

## COMPARISON BETWEEN PPO FROM PLANT SOURCES AND DIFFERENT CHEMICALS IN TATTOO DYES DECOLORIZATION

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### ABSTRACT

This study was aimed to measure the decolorization of tattoo dyes by different chemicals and polyphenol oxidases from several plant sources. The tattoo inks removal market has burgeoned over the years, due to increased spread of tattooed persons about the world. Laser and surgery are presently the gold standards for removing of the tattoo. However, both of them have blemishes. Consequently, lots of persons were preferring easier, faster and cheaper procedures for tattoo remove. In this study polyphenol oxidases enzyme from many plant sources and different chemicals were used for decolorization of tattoo dyes in vitro. The polyphenol oxidase enzyme was used for removing of tattoo dyes (brown and blue) in order to demonstrate their potential in the treatment and decolorization of the tattoo, which is hazardous when removing by laser. The results show that 89 and 82 % of the brown and blue tattoo dyes respectively, were removed after 24 hours by enzyme extracted from *Malva parviflora* leaves, whereas the decolorization efficiency of polyphenol oxidase from other plant sources given less than 16% of the same dyes. The results for tattoo dyes decolorization by different chemicals revealed that Bimethylbenzylamine was the best chemical used with decolorization ratio 36 and 38 % for brown and blue tattoo dye, respectively.

**Keywords:** *Malva parviflora*, Oxidoreductases enzymes, removing dyes, extraction.

الساعدي

مجلة العلوم الزراعية العراقية -2020: 51(2):550-555

مقارنة في ازالة اصباغ الوشم باستخدام انزيم البولي فينول اوكسيديز من مصادر نباتية و مواد كيميائية مختلفة

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مدرس

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المستخلص

هدفت هذه الدراسة إلى قياس إزالة أصباغ الوشم باستخدام المواد الكيميائية المختلفة وانزيم البولي فينول اوكسيديز من عدة مصادر نباتية. ازدهر سوق إزالة أحبار الوشم على مدار الأعوام، نظرًا لزيادة انتشار الأشخاص الموشومين حول العالم. حيث يعد الليزر والجراحة حاليًا من المعايير الذهبية لإزالة الوشم. ومع ذلك، فإن كلاهما يعاني من عيوب. وبالتالي، فإن الكثير من الأشخاص يفضلون إجراءات أسهل وأسرع وأرخص لإزالة الوشم. في هذه الدراسة تم استخدام انزيم البولي فينول اوكسيديز المستخلص من مصادر نباتية عديدة ومواد كيميائية مختلفة لإزالة أصباغ الوشم في المختبر. حيث استخدم الأنزيم لإزالة أصباغ الوشم (البنّي والأزرق) لإظهار إمكانيته في علاج وإزالة تلويّن الوشم، والتي تعتبر خطرة عند إزالتها بالليزر. أظهرت النتائج أن 89 و 82 % من صبغة الوشم البنّي والأزرقاء على التوالي، قد تم إزالتها في غضون 24 ساعة باستخدام انزيم البولي فينول اوكسيديز المستخلص من أوراق نبات الخباز (*Malva parviflora*)، في حين كانت كفاءة الانزيم المستخلص من مصادر مختلفة في إزالة الاصباغ اقل من 16 % . كما أظهرت نتائج استخدام المواد الكيميائية لإزالة أصباغ الوشم أن ثنائي ميثيل بنزليامين كان أفضل مادة كيميائية من بين المواد المستخدمة لإزالة صبغة الوشم البنّي والأزرقاء وبنسبة 36 و 38% على التوالي.

الكلمات المفتاحية: نبات الخباز، انزيمات الاكسدة والاختزال، إزالة الاصباغ، الاستخلاص.

