

## A FACILE AND ENVIRONMENT FRIENDLY SYNTHESIS OF ARYLALDIMINES AND THEIR SIGNIFICANT ANTIMICROBIAL, ANTIOXIDANT AND ENZYME INHIBITION ACTIVITIES.

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### خلاصہ

بینز الڈہائیڈ (sp-1 سے sp-10)، سینٹیم الڈہائیڈ (sp-2 سے sp-15) اور سیلی سائیل الڈہائیڈ (sp-16 سے sp-24) کے شف اساس ماحول دوست طریقہ کار کو استعمال کرتے ہوئے قدرتی تیزاب (لیموں کارس) بطور کیٹالسٹ سوئیٹھ میں تیار کئے گئے۔ ان حاصلات کی ساختی وضاحت کے لیے مختلف سپیکٹرواسکوپک تکنیک جیسے عنصر تجزیہ کاری، انفراریڈ (IR)، الیکٹران اسپیکٹ ماس اسپیکٹرواسکوپ (EIMS) اور پروٹان نیوکلیر میگنیٹک ریزونانس (HNMR) استعمال کی گئی۔ حاصل کردہ پروڈکٹ کی اینٹی بیٹیئرل، اینٹی آکسیڈینٹ اور اینزائم انہیبیشن (ممانعت) سرگرمیوں کو جانچا گیا۔ اینٹی بیٹیئرل سرگرمی کے نتائج کے تحت مرکبات sp-10، sp-12، sp-18 اور sp-18 نے سب سے زیادہ علاقے میں جو 10-35 ملی میٹر تھی مختلف قسم کے گرام مثبت اور منفی بیٹیئر یا میں اثرات دکھائے۔ سب سے زیادہ بہترین اینٹی فنگل سرگرمی مرکب sp-7 اور sp-9 سے sp-12 میں دیکھی گئی جو 11 سے 33 ملی میٹر تھی۔ شف اساس sp-6، sp-7، sp-15 اور sp-21 نے 80 سے 90 فیصد انہیبیشن (ممانعت) کے ساتھ بہترین اینٹی آکسیڈینٹ سرگرمی دکھائی۔ مرکبات sp-2 اور sp-19 نے ٹائیر و سینیز کے خلاف قابل ذکر سرگرمی دکھائی جو IC<sub>50</sub> کے ساتھ بالترتیب 95.2 ± 0.59 اور 97.2 ± 0.55 μM تھی جو کوجک تیزاب (IC<sub>50</sub> = 16.67 ± 0.06) کو بطور معیار موازنہ کے لیے جانچی گئی۔ مرکبات sp-4، sp-7 اور sp-10 نے یوری ایز انہیبیشن کے خلاف سرگرمی دکھائی جو IC<sub>50</sub> کے ساتھ بالترتیب 42.7 ± 0.56، 38.6 ± 0.86 اور 49.3 ± 0.38 μM تھی جو تھائیو یوریا (IC<sub>50</sub> = 21.6 ± 0.12 μM) کو بطور معیار موازنہ کے لیے جانچی گئی۔

### Abstract

Environmental benign method was used to synthesize Schiff bases of benzaldehyde (**sp-1 to sp-10**), cinnamaldehyde (**sp-11 to sp-15**) and salicylaldehyde (**sp-16 to sp-24**) by using natural acid (Lemon extract) as catalyst in sonicator. The structural elucidation of these products were performed by various spectroscopic techniques like elemental analysis, Infrared (IR), Electron impact mass spectroscopy (EIMS) and Nuclear magnetic resonance (<sup>1</sup>H-NMR). Title products were also examined for their antimicrobial, antioxidant and enzymes inhibition activities. The results of antibacterial activity revealed that compounds **sp-10, sp-12 and sp-18** showed highest zone of inhibition ranging between 10-35 mm against both kinds of bacteria gram positive and negative. The most remarkable antifungal activity was observed in compounds **sp-7 and sp-9 to sp-12** with zone of inhibition in the range between 11-33 mm. The Schiff bases **sp-6, sp-7, sp-15 and sp-21** showed excellent antioxidant activity with 80-90% inhibition. In enzyme inhibition assay compounds **sp-2 and sp-19** showed significant activity against tyrosinase showing IC<sub>50</sub> values 95.2 ± 0.59 and 97.2 ± 0.55 μM, respectively as compared to Kojic acid (IC<sub>50</sub> = 16.67 ± 0.06) as standard. The compounds **sp-4, sp-7 and sp-10** appeared active for urease inhibition with IC<sub>50</sub> values 42.7 ± 0.56, 38.6 ± 0.86 and 49.3 ± 0.38 μM against standard thiourea (IC<sub>50</sub> = 21.6 ± 0.12 μM).

### Introduction

Imines are chemical compounds which contain a double bond between carbon and nitrogen having different biological activities like anticancer (Popp, 1961), anti-inflammatory (Hadiipaylou-litina and Geronikaki, 1996), antitumor (Verma *et al.*, 2004), insecticidal (Murthy *et al.*, 1998), antituberculosis (Solak and Rollas, 2006), antibacterial (Venugopala and Jayyashree, 2004), antimicrobial (Wadher *et al.*, 2009) and anticonvulsant (Cates and Rasheed, 1984) activity.

A classical method of these compounds involves the condensation of primary amines with carbonyl compounds (Asiri and Khan, 2010). The combination of organic solvent, mineral acid and prolong reaction time makes this technique unsafe to environment. Schiff bases (**sp-1 to sp-24**) were synthesized by the green method of sonication by using natural acid as a catalyst.

**Grapefruit (*Citrus. paradisi*):** The grapefruit has sour to semi-sweet taste. It is acidic in nature, contain tartaric, malic and citric acid. Its pH is 3.00 - 3.75 (Anon, 1962). It is a big source of vitamin C (Feller *et al.*, 1990) and the pectin fiber is also found in it (Cerda *et al.*, 1988). Its main constituents are sodium 0.001 g, potassium 0.162 g, total fat 0.100 g, carbohydrate 9.00g (3%), protein 0.500 g (1%), vitamin A (8%) vitamin C (63%) iron (1%) magnesium (3%).

**Orange (*Citrus. xsinensis*):** *C. xsinensis* is acidic in nature like all citrus fruits. Its pH is 3.13 - 4.19 (Cerda *et al.*, 1988). It is enriched with vitamin C, A, Thiamine, Niacin and Ascorbic acid. Other acids are malic, oxalic acid but citric is major acid (0.6 – 1.0 wt %).

**Lemon (*Citrus. limon*):** Among fruits, citric acid is most enriched in lemons. Its pH is 2.00 - 2.60 (Cerda *et al.*, 1988). It has 8% of the dehydrated fruit mass. It also contains sugar, gum, and a very little potash. Per ounce of fresh lemon, orange and grape fruit juices contain 1.44, 0.25 and 0.0068 g of citric acid, respectively (Penniston *et al.*, 2008).

In the present work extract obtained from grape fruit, lemon and orange were used as a catalyst. All the synthesized Schiff bases (**sp-1** to **sp-24**) were assessed for their antimicrobial, enzyme inhibition and antioxidant activities.

## Materials and Methods

All the reagents used for the chemical reaction were acquired from Merck Germany. Reaction were examined by thin layer chromatography (TLC) which was accomplished on aluminum sheets of E. Merck, Germany pre-coated with silica F254 gel (Kiesel gel 60). Melting point apparatus, Gallen kamp was used to find the melting point in glass capillary. EIMS were taken on JEOL JMS-600H mass spectrometer. <sup>1</sup>H NMR spectra were noted on Advance AV-200, 300, 400 and 500 MHz in Chloroform (CDCl<sub>3</sub>) and Dimethylsulphoxide (DMSO-d<sub>6</sub>) with respect to Tetra methyl silane(TMS) which was used as an internal standard. JASCO-302-A spectrophotometer was utilized to note IR spectra. For Elemental analysis a Carlo Erba Strumentazione-Mod-1106 (Italy) was used. Elmasonic E-15H 2-Quart Ultrasonic Cleaner with high performance 37kHz transducer system is used for sonication.

