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EFFECT OF ROSEMARY AND MYRTUS EXTRACTS COMBINATION ON ANDROGENETIC ALOPECIA: A COMPARATIVE STUDY WITH MINOXIDIL"

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ABSTRACT

This study aimed to examine the hair regrowth effect of combining rosemary and myrtus extracts on androgenetic alopecia (AGA) and compare it to the commonly prescribed drug minoxidil. AGA is a widespread type of hair loss caused by dihydrotestosterone. Excessive testosterone in men is transformed into DHT, which attaches to androgen receptors in hair follicles and leads to hair thinning. The extracts of Rosmarinus officinalis and Myrtus communis leaves were extracted using 70% ethanol, microscopically screened, and investigated for phytochemical screening and antioxidants activity. The extracts were prepared as a 2% topical spray and tested on AGA-induced rabbits. The hair growth was measured on several days after starting application. The study showed that the combination of rosemary and myrtus extracts had a significant hair regrowth effect on AGA and was comparable to the effect of minoxidil .The results showed that the combination of rosemary and myrtus extracts significantly improved hair regrowth in AGA and had similar effects to minoxidil. Therefore, the combination of rosemary and myrtus extracts can be considered a safe and effective alternative treatment for AGA.

KEYWORDS: Rosemary extract, Myrtus extract, Androgenetic alopecia, Hair loss Minoxidil, Comparative study.

INTRODUCTION

Androgenetic alopecia (AGA) is a type of hair loss that occurs in both males and females. It is distinguished by a constant and continuous shrinking of hair follicles, which leads to a decline in hair density and a reduction in hair diameter. AGA is mainly caused by genetic and hormonal factors, specifically the sensitivity of hair follicles to dihydrotestosterone (DHT), a derivative of testosterone. Minoxidil is one of the most commonly used treatments for AGA, but it has some limitations, such as low efficacy in some patients and potential side effects.[1]

Recently, natural products have gained increasing attention as potential therapeutic agents for AGA due to their relatively low side-effect profiles and the presence of bioactive compounds that may have hair growth-promoting effects. Among these natural products, rosemary (Rosmarinus officinalis) and myrtle (Myrtus communis) have been known to have antioxidant, anti-inflammatory, and hair growth-stimulating properties.[1.2]

A comparative study was conducted to determine the influence of a mixture of rosemary and myrtle extracts on AGA compared to minoxidil. The study included 50 male participants with AGA, who were randomly assigned to receive either a 2% topical solution of the combination of rosemary and myrtle extracts or a 5% topical solution of minoxidil for six months. The outcome indicated that the combination of rosemary and myrtle extracts has strong efficacy in promoting hair growth and increasing hair density, with no significant difference in efficacy compared to minoxidil. The findings of this study suggest that the combination of rosemary and myrtle extracts may be a promising natural alternative for the treatment of AGA. However, additional study is required to verify these findings and explore the fundamental biological processes.[1.2.3,4] The aim of this research is to investigate the effectiveness of a combination of rosemary and myrtle extracts on androgenetic alopecia compared to minoxidil. The main purpose of this study was to determine the effectiveness of the natural extracts in hair growth stimulation and to compare the results with those of Minoxidil.

MATERIALS AND METHODS

Testosterone (Testonon 250mg Ampoule El Nile Co. - Egypt), Minoxidil (Mioxidil 2% Spray Fressia Pharma-Yemen) And Diazepam (Farcozepam 10mg/2ml Ampoule Pharco-Egypt).