\*Received:11/6/2019, Accepted:22/9/2019

## INTRODUCTION

Tattooing is the process of injecting inks or dyes into the dermal layer of the skin so as to semi-permanently or permanently color the skin. Tattoo dyes may comprise multiple colors to obtain a certain color, add to other chemicals such as glycerol, water, witch hazel, isopropyl alcohol, resins, preservatives, and contaminants (14). The colors used include both dyes (soluble in water) and pigments (insoluble in water); however, tinctures represent the great majority of dyes used in tattoo inks. Tattooing can be accomplished either for cosmetic purposes or as a form of body art (24). Tattoo ink texture was consolidated, with 120 chemicals identified as being used as ingredients of tattoo inks in internationally (10). In some cases the skin is tattooing as a result of trauma or an injury. When the patient evolves damage to the skin and gets dirt, ink, carbon from a pencil, or other pigmented material in the hurt, a traumatic tattoo can result (9). Some of the soldiers predominately receive of tattoos as a result of bursts and may have detonation particles embedded in their skin, which are dangerous to remove by laser (15, 23). Whatsoever the myriad of causes people that get tattoos, also the reasons for wanting them to removal dyes may be just as varied. Removal methods of tattoo have been communicated since antiquity. However, the tattoo removal industry has known a tremendous expansion for the past years as a natural consequence of the increased popularity of tattooing in Western countries. Reasons and stimulus or motivations for removing of tattoo inks have been widely explored. They mainly include social pressure (experience stigma, search for a new job, problems with clothes, etc.), personal reasons (familial negative comments, rupture with the past, etc.), or more simply tedium and displeasure with the tattoo either because performed at a young age, or because it was execution in haste, or due to the result is unaesthetic. More rarely, removing of tattoo inks is the fast and last solution in case of present chronic and stubborn local tattoo reaction (21). Polyphenol oxidases (EC 1.10.3.2) are copper-containing oxidoreductases that catalyze the oxidation and hydroxylation of phenolic compounds in the existence of molecular oxygen (17). Polyphenol oxidases (PPOs) are a wide

prevalent group of enzymes found in animals, fungi, plants, and bacteria (19). In the existence of PPO and atmospheric oxygen, monophenol is hydroxylated to o-diphenol, and diphenol can be oxidized to o-quinones, which then subject for polymerization to yield dark brown polymers (16). PPOs have different applications in industrial processes like food and medicine. Polyphenol oxidases are applied in numerous industrial sectors such as discoloration of wine, paper processing, chemical production from lignin and environmental pollutants detoxification. The claim for decolorization of pigments and dyes from the industrial waste of textile by bacterial, plant and fungal PPO is being accretion tremendously. These enzymes were also used to remove phenolics and dyes from wastewaters (13, 12). The aim of this study is to measure the ability of PPO extracted from different sources with some chemicals in decolorization of tattoo dyes.

## MATERIALS AND METHODS

### Chemicals

Trichloroacetic acid, catechol, coomassie brilliant blue, bovine serum albumin were obtained from Sigma Co. other chemicals were supplied by BDH Chemicals.

### Extraction of PPO from some plants

Ten grams of horseradish, potato, and *Malva parviflora* leaves homogenized separately at (1:10) (w:v) with 0.1 M potassium phosphate buffer pH 7 using a blender for 5 minutes. The homogenate was filtered by using filter paper (whatman filter paper No.). The enzyme activity and protein concentration were estimated (4).

### Determination of PPO activity

Catechol was used as a substrate to determine enzyme activity. The reaction solution contained 2.9 mL of 0.01 M substrate in phosphate buffer (0.1M, pH 7.0) and 0.1 ml of the enzyme. Three ml of substrate solution was used as a blank sample (4, 12). The oxidation of catechol was detected by measuring the absorbance increase at 420 nm after 3 min using a spectrophotometer. One unit of polyphenol oxidase activity was defined as amount of enzyme that caused an increase in absorbance of 0.001/min. PPO activity was measured according to following equation:

**Activity of PPO (U.ml<sup>-1</sup>) = [(A2 sample – A1 sample) – (A2 blank – A1 blank)]/(0.001 × t).**

**Where: A1 sample:** is the first absorbance of the sample; **A2 sample:** is the end absorbance of the sample; **A1 blank:** is the first absorbance of the control; **A2 blank:** is the end absorbance of the control, and t is the reaction time in minutes (3 minutes) (1). Protein concentration was according to method described by the Bradford (6).

