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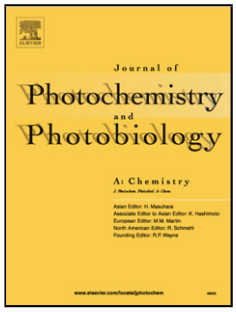

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Photosonoelectrochemical analysis of Lawsonia inermis (henna) and artificial dye used in tattoo and dye industry

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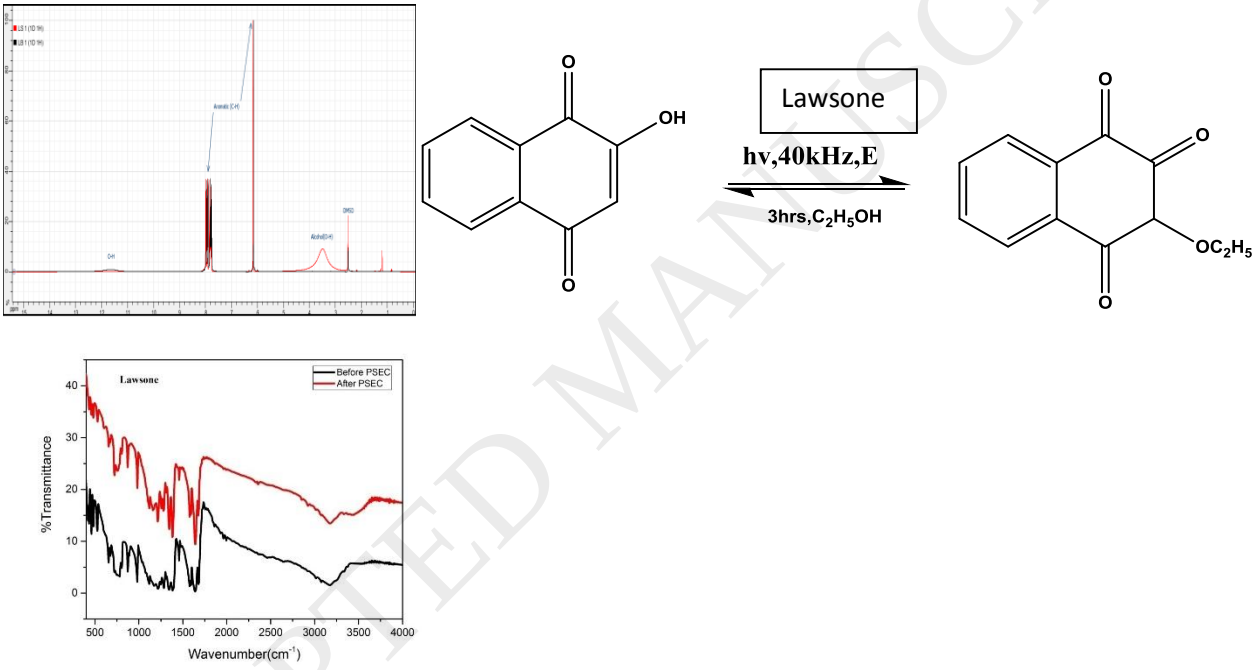
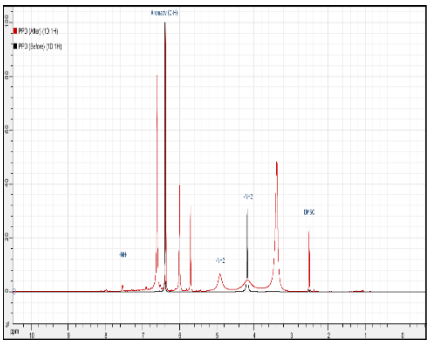
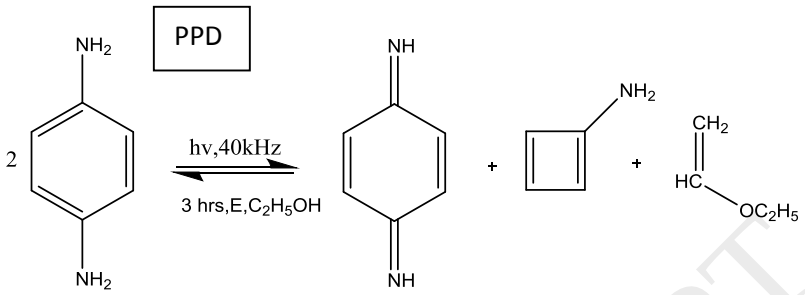
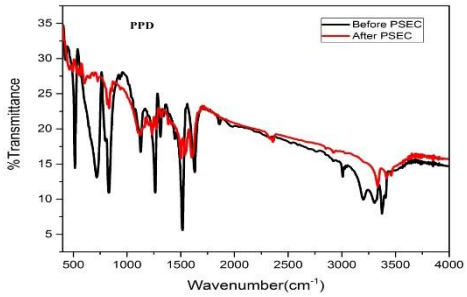
Graphical Abstract









Highlights

- Lawsonia inermis donot undergo degradation upon exposure to PSEC perturbation
- commercial lawsone undergo oxidation upon PSEC perturbation forming ethoxy substituted napthaquinone
- PPD, an artificial dye resulted in the formation of amino substituted cyclobutadiene upon PSEC perturbations.
- Formation of carcinogenic byproducts by lawsone and PPD respectively demonstrated the harmfulness in utilizing these artificial dyes as colour enhancing agent to henna by dye and tattoo industries.

Abstract.

Photosonoelectrochemical (PSEC) analysis of Lawsonia inermis, lawsone and β-Phenylenediamine were investigated in ethanol to understand the degradation mechanism and harmful byproducts. To simulate the operating conditions of the tattoo ink, dye solutions were exposed to appropriate sonication, UV irradiation and electrochemical perturbations. Analyses of the dyes were done before and after PSEC perturbations employing UV-Vis, FTIR, HPLC, ^{1}H NMR and ^{13}C NMR studies and degradation pathway is provided. The PSEC studies inconjunction with the spectroscopic and HPLC analysis identified formation of byproducts such as 3-ethoxy naphthaquinone from lawsone and 1-Amino cyclobutadiene from β-Phenylenediamine demonstrating the harmfulness of utilizing these dyes over henna by tattoo and hair dye industries.

Keywords: Lawsonia inermis; lawsone; β-Phenylenediamine; Cyclic Voltametry and UV-Visible; FTIR; ^{1}H NMR spectroscopy; sonication.

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1. Introduction:

Natural dyes are extensively used in cosmetic industries**[1]** in the form of biodegradable commodities due to its non-toxic, non-carcinogenic and biodegradable nature. For centuries, henna had been used by cosmetic and tanning industries to dye skin, hair, fingernails, silk, wool, and leather **[2-4].** The coloring property of henna (Lawsonia inermis) was due to lawsone**[5]**. Several studies were reported**[3]** on the antimicrobial activity and effect on hair growth and *invivo* injury recovery**[6]** by henna. The utility of commercial lawsone (2-hydroxy-1,4-naphthoquinone) ranges from traditional tattoo ink (henna) to medicinal remedies. It was used as artificial-tanning, hair colorants**[7]**, antifungal agent**[8,9]** and antioxidant in rubber industry**[10]**. In general, henna is harmless except for people with glucose-6-phosphate dehydrogenase deficiency**[11].** Most of the commercial henna body art products were mixtures of henna and other dye compounds such as silver nitrate, carmine, pyrogallol, disperse orange dye, β-phenylenediamine (PPD) and chromium**[12,13].** PPD is a skin sensitizer which causes Contact Allergy Dermatitis **[14,15].** Pure henna was a weak sensitizer, but the addition of PPD made tattoos darker and long-lasting. Thus obtaining black henna, by mixing henna with various concentrations of PPD resulted in contact sensitization to PPD[**16,17].** Ultrasound was found to be an attractive advanced oxidation technology for the degradation of hazardous organic compounds**[18].** In general the combination of photocatalysis and ultrasound was considered to enhance the degradation rate **[19-20].** Sonoelectrochemical technique is not new to scientists as most of the electroanalytical and electrosynthesis utilize sonoelectrochemistry for homogeneous electrochemical reaction and uniform mass transport to the electric double layer from bulk of the electrolyte solution**[21].** Although several techniques and methodology had been followed by researchers to study and understand the degradation mechanism of artificial dyes, none so far on PSEC perturbations had been investigated. As the tattooing machine utilizes electric or electromagnetic impulses to perform tattoo art on human

skin and the machine vibrates during the process, simulating these conditions via PSEC perturbations can provide a realistic approach to identify the degradation products. Most of the tattoo inks utilize solvents such as ethanol, water, methanol or mixture of water and ethanol/water and methanol, hence we conducted the PSEC experiments in 50% ethanol solution. 3% of solar radiation constitutes UV rays (42 W/m^2), the maximum threshold for human skin tolerance towards UV radiation received by earth is 25%. Thus in the current study, the dyes were exposed to 10.4mW/cm^2 of UV radiation. Thus the major objective of the present work are PSEC analysis of henna, lawsone and PPD in ethanol to understand the mechanistic pathway of their degradation, spectroscopic analysis of the dyes before and after PSEC treatment to understand the degradation products formed and the mechanism of degradation, HPLC and ^{13}CNMR analysis of the products formed and to insist on the usage of henna rather than other artificial dyes in tattoo and dye industries.

2. Experimental details:

All reagents were of analytical grade and used without further purification. Herbal henna was purchased from Aravindh Herbal Labs (P) Ltd., Rajapalayam, India. 2-hydroxy -1,4-napthoquinone (Lawsone) was purchased from Sigma Aldrich India Pvt. Ltd. PPD and ethanol were purchased from loba chemie pvt. ltd., India.

Photolytic activity

Photolytic activity of 0.1% and 0.5% concentrations of Lawsonia inermis, lawsone and PPD were tested and compared for their degradation. These solutions were exposed to UV light (125W Hg arc, 10.4 mW/cm^2) under constant sonication (40 kHz) for 3hrs.

Electrochemical studies

Zahnner-pp 211 electrochemica1 workstation was used for electrochemical analysis. Cyclic Voltammetry(CV) and Linear Sweep Voltammetry(LSV) were used to study the mechanism of electron transfer in Lawsonia inermis, lawsone and PPD. The electrochemical experiments were carried out using three electrode assembly with Ag/AgCl electrode as reference electrode, Pt wire as counter electrode and glassy carbon as working electrode. The concentration range of henna, lawsone

and PPD were 0.1% and 0.5% as these were the concentrations used in artificial dye. The potential range was -1 to +1V, at scan rates of 25, 50 and 100mV/s.

Spectroscopic studies

UV-Vis, FTIR, ^{1}H NMR and ^{13}C NMR analysis were studied to investigate the byproducts formed after PSEC perturbations. The UV–Vis absorption spectra of the samples were measured using SPECORD-210 UV spectrophotometer at room temperature in the wavelength range of 190–1100 nm in ethanol. The samples were analyzed by BRUKER ALPHA E&T FTIR spectrophotometer using KBr pellette. The absorption of electromagnetic radiation is in the wave number range of 400-4000 cm^{-1}. Solution state ^{1}H NMR spectra was recorded by Bruker 500 MHz standard bore (SB) NMR spectrometer equipped with BBO probe head, while solid state ^{13}C NMR Cross Polarization Magic Angle Spinning (CPMAS) spectra were recorded on a Bruker 500 MHz wide bore (WB) NMR spectrometer equipped with a solid state MAS probe head before and after electrochemical experiments to understand the structural shift due to perturbations.

HPLC experiments:

HPLC-DAD Column Xbridge-C18 with mobile phase of water-ethanol (50-50) mixture, at a flow rate of 1 ml/min was used to perform HPLC studies. The injection volume is 10ul. The column oven temperature was maintained at 35^{o}C. Different wavelengths were specified for lawsone and PPD. Lawsone at 280 nm, PPD at 242 nm. These wavelengths allow a sufficient sensitivity of detection for the determined compounds to be obtained.

3. Results and discussion

3.1 Electrochemical studies

The electrochemical studies of the sample were done using three electrode assembly (i) during UV irradiation and sonication and (ii) after UV irradiation and sonication.

3.1.1 Cyclic Voltammetry

a) Lawsonia inermis

The absence of redox behavior for 0.1% and 0.5% Lawsonia inermis in ethanol (Figs.1a&b), indicated that the dye component (lawsone) was not freely available to be extracted in ethanol due to the presence of other constituents such as chlorophyll. Thus degradation of lawsone, the basic colorant in

Lawsonia inermis was not possible via UV-irradiation, sonication and electrochemical treatment, hence the dye remain longer on skin without degradation and harmful effects.

b) Lawsone

Sonicating the ethanolic dye solution reduced the particle size with increase in the reactivity of lawsone with ethanol. Therefore oxidation peak at 0.5 V was observed (Figs 1c&d) and the peak current increased with scan rate. The oxidation peak could be attributed to the formation of quinonoid ring in the solution due to electrochemical perturbation. As peak current is directly proportional to concentration of the reactive species and square root of the scan rate, with scan rate the peak current increases. Due to oxidation of lawsone, concentration of quinonoid species in solution increases leading to increased peak current. Thus due to PSEC perturbation lawsone is oxidized to quinonoid compound as supported by spectroscopic and HPLC analysis in the following sections.