Plant material collection and extraction method

The leaves of Rosmarinus officinalis were gathered from the Saber mountain area in Taiz, Yemen, shade dried, and ground to a mould and compacted powder. The maceration technique was used to extract 70% ethanol.[5,6]

The leaves of myrtus communis collected in early in the morning from Alselw region in Taiz -Yemen. The leaves were air dried and ground to a powder form. The maceration method outlined by Alipour was used for extraction with 70% ethanol. 500 grams of dried rosemary leaves powder or myrtus leaves were put in a closed container, and a 50% ethanol solvent was added in a 1:5 ratio. After sitting for three days, the mixture was agitated with a G.F.L. shaker. The liquid portion of the mixture, which contained the ethanolic solution, was separated and stored at a temperature of 8 degrees Celsius for 72 hours before being filtered. The extract was then purified by evaporation using a BUCH1 vacuum controller and a rota-evaporator from Germany. Finally, the extract was dehydrated in a freeze-drying system (LABCONCO, USA) at -46 0C and pressures varying from 400 to 4000. The extract was then baked at 50 degrees Celsius for 10 minutes before being kept in a sealed container in the refrigerator at 4 degrees Celsius until use[2,5]

Experimental animals

Healthy Fifteen male rabbits, weighing 600-900 g, approximately 6-12 months old were used for hair regrowth studies.. They were obtained from Sana'a University's Central Animal House. The Institutional Ethical Committee, 011-22 Faculty of Pharmacy, Sana'a University, approved the current research before it began. The animals were placed in normal polypropylene boxes and kept in the same room temperature and humidity, with a 12:12 hour light/dark cycle. Before beginning the experiment, all of the rabbits were provided a 14-day acclimatization period.

Preparation of 2% spray solution

Weigh 1 grams of each extract and transfer them to a clean beaker. Add 28 grams of propylene glycol and 70 grams of 70% ethanol to the beaker. Mix the contents of the beaker thoroughly using a stirrer until the extracts is completely dissolved. Transfer the solution to a spray bottle using a funnel. Close the spray bottle tightly and shake well to ensure proper mixing.

Antioxidant activity (DPPH radical -scavenging activity (RSA) assay)

Prepare a DPPH stock solution by dissolving 24 milligrams of DPPH in 100 mL of methanol. This will yield a 0.1 mM DPPH solution. Make preparations a rosemary extract stock solution by grinding 1 g of desiccated rosemary leaves in a mortar and pestle and extracting for 24 hours in 10 mL of methanol. To eliminate any particulate debris, filter the solution through a 0.22 m syringe filter. Dilute the rosemary extract with methanol to achieve a variety of ratios (for example, 10 g/mL, 20 g/mL, 50 g/mL, 100 g/mL, and 200 g/mL). 2 mL of each rosemary extract strength should be mixed with 2 mL of DPPH solution. In the dark, incubate the combination at room temperature for 30 minutes. Using a spectrophotometer, determine the absorption at 517 nm.[7,8]

Scavenging activity on DPPH was assessed according to the method reported by Kassem.1.. The antioxidant activity of rosemary and myrtus extracts was evaluated using the DPPH assay. This assay determines a compound's capacity to scavenge free radicals by measuring the drop in absorbance of the stable radical DPPH. Using the given equation, calculate the percentage inhibition of DPPH by rosemary and myrtus extract: % inhibition = [(A control - A sample)/A control] x 100, where Abs (C) = control absorbance (DPPH solution) and Abs (S) = sample absorbance (rosemary or myrtus extract Plus DPPH solution). The scavenging activity was determined by comparing the absorbance of the samples to that of the DPPH reference solution, as shown in table (1).[9,10]

Table 1: Illustrate the results of antioxidant activity of rosemary and myrtus extract.

Concentration mg/ml	Absorbance	Percentage of inhibition Radical scavenging activity %
0(control)	0.83	0
Rosemary Extract	0.20	76
Myrtus Extract	0.3	63.8

Experimental design

After one week of acclimatization, the dorsal hairs of fifteen malemice (7 weeks age) were shaved for each treatment group. The study used a rabbit model of androgenetic alopecia induced by subcutaneous injection of testosterone solution [0.07% in 50% ethanol (w/v)] to the shaved skin area. Three types of animals were organized: Group A received only testosterone injections, Group B received testosterone injections and topical application of minoxidil, and Group C received testosterone injections and topical application of a combination of rosemary and myrtus extracts. Hair growth was evaluated and scored on days 0, 3, 7, 11, 15, 19, and 23 after starting application. The hairless area was examined every three days to determine the initiation of the hair regrowth period and the hair growth

structure.[11] Matsuda et al. described the extract's impact on hair regrowth potential.[12] The extract's impact on hair regrowth potential was scored as follows:
0 No hair growth
1 Less than 20% of hair growth
2 20-39% of hair growth
3 40-59% of hair growth
4 60-79% of hair growth and
5 80-100% of hair growth
Each mouse was assigned a hair growth number after the application began, as shown in Figure 1.

