Effect of *Pleurotus nebrodensis* extract on melanin synthesis: A natural alternative for cosmetics

D. M. DANGRE1*, L. P. DAFNE1, R. P BHAGAT2, C. J. CHANDEKAR3

1Department of AYUSH, Maharashtra University of Health Sciences, Vani-Dindori Road, Mhasrul, Nashik, Maharashtra, India.

2Department of Biotechnology, Rajarshi Shahu College, Latur, Maharashtra, India.

3Department of Biotechnology, Shri Shivaji Science College, Congress Nagar, Nagpur, India.

*Corresponding Author, Tel: +91- 253-2539125, Fax: +91-253-2539125

Article History: Received 6th October 2012, Revised 27th December 2012, Accepted 28th December 2012.

Abstract: The mushroom *Pleurotus nebrodensis* is an edible mushroom widely used for nutritional and medicinal purposes. It was observed that the skin of mushroom farmers is turn out to be fairer as they were regularly involved in mushroom handling and process. In order to examine this effect and as a preliminary step to apply for cosmetic ingredients, the extracts as well as its fractionates with certain organic solvents have been prepared to characterize the effect by tyrosinase inhibition assay and melanogenesis inhibition capability in cultured human melanocytes as an *in vitro* model. The investigation revealed that certain extracts from *P. nebrodensis* are more effective in skin whitening than that of Arbutin, a widely used whitening ingredient in functional cosmetic products.

Keywords: Cosmetic ingredients; edible mushroom; melanogenesis inhibition; tyrosinase inhibition assay.

Introduction

The mushroom *Pleurotus nebrodensis* is an edible mushroom widely used for nutritional and medicinal purposes. The extract of this mushroom with hot water have been reported to have medicinal effects such as the reduction of cholesterol level in blood and the activation of macrophages and lymphocytes in human beings (Alam et al. 2011). Recently, it was observed that among the woman workers directly involved in handling of mushrooms at mushroom farm were having whitening effect on their skin. In order to examine this effect and as a preliminary step to apply for cosmetic ingredients, the extracts as well as its fractionates with certain organic solvents have been prepared to characterize the effect by tyrosinase inhibition assay and melanogenesis inhibition capability in cultured Melanocytes or in human melanocytes as an *in vitro* model.

Melanin is one of the most widely distributed pigments and is found in bacteria, fungi, plants as well as animals. It is a heterogeneous polyphenol-like biopolymer with a complex structure and color varying from yellow to black (Prota 1988). The color of mammalian skin and hair is determined by a number of factors, the most important of which is the degree and distribution of melanin pigmentation. Melanin is secreted by melanocytes distributed in the basal layer of the dermis (Spritz and Hearing 1994). The role of melanin is to protect the skin from ultraviolet (UV) damage by absorbing UV sunlight and removing reactive oxygen species (ROS).

Great interest has been shown in the involvement of melamins in malignant Melanoma, the most life-threatening skin tumors. Melanin is formed through a series of oxidative reactions involving the amino acid tyrosine in the presence of tyrosinase. Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme that catalyzes two distinct reactions of melanin synthesis: the hydroxylation of tyrosine by monophenolase action and the oxidation of 3,4-dihydroxyphenylalanine (L-DOPA) to o-dopaquinone by diphenolase action. However, if
L-DOPA is an active cofactor, its formation as an intermediate during o-dopaquinone production is still controversial. o-Dopaquinone is unstable in aqueous solution and rapidly suffers a non-enzymatic cyclization to leukodopachrome, which is further oxidize enzymatically by another molecule of o-dopaquinone to yield dopachrome and one molecule of regenerated L-DOPA (Garcia-Canovas et al. 1982; Rodriguez-Lopez et al. 1991; Cooksey et al. 1997).

