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STUDY ON AZO-ALDEHYDE. PART-IX: SYNTHESIS, CHARACTERIZATION,LIQUID CHROMATOGRAPHY AND BIOLOGICAL SCREENING OF AZOSALICYLALDEHYDE FROM BROMO AND CHLORO-ANILINES

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ABSTRACT

Azo salicylaldehyde (**I-II**) were prepared by reaction of Salicylaldehyde with diazonium salt of variedly substituted aniline by diazo coupling route. These were characterized by colour, physical constant, TLC, Schiff reagent test, UV-Vis, FTIR and HPLC. A simple, fast and accurate method has been developed and reported for the identification of azo aldehydes by chromatographic technique, High-performance liquid chromatography (HPLC). The synthesized Azo-aldehydes were screened for biological properties.

KEYWORDS: Azo Aldehydes, Spectral method, Schiff reagent test, HPLC and Biological Activity.

INTRODUCTION

The phenolic compounds showed reaction like diazotization and hence, due to variety applications of the azo compounds, it is interesting to study the synthesis of such azo phenolic derivatives. The extensive literature study shows the scope for study on their derivatives in order to explore the newer potentials of similar compounds. In the literature few times these azo compounds are many often described as the chromogen.[1] The amino-[2] and hydroxyl-[2-6] are the most common functional group of organic compounds used as coupling agents.[7] A review on the azo compounds of amino benzothiazoles and salicylic

acid with aromatic or heteroaromatic mofits and its biological activities has been reported[8-10] in the literature. Very recently an azo group containing Beta-Lactams[11] can be synthesized from azo aldehyde, to Schiff bases[12] as reported. Recently, from our lab, synthesis of azo-azomethine was synthesized from azo-aldehydes.[13-14]

While surveying the literature, it is marked that there are many studies on thesynthesis but its analytical (limited to spectral and elemental) studies were less attended or very scare studies reported on the advanced analytical methods like HPLC, AAS, GC, electrochemistry and TGA.

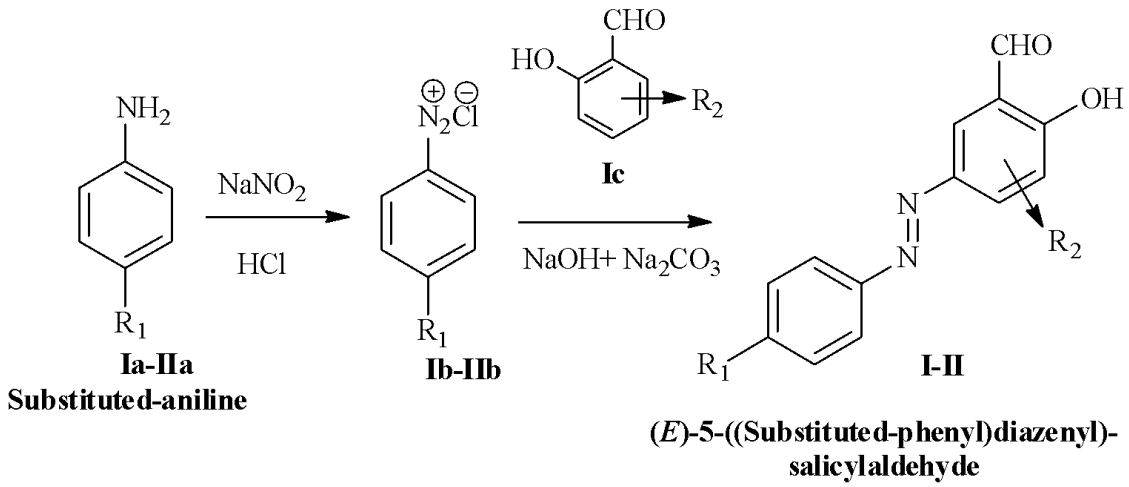
As per the literature survey[15-18] with respect to azo-aldehyde synthesis we have proposed following work and depicted in **Scheme-I**. Here, we proposed the synthesis as well as their advance analytical method like HPLC thought to undertake this initiative and studied the HPLC method development parameters in detail as per ICH guidelines.[17] Such methodology would help to research and developmental activities in exploring further, the estimation of azo aldehydes by HPLC method. Also, these synthesized compounds were screened for the antifungal potential evaluation against two fungal strains.

Scheme of the Present Work

The following **Scheme-I** indicates the one step involved for the synthesis of particular Azo-aldehyde in the present work.

