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Novel Test for Developing Drugs- Hyper/Hypopigmentation

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Abstract: *In the present investigation we have invented and used 'Pityrosporum ovale- Potato' method to evaluate tyrosine analogue compounds. We have discovered the tyrosine analogue behaviour of Psoralea corylifolia through our experiment which is quite new to our knowledge. The finding of our study has great scientific significance although it may require further investigation.*
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I. Introduction

Pityrosporum ovale, is a lipophilic yeast known to cause dandruff and Pityriasis versicolor in human being [1 & 2]. Hypopigmented patches do occur during skin infection due to the above organism. The azelaic acid produced by the organism is known to suppress tyrosinase enzyme and the enzyme is known to downregulate melanogenesis [3]. Due to the above benefit of Azelaic acid, it is being used widely in several skin lightening/whitening/fairness creams and lotions [4].

Most fruits and vegetables including the underground tuber –Potato are rich source of phenol oxidase enzyme [5]. The phenol oxidase is an analogue of tyrosinase enzyme [6]. In the present research work we have attempted a novel approach by bringing together '*Pityrosporum ovale* and Potato' as a potential tool to screen various herbs that might have great treatment value for hypo and hyperpigmentation problems.

In the first embodiment of research, we have made an assumption that when the azelaic acid can inhibit tyrosinase, it does inhibit phenol oxidase released by potato. To confirm the above concept we have prepared the cell filtrate of *Pityrosporum ovale* through sonication. The cell filtrate was used as source of azelaic acid. The findings are presented in the paper.

II. Materials and methods

a) Preparation of cell filtrate of *Pityrosporum ovale*

Five day old culture of *Pityrosporum ovale* grown in oleic acid supplemented media was scooped out and washed thoroughly with distilled water. The washed cells were re-suspended in distilled water and sonicated for 45 min at 26˚C. After sonication the soup was centrifuged at 5000rpm for 15min and the supernatant was separated and stored at 4˚C until use.

b) Preparation of potato juice

Freshly harvested potato was procured and washed thoroughly in chill water. The skin of the potato was gently peeled and the potato just beneath the skin was crushed and the juice was prepared. The juice was then filtered and stored 4˚C until use.

c) Experiment

One millilitre of the potato juice was pipetted into an Eppendorf tube and was kept as control. In the next Eppendorf tube that contains 1ml of potato juice was treated with 0.25ml of cell filtrate of *P.ovale*. This served as reaction control.

In the third tube along with potato juice and culture filtrate 0.01g of freshly prepared tyrosine was maintained. In the fourth tube only potato juice and 0.01g of freshly prepared tyrosine was maintained.

The tubes from fifth to ninth were maintained as potato juice+culture filtrate+aqueous extract of *Psoralea corylifolia*, Potato juice+ 0.1ml of aqueous extract of *Psoralea corylifolia* , culture filtrate+0.1ml of aqueous extract of *Psoralea corylifolia*, Potato juice+0.1ml of aqueous extract of *Psoralea corylifolia* +0.01g of tyrosine, Potato juice+0.1ml of aqueous extract of *Psoralea corylifolia* +tyrosine+cell filtrate respectively.

To revalidate the findings we denatured both the likely azelaic acid in cell filtrate and phenol oxidase in potato juice and conducted the above experiments. The colour changes in each tube at different time intervals were recorded visually by a panel of trained scientists to avoid any bias in the recordings.

III. Results

As expected the potato juice has turned to deep black in colour in 1 hour incubation. When the cell filtrate was added to the above solution the coloration was brown than deep black. However when the substrate tyrosine was added to the above mixture the coloration turned to deep black. The addition of aqueous extract of *Psoralea corylifolia* has resulted in deep black coloration. Interestingly when the aqueous extract of *Psoralea corylifolia* was added into the potato juice the colour turned to deep black within 30 min instead of one hour incubation time. Instead of potato juice, the cell filtrate was when added to the plant extract the colour become brown than black.

To understand the likely role of aqueous extract of *Psoralea corylifolia* acting like substrate (analogue to tyrosine) we heat denatured both the cell filtrate and potato juice and did the above experiment. Interestingly when tyrosine was allowed to react with potato juice in the presence of heat denatured cell filtrate the reaction mixture turned to deep black in colour. When we replaced the aqueous extract of *Psoralea corylifolia* instead of tyrosine to the reaction mixture that contains potato juice and heat denatured cell filtrate the coloration turned to be deep black. When we treated the heat denatured potato juice with aqueous extract of *Psoralea corylifolia* the resultant coloration was white, and the result was same for tyrosine as well when used separately. When we allowed the cell filtrate to react with tyrosine and aqueous extract of *Psoralea corylifolia* in the presence of heat denatured potato juice the resultant coloration was light brown (Table 1).