**General extraction procedure for the orange, lemon and grape fruit juices:** Fresh orange, lemon and grape fruit squeezed by using presser and suspended solid material was removed through filtration. Clear juice was utilized as a catalyst.

**General method for the synthesis of Schiff base (sp-1 to sp-24):** Preparation of Schiff base **sp-05** is defined as an illustrative example: A mixture of benzaldehyde (11.77m.mol, 1.20mL) and Sulphanilamide (12.07m.mol, 2.08g) and 0.50ml lemon juice were taken in a flask. Ultrasonic bath is used to sonicate the reaction mixture (Guzen *et al.*, 2007) for 12 min. The reaction development was observed by taking TLC. The reaction mixture was sieved and the attained residue was washed through water and dried. The gained product was re-crystallized from alcohol and gave the pure Schiff base **sp-5**. The same procedure was adopted to synthesize Schiff bases with orange and grape fruit (**Scheme-1**).

## Bioassay protocol

### Assortment of pathogens

Microbiology Department, FUUAST, Karachi, Pakistan provided all fungal and bacterial strains.

### Culture preparation

To culture bacterial stains for media, Muller Hinton broth (Oxoid) and Muller Hinton agar (Oxoid) and for fungal strains Sabour dextrose agar (SDA) plates were used.

### Antibacterial activity

Antibacterial activity of the synthetic compounds (sp-1 to sp-24) was determined by disc diffusion method (Bauer *et al.*, 1966). Bacterial pathogens were maintained for 2 h in log phase by using Autoclaved Muller Hinton broth with constant mixing. Pure compounds were dissolved in DMSO to prepare 100mg/mL of stock solution. For screening, sterile filter discs (7 mm in diameter) were used which contain 10 µL of stock solution. 24 h old culture developed in Mueller Hinton broth (Oxoid) was utilized to seed the Mueller Hinton agar (Oxoid) plates. The ready discs were attuned on to the surfaces at numerous locations and plates were placed for incubation at 37 °C for 24 h. Outcomes were noted by finding the inhibition zone in mm. Negative control was DMSO and gentamycin was utilized as positive control.

**Antifungal activity**

Antifungal activity of synthetic products (sp-1 to sp-24) was also obtained by disc diffusion strategy as defined previously (Bauer *et al.*, 1966). A homogeneous blend of fungal culture was readied and the SDA plates were sowed with this suspension. Germ-free filter discs which contain 10  $\mu$ L volume of stock solution were put on to the surfaces at various positions. Plates were placed for incubation for one week at room. After incubation distance across of zone of inhibitions was noted in mm. The gresiofulvin was utilized as standard.

**Finding of Minimum inhibitory concentration (MIC)**

Those synthesized derivatives demonstrating antibacterial activity were also investigated to calculate MIC values by disc diffusion method (Bauer *et al.*, 1966). For this reason sterile discs of various concentrations ( $\mu$ g/mL) of compounds and standard were set. The MIC was obtained at the lowest concentration of test compounds indicating zone of inhibition. Results were recorded in an average of triplicate.

**Antioxidant activity**

Antioxidant activity of the compounds (sp-1 to sp-24) was found by utilizing the defined technique in (Lee *et al.*, 1998). Ethanol is used to make 300  $\mu$ M stable radical solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This solution and test samples 90  $\mu$ L and 10  $\mu$ L respectively were added in 96-well microtiter plates. For 30 min 396-well microtiter plates were placed for incubation for at 37  $^{\circ}$ C. Spectrophotometer was used to find absorbance at 515 nm. Standard was Ascorbic acid. DMSO was utilized as negative control. Inhibition of radicals in term of percentage was determined by the action of test sample and contrast with DMSO.

$$\% \text{ Inhibition} = \frac{(\text{control absorbance} - \text{test sample absorbance})}{\text{control absorbance}} \times 100$$

Calculated EC50 value represents the concentration (in  $\mu$ g/ml) of sample essential to scavenge 50% of DPPH.

**Tyrosinase Inhibition Assay**

Spectrophotometre was used to find tyrosinase inhibition activity by following the process defined in literature (Fais *et al.*, 2009). DMSO was used as solvent to dissolve prepared compounds. Tyrosinase o-diphenolase inhibitory activity of synthesized compounds was investigated. Substrate was L-Dopa. Standard inhibitor was kojic acid. Preincubation of 28mM mushroom tyrosinase (30 units) was done at room temperature by utilizing 50mM sodiumphospahte buffer of pH 6.8. L-Dopa of 0.5 mM was added in it. L-DOPA chrome was produced due to enzyme reaction, was checked by determining absorbance for 10 min in spectramax 340 microplate reader (Molecular Devices USA) at 475nm (at 37 $^{\circ}$ C). It was linked to the curve of standard. Following formula was used to find percent inhibition :

$$\% \text{ Inhibition} = \frac{(\text{blank absorbance} - \text{sample absorbance})}{\text{blank absorbance}} \times 100$$

The results were in a mean of triplicate and indicate average  $\pm$  SEM (standard error of the mean).

**Urease Inhibition Assay**

The indophenol process was used to investigate urease activity (Weatherburn, 1967). Ammonia was produced through this reaction. By measuring produced ammonia results were obtained. The reaction mixtures containing 100 mM urea, 25  $\mu$ L solution of Jack bean Urease enzyme and 8.2 pH buffer of volume 55  $\mu$ L containing  $K_2HPO_4 \cdot 3H_2O$ , EDTA and LiCl 10, 1 and 10 mM respectively. For 15 min, 96-well plates were placed for incubation along with 1 mM of test compounds (5  $\mu$ L) at 30 $^{\circ}$ C. Shortly, alkali reagent of volume 70  $\mu$ L containing NaOH and active chloride NaOCl of w/v 0.5 and 0.1% respectively. Each of 45  $\mu$ L phenol reagent containing phenol and sodium nitroprusside of w/v 1 and 0.005 %, respectively. All were mixed to each well thoroughly. At 630 nm microplate reader (Molecular Device, USA) was utilized to find the increasing absorbance after 50 min. Reactions were finished in the form of triplicate of volume 200  $\mu$ L. The consequence is alteration in absorbance in a min., were managed on a software named SoftMax Pro (Molecular Device, USA). Standard was thiourea for contrast. Following formula was used to get Percentage inhibition:

$$\% \text{ inhibition} = 100 - (\text{OD}_{\text{testwell}} \div \text{OD}_{\text{control}}) \times 100$$

**(E)-1- benzylideneaniline (sp-1)**

M.P: 49-50 $^{\circ}$ C; FT-IR (potassium bromide)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3032 (Aromatic-CH), 1637 (C=N), 1600, 1472 (Aromatic, C=C), 1192 (C-N);  $^1\text{H}$  NMR ( $CDCl_3$ , ppm, 500 MHz):  $\delta$  7.32-8.12 (10H, m,  $C_6H_5$ ), 8.60 (1H, s, CH=N); EI-MS:  $m/z$  (relative abundance in %), 181 ( $M^+$ , 30); Elem. Anal.: Calculated for  $C_{13}H_{11}N$  (181.23); C, 86.16; N, 7.74; H, 6.13 %; found: C, 86.17; N, 7.75; H, 6.14% (Ibrahim *et al.*, 2006).