Aniline to Azo-aldehyde,

(E)-5-[(Substituted-phenyl)diazenyl]-2-hydroxy-benzaldehyde



Ia-IIa
Substituted-aniline

Ib-IIb

I-II

(*E*)-5-((Substituted-phenyl)diazenyl)-salicylaldehyde

Where, R_1 = Br-, R_2 = H; R_1 = Cl-, R_2 = H;

Scheme I: The reaction involved in the synthesis of Azo-aldehydes, **I-II**.

EXPERIMENTAL

A. MATERIAL AND METHODS

All the reagents and solvents used are of synthesis grade and were purchased from Sigma Aldrich and Merck. Solvents were of synthesis and analytical grade.

B. SYNTHESIS

a) Synthesis of Substituted-azo-salicylaldehyde

The diazonium chloride from substituted aniline was synthesized and reacted with substituted-salicylaldehyde as per the following procedure.[13]

a) Preparation of diazonium salt

In 100 ml beaker charged 0.035 mole substituted-aniline, **Ia** further added to it 40 ml concentrated HCl and 15 ml distilled water, and the solution is cooled up to 0°C by keeping it in the ice-salt bath (**Solution-X**).

In a 250 ml RB flask charged, 0.045 mole of Sodium nitrite and dissolved 15 ml water and the solution cooled up to 0 ^{0}C by keeping it in ice-salt bath (**Solution-Y**).

When both the solutions attained 0 ^{0}C temperature, solution-Y is added dropwisely in solution-X with constant stirring over one hour. During addition temperature is maintainedup to 5 ^{0}C. The diazotize solution was tested using starch iodide paper, which turns to blue colour. Pinch of solid urea was added to decompose the excess of Nitrous acid. Filter the solution through cotton to get clear diazonium salt and is used for the next step of synthesis.

b) Synthesis of Azo-aldehyde (I-II)

In 250 ml capacity beaker prepare a solution of 0.123 mol, Na_2CO_3 and 0.034 mol NaOH in 125 ml distilled water(if required add excess of Na_2CO_3 and NaOH). In the clear solution charge Substituted-salicylaldehyde, **Ic** 0.025 mol. The solution is cooled the up to0 ^{0}C by keeping it in ice-salt bath. Above filtered diazonium solution is added slowly to this content with vigorous stirring and during addition temperature was maintained up to 5°C. After complete addition, allowed the charged mass to stand for 75 minutes in ice bath.

Filtered the dye, washed with cold water with pH check to neutral, dried, recrystallize the crude product, weighed and stored in an air tight container till to get a constant weight. It is assigned as **I**. This synthesis is in accordance to the modification in reported procedure.[13] The derivative, **II** also prepared using similar procedure by changing the initial aniline.

C. CHARACTERIZATION

The characterization of Azo-aldehyde derivatives was done by primary method like TLC and colour and instrumental methods viz. UV-Vis, FT-IR and by usual HPLC Characterization.

I. Primary Characterization

Colour by visual method; Nature solid or liquid; Physical Constant (m.p.) by instrument recorded on Equiptronics digital m.p/ b.p. apparatus in degree calculus on model EQ-730 and TLC using TLC plates of silica gel coated aluminum plates made by Merck. The progress of reaction was monitored by TLC method.

a) Schiff's reagent Colour Test for the Azo-aldehyde

Schiff's reagent is prepared by using 0.2 gm of Rosaniline hydrochloride and 100 ml water is mixed and this solution is decolourized by aqueous SO_2 or H_2SO_3. Take powdered compound, in a test tube and add 2 ml of above prepared Schiff's reagent, shake well and observe the colour on standing.

II. Secondary Characterization (Instrumental)

The UV-Vis spectra were recorded on Schmadzu UV 1800 series spectrophotometer in the range of wavelength 800-200 nm. FT-IR spectra were recorded on (KBr pellets) FT-IR spectrometer (Shimadzu, 4000-600 cm^{-1}). The instrumental work viz. analytical HPLC work was performed at a public testing laboratory.

i) HPLC Characterization

A simple, fast and cost-conscious method has been developed for the determination of all below compounds by HPLC. The analysis was carried out on Agilent 1100 separationmodule HPLC system with wavelength (UV-Vis) Absorbance detector. The column used was Agilant make C-18 Eclipse stainless steel column of dimension 250 mm x 4.6 mm, packed with octadecylsilane bonded to porous silica. The detector used was UV-Vis and Photodiode detector.