Overview of melanin biosynthesis in mammals

Mammalian melanocytes can produce two types of melanin; eumelanin is black or brown and pheomelanin is red or yellow in color (Prota 1992; Ito 1993). Switching between these two types of melanins in follicular melanocytes elicits a temporary shift from eumelanogenesis to pheomelanogenesis, which is responsible for the wild-type agouti pigment of murine hair color (Hirobe 1991). For many decades, melanosomal proteins that regulate melanin biosynthesis have been studied and characterized, especially those required for eumelanogenesis (Hearing and King 1993). The pathway of eumelanogenesis may be divided into two phases, one proximal and the other distal (Alam et al. 2011; Kobayashi et al. 1995). The proximal phase consists of the enzymatic oxidation of tyrosine or L-DOPA to its corresponding o-dopaquinone catalyzed by tyrosinase. This nascent o-dopaquinone can undergo two different types of reactions; intramolecular 1,4-addition to the benzene ring which causes its cyclization into leukodopachrome. This intermediate is quickly oxidized to dopachrome by another o-dopaquinone, which is reduced back to L-DOPA (Brun and Rosset 1974; Pawelek 1991; Araca et al. 1990). The second reaction occurs with cyclizable and noncyclizable quinines and consists of a water addition to the benzene ring, which leads to the formation of a three-hydroxylated phenol, 2,4,5-trihydroxyphenylalanine (TOPA), which is chemically oxidized to p-topaquequinone by another o-dopaquinone (Garcia-Canovas et al. 1982; Brun and Rosset 1974). This p-topaquequinone evolves through a series of slow reactions to dopachrome, which is the final product of the proximal phase. The distal phase is represented by chemical and enzymatic reactions which occur after dopachrome formation and lead to the synthesis of eumelanins. This phase starts with the slow chemical decarboxylation of dopachrome to 5,6-dihydroxyindole (DHI) and its subsequent oxidation to indole-5,6-quinone. As an alternative to this chemical evolution in the distal phase, dopachrome may be enzymatically transformed into 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by dopachrome tautomerase (Pawelek 1991 and Aroca et al. 1990). DHICA is further oxidized by a redox reaction with o-dopaquinone to form indole-5,6-quinone carboxylic acid, which can exist in three tautomeric forms, including the quinone-imine and the corresponding highly reactive quinone-methide (Lambert et al. 1989; Sugumaran and Semesi 1991). Properties of DHI-derived and DHICA-derived melanins differ in each other; the former are black and flocculent, while the latter are yellowish-brown and finely dispersed (Aroca et al. 1990). During pheomelanogenesis, the thiol group of Sulphydryl compounds such as glutathione and Cysteine nucleophilically attack o-dopaquinone made enzymatically by tyrosinase to produce Cysteinyldopa or Glutathionylida, this thiol group can be added to different ring positions, although the 5-position is the favored position. Subsequent cyclization and polymerization of Cysteinyldopa or Glutathionylida in an uncharacterized series of reaction result in the production of pheomelanins and trichochromes (Hearing and King 1993; Deshpande et al. 1984). The interaction between the eumelanin and pheomelanin compounds gives rise to a heterogeneous pool of mixed-type melanins.

Tyrosinase inhibitors

Melanin plays a crucial protective role against skin photocarcinogenesis. However, the production of abnormal melanin pigmentation is a serious esthetic problem in human beings (Preiestley 1993). The cytotoxicity of L-DOPA has been attributed to its selective uptake by melanocytic cells and to the formation of reactive quinines and semiquinones formed in situ during metabolic activation of L-DOPA by tyrosinase (Wick et al. 1977; Graham et al. 1978; Korytowski et al. 1987). The truphenolic
Tyrosinase inhibitors application in cosmetics

Use of tyrosinase inhibitors is becoming increasingly important in the cosmetic and medicinal industries due to their preventive effect on pigmentation disorders. Tyrosinase inhibitors may result in a reduction in melanin biosynthesis and are used in cosmetic products for hyperpigmentation-related concerns, including the formation of freckles. Tyrosinase and its inhibitors may also be targets for developing medicines to treat hypopigmentation-related problems, such as albinism and piebaldism. A number of tyrosinase inhibitors have been reported from both natural and synthetic sources, but only a few of them are used as skin-whitening agents, primarily due to various safety concerns.

Currently Arbutin and Aloesin are used in the cosmetic industry as skin-whitening agents because they show strong inhibition toward tyrosinase, which is responsible for pigmentation in human beings. On the other hand the co-treatment of Arbutin and Aloesin exhibited the inhibitory effect on tyrosinase in a synergistic manner by acting through different mechanisms: arbutin inhibited competitively, whereas aloesin inhibited noncompetitively (Jin et al. 1999). Taken together they inhibit melanin production synergistically by a combined mechanism of competitive and noncompetitive inhibition. This result indicates that it is beneficial to use Arbutin and Aloesin as a mixture in depigmentation because the co-treatment reduces the doses of these agents for the same inhibitory effect on tyrosinase activity and can diminish adverse side effects.