The diffusion coefficient of lawsone could be calculated using Randles-Sevick equation given below.

$$i_p = Kn^{3/2} A D^{1/2} C v^{1/2} \quad (1)$$

where i_p represent peak current in amps, K being 2.69×10^5 C mol^{-1} V$^{-1/2}$, n denoted number of electrons transferred in the redox reaction (n=1), A depicted area of electrode in cm^2, D indicated the diffusion coefficient in cm^2/s, C being the concentration of lawsone (mol/cm^3) and ν represent the scan rate in mV/s. On substituting i_p values at different scan rates (25,50 and 100mV/s), for 0.1% and 0.5% lawsone, D values were calculated using eqn (1). At low concentration the diffusion coefficient was higher as can be seen from Fig 2a. In addition, the peak current i_p, for the oxidation peak is lower at all scan rates for 0.5% lawsone after PSEC experiments (cf. Fig 2b). Thus at lower concentrations, the degradation of lawsone was faster in comparison to the higher concentrations. The reason for this facile degradation at lower concentration is the surplus availability of solvent which acts as chromophore and initiates degradation of lawsone.

c) PPD

In general, PPD easily losses proton from amine group in water and form conjugated product. Since ethanol is less polar than water, PPD gets involved in H-bond instead of conjugated product formation.

This could be attributed to aromatic ring opening during sonication and UV irradiation. The redox behavior was not seen due to the formation of electro inactive byproducts via aromatic ring opening (cf. Figs. 1e&f) and rearrangement reaction as can be deduced and supported by FTIR, ^{1}H NMR, HPLC and ^{13}C NMR analysis (cf.section 3.2). Although redox reaction is not seen, electrochemical perturbations facilitates the reaction between the byproducts formed due to UV irradiation and sonication leading to further degradation of the intermediates into substituted dienes and conjugated aromatic diamines as discussed in following sections.

3.1.2. Linear Sweep Voltammetry (LSV)

Lawsone depicts a significant peak current with the concentration of the dye in LSV(Fig 3). In PPD the intensity of the peak increased with concentration(Fig 3). The diffusion coefficient of lawsone for all scan rates at 0.1% and 0.5% concentrations were calculated as mentioned earlier and plotted (cf. Figure 4 a and b). Analogous to CV analysis lawsone demonstrated peak current proportionality to scan rate (cf. section 3.1 and Fig 4b).

a) Lawsone

Figs 3 (a & b) show an anodic oxidation peak for 0.1% and 0.5% of lawsone in ethanol. The effect of scan rate on the PSEC process was evaluated (25,50 and 100mV/s). The purpose of the experiment was to determine whether the oxidation of species in solution was limited by electron transfer or anolyte diffusion**[22]** process. The results indicate that 0.1% lawsone had higher peak current than 0.5% of lawsone. Each curve has the same form but it was apparent that the total current increased with increasing scan rate. Due to sonication the peak current at 0.1% is as significant as 0.5%. Sonication helps in homogeneous supply of electroactive species from bulk of the solution to the electrical double layer (where the electron transfer occurs). Even though the amount of solute (lawsone) is less in 0.1%, sonication of the solution during electrochemical analysis provides continuous supply of solute to the interface. On the contrary, at 0.5% as the solute is saturated in the

solution, sonication creates disturbance to the mass transfer from bulk to interface and leads to undesired electrochemical activity such as hydrogen evolution and oxygen reduction reactions.

Figs 4 (a & b) depict $D^{1/2}$ vs ν and i vs $\nu^{1/2}$ results of 0.1% and 0.5% of lawsone . Here the peak current was high at 0.1% concentration than 0.5% due to the reduction of lawsone particle size during sonication. Thus lawsone was highly active at low concentration with high ion transfer rate compared with higher concentration. Thus CV and LSV data of lawsone demonstrate that, at 0.1% concentration lawsone is electro active and the peak current is higher than 0.5% concentration. Hence no products and ring open reaction occurred at 0.5% due to solubility effect as discussed above and supported by spectroscopic studies (cf.section 3.2).

b) PPD

Figs 3 (c & d) represent the LSV of 0.1% and 0.5% PPD in ethanol. The purpose of the experiment was to determine the oxidation of PPD upon PSEC perturbations. PPD has no oxidation peak but only semiconductor behavior in ethanol due to quenching effect by the solvent (cf.section 3.2). As discussed in CV studies, PPD undergo further degradation of the conjugated aromatic diamines into dienes. This reaction does not show oxidation/reduction profile, instead utilizes the electrochemical impulses to initiate degradation process via aromatic ring opening. As the products formed did not adsorb on the electrode surface, it did not show any peak in CV or LSV. In addition sonication will deter any weak interaction between the products and the glassy carbon electrode thereby accumulating the product in the reaction solution itself.

3.2. Spectroscopic studies

3.2.1. UV-Vis analysis

a) Lawsonia inermis

Fig. 5a presents optical absorption band from 400 to 750 nm for 0.1% and 0.5% concentrations of Lawsonia inermis in ethanol before and after PSEC studies**.** The major portion of henna leaf extract is constituted by lawsone (2-hydroxy-1, 4-napthaquinone), resin, tannin, coumarins, gallic acid and sterols **[23, 24]**. Lawsonia inermis in ethanol showed four absorbance peaks at 275, 334, 412 and

665nm before PSEC perturbation. Peak at 412nm (blue light) was due to chlorophyll-B and 665nm (red light) was caused by chlorophyll-A **[25]**. The intensity of the absorbance peak for higher concentration decreased due to dye degradation after PSEC studies.

b) Lawsone

Khan et al reported that the absorption wavelengths of lawsone in ethanol, involved two bands at 284 and 332 nm [**26**]. In the current study, lawsone absorbed at 246, 277 and 333nm in ethanol (Fig 5b). The first absorption peak at 246nm was attributed to the intramolecular hydrogen bonding of benzoquinone [27]. At 277 nm, the peak was due to the delocalization of electrons from benzonoid ring to quinonoid ring. The peak at 333 nm accounts for the presence of naphthoquinone in ethanol**,** forming hydrogen bonds with the carbonyl groups, as reported in the literature **[28-30]**.

c) PPD

We infer from Fig. 5c that the intensity of longer wavelength peaks decreased after the PSEC experiments. The peaks were obtained at 244 and 311nm. With increase in PPD concentration the peak intensity increased without peak shift. Thus one can come to the conclusion that -NH_2 group as an electron releasing entity is involved in the reaction via π electrons conjugation. The band gap energy of 3.99eV is observed at longer wavelength (311 nm). This is in agreement with the band energy of natural dye lawsone sharing electronic transition state from HOMO to LUMO. Thus the natural dye lawsone and synthetic dye PPD has almost similar energy levels in alcoholic medium**[31**]. Due to this similarity in their property, PPD is used as colour enhancement agent (adulterant) in henna powder or henna based hair dye [as discussed in the following section].

d) Band gap energy calculation

When the dye is illuminated with light, it absorbs photon (*hν*) equal or greater in energy than the band gap and electron gets promoted from the valence band (VB) to the conduction band (CB) [**32**]. Upon PSEC perturbation, the dye molecules were excited from the highest occupied molecular orbitals (HOMOs) to the lowest unoccupied molecular orbital (LUMO) states in ethanol. When electron gets excited from HOMO to LUMO under PSEC reaction, the excited state of the dye species was further

converted into a semi oxidized radical cations (Dye•+). These radical cations are highly reactive and short lived. It possess the ability to easily absorb UV light via conjugation and react with ethoxy group resulting in oxidation product. Based on the optical studies of Lawsonia inermis, lawsone and PPD, the energy corresponding to different electron transitions in the molecule could be identified. Lawsone exhibit common peak with both henna and PPD. Peak at 3.72eV for lawsone and 3.99eV for PPD correspond to the hydrogen bonding contributions (Fig 6 and Table 1). In the case of lawsone, peak at 333nm was due to -C=O, -OH hydrogen bonding, whereas for PPD it was due to 311 nm peak caused by H-N-H ($-NH_2$) hydrogen bonding. The peak wavelength or energy difference was caused by variation of H-bonding species in both the compounds. The lower wavelength peak at 246nm for lawsone is attributed to the intramolecular hydrogen bonding leading to conjugation. On the contrary, peak at 244nm for PPD is due to π electron conjugation. This similarity in the optical energy bands of lawsone and PPD makes them complimentary to each other in the application of dyes for human usage.

3.2.2 FTIR analysis

a) Lawsonia inermis

The broad peak obtained at 3419cm^{-1} is allocated to phenolic -OH stretching frequency. The aromatic C-H stretching band is observed near 3000cm^{-1}. The acidic O-H stretching frequency is identified at 2361 and 2339 cm^{-1}, but after PSEC reaction the acid O-H stretching at 2339 cm^{-1} vanished. The α-β unsaturated C=O stretching band is seen at 1633cm^{-1}. The aromatic C=C stretching peak at 1449cm^{-1} is shifted to 1447 cm^{-1} after PSEC perturbation. From 1400-1600cm^{-1}, small peaks due to aromatic C=C stretching were observed. sp^3 hybridized C-H bending peak recorded at 1383 and 1316cm^{-1}. The alkoxy C-O stretching is noticed at 1194 and 1102cm^{-1} and the aromatic CH_2 bending at 780 and 658cm^{-1}. The results show that the aromatic C=C and C-H stretching peaks were observed at low transmittance. It indicates that the intensity of the peaks after PSEC perturbations decreased without any change in the peak positions as shown in Fig 7 and Table 2. Thus, we can conclude that upon PSEC treatment Lawsonia inermis does not undergo any degradation thereby making it a harmless constituent for dying and tattooing purposes.

b) Lawsone

The O-H stretching peak before and after PSEC perturbations appeared at 3175cm^{-1}. The aromatic C-H stretching were seen before PSEC at 3071 and 3015cm^{-1} and after PSEC at 3071 and 3010cm^{-1}. The α-β unsaturated C=O stretching band had been noticed respectively at 1678 and 1641cm^{-1} before and after PSEC reactions. The aromatic C=C stretching frequency before and after PSEC reaction is observed at 1591, 1577 and 1458cm^{-1}. After PSEC reaction, two broad peaks were noticed at 1382 and 1345cm^{-1}, demonstrating the presence of sp^3 hybridized C-H bending peak of ethanol attached to the meta position of the aromatic ring (refer section 3.6). The alkoxy C-O stretching peaks were seen after PSEC reaction from 1100-1300 cm^{-1} range. Hence, FTIR confirmed ethoxy group substitution to the aromatic ring. Aromatic CH_2 bending is depicted at the frequency range of 1000-600 cm^{-1} before and after PSEC reaction. Thus additional peaks were noticed after PSEC perturbations. Peaks at 1345, 1382, 1282, 1257, 1215 and 1119 cm^{-1} corresponds to the C-H bending and alkoxy C-O stretching modes. These additional peaks appear due to degradation products. Thus one may conclude that usage of lawsone as colouring agent in high concentration would lead to carcinogenic aromatic compounds as degradation byproducts, leading to skin diseases and other health hazards (Fig 8 and Table 3).

b) PPD

FTIR spectra of reacted PPD possess lower frequency and low transmittance peaks due to dye degradation caused by PSEC perturbations. In the frequency range 3500-3200 cm^{-1}, NH_2 stretching peak were observed before and after PSEC reaction, the transmittance of the peak decreased due to degradation causing high frequency red shift. The aromatic -C-H stretching frequency noticed before reaction at 3008 cm^{-1} vanished for reacted sample. Thus the aromatic ring was broken after PSEC reaction and resulted in byproducts (refer section 3.6). In 2000-1600 cm^{-1} range, two peaks were identified at 1850 and 1630 cm^{-1}. The para substituent observed before reaction at 1850cm^{-1} is not visible after PSEC reaction. At 1630cm^{-1} N-H bending peak seen before reaction split into two peaks at 1604 and 1539 cm^{-1} after reaction. Sharp aromatic C=C stretching peaks were noticed at 1600-1400

cm^{-1} before and after PSEC reaction. Alkene C=C (cyclobutadiene) stretching peak appeared at 1556cm^{-1} with high transmittance after PSEC reaction. Peak at 1516 cm^{-1} frequency range indicated the position of aromatic C-C stretching observed with low transmittance, before reaction and shifted to 1501cm^{-1} after reaction. Aromatic C-N stretching frequency seen in the range 1340-1300cm^{-1} before reaction shifted to 1350-1250cm^{-1} indicating aliphatic C-N stretching frequency after reaction. Before reaction two broad peaks were identified at 831 and 718 cm^{-1} due to β-disubstituted aromatic C-H bending, whereas after reaction small peaks for alkene, CH_2 bending frequency is noticed. Also, the shifting of N – H bending band from 798 cm^{-1} to 813 cm^{-1} was seen after the PSEC reaction. Thus these results indicate dye degradation via aromatic ring opening due to UV irradiation, sonication and electrochemical perturbations.