ii) HPLC Method Development

In the present competitive world, the cost and time are very important points in the Good Manufacturing Practices.[16] The objective of method development[17] is user friendly approach quality aspects are not compromised. Use of buffer powders is avoided because it affects the column life. Many different methods were also tried but amongst that the present

method is selected for the analysis as it is fast, more simple and accurate. The sample is freely soluble in water: methanol (50:50). The purity of peak is confirmed with Agilant system which proves that there is no co-elusion except the raw materials (amine) used.

iii) Reagents and chemicals

Methanol HPLC grade : Merck Ltd.
Acetonitrile HPLC grade : Merck Ltd.
Orthophosporic acid HPLC grade : Merck Ltd.
Puirified Water : Double distilled

iv) Preparation of Buffer solution

Transfer 500 ml of purified water to 1000 ml volumetric flask, add 0.1 ml of pure o-phosphoric acid in it with constant stirring, diluted upto the mark with purified water.
Mix and continue the stirring (pH to 3.00 ± 0.05).

v) Preparation of Mobile phase

A mixture of 38 volumes of Methanol, 12 volume of acetonitrile and 50 volume of buffer solution (prepared as above). Filter through 0.45 µm filter paper and degased for 45 min.

vi) Preparation of Test Solution

Weigh accurately about 100 mg of azo aldehyde, **I** / **II** transfer into a 100 ml volumetric flask, added sufficient amount of diluent, sonicate to dissolve, allow it cool at room temperature and diluted upto the mark with the same diluent. Further dilute 10 ml of this solution to 100 ml with diluent. Filter through Millipore Millex-HN Nylon 0.45 µm syringe filter.

a) Instruments used

Agilant 1100 separation module HPLC system was used with UV-Vis wavelength Absorbance detector (DAD) with autosampler and quaternary gradient system.

b) Column used

Agilant make C-18 Eclipse 250 mm x 4.6 mm (Batch number 990967-902, Sr. No. USN H061662)

c) Conditions or Method: The method details are as follows.

Detector, Wavelength **:** 254 nm,

Column oven temperature: Not used

Mobile phase **:** Methanol: ACN: Water (38:12:50 proportions in volume).

Flow rate **:** 1.0 ml/min

Diluent **:** Ethanol

Injection volume **:** 20 µL

Run time of each sample **:** 20 min.

d) Procedure

Sequence of injections

Sequence I: 1) Blank, to check baseline

Sequence II: 1) Sample **I** and **II**.

Inject separately equal volumes (20 µl) of the Blank solution (i.e. Diluent) and desired sample solutions of **I** and **II** into the chromatograph. Record the chromatograms & measure the responses for the major peaks. Record and print the output result.

D. Evaluation of Biological Activity

These synthesized Azo-aldehydes were screened for the antifungal potential evaluation against two fungal strains viz. *A. flavus (Aspergillus flavus) and C. albicans (Candida albicans).*

E. RESULTS AND DISCUSSION

In the present work, substituted-phenyldiazonium chloride reacted with Salicylaldehyde or substituted salicylaldehyde to obtain Azo-salicylaldehyde, as per the **Scheme 1**.

Schiff's Colour Test for the Azo-aldehyde

Schiff's reagent test, indicates, development of pink colour solution on standing. This confirms the presence of –CHO group in the newly synthesized compound **I** (**Fig. 1**).

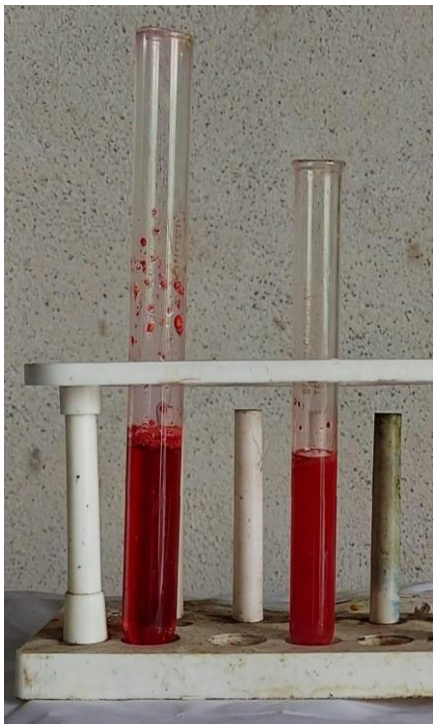


Fig. 1: Colour test – Schiff's Reagent test for the synthesized azo-aldehyde respectively (I and II).