**(E)-1-benzylidene-2-phenylhydrazine (sp-2)**

M.P: 151-152 $^{\circ}$ C; FT-IR (potassium bromide)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3322(N-H), 3033(Aromatic-CH), 1650(C=N), 1600, 1465 (Aromatic, C=C), 1134 (C-N);  $^1\text{H}$  NMR ( $CDCl_3$ , ppm, 500 MHz):  $\delta$  7.67 (1H, s, CH=N), 7.65-7.26 (5H, m,  $C_6H_5$ ), 7.11-6.83 (5H, m, NH- $C_6H_5$ ), 3.47 (1H, brs, NH); EI-MS:  $m/z$  (relative abundance in %),

196(M<sup>+</sup>, 28); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub> (196.25); C, 79.57; H, 6.15; N, 14.26%; found: C, 79.60; H, 6.18; N, 14.29% (Khan *et al.*, 2014)

**(E)-1- benzylidene urea (sp-3)**

M.P: 75-76 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3450 (NH<sub>2</sub>), 3040(Aromatic-CH), 1674 (C=O), 1456 (C=N); <sup>1</sup>H-NMR (*DMSO-d*<sub>6</sub>, ppm, 400 MHz):  $\delta$  9.50 (1H, s, CH=N), 8.10-7.50, (5H, m, C<sub>6</sub>H<sub>5</sub>), 5.70 (2H, s, NH<sub>2</sub>, amide); EI-MS: *m/z* (relative abundance in %), 148(M<sup>+</sup>, 34); Elem. Anal.: Calculated for C<sub>8</sub>H<sub>8</sub>N<sub>2</sub>O (148.20); C, 64.86; H, 5.43; N, 18.92%; found: C, 64.92; H, 5.46; N, 18.89% (Kshash *et al.*, 2011).

**(E)-1- benzylidene thiourea (sp-4)**

M.P: 110-111°C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3247 (NH<sub>2</sub>), 3032 (Aromatic-CH), 1615 (C=N), 1600,1450 (Aromatic, C=C), 1245 (C=S),1190 (C-N); <sup>1</sup>H NMR (*DMSO-d*<sub>6</sub>, ppm, 400 MHz):  $\delta$  9.00 (1H, s, CH=CN), 8.00-7.50 (5H, m, C<sub>6</sub>H<sub>5</sub>), 2.20 (2H, s, S=C-NH<sub>2</sub>); EI-MS: *m/z* (relative abundance in %), 164 (M<sup>+</sup>, 32); Elem. Anal.: Calculated for C<sub>8</sub>H<sub>8</sub>N<sub>2</sub>S (164.22); C, 58.52; H, 4.90; N, 17.07; S19.52 %; found: C, 58.53; H, 4.92; N, 17.07; S19.54 % (Al-Obaidi, 2012).

**4-amino-N-[(E)-phenylmethylidene] benzene sulfonamide (sp-5)**

M.P: 178-179 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3240(NH<sub>2</sub>), 3102(Aromatic-CH),1650(C=N), 1620, 1472(Aromatic, C=C), 1322(O=S=O), 823(C-S); <sup>1</sup>H NMR (*DMSO-d*<sub>6</sub>, ppm, 400 MHz):  $\delta$  8.60 (1H, s, CH=N), 7.90-7.40 (4H, m, C<sub>6</sub>H<sub>4</sub>), 8.20-7.35 (5H, m, C<sub>6</sub>H<sub>5</sub>), 6.85 (2H, s, SO<sub>2</sub>NH<sub>2</sub>); EI-MS: *m/z* (relative abundance in %), 260(M<sup>+</sup>, 27); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S (260.30); C, 59.97; H, 4.66; N, 10.75; S, 12.32%; found: C, 60.56; H, 4.66; N, 10.75; S 12.30% (Singh *et al.*, 2010).

**4-[(E)-benzylideneamino] phenol (sp-6)**

M.P: 150 -152 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3449 (O-H), 1627(C=N), 1589, 1453 (C=C), 1377 (C-O); <sup>1</sup>H NMR (*DMSO-d*<sub>6</sub>, ppm, 200 MHz):  $\delta$  9.55(1H, s, OH), 8.45(1H, s, CH=N), 7.45-7.87(5H, m, -C<sub>6</sub>H<sub>5</sub>), 7.16(2H, d, J= 8.8Hz, C<sub>6</sub>H<sub>4</sub>OH), 6.84(2H, d, J= 8.8Hz, -C<sub>6</sub>H<sub>4</sub>OH); EI-MS: *m/z* (relative abundance in %), 197(M<sup>+</sup>, 36); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>11</sub>NO (197.20); C, 79.17; H, 5.61; N, 7.11%; found: C, 79.19; H, 5.64; N, 7.11% (da Silva *et al.*, 2011).

**2-[(E)-benzylideneamino] phenol (sp-7)**

M.P: 57-58 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3375 (O-H), 1625 (C=N), 1584, 1482 (C=C), 1380 (C-O); <sup>1</sup>H NMR (*DMSO-d*<sub>6</sub>, ppm, 200 MHz):  $\delta$  9.05(1H, s, OH);8.69(1H, s, CH=N); 7.92-7.47(5H, m, -C<sub>6</sub>H<sub>5</sub>), 7.18-6.87(4H, m, -C<sub>6</sub>H<sub>4</sub>OH); EI-MS: *m/z* (relative abundance in %), 197 (M<sup>+</sup>, 30); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>11</sub>NO (197.23); C, 79.17; H, 5.61; N, 7.11%; found: C, 79.18; H, 5.63; N, 7.11% (da Silva *et al.*, 2011).

**(E)-N-(3-chlorophenyl)-1-phenylmethanimine (sp-8)**

M.P: 101-102 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3030(Aromatic-CH), 1635(C=N),1600,1470 (Aromatic, C=C),1192 (C-N); <sup>1</sup>H NMR (*CDCl*<sub>3</sub>, ppm, 400 MHz):  $\delta$  8.68 (1H, s, CH=N), 7.90-7.46 (5H, m, -C<sub>6</sub>H<sub>5</sub>), 7.30-7.00 (4H, m, -C<sub>6</sub>H<sub>4</sub>Cl); EI-MS: *m/z* (relative abundance in %), 216(M<sup>+</sup>, 30); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>11</sub>N (215.68); C, 72.38; H, 4.68; Cl, 16.45; N, 6.48; found: C, 72.41 ; H, 4.69; Cl, 16.45; N, 6.51% (Dragone *et al.*, 2013).

**(E)-N-(1,3-benzothiazol-2-yl)-1-phenylmethanimine (sp-9)**

M.P: 121-122 °C; FT-IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3057 (Aromatic-CH), 1607-1597 (C=N), 1542, 1442(Aromatic, C=C); <sup>1</sup>H NMR (*CDCl*<sub>3</sub>, ppm, 400 MHz):  $\delta$  9.06(1H, s, CH=N), 8.03-7.09(9H, m, -C<sub>6</sub>H<sub>5</sub>, benzothiazole-H); EI-MS: *m/z* (relative abundance in %), 238(M<sup>+</sup>, 28); Elem. Anal.: Calculated for C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>S (238.31); C, 70.56; H, 4.23; N, 11.76; S, 13.46%; found: C, 70.58; H, 4.25; N, 11.78; S, 13.47% (Vicini, *et al* 2003).

**(E, E)-N, N'-benzene-1,4-diylbis(1-phenylmethanimine) (sp-10)**

M.P: 155-156 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3052(Aromatic, CH), 1616(C=N), 1574, 1495(Aromatic, C=C); <sup>1</sup>H NMR (*CDCl*<sub>3</sub>, ppm, 400 MHz):  $\delta$  8.50 (1H, d, CH=N), 7.91-7.46 (9H, m, -C<sub>6</sub>H<sub>5</sub>, -C<sub>6</sub>H<sub>4</sub>); EI-MS: *m/z* (relative abundance in %), 284 (M<sup>+</sup>, 30); Elem. Anal.: Calculated for C<sub>20</sub>H<sub>16</sub>N<sub>2</sub> (284.35); C, 84.48; H, 5.67; N, 9.85; found: C, 84.46; H, 5.69; N, 9.83% (Gomaa *et al.*, 2012).

