



Formulation and Anti-alopecia Studies on the Stem-bark of *irvingia gabonensis* (aubry-lecomte ex o'rorke) baill (IRVINGIACEAE)**Troy Salvia Malgwi ¹, Cletus Anes Ukwubile ², Musa Yusuf Dibal ³**^{1,2,3} *Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria**** Corresponding Author E-mail:** troysm2013@unimaid.edu.ng, doi.org/10.55639/njpbr.1071.007

Abstract

Alopecia has exacted significant physical and emotional damage that might ultimately lead to death in uncontrolled and severe cases. Lack of covering on the scalp can expose individuals to mechanical injury and in many cases significant psychosocial stress, causing intense emotional and social problems. Irvingia gabonensis has been used in traditional medicine by women in Hawul local government area of Nigeria to treat alopecia which necessitated this research. The stem-bark of Irvingia gabonensis were often sort for from Osun state and used in Hawul local government area to stimulate hair growth. The research aimed to evaluate the anti-alopecia properties of a cream formulation of the stem bark extract of Irvingia gabonensis. The study assessed the plant extracts' potential for treating alopecia (hair loss) in animal models (rabbits) by topically applying various strengths of the prepared herbal cream extracts. The anti-alopecia study revealed that the ethyl acetate extract showed the most activity of the extracts and is appreciably comparable to the standard treatment. Statistics showed no significant difference between the ethyl-acetate extract and the standard drug.

Key: Alopecia, Cream Formulations, Irvingia gabonensis, ethyl acetate extract, Minoxidil cream(std)

Introduction

Traditional medicine continues to play an essential role in health care, with about 80% of the world population relying mainly on traditional medicine as the primary source of health care (Owolabi *et al.*, 2007). It has been well documented that higher plants and their extracts have been used in treating of several diseases in traditional African medicine as an age old practice. These plants have been useful in the development of new drugs and continue to play an important role in the drug discovery process (Farnsworth and Fabricant, 2001; Cragg *et al.*, 1997).

There is a renewed interest in drugs of natural origin due to their easy availability, accessibility and little or no side effects (Chanda, 2014), coupled with a growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Sharma *et al.*, 2017). A number of natural resources such as herbs (*Panax ginseng*, *Echinacea purpurea* (*Echinacea*), *Curcuma longa* (*Turmeric*), *Salvia officinalis*, *Aloe vera*, *Zingiber officinale*, *Mentha piperita*, *Camellia sinensis*, *Ginkgo biloba*, *Lavandula angustifolia*) (Niazi and Monib, 2024); (Al-Achi, 2020), are used in the traditional medical systems in many

countries for the treatment of alopecia. Many medicinal plants provide relief of symptoms of diseases including malaria, inflammation, pain and pyrexia. Some have also been used for the treatment of alopecia (Kim, 2014).

Hairs are modified epithelial structures formed as a result of the keratinization of germinative cells. Hairs are the outgrowths from the follicles present on the skin. These follicles are situated on the dermis, the second layer of the skin and extend up to the epidermis, the outermost layer of the skin. Hair is composed of keratin with chemical constituents such as carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O). Hair growth varies from person to person but on average hair grows about 5-10 mm/month. Maximum growth of hairs takes place at about 15-30 years in humans (Jain *et al.*, 2012).

Hair is one of the body's vital parts derived from the ectoderm of skin. It is a protective appendage on the body and considered an accessory structure of the integument along with sebaceous glands, sweat glands and nails (Marty and Price, 1997). They are known as epidermal derivatives from the epidermis during embryological development. Hair is important to the overall appeal of the human body. A hair growth cycle has three main phases: Anagen, catagen, and telogen. The anagen phase is the growth cycle that typically lasts 3-5 years. (Harada *et al.*, 2007) On a healthy scalp, there are approximately 1,000,000 hairs and 90% of the follicles are continually in the anagen phase of hair growth (Sinclair and Jolliffe, 2013). The catagen stage follows the end of the growth period when a follicle begins to become dormant. However the telogen stage is a dormant or resting period

lasting 3-4 months. When the dormant phase ends, an old hair falls out. A hair follicle then returns to the anagen stage, and new hair grow. An average rate of hair growth is about half an inch per month depending on hair follicles and the age of an individual. On average, 50-100 scalp hairs are lost daily in a normal hair growth cycle and new hairs begin to grow from these follicles. Hair loss begins when less new hair begins the re-growth stage (Jain *et al.*, 2016).