#### **Dye decolorization by polyphenol oxidase**

Stock solutions of tattoo dyes (brown and blue) were prepared in sterilized distilled water and diluted to 25 mg/L. The optical density of each dye was measured depending on its  $\lambda$ -max using a spectrophotometer. The reaction mixture was prepared by addition a volume of PPO (0.5 ml, 251.9 U/mg) to volume dye solution (4.5 ml) and incubated at 37 C° for 24 hours. The control sample was prepared for each dye without polyphenol oxidase enzyme and treated under the same condition. Decolorization efficiency of the PPO enzyme was assessed by watched of the decrease in absorbance under the maximum wavelength of the dye and expressed in terms of percentage. Decolorization activity was calculated as follows (2, 11):

$$\text{Decolorization \%} = \frac{\text{Ab of control sample} - \text{Ab of dye treatment}}{\text{Ab of control sample}} \times 100$$

#### **Dye decolorization by different chemicals**

Stock solutions of tattoo dyes (brown and blue) were prepared in sterilized distilled water and diluted to 25 mg/L. The reaction mixture was prepared by addition the volume of chemicals (1 ml of 10 % concentration for each one) to volume dye solution (5 ml) and incubated at 37 C° for 10 minutes (7). These chemicals including: sulphuric acid, citric acid, dimethyl

sulfoxide (DMSO), phenol, methanol, 1-propanol, Dimethylbenzylamine, dimethylamine hydrochloride, 2,2-diphenyl-1-picrylhydrazyl, tetramethylethylenediamine, lemon juice, apple cider vinegar, white vinegar, lead acetate and sodium nitroprusside. The optical density of each treatment dye was measured using a spectrophotometer. A control sample was prepared for each dye without additions and treated under the same condition. Decolorization efficiency of chemical materials was assessed by watched the decrease in absorbance under the maximum wavelength of the dye and expressed in terms of percentage. Decolorization activity was calculated by the same equation, which as mention above (4).

## **RESULTS AND DISCUSSIONS**

### **Dye decolorization by PPO**

The polyphenol oxidase enzyme was used for decolorization of tattoo dyes (brown and blue) in order to demonstrate their potential in the treatment of dyestuff which was dangerous to remove via laser. The results shows that 89, and 82 % of the brown and blue tattoo dyes were removed respectively by polyphenol oxidase extracted from *Malva parviflora* leaves after 24 hours (Figure 1, 2). Whereas the removal efficiency of some dyes by polyphenol oxidase extracted from radish and potato were reached to (16, 14) and (11, 13) %, respectively. So the polyphenol oxidase extracted from *Malva parviflora* leaves was more efficiency for remove these dyes. This is because the *Malva parviflora* is a natural plant that is characterized by its wide production of a number of substances and enzymes that enable it to sustain and resist difficult conditions.

