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Comparative antifungal potential of six formulated herbal shampoos against *Candida albicans* causing Seborrheic dermatitis

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ABSTRACT

Cosmetics have been used by humans since centuries for variety of purposes, including enhancing appearance, protection and cleansing. Herbal ingredients have been used in cosmetics for their natural and beneficial properties. When combined, herbal ingredients and cosmetics can provide a number of benefits, including improved skin health, protection from the sun and a more youthful appearance. Shampoo is a key part of hair cosmetics which is used to clean scalp and hair. There are many scalp infections such as Seborrheic Dermatitis, Scalp Psoriasis and Candidiasis which are caused by the fungus *Candida albicans* and are treated by using antifungal shampoos. But it was observed that marketed antifungal shampoos cause various side effects such as alopecia, eye irritation, dry hair, abnormal hair texture, dry scalp and headache etc. The current study focuses on comparison of antifungal potential of six herbal shampoos developed using isolated phytoconstituents such as Rutin, Eugenol, Camphor and Menthol; as well as their combination. The results showed that antifungal potential of all the six developed herbal shampoos against *Candida albicans* causing Seborrheic dermatitis is comparable to the marketed synthetic antifungal shampoo. Hence, it is concluded that the six developed shampoos can be successfully used to treat Seborrheic dermatitis caused by *Candida albicans*.

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Introduction

Plants have been used for medicinal and cosmetic purposes for centuries. They are known to have a variety of benefits for the skin, including soothing, treating and improving a variety of skin conditions. The herbal sector has been improved a significant growth in recent years, as more and more people are turning to natural products for their health and beauty needs (Hussain et al. 2022). The scalp and hair are vulnerable to external factors such as UV rays, pollution and contaminants etc. This can lead to a variety of common problems, including alopecia, an itchy scalp, greying, hair loss, oily or dry hair and dandruff. Shampoos are an essential part of individual care and are used daily to cleanse the scalp and hair. The main purpose of shampoo is to cleanse the scalp and hair while also keeping the hair soft, manageable and lustrous. However, there are also special shampoos that contain unique ingredients with specific purposes, such as anti-dandruff, baby care, hair-nourishing, medicated and premium conditioning shampoos. Shampoos can be transparent or opaque and they are available in a variety of dosage forms, including creams, gels, liquids, lotions, dry-powder aerosols and pastes (Al-Quadeib et al. 2018; Singh et al. 2020; Banduke et al. 2022).

Candida albicans is the most common fungus that can cause infections in humans. It is a serious health and economic burden because it can cause high mortality rates (over 70%) in patients, especially those who are immunocompromised. *C. albicans* is an opportunistic fungus that has evolved a variety of mechanisms to survive and spread in the human body. These mechanisms include forming biofilms, which are protective clusters of cells that are difficult to kill, switching between yeast and hyphal forms, which allows it to adapt to different environments and producing hydrolytic enzymes, which can break down tissues and allow the fungus to spread. *C. albicans* can also develop resistance to antifungal drugs by increasing the expression of certain genes such as CDR1, a gene that encodes a protein that helps *C. albicans* to repair DNA change and ERG11, a gene that encodes a protein that is involved in synthesis of ergosterol, a molecule that is essential for the cell membrane of *C. albicans* (Ivanov et al. 2020).

Candida albicans is most associated with scalp infections such as seborrheic dermatitis (Buslau et al. 1989), scalp psoriasis (Elsner et al. 2022) and candidiasis. Seborrheic dermatitis also named as dandruff, which is a skin condition characterized by pruritic, erythematous patches and dry, flaky scales on the areas containing sebaceous glands such as scalp, axilla, upper chest, groin, back and face (Berk et al. 2010). Scalp Psoriasis is a skin condition that causes red, thickened patches of skin with silvery-white scales, mainly confined to the scalp or they

can extend onto the ears, posterior neck and forehead. In most of the cases, scalp psoriasis is also leads to dandruff, alopecia or intense pruritus (Blakely et al. 2016). Candidiasis is a scalp yeast infection that causes an itchy rash on head that can be purple or red. The rash may also be flaky and crusty. Treatment for these scalp infections may include an antifungal medication, that target DNA repair or ergosterol synthesis, such as an antifungal shampoo. There is a need to find alternative herbal formulation against *Candida albicans* because herbal formulations are increasing in demand due to widespread and side effects of marketed synthetic antifungal shampoos (Hsu et al. 2021).

Traditional medicine offers many natural ingredients that can be used in hair care products. These ingredients are vitamins, fruit acids, sugars, phytohormones, glycosides, bioflavonoids, amino acids and essential oils. These nutrients have positive effects on the skin as well as on hair and can help to improve scalp as well as hair health (Banduke et al. 2022).

The current study aims to compare the antifungal potential of six herbal shampoos formulated with single phytoconstituents such as Rutin, Eugenol, Camphor, and Menthol, as well as their combinations against *C. albicans* causing Seborrheic dermatitis.

Materials and Methods

Sample collection

The two isolated phytoconstituents Rutin and Eugenol were obtained from Yucca Enterprises (Mumbai, India). The other two isolated phytoconstituents Camphor and Menthol were purchased from the local market in Pune, India. Excipients such as sodium lauryl sulphate (SLS), carbomer, bezalkonium chloride, triethylamine and Sabouraud dextrose agar were purchased from the local suppliers in Pune, India. Rose water was purchased from the local market in Pune, India. The strain *Candida albicans* MTCC-183 was procured from MTCC (Chandigarh, India).