The physical and analytical data such as physical constant viz. m.p. range, colour and yield, for the synthesized Azo Salicylaldehyde and Azo substituted Salicylaldehyde …

The C, H and N data for azo-aldehyde, **I- II** is depicted in **Table 1**.

Table 1: The elemental analysis (C,H,N) data for varied Azo-aldehyde (I-II) (Scheme-I).

Code	R_2-	Calc.(Obs.)		
		% C	% H	% N
I	H-	51.17 (51.13)	2.97 (3.00)	9.18 (9.16)
II	H-	59.90 (59.85)	3.48 (3.47)	10.75 (10.71)

The results in **Table 1**, indicated satisfactory CHN analysis, as per the records previous records.[4,5,11,15]

The Physical and Analytical data of azo-aldehyde, **I - II** is given in **Table 2**.

Table 2: The Physical and Analytical data for Substituted-azo-aldehyde, I-II(Scheme-I).

Code	Compound.	Mol.Wt. (g/mol)	Colour	m.p. ^oC	*R_f value	% Yield
I	$C_{13}BrH_9N_2O_2$	303.98	GoldenYellow	170-176	0.70	80.69
II	$C_{13}H_9ClN_2O_2$	260.04	Yellow	130-135	0.42	89.03

*Ethyl Acetate: n-Hexane 2.5:0.5

The UV-Vis and FTIR spectral data for azo-aldehyde, **I-II**, is given in **Table 3**, UV spectra of **I-II** is shown in **Fig. 2** and the FTIR spectra of **I-II,** is shown in **Fig. 3 and Fig. 4** respectively.

The UV-Vis and FT-IR spectral data of Azo salicylaldehyde (**I-II**) in **Table 3**.

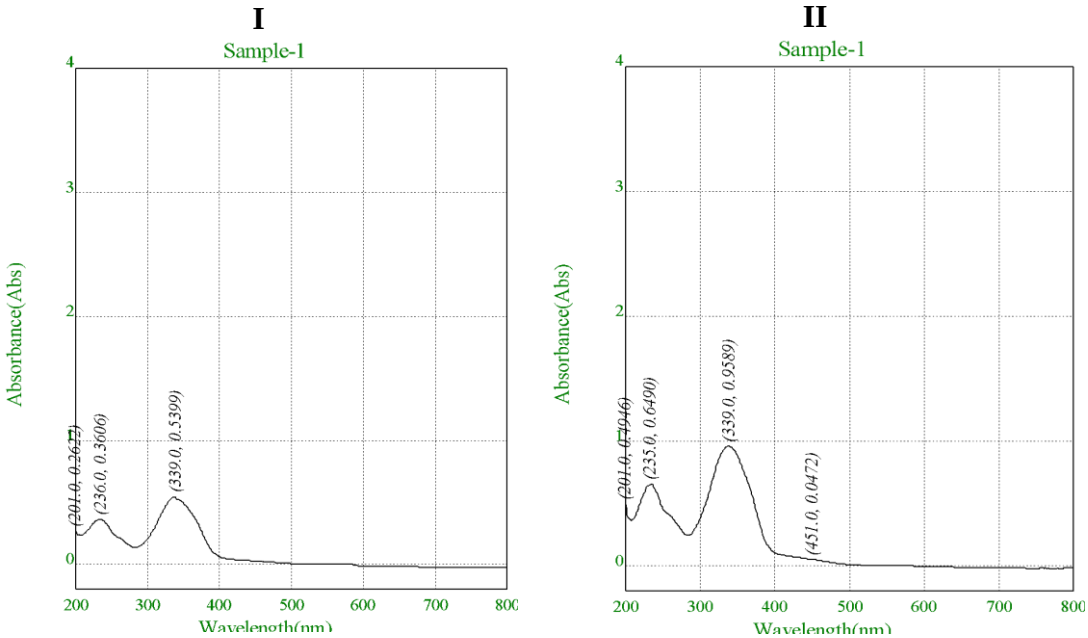


Fig. 2: UV-Vis spectrum of the Substituted-azo-aldehyde, I-II.