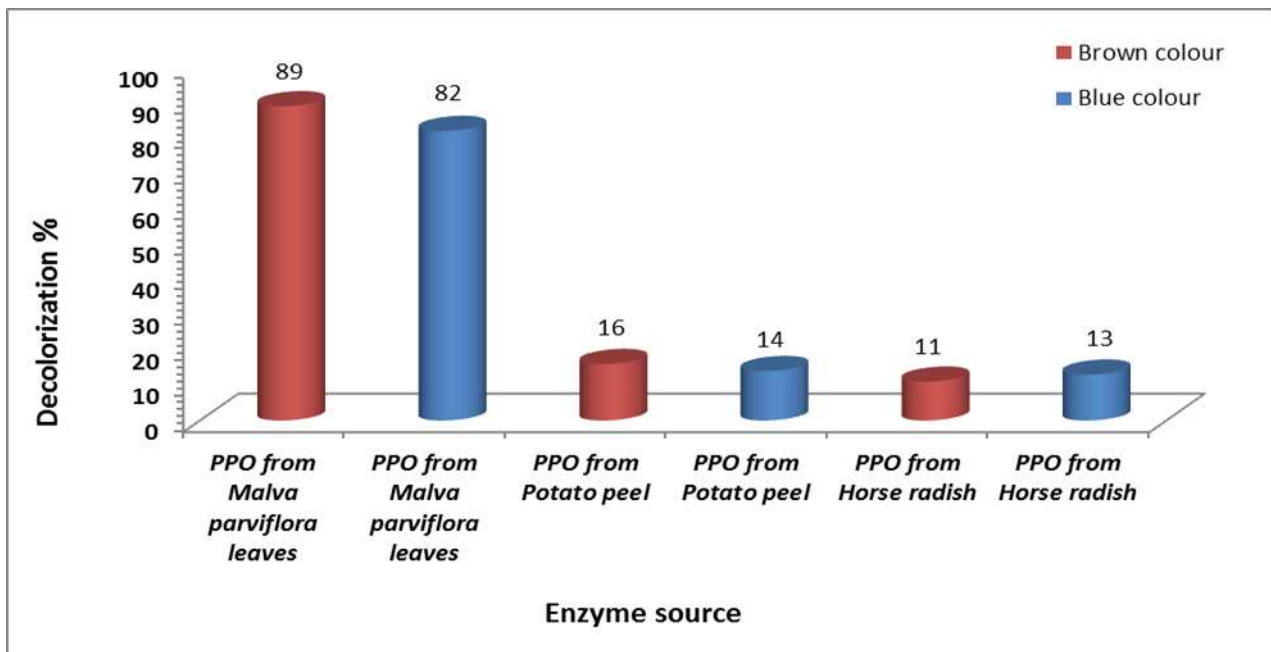


Figure 1. Decolorization of tattoo dyes by polyphenol oxidase extracted from different plant sources incubated at 37 C° for 24 hours

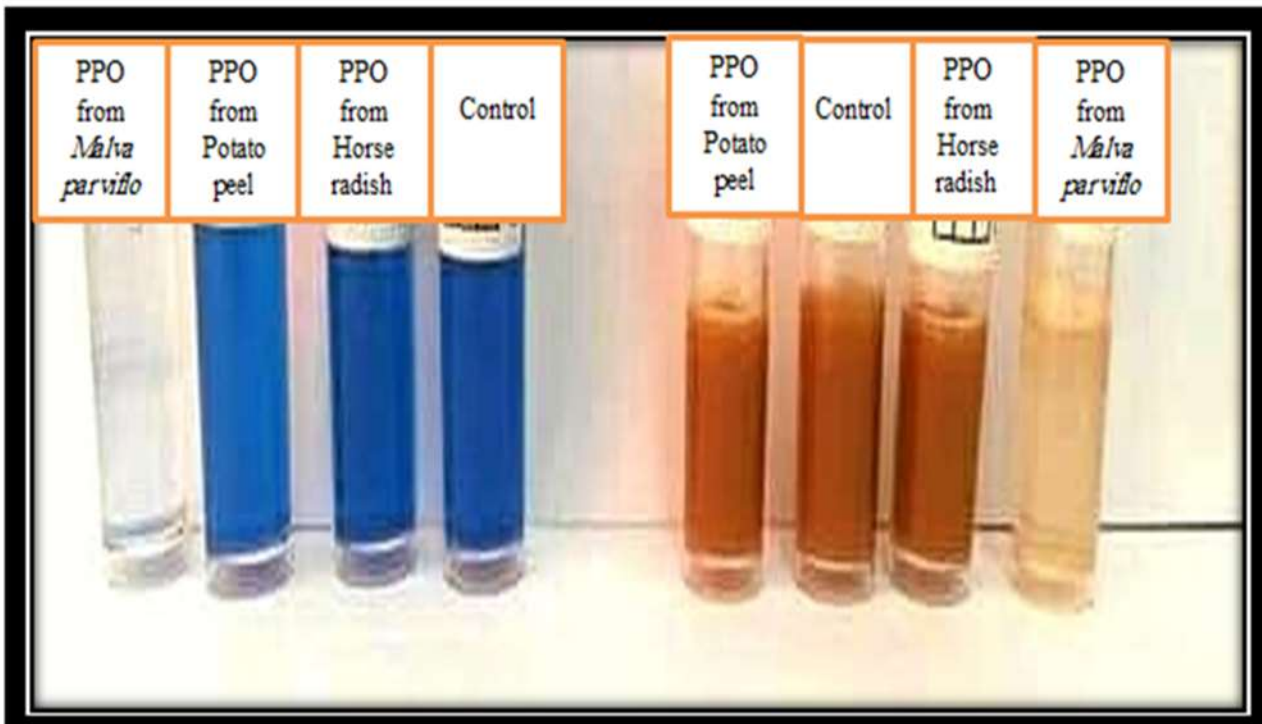


Figure 2. Decolorization of tattoo dyes by polyphenol oxidase extracted from different plant sources incubated at 37 C° for 24 hours

There are several studies in which a different types of polyphenols oxidases, like laccase enzyme, were used to remove of many dyes. Wang, *et al.*, (22), were found that 90% of the remazol brilliant blue R and alizarin red was removed by laccase spore from *Bacillus subtilis* when incubated at 37 C° for 5 days, while 50 - 70% in the treatments observed for other dyes (congo red, methyl orange, and methyl violet). Hussein (12), found that immobilized polyphenol oxidase has the ability to remove