Alopecia is a dermatological disorder that has been recognized for more than 2000 years It is common worldwide and has been estimated to affect between 0.2 % - 2% of the world population (Alhanshali *et al.*, 2023).

There are various causes of alopecia such as genetic tendencies, environmental triggers, exposure to chemicals, medicines, nutritional deficiency, oxidative stress, or long-term illness (Hlail, 2020). There are various types of alopecia but the two major types of concern are alopecia areata and androgenetic alopecia (Darwin *et al.*, 2018).

Androgenetic alopecia, commonly referred to as Male pattern baldness or Female pattern baldness maybe is characterized by the loss of hair in a defined pattern. It is usually due to an excessive concentration of the androgenic hormone; testosterone, and is often permanent. In women, Androgenetic alopecia appears as diffuse hair loss occurring over most scalp. In men however, the pattern of loss usually starts with a receding hairline which then advances to thin the top of the head (Chen, 1996); (Sinclair and Jolliffe, 2013).

Conversely, Alopecia areata is a highly unpredictable autoimmune disease resulting in

hair loss on the scalp and elsewhere on the body. It usually starts with one or more small, round, smooth patches on the scalp and can progress to total scalp hair loss (Alopecia totalis) or complete body hair loss (Alopecia universalis). Alopecia universalis is the rarest form of Alopecia areata (Prager *et al.*, 2002).

Natural products are being used in the cosmetics industry especially in hair care products. It is worth noting that they are used in hair care products. It is worth noting that different plant extracts have been researched in relation to hair growth (Rathi *et al.*, 2008)

Alopecia, even though not life-threatening, can cause a series of devastating conditions. Loss of hair on the scalp has resulted in mechanical injury due to lack of covering, and skin cancer of the scalp has resulted from continuous exposure of the bare scalp to ultraviolet radiation from the sun (de Galvez *et al.*, 2015). Alopecia can cause significant emotional distress and lead to personal, social, and work-related issues. Around 40% of women with alopecia experience marital problems, and about 63% face career-related challenges. The condition is linked to depression, anxiety, and social phobia. Stress can both trigger alopecia and be exacerbated by it, creating a complicated cycle of distress (Davis, 2014).

Available treatments include medications like Minoxidil and Finasteride, which are widely used to stimulate hair growth and reduce hair loss by targeting specific hormones. Additional therapies such as corticosteroid injections and Platelet-Rich Plasma (PRP) therapy are employed to reduce inflammation and promote hair growth (York *et al.*, 2020). Surgical options, including hair transplant surgery, provide a more permanent solution by

relocating hair follicles from one part of the body to balding areas (Pothula and Jayanth, 2021). Natural compounds, including essential oils like rosemary sage, and aloe vera are also explored for their potential benefits in treating alopecia due to their holistic approach and fewer severe side effects compared to pharmaceuticals (González-Minero *et al.*, 2020).

However, conventional alopecia treatments have notable drawbacks, such as scalp irritation from Minoxidil and sexual side effects from Finasteride (Nestor *et al.*, 2021). Corticosteroid injections can cause skin thinning and are not suitable for long-term use, while PRP therapy is costly and variably effective (Gupta *et al.*, 2021). Hair transplant surgery is expensive, time-consuming, and carries risks like scarring (Anastassakis, 2024). Research into natural remedies aims to find safer, cost-effective, and accessible treatment options with minimal side effects, offering a promising alternative to conventional therapies by leveraging the body's natural healing processes (Kaiser *et al.*, 2023)