The UV-Vis spectra of Azo-aldehyde, **I-II,** in ethanol shows 3 bands at about 339.0, 235.0-236.0 and 201.0 nm. The excitation at 339.0 nm shows n→π* transition, 235.0 nm and 201.0 nm arising due to π→π* transition, as shown in **Fig. 2** and the data is depicted in **Table 2.** These UV-Vis observations are coinciding with the observations with earlier reports.[12-14]

Table 3: The UV-Vis and FT-IR spectral data for Substituted Azo-aldehyde, I-II.

Code	UV-Vis, λ_{max}, (nm)	FT-IR data (cm^{-1})			
I	339.0 236.0 201.0	3410 1664 1461 831	OH >C=O -N=N- -C-Br	2880 1567 1064	H>C=O ->C=C<(ar) -C-O-C
II	451.0w 339.0 235.0 201.0	3232 1657 1478 723	OH >C=O -N=N- -C-Cl	2870 1578 1087	H>C=O ->C=C<(ar) -C-O-C

(w = weak)

The FTIR spectra of Azo-aldehyde, **I-II,** are depicted in **Fig. 3** and **Fig. 4,** respectively.

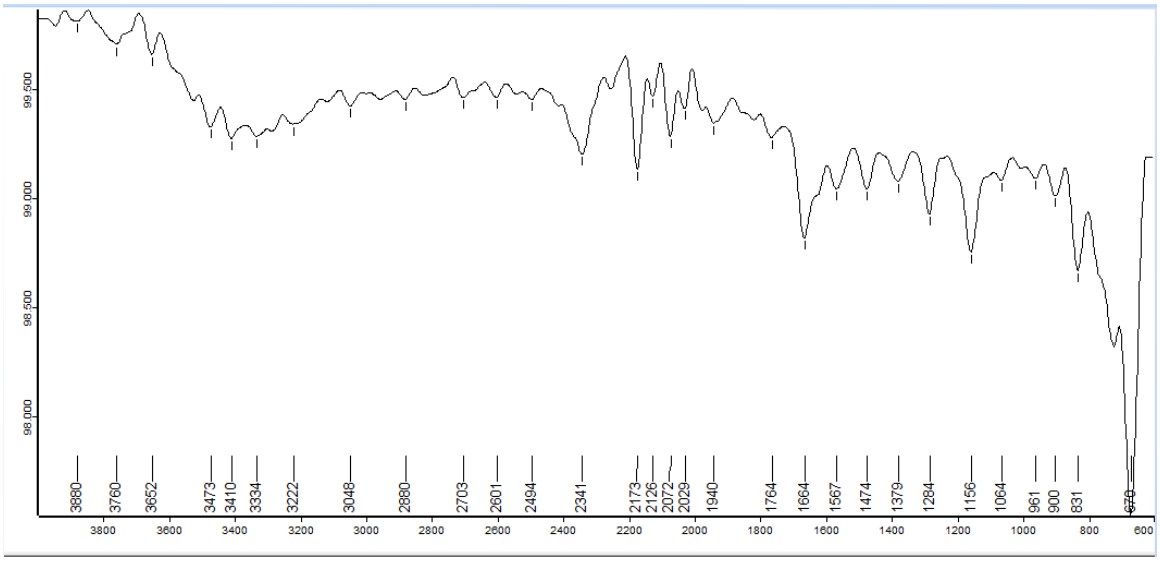


Fig. 3: FTIR spectrum for Substituted-azo-aldehyde, I.

