Lemon Juice as a Natural Catalyse for Synthesis of Shiff’s base: A Green Chemistry Approach

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Abstract—The advantages of the use of natural catalysts are eco-friendly, inexpensive, high yields, nonhazardous and short reaction times. The utilization of green chemistry techniques is the elimination of solvents during chemical processes or the replacement of hazardous solvents with environmentally friendly solvents. Green chemistry has used for the synthesis of Schiff bases. Synthesis of Schiff base is carried out the mixture of aldehyde (or ketone) and amine in organic medium with or without an acid catalyst. He presents study Uses Lemon juice as a natural and eco-friendly catalyst in the green chemistry investigated. The synthesized product was characterized by its physical properties, melting point, TLC and then subjected to the in vitro antibacterial activities against gram-positive and gram-negative strains of microbes.

Keywords—Antibacterial activity, Green chemistry, Lemon juice, Shiff’s base

I. INTRODUCTION

Green chemistry is defined as environmentally benign chemical synthesis. The synthetic schemes are designed in such a way that there is least pollution to the environment [1]. Green chemistry is focused on the designing of products and processes that minimize the use and generation of hazardous substances. Green chemistry is focused on technological approaches to preventing pollution and reducing consumption of non-renewable resources [2,3,4,5,6].

Green solvents, are normally derived from renewable resources and biodegrade to harmless, often naturally occurring product. [7,8]

Recently fruit juice is used in organic solvents for the synthesis of compounds of pharmaceutical interest. [9]. Fruit juices are inimitable solvent because they are readily available, inexpensive, nontoxic, safer, and environmentally benign. Lemon juice is a green alternative to hazardous solvents and natural catalyst for synthesis of Schiff bases. [10,11] A Schiff base is a compound with the general structure R2C=NR’ (R’ ≠ H) [12]. The formation of carbon-nitrogen double bond plays important role in organic synthesis. Schiff bases can be synthesized from an aliphatic or aromatic amine and a carbonyl compound [13,14]. Schiff bases are known as organic chemicals due to significant biological activity such as anticancer [15], antitumor [16], anti-inflammatory agents [17], antibacterial [18], antibiotics [19], antimicrobial [20], anticonvulsant activity [21].

II. EXPERIMENTAL

2.1 Material and Methods

All chemical purchased from Merck and Aldrich Company and used without further purification. The IR spectra were taken with a Shimadzu 300 spectrometer using potassium bromide pellets. 1HNMR (nuclear magnetic resonance) spectra of ligand were recorded on a Bruker AMX 250 MHz spectrometer in the DMSO-d6 solvent using tetramethyl silan as an internal reference. Melting points of compounds were measured with an electro thermal melting point apparatus and were not corrected. The molar conductance of the complexes in DMSO (1×10⁻³ M solution) was performed at 25 °C using Oakton ECTestr 11 dual-range, conductivity tester. The progress of the reactions was monitored by thin-layer chromatography (TLC) on silica gel Polygram precoated TLC sheets.

2.2 Preparation of catalyst

Fresh lemon was taken and washed it thoroughly with Water and cut by using a knife and then pieces were pressed manually. Then the juice was filtered through cotton to remove solid material and to get clear juice which was used as a catalyst.

2.3 General procedure for synthesis of pyrimidine compounds

A mixture of the selected aldehyde (0.1mmol) and 4,6-diamino 2-thiol pyrimidine (0.1 mmol) and catalyst juice (lemon juice) (10ml) were added and stirred at 55 °C for the appropriate time. The progress of the reaction was monitored by TLC. The product was dried and recrystallized from hot alcohol to obtain the pure product. The product was characterized by melting point, 1H NMR, IR.

Fig. 1: Synthesis of pyrimidine compounds
2.4 synthesis of compound and analytical and spectral data of products

2.4.1 4-amino-6-(benzylideneamino)-2-thiol – pyrimidine

(3a)

White solide. Yield 75%, mp 240-242°C.