Table 1: Findings of Pityrosporum and potato based screening

Sl.no	Experiment	After 1 hour Visual observation
1	Potato juice	Deep black
2	Potato juice+cell filtrate	Brown
3	Potato juice +cell filtrate+Tyrosine	Deep black
4	Potato juice+Tyrosine	Deep black
5	Potato juice+cell filtrate+*P.corylifolia*	Deep black
6	Potato juice+ *P.corylifolia*	Deep black
7	Cell filtrate+ *P.corylifolia*	Brown
8	Cell filtrate+Tyrosine	White
9	Potato juice+ *P.corylifolia*+Tyrosine	Deep black
10	Potato juice+ *P.corylifolia*+Tyrosine+ cell filtrate	Deep black
11	Heat denatured cell filtrate+potato juice	Brownish black
12	Heat denatured cell filtrate+potato juice+ *P.corylifolia*	Brown
13	Heat denatured cell filtrate+potato juice+Tyrosine	Deep black
14	Heat denatured cell filtrate+potato juice+Tyrosine+*P.corylifolia*	Deep black
15	Heat denatured cell filtrate+potato juice+ *P.corylifolia*	Deep black
16	Cell filtrate+ heat denatured potato juice	White
17	Cell filtrate+ heat denatured potato juice+ *P.corylifolia*	Brown
18	Cell filtrate+ heat denatured potato juice+Tyrosine	White
19	Cell filtrate+ heat denatured potato juice+Tyrosine+*P.corylifolia*	Light brown

IV. Discussion

In line with our initial concept, the cell filtrate of *Pityrosporum ovale* has slightly inhibited the browning of potato juice. This may be due to the difference in the operating mechanism of either the azelaic acid or the phenol oxidase enzyme. Time, temperature, extent of oxidation etc., do influence the browning of potato [7]. When we added freshly prepared tyrosine into potato juice the colour of the juice turned to deep black whereas when the cell filtrate was added, only slight variability in the colour was observed. We presume that the phenol oxidase may be competitively utilising the substrate tyrosine and hence it becomes deep black in colour. The mechanism of action of azelaic acid may be more specific towards tyrosinase and the receptor site for azelaic acid may not be effective in phenol oxidase.

Surprisingly when the aqueous extract of *Psoralea corylifolia* was incorporated in the reaction mixture of potato juice that contains azelaic acid and tyrosine, we have obtained brown pigmentation. When we replaced the substrate tyrosine with the aqueous extract of *Psoralea corylifolia*, the coloration of potato juice became deep black. In order to confirm the likely role of the aqueous extract of *Psoralea corylifolia* as substrate equivalent of tyrosine, we have used the denatured cell filtrate and potato juice for the experiment. None of the above colour changes could be observed when heat denatured cell filtrate or potato juice was used. The aqueous extract of *Psoralea corylifolia* when treated with cell filtrate, contrary to our expectation a brown coloration was formed. The question is how an enzyme inhibitor when treated with an aqueous extract of *Psoralea corylifolia* (substrate analogue) in the absence of an enzyme can result in colour formation. Neither the culture filtrate is pure nor the herbal extract. Therefore we presume some other mechanism would have resulted in the brown coloration. Perhaps this may be the first scientific observation in the world to the best of our knowledge that the

aqueous extract of *Psoralea corylifolia* can occupy the niche of tyrosine. However this needs further investigation.

Photosensitivity of *Psoralea corylifolia* is well known however its ability to upregulate melanogenesis as well by acting as substrate analogue of tyrosine is not yet reported. The role of tyrosine supplementation in the treatment of vitiligo is well known but which is less exploited and explored [8 & 9].

Psoralea corylifolia being a well-known herb used even in the modern scientific medicine for the treatment of vitiligo and Psoriasis and the best example is PUVA therapy [10]. The present finding gives a nascent hope to the treatment of vitiligo.

The experimental tool we have innovated and used in the present experiment has tyrosinase inhibitor azelaic acid, the analogue of tyrosinase - the phenol oxidase, the substrate tyrosine and *Psoralea corylifolia* and other conditions like light, heat, oxygen etc. This complex system is extremely effective for screening drug intermediates that may have substrate analogue property.

The finding of our study is not only startling but has great scientific value as well although the findings may require further investigation.

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