3.2.3 HPLC analysis

(a) Lawsone

HPLC analysis of lawsone in 50% ethanol show peaks at 0.987 and 1.537 mins at the excitation wavelength of 280nm. The high intensity peak at 1.537min corresponds to lawsone whereas the low intensity peak at 0.987 min accounts for the resonance structures (cf. section 3.2.3 b). After PSEC perturbations there is a shift in the peaks towards higher retention time. The intensity of 1.778 min peak is 20 times higher than 1.537 min peak (before PSEC). The 1.778 min peak corresponds to 3-ethoxy naphthaquinone (cf. section 3.3a). The peaks at 1.030 and 1.166 mins (couldn't identify the peaks in Fig 10) are due to resonance structures of lawsone in its native state. Thus HPLC data supports the mechanism of degradation of lawsone proposed in section 3.3a. The amount of 3-ethoxy naphthaquinone present in the solution after PSEC is 85%, thereby demonstrating the near complete degradation of lawsone to harmful 3-ethoxy naphthaquinone (Fig10).

(b) PPD

HPLC of pure sample of PPD in 50% ethanol showed two characteristic peaks at retention time 4.49 min and 3.21 min at an excitation wavelength of 242nm and is in agreement with the literature [36]. Here peak at 4.49min corresponds to PPD and 3.21 min indicates presence of conjugated PPD[37].

After PSEC experiments, the solution containing products were eluted in HPLC at 242nm and in 50% ethanol. The peaks shifted drastically. Two peaks were noticed at 1.663 and 2.042 retention time. This lower retention time peaks at 1.663 and 2.042 for the solution after PSEC demonstrates the conversion of aromatic diamines into aliphatic amino dienes and the absence of peaks at 4.491 and 3.21 indicates the absence PPD in the product(Fig 11). Thus it is evident that the mechanism proposed in section 3.3b is highly possible for the degradation of PPD in ethanol after PSEC perturbations. The total quantity of 1-amino cyclobutadiene is 79% and remaining anolyte is in conjugated PPD form with 20% at 242 nm. Thus after PSEC perturbations the degradation is almost complete with 79% of harmful substituted cyclobutadiene product.

3.2.4. ^{1}H NMR analysis

a) Lawsonia inermis

In Lawsonia inermis before and after PSEC perturbation, two peaks were observed at 2.5 and 3.4ppm, due to solvent DMSO (Fig 12). In ^{1}H NMR analysis, aromatic and O-H group peaks were observed at low intensity due to sparing solubility of Lawsonia inermis in DMSO.

b) Lawsone

^{1}H NMR of lawsone in DMSO recorded (Fig 13) three sharp peaks and one less intensity peak. The peak at 6.14ppm indicated H_3 proton in quinonoid structure. 7.75-7.96 ppm denoted the aromatic protons H_5, H_6, H_7, H_8 as doublet and multiplet. 11.64 ppm represented the -OH proton as substituent to quinonoid ring with weak C-O bond formation. This pure lawsone solution data is compared with sample after the PSEC perturbation. The chemical shift for lawsone after PSEC reaction being a singlet peak H_3, shifted to 6.16 ppm, aromatic protons were shifted to 7.77-7.98 ppm but the O-H proton chemical shift vanished. This indicated that after PSEC reaction, proton belonging to O-H group was terminated from the quinonoid ring in lawsone. Instead ethoxy group protons were observed at 3.5 and 1.5 ppm (Table 5 and Scheme 1).

c) PPD

^{1}H NMR of PPD (Fig 14 and Table 6) before and after PSEC perturbations were analyzed. Before reaction two sharp peaks were noticed, one for -NH_2 group at 4.18ppm and another peak for aromatic protons at 6.39 ppm with all aromatic protons of same type. After PSEC perturbations the final product gave the ^{1}H NMR data with 6 sharp peaks. Two peaks for solvent, ethoxy group protons were observed at 1.5 and 3.5 ppm, remaining 4 sharp peaks had protons at different chemical environment due to intramolecular hydrogen shifting in the aromatic ring. The resonance structures of PPD are depicted in Scheme 2.

3.2.5 ^{13}C NMR analysis

(a) Lawsone:

The chemical shift of lawsone (Fig 15 a) C1 is about 138 ppm, C3 is observed at 152 ppm which is on higher field. On the other hand the chemical shift of C2 is observed on lower field (114 ppm). The chemical shifts of C3 & C4 are noticed at 151 and 145 ppm. The considerable lower field shift for C4 is reasonable due to C=O. The chemical shifts of C5 to C10 were observed at 114, 140, 141, 128, 129, 142 ppm. These data agree well with the ^{13}C NMR spectra of lawsone reported in the literature**[39]**. After PSEC perturbations, the samples were analysed for ^{13}C NMR, the chemical shifts were almost identical to lawsone, except for ethoxy carbon peaks at lower field of 116ppm, C7 to C10 carbons grouping at 128ppm. This grouping of C7-C10 carbons indicate identical chemical environment for the product obtained. C2 at 152ppm, showing the shift of hydroxyl group attached to C2 into carbonyl group. C3 chemical shift is at 114ppm whereas the chemical shift of remaining carbons (C1, C3-C6) appears at the same position as lawsone (Fig 15b). Thus from these chemical shifts, we can infer that the product formed is identical in structure to lawsone except for an carbonyl group at C2 and ethoxy group at C3. Thus ^{13}C NMR analysis confirms the product obtained after PSEC perturbation as 3-ethoxy naphthaquinone.

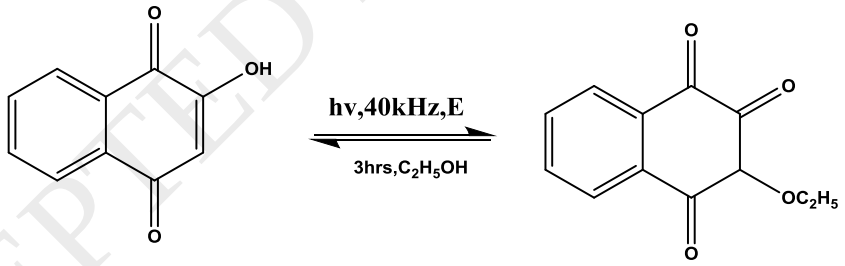
(b) PPD:

Pure PPD standard show two environments in ^{13}C NMR. One peak at 138 ppm contributed by ρ-carbons to which amino groups are attached. The o- and m- position carbons appear at 117ppm. ^{13}C

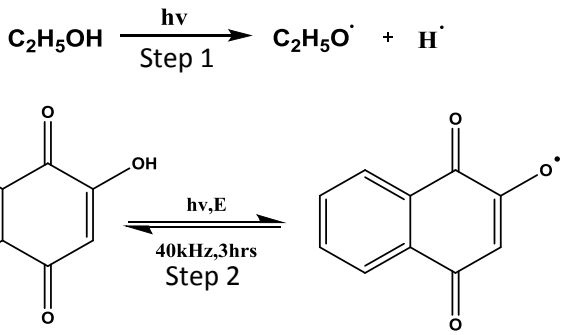
NMR spectra of PPD in 50% ethanol after PSEC perturbations displayed peaks corresponding to different carbon environments in different positions. Here two sharper and small peaks are identified, one at 115ppm and the other at 138ppm. These small intensity peaks are attributed to the unreacted conjugated PPD present in the solution after PSEC perturbations. The sharp peak at 40ppm, when analyzed carefully is found to split into septet (40.3, 39.9, 39.7, 39.5, 39.2, 39, 38 ppm). These peaks correspond to different carbon environments in 1-amino cyclobutadiene. The amino group substituted carbon (C1) appeared at 40ppm, the neighbouring carbon atoms (C2 and C4) are affected by C3 carbon as well as C1 carbon leading to a triplet, C3 carbon inturn is affected by C1, C2 and C4 environment leading to triplet. Thus a singlet peak at 40ppm is split further with very little chemical shift forming a septet. Thus ^{13}C NMR analysis supports the formation of 1-amino cyclobutadiene as the predominant product from PPD upon PSEC perturbations (cf. Fig 16).

3.3. Degradation mechanism based on PSEC perturbations and spectral analysis

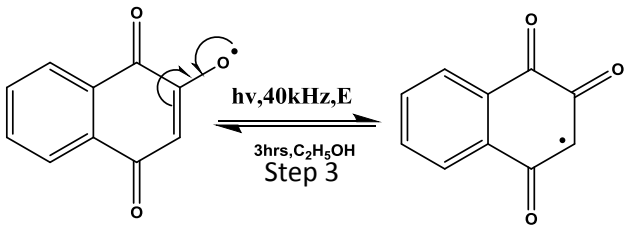
a) Degradation pathway for Lawsone:



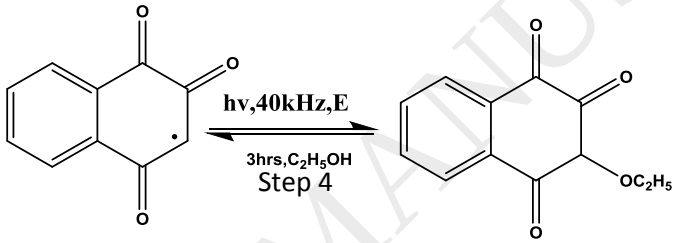
The possible degradation mechanism for lawsone is obtained based on HPLC, UV-Vis, FTIR and ^{1}H NMR and ^{13}C NMR spectroscopic analysis as follows:



Lawsone being a photo sensitive dye upon UV irradiation, hydrogen radical elimination occurred as supported by ^{1}H NMR analysis data. As the radical species is short lived intermediate and get extensively involved in the resonance structure (step 3), peaks corresponding to the radical is not observed in UV-Vis spectra.

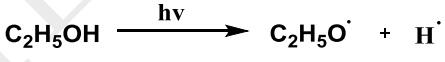


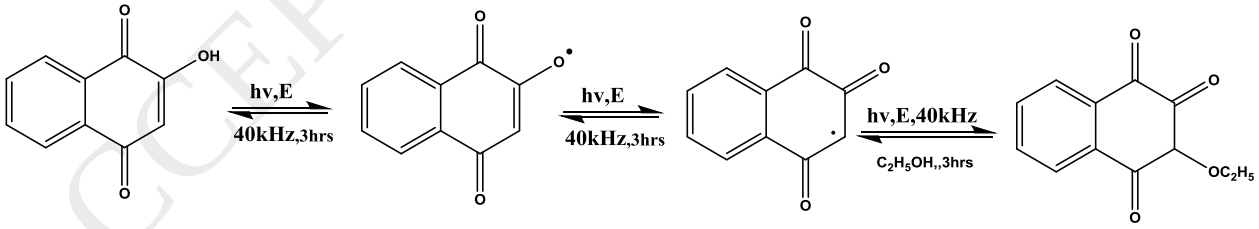
Lawsone undergo electron shifting due to the stabilization of oxygen radical by taking the π electrons in the naphthoquinone group via π electron conjugation.



Naphthoquinone radical reacted with ethoxy radical (chromophore) and form new bond.

Hence the degradation mechanism is shown as given below.

