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SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT, DNA CLEAVAGE AND CYTOTOXIC STUDIES OF AMINO DERIVATIVES OF LAWSONE

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ABSTRACT

2-Hydroxy-1,4-naphthaquinone (Lawsone) was isolated from the leaves of *lawsonia inermis*. Two amino derivatives, 2-[(Para –methyl) anilino]-1, 4-naphthoquinone (PAN) and 2-(thiol anilino)-1, 4- naphthoquinone (TAN) were synthesized from lawsone by ultrasound accelerated technique, a new method which have not been so far reported. These compounds were characterized using various spectral techniques and elemental analysis. Its fluorescence emission wavelength was measured in acetonitrile solution. The synthesized compounds were screened for their antioxidant activity. The in vitro cytotoxic effect was studied by using trypan blue dye exclusion method. DNA Cleavage activity of the compounds was also investigated on pET20b plasmid DNA by gel electrophoresis experiments. These derivatives exhibited significant activity in all the studies conducted. In addition, the fluorescent property of the compounds may be useful as molecular fluorescent probes for antibody- antigen biochemical species.

Key words: Lawsone, Antioxidant study, DNA cleavage, Cytotoxic activity.

INTRODUCTION

A great variety of hydroxy quinones are found in nature. Quinones with hydroxy group on the quinone ring comprise an interesting class of organic compounds (Thomson RH, 1987). The quinone and their substituted derivatives have long been known to posses numerous chemical and biological significant properties with many important applications in several areas (Patai, 1998). The cytotoxicity of quinone compounds are mainly through intercalation, inhibition of DNA and RNA and breaking of DNA (Gokhale N *et al*., 2000). Antioxidant-based drug formulations are used for the prevention and treatment of complex disease like Atherosclerosis, Stroke, Diabetes, Alzheimer's disease and Cancer (Deasagayam *et al*., 2004). The antioxidative activity of henna extract was found to be the highest as compared to vitamin E or tocopherol. The strong cytotoxic properties of this extract could be due to its high antioxidant activities (Varghese JK *et al*., 2010). Quinone derivatives were reported as fluorescent compounds (Dabiri M *et al*., 2011). 1, 4-naphthaquinone possessing an amine or a substituted amine group in the 2-position have been used in variety of medicinal and biological application (Franco C *et al*., 2011). The preparation of amino derivatives of quinone which have already been reported (Feizi N *et al*., 2010) are by very tedious procedures. The development of a method for their preparation in mild condition is of great importance. Here we have employed an easy method using ultrasound irradiation for the preparation of the amino derivatives of lawsone.

The present communication deals with the preparation of two amino derivatives of lawsone and these compounds were characterized by spectral data (IR, UV, ^{1}H and ^{13}C NMR and mass spectra.). These compounds were screened for antioxidant, nuclease and cytotoxic activities. It is possible that these new substance can constitute a new class of bioactive naphthaquinone derivatives.

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MATERIALS AND METHODS

All chemicals except lawsone were obtained from commercial suppliers and used as received without further purification. Melting points were obtained using a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR Prestige-21 FT-IR spectrometer as KBr pellets in the case of solids. ^{1}H and ^{13}C NMR spectra were measured on a 500 MHz Bruker advanced DPX spectrometer. Internal standard used for ^{1}H and ^{13}C NMR is 1,1,1,1- tetramethyl silane (TMS). CHN analyses were carried out on an Elemental vario MICRO cube Elemental Analyzer. All values recorded in elemental analyses are given in percentages. Absorption spectra and emission were recorded on Shimadzu UV-3600 UV-VIS-NIR.and Horba jobin Yvon Flurolog spectrometer respectively. Mass spectra were recorded on a JEOL JMS600H mass spectrometer operating at an ionization mode FAB. TLC analyses were performed on precoated aluminum plates of silica gel 60 F254 plates (0.25 mm, Merck) and developed TLC plates were visualized under short and long wavelength UV lamps. Column chromatography (CC) was performed using silica gel G (mesh 100-200) and silica gel G (Merck) respectively. DNA cleavage activity was performed on an agarose gel electrophoresis method against plasmid DNA pET20b. In vitro cytotoxicity was studied using Dalton's Lymphoma ascites cells by tripan blue method and number of dead cells was determined by using haemocytometer.