Optimization of formula

After many attempts, a gel with the desired consistency was created by Carbomer solution (1% w/v) in water with triethylamine as a pH neutralizer. The desired foam was obtained by SLS solution (4% w/v) in water. Seven different shampoos were formulated, one without any active ingredient i.e., Control and six with different active ingredients (Isolated Phytoconstituents). The active ingredients were Rutin (0.5 gm in 20 ml methanol), Eugenol (2 ml), Camphor (0.5 gm in 2 ml ethanol) and Menthol (1 gm in 2 ml ethanol). Two of the shampoos contained combinations of active ingredients, one with Eugenol and Rutin and other with Menthol and

Camphor. The developed shampoos were evaluated using standard protocols.

Our study focuses on development of six herbal antifungal shampoos using isolated phytoconstituents such as Rutin, Eugenol, Camphor and Menthol and comparison of antifungal potential of these herbal shampoos against *Candida albicans* causing Seborrheic dermatitis.

The IC₅₀ values were calculated using a linear regression method. Various concentration such as, 5, 10, 20 and 40 % of all the shampoos were prepared and their antifungal activity was performed using well diffusion method. The zone of inhibitions obtained for each concentration of the shampoos were measured as mean diameter in cm.

A scatter graph was obtained by plotting concentration versus zone of inhibition in Microsoft Excel. Linear trend line was chosen to obtain the slope value ($y = mx + C$) and R² value. The concentration required for 50 % inhibition (IC₅₀) was calculated using the slope equation obtained from the graph.

Antifungal activity of herbal shampoos

The test pathogenic fungi *Candida albicans* was grown on Sabouraud dextrose agar (SDA). Firstly, a fungal suspension (inoculum) was prepared by dissolving a small number of spores of fungus in 0.2 ml sterile water. The suspension was then spread onto SDA plate using a glass spreader and plate was incubated at 32°C for 2 days. Then the plate was observed for fungal growth. After the fungus grew, the plate was stored in refrigerator until further use.

In-vitro antifungal assay: Sterile, liquefied SDA (30 ml) was aseptically poured into each sterile plate with an inner diameter of 10 cm. The plates were allowed to solidify completely. After solidification, 0.2 ml of fungal suspension (freshly prepared from the grown fungus) was inoculated onto each plate and was spread on SDA surface using a glass spreader. The plates were then set aside for 10 minutes. After that, 3 wells were made in each SDA plate in an aseptic manner using a sterilized stainless steel cork borer. Each well was loaded with 0.1 ml of shampoo solution. The plates were then incubated at 32°C for two days. After incubation, the zones of inhibition around the wells were measured.

The assay was repeated twice for each shampoo for four different concentrations- 5%, 10%, 20% and 40%. Ketoconazole (Ketocip) shampoo was used as positive control and Control shampoo was used as negative control (Kulkarni et al. 2020; Dhanablan et al. 2022; Girma et al. 2021; Vijayakumar et al. 2006; Massiha et al. 2015).

Results

The developed six herbal shampoos were evaluated using standard protocols and it passes the standard limits. It was further evaluated for their antifungal potential against *Candida albicans* and compared with marketed antifungal shampoo i.e. Ketocip.

Antifungal activity of herbal shampoos

After incubation, complete fungal growth was observed. This grown culture of *Candida albicans* was maintained by subculturing it regularly and was stored in refrigerator until use.

In-vitro antifungal assay: After incubation, it was observed that the shampoos of Rutin, Eugenol, Eugenol and Rutin combination, Camphor, Menthol, Menthol and Camphor combination showed good zone of inhibition when compared to the standard shampoo (Figure 1 A-C). A zone of inhibition is a clear area around a substance that inhibits the growth of fungus. Later on, zone of inhibition of shampoos were measured by calculating their mean and IC₅₀ value was determined (Figure 2 A-G). The results are shown in **Table 1**. The shampoos of Eugenol and Rutin combination, Camphor, Menthol, Menthol and Camphor combination were found to be as effective at inhibiting growth of fungus as standard marketed shampoo. This was determined by measuring the IC₅₀ (Half-maximal inhibitory concentration) value of each shampoo, which is a measure of how much amount of drug is needed to inhibit a biological process by half. A lower IC₅₀ value indicates a more potent drug.

Discussion

Cosmetics have become increasingly popular in recent decades. Shampoos, face powders, eye liner, eye-shadow, creams, lotions, lipsticks and nail lacquers are some of the most popular products. However, the use of synthetic ingredients in these products has been linked to health problems, such as allergies, irritation, cancer, skin, scalp and hair damage etc. Herbal cosmetics are becoming increasingly popular as a healthier alternative. Herbal cosmetics are made with natural ingredients that are safe for the skin and hair. They are also effective in providing skin care benefits (Banduke et al. 2022). Shampoos are viscous solutions of detergents that are used to clean, condition and protect scalp and hair. They may also contain additional ingredients such as preservatives, fragrances and active substances that provide additional benefits (Vijayalakshmi et al. 2018). The use of herbal components in cosmetics is a growing trend. This is because herbal ingredients are seen as being less toxic than synthetic ingredients and are also more likely to be accepted by consumers (Latifah-Munirah et al. 2015; Ivanov et al. 2021).