FTIR (vmax, KBr): 3436 (NH$_2$), 3392 (CH$_{aromatic}$), 2170 (SH), 1663 (C=N), 1585 (C=C), 1313 (C-N) cm$^{-1}$

1HNMR: (DMSO): $\delta$=5.84 ppm (s,1H, H pyrimidine), 6.70 ppm (s,2H, NH$_2$), 7.58-7.72 ppm (m,3H, Haromatic), 7.89-7.91 ppm (d of d,2H, Haromatic), 8.30 ppm (s,1H, C=NH), 12.71 ppm (s,1H, SH), 13CNMR (DMSO): $\delta$=97.90, 128.80, 129.33, 132.67, 137.20, 158.85, 167.02, 181.74, 184.82 ppm

4-amino-6-(benzylideneamino)-2-thiol - pyrimidine

4-amino-6-(2-hydroxybenzylidene) amino -2-thiol – pyrimidine

Brown solid. Yield 80%, mp 226-228°C.

FTIR (vmax, KBr): 3435 (OH), 3392 (NH$_2$), 3259 (CH$_{aromatic}$), 2715 (SH), 1665 (C=N), 1423 (C=C), 1312 (C-N) cm$^{-1}$

1HNMR: (DMSO): $\delta$=5.84 ppm (s,1H, H pyrimidine), 6.79 ppm (s,1H, NH$_2$), 7.47-7.58 ppm (t,1H, Haromatic), 7.90-7.99 ppm (d,1H, Haromatic), 8.11-8.14 ppm (d,1H, Haromatic), 8.37 ppm (s,1H, Haromatic), 8.92 ppm (s,1H, OH), 12.04 ppm (s,1H, SH).

4-amino-6-(3-nitrobenzylidene) amino -2-thiol – pyrimidine

Orange solide. Yield 75%, mp 168-170°C. FTIR (vmax, KBr): 3333 (NH$_2$), 3172 (Charomatic), 2975 (SH), 1738 (C=C), 1637 (C=N), 1528 (NO$_2$), 1351 (C-N) cm$^{-1}$

1HNMR: (DMSO): $\delta$=5.78 ppm (s,1H, H pyrimidine), 6.50 ppm (m,3H, NH$_2$, H aromatic), 6.82-6.83 ppm (t,1H, H aromatic), 7.09-7.11 ppm (t,1H, H aromatic), 7.40 ppm (d,1H, H aromatic), 8.80 ppm (s,1H, C=NH), 10.98 ppm (s,1H, OH), 11.80 ppm (s,1H, SH).
4-amino-6-(3,4-dimethoxybenzylidene) amino -2-thiol – pyrimidine(3d)
Dark brown solide. Yield 75%, mp 110-112 0C.
FTIR (vmax, KBr):3435 (NH$_2$), 2924(CH$_\text{aromatic}$), 2852 (SH),1730 (C=C), 1609 (C=N),1513 (OCH$_3$),1463 (OCH$_3$), 1258 (C-N) cm$^{-1}$
1HNMR: (DMSO): δ=3.65-3.67ppm(m,6H,2CH$_3$),5.83ppm (s,1H, H pyrimidine) ,6.69ppm (s,2H, NH$_2$) ,6.95-6.97ppm (d,1H, Haromatic),7.20ppm (d,1H, Haromatic), 7.39ppm(s,1H,Haromatic), 8.84ppm (s,1H,C=NH),11.99ppm(s,1H,SH).

4-amino-6-(4-chlorobenzylidene) amino -2-thiol – pyrimidine(3e)
Gray solide. Yield 75%, mp 263-265 0C.
FTIR (vmax, KBr):3402 (NH$_2$) ,3162 (CH$_\text{aromatic}$) ,2996 (SH), 1657(C=N),1555(C=C), 1177(C-N) cm$^{-1}$
1HNMR: (DMSO): δ=5.94 ppm (s,1H, H pyrimidine),6.8 ppm (s,2H, NH$_2$), 7.46-7.48ppm (d,2H, Haromatic),7.82-7.86ppm (d,2H, Haromatic), 9.02ppm (s,1H, C=NH),12.02ppm (s,1H, SH).