**(1E, 2E)-N,3-diphenylprop-2-en-1-imine (sp-11)**

M.P: 141-142 °C; FT-IR (potassium bromide)  $\nu_{\max}$  cm<sup>-1</sup>: 3010 (Aromatic-CH), 1599(C=C) 1610(C=N),1600,1455 (Aromatic, C=C); <sup>1</sup>H NMR (*CDCl*<sub>3</sub>, ppm, 400 MHz):  $\delta$  8.25 (1H, d, J=10.0Hz, CH=N), 7.53-7.19(9H, m, -C<sub>6</sub>H<sub>5</sub>, -N-C<sub>6</sub>H<sub>5</sub>), 7.22 (1H, d, J=17.0Hz, =CH), 6.70 (1H, dd, J=17.0, 10.0Hz, =CH); EI-MS:

$m/z$  (relative abundance in %), 207( $M^+$ , 30); Elem. Anal.: Calculated for  $C_{15}H_{13}N$  (207.27); C, 86.93; H, 6.31; N, 6.75; found: C, 86.94; H, 6.34; N, 6.75% (Vineetha and Parasad, 2013).

**(1E, 2E)-N-(3-chlorophenyl)-3-phenylprop-2-en-1-imine (sp-12)**

M.P: 185-186 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3030(Aromatic-CH), 1590(C=C) 1605(C=N), 1600,1450 (Aromatic, C=C);  $^1H$  NMR ( $CDCl_3$ , ppm, 400 MHz):  $\delta$  8.22 (1H, d, J= 10.20, CH=N), 7.45-7.30(5H, m,  $-C_6H_5$ ), 7.38-7.25 (4H, m,  $C_6H_4Cl$ ), 7.28 (1H, d, J= 17.90Hz, =CH), 6.70(1H, dd, J=17.90, 10.20Hz =CH); EI-MS:  $m/z$  (relative abundance in %), 241( $M^+$ , 36); Elem. Anal.: Calculated for  $C_{15}H_{12}ClN$  (241.71); C, 74.54; H, 5.01; Cl, 14.68 N, 5.78 found: C, 74.54; H, 5.02; Cl, 14.69 N, 5.81% (Vineetha *et al.*, 2013)

**(2E)-1-phenyl-2-[(2E)-3-phenylprop-2-en-1-ylidene] hydrazine (sp-13)**

M.P: 140-141 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3312 (N-H), 3040 (Aromatic, C-H), 1610 (C=N), 1600, 1455 (Aromatic, C=C);  $^1H$  NMR ( $DMSO-d_6$ , ppm, 400 MHz):  $\delta$  7.41-7.23 (5H, m,  $C_6H_5$ ), 7.35 (1H, d, J= 10.0Hz, CH=N), 7.19-7.08 (4H, m,  $-NHC_6H_5$ ), 6.85 (1H, d, J= 15.0Hz, =CH), 6.50 (1H, dd, J= 15.0, 10.0Hz, =CH), 7.28; EI-MS:  $m/z$  (relative abundance in %), 222( $M^+$ , 30); Elem. Anal.: Calculated for  $C_{15}H_{14}N_2$  (222.28); C, 81.04; H, 6.36; N, 12.61; found: C, 81.03; H, 6.42; N, 12.55% (Khan *et al.*, 2014)

**2-[(E)-[(2E)-3-phenylprop-2-en-1-ylidene] amino] phenol (sp-14)**

M.P: 189-190 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3335 (O-H), 3082(Aromatic, C-H), 1600, 1450 (Aromatic, C=C), 1582 (C=C), 1610 (C=N);  $^1H$  NMR ( $DMSO-d_6$ , ppm, 300 MHz):  $\delta$  8.40 (1H, d, J= 10.2Hz, CH=N), 7.38-7.24(5H, m,  $-C_6H_5$ ), 7.34-6.90 (4H, m,  $C_6H_4OH$ ), 7.25 (1H, d, J= 17.0Hz, =CH), 6.60 (1H, dd, J= 17.0Hz, 10.2Hz, =CH); EI-MS:  $m/z$  (relative abundance in %), 223( $M^+$ , 36); Elem. Anal.: Calculated for  $C_{15}H_{13}NO$  (223.27); C, 80.68; H, 5.88; N, 6.28; found: C, 80.67; H, 5.85; N, 6.26% (Mohammad, 2011)

**4-[(E)-[(2E)-{3-phenylprop-2-en-1-ylidene] amino] phenol (sp-15)**

M.P: 125-126 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3362(O-H), 3066(Aromatic, C-H), 1580, 1430 (Aromatic, C=C), 1582 (C=C), 1556 (C=N);  $^1H$  NMR ( $DMSO$ , ppm, 500 MHz):  $\delta$  8.38 (1H, d, J= 10.3 Hz, CH=N), 7.37-7.24 (5H, m,  $-C_6H_5$ ), 7.27-7.02(4H, m,  $-C_6H_4OH$ ), 7.24( 1H, d, J=15.0Hz, =CH), 6.50(1H, dd, J=15.0, 10.3Hz, =CH); EI-MS:  $m/z$  (relative abundance in %), 223( $M^+$ , 34); Elem. Anal.: Calculated for  $C_{15}H_{13}NO$  (223.27); C, 80.68; H, 5.88; N, 6.26; found: C, 80.70; H, 5.89; N, 6.29% (Chandramohan *et al.*, 2012 )

**2-[(E)-(phenylimino) methyl] phenol (sp-16)**

M.P: 50-51 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3480 (OH), 3062(Aromatic, C-H), 1622 (C=N), 1590, 1470 (Aromatic, C=C);  $^1H$  NMR ( $DMSO-d_6$ , ppm, 400 MHz):  $\delta$  11.00 (1H, s, OH), 8.70 (1H, s, CH=N), 7.76–6.90(4H, m,  $-C_6H_4OH$ ), 7.10-7.30(5H, m,  $-C_6H_5$ ); EI-MS:  $m/z$  (relative abundance in %), 197( $M^+$ , 29); Elem. Anal.: Calculated for  $C_{13}H_{11}NO$  (197.23); C, 79.17; H, 5.63; N, 7.11; found: C, 79.18; H, 5.61; N, 7.12% (Amame and Bouhdada, 2014).

**1-[(E)-(2-hydroxyphenyl) methylidene] urea (sp-17)**

M.P: 149-150°C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3470 (O-H), 3060(Aromatic, C-H), 1632 (C=O), 1610 (C=N), 1590-1470 (Aromatic, C=C);  $^1H$  NMR ( $DMSO-d_6$ , ppm, 500 MHz):  $\delta$  11.00(1H, s, OH), 9.65(1H, s, 5H, CH=N), 8.00-6.85(4H, m,  $-C_6H_4OH$ ), 5.60(2H, s,  $-CONH_2$ ); EI-MS:  $m/z$  (relative abundance in %), 164( $M^+$ , 34); Elem. Anal.: Calculated for  $C_8H_8N_2O$  (164.20); C, 58.52; H, 4.92; N, 17.05; found: C, 58.55; H, 4.93; N, 17.07% (El-Ajaily and El-Saied, 2007).

**4-[(E)-(2-hydroxyphenyl) methylidene] amino} benzene sulfonamide (sp-18)**

M.P: 207-208 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3352 (O-H), 3248 ( $NH_2$ ), 3043 (Aromatic, C-H), 1680 (C=N), 1590 (Aromatic, C=C), 1330 (O=S=O), 753 (C-S);  $^1H$  NMR ( $CDCl_3$ , ppm, 300 MHz):  $\delta$  12.00 (1H, s, OH), 10.20 (2H, s,  $-SONH_2$ ), 9.03(1H, s, CH=N), 8.01-6.92(4H, m,  $-C_6H_4OH$ ); EI-MS:  $m/z$  (relative abundance in %), 276( $M^+$ , 30); Elem. Anal.: Calculated for  $C_{13}H_{12}N_2O_3S$  (276.31); C, 56.50; H, 4.39; N, 10.13; S, 11.61; found: C, 56.52; H, 4.39; N, 10.16; S, 11.63% (Omar, 2007).