Scalp infections such as seborrheic dermatitis, scalp psoriasis and candidiasis are caused by a fungus called *Candida albicans*. Its treatment includes an antifungal medication, such as an antifungal shampoo, targeting DNA repair or ergosterol synthesis (Norouzi et al. 2021; Ivanov et al. 2021).

Table 1: IC₅₀ values of herbal antifungal shampoos

Sr no	Shampoo Formulation	IC ₅₀
1	Std	16.4479
2	Eugenol	19.0537
3	Rutin	14.4428
4	E+R	20.9219
5	Menthol	14.8622
6	Camphor	12.871
7	M+C	32.1734

Std: Standard; E+ R: Eugenol+ Rutin; M+C: Menthol + Camphor

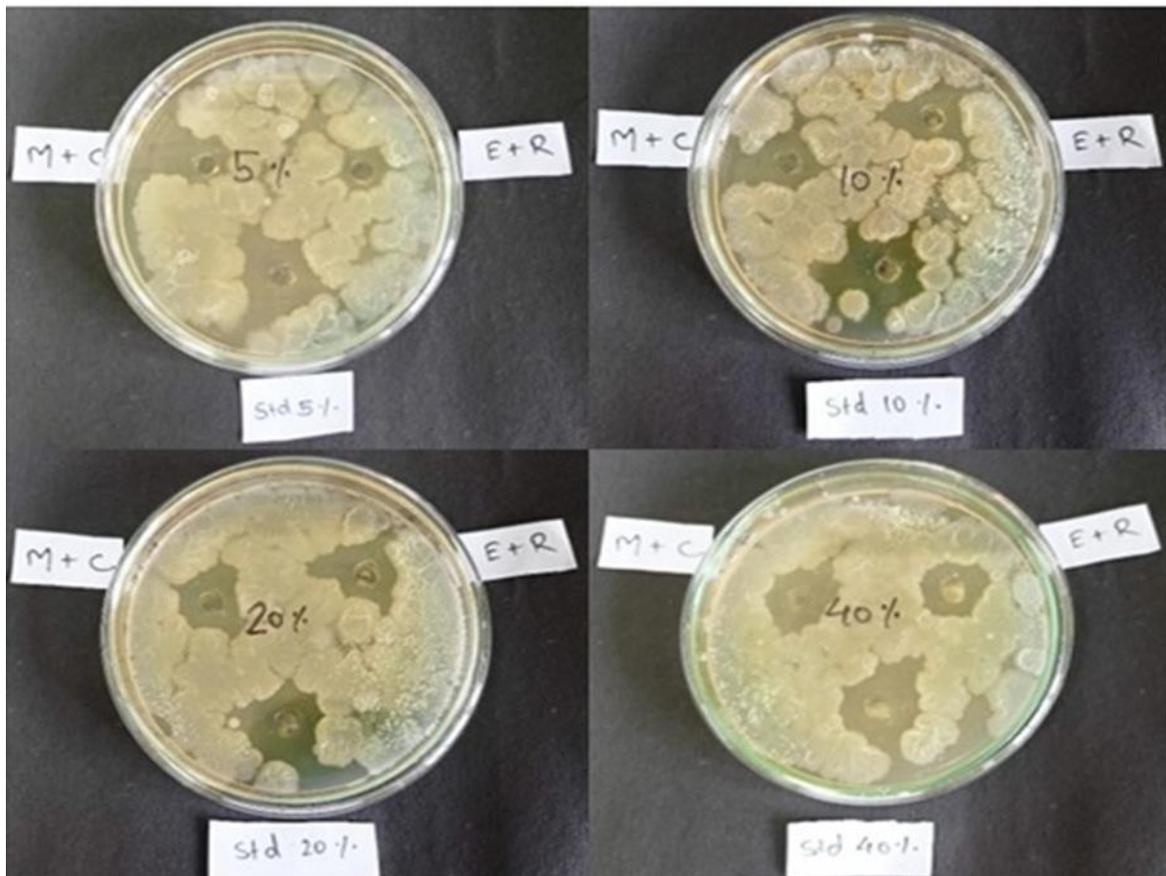


Fig 1 A. Antifungal evaluation of herbal shampoos containing Eugenol, Control and Standard (Std).