Isolation and Purification of Lawsone

Shade dried and powdered henna was extracted by pH gradient method **(**Ashnagar A *et al*., 2010) and then further extracted using diethyl ether. The ether layer was removed completely in vacuum to get reddish brown solid as crude product. The crude product was subjected to column chromatography. The extract was eluted with ethanol-ethyl acetate (1:2 v/v). The residue was recrystalised using glacial acetic acid to give orange red crystals and the purity was checked by TLC .The red spot obtained, had Rf value 0.06 and its melting point was obtained as 195^{o}C

2-Hydroxy- 1, 4 –naphthaquinone (Lawsone)

IR (KBr) (cm^{-1}): 3410(OH), 1676(C4=O), 1644(C1=O), 1231(C-O); ^{1}HNMR ($CDCl_3$)
(δ)8.22(d,2H,H5,H8),7.65(t,2H,H7,H6),13(s,1H,OH)^{13}CNMR184(C4),181(C1),156(C2),
134(C6), 133(C7), 125(C8), 110(C3): MS, m/z (M+) 174.

PREPARATION OF AMINO DERIVATIVES OF LAWSONE USING ULTRASOUND IRRADIATION TECHNIQUE

General procedure

Two derivatives were prepared by addition of appropriate amines (para toluidine and ortho amino thio phenol) to lawsone in methanol in equi:molar concentration followed by ultrasound irradiation for 2-hours. The reaction mixture was kept at room temperature overnight. The precipitate was purified by column chromatography (gradient elution, 1:3 ethyl acetate and hexane) and the purity was checked by TLC. The derivative 2-[(Para –methyl) anilino]-1, 4-naphthoquinone (PAN) was obtained as dark brown crystals with m.p 174^{0}C and the derivative, **2**-(thiol anilino) - 1, 4-naphthoquinone(TAN) as dark violet crystals with m.p 220 ^{0} C.

2-[(Para – methyl) anilino]-1, 4-naphthoquinone (PAN).

IR(KBr)(cm-1):
3325(NH),1678(C4=O),1639(C1=O),1560C-N);1HNMR (CDCl3) (δ) 8.12(d, 2H ,H5,H8),7.75(t, 1H,H7),7.54(t ,1H,H6),7.54(sb,1H NH)7.3(t,2H H15), 7.10(d, 2H, H16,H12) 6.42(S,1H,H3); 13CNMR 183 (C4) 182(C1) 144(C2) 139(C11) 129(C4) 21(CH3);)264 Anal Calcd for C17H13O2N: C, 77.56; H, 4.94; N, 5.32. Found: C, 77.28; H, 5.10; N, 5.28.

2-(Thiol anilino) - 1, 4- naphthoquinone(TAN)

IR(KBr)3325,3261(NH),1676(C4=O),1636(C1=O),720(C-S); ^{1}HNMR 8.12(d 2H H5,H8), 7.75(t, 1H, H7) ,7.64(t ,1H, H6),6.42(s,1H,H3),7.02(d, 2H,H16,H13) ,7.30(t, 2H,H15, H14); ^{13}CNMR 183 (C4),182 (C1),135 (C2)134 (C10);MS, m/z(M-1) 280 Anal Calcd for $C_{16}H_{11}NO_2S$: C, 68.32: H, 3.91; N, 4.98; S, 11.38 Found: C, 68.42;H, 382; N, 5.01; S, 11.29.

DPPH Free Radical Scavenging Activity

DPPH radical scavenging activity was studied using the derivatives. For this, a solution of 0.1mM DPPH was prepared. Different concentrations (20-100µg/mL) of the compounds were prepared in methanol. The sample was made up to 0.5mL with methanol. 5mL of DPPH solution was added to each test tube and shaken well. The tubes were kept at (30±1) ^{o} C in dark for 30 min. A control was prepared with 5mL DPPH solution and 0.5mL methanol. Ascorbic acid was used as a reference to antioxidant compounds. Absorbance was measured after 30min at 517nm in a UV-visible spectrophotometer. Percentage of radical scavenging activity was calculated using the formula,

$$\%\ \text{of Radical scavenging activity} = \frac{\text{Absorbance of control - absorbance of sample}}{\text{Absorbance of control}} \times 100$$

DNA Cleavage Studies

Preparation of culture media and DNA isolation of *E.coli* microbial strains were done according to the reported procedure (Sangamesh *et al*., 2011).

Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/ml) were prepared in DMSO. 0.3µg of isolated plasmid DNA pET20b was incubated with 50µM of the derivatives in 50 µM Tris buffer pH 8.0. The reaction mixture was incubated at 37 ºC for 3 hours. After incubation the samples were mixed with gel loading dye and a constant 50V of electricity passed for around 30 min. Then it was analyzed by agarose gel electrophoresis along with untreated control plasmids and photographed to determine the extent of DNA cleavage.

***In Vitro* Cytotoxicity Studies**

In vitro cytotoxicity of derivatives was studied using Dalton's Lymphoma ascites cells. 10mg of derivatives were taken in an eppeandorf vial of capacity 1ml and diluted to six different concentrations with its duplicate and control (50%) using DMSO as a solvent. The tumour cells aspirated from peritoneal cavity of tumour bearing mice were washed thrice with normal saline and the cell viability was checked by trypan blue dye (1%). The cell suspension (1x 10^6 cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made upto 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 hour at 37^0C. After incubation, 0.1 ml trypan blue was added and number of dead cells determined by using haemocytometer.

RESULT AND DISCUSSION

Spectral studies of the extracted lawsone were carried out and the data were consistent with the reported data (Stefen et. al Classic spectroscopy.)The structure of lawsone is as shown in figure. 1

From the IR spectral data of derivatives, hydrogen bonded OH peak of the parent compound (Lawsone) is absent in the derivatives indicating that coupling has taken place at the hydroxyl group. In the derivative, 2-(thiol anilino) 1, 4 naphthoquinone (TAN), the coupling has taken place between OH group of Lawsone and SH group of ortho amino thio phenol which leads to C-S bond formation rather C-N bond formation (Marjit W Singh *et al*., 2007). This is supported by the strong C-S band observed at 721 cm^{-1} .The absorption bands observed at 3240 and 3260 cm^{-1} are due to NH_2 vibrations of TAN while in PAN this is observed at 3325 cm^{-1} which is attributed to the N-H vibration. The C-1 and C-4 carbonyl bands have no remarkable change in frequencies.

UV- visible spectra gave a band in the 250- 271 nm region corresponding to the intense benzene and quinone π-π* electron transitions and the second band observed at 477 nm may be attributed to n-π* transitions of carbonyl group in the quinone ring (Aguilar-Martinez M *et al*., 1999).

In the ^{1}HNMR spectral data of the derivative PAN, the NH_2 signal at 4.76 of the parent para toluidine was absent. The signal observed at 7.503 is attributed to NH proton in the derivative. Hence it is observed that the NH2 signal had suffered a downfield shift to7.503 in the derivative. While in TAN the NH_2 signal observed at 4.12 in ortho amino thiophenol remains in the same position in the derivative. The above spectral data suggest the structure of the compounds as shown in Figure 2 and Figure 3.

Fluorescence Spectra

The fluorescence emission was recorded at the absorption maxima. Optical density (OD) of the samples was adjusted to be equal. The compounds showed interesting fluorescence emission in polar aprotic acetonirile solution. $\lambda_{f\ max}$ for the derivative TAN is observed at 580nm and for PAN, it is observed at 656nm. The alkyl group is an electron donor while the carbonyl is an electron acceptor thus the compound form a donor – acceptor complex upon excitation. The methyl substituted compound (PAN) shows higher intensity than the other (Kamakshi R *et al*., 2010). Thus the compound PAN may be useful as a fluorescent probe for exploring how the stronger non-covalent interactions (hydrogen bonds, donor- acceptor or charge transfer interactions.) behave for bio molecules such as glycoproteins, glycolipids, lectins (Marioara Bem *et al*., 2007).

DPPH Radical Scavenging Activity

The scavenging of the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals were determined by decrease in its absorbance max at 517 nm.

