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

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خلاصہ

بینزایلڈ ہائیڈ (sp-1 سے sp-10)، سینم ایلڈی ہائیڈ (sp-2 سے sp-15) اور سیلی سائیل ایلڈ ہائیڈ (sp-16 سے sp-24) کے شف اساس ماحول دوست طریقہ کار کو استعمال کرتے ہوئے قدرتی تیزاب (لیموں کا رس) بطور کیٹالسٹ سونیکیٹر میں تیار کئے گئے۔ان حاصلات کی ساختی وضاحت کے لیے مختلف سپیکٹرواسکوپک تکنیک جیسے عنصر تجزیہ کاری، انفراریڈ (IR)، الیکٹران امپیکٹ ماس اسپیکٹرواسکوپی (EIMS) اور پروٹان نیوکلر میگنیٹک ریزونینس (^{1}HNMR) استعمال کی گئی۔ حاصل کردہ پروڈکٹ کی اینٹی بیکٹیریل، اینٹی آکسیڈینٹ اور اینزائم اِنہیبیشن (ممانعت) سرگرمیوں کو جانچا گیا۔اینٹی بیکٹریل سرگرمی کے نتائج کے تحت مرکبات sp-10، sp-12 اور sp-18 نے سب سے زیادہ علاقے میں جو 35-10 ملی میٹر تھی مختلف قسم کے گرام مثبت اور منفی بیکٹیریا 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 آکسیڈنٹ سرگرمی دکھائی۔ مرکبات sp-2 اور sp-19 نے ٹائیروسینیز کے خلاف قابل ذکر سرگرمی دکھائی جو IC_{50} کے ساتھ بالترتیب 95.2±0.59 اور 97.2±0.55 مائیکرومولر تھی جو کوجک تیزاب (IC_{50}=16.67±0.06) کو بطور معیار موازنے کے لیے جانچی گئیں۔ مرکبات sp-4، sp-7 اور sp-10 نے یوری ایز انہیبیشن کے خلاف سرگرمی دکھائی جو IC_{50} کے ساتھ بالترتیب 42.7±0.56، 38.6±0.86 اور 49.3±0.38 مائیکرومولر تھیں جو تھائیوریا (IC_{50}=21.6±0.12µM) کو بطور معیار موازنہ کے لیے جانچی گئیں۔

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 estract) 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 (^{1}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_{50} values 95.2 ± 0.59 and 97.2 ± 0.55 µM, respectively as compared to Kojic acid (IC_{50}=16.67 ± 0.06) as standard. The compounds **sp-4**, **sp-7** and **sp-10** appeared active for urease inhibition with IC_{50} values 42.7 ± 0.56, 38.6 ± 0.86 and 49.3 ± 0.38 µM against standard thiourea (IC_{50}=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 Jayyshree, 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 appartus, Gallen kamp was used to find the melting point in glass capillary. EIMS were taken on JEOL JMS-600H mass spectrometer. 1H NMR spectra were noted on Advance AV-200, 300, 400 and 500 MHz in Chloroform (CDCl3) and Dimethylsulphoxide (DMSO-d6) 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 Sabourd 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 µL 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 (µg/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 µM stable radical solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This solution and test samples 90 µL and 10 µL respectively were added in 96-well microtiter plates. For 30 min 396-well microtiter plates were placed for incubation for at 37 ºC . 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.

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

Calculated EC50 value represents the concentration (in µg/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 investegated . 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˚C). It was linked to the curve of standard. Following formula was used to find percent inhibition :

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

The results were in a mean of triplicate and indicate average ± 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 µL solution of Jack bean Urease enzyme and 8.2 pH buffer of volume 55 µL containing $K_2HPO_4.3H_2O$, EDTA and LiCl2 10, 1 and 10 mM respectively. For 15 min, 96-well plates were placed for incubation along with 1 mM of test compounds (5 µL) at 30ºC. Shortly, alkali reagent of volume 70 µL containing NaOH and active chloride NaOCl of w/v 0.5 and 0.1% respectively. Each of 45 µL 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 µL. 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:

$$\% \text{ inhibition} = 100 - (\text{ODtestwell} \div \text{ODcontrol}) \times 100$$

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

M.P: 49-50ºC; FT-IR (potassium bromide) ν_{max} cm^{-1}: 3032 (Aromatic-CH), 1637 (C=N),1600,1472 (Aromatic, C=C),1192 (C−N); ^{1}H NMR (*$CDCl_3$*, ppm, 500 MHz): δ 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:151-152ºC; FT-IR (potassium bromide) ν_{max} cm^{-1}: 3322(N-H), 3033(Aromatic-CH), 1650(C=N), 1600,1465 (Aromatic, C=C),1134 (C−N