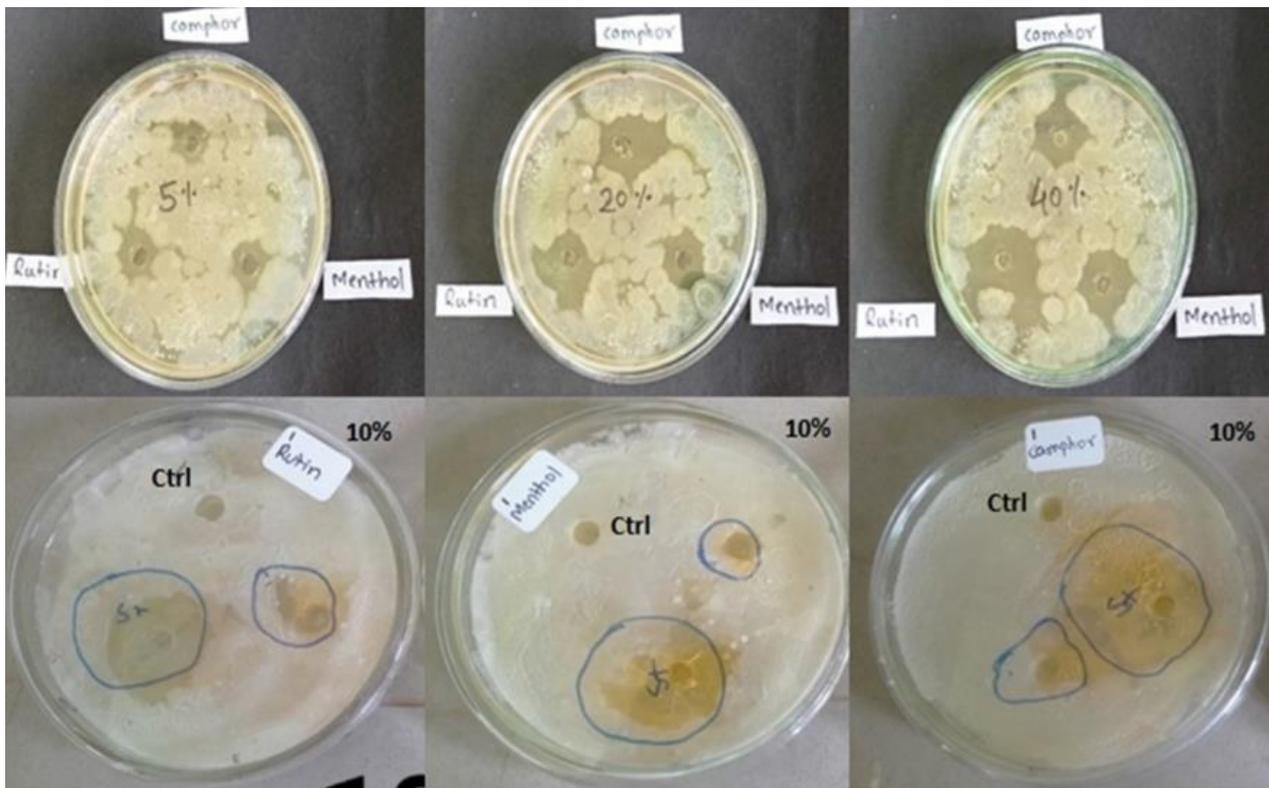


Fig 1 B. Antifungal evaluation of herbal shampoos containing Rutin, Menthol, Camphor, Control(Ctrl) and Standard (Std).

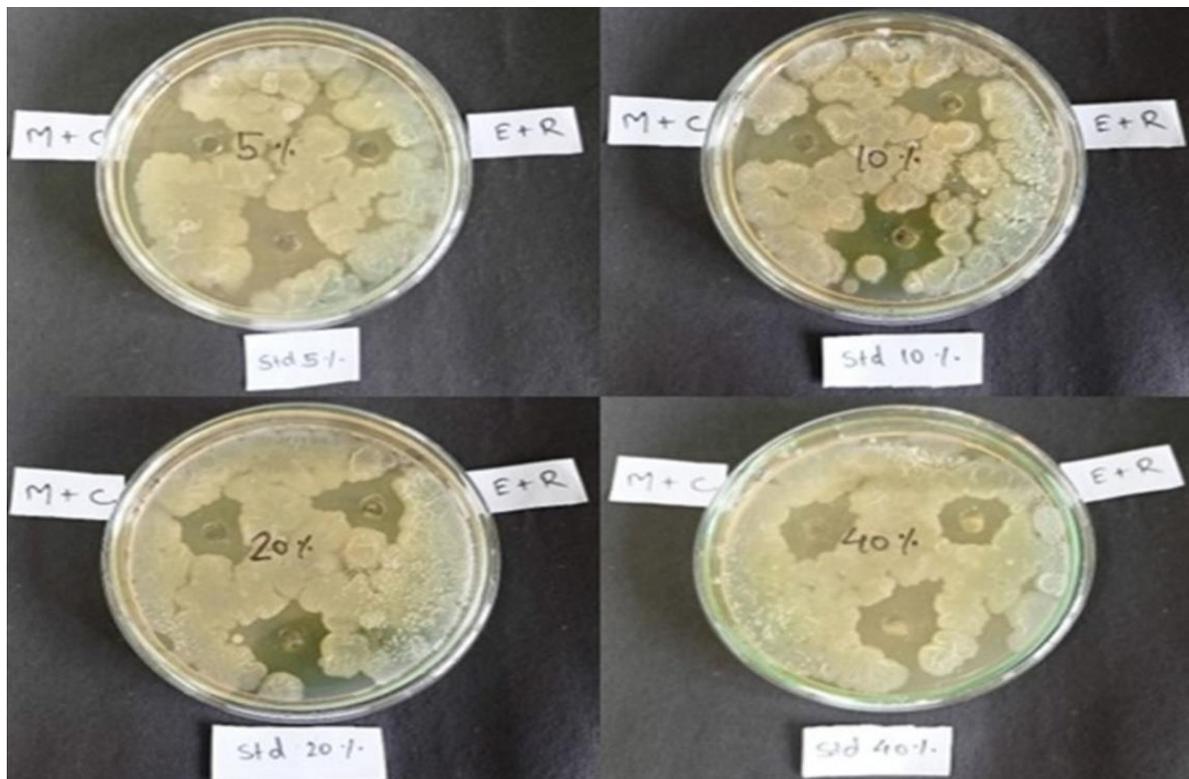


Fig 1 C. Antifungal evaluation of herbal shampoos containing Eugenol and Rutin Combination (E+R), Menthol and Camphor combination (M+C), Standard (Std).