In this study, we investigated tyrosinase inhibition activity of the samples extracted from Pleurotus nebrodensis directly by using enzyme assays and indirectly through melanin synthesis in Melanocytes.

Materials and methods

The mushroom Pleurotus nebrodensis was sliced and completely removed the moisture by freeze dry at -70 °C for 3 days. The 80% or 100% pure Methanol (MeOH) were used for extraction under cold and hot temperature condition. Every sample extracted in MeOH for 3 hours and repeated 3 times the extraction with each sample.

The organic solvents (n-Hexane, Chloroform and Butanol) were used for fractionation. From these solvents, we obtained n-Hexane fractionate sample, Chloroform fractionate sample and Butanol fractionate sample for further study. Samples were dissolved in DMSO.
Enzyme assay

The tyrosinase activity, using L-Tyrosine as a substrate was measured according to the methods of Kubu and Kinst-Hori, 2000 with slight modifications. The mushroom tyrosinase (EC 232-653-4) used for bioassay was purchased from Sigma-Aldrich Co. 40 µl of 1.5mM L-tyrosine, 190 µl of 0.1M phosphate buffer (pH 6.8) and sample dissolved in DMSO were mixed, and 20 µl of mushroom tyrosinase (1,500 U/ml) was added. The enzyme-reacted solution was incubated for 15 min at 37 °C and the solution was immediately monitored for the formation of dopachrome by measuring the linear increase in absorbance at 490 nm. Arbutin was taken as a positive control to get comparative output.

The percentage of inhibition of enzyme activity was expressed as

\[ 1 - (C - D) / (A - B) \times 100 \]

(A = absorbance at 490 nm with enzyme and substrate but without test sample; B = absorbance at 490 nm without test sample and enzyme; C = absorbance at 490 nm with test sample, enzyme and substrate; D = absorbance at 490 nm with test sample and substrate, without enzyme).

Means of triplicates were determined and the 50% inhibition (IC_{50}) of tyrosinase activity was calculated as the concentrations of test samples that inhibited tyrosinase activity by 50% under the experimental conditions.

The samples were gone through Thin Layer Chromatography (TLC) to separate the compounds in various conditions. For each samples we choose a suitable liquid phase such as Hexane: Ethyl Acetate with ratio was 2:1 and 4:1, Methanol: Chloroform with ratio 1:20.

Cell viability and proliferation test

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to determine cellular viability and proliferation after the treatment of cells by the samples for 24 hrs as described. The culture plate was further
incubated in a humidified atmosphere containing 5% CO\textsubscript{2} at 37 °C for 24 hrs, after 24hrs the old media was replaced by fresh media and incubated for 24hrs at similar condition mentioned above. MTT was dissolved in H\textsubscript{2}O at a concentration of 5 mg/ml, and added 20µl to 200µl culture medium. After removing the medium, 150µl DMSO was added, and the absorbance was measured at 570 nm.

Melanogenesis inhibition analysis with cultured cells

Melanocytes were grown in plastic tissue culture flasks and plates in DMEM supplemented with 10% FBS and 1% penicillin and streptomycin. Cells were incubated at 37 °C in an atmosphere of 5% CO\textsubscript{2}. The cells were placed in 24-well plastic culture plates at a density of 1 x 10\textsuperscript{5} cells/well and incubated for 24 hrs in medium prior to treatment with the sample. After 24 hrs, the medium was replaced with 990µl of fresh medium. To this was added 30µl of sample dissolved in DMSO or DMSO only (control). The cells were exposed to the test compounds for 4 days at 37 °C and 5% CO\textsubscript{2}. The melanin content of cells after treatment was determined as follows. After removing the medium, cells were washed with PBS and lysed by adding 0.3 ml of 1N NaOH. The crude cell extracts were assayed and analyzed using a microplate reader (Bio-Rad, USA) at 490 nm to determine the melanin content. Results from samples were analyzed with percent of the control.