2.4 In vitro antibacterial activity
Bacillus subtilisin (ATCC: 6633) and Staphylococcus aureus (ATCC: 6838) as gram-positive bacteria Escherichia coli (ATCC: 25922), Serratia marcescens
(ATCC: 13880) as gram-negative bacteria as well as were used for the test of antibacterial activity of synthesized compounds.

Microorganisms were cultured onto Muller Hinton Agar (MHA) plate and incubated for 18-24 h at 35 °C. The density of bacteria cultures required for the test was adjusted to 0.5 McFarland (1.5 x108 CFU/ml) (CFU = Colony Forming Unit). The antibacterial activity of the synthesized compounds was determined with two methods: minimum inhibitory concentration (MIC) of antibiotic for that bacteria and the disc diffusion methods. The tests were repeated three times to ensure reliability.

2.5.1- Disc diffusion method

The disk diffusion method tests the effectiveness of antibiotics on a specific microorganism. The compounds (0.02 g) were dissolved in 1 mL DMSO. A bacterial culture (which has been adjusted to 0.5 McFarland) was used to lawn Hinton agar plates using a sterile swab. The discs had been impregnated with synthesized compounds were placed on the Muller-Hinton agar surface. Tetracycline and cephradine were used as standards for antibacterial measurements. DMSO showed no activity against any bacterial strains. After incubation for 18 h, the diameter of each zone of inhabitation was measured (mm). the disk diffusion method values are presented in Table 1.

**Table 1: Inhibition zone of Compounds against bacterial strains.**

| Compounds | G (+) | G (-) |
|-----------|-------|-------|
| E.coli | S.marcescen | S.aureus |
| 3a | 13 | 14 |
| 15 | N.A | N.A |
| 3b | N.A | 10 |
| N.A | N.A | 12 |
| 3c | N.A | 15 | 14 |
| 3d | N.A | 12 |
| 13 | N.A | 15 |
| 3e | N.A | 15 |
| 15 | N.A | 15 |
| 3f | N.A | 15 |
| 11 | 14 | 10 |
| Tetracycline | 10 | 21 |
| Polymixin | 9 | 10 |
| DMSO | 0 | 0 |
| 0 | 0 | 0 |

2.5.2- Minimal Inhibitory Concentration (MIC) method

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents the visible growth of a bacterium. MIC is the lowest concentration of the antimicrobial compound, which inhibits the visible growth of a microorganism after overnight incubation. In this method, the various concentrations of synthesized compounds were made from 2000 to 1.95µg/ml in a sterile tube. A 1 ml sterile Muller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 1 ml test compound in tube 1. Two-fold serial dilutions were carried out from all the tubes and excess broth (1ml) was discarded from the last tube. To each tube 0.1 ml of the standard microorganism (1.5 x108 CFU/ml) was added. Turbidity was observed after incubating the inoculated tubes at 35 °C for 24 h. the MIC values are presented in Table 2.

**Table 2: Minimal Inhibitory Concentration, µg/ml of Compounds against bacterial strains.**

| Compounds | G(+) | G(+) | G(-) | G(-) |
|-----------|------|------|------|------|
| E.coli | S.marcescen | S.aureus | E.coli | S.marcescen |
| 3a | 250 | 500 | 1000 | 16.72 |
| 3b | 15.62 | 125 | 60.5 | 15.62 |
| 3c | 15.62 | 125 | 15.62 | 17.50 |
| 3d | 1000 | 500 | 500 | 15.62 |
| 3e | 17.50 | 125 | 1000 | 15.62 |
| 3f | 1000 | 1000 | 125 | 16.72 |

**III. CONCLUSION**

The present study concentrates on the importance of fruit juice in organic transformations with natural and biocatalyst exclusivity. The benefit of fruit juice in organic synthesis is based on acidic properties, enzymatic activity, benign environmental nature, cheap material, and commercial usability. The catalyst based activity is consisting of the benefit of fruit juices in various organic transformations including the formation of C-C, C-N bonds in different synthetically important organic compounds that researched before. We can imagine that in next years the chemistry of natural catalysts will continue to attract remarkable research activity.

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