**2-[(E)-(2-phenylhydrazinylidene) methyl] phenol (sp-19)**

M.P: 170-171 °C; FT-IR (potassium bromide)  $\nu_{max}$   $cm^{-1}$ : 3450 (O-H), 3053 (Aromatic, C-H), 1622 (C=N), 1580, 1470 (Aromatic, C=C);  $^1H$  NMR ( $DMSO-d_6$ , ppm, 400 MHz):  $\delta$  7.90(1H, s, CH=N), 7.45-6.90(4H, m,  $-C_6H_4OH$ ), 7.42-7.05(5H, m,  $-HN-C_6H_5$ ); EI-MS:  $m/z$  (relative abundance in %), 212( $M^+$ , 28); Elem. Anal.: Calculated for  $C_{13}H_{12}N_2O$  (212.25); C, 73.57; H, 5.71; N, 13.19%; found: C, 73.59; H, 5.73; N, 13.22% (Khan *et al.*, 2014).

**(2-Hydroxy- benzylidene) -thiourea (sp-20)**

M.P: 165-166°C; FT-IR (potassium bromide)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3470 (O-H), 3025 (Aromatic-CH), 1590, 1470 (Aromatic, C=C), 1622 (C=N), 1242(C=S);  $^1\text{H NMR}$  (*DMSO-d*<sub>6</sub>, ppm, 300 MHz):  $\delta$  11.05 (1H, s, OH), 9.08 (1H, s, CH=N); 7.98-6.90(4H, m, -C<sub>6</sub>H<sub>4</sub>OH), 2.2(2H, s, -CSNH<sub>2</sub>); EI-MS: *m/z* (relative abundance in %), 180(M<sup>+</sup>, 28); Elem. Anal.: Calculated for C<sub>8</sub>H<sub>8</sub>N<sub>2</sub>OS (180.23); C, 53.30; H, 4.46; N, 15.55%; found: C, 53.33; H, 4.49; N, 15.53% (Baiu *et al.*, 2009).

**2-[(E)-(2-hydroxybenzylidene) amino] phenol (sp-21)**

M.P: 175-176 °C; FT-IR (potassium bromide)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3429 (O-H), 3062(C-H), 3040 (Aromatic, C-H), 1628 (C=N), 1540-1470 (Aromatic, C=C);  $^1\text{H NMR}$  (*DMSO-d*<sub>6</sub>, ppm, 500 MHz):  $\delta$  13.73 (2H, s, -OH), 8.90 (1H, s, CH=N), 7.65-6.82 (8H, m, -C<sub>6</sub>H<sub>4</sub>OH); EI-MS: *m/z* (relative abundance in %), 213. (M<sup>+</sup>, 31); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>11</sub>NO<sub>2</sub> (213.23); C, 73.22; H, 5.19; N, 6.58%; found: C, 73.25; H, 5.21; N, 6.59% (Aziz *et al.*, 2012).

**2-[(E)-(4-hydroxyphenyl imino) methyl] phenol (sp-22)**

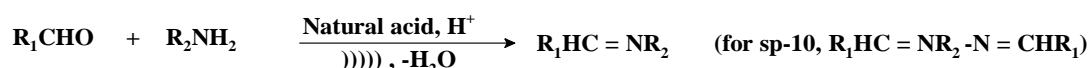
M.P: 149-150 °C; FT-IR (potassium bromide)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3425 (O-H), 3058(Aromatic, C-H), 1620 (C=N), 1590-1470 (Aromatic, C=C);  $^1\text{H NMR}$  (*DMSO-d*<sub>6</sub>, ppm, 400 MHz):  $\delta$  9.72 (1H, s, -OH), 8.65(1H, s, CH=N), 7.54-6.98(8H, m, -C<sub>6</sub>H<sub>4</sub>OH); EI-MS: *m/z* (relative abundance in %), 213(M<sup>+</sup>, 30); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>11</sub>NO<sub>2</sub> (213.23); C, 73.22; H, 5.15; N, 6.56; found: C, 73.24; H, 5.18; N, 6.68% (Savalia *et al.*, 2013).

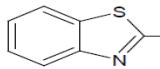
**2-[(E)-(3-chlorophenyl imino) methyl] phenol (sp-23)**

M.P: 179-180 °C; FT-IR (potassium bromide)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3062(Aromatic, C-H), 1640 (C=N), 1590, 1470 (Aromatic, C=C);  $^1\text{H NMR}$  (*DMSO-d*<sub>6</sub>, ppm, 400 MHz):  $\delta$  12.90 (1H, s, -OH), 8.66 (1H, s, CH=N), 7.72-6.91 (4H, m, -C<sub>6</sub>H<sub>4</sub>OH), 7.41-7.15 (4H, m, -C<sub>6</sub>H<sub>4</sub>Cl); EI-MS: *m/z* (relative abundance in %), 231 (M<sup>+</sup>, 32); Elem. Anal.: Calculated for C<sub>13</sub>H<sub>10</sub>ClNO (231.68); C, 67.40; H, 4.36; N, 6.06; Cl, 15.31; found: C, 67.11; H, 4.10; N, 5.88; Cl, 15.36% (Kumar *et al.*, 2016).

**2-[(E)-(1,3-benzothiazol-2-ylimino) methyl] phenol (sp-24)**

M.P: 150-151 °C; FT-IR (potassium bromide)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3430 (O-H), 3050 (Aromatic, C-H), 1605 (C=N), 1597, 1470 (Aromatic, C=C);  $^1\text{H NMR}$  (*DMSO-d*<sub>6</sub>, ppm, 500 MHz):  $\delta$  11.55(1H, s, OH), 9.25 (1H, s, CH=N), 7.90-7.35 (4H, m, benzothiazole-H); EI-MS: *m/z* (relative abundance in %), 254 (M<sup>+</sup>, 36); Elem. Anal.: Calculated for C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>OS (254.31); C, 66.11; H, 3.97; N, 11.01; S, 12.60. Found: C, 66.14; H, 3.98; N, 11.03; S, 12.63(Zaki *et al.*, 1998).

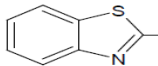
**Scheme-1: Reaction to display synthesis of Schiff bases (sp-1 to sp-24).**(a) If R<sub>1</sub> is C<sub>6</sub>H<sub>5</sub>-

Compounds code	Compound name	R <sub>2</sub>
sp-1	(E)-1-benzylideneaniline	C <sub>6</sub> H <sub>5</sub> -
sp-2	(E)-1-benzylidene-2-phenylhydrazine	C <sub>6</sub> H <sub>5</sub> -NH-
sp-3	(E)-1-benzylidene urea	NH <sub>2</sub> CO-
sp-4	(E)-1- benzylidene thiourea	NH <sub>2</sub> CS-
sp-5	4-amino-N-[(E)-phenylmethylidene] benzene sulfonamide	<i>p</i> -(NH <sub>2</sub> ) C <sub>6</sub> H <sub>5</sub> -SO <sub>2</sub> -
sp-6	4-[(E)-benzylideneamino] phenol	<i>p</i> -(OH) C <sub>6</sub> H <sub>5</sub> -
sp-7	2-[(E)-benzylideneamino] phenol	<i>o</i> -(OH) C <sub>6</sub> H <sub>5</sub> -
sp-8	(E)-N-(3-chlorophenyl)-1-phenylmethanimine (SP-08)	<i>m</i> -(Cl) C <sub>6</sub> H <sub>5</sub> -
sp-9	(E)-N-(1,3-benzothiazol-2-yl)-1-phenylmethanimine	
sp-10	(E, E)-N, N'-benzene-1,4-diylbis(1-phenylmethanimine)	C <sub>6</sub> H <sub>5</sub> -