Results and discussion

Concentration of sample to inhibit 50% of tyrosinase activity (IC\textsubscript{50})

To investigate the tyrosinase activity inhibition, enzyme assay technique was carried out with L-tyrosine as a substrate. The enzyme-reacted solution was incubated for 15 min at 37 °C and the solution was immediately monitored for the formation of dopachrome by measuring the linear increase in absorbance at 490nm. The results bring out that the inhibition ability of samples extracted under cold condition were lower than that of hot extracts (table 1 and figure 2). Among the 3 fractionates of the extract, Chloroform fractionate have lower IC\textsubscript{50} value (0.42mg) which indicates its highest inhibition performance towards tyrosinase activity (Table 1). The inhibition by methanol extract was poorer, while its fractionates had shown the better inhibitory effect (Figure 2). Arbutin, a positive control had IC\textsubscript{50} of 0.55mg.

Table 1: Sample description and IC\textsubscript{50} value.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Samples</th>
<th>IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arbutin</td>
<td>0.55</td>
</tr>
<tr>
<td>2</td>
<td>80% MeOH extract</td>
<td>0.53</td>
</tr>
<tr>
<td>3</td>
<td>n-Hexane fractionate</td>
<td>0.47</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform fractionate</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>Butanol fractionate</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Figure 2: Comparison of IC\textsubscript{50} values among various extracts and fractionate samples

Separation of compounds by Thin Layer Chromatography

To know our extraction sample has single or multi compound that inhibited tyrosinase activity, we were applied Thin Layer Chromatography (TLC) to identified.

We detected 2 different compounds in 80% MeOH under cold extraction, 3 different compounds in 80% MeOH under hot extraction, 100% MeOH under cold and hot extraction were detected 3 different compounds of each. In each organic solvent fraction, sample were also detected for finding the compounds by TLC technique, the results were revealed and described as shown in figure 3.
Mycoanalysis of Pleurotus nebrodensis against melanin synthesis

Dangre et al.

http://www.openaccessscience.com

http://ijmap@openaccessscience.com

Fractionation of 80% hot MeOH extract shows 5 compounds spots; Chloroform fractionate of 80% cold MeOH extract and 80% hot MeOH extract showed 5 and 4 compound spots respectively, while Butanol fractionate of 80% cold and hot MeOH extracts revealed 3 and 1 spots for separated compounds respectively. In n-Hexane fractionate of absolute cold and hot MeOH extracts 4 and 2 spots of compounds were observed respectively, however, in Chloroform fractionate of absolute cold and hot MeOH extracts, 4 and 2 spots observed respectively, while 3 and 2 compounds spots were observed in Butanol fractionate of absolute cold and hot MeOH extract.

Figure 3: Thin Layer chromatography of test fractionates.

a1 = n-Hexane fractionate of 80% cold MeOH extract, a2 = n-Hexane fractionate of 80% hot MeOH extract; b1 = Chloroform fractionate of 80% cold MeOH extract, Chloroform fractionate of 80% hot MeOH extract; c1 = Butanol fractionate of 80% cold MeOH extract, c2 = Butanol fractionate of 80% hot MeOH extract; d1 = n-Hexane fractionate of absolute cold MeOH extract, d2 = n-Hexane fractionate of absolute hot MeOH extract; e1 = Chloroform fractionate of absolute cold MeOH extract, e2 = Chloroform fractionate of absolute hot MeOH extract; f1 = Butanol fractionate of absolute cold MeOH extract, f2 = Butanol fractionate of absolute hot MeOH extract.

Sample stabilization against high temperature and UV irradiation

a) Against high temperature: High temperature and UV radiation are the agents who can affect on some compounds which result in chemical structure which leads to lose in function. To find the compounds which can inhibit tyrosinase activity but also stable with high temperature and UV light are important to apply sample for life such as whitening agent or food industry. Therefore, we were exposed sample with high temperature (60 °C) and UV light, the sample was exposed for 1, 2, 3, 4, 5, 10 days. The effect on sample function was determined by measuring IC_{50} value.

High temperature revealed no effect on sample when exposed for 5 days, the IC_{50} value was similar as compare with control sample (not exposed with high temperature), but the sample exposed in 60 °C up to 10 days decreased tyrosinase inhibition ability indicated by increase in the IC_{50} value (Table 2a; Figure 4).

b) Against UV irradiation: The UV radiation is also important agent causes break to some compound. The sample was exposed on UV light for 1, 2, 3, 4, 5 and 10 days. The stabilization of sample was observed until day 3 exposed, but up to 5 and further 10 days sample was showing decreased tyrosinase inhibition activity indicated by increase in IC_{50} value (Table 2b; Figure 4).