Method

Extraction of the stem bark of I. gabonensis

The plant materials after collection was air dried, powdered and 3 kilograms of the powdered plant material was extracted sequentially at room temperature with n-hexane, ethyl acetate and methanol by maceration for a period of 48 hours, 72 hours and then 72 hours respectively. All the extracts were filtered and the filtrates were concentrated and stored properly in a desiccator for further analysis. The percentage yield was calculated as well with reference to the weight of the powdered plant material.

$$\text{Percentage yield of extracts} = \frac{\text{Weight of total extract}}{\text{Weight of powdered material}} \times 100$$

Experimental Animals

Male rabbits weighing 1112 g to 1370 g were obtained and kept in well-ventilated cages and maintained under standard laboratory conditions. All experiments were carried out in compliance with the Ahmadu Bello University Ethics on Animal Use and Care (ABUCAUC) with an approval number: ABUCAUC/2023/068 and following the regulation for the Care and Use of Laboratory Animals as accepted internationally.

The leaves, fruits, stem-bark and roots of the plant were collected in October, 2018 at Iragbiji community, Bori Local Government Area of Osun State, Nigeria. The plant was identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria and a voucher number ABU06926 was obtained.

a. Test Animal Preparation:

Testing of anti-alopecia activity was carried out based on the method developed by Jufri *et al.*, (2017), in this research the dorsal area of four-five (4-5) month old Chinchilla rabbits was shaved clean and disinfected. The experiment steps was as follows;

A total of thirty- one (31) adult four to five (4-5) month old male chinchilla rabbits were used for this study. The selection of male rabbits for this study is because the male rabbit physiological conditions were relatively more stable when compared with female rabbits which are usually affected by the hormonal factor such as pregnancy, which ultimately affects hair growth. Meanwhile, rabbits aged between four and five months, in general, were

used because rabbits between this age limit had entered adulthood, and their physiology and anatomy had fully developed and were therefore, suitable for this study. Before the treatment was given, the rabbits were acclimatized first for 7 days to adapt to the surrounding environment's condition to reduce potential stress. On day 6 of acclimatization, the rabbits were shaved on the dorsal side and then smeared with 70% alcohol as an antiseptic, after which the rabbits were rested for 24 hours.

i. Positive and Negative Controls

The cream base without medicament served as the negative control, 5% Minoxidil as positive control and untreated shaved rabbits as the normal control.

ii. Rabbit Grouping

A total of three (3) male rabbits were used in each group for this assay. The dorsal side of the rabbits was shaved and then smeared with 70% alcohol as an antiseptic. The various groups are; the positive control, negative control and normal control. For the three extracts, the rabbits were grouped in threes for the 5% strength, 10% strength, 15% strength and 20% strength respectively.

b. Formulation of the crude extract cream :

The composition and amounts of the formulation ingredients used in the crude extract cream, describes an oil-in-water type of cream preparation. The cream was formulated according to a reported method by Gyawali *et al.* Briefly the oily phase was prepared by melting stearic acid and cetyl alcohol in a water bath, adding mineral oil and heating to 70°C. The aqueous phase was prepared by

mixing the water-soluble ingredients (triethanolamine, glycerin, benzyl alcohol, crude extract and water) and heating the mixture to 70°C. The oily phase was then added to the aqueous phase with rapid stirring to avoid phase separation (Gyawali *et al.*, 2020).

c. Assay

The anti-alopecia assay was conducted following a protocol established by Jufri *et al.* (2017). In this procedure, approximately 1 mL of the herbal cream—prepared for the standard drug and various extracts—was applied to the shaved dorsal side of each test animal according to their respective groups. The groups were organized as follows: the first group received a 5% Minoxidil cream as a positive control, the second group was treated with the cream base only, and the third group received no treatment. The remaining groups were treated with different concentrations (5%, 10%, 15%, and 20%) of three extracts (n-hexane, ethyl acetate, and methanol).

The treatment was administered daily over a period of 27 days. Hair growth measurements

were taken every three days using a Vernier caliper

Statistical Analysis

A repeated measure spilled analysis of variance (ANOVA) with Greenhouse Geisser correction was used to analyze the data between groups and across groups obtained from the anti-alopecia study. Statistical significant differences were considered at a 95% confidence interval ($p \leq 0.05$).