(b) If R<sub>1</sub> is C<sub>6</sub>H<sub>5</sub>-HC=CH-

Compounds code	Compound name	R <sub>2</sub>
sp-11	(1 <i>E</i> , 2 <i>E</i> )- <i>N</i> ,3-diphenylprop-2-en-1-imine	C <sub>6</sub> H <sub>5</sub> -
sp-12	(1 <i>E</i> , 2 <i>E</i> )- <i>N</i> -(3-chlorophenyl)-3-phenylprop-2-en-1-imine	<i>m</i> -(Cl) C <sub>6</sub> H <sub>5</sub> -
sp-13	(2 <i>E</i> )-1-phenyl-2-[(2 <i>E</i> )-3-phenylprop-2-en-1-ylidene] hydrazine	C <sub>6</sub> H <sub>5</sub> -NH-
sp-14	2-[( <i>E</i> )-[(2 <i>E</i> )-3-phenylprop-2-en-1-ylidene] amino] phenol	<i>o</i> -(OH) C <sub>6</sub> H <sub>5</sub> -
sp-15	4-[( <i>E</i> )-[(2 <i>E</i> )-{3-phenylprop-2-en-1-ylidene} amino] phenol	<i>p</i> -(NH <sub>2</sub> ) C <sub>6</sub> H <sub>5</sub> -

(c) If R<sub>1</sub> is *O*-(OH) C<sub>6</sub>H<sub>5</sub>-

Compounds code	Compound name	R <sub>2</sub>
sp-16	2-[( <i>E</i> )-(phenylimino) methyl] phenol	C <sub>6</sub> H <sub>5</sub> -
sp-17	1-[( <i>E</i> )-(2-hydroxyphenyl) methylidene] urea	NH <sub>2</sub> CO-
sp-18	4-[[( <i>E</i> )-(2-hydroxyphenyl) methylidene] amino] benzene sulfonamide	<i>p</i> -(NH <sub>2</sub> ) C <sub>6</sub> H <sub>5</sub> -SO <sub>2</sub> -
sp-19	2-[( <i>E</i> )-(2-phenylhydrazinylidene) methyl] phenol	C <sub>6</sub> H <sub>5</sub> -NH-
sp-20	1-[( <i>E</i> )-(2-hydroxyphenyl) methylidene] thiourea	NH <sub>2</sub> CS-
sp-21	2-[( <i>E</i> )-(2-hydroxybenzylidene) amino] phenol	<i>o</i> -(OH) C <sub>6</sub> H <sub>5</sub> -
sp-22	2-[( <i>E</i> )-[(4-hydroxyphenyl) imino] methyl] phenol	<i>p</i> -(NH <sub>2</sub> ) C <sub>6</sub> H <sub>5</sub> -
sp-23	2-[( <i>E</i> )-[(3-chlorophenyl) imino] methyl] phenol	<i>m</i> -(Cl) C <sub>6</sub> H <sub>5</sub> -
sp-24	2-[( <i>E</i> )-(1,3-benzothiazol-2-ylimino) methyl] phenol	

**Table 1.** Yield and sonication time of Schiff bases (sp-1sp-24) obtained with 0.5mL of different natural acid catalysts.

Compounds	Lemon juice		Grape juice		Orange juice	
	Time of Sonication (min)	Yield (%)	Time of Sonication (min)	Yield (%)	Time of Sonication (min)	Yield (%)
sp-1	08	90	10	90	10	89
sp-2	09	59	10	58	11	58
sp-3	11	58	12	59	12	58
sp-4	12	70	12	69	12	69
sp-5	12	66	13	66	15	66
sp-6	08	80	10	81	11	78
sp-7	10	84	10	83	10	79
sp-8	10	97	12	95	11	95
sp-9	09	72	11	72	10	72
sp-10	07	68	09	68	12	68
sp-11	06	70	08	70	10	69
sp-12	06	88	09	87	10	86
sp-13	07	48	09	47	10	47
sp-14	05	82	07	80	10	80
sp-15	10	78	08	79	12	77
sp-16	04	85	06	83	07	82
sp-17	05	74	07	74	08	74
sp-18	07	69	10	69	10	69
sp-19	06	62	10	60	10	60
sp-20	08	80	10	79	08	78
sp-21	05	78	07	77	08	77
sp-22	08	74	10	74	12	74
sp-23	06	72	08	71	10	71
sp-24	07	80	10	79	10	79





Continue Table -2

<i>Microorganism</i>	sp-1	sp-2	sp-3	sp-4	sp-5	sp-6	sp-7	sp-8	sp-9	sp-10	sp-11	sp-12	sp-13
<b>Gram negative bacteria</b>													
<i>Acinetobacter baumannii</i>	-	-	11	-	-	18	-	-	-	-	-	-	-
<i>Aeromonas hydrophila</i>	-	-	13	-	-	13	-	-	-	-	-	-	-
<i>Campylobacter jejuni</i>	-	-	-	-	-	-	15	-	-	14	-	-	-
<i>Campylobacter coli</i>	-	-	-	-	-	-	15	-	-	17	-	-	-
<i>Enterobacter aerogenes</i>	-	-	18	-	-	-	27	-	19	27	-	35	-
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	18	-	-	34	-
<i>Escherichia coli</i> ATCC 8739	-	-	-	-	-	-	-	-	20	-	-	31	-
<i>E. coli</i> MDR	-	-	-	-	-	-	-	-	17	-	-	31	-
<i>Enteropathogenic Escherichia coli</i> III	-	-	-	-	-	-	-	-	-	-	-	-	24
<i>Helicobacter pylori</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hemophilus influenza</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	-	-	34	-	-	10
<i>Salmonella paratyphi A</i>	-	-	-	-	-	-	-	-	-	32	-	-	25
<i>Salmonella paratyphi B</i>	-	-	-	-	-	-	-	-	-	34	10	-	27
<i>Shigella flexeneri</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Shigella sonnei</i>	-	-	-	-	-	-	-	-	-	-	10	15	25
<i>Serratia marcesens</i>	-	-	-	-	-	-	-	-	-	-	-	15	-
<i>Klebsiella pneumonia</i>	-	-	-	-	-	-	-	-	-	28	10	-	15
<i>Vibrio cholera</i>	-	-	13	-	-	-	15	-	31	16	11	17	12

Microorganism	sp-14	sp-15	sp-16	sp-17	sp-18	sp-19	sp-20	sp-21	sp-22	sp-23	sp-24	Gentamycin
<i>Acinetobacter baumannii</i>	12	-	21	-	30	-	-	-	-	-	-	22
<i>Aeromonas hydrophila</i>	-	-	17	-	-	-	-	-	-	-	-	10
<i>Campylobacter jejuni</i>	-	-	15	-	-	-	-	-	-	-	-	18
<i>Campylobacter coli</i>	-	-	13	-	-	-	-	-	-	-	-	15
<i>Enterobacter aerogenes</i>	-	-	-	-	-	-	-	-	-	-	-	16
<i>Escherichia coli</i>	14	-	-	-	09	-	-	-	-	-	-	15
<i>Escherichia coli</i> ATCC 8739	-	-	-	-	-	-	-	-	-	-	-	16
<i>E. coli</i> MDR	-	08	-	-	-	-	-	-	-	-	-	17
Enteropathogenic <i>Escherichia coli</i> III	-	-	-	26	25	-	-	-	-	-	-	20
<i>Helicobacter pylori</i>	-	-	-	-	-	-	-	-	-	-	-	10
<i>Hemophilus influenza</i>	-	-	-	-	-	-	-	-	-	-	-	10
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	12
<i>Proteus mirabilis</i>	25	10	-	-	-	-	-	-	-	-	-	18
<i>Salmonella typhi</i>	12	11	-	-	32	11	-	-	-	-	-	18
<i>Salmonella paratyphi A</i>	16	14	-	25	20	10	-	-	-	-	-	18
<i>Salmonella paratyphi B</i>	14	25	-	-	25	10	-	-	-	-	-	20
<i>Shigella flexeneri</i>	-	-	-	-	-	-	-	-	-	-	-	10
<i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	10
<i>Shigella sonnei</i>	13	23	-	20	26	10	-	-	-	-	-	17
<i>Serratia marcesens</i>	-	-	-	-	-	-	-	-	-	-	-	12
<i>Klebsiella pneumoniae</i>	20	25	25	07	25	17	-	-	-	-	-	10
<i>Vibrio cholerae</i>	25	-	-	-	-	15	-	-	-	-	-	18

key: values are in term of zone of inhibition (mm) and an average of triplicate, (-) indicates inactivity.