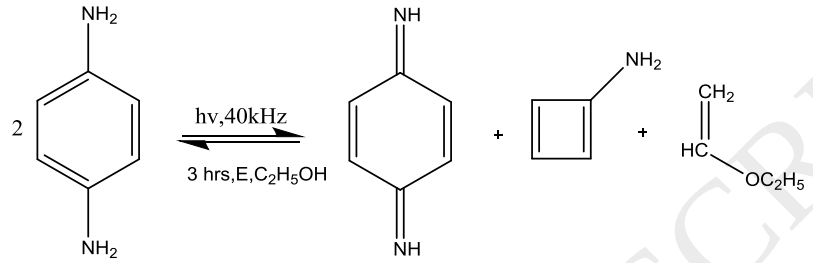




The above degradation mechanism is deduced and supported by HPLC, UV-Vis, FTIR, ^{1}H NMR and ^{13}C NMR spectroscopic analysis. In UV analysis the peak intensity was increased due to chromophore attachment to lawsone by the removal of hydrogen radical. In FTIR ethoxy group attached to the naphthoquinone after PSEC perturbation showed peaks at 1345, 1382, 1282, 1257, 1215 and 1119 cm^{-1}. ^{1}H NMR results showed the alcohol peak at 3.5 ppm and hydroxyl peak at 11.6 ppm before the PSEC reaction disappeared after PSEC perturbations. HPLC accounted for the formation of 3-ethoxy

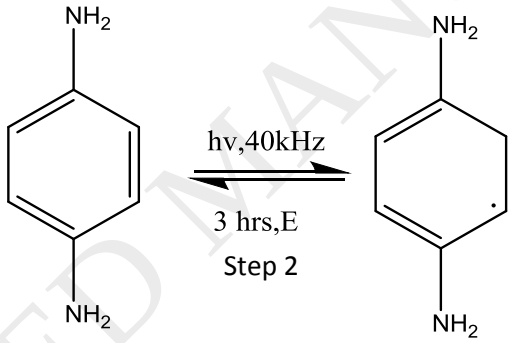
naphthaquinone as predominant product via retention time peak at 1.778 min. ^{13}C NMR analysis proves this product formation via the chemical shift of C2 and C3 carbon dissimilar to lawsone (presence of C2 carbonyl and C3 ethoxy carbons).

b) Degradation pathway for PPD

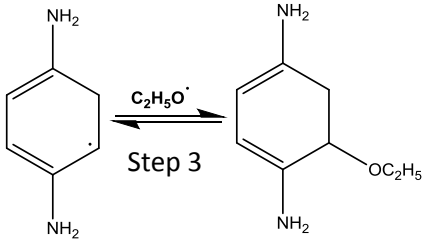


$C_2H_5OH \xrightarrow{hv} C_2H_5O^{\cdot} + H^{\cdot}$

Step 1

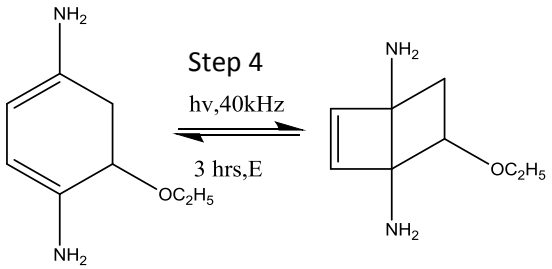


Step-2 shows that the π electrons were excited due to PSEC treatment. So PPD resulted in radical formation.



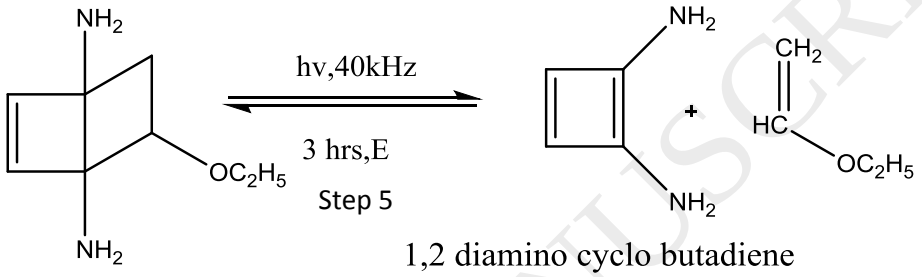
PPD 1,4 diamino,3-ethoxy,cyclohexadiene

In step-3 the radical form of PPD reacted with ethoxy radical to form a new compound. This ethoxy group attachment to aromatic ring at meta position was supported by ^{1}H NMR data via the splitting of aromatic C-H peaks due to change in the environment.



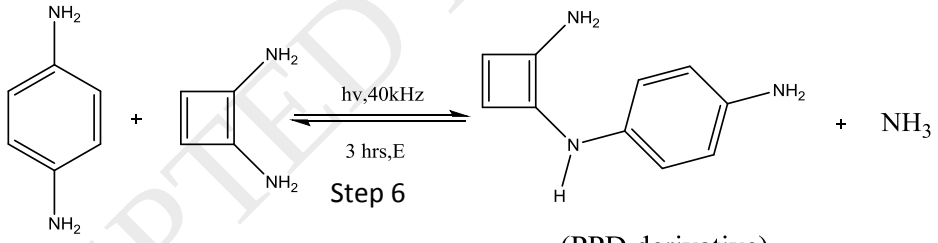
1,4 bicyclo,3-ethoxy,cyclohexaene

In step-4 formation of a bridged compound due to PSEC perturbation is noticed.



1,2 diamino cyclo butadiene

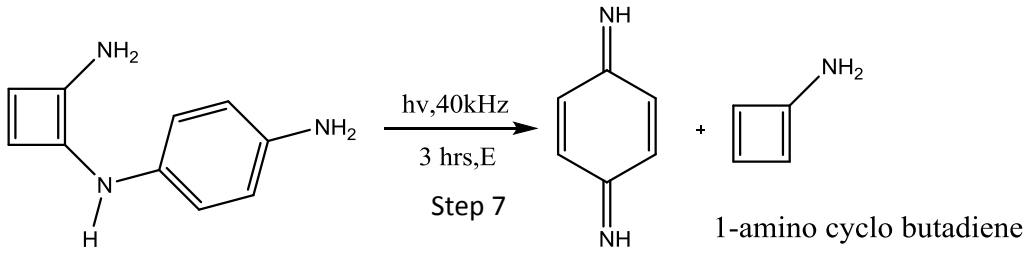
In step-5 upon PSEC perturbation, 1,2 diamino cyclobutadiene and ethoxy ethylene were formed as evident from FTIR results at 1234 cm^{-1} due to aliphatic C-N-C bending. Ethoxy group is observed in ^{1}H NMR analysis at 3.5ppm.



(PPD derivative)

1-amino,2-substituted PPD,cyclobutadiene

In step-6 another mole of PPD reacted with 1,2 diamino-cyclobutadiene to form an addition compound with removal of ammonia. The product formed is supported by ^{1}H NMR analysis at 4.1, 4.9(-NH_2), 7.5-7.9ppm(-NH-) and the path followed is similar to Diels Alder reaction.

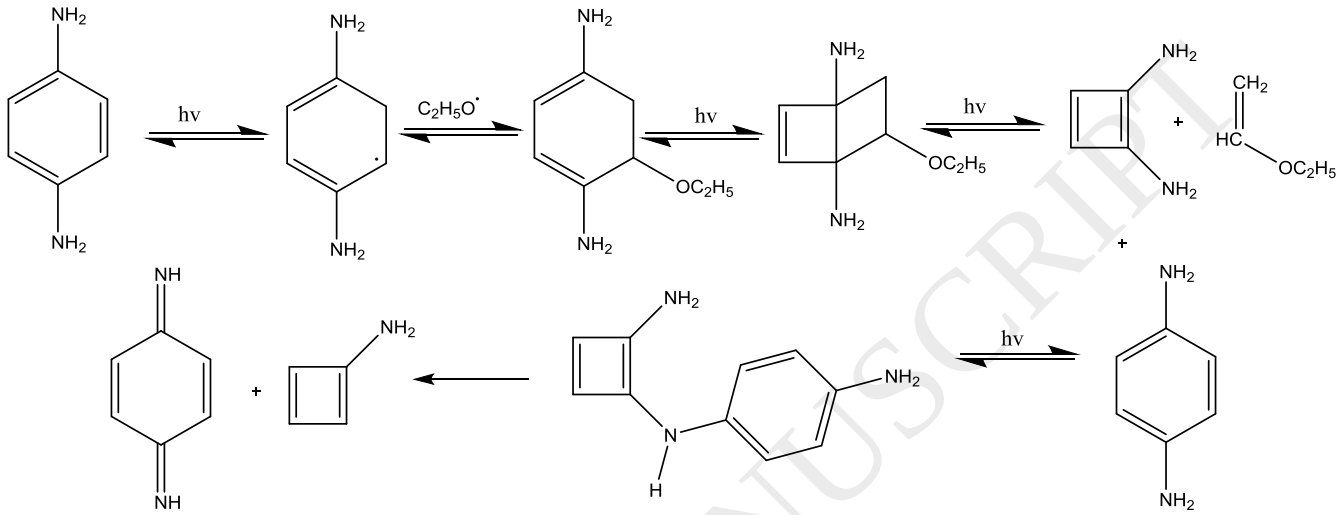


1-amino cyclo butadiene

Conjugated PPD

Upon further perturbation via PSEC experiments, in step-7 the bond breaking occurred resulting in resonance structure of PPD and 1-amino cyclobutadiene.

The overall mechanism is displayed below with the support of spectroscopic analysis.



The mechanism was deduced based on FTIR and ^{1}H NMR analysis. In FTIR analysis, after PSEC perturbations the peak shift was observed from aromatic to aliphatic C-N-C bending from 1262 to 1234 cm^{-1}, in ^{1}H NMR analysis new peaks were identified at low intensity due to formation of new compound and aromatic peaks were also observed at 4.9 ppm, 7.5-7.9 ppm, and 5.7-6.7 ppm. The new peaks in ^{1}H NMR were due to formation of products such as ethoxy group, -NH group and -NH_2 group after PSEC reaction. HPLC analysis of PPD after PSEC perturbations revealed two peaks at 1.663 and 2.042 retention time. This lower retention time peaks at 1.663 and 2.042 for the solution after PSEC demonstrates the conversion of aromatic diamines into aliphatic amino dienes and the absence of peaks at 4.491 and 3.21 indicates the absence PPD in the product. ^{13}C NMR analysis peaks correspond to different carbon environments in 1-amino cyclobutadiene. The amino group substituted carbon (C1) appeared at 40ppm, the neighbouring carbon atoms (C2 and C4) are affected by C3 carbon as well as C1 carbon leading to a triplet, C3 carbon in turn is affected by C1, C2 and C4 environment leading to triplet. Thus a singlet peak at 40ppm is split further with very little chemical shift forming a septet. Thus ^{13}C NMR analysis supports the formation of 1-amino cyclobutadiene as the predominant product from PPD upon PSEC perturbations.

Summary

PSEC perturbation studies of Lawsonia inermis (natural dye, henna), lawsone and PPD were carried out in ethanol. The PSEC studies demonstrated that (i) lawsone, a major constituent of Lawsonia inermis donot undergo degradation upon exposure to UV – irradiation, sonication and electrochemical perturbation and hence the natural dye can stay longer on the skin or fabric without any harmful effects, (ii) commercial lawsone added as colouring agent to henna by dye and tattoo industry responded as an electroactive compound undergoing oxidation upon PSEC perturbation resulting in the formation of ethoxy (at meta position) substituted naphthaquinone as demonstrated by the spectroscopic analysis and (iii) PPD, an artificial dye added as adulterant to henna to enhance its colouring property resulted in the formation of amino substituted cyclobutadiene upon PSEC perturbations. Thus from the present findings, formation of carcinogenic **[40,41]** ethoxy substituted naphthaquinones and amino substituted cyclobutadiene by lawsone and PPD respectively demonstrated the harmfulness in utilizing these artificial dyes as colour enhancing agent to henna by dye and tattoo industries.

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Authors gratefully acknowledge the valuable comments and suggestions by the reviewers that substantially improved the manuscript.

References

1. Bhuyan R, Saikia CN, Das KK,2004. Extraction of identification of colour components from the barks of mimusops elengi and Terminalia arjuna and evaluation of their dyeing characteristic on wool. *Indian Journal of Fibre and Textile Research*.**29**,470-476.

2. Jyotshna, Gaura P, Singh D. K, Luqman S, Shanker K, 2017. Validated method for quality assessment of henna (Lawsonia inermisL.) leaves after postharvest blanching and its cosmetic application, *Industrial Crops and Products* **95**, 33–42

3. Meiti W, Dallali S, Belkhir M, Fattouch S, 2015. Commercial Lawsonia inermis dried leaves and processed powderiphyto chemical composition, antioxidant, antibacterial and allopathic activities *Industrial Crops and Products* **77**, 544-552.

4. Brauer E, Raphael P, 1993. The Jews of Kurdistan. Detroit (Wayne State University Press).

5. Ashnagar, A, Shiri A, 2011. Isolation and characterization of 2-hydroxy-1,4-naphthoquinone (lawsone) from the powdered leaves of henna plant marketed in Ahwaz city of Iran. *International Journal of Chemical Technology Research*. **3**, 1941-1944.

6. Reddy S. H, Al-Aamri S. H, Al-Syabi, W.I, 2015. Study on Phytochemical Screening, HPLC Analysis of Phenols and In vivo Assay on Mice by Using Traditional Herbal Medicinal Plant in Oman, *International Journal of Scientific and Research Publications*, **5**, 4-12.

7. Pallipurath A, Skelton J.M, Delori, A, Duffy C, Erxleben A, Jones W, 2015. Crystalline adducts of the Lawsone molecule (2-hydroxy-1,4- naphthaquinone): optical properties and computational modeling. *The Royal Society of Chemistry, Crystal Engineering Communication*, **17**, 7684–7692.

8. Rahmoun N.M, Atmani Z.B, Benabdallah M, Boucherit K, Villemin D, Braham N. C, 2013. Antimicrobial Activities of the Henna Extract and Some Synthetic Naphthoquinones Derivatives , *American Journal of Medical and Biological Research*, **1**,16-22.

9. Garg R. K, Tripathi R, Batav N, Singh RK, 2017. Phytochemical High Performance Thin Layer Chromatography based Estimation of Lawsone in *Lawsonia inermis* (Henna) obtained from Two Natural Habitats and Dye Products Collected from Local Market. *Med Aromat Plants (Los Angels)* **6,** 290.