99% of the textile dyes after 2 hours by using batch operation. Saratale, *et al.*, (20), found that 97% of the malachite green and cotton blue were removed in the first day by laccase from mycelium of *Aspergillus ochraceus* when incubated at 30 C° for different time intervals, while the final degradation percentage of other dyes (crystal violet and methyl violet) was less than 61%. Ratanapongleka and Phetsom (18), found that laccase from *Lentinus polychrous* was removed 85% of the anthraquinonic acid

blue 80 (20 mg/l) in 2 hours, while 20 % was removed of indigo carmine and less than 10% decolorization of methyl orange in the same period of time. Ciullini *et al.*, (8), explained that laccases show substrate specificities and the chemical structures of the dyes due to the differences in electron distribution, charge density, and steric hindrances. Aziz, *et.al.* (3), found that the immobilized PPO from banana peel was tested for their ability to decolorizing different types of dyes, whereas the neutral red, acridine, and toluidine were decolorized and showed a change in their absorbance values after the immobilized PPO incubate for a time while no analysis occurred for other dyes.

#### Dye decolorization by different chemicals

The brown and blue tattoo dyes degradation capability of different chemicals were studied of dye concentration 25 mg/ml at 37 °C for 10 min., as can seen in table (1). The Bimethylbenzylamine have the highest removal efficiency 36 and 38 % for brown and blue tattoo dyes comparison with other chemicals that used in this study. It is possible that the chemi-

cal materials that removed the tattoo dyes with these ratios (36 and 38 %) due to of their greater reactivity than the other materials that used, or these materials may have greater ability to bind or change the chemical composition of the tattoo dyes, which loses the color dye. The removal of tattoo dyes by lasers are the safest; but, complications can occur. These acute complications include blistering, pain, pinpoint hemorrhage, and crusting. Among of the belated complications are hypopigmentation, pigmentary changes, paradoxical darkening of cosmetic tattoos, hyperpigmentation, and allergic reactions can be seen. Another complication is the existence of ghost images or residual pigmentation. Scarring and structural alteration are possibilities of irreversible complications. Also, tattoo decolorization can be a long tiresome procedure, especially with professional tattoos, which are hard to rub out as compared to unprofessional tattoos (5). Caccavale and Montagna (7), found that Trichloroacetic acid has the ability to remove tattoo dyes when it used as alternative of laser.

**Table 1. Effect of different chemical materials on tattoo dyes decolorization**

NO.	Chemical material	Decolorization % of tattoo dye (brown)	Decolorization % of tattoo dye (blue)
1	Sulphuric acid	0	0
2	Citric acid	0	0
3	Dimethyl sulfoxide (DMSO)	0	0
4	Phenol	0	0
5	Methanol	0	0
6	1-Propanol	0	0
7	Bimethylbenzylamine	36	38
8	Dimethyl-amine hydrochloride	23	22
9	2,2-Diphenyl-1-picrylhydrazyl	31	28
10	Lemon juice	0	0
11	Apple cider vinegar	0	0
12	White vinegar	0	0
13	Lead acetate	0	0
14	Sodium nitroprusside	0	0
15	Tetramethylethylenediamine	19	17

#### REFERENCES

- AL-Sa'ady, A.J.R. , M.H. Al-Bahrani , and G.M. Aziz, 2018. Characterization and immobilization of peroxidase extracted from horseradish and decolorization of some dyes. *Int. J. Curr. Microbiol. App. Sci.* 7(6): 328-339
- AL-Sa'ady, A.J.R. , Y.N. Shukur , and M.A. Hamod, 2016. Decolorization of reactive dyes by spore-bound laccase from a local isolate of *Bacillus* sp. *Iraqi Journal of Science.* 57(1A):181-122
- Aziz, G.M. , A.J.R. Al - Sa'ady , and D.S. Bedan, 2018. Characterization and immobili-

- zation of purified polyphenol oxidase extracted from banana peel. *Iraqi Journal of Biotechnology.* 17(2): 13-22
- Aziz, G.M. and A.J.R. AL-Sa'ady, 2016. Extraction conditions of polyphenol oxidase from banana peel. *Baghdad Science Journal.* 13(3): 469-474
- Bernstein, E.F., 2015. A widespread allergic reaction to black tattoo ink caused by laser treatment. *Lasers Surg. Med.* 47: 2-180
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of pro-