<i>Campylobacter jejuni</i>	-	-	-	84	-	80	-	-	-	-	-	74	-	-	-	-	-	-	0.8
<i>Campylobacter coli</i>	-	-	-	84	-	60	-	-	-	-	-	54	-	-	-	-	-	-	12.5
<i>Enterobacter aerogenes</i>	-	24	-	20	26	22	-	34	-	-	-	-	-	-	-	-	-	-	12.5
<i>Escherichia coli</i>	-	-	-	-	24	-	-	22	-	40	-	-	-	-	-	-	-	-	12.5
<i>Escherichia coli ATCC 8739</i>	-	-	-	-	20	-	-	14	-	-	-	-	-	-	-	-	-	-	12.5
<i>E. coli MDR</i>	-	-	-	-	40	-	-	88	-	-	-	-	-	-	-	-	-	-	0.8
<i>Enteropathogenic Escherichia coli III</i>	-	-	-	-	-	-	-	27	-	-	-	-	25	28	-	-	-	-	0.4
<i>Helicobacter pylori</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
<i>Hemophilus influenzae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	30	86	-	-	-	-	-	-	-	0.8
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	-	40	25	30	-	-	30	45	-	-	-	0.8
<i>Salmonella paratyphi A</i>	-	-	-	-	-	50	-	-	30	60	70	-	30	40	88	-	-	-	0.8
<i>Salmonella paratyphi B</i>	-	-	-	-	-	52	88	-	20	60	30	-	-	32	80	-	-	-	0.4
<i>Shigella flexeneri</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
<i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
<i>Shigella sonnei</i>	-	-	-	-	-	-	88	34	28	88	28	-	30	28	86	-	-	-	0.8
<i>Serratia marcesens</i>	-	-	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	12.5
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	90	90	-	40	25	30	84	-	30	45	-	-	-	12.5
<i>Vibrio cholerae</i>	-	84	-	82	22	42	88	48	78	20	-	-	-	-	80	-	-	-	0.8

key: values are in zone of inhibition ( $\mu\text{g/mL}$ ), (-) indicates inactivity.



Microorganism	sp-14	sp-15	sp-16	sp-17	sp-18	sp-19	sp-20	sp-21	sp-22	sp-23	sp-24	Gresiofulvin
<i>Aspergillus flavis</i>	16	-	-	18	-	12	-	-	-	08	-	18
<i>Aspergillus nigar</i>	17	-	15	20	-	12	-	-	08	08	-	18
<i>Penicillium species</i>	15	10	18	18	-	09	-	-	-	-	-	22
<i>Rhizopus species</i>	-	-	-	15	-	-	-	-	-	-	-	22
<i>Candida albicans</i>	-	-	13	-	-	-	-	-	10	10	-	18
<i>Candida albicans ATCC</i>	-	-	15	-	-	-	-	-	-	-	-	22
<i>Candida galbrata</i>	-	-	-	-	-	-	-	-	-	-	-	22
<i>Candida tropicalis</i>	20	12	-	-	-	14	-	-	-	-	-	22
<i>Candida kruzei</i>	-	-	-	-	-	-	-	-	-	-	-	22
<i>Sachromyces cerevisiae</i>	-	-	-	-	-	-	-	-	16	08	-	22
<i>Fusarium species</i>	-	-	-	-	-	-	-	-	-	-	-	18
<i>Helminthosporum</i>	-	-	-	-	-	-	-	-	-	-	-	18
<i>Microsporium canis</i>	-	-	-	-	-	-	-	-	-	-	-	18
<i>Microsporium cereus</i>	16	-	-	15	-	20	-	-	-	-	-	22
<i>Microsporium gypsium</i>	-	-	-	-	-	-	-	-	-	-	-	22
<i>Neurospora</i>	-	-	-	-	-	-	-	-	-	-	-	22
<i>Trichophyton rubrum</i>	-	-	-	-	-	-	-	-	-	-	-	10
<i>Trichophyton tonsurans</i>	-	-	-	-	-	25	-	-	-	-	-	10
<i>Trichophyton mentagrophytes</i>	-	-	-	-	-	-	-	-	-	-	-	22

key: values are zone of inhibition (**mm**) and an average of triplicate, (-) indicates resistance.

**Table-5:** MIC values of compounds (sp-1 to sp-24).

Microorganism	MIC values of compounds ( $\mu\text{g/ml}$ )															Gresiofulvin ( $\mu\text{g/Disc}$ )
	sp-1	sp-5	sp-7	sp-9	sp-10	sp-11	sp-12	sp-13	sp-14	sp-15	sp-16	sp-17	sp-19	sp-22	sp-23	
<i>Aspergillus flavis</i>	40	62	-	-	-	100	120	-	200	-	-	200	78	-	-	0.8
<i>Aspergillus nigar</i>	44	60	-	-	-	54	200	32	120		60	166	78	-	-	0.8
<i>Penicillium species</i>	-	66	-	-	-	60	44	-	200	180	94	18	-	-	-	0.4
<i>Rhizopus species</i>	-	-	-	-	-	94	50	-	-	-	-	24	-	-	-	0.4
<i>Candida albicans</i>	78	88	14	120	94	-	242	-	-	-	72	-	-	180	200	0.8
<i>Candida albicans</i> ATCC	-	84	16	88	48	-	320	-	-	-	40	-	-	-	-	0.4
<i>Candida galbrata</i>	-	-	52	90	180	-	214	-	-	-	-	-	-	-	-	0.4
<i>Candida tropicalis</i>	-	-	44	210	200	-	204	30	80	70	-	-	50	-	-	0.4
<i>Candida kruzei</i>	-	-	40	22	80	-	144	-	-	-	-	-	-	-	-	0.4
<i>Sachromyces cerevisiae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	55	-	0.4
<i>Fusarium species</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8
<i>Helminthosporum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8
<i>Microsporium canis</i>	-	32	-	140	12	220	10	-	-	-	-	-	-	-	-	0.8
<i>Microsporium cereus</i>	-	-	-	-	-	-	-	22	180	-	-	30	40	-	-	0.4
<i>Microsporium gypsium</i>	98	-	-	-	-	55	200	-	-	-	-	-	-	-	-	0.4
<i>Neurospora</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4
<i>Trichophyton rubrum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
<i>Trichophyton tonsurans</i>	-	98	-	-	-	20	-	20	-	-	-	-	23	-	-	12.5
<i>Trichophyton mentagrophytes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4

key: values are in zone of inhibition ( $\mu\text{g/mL}$ ), (-) indicates inactive.

**Table-6:** *In vitro* anti-oxidant activity of compounds (sp-1 to sp-24).

Compounds	% Inhibition $\pm$ SD	EC <sub>50</sub> ( $\mu$ g/ml)	Compounds	% Inhibition $\pm$ SD	EC <sub>50</sub> ( $\mu$ g/ml)
sp-1	69.0 $\pm$ 0.01	93.5	sp-14	12.0 $\pm$ 0.01	-
sp-2	68.0 $\pm$ 0.02	95.2	sp-15	80.0 $\pm$ 0.01	73.5
sp-3	46.0 $\pm$ 0.01	-	sp-16	57.9 $\pm$ 0.02	100.0
sp-4	47.0 $\pm$ 0.01	-	sp-17	71.0 $\pm$ 0.01	37.5
sp-5	59.0 $\pm$ 0.02	93.8	sp-18	16.0 $\pm$ 0.01	-
sp-6	80.0 $\pm$ 0.02	38.5	sp-19	36.0 $\pm$ 0.02	-
sp-7	80.0 $\pm$ 0.02	37.5	sp-20	60.0 $\pm$ 0.01	98.2
sp-8	15.0 $\pm$ 0.01	-	sp-21	88.0 $\pm$ 0.01	39.2
sp-9	58.0 $\pm$ 0.02	100.0	sp-22	15.0 $\pm$ 0.01	-
sp-10	18.0 $\pm$ 0.01	-	sp-23	13.0 $\pm$ 0.01	-
sp-11	32.0 $\pm$ 0.02	-	sp-24	18.0 $\pm$ 0.02	-
sp-12	70.0 $\pm$ 0.02	37.5	Ascorbic acid	80.0	8.3
sp-13	13.0 $\pm$ 0.02	-			

Key: SD= standard deviation, EC<sub>50</sub>= effective concentration of compounds that scavage 50% radical, (-) indicates resistance.