10. El-Basheir Z.M, Fouad M.A, 2002.A preliminary pilot survey on head lice, pediculosis in Sharkia Governorate and treatment of lice with natural plant extracts., *Journal of Egyptian Society Parasitol*. **32**,725-736.

11. Prisdiani A, Tabri F, Muchtar S. V, Bahar B, Wirohadidjojo Y.W, Bukhari A, 2017. Study of Para-Phenylenediamine (PPD) in the Clothes Textile Materials, *American Journal of Clinical and Experimental Medicine*. **5,** 36-40

12. Baldwin R.P, Ravichandran K, Johnson R. K, 1984. A Cyclic Voltammetry Experiment for the Instrumental Analysis Laboratory. *Journal of Chemical Education,* **61**,820-823

13. Hussain, I, Zhang Y, Huanga S, 2014. Degradation of aniline with zero valent iron as an activator of persulfate in aqueous solution. *RSC Advances*. **4**, 3502-3511.

14. Al-Suwaidi, A., Ahmed, H, 2010. Determination of para- Phenylenediamine (PPD) in Henna in the United Arab Emirates. *International Journal of Environmental Research and Public Health,* **7,** 1681-1693.

15. Dornbusch, D.A, Hilton,R, Gordon, M.J, Suppes, G.J, 2013, Effects of Sonication on EIS Results for Zinc Alkaline Batteries, *ECS Electrochemistry Letters*, **2**, A89-A92.

16. Khan M.S, Khan Z.H, 2005.Ab initio and semiempirical study of structure and electronic spectra of hydroxy substituted naphthoquinones, *Spectrochemica Acta Part A,* **61**,777-790.

17. Hassan S.F, Arif S.S, Ghazanfar M, 2017. Para Phenylene Diamine Poisoning: Hepatic and Renal Damage. *Journal of Women's Health Care*, **6,** 364-369.

18. Kobayashi, D., Shimakage, K., Honma, C., Matsumoto, H., Otake, K. and Shono, A. 2015. Effect of Particle Addition on Ultrasonic Degradation Reaction Rate. *Open Journal of Acoustics.* **5**, 67-72.

19. Cho E, Park B, Na S, Khim J, 2011. Effects of Power Density and TiO2 Dose in the Sonocatalytic Degradation of Diethyl Phthalate Using High Frequency, *Japanese Journal of Applied Physics*, **51**, 1-4.

20. Park, B., Cho, E., Park, H. and Khim, J, 2011. Sonophotocatalytic Destruction of Chloroform: Comparison of Processes and Synergistic Effects. *Japanese Journal of Applied Physics,* **50**, Article ID: 07HE10.

21. Richard G. Compton, John C. Eklund , Frank Marken, 1997, Sonoelectrochemical processes: A review. *Electroanalysis*, **9**, 509-522. (online by 25 April 2005)

22. Luiz Fernando M. de Azevedo,***a*** Marcello G. Trevisan,***a,b*** Jerusa S. Garcia***a*** and Alzira M. S. Lucho***,2014.a*** Electrochemical Oxidation, Adsorption and Quantification of 1,2-Benzopyrone**,** Journal of Brazilian Chemical Society, **25**, 469-477.

23. Al-Etre A.Y., Abdallah M., Al-Tantawy Z.E., 2005.Corrosion inhibition of some metal using lawsonia extract, *Corrosion Science,* **47**, 385-395.

24. Hamdy A., El-Gendy N.S., 2013.Thermodynamic, adsorption and electrochemical studies for corrosion inhibition of carbon steel by henna extract in acid medium, *Egyptian Journal of Petroleum,* **22**, 17-25.

25. Torchani A., Saadaoui S., Gharbi R., Fathallah M., 2015, Sensitized solar cells based on natural dyes. *Current Applied Physics,***15**,307-312.

26. Pedersen A.J, 2002, On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **58**, *1*257-1270

27. Wilke T, Schneider M, Kleinermanns K, 2013, 1,4-Hydroquinone is a Hydrogen Reservoir for Fuel Cells and Recyclable via Photocatalytic Water Splitting ,*Open Journal of Physical Chemistry.* **3**, 97-102.

28. Eric A. Perp`ete a,1, Lambert C, Wathelet V, Preat J, Jacquemin D, 2007. *Ab initio* studies of the λmax of naphthoquinones dyes. *Spectrochimica Acta Part A.* **68**, 1326–1333.

29. Weast R.C., 1970. Handbook of Chemistry and Physics, 51st ed., The Chemical Rubber Company, Cleveland, OH.

30. Grasselli J.G., 1973, Atlas of Spectral Data and Physical Constants for Organic Compounds, The Chemical Rubber Company, Cleveland, OH.

31. Rumble, J.R, 2018, CRC handbook of Chemistry and Physics, 98th Edition, CRC press.

32. Trivedi M.K, Branton A, Trivedi D, Nayak G, Singh R, Jana S, 2015.Characterization of Physical, Thermal and Spectroscopic Properties of Biofield Energy Treated β-Phenylenediamine and p-Toluidine, *Journal of Environmental Analytical Toxicology*, **5**, 6-12.

33. Mahlambi M P M, Catherine J, Ngil, Mamba B.B, 2015. Recent Developments in Environmental Photocatalytic Degradation of Organic Pollutants: The Case of Titanium Dioxide Nanoparticles—A Review, *Journal of Nanomaterials*, 29.

34. Ashnagar A., Shiri A., 2011. Isolation and characterization of 2-hydroxyl-1,4 naphthoquinone (lawsone) from the powdered leaves of henna plant marketed in Ahwaz city of Iran,International *Journal of Chemical Technology Research*, **3**

35. Sangeetha J, Philip J, 2013. Synthesis, characterization and antimicrobial property of Fe_3O_4-Cys-HNQ nanocomplex, with L-cysteine molecule as a linker, *RSC Adv*ances., 3, 8047–8057..

36. Ikarashi Y, Kaniwa M, 2000, Determination of p-Phenylenediamine and Related Antioxidants in Rubber Boots by High Performance Liquid Chromatography. Development of an Analylical Method for N-(1-Methylheptyl)- N'-Phenyl-p-Phenylenediamine, *Journal of Health Science*, **46**, 467-473.

37. Meyer A, Fischer K, 2015, Oxidative transformation processes and products of para-phenylenediamine (PPD) and para-toluenediamine (PTD)—a review, *Environmental Sciences Europe,* **27,** 11-20.

38. El-Hendawy, El-Ghany, El-Kourashy, Shanab M.M, 1991, Schiff base complexes of Ruthenium(III),Molybdenum(VI) and Uranium(W) and use of the former as catalytic organic oxidants. *Polyhedron,* **II**, 523-530.

39. Pawar A.B., Jadhav K.D., Wadekar M.P., Sarawadekar R.G., 2011, Spectral and theoretical investigation on 2- hydroxy- 1,4,naphthoquinone (Lawsone), *Journal of Pharmacy Research,**4***,1745-1747.

40. Khandagale P, Chikate R, Joshi S.B, Kulkarni B.A., 2005, .Binuclear lanthanide complexes of 2,3-dihydroxy-1,4-naphthoquinone, *Journal of Alloys and Compounds*, **392**, 112–119.

41. Bajer I.K, 2004.Synthesis and spectroscopic properties of aniline tetramers. Comparative studies, *New Journal of Chemistry*, **28**, 669 – 675.

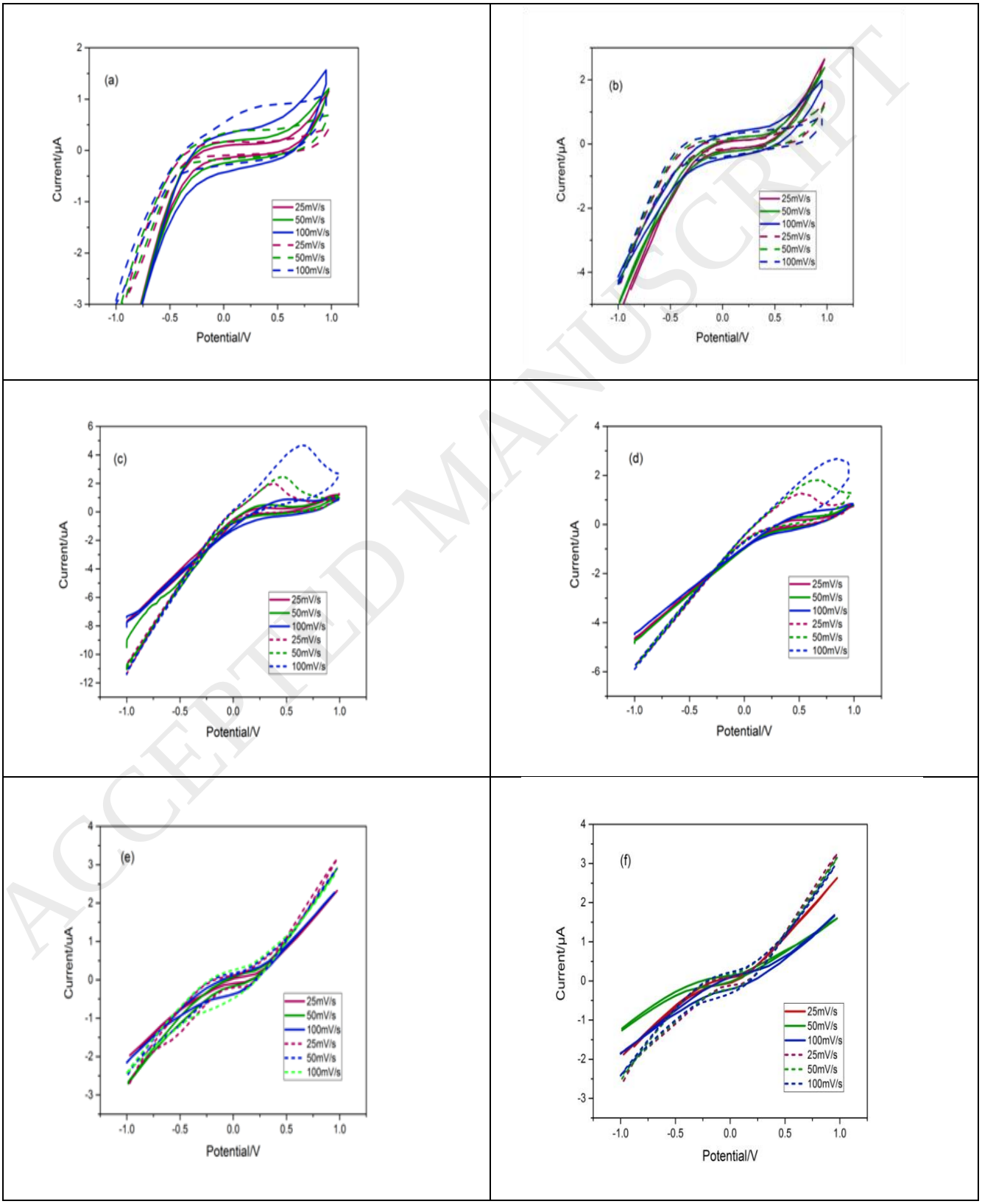


Fig.1. Cyclic Voltammograms in ethanol under UV irradiation with sonication (solid lines) and after 3 hrs of UV irradiation and sonication (dotted lines) of (a) 0.1% Lawsonia inermis (b)) 0.5% Lawsonia inermis (c) 0.1% Lawsone (d) 0.5% Lawsone (e) 0.1% PPD and (f) 0.5% PPD.