Results

Percentage Yield of the Stem Bark Extract of *Irvingia gabonensis*

The yield obtained from n-Hexane was 110.16g and the percentage yield was calculated as 3.67%, ethyl acetate gave a total yield of 160.86g which was 5.36%. Methanol extract had a weight of 280.22 with a percentage yield of 9.34 as presented in Table 1

Table 1: Percentage Yield for the Extract of *Irvingia gabonensis*

Extract	Weight (g)	Percentage Yield (%w/w)
n-Hexane	110.16	3.67
Ethyl acetate	160.86	5.36
Methanol	280.22	9.34

Anti-Alopecia activity of *Irvingia gabonensis* Stem Bark Extract

The findings from the comprehensive investigation on the efficacy of various extracts as anti-alopecia agents are detailed and presented in Figures 1, 2, 3 and 4. Strengths of 5%, 10%, 15% and 20% of each extract were used in this study.

Figure 1, presented in a line chart gives the outcome of the control groups while Figure 2 collectively elucidates the outcomes associated with the n-Hexane extract at various strengths. This was compared statistically with the standard drug. These results notably

underscore the superior effectiveness of the 15% strength of the n-Hexane extract. Figure 3 shows a comprehensive analysis of the diverse concentrations employed within the ethyl acetate extracts. This data prominently indicates that the 10% concentration within the ethyl acetate extract was observed as the most effective and showed no significant statistical difference with the standard drug. Similarly, Figure 4 provides an intricate examination of the varying concentrations within the methanol extract, which was compared statistically with

the standard drug; within this framework, it is evident that the 10% strength of the methanol extract exhibited the highest level of efficacy among the diverse concentrations tested. Furthermore, statistical analysis of the results within groups and across groups based on efficacy compared with the standard drug showed that the ethyl acetate extract was the most potent compared to other extracts, and showed no statistical difference with the standard medication.

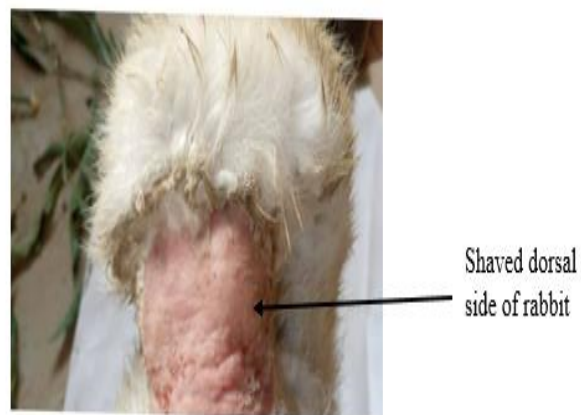
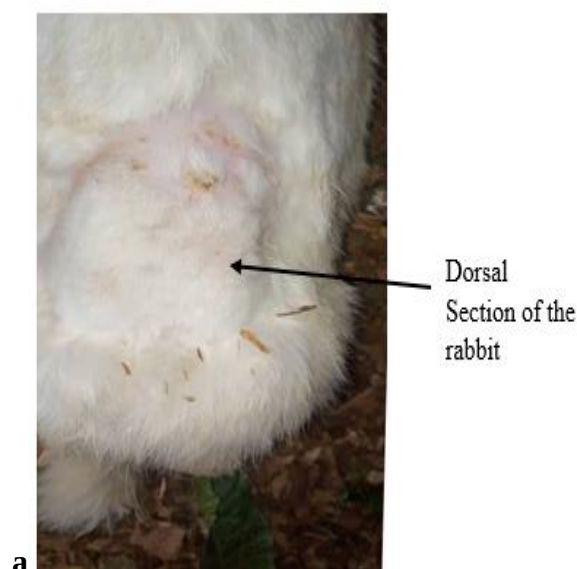




Plate 1: Picture of Dorsal Section of Experimental Animal

a: Dorsal section of the Rabbit b: Shaved Dorsal section of Rabbit c: Herbal Cream