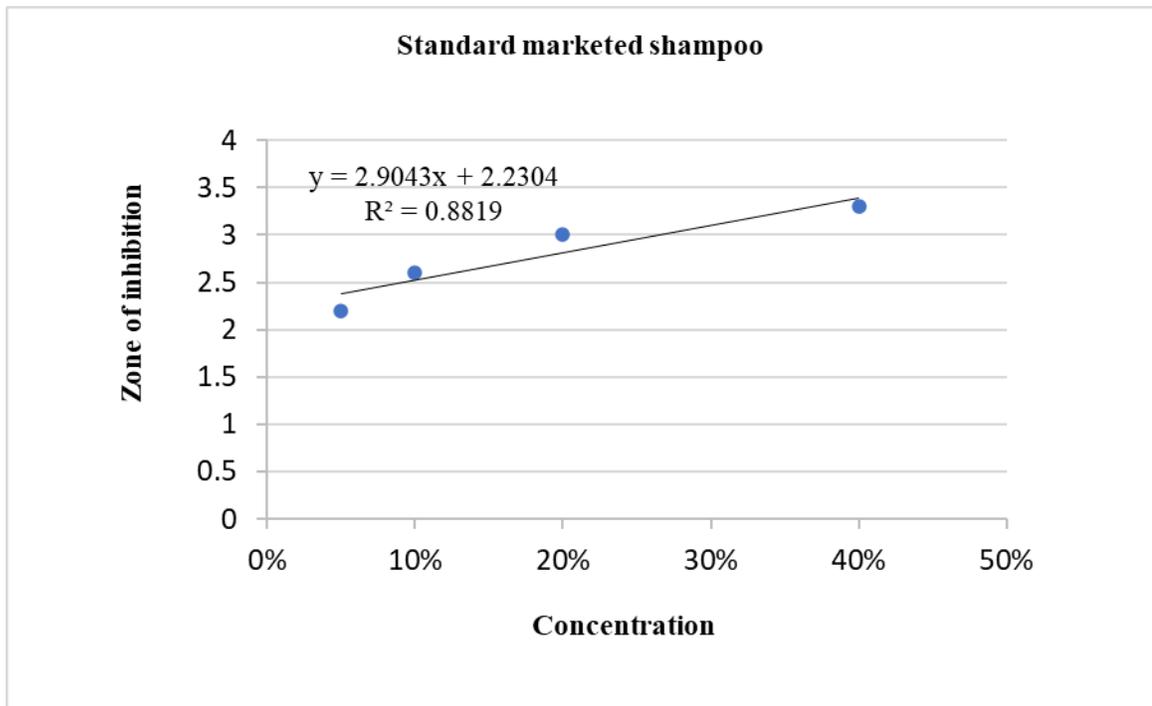


Fig 2A. Graph of concentrations versus zone of inhibition for standard marketed shampoo. The values represented are the means.

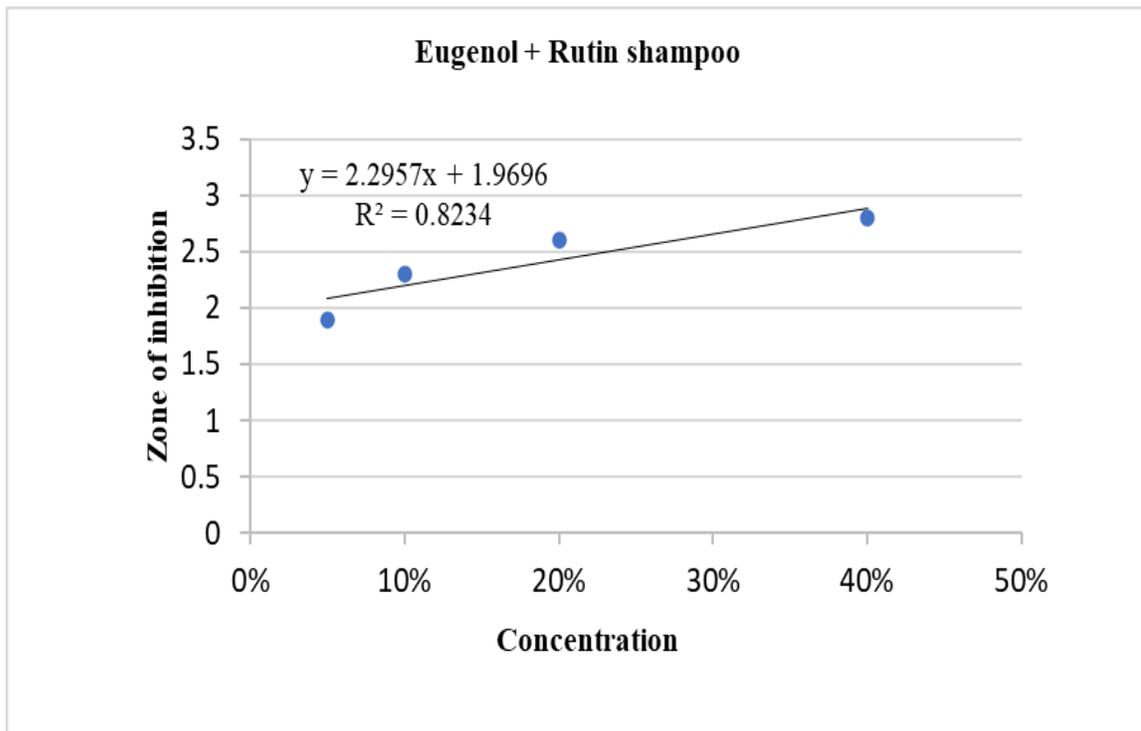


Fig 2B. Graph of concentrations versus zone of inhibition for Eugenol and Rutin combination shampoo.