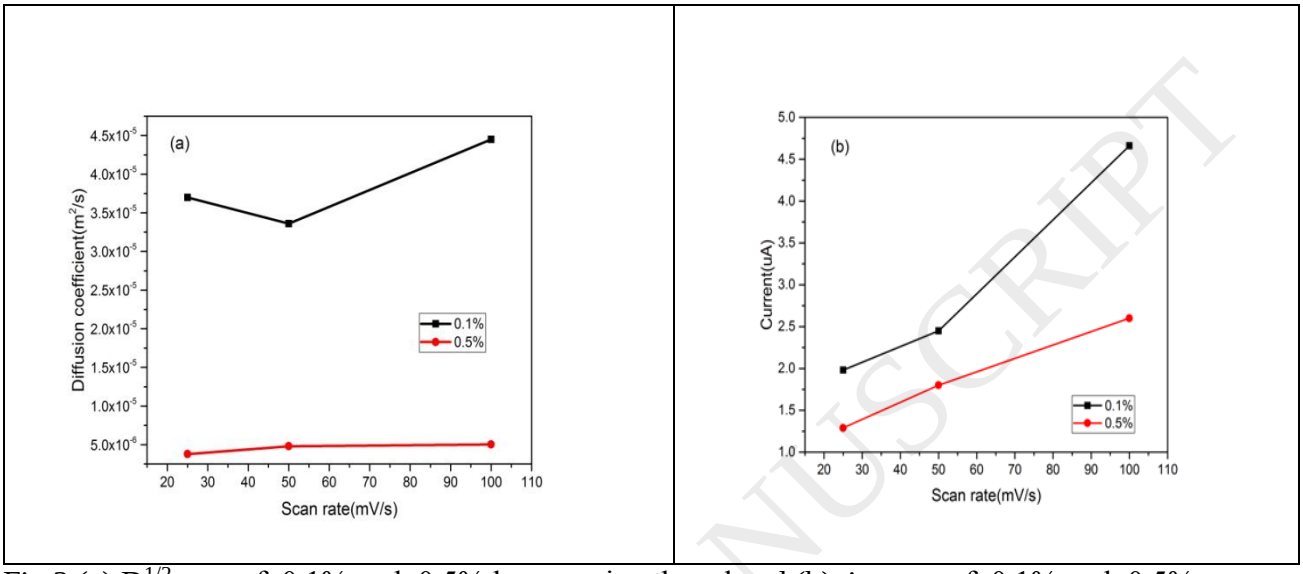


Fig.2 (a) $D^{1/2}$ vs ν of 0.1% and 0.5% lawsone in ethanol and (b) i_p vs ν of 0.1% and 0.5% lawsone in ethanol

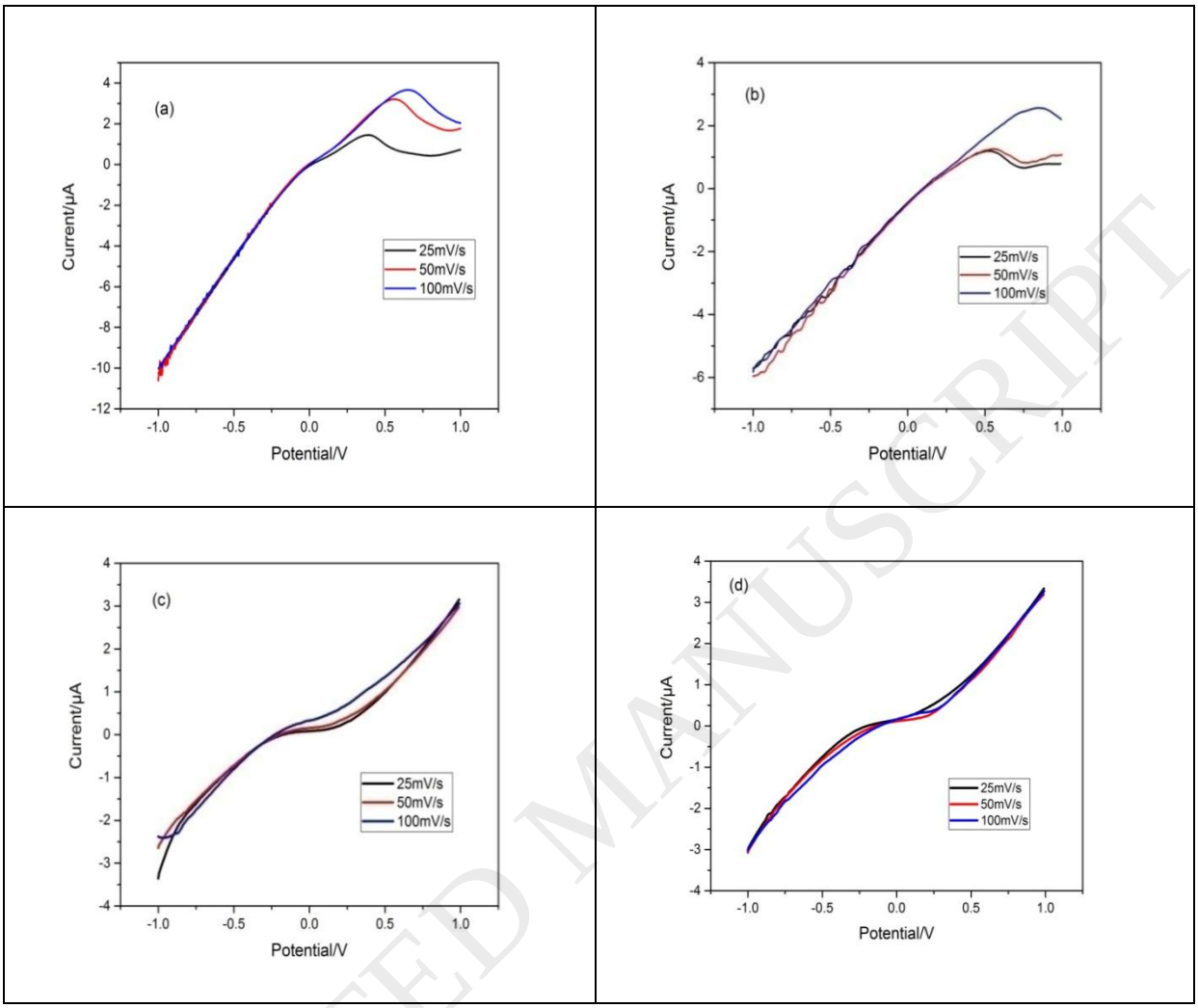


Fig.3. Linear sweep voltammetric analysis in ethanol of (a) 0.1% Lawsone (b) 0.5% Lawsone (c) 0.1% PPD (d) 0.5% PPD

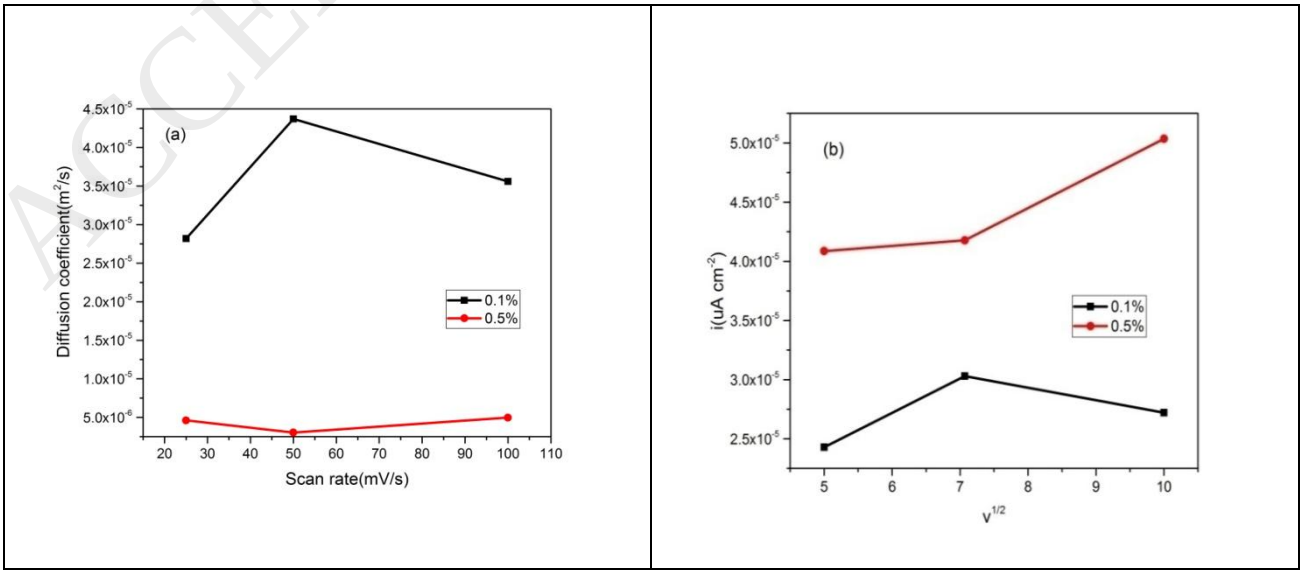
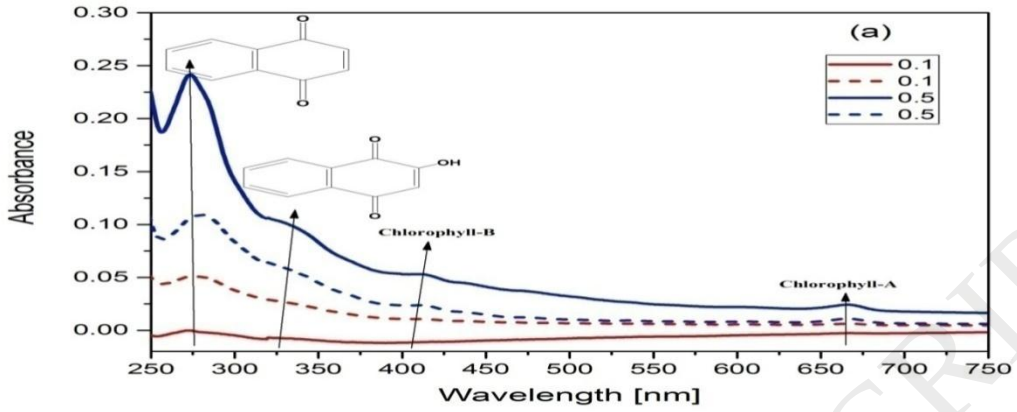
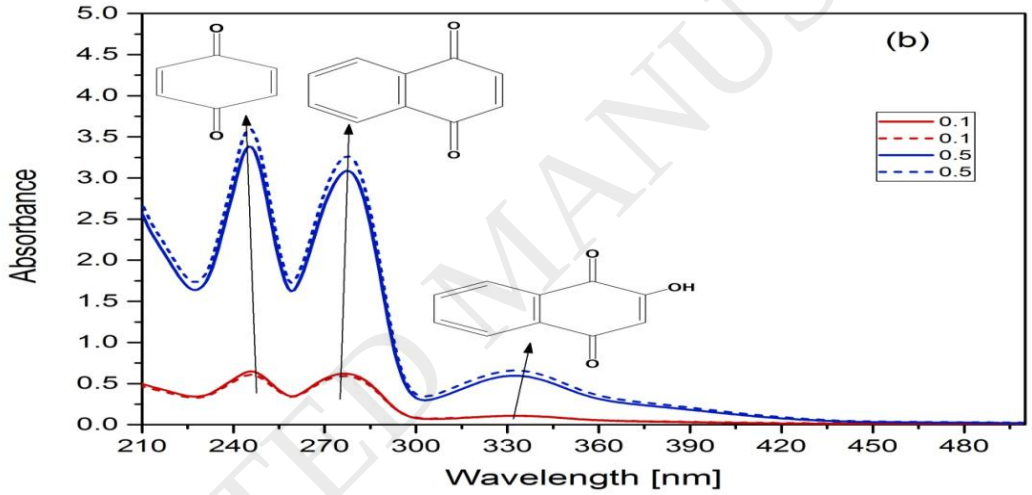


Fig.4 (a) $D^{1/2}$ Vs ν of 0.1% and 0.5% lawsone in ethanol and (b) i Vs $\nu^{1/2}$ of 0.1% and 0.5% lawsone in ethanol





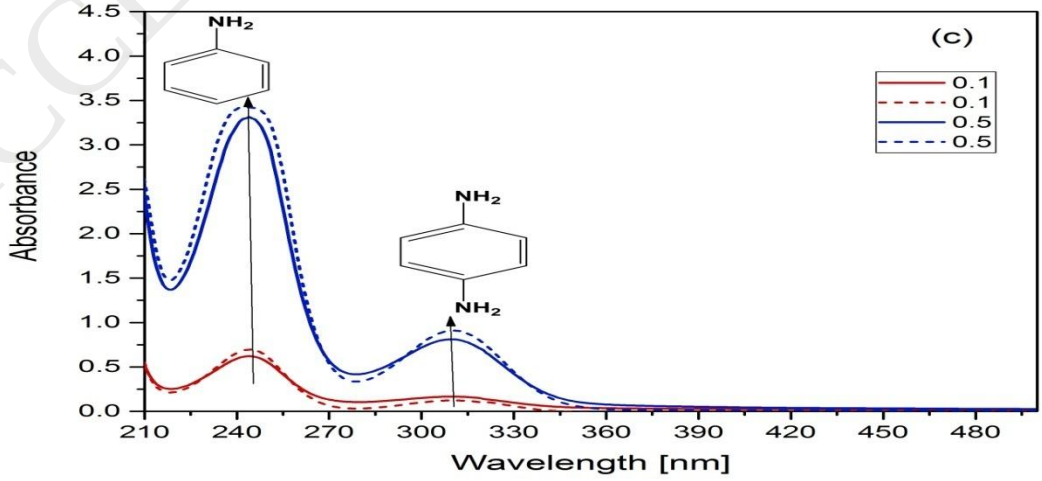


Fig 5. UV-Vis spectrum in ethanol 0.1% and 0.5% concentrations before (solid lines) and after PSEC perturbations (dotted lines) of (a) Lawsonia inermis (b) Lawsone (c) PPD.