The decrease in absorbance of DPPH radical was caused by antioxidants, because of reaction between antioxidant molecules and radical, which result in the scavenging of the radical by hydrogen donation. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Alkan M *et al*., 2008). Ascorbic acid was used as a reference to antioxidant compounds. The compounds tested with this method exhibited marked DPPH free radical scavenging activity in a concentration-dependent manner. Figure 5 illustrate a decrease in the concentration of DPPH radical due to the scavenging ability of the compound. These results indicate that the derivative (TAN) exhibited more

antioxidant activity than the other. It may be due to the presence of free NH_2 group present in TAN, which enhance the radical scavenging activity by hydrogen donation.

DNA Cleavage Studies

DNA cleavage experiments using pET20b plasmid DNA were performed with these derivatives (PAN and TAN) at 50 μM concentration. On incubation with the plasmid DNA for 3 hrs at 37 ºC, the nuclease activity was enhanced. It is evident from figure.6, TAN exhibited more DNA cleavage property than PAN. This observation suggests that the compound have ability to cleave DNA by non oxidative mechanism. Oxidative cleavage agents require the addition of external agent (e.g. light or H_2O_2) to initiate cleavage and thus limited to in vitro applications. Hydrolytic cleavage does not require co-reactants and therefore, may be more useful in drug design (Reddy *et al*., 2010).

***In Vitro* Cytotoxicity Studies**

The derivatives exhibited DNA cleavage property in plasmid DNA. Further studies in vitro cytotoxic effect in DLA cell line was done by tripan blue exclusion method. The various concentrations of samples were used. For both the derivatives, a decrease in cell count was observed with increase in concentration shown in Figure.7. There was a dose dependent increase in cytotoxic activity for all the concentrations tested. On the basis of above result it can be concluded that these derivatives, TAN and PAN posses significant cytotoxic activity studied by in vitro cells (Kumar S *et al*., 2011).

Figure 1. 2-Hydroxy -1, 4- naphthaquinone

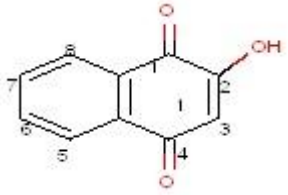


Figure 3. 2- [(Para –methyl) anilino]-1, 4-naphthaquinone (PAN)

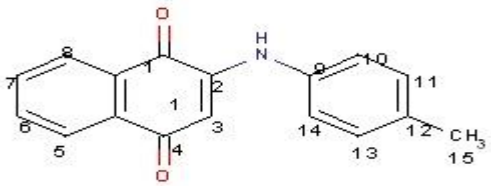


Figure 4. 2-(thiol anilino) 1, 4- naphthoquinone(TAN)

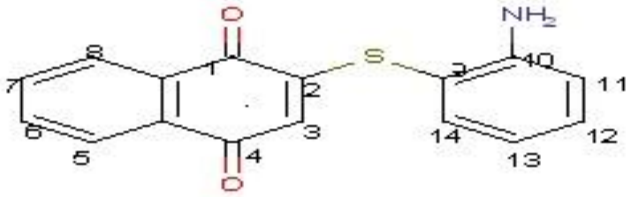
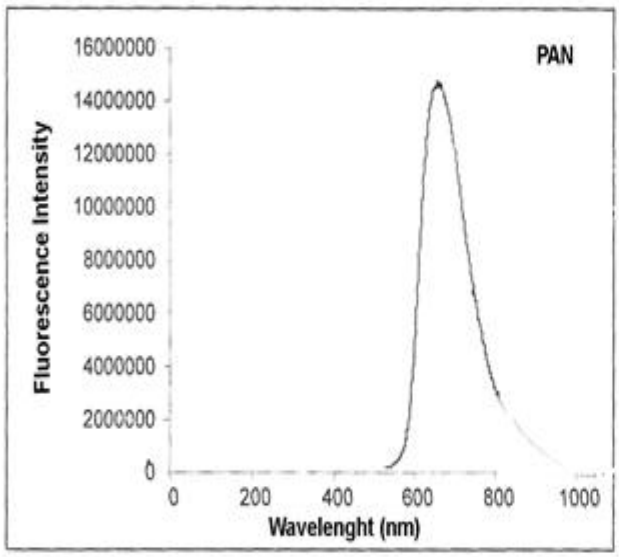