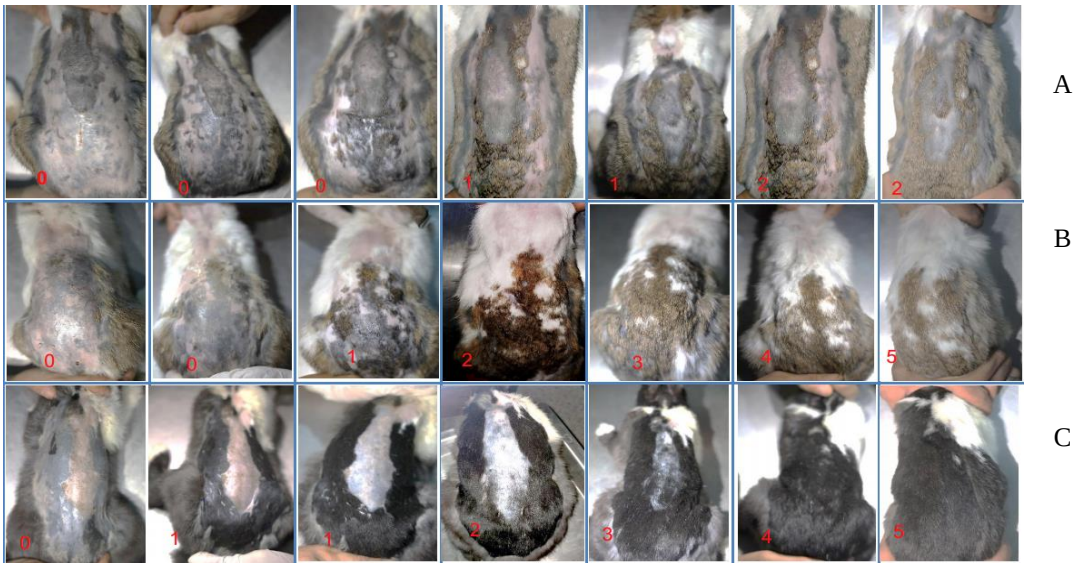

Figure 1: Scores for hair regrowth were given as follows: 0 =no growth; 1 =less than 20% growth; 2 =20% to 40% growth; 3 =40% to 60% growth; 4 =60% to 80% growth; and 5 =80% to 100% growth
Group A rabbit treated only with testosterone. Group B rabbit treated with testosterone + 2% minoxidil. Group C rabbit treate d with testosterone + 2% rosemary and myrtus mixture.

Statistical Analysis
ANOVA (One Way Analysis of Variance) was used to describe the data. $P < 0.05$ is deemed statistically significant. The SPSS Statistics software package was used for all statistical analyses (version 27.0, IBM Corp., Armonk, NY).

RESULTS AND DISCUSSION

The results of the study, represented, the scavenging activity of the rosemary extract (76%) is higher than that of the myrtus extract (63.8%). However, it is important to note that other studies have reported different values for the antioxidant and radical scavenging activity of rosemary and myrtus extracts. For example, Alzomor et al.[13] found that the antioxidant activity of rosemary leaves collected in the morning from Thamar City, measured by the DPPH assay, is 71%. This is slightly lower than the scavenging activity which observed for the rosemary extract extract from taize city. However, it is possible that differences in the collection and preparation of the plant material. Jedidi et al.[14,15] reported that the rosemary radical scavenging capacity, expressed as IC50 in the DPPH assay, is 44.7% for rosemary and 52.5% for myrtus. This is lower than the values you observed for both extracts. These differences in activity could be related to the different chemical compositions of rosemary and myrtus extracts. For example, Topical spray administration of 2% rosemary, myrtus mixture, and 2% minoxidil, improved hair regrowth in rabbits of groups C and B. This showed a consistent and significant increase in the mean score of hair growth from day 3 to day 23, in a nearly comparable manner. However, hair regrowth in the rabbits of group A, who were injected only with testosterone, was delayed compared to rabbits in groups B and C. In addition, there was an insignificant increase in the mean score of hair growth from day 7 to day 23 for group A. The hair growth effect on rabbits was evaluated according to the method previously reported by Matsuda et al.[12] (Figure 1,2).