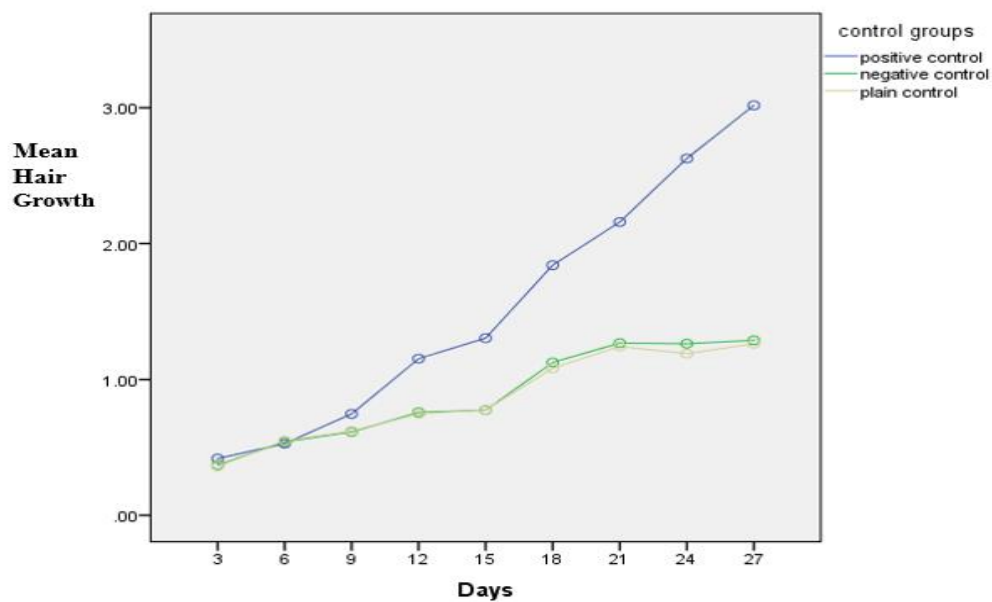


Figure 1: Hair Growth Response in Rabbits: Positive, Negative, and Baseline Controls

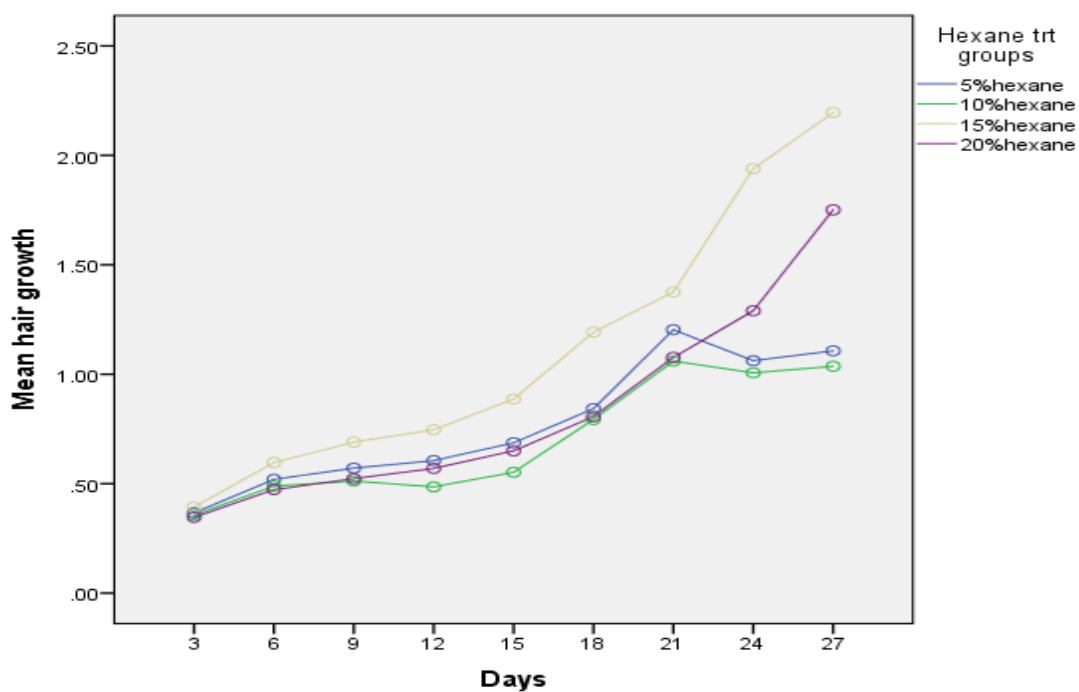


Figure 2: Hair Growth (cm) in Response to N-Hexane Extract of *Irvingia gabonensis*