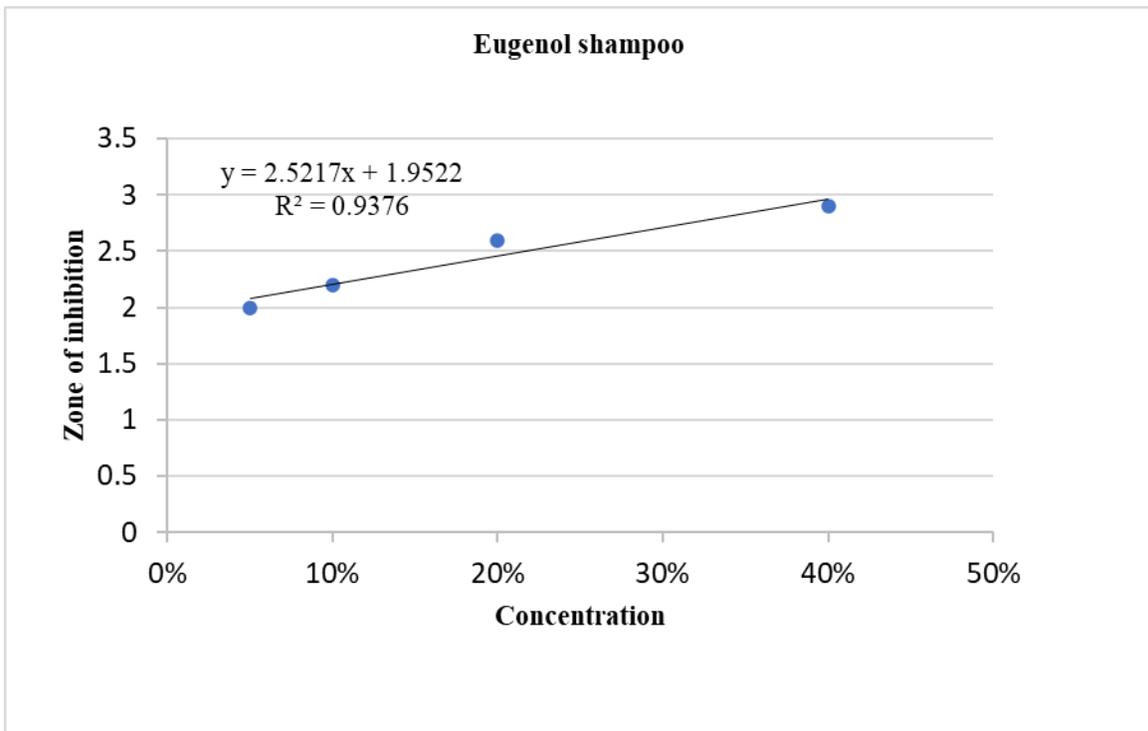


Fig 2C. Graph of concentrations versus zone of inhibition for Eugenol shampoo.

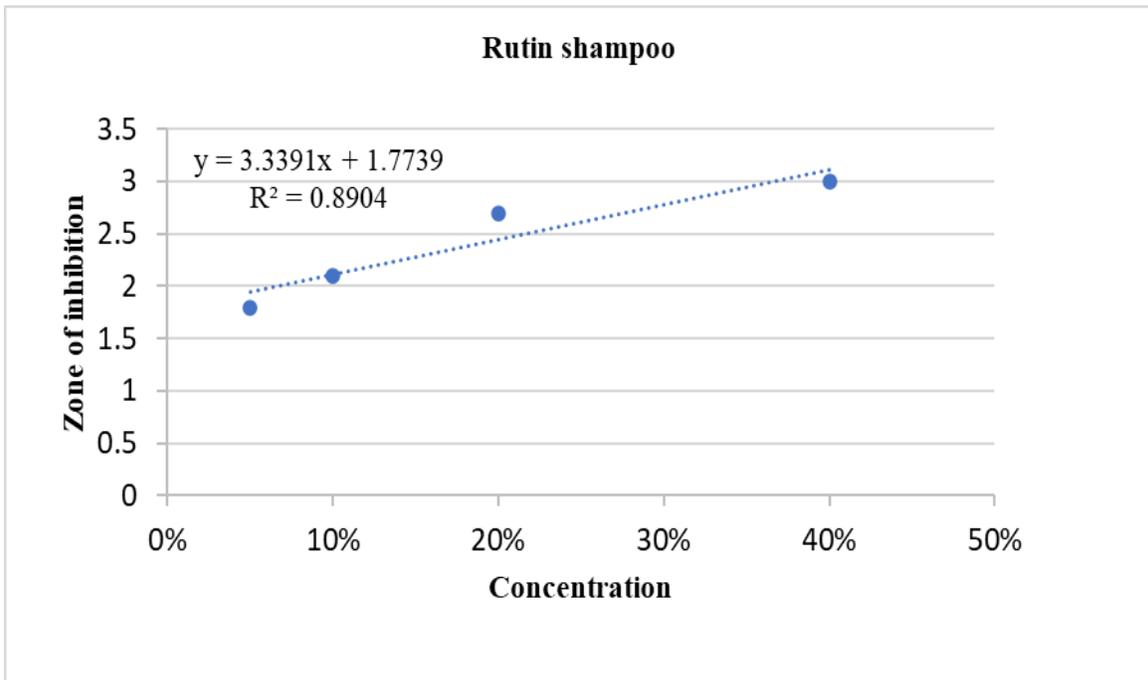


Fig 2D. Graph of concentrations versus Zone of inhibition for Rutin shampoo.