Figure 4. Fluorescence emission spectra of PAN and TAN



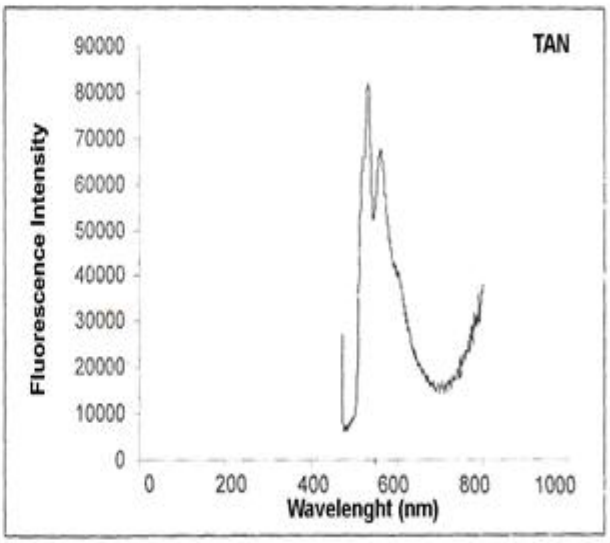


Figure 5. DPPH radical scavenging

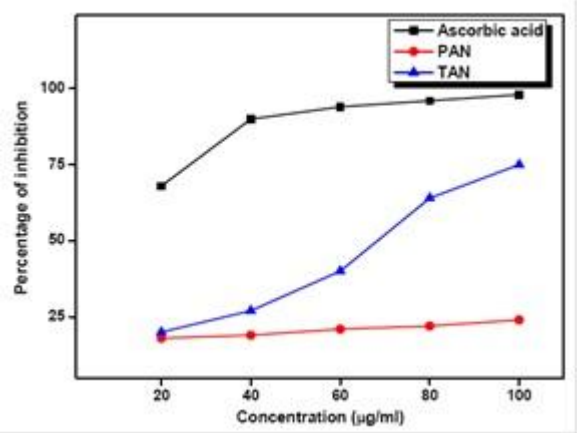


Figure 6. Agarose gel showing result of electrophoresis of pET20b plasmid DNA,lane 1: DNA control ,lane 2: DNA+ PAN, lane 3: DNA+TAN)

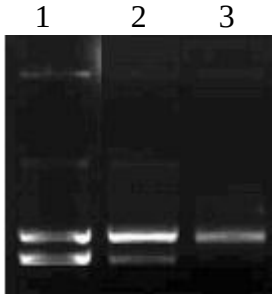
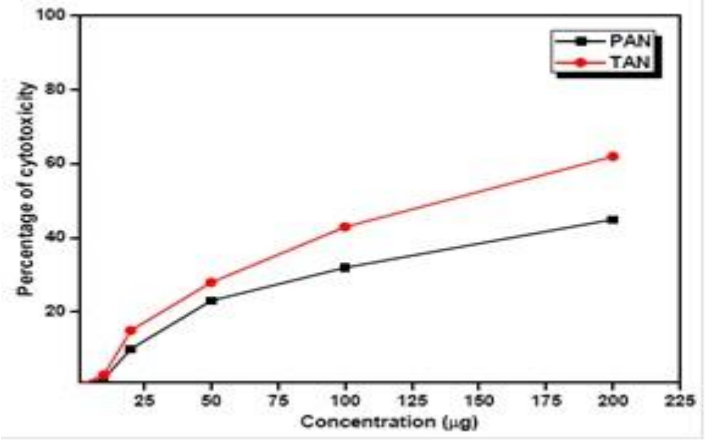


Figure 7. Cytotoxic activity of TAN and PAN using DLA cell lines



CONCLUSION

In the present study, the amino derivatives of lawsone were synthesized by ultrasound irradiation technique and they exhibited interesting fluorescence emission and hence they may be used as fluorescent probes. The DNA cleavage, cytotoxic and antioxidant activities were studied and these derivatives demonstrated good activities.

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REFERENCE

Aguilar-Martinez M, Cuevas G, Jimenez-Estrada M, Gonzalez I, Lotina Hennsen B, Macias-Ruvalcaba N. An Experimental and Theoretical Study of the Substituent Effects on the Redox Properties of 2-[(R-phenyl) amine]-1, 4-naphthalenediones in Acetonitrile. *J.Org Chem*, 64, 1999, 3684-3694.