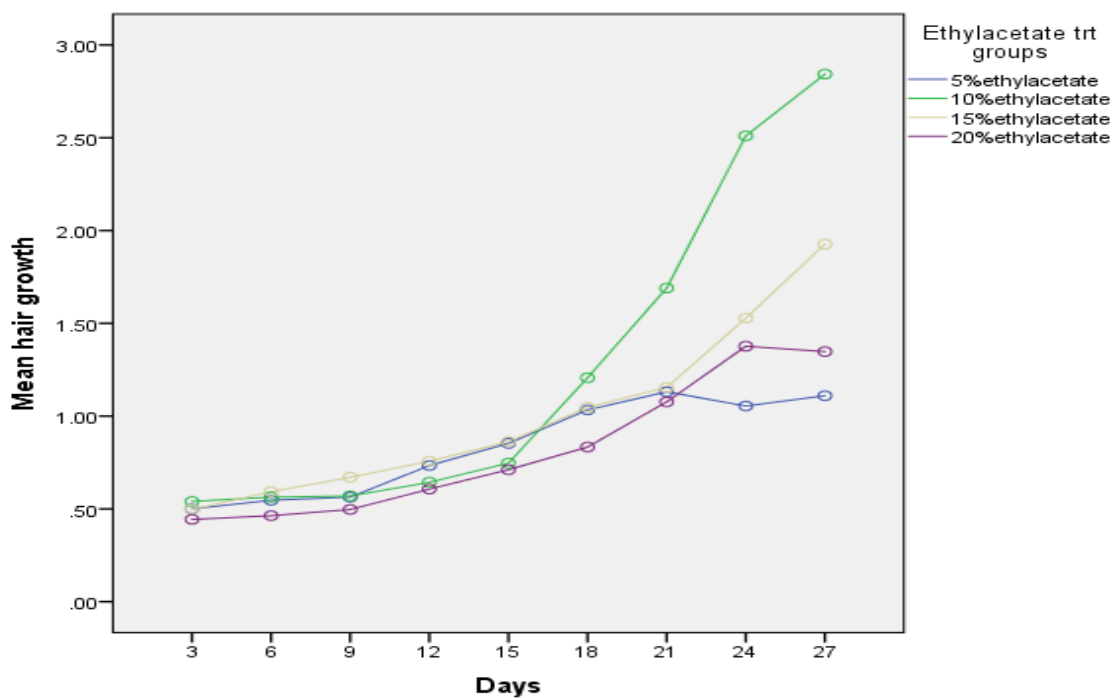


Figure 3: Hair Growth (cm) in Response to Ethyl-acetate Extract of *Irvingia gabonensis*