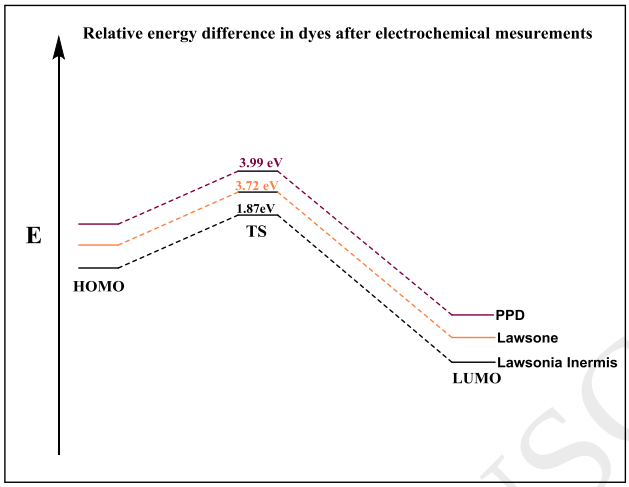


Fig.6 Relative energy diagram of Lawsonia inermis, Lawsone and PPD

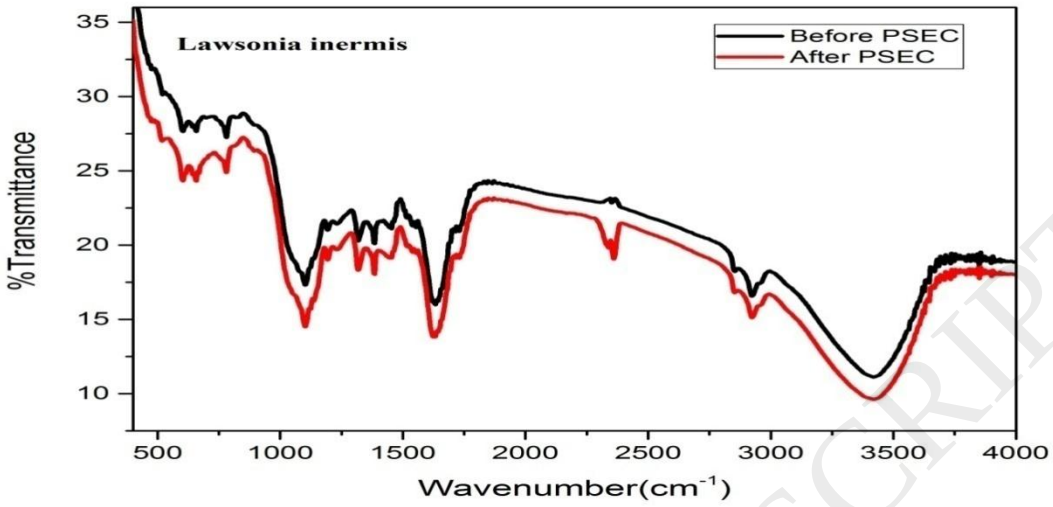


Figure 7. FTIR spectrum of Lawsonia inermis

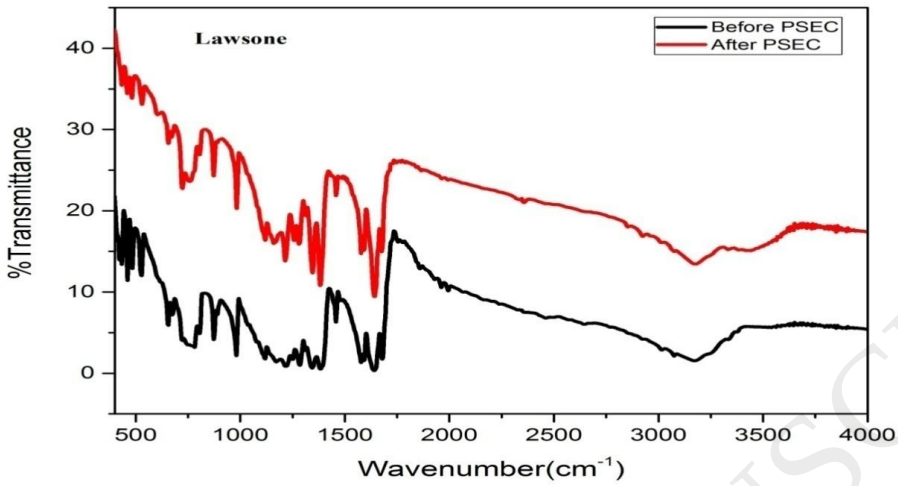


Figure 8. FTIR spectrum of Lawsone

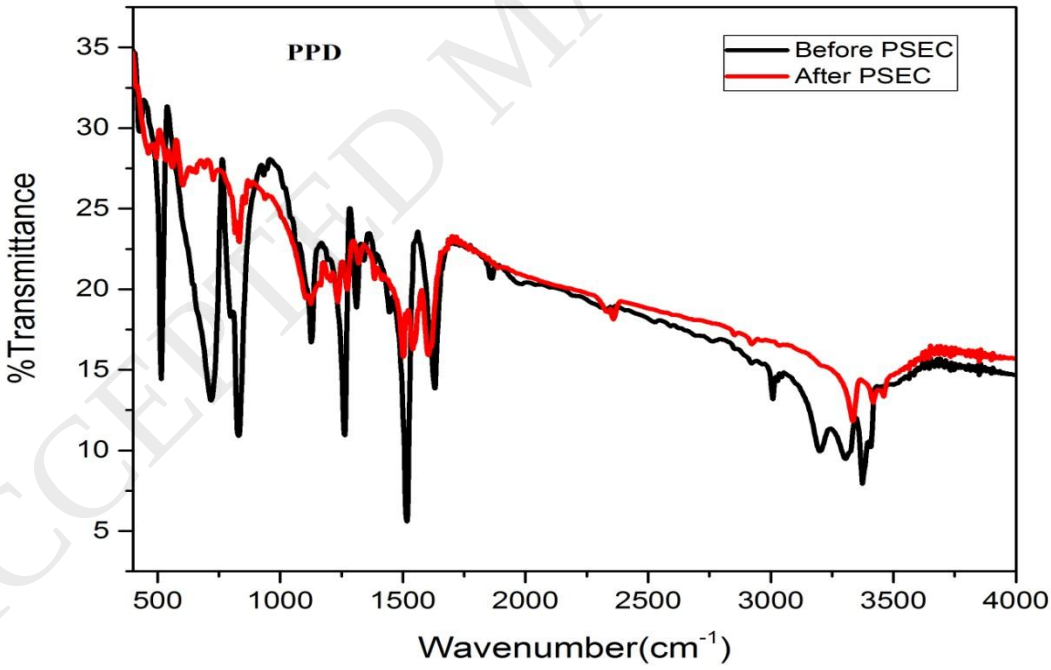


Figure 9 FTIR spectrum of PPD

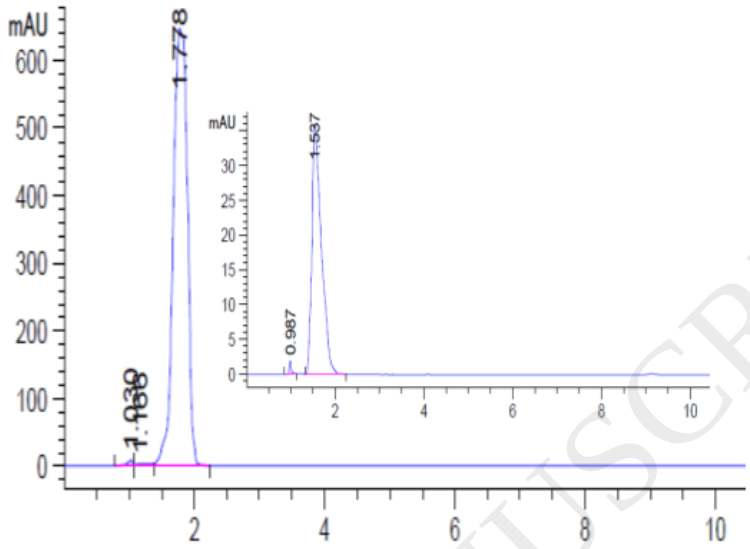


Figure 10: HPLC of Lawsone after PSEC perturbations and inset is before PSEC perturbations

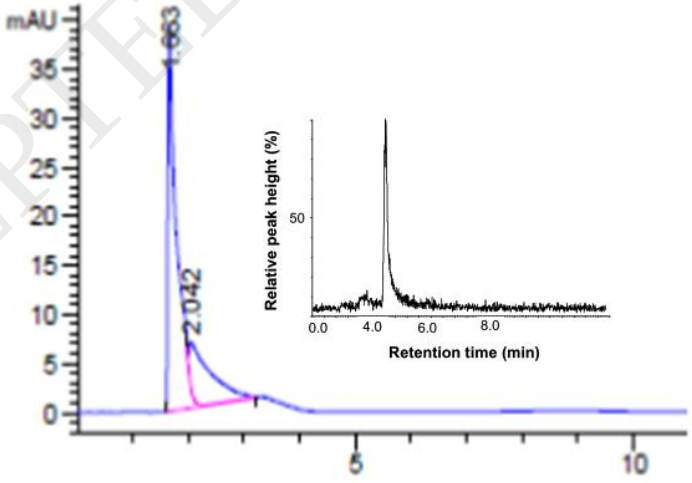


Figure 11 HPLC of PPD in 50% ethanol after PSEC perturbations and inset is before PSEC