Table 2a: Effect of high temperature (60 °C) on sample.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Days</th>
<th>Arbutin 80% MeOH extract</th>
<th>n-Hexane fractionate</th>
<th>Chloroform fractionate</th>
<th>Butanol fractionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.55</td>
<td>0.53</td>
<td>0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.56</td>
<td>0.55</td>
<td>0.48</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.57</td>
<td>0.55</td>
<td>0.48</td>
<td>0.45</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.57</td>
<td>0.57</td>
<td>0.50</td>
<td>0.45</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.59</td>
<td>0.57</td>
<td>0.51</td>
<td>0.47</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.70</td>
<td>0.72</td>
<td>0.69</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Table 2b: Effect of UV irradiation on sample.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Days</th>
<th>IC$_{50}$ of samples in mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arbutin</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.53</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.59</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.66</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Figure 4: Relative samples stabilization and activity check on exposure of (A) Temperature at 60 °C and (B) UV.

Cell viability and proliferation test on melanocytes

The MTT assay was used to measure viability and proliferation of cells. The culture cells were treated with the extracts for 24 hours as described in methods. The degree of cell growth was determined by means of the MTT assay. We applied our samples to investigate the cytotoxicity of the sample on melanin cells. Dimethyl sulfoxide (DMSO), arbutin, n-Hexane fractionate, Chloroform fractionate and Butanol fractionate were used for MTT test as toxic agents, with different concentrations as 0.5 µl/ml, 1.0µl/ml, 2.0µl/ml, 4.0µl/ml and 8µl/ml for each. The results were obtained by measuring the OD at 570 nm with microplate reader (Bio-Rad).

a) DMSO: Dimethyl Sulfoxide (DMSO), a colorless liquid, is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water. All the samples were dissolved in DMSO and to know the toxicity, DMSO was applied for MTT test on melanocytes as a control. The results were obtained by measuring the OD at 570 nm, the results indicated no effect on cell viability (figure 5).

b) Arbutin: Arbutin is the inhibitor of tyrosinase and thus prevent formation of melanin. Arbutin is therefore used as a skin-lightening agent. Arbutin was added in culture media at different concentration as previously mentioned. In these concentrations range, the results revealed that Arbutin does not affect the melanocytes viability (figure 5), up to 2µg/ml of concentration but there are a decline in viability which indicates the cytotoxicity with increase in concentration of Arbutin.

c) n-Hexane fractionate of P. nebrodensis: n-Hexane is a non-polar solvent was used for fractionation. The Hexane fractionate samples dissolved in DMSO was added in culture media at different concentration as mentioned above. The effects on melanocyte viability were observed by measuring the reduction of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple Formazan in the mitochondria of living cells at OD$_{570nm}$. We added n-Hexane sample to culture media with concentration...
range (0.5µl/ml, 1µl/ml, 2µl/ml, 4µl/ml, 8µl/ml). From 0.5µl/ml to 2µl/ml did not affect the Melanocytes, but 4µl/ml sample was affecting on viability of cells by 41% and 93% when up to 8µl/ml sample.

d) Chloroform fractionate of P. nebrodensis: The Chloroform fractionate was also applied to investigate cell viability. The testing process was similar with n-Hexane fractionate. The concentration up to 4µl/ml shows better tolerance with no significant effect. However, the culture media having 8µl/ml of sample showed a sharp decline by 90% of cell quantity as compare to the control (figures 5).

e) The Butanol fractionate of P. nebrodensis: Sample was applied for cell viability test at similar concentrations mentioned above. Sample concentrations up to 2µl/ml were not cytotoxic for melanocytes, but with 4µl/ml concentration, the sample showed a successive decline with increase in concentration for toxicity (Figure 5).

According to MTT test results, we expected suitable concentration of each samples to be applied for investigating melanogenesis inhibition in melanocytes. Tyrosinase is a key enzyme in melanogenesis pathway in organisms, if there is any agent that interrupted this enzyme, cause effect on melanin synthesis. Based on tyrosinase assay, that determined our sample has strongly inhibited tyrosinase activity, we applied the sample on melanocytes to observe the effect on melanogenesis (Figure 6).

Figure 5: Comparative analysis of melanocyte viability against different concentrations of samples.