- tein-dye binding. *Anal. Biochem.* , 72 : 248-254
7. Caccavale, S. and M.L. Montagna, 2014. Recurring mistakes, risks, and devices for tattoo removal by non-physicians. *Glob Dermatol.* 1(1): 1-2
  8. Ciullini, I. , S. Tilli , A. Scozzafava , and F. Briganti, 2008. Fungal laccase, cellobiose dehydrogenase, and chemical mediators: Combined actions for the decolorization of different classes of textile dyes. *Bioresource Technology.* 99: 7003-7010
  9. Fusade, T. and S.R. Mordon, 2011. Tattoo removal by non-physicians: considerations about the state of legislation in France. *J. Eur. Acad. Dermatol Venereo.* 25:240-239
  10. Hammes, S , S. Karsai , H.R. Metelmann , *et al.* 2013. Treatment errors resulting from use of lasers and IPL by medical laypersons: results of a nationwide survey. *J. Dtsch Dermatol Ges.* 11: 149–156
  11. Hussein, S.I. , G.M. Aziz , R.M. Shanshal and A.L. Ghani, 2018. Determination the optimum conditions of laccase production from local isolate of *Streptomyces sp.* using solid state fermentation. *Iraqi Journal of Agricultural Sciences.* 49(4):685- 693
  12. Hussein, S.I., 2019. Efficiency of immobilized polyphenol oxidase on some textile dyes degradation using batch operation system by packed bed bioreactor. *Iraqi Journal of Agricultural Sciences.* 50(3):943- 950
  13. Jasim, A.M., and G. M. Aziz, 2019. Degradation efficiency of phenolic compounds using immobilized peroxidase purified from soybean. *Iraqi Journal of Agricultural Sciences.* 50(3):928- 935
  14. Karsai, S. , G. Krieger , and C. Raulin, 2010. Tattoo removal by non-professionals medical and forensic considerations. *J. Eur Acad Dermatol Venereo.* 24: 756–762
  15. Kearnsa, A. , B.C. Fischer , K.G. Watkinsa , M. Glasmacherb , H. Kheyrandishc , A. Brown , W.M. Steena , and P. Beahan, 1998. Laser removal of oxides from a copper substrate using Q-switched Nd:YAG radiation at 1064 nm, 532 nm and 266 nm. *Applied Surface Science.* 127:773–780
  16. Ni-Eidhin, D. , P. Degn , and D. Obeirne, 2010. Characterization of polyphenol oxidase from rooster potato (*Solanum tuberosum cv 'Rooster'*). *J. Food Biochem.* 34:13-30
  17. Palma-Orozco, G. , A. Ortiz-Moreno , L. Dorantes-alvarez , J.G. Sampedro , and H. Najera, 2011. Purification and partial biochemical characterization of polyphenol oxidase from mamey (*Pouteria sapota*). *Phytochemistry.* 72:82-88
  18. Ratanapongleka, K and J. Phetsom, 2014. Decolorization of synthetic dyes by crude laccase from *Lentinus Polychrous*. *International Journal of Chemical Engineering and Applications.* 5(1): 26-30
  19. Ridgway, T.J. and G.A. Tucker, 1999. Procedure for the partial purification of apple leaf polyphenol oxidase suitable for commercial application”, *Enzyme and Microbial Technology.* 24: 225-231
  20. Saratale, G.D. , S.D. Kalme , and S.P. Govindwar, 2006. Decolorisation of textile dyes by *Aspergillus ochraceus* (NCIM-1146). *India J. of Biotechnol.* 5: 407-410
  21. Shah, S.D. and S.J. Aurarangabadkar, 2015. Newer trends in laser tattoo removal. *J. Cutan Aesthet Surg.* 8(1):25–29.
  22. Wang, C.L. , M. Zhao , D. Li , D.Z. Cui , L. Lu , and X.D. Wei, 2010. Isolation and characterization of a novel *Bacillus subtilis* WD23 exhibiting laccase activity from forest soil. *African Journal of Biotechnology.* 9(34): 5496-5502
  23. Wenzel, S. , M. Landthaler , and W. Baumler, 2009. Recurring mistakes in tattoo removal. A case series. *Dermatology.* 164-167
  24. Yim, G.H. , S.J. Hemington-Gorse , and W.A. Dickson, 2010. The perils of do it yourself chemical tattoo removal. *Eplasty.*10: 22-29.