

## Short communication



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## Combined activity of selenite-resistant bacteria and *Pisum sativum* sprout extract against *Malassezia furfur*: A preliminary qualitative evaluation

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**Keywords:** *Malassezia furfur*, *Pisum sativum* sprout extract, antifungal activity, dandruff, selenium resistant bacteria

**Introduction:**

The lipid-dependent basidiomycetes yeasts of the genus *Malassezia*, have eighteen species and numerous functionally distinct strains, are vital to the skin microbiome. Further long read sequencing and chromosome-level genome assembly will be required to refine or examine *Malassezia* evolution because many species of the genus *Malassezia* lack entire genomes. *Malassezia* are more common in sebaceous zones (the breast, back, and head) due to the abundance of fatty resources in these areas (1). Skin health maintenance is linked to *Malassezia* density. An active partnership in which both species benefited is referred to as mutualism. *Malassezia* may not only be able to thrive on human skin, but they may also be able to defend against potentially harmful microorganisms like *S. aureus* (2). Only for dandruff and pityriasis versicolor has a causal relationship been

**Abstract:**

Selenium has been an essential micronutrient required for human's biological and dermatological health including prominent antifungal properties. The fungal strain *Malassezia furfur* is associated with skin problems such as hair scalp dryness more particularly dandruff. This preliminary evaluation involved isolation of fungal strain from dried, scaly hair scalp and identified through growth characteristics, catalase reaction and microscopic morphology. *Pisum sativum* sprout extract possess potential hair growth properties. The selenium treated pea sprouts and controls were grown in favorable conditions. The experimental groups were combining pea sprouts treated with selenium and selenite resistant bacterial extracts, pea sprouts treated with selenium and control with no treatment to peas and bacterial extracts alone. The findings suggest that selenium resistant bacteria treated pea sprout extract, pea sprouts treated with selenium displayed minimum inhibition against *Malassezia furfur* as compared to controls under tested conditions. The maximum inhibition was observed in group in which *Malassezia* was treated with extract of selenium resistant bacteria treated pea sprouts. Since this is an exploratory study and did not measure any quantitative inhibition, additional research is required in order to determine the effectiveness of its treatment for *Malassezia* infections associated with dandruff problems.

established between *Malassezia* and the development of the disease; its involvement in atopic dermatitis (AD) is still largely correlative and unclear (3). Selenium is an essential micronutrient involved in various physiological processes and strengthens antioxidant defense mechanisms, neuroprotective properties, regulate reproductive processes, and take role in the metabolism of thyroid hormones and is a component of the antioxidant enzyme glutathione peroxidase (4). During respiration, selenite is physiologically converted into elemental selenium (Se<sup>0</sup>) by selenite-reducing bacteria (5). In addition to being used in the creation of nutritional supplements containing selenium, it is also used in the manufacturing of cosmeceuticals and nano-cosmeceuticals that are intended to protect the skin, hair, nails, and lips from wrinkles, photo aging,

dandruff, and hair damage (6). Pea sprout extract's rich nutritional content and bioactive components nourish the scalp and encourage hair development. It prolongs the anagen (growth) phase of the hair cycle, stimulates hair follicles, and improves scalp health and also demonstrated that in general exposure of 100mg of pea sprout extract to hair roots efficiently decrease hair loss in the individuals facing hair fall problems (7). However, finasteride and minoxidil function differently, with finasteride preventing the formation of dihydro testosterone and minoxidil boosting blood flow to the scalp. Pea sprout extract is rich in

#### Material and methods:

The selenite resistant bacteria were isolated, cultured and tested for their biochemical properties separately (10). The fungal strain that is *Malassezia* was isolated from scalp bearing dandruff problem via swabbing and was inoculated at Sabouraud Dextrose Agar. Before pouring, the 100ml media was added with 25mg/5ml chloramphenicol solution (11). The media is overlaid with lipid source as the desired dandruff agent is lipid dependent. Then it is subjected to incubation for 3 days. The strains were fresh in Sabouraud Dextrose broth with similar protocol with the exception of agar. The strain was grown at 30°C, 37°C and 41°C. Then fungal strain was stained with methylene blue and catalase test was performed with 3% $H_2O_2$ .

The peas were germinated by treating them with 1% selenium solution and growth was observed. The bacterial extracts were prepared separately by simple

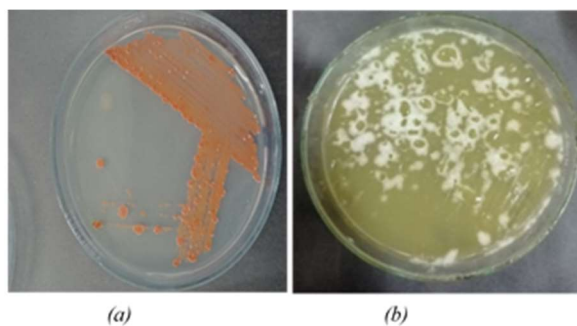
#### Results:

The isolated fungal strain demonstrated maximum growth on Sabouraud Dextrose Agar (figure 1). Microscopic examinations with methylene blue revealed the fungal strain with long hyphae with clustered cells consistent with *Malassezia* morphology (figure 2). The fungal strains were catalase positive

flavonoids and phenolic compounds, which protect hair follicles from oxidative stress and damage caused by free radicals. Antioxidants support the growth of robust, healthy hair and enhance the health of the scalp (8). And methods to treat dandruff like problems efficiently include selenium in the procedure (9). So this study aims to investigate the combined effect of selenite resistant bacteria and selenium treated *Pisum sativum* sprout extract and results were expected in such a way that such a substance be able treat dandruff, dryness as well as hair loss problems.

obtaining the supernatant of bacterial culture after centrifugation at 6000rpm for 15 minutes. The experimental groups included; peas treated with selenium, combining pea sprouts treated with selenium and selenite resistant bacterial extracts while controls were peas with no treatment and bacterial extracts alone. The peas were grind in all four groups that is two controls, bacterial treated peas and selenium treated peas and extract was obtained first by vortexing and then after centrifugation at 5000 rpm for 10 minutes and supernatant was separated. Then the antifungal activity assay was established. The wells were filled with 50 $\mu$ L of the extract and for experimental group to determine combined activity the extracts were mixed in 1:9 i.e., 10 $\mu$ l of bacterial extract and 90 $\mu$ l of pea sprout extract treated with selenium respectively.

and can grow on temperature varying from 30°C to 41°C (table 1). The *Malassezia* strain utilizes ghee as the potential source of lipid for its efficient growth. The fungal strain showed maximum inhibition was observed when extracts from selenium treated peas and bacterial extracts were mixed as compare to other experimental group and controls (figure 3).



**Figure 1:** Selenite resistant bacteria on Nutrient agar plate supplemented with sodium selenite (a), fungal strain isolated from sample (b)



**Figure 2:** Fungal strain subjected to grow at 30°C, 37°C and 41°C (a) Catalase positive fungal strain (b), long hyphae of fungal strain visible on staining with methylene blue (c)

**Table 1:** Morphological, biochemical and growth parameters of *Malassezia furfur*

Sr.no	Catalase test	Temperature			Staining	Lipid
		30°C	37°C	41°C		
	3% H <sub>2</sub> O <sub>2</sub>	30°C	37°C	41°C	Methylene blue	Ghee
1	+ve	+ve	+ve	+ve	Clusters, long hyphae	+ve



**Figure 3:** Peas sprouts treated with selenium as compare to control (a), peas sprout extracts (b), selenite resistant bacteria extract (c)

### Discussion:

A study by (12) demonstrated that 1.8% selenium sulfide has activity against dandruff related *Malassezia furfur*. The use of selenium sulfides, zinc pyrithiones and azoles has been explained as potential treatments for curing *Malassezia furfur* like skin illnesses (13). While selenium not itself was utilized in the current study but selenite resistant bacterial extract efficacy combined with pea extracts treated with selenium against *Malassezia* was determined. As biological produced monolayers established by bacterial strains possess antibacterial and antifungal characteristics. And is less harmful to humans and animals than synthetic fungicides, it is recommended for use as a fungicide in various fields of life (14). Various studies in the field of herbal and natural cosmetics has discussed that *Pisum sativum* sprout

extract improve and nourish the hair health by enhancing the ratio of anagen and telegenic hair (15). The pea sprout extracts were obtained first by washing, cleaning and drying the sprouts. After drying they were subjected to mix with aqueous solvents, then vortexed and centrifuged (16). It has been established by previous studies that the antifungal properties of selenite derivatives, especially their application in the management of dandruff through selenium sulfide, have been remarkable. Likewise, the antifungal properties of biologically active compounds synthesized from microorganisms and plants have also been studied (17). Although the results observed from these experiments indicated that the concentrations or extraction processes may not be enough to exhibit antifungal activity, there is some truth to these studies. Selenite resistant bacteria were isolated from soil

samples and cultured on N-agar. The strains were allowed to culture in nutrient broth then subjected to centrifugation. The supernatant was used as bacterial extracts. The antifungal activity of combined extracts was determined by cylindrical plate method. After swabbing the pure cultured *Malassezia* strain, the extracts were poured in the wells and incubation was done. The plates were checked for zones after every 24 hours. The idea of direct using selenite resistant bacterial strains with pea sprout extracts was thought to be novel. The zone of inhibition was observed against selenium treated *Pisum sativum* sprout extract combined with selenium resistant bacteria as compared to control. Still research is needed to determine exact amounts of both extracts efficacy against *Malassezia furfur*.

### Conclusion

In conclusion, the preliminary qualitative findings demonstrated limited antifungal activity of selenium-treated *Pisum sativum* sprout extract combined with selenite-resistant bacterial extract against *Malassezia furfur*. Since, only little visible inhibition was observed, the study presents a potentially novel biological approach for exploring alternative anti-dandruff strategies. Further studies involving optimized extract concentrations, quantitative antifungal assays, and statistical analysis are required to confirm the therapeutic potential of the combined formulation.

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### Conflict of interest:

The authors have no competing interests.

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