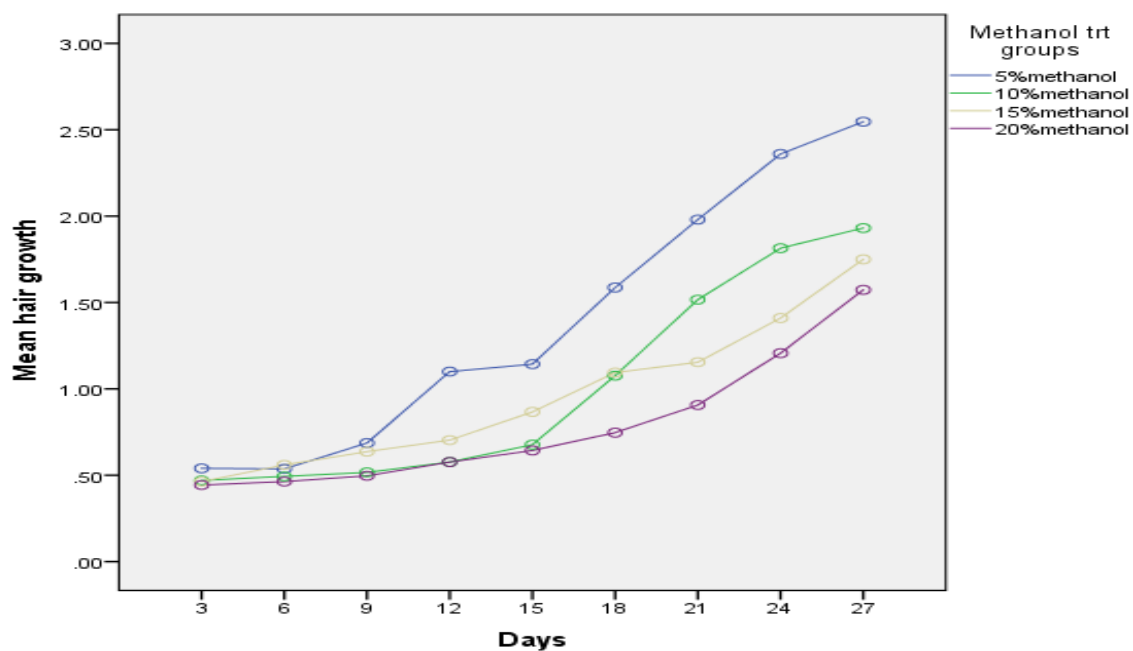


Figure 4: Hair Growth (cm) in Response to Methanol Extract of *Irvingia gabonensis*

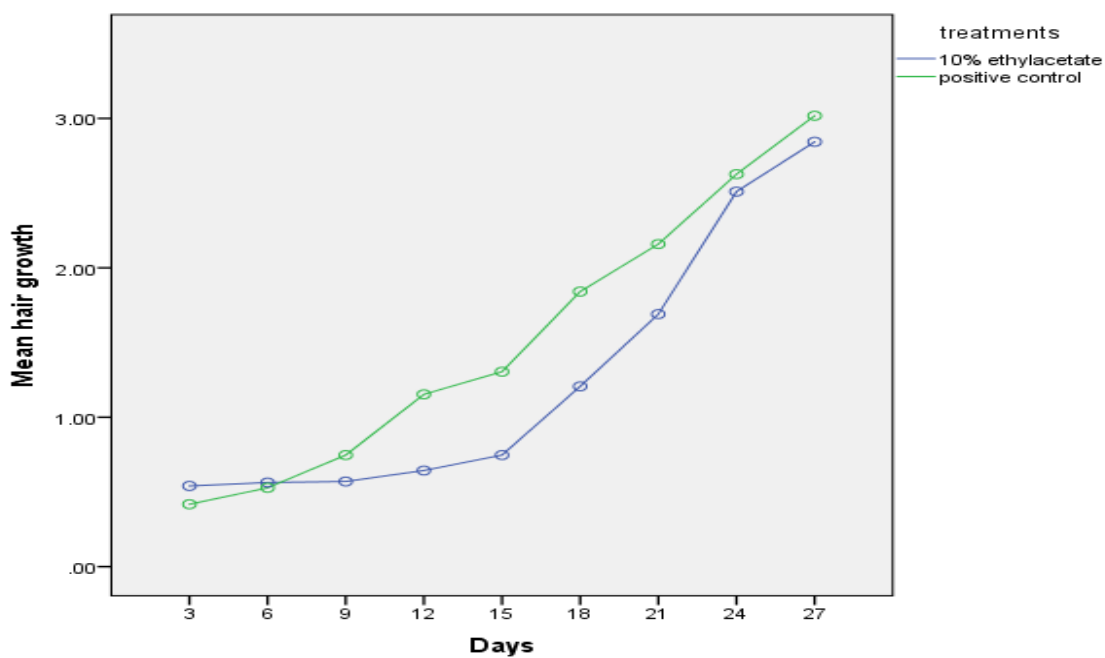


Figure 5: The most effective concentrations of the three Extracts alongside the standard drug

Discussion

The extraction of the powdered stem bark of *Irvingia gabonensis* with n-hexane yielded 3.67% (w/v) of extract, successive extraction with ethyl acetate and methanol yielded a further 5.30% (w/v) and 9.34% (w/v) respectively. This shows that the stem bark of *Irvingia gabonensis* possesses more polar constituents than non-polar ones. This is in agreement with a research conducted by Kuete and co-workers in 2007, where the yield was more polar constituents than non-polar. This polar phytochemical might be implicated in the activity seen in this research.