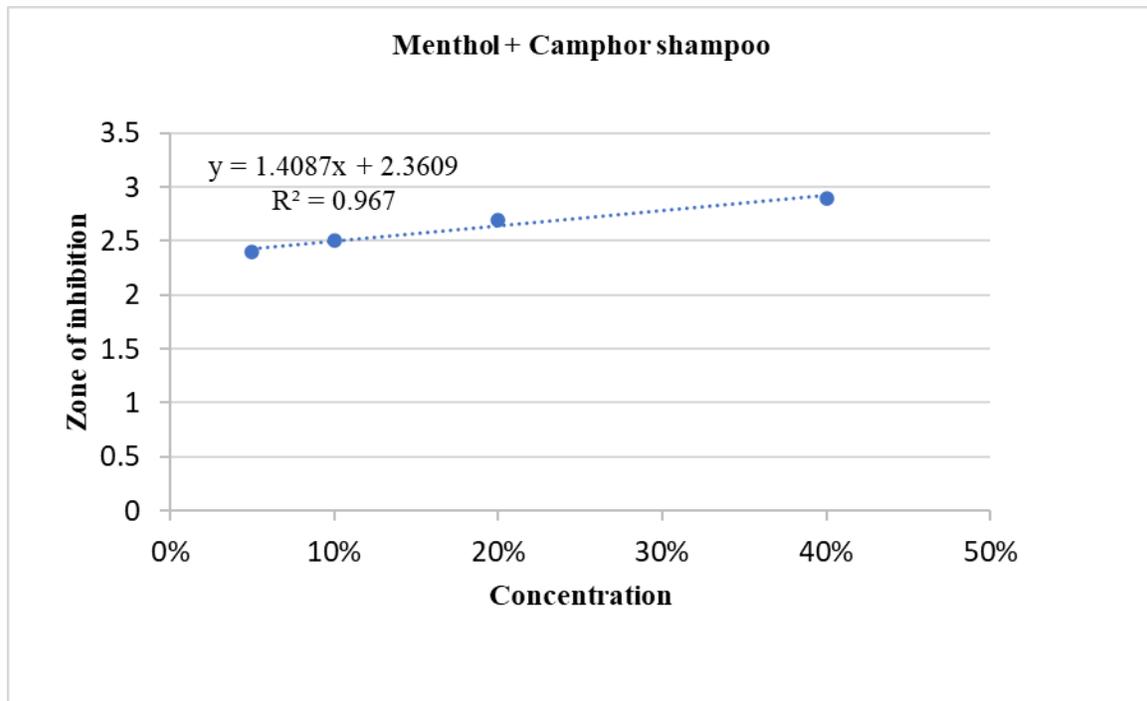


Fig2E. Graph of concentrations versus zone of inhibition for Menthol and Camphor combination shampoo.