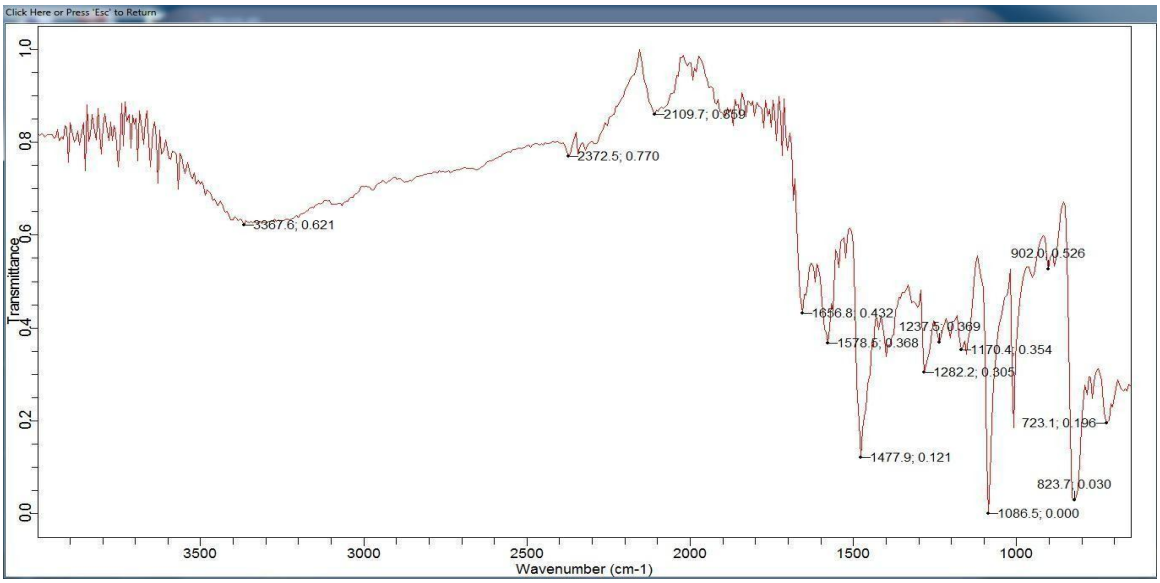
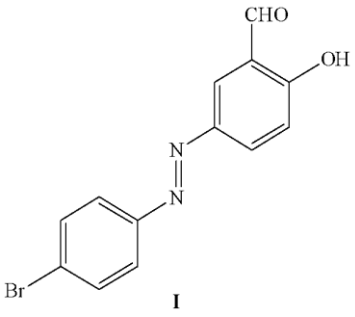


Fig. 4: FTIR spectrum for Substituted-azo-aldehyde, II.

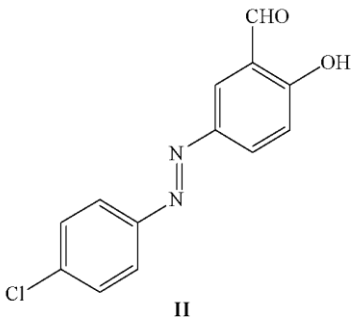
The FTIR spectra of Azo-aldehyde, **I-II,** showed the observations as 1567 and 1578 cm^{-1} are >C=C< in aromatic ring, 1664 and 1657 cm^{-1} are >C=O stretching for aldehyde, 2880 and 2870 cm^{-1} are >CH=O stretching for aldehyde, 3410 and 3232 cm^{-1} are – OH stretching, 1064 and 1087 cm^{-1} for $_{-C-O-C}$ stretching, whereas 831 cm^{-1} $_{>C-Br}$ and 723 cm^{-1} $_{>C-Cl}$ for bromo and chloro substituents, on aromatic ring. Similar observations were reported previously.[12-14]

From the results of primary observations, based on the reactants and method used in the reaction and the spectral data of UV-Vis and FTIR one arrives at the following structures of the compounds.



I

(*E*)-5-((4-Bromo-phenyl)diazenyl) -2-hydroxybenzaldehyde



II

(*E*)-5-((4-Chloro-phenyl)diazenyl) -2-hydroxybenzaldehyde

HPLC Characterization

The results of the HPLC analysis are depicted in **Table 4**. The results are explained on the basis of the spectral purity of the peak.

Table 4: The observed Peak Purity (% assay) determined by HPLC.

Compound code	HPLC Peak Purity for Anilines used for synthesis of Azo-aldehyde	HPLC Peak Purity for Azo-aldehyde	Remark
I	99.02	95.03	Peak spectrally pure
II	98.26	96.01	Peak spectrally pure

The method of analysis used for Azo-aldehyde, **I-II** (in % w/w) on as is basis by using High performance liquid chromatography method is developed here in this work.

The representative HPLC chromatogram indicating purity of amines used for the synthesis of azo-aldehyde are depicted in the HPLC **Fig. 5** (blank), **Fig. 6** (HPLC Graph - Azo aldehyde**)**, respectively.