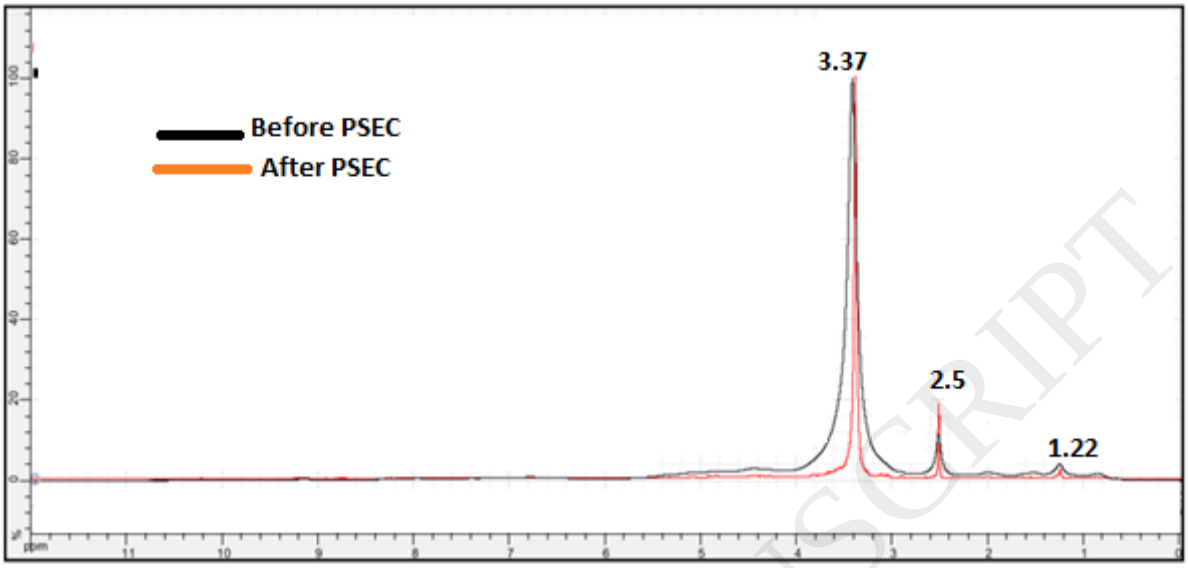


Figure 12 ^{1}H NMR spectrum of Lawsonia inermis before and after PSEC reaction

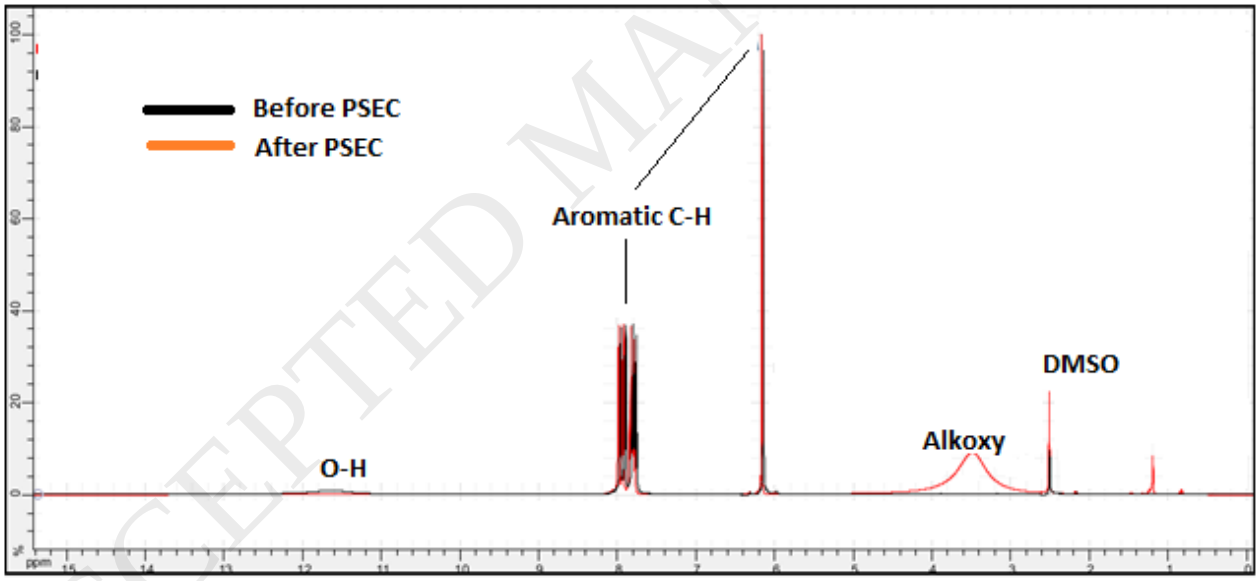


Figure 13. ^{1}H NMR spectrum of Lawsone before and after PSEC perturbations

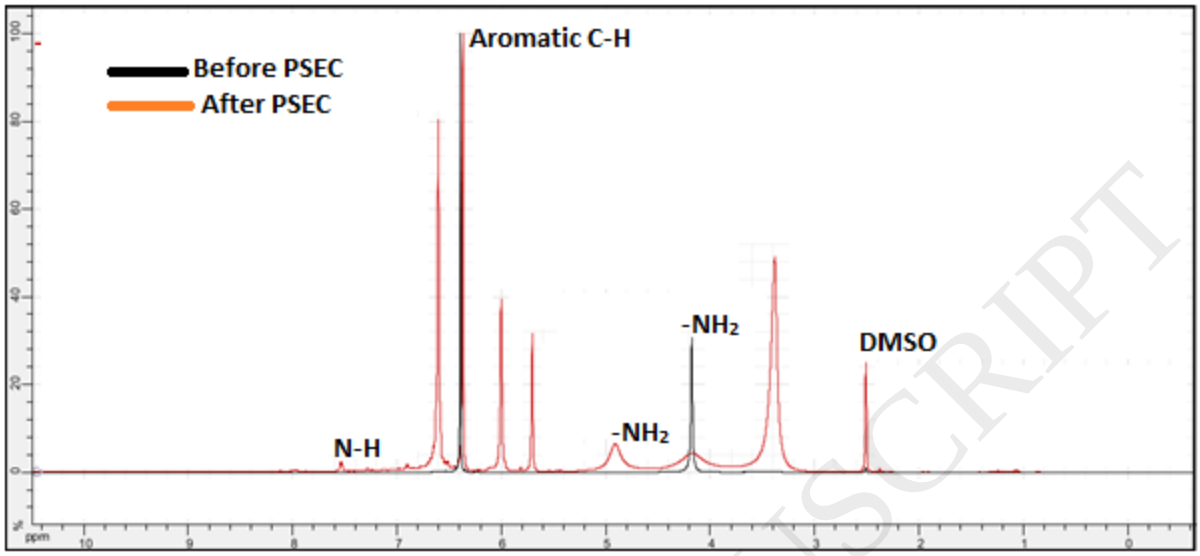


Figure 14. ^{1}H NMR spectrum of PPD before and after PSEC perturbations

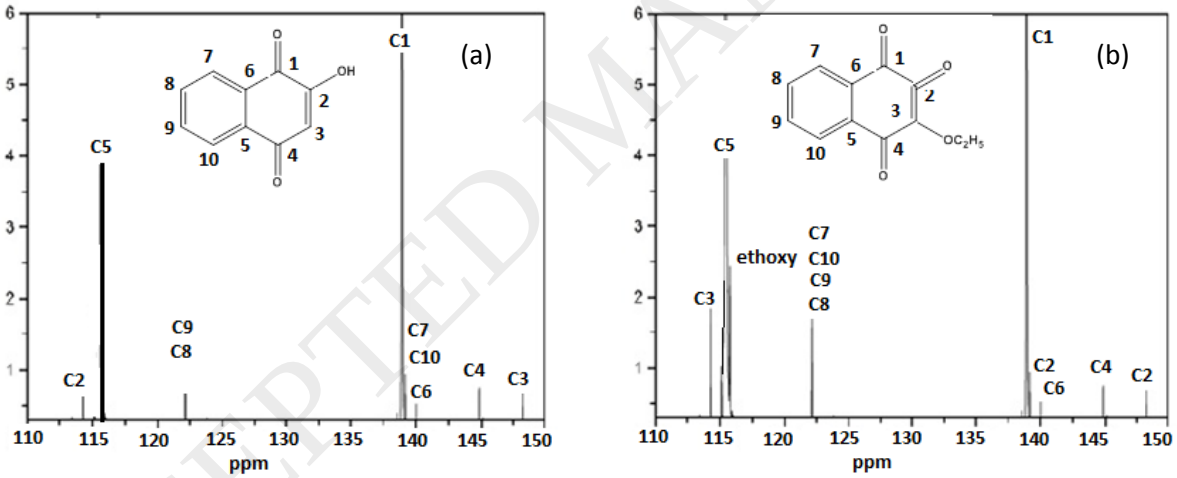


Figure 15 ^{13}C NMR of Lawsone (a) before PSEC and (b) after PSEC, solvent is DMSO

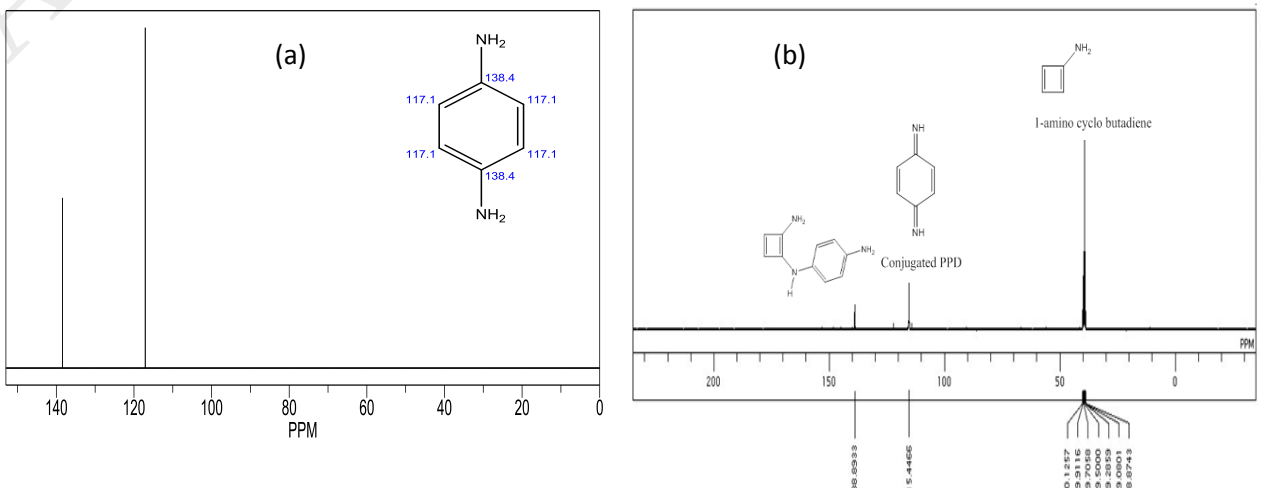
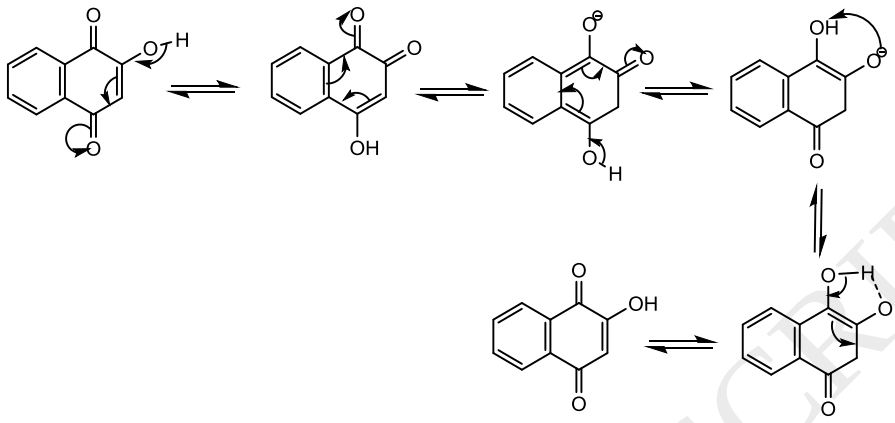


Figure 16: ^{13}C NMR of (a) pure standard PPD and (b) after PSEC

Scheme 1: The resonance structure of lawsone responsible for the observed NMR



Scheme 2

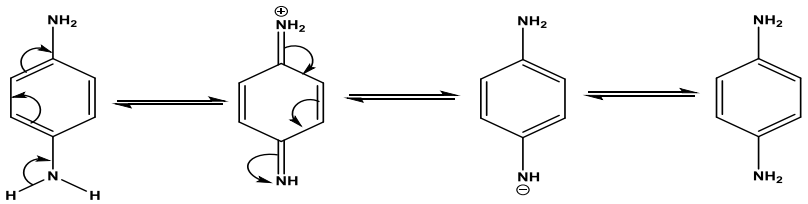


Table:1 Band gap energy from Fig 5

S.no	Name of the Dye	λmax (nm)	Band gap(eV)
1	Lawsonia inermis	275, 334, 412, 665	4.51, **3.72,** 3.01, 1.87 eV
2	Lawsone	246, 277, 333	5.04, 4.51, **3.72** eV
3	PPD	244, 311	5.04, **3.99** eV

Table 2. FTIR of Lawsonia inermis before and after PSEC reaction

S.No	Functional group	Wave number (cm^{-1})		
		Before	**After**	**Literature[33]**
1	OH streatching	3419	3419	3309
2	Ar C-H stretching	2928,2852	2928,2852	2953
3	Acid OH	2361,2339	2361	Absence of 2339cm^{-1} peak after PSEC perturbation findings of present work
4	α-β unsat-C=O stretching	1633	1633	1655
5	Ar C=C stretching	1449	1447	1403
6	C-H bending	1383,1316	1383,1316	-
7	Alkoxy C-O stretching	1194,1102	1194,1102	1000
8	Ar CH_2 bending	780,658	780,658	-

Table3. FTIR of Lawsone before and after PSEC reaction

S.No	Functional group	Wave number(cm^{-1})		
		Before	**After**	**Literature[34]**
1	OH streatching	3175	3175	3442,3174
2	Ar C-H stretching	3071,3015	3071,3010	-
3	α-β unsat-C=O stretching	1678,1641	1678,1641	1591,1642,1678
4	Ar C=C stretching	1591,1577,1458	1591,1577,1458	1458,1383,1345
5	C-H bending	-	**1382, 1345** Findings of present work	-
6	Alkoxy C-O stretching	-	**1282,1257,1215,1119** Findings of present work	-
7	Ar CH_2 bending	1000-600	1000-600	-

Table 4. FTIR of PPD before and after PSEC reaction

S.No	Functional group	Wavenumber (cm^{-1})		
		Before	**After**	**Literature [35]**
1	N-H stretching	3373,3304,3200	3417,3334	3410,3375
2	C-H stretching(aromatic)	3008	Absence of 3008cm^{-1} peak findings of present work	3009
3	Para substituted	1850	Absence of 1850 cm^{-1} peak findings of present work	-
4	C-N-H bending	1630	1604,1539 Splitting of peaks findings of present work	1633
5	Alkene C=C stretching	-	New peak at 1556cm^{-1} findings of present work	-
6	C-C stretching(aromatic)	1516	1501	1516,1456
7	C-N stretching	1340,1311	1320,1274	1340
8	C-N-C bending	1262	**1234**	1263
9	C-H deformation (in plane)	1127,1065	1124,absence of 1065cm^{-1} peak	1130,1066
10	Distributed ring	831	832	831
11	N-H bending	798	813	798
12	C-H bending (out of plane)	718	602	721

Table 5 : ^{1}H NMR data for Lawsone pure and after PSEC perturbation

Compound	Data
Lawsone [**36]**	6.15 (s; H3), 7.79 (q; H6,7), 7.90 (d; H5), 7.95 (d; H8) 11.63 (b; O H)

Lawsone (Before)	6.14 (s; H3), 7.75 (m; H6,7), 7.90 (d; H5), 7.96 (d; H8) 11.64 (b; O H)
Lawsone (After)	6.16 (s; H3), 7.77 (m; H6,7), 7.93 (d; H5), 7.98 (d; H8)

Table 6 . ^{1}H NMR data for PPD pure and after PSEC perturbations

Compound	Data
PPD **[38]**	4.18(-NH_2), 6.8-7.9(s;$H_{2,3,5,6}$)
PPD (Before)	4.18(-NH_2),6.3 (s;$H_{2,3,5,6}$)
PPD (After PSEC)	7.5-7.9(-NH-),4.1 and 4.9 (-NH_2), 5.7-6.7(s;H s;$H_{2,3,5,6}$)