.

In addition, in comparison to control groups, PKR ointment showed an increase on hair mass of the rabbits male treated (44.25±0.022 mg) and female treated (41.75±0.051 mg) against control male (39.65±0.015 mg) and control female (40.40±0.003) (Table V). It demonstrated that the application of the ointment influenced probably hair density on rabbits and this ointment was effective as a potent hair grower.

PKR ointment had a stronger promoting hair growth in male rabbits than female rabbit. The effect of this ointment on hair growth in male rabbits could be explained by the presence of omega 9 in the composition of castor oils. Omega 9 are monounsaturated fatty acids with a double bond in the C9 position, and possess hair growth promoting activity by strengthening hair structure and the capillary wall of smaller blood vessels supplying hair follicles, improve blood circulation to nourish the hair follicles and thereby promote the hair growth (Mboui, 2003, Maroyi, 2007). In addition, castor oil contained 85% glycerids, including the main fatty acid; Ricinoleic acid (90%) which is an omega 9 (Dumeignil, 2012). Considering the particular chemical composition of shea butter (excipient) which is known to easily release the active compounds of the preparations in which it is included (Toe, 2007). The effectiveness of this ointment in male rabbits may suggest its use to prevent pre-mature graying among younger men.

This study evidently showed positive promotion effects of vegetables oils as ointment on the hair growth in rabbits. Vegetables oils, as indicated by

many authors, contained majority or at least some of the phytochemicals that were found to show hair growth promoting activities. Semwal *et al*. (2011) stated there are more than ten substances suspected effect on hair growth, including saponin, alkaloids, ecliptine, wedelic acid, lauric and myristic acids, luteolin, triterpine, glycosides, β- sitosterol, hentriacontanol, vitamin A, vitamin C, iron calcium oxalic, malic acid; α pinene, β pinene, fatty acid, sterol compounds, polyphenols, steroids, volatile oil and essential oil.

Moreover, according to Begum et al. (2014), hair growth is coordinated by hormones and It commands the follicle to undergo appropriate changes during this process. The hormone androgens may cause stimulation of hair growth. This process is followed by a specific cyclic order and characterized by anagen (growth phase), catagen (regression), and telogen (resting phase). The PKR ointment contained probably compounds that act like the hormonal activity.

In vitro study revealed that vegetable oils used for this ointment formulation had direct impact on hair follicles and thus may improve the hair growth. It is expected that several fatty acids, e.g. palmitic, oleic, linoleic, linolenic and arachidonic acids, as well as mixture of these acids showed a significant anti-androgenic effect owing to their testosterone 5-α-reductase inhibitory activity (Yoon *et al*., 2010; Jain *et al*., 2012)

Data of the study provide a significant contribution to the pharmaceutical science, particularly in the field of hair care. It opens the insight that in nature there are too many plants which could potentially be used as alternative ingredients for enhancing hair growth, including vegetable oils.

Conclusion

The species of *Ricinus communis* and *Butyrospermum parkii* have made it possible to formulate from their vegetable oils an ointment which promote hair growth in male rabbits and would not be irritating. This ointment was very homogeneous with very good physical parameters. The effectiveness of this ointment suggests that its application could be extended to humans in order to prevent early hair loss. To do this, additional studies including methods of optimizing its activity against some germs responsible for diseases of the scalp and clinical trials should be carried out.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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