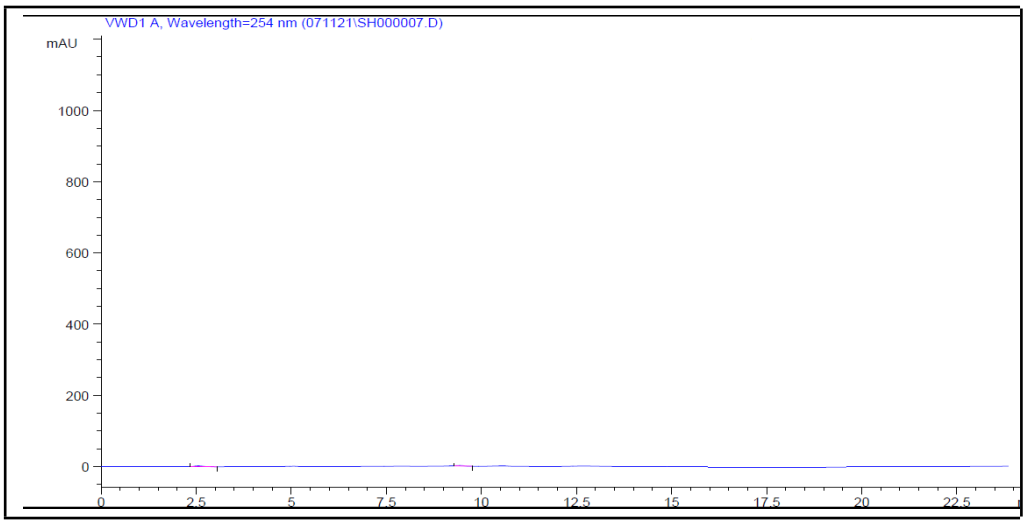


Fig. 5: Chromatogram for Blank.

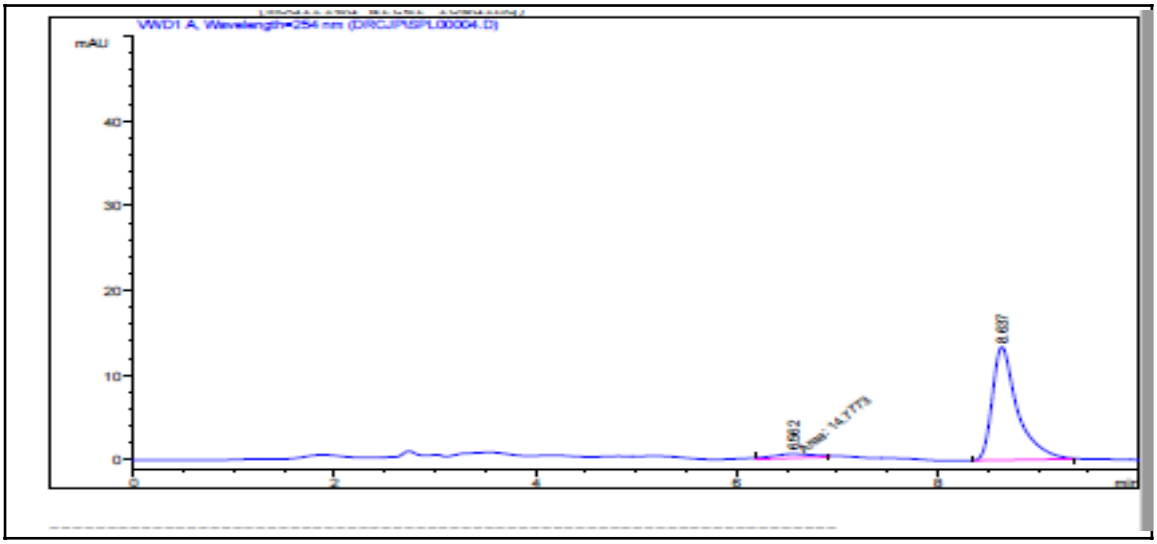


Fig. 6: Representative Liquid Chromatogram for Azo aldehyde, I.

Fig. 5, depicts the blank HPLC graph as obtained on running the blank mobile phase without the sample. **Fig. 6** indicates the chromatogram of the Azo-aldehyde derivatives (e.g. **I**). Thus, the peaks are spectrally pure (99.02 %).

Antifungal Activity

The synthesized Azo-aldehyde compounds were screened for the antifungal potential evaluation against two fungal strains viz. viz. *A. flavus and C. albicans* and compaired with standard drug, Fluconazole, after 48 hrs.

Table 5: The Antifungal Activity data for Azo aldehyde (I – II) (Scheme-1).

Antifungal Activity		
Compound Code andConcentration	***A. flavus***	***C. albicans***
	Zone of Inhibition After 48 hrs. of Incubation	
I (1000 µg/ml)	21	16
II (1000 µg/ml)	22	22
Fluconazole (1000 µg/ml)	22	19
+ ve control	+ ve	+ ve
(Distilled water)		

Glimpses of the Antifungal Activity of Azo aldehyde

- All the azo-aldehydes showed positive activity at 1000 mg/ml against *A. flavus and C. albicans.*
- All the azo-aldehyde **I**, showed higher activity against *A. flavus* than *C.albicans* at 1000 mg/ml.
- In this study highest activity is depicted by **I** against *A. flavus and* **II** against *C.albicans*

at (1000 mg/ml).