Results from the anti-alopecia study showed that ethyl acetate extract gave the most anti-alopecia activity, suggesting that the phytochemical implicated in the activity is likely polar, which was also reported by Mustarichie *et al.* (2017); in research to investigate the Anti-alopecia activity of *Erythrina variegata* leaves extract. Also another research conducted by Mustarichie and co-workers in 2017, titled Characteristics and alopecia activity of *Angiopteris evecta* (G. forst) hoffm.) Growing in galunggung mountainside, West Java found anti-alopecia activity in all the polar fractions. Susanti *et al.* (2022), identified a class of alkaloid compounds from the fruits of the Noni plant (*Morinda citrifolia*) as potent anti-alopecia compounds, in this research, it was observed that the ethyl acetate extract showed the most promising hair growth-promoting activity. Mustarichie *et al.* (2018), in a study aimed at scientifically validating the effectiveness of the leaves of *Malvaviscus arboreus* in promoting hair growth. The ethanol extract was tested for its ability to stimulate hair growth, using a modified Tanaka method, with minoxidil 2%

serving as a positive control for comparison. The findings revealed that the *Malvaviscus arboreus* leaf extract, particularly at a 15% concentration, displayed significant hair growth-stimulating activity comparable to the positive control.

Kasmawati *et al.* (2022), conducted a study aimed at evaluating the hair growth-promoting properties of a subfraction obtained from *Sansevieria trifasciata* and to characterize the isolate using infrared spectroscopy. To achieve this, the researchers separated the fraction from *Sansevieria trifasciata* through column chromatography. They assessed hair growth activity using a modified Tanaka method and examined the effects of the subfraction on rabbit skin through hematoxylin and eosin staining. Additionally, they used infrared spectroscopy (FT-IR) to analyze and characterize the isolate. The study revealed that the subfraction known as subfraction-C which was the fraction separated by n-hexane and ethylacetate ratio 1:1 demonstrated the highest hair growth activity. Abdurrahman *et al.* (2023), Conducted an in-vivo study on rabbits to determine the anti-alopecia activity of the leaves of *Merremia peltata*. Compounds isolated from the ethyl acetate fraction were tested in-silico with minoxidil as a comparison ligand. Scopolin and scopoletin were identified as potential anti-alopecia compounds using docking, molecular dynamics simulations, and predicting absorption, distribution, metabolism, excretion, and toxicology (ADME-Tox). Jang *et al.* (2022), in a study indicated that the ethyl acetate extract of the plant *Connarus semidecandrus* has the potential to be an effective and relatively safe treatment for androgenic alopecia by promoting hair growth, reducing androgenic

effects, and supporting the survival of hair-follicle cells.

The study on the anti-alopecia properties of *Irvingia gabonensis* stem bark extract provides compelling evidence for its efficacy in treating hair loss. The research, conducted using animal models (rabbits), demonstrated that the ethyl acetate extract of the plant exhibited significant anti-alopecia activity. This extract's performance was found to be statistically comparable to that of standard treatments, with statistical analysis indicating no significant difference in efficacy between the ethyl acetate

extract and the established treatment options. The findings suggest that *Irvingia gabonensis* could be a viable alternative for managing alopecia, particularly in areas where traditional medicine practices are prevalent. This study underscores the potential of ethno medicinal plants in developing effective treatments for conditions that have substantial physical and emotional impacts. Further research is warranted to confirm these findings in human trials and to explore the underlying mechanisms of the plant's anti-alopecia effects.

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