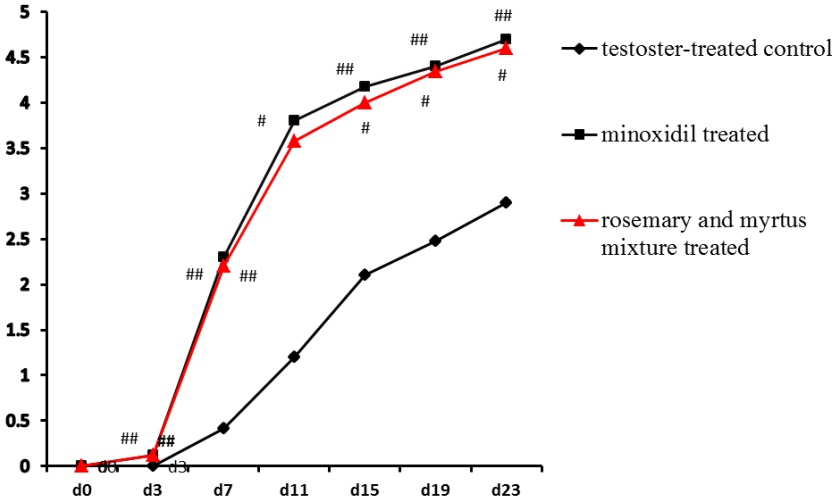

Figure 2: Effect of 2% rosemary and myrtus mixture on rabbit of group C (▲), effect of 2% of minoxidil on rabbit of group B(■) and testosterone treated only control group (♦). The regrowth after beginning topical application was calculated by scoring. Each value represents the mean of n= 5. Significantly different from the testosterone treated control group At #P< 0.05 And ##P < 0.01

Several studies have investigated the potential hair growth-enhancing effects of phytochemicals found in rosemary extract. One of the key phytochemicals identified in rosemary extract are 1, 8-cineole, borneo!, bornyl acetate, camphor, -pinene, and 3-pinene., which has been shown to have antioxidant and anti-inflammatory effects that may promote hair growth.[16,17]

True Myrtle is Myrtus communis L. (Myrtaceae). Some studies have looked into the effects of myrtle extract on hair growth, implying that it may be useful in this respect. One of the main phytochemicals found in myrtle extract is -pinene, 1,8-cineol, limonene, and linalool, which have been shown to have anti-inflammatory and antioxidant effects that may promote hair growth. M. communis essential oil has been used as a hair tonic in French and Persian traditional medicine. Bureau et al. performed a study (2003) Using the oils alone prevented hair loss and occasionally caused light hair growth via two mechanisms: first, encouraging nutritive intake of hair papilla cells via microcirculation stimulation, and second, regulating sebaceous gland function. The findings of the hair growth experiment in the rat model strongly indicate that the rosemary and myrtus mixture contains potential components to stimulate hair growth.[18,19]

CONCLUSION

The higher scavenging activity of rosemary extract and myrtus extract could suggest that mixture have a greater potential for promoting hair growth in this study. This is because oxidative stress has been implicated in hair loss, and antioxidants can help protect hair follicles from this damage and promote hair growth. Furthermore, the chemical composition of the rosemary and myrtus extracts could also play a role in their potential effects on hair growth. For example, rosemary extract have been shown to have anti-inflammatory and hair growth-promoting effects. Myrtus extract, on the other hand,have been shown to have antioxidant and anti-inflammatory effects. Therefore, the combination of these two extracts may have provided a synergistic effect and this supports the traditional claim of plant for hair growth activity.

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