- The azo-aldehyde **II**, showed higher activity than the standard drug Fluconazoleat studied concentration (1000 mg/ml).

These antifungal activities are represented in the histogram form as in **Fig. 7**.

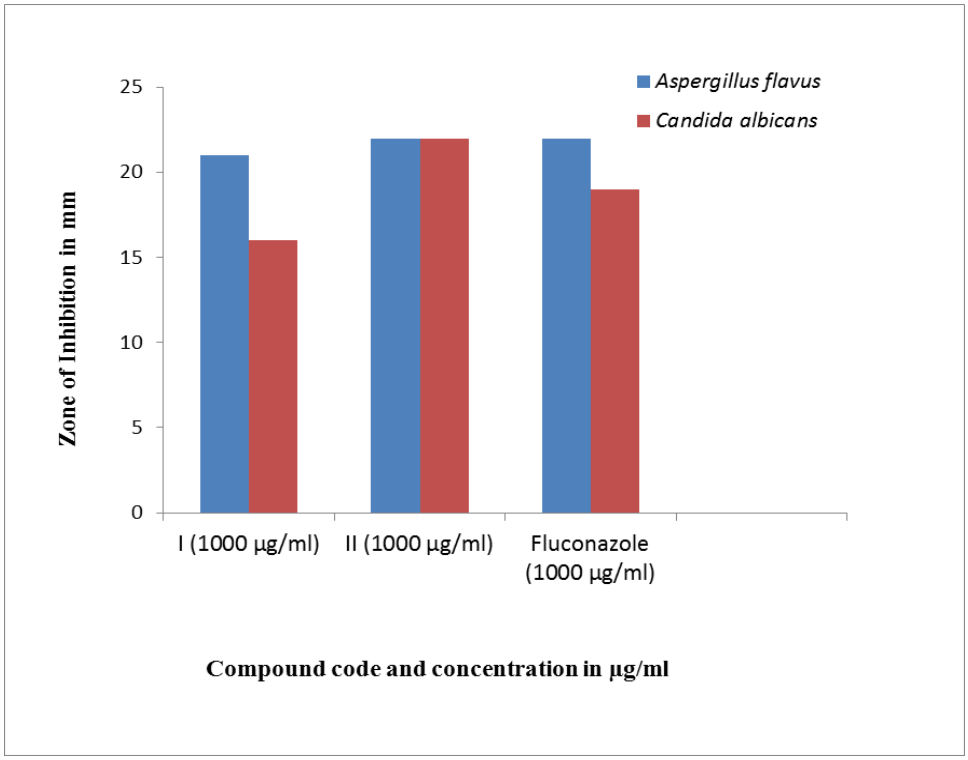


Fig. 7: The antifungal activities of the azo-aldehyde, (I to II) against *A. flavus and C. albicans* in the graphical form.

ABBREVIATIONS

UV-Vis : Ultra Violet Visible

FTIR : Furirer Transform Infra Red

HPLC : High performance liquid chromatography

CONCLUSION

The present scientific article gives an overview or detailed account of synthesis of azo-aldehyde and its usefulness in reaction forming organic compounds. The compounds synthesized from the azo-aldehyde are useful for many applications such as colours of azo-dyes include different shades of yellow, red, orange, brown, and blue used for textile, dying industry, for the chelation ion-exchanging properties of the polymers. These types of compounds have been used as a building block for many intermediates. Few compounds **I**

against *A. flavus and* **II** against *C. albicans* also which is respectively similar and more than the standard drug used (at 1000 mg/ml).

The analytical method used for analysis of Azo-aldehyde (**I, II**), HPLC analysis data is useful for the estimation of metals in the solution of say waste samples using HPLC analysis of ligands as seen in the scientific literature.

FUTURE SCOPE

Thus, this scientific output may be useful to many researchers for further developments of varied compounds in particular Azo-aldehyde and Azo-schiff bases and their varied reactions to form different applied derivatives in near future. HPLC analysis method and data made available will be useful to the analytical as well as organic chemist to identify the molecule, for the estimation of various metals. Further, these compounds canbe screened for the antibacterial activity.

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