**Table -7:** *In vitro* enzyme inhibition activity of compounds (sp-1 to sp-24).

Compounds	Enzyme inhibition activity		Compounds	Enzyme inhibition activity	
	Inhibition of tyrosinase IC <sub>50</sub> $\pm$ SEM ( $\mu$ M)	Inhibition of ofurease IC <sub>50</sub> $\pm$ SEM ( $\mu$ M)		Inhibition of tyrosinase IC <sub>50</sub> $\pm$ SEM ( $\mu$ M)	Inhibition of ofurease IC <sub>50</sub> $\pm$ SEM ( $\mu$ M)
sp-1	100.0 $\pm$ 0.14	NS	sp-14	NS	NS
sp-2	95.2 $\pm$ 0.59	135.1 $\pm$ 0.69	sp-15	NS	NS
sp-3	231.0 $\pm$ 0.12	NS	sp-16	NA	NS
sp-4	NA	42.7 $\pm$ 0.56	sp-17	NA	NS
sp-5	NA	NS	sp-18	98.2 $\pm$ 0.53	100.0 $\pm$ 0.94
sp-6	NA	98.0 $\pm$ 0.36	sp-19	97.2 $\pm$ 0.55	NS
sp-7	100.0 $\pm$ 0.22	38.6 $\pm$ 0.86	sp-20	98.2 $\pm$ 0.09	NS
sp-8	NA	NS	sp-21	NS	68.7 $\pm$ 0.62
sp-9	NA	NS	sp-22	NS	NS
sp-10	NA	49.3 $\pm$ 0.38	sp-23	NS	55.5 $\pm$ 0.31
sp-11	NA	NS	sp-24	122.2 $\pm$ 0.31	NS
sp-12	NA	NS	Kojic acid	16.67 $\pm$ 0.06	-
sp-13	NS	NS	Thiourea	-	21.6 $\pm$ 0.12

Key: SEM= standard error of the mean, IC<sub>50</sub>= concentration of compound that inhibit 50% enzyme activity, NS= not significant.



## Results and discussion

Schiff bases (**sp-1** to **sp-24**) were synthesized by the condensation of primary aromatic amines with appropriate aromatic aldehydes by using natural acid as a catalyst found in the extract of grape fruit, orange and lemon (see experimental). Green methodology was adopted to synthesize these products in sonicator under the influence of sound waves. Yield and sonication time of all the synthesized compounds (**sp-1** to **sp-24**) are presented in **Table 1**.

All the prepared imines (**sp-1** to **sp-24**) were investigated for their anti-bacterial activity (**Table 2** and **3**) against standard gentamycin. Fourteen gram positive (+ve) and twenty-two gram negative (-ve) bacterial pathogens were used. On the basis of diameter of inhibited zone in mm outcomes were reported that was appeared around the disc (7mm). Compounds **sp-10**, **sp-12** and **sp-18** displayed strong antibacterial activity against several gram +ve and -ve bacterial strains, **sp-10** showed 34 mm (MIC=25 µg/ mL) as maximum zone of inhibition against *bacillus subtilis* (gram +ve bacteria) whereas same zone of inhibition was observed (MIC= 50 µg/mL) against *salmonella typhi* (gram -ve bacteria).

**sp-12** showed highest zone of inhibition of 29 mm (MIC= 28 µg/mL) against *bacillus cereus* (gram +ve bacteria) where as in gram -ve bacteria its greatest zone of inhibition appeared at 35 mm (MIC =34µg/mL) against *enterobacter aerogenes*.

In case of gram positive bacteria, **sp-18** displayed its zone of inhibition with maximum value of 35 mm against *staphylococcus aureus* and *staphylococcus aureus* (MRSA) having MIC of 18 and 15 µg/ mL respectively. Where as in gram -ve bacteria it showed highest inhibited zone of 32 mm (MIC=30 ug/mL) for *salmonella typhi*.

Compounds **sp-1**, **sp-4**, **sp-5**, **sp-8** and **sp-24** were found inactive against all the applied bacterial strains whereas **sp-2** and **sp-20** to **sp-23** showed moderate activity for gram +ve bacteria and no activity for gram -ve bacteria.

All the synthesized products (**sp-1** to **sp-24**) were tested for their *in vitro* antifungal activity (**Table 4** and **5**) taken gresiofulvin as standard against nineteen fungal strains. Compounds **sp-7** and **sp-9** to **sp-12** showed strong activity against various fungal strains. **sp-7** showed maximum inhibition zone of 33 mm (MIC=14 µg/mL) against *candida albicans*. **sp-9** displayed highest inhibition zone of 32 mm (MIC = 22 µg/mL) for *candida kruzei*. **sp-10** indicated greatest inhibited zone of 29 mm (MIC = 200 µg /mL) against *candida tropicalis*. **sp-11** showed maximum inhibited zone of 25 mm (MIC =20 µg/mL) against *trichophyton tonsurans*. **sp-12** presented significant inhibition zone of 24 mm (MIC =44 µg/mL) against *penicillium species*. Compounds **sp-3**, **sp-4**, **sp-6**, **sp-8**, **sp-18**, **sp-20**, **sp-21** and **sp-24** were found inactive against any fungal strain.

The prepared imines (**sp-1** to **sp-24**) were also screened for their *in vitro* antioxidant activity against DPPH radical along with Ascorbic acid as a standard. **sp-6**, **sp-7**, **sp-15** and **sp-21** showed remarkable antioxidant activity having % inhibition of 80 to 88 % with EC<sub>50</sub> values 38.5, 37.5, 73.5, and 39.2 µg/mL respectively. While compounds **sp-1**, **sp -2**, **sp -5**, **sp -9**, **sp -12**, **sp -16**, **sp -17** and **sp -20** showed moderate anti-oxidant activity (**Table-6**).

All the synthetic compounds (**sp-1** to **sp-24**) were also subjected for their *in vitro* tyrosinase inhibitory activity against standard Kojic acid. Out of these twenty-four compounds, **sp-2** and **sp-19** exhibited moderate tyrosinase inhibitory activity with IC<sub>50</sub> = 95.2±0.59 and 97.2±0.55 µM respectively.

In screening for *in vitro* urease inhibitory activity taking thiourea as a standard, among these synthesized products (**sp-1** to **sp-24**), three compounds **sp-4**, **sp-7** and **sp-10** were found to be the most active for urease inhibition having IC<sub>50</sub> values of 42.7±0.56, 38.6±0.86 and 49.3±0.38 µM respectively whereas **sp-6**, **sp-21** and **sp-23** showed moderate enzyme inhibitory activity (**Table-7**).

## Conclusion

Synthesis of Schiff bases (**sp-1** to **sp-24**) by utilizing natural acid as a catalyst in sonicator under the influence of sound waves was found to be most effective method having few advantages over classical procedure such as short reaction time, high yield and greener reaction conditions. The biological investigations of the above synthesized imines revealed that compounds **sp-2**, **sp-4**, **sp-6**, **sp-7**, **sp -9**, **sp-10**, **sp-12**, **sp-18** and **sp-19** showed noteworthy antimicrobial, antioxidant and enzyme inhibition activities. It is concluded that these products have potential of curing numerous microbial infections and can have opportunity in pharmaceutical level.

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