Alkan M, Yuksek H, Özlem Gürsoy-Kol , Calapoğlu M. Synthesis, Acidity and Antioxidant Properties of Some Novel 3,4-disubstituted-4,5-dihydro-1*H*-1,2,4 triazol-5-one Derivatives. *Molecules*, 13, 2008, 107-121

Ashnagar A, Shiri A. Isolation and characterization of 2-hydroxy, naphthaquinone, lawsone from the powdered leaves of henna plant marked in Ahwaz city of Iran. *International journal of chem. Tech research*, 3(4) , 2011, 1941-1944.

Berger S, Sicker D. Classic Spectroscopy Isolation and structure elucidation of natural products, Wiley –vch, 190.

Dabiri M, Tisseh ZN, Bazgir A . Synthesis of fluorescent hydroxyl naphthalene-1,4-dione derivatives by a three-component reaction in wate. *Dyes and Pigments*, 89, 2011, 63-69.

Devasagayam TPA, Tilak JC, Boloor KK. Review: Free radical and antioxidants in human health. *Curr Stat Fut Pros JAPI*, 53, 2004, 794-804.

Devi KS, Mohanan PV. Cytotoxicpotential of the preparation from Solanum trilobatu on tumour reduction in mice. *Cancer.lett*, 110(1-2), 1996, 71-76.

Franco C FJ, Jordão AK, Ferreira VF, Pinto AC, Maria de Souza CBV, Jackson ALC. Resende**,** Cunha AC. Synthesis of new 2-Aminocarbohydrate -1,4 Naphthaquinone Derivatives Promoted by Ultrasound Irradiation. *J. Braz. Chem. Soc*, 22(1), 2011, 187-193.

Feizi N, Rahul VP, Shridhar PG, Fareed BS, Rajesh G, Rane SY. Crystal structure, NMR and theoretical investigations on 2-(o- hydroxyl-anilino)- 1,4 naphthaquinone. *Journal of Molecular Structure*, 966, 2010, 144-151.

Gokhale N, Padhye S, Newton C, Pritchard R. Hydroxy naphthaquinone metal complexes as antitumor agents. *Metal based drugs*, 13, 2000, 122-127.

Kamakshi R,Swarna S, Latha , Reddy BSR .An efficient synthesis of bio active fluorescent benzylidine tetralones. *Indian Journal of Chemistry*, 49B, 2010, 944-947.

Kumar SB, Bhat IK. *In vitro* cytotoxic activity studies of clitoria ternatea linn flower extracts. *International Journal of Pharmaceutical Sciences Review and Research*, 6**,** 2011, 120-124.

Marioara Bem ,Badea F, Draghici C, Caproiu TM, Vasilescu M, Beterinhe A, Caragheorgheopol A, Maganu M, Constantinescu T, Balaban TA. Synthesis and fluorescent properties of new derivatives of 4-amino-7-nitrobenzofurazan. Arkivoc, 13, 2007, 87-104.

Patai S. The Chemistry of the Quinoid Compounds; 17, Part 1 Wiley: New York 1998.

Reddy PR, Shilpa A. Interaction of DNA with Small molecules: Role of copper histidyl peptide complexes in DNA biniing and hydrolytic cleavage. *Indian Journal of Chemistry*, 49(A), 2010, 1003-1015.

Sangamesh AP, Naik VH, Ajakumar DK, Badami SP. DNA cleavage antimicrobial, spectroscopic and fluorescence studies of Co(II), Ni(II) and complexes with SNO donor coumarin Schiff base. *Spectrochimica Acta Part A molecular and bio molecular spectroscopy*, 2010, 75, 347-354.

Singh WM, Kurmakar A, Nilotpal Barooah Baruah BJ. Variation in product in reaction of naphthaquinone with primary amines, *Beilstein J of organic Chem*., 3(10), 2007, 1-6.

Thomson RH. Naturally occurring quinone, IV Blackie Academic and Professional Landon, 1997.

Varghese JK, Silvipriya KS, Resmi S, Jolly CI. Lawsonia Inermis (Henna): A natural dye of various therapeutic uses-A review. *Inventi Rapid: Cosmeceuticals*, 1, 2010.