![Figure 5: Comparative analysis of melanocyte viability against different concentrations of samples.](image1)

Analysis of melanogenesis inhibition in human melanocytes with various extracts

The melanocytes after treatment with sample were lysed by 0.1N NaOH to release melanin content and OD was taken at 490 nm. Among the observation, the sample 6 and 7 (n-Hexane fractionate) have revealed high inhibition towards the melanogenesis at 42% and 39% respectively. The samples 8 and 9 (Chloroform fractionate) showed inhibition towards melanogenesis at 46% and 45% respectively (Figure 6). Similarly, sample 10 and 11 (Butanol fractionate) showed highest inhibition within the test with 49% each. These samples (6, 7, 8, 9, 10 and 11) were confirmed one more time at 0µl/ml, 0.25µl/ml, 0.50µl/ml, 1.0µl/ml and 2.0µl/ml each. The resultant observation was that the rate of inhibition was increased with the increase in concentration of sample. A similar result was observed with Arbutin also.

Figure 6: Melanogenesis inhibition by different samples.

![Figure 6: Melanogenesis inhibition by different samples.](image2)

Table 3: Melanocyte viability testing against various fractionates.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration</th>
<th>Control</th>
<th>Positive control (Arbutin)</th>
<th>n-Hexane Fractionates</th>
<th>Chloroform</th>
<th>Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.60</td>
<td>1.20</td>
<td>1.49</td>
<td>1.46</td>
<td>1.42</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>0.75</td>
<td>1.24</td>
<td>1.48</td>
<td>1.52</td>
<td>1.47</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>0.57</td>
<td>1.15</td>
<td>1.30</td>
<td>1.40</td>
<td>1.27</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>0.51</td>
<td>1.04</td>
<td>0.70</td>
<td>1.42</td>
<td>0.45</td>
</tr>
<tr>
<td>5</td>
<td>8.0</td>
<td>0.48</td>
<td>0.30</td>
<td>0.21</td>
<td>0.19</td>
<td>0.23</td>
</tr>
</tbody>
</table>

http://www.openaccessscience.com

ijmap@openaccessscience.com
Conclusion

The mushroom *Pleurotus nebrodensis* was used as main source to extract the compounds which were expected to inhibit tyrosinase activity involved in melanogenesis during pigmentation. In order to obtain the effective compounds, the fruit body of the mushroom was extracted with Methanol followed by fractionation of the extract with various organic solvents. The extracted compounds were analyzed by enzyme assay with mushroom tyrosinase and L-tyrosine as substrate to screen the effective ones before investigating their effectiveness with cultured cells. The enzyme assay revealed that among fractionates with organic solvents using n-Hexane, Chloroform and Butanol, the Butanol fractionate was more effective on tyrosinase inhibition.

To identify the relative amounts of each compound in samples, Thin Layer Chromatography was carried out. Relative to the inhibition assay, it is necessary to characterize spots observed in Butanol fractionates and Chloroform fractionates extensively as we assumes that these spots contains the responsible compound for melanogenesis inhibition. All the samples were chosen to test for stabilization against high temperature and UV exposed. Samples were exposed to 60 °C for 10 days. They showed 90% of initial activity remained up to 5 days and then the activity was decreased up to 50%. Similarly, 80% of activity was observed by 3 days of UV exposure on the samples, but dropped to 50% after 5 day exposure.

The ultimate goal of this study is to apply the extracts to cosmetic for whitening human skin with safety on human health and environment. MTT test was carried out to analyze the cytotoxicity of samples with melanocytes and with human melanocytes as well. The results revealed that the n-Hexane, Chloroform and Butanol fractionates have no cytotoxicity up to 2µl/ml in average. Melanogenesis inhibition was investigated by measuring the melanin content in melanocytes. The fractionates with n-Hexane, Chloroform and Butanol had shown high melanogenesis inhibition in melanocytes as 41%, 46% and 49% respectively. The Butanol fractionate has revealed a high inhibition in the melanin synthesis within the cells. The fractionate with Chloroform and Butanol showed the highest melanogenesis inhibition effect among the tests, and more efficient than arbutin in melanogenesis inhibition even in cell cultures of melanocytes.

In this study, though potent melanogenesis inhibiting materials were successfully fractionated with n-Hexane, Chloroform and Butanol from mushroom, *Pleurotus nebrodensis*, still the research is under development for determination of responsible compound(s) from this mushroom for whitening the skin.

Acknowledgement: The authors are grateful to Dr. Arun Jamkar, Hon’ble Vice-Chancellor, MUHS, Nashik, for his continuous encouragement towards the research work we have done.

References