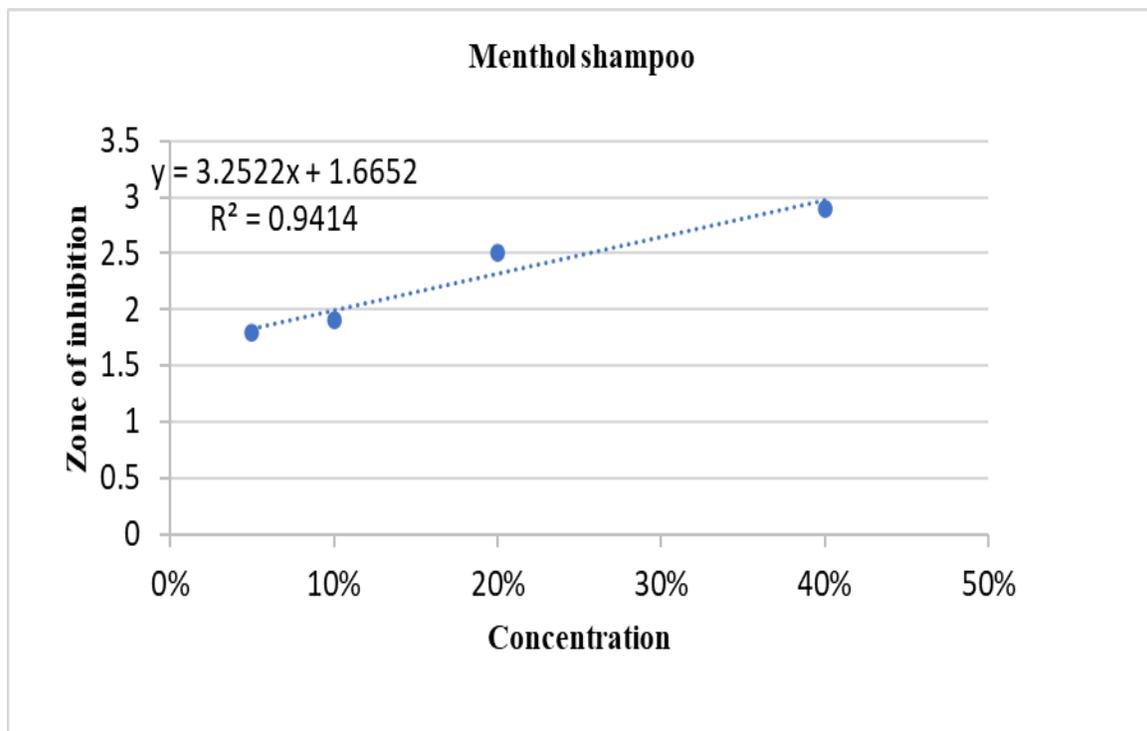


Fig2F. Graph of concentrations versus zone of inhibition for Menthol shampoo.

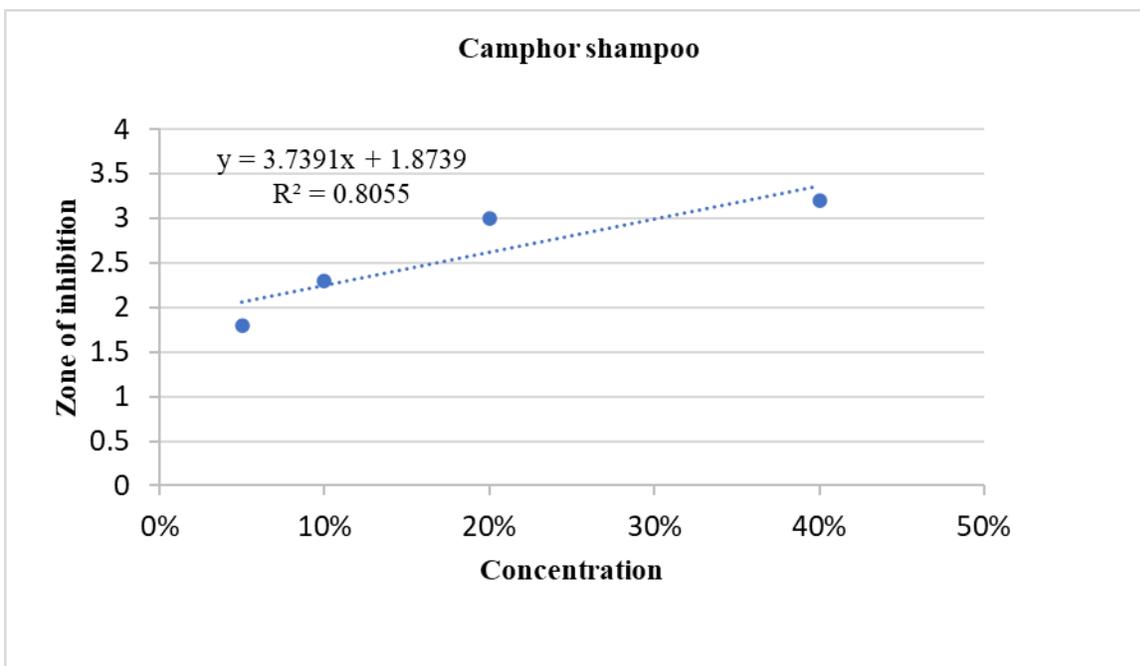


Fig 2G. Graph of concentrations versus zone of inhibition for Camphor shampoo.

Marketed synthetic antifungal shampoos which contain ketoconazole or ciclopirox olamine as an active ingredient can have a number of side effects such as alopecia, eye irritation, dry hair, abnormal hair texture, dry scalp and headache etc. Natural ingredients, on the other hand, are less likely to cause side effects (Gubitosa et al. 2019). The development of shampoos using natural ingredients with antifungal properties is a promising area of research. These shampoos could offer a safe and effective alternative to synthetic antifungal shampoos. There are a number of natural ingredients that have antifungal properties and these ingredients can be effectively used to develop shampoos that are effective to treat scalp infections without any side effects. In the present study, six herbal antifungal shampoos were developed using isolated phytoconstituents such as Rutin, Eugenol, Camphor and Menthol and also compared antifungal potential against *Candida albicans* causing Seborrheic dermatitis. The antifungal activity of all six developed shampoos towards the fungus *Candida albicans* was effectively performed and the results showed that these shampoos can be used to treat Seborrheic dermatitis. The shampoos containing Rutin, Eugenol, Camphor and Menthol showed significant results for antifungal potential as compared to the marketed antifungal shampoo. The antifungal effect of these ingredients is due to their ability to disrupt the cell walls of *Candida* yeast cells targeting DNA repair or ergosterol synthesis. This causes the cells to die, which helps to clear up the infection.

Conclusion

A comparative antifungal potential study of six formulated herbal shampoos was effectively performed against *Candida albicans* causing Seborrheic dermatitis. The isolated phytoconstituents like Rutin, Eugenol, Camphor and Menthol can be effectively used to develop herbal antifungal shampoos as they actively showed antifungal activity against *Candida albicans* which means that these ingredients can effectively treat Seborrheic dermatitis. Hence, we conclude that all the six developed shampoos can be used as herbal antifungal shampoos against *Candida albicans* causing Seborrheic dermatitis.

Conflict of interest

The authors declare that they have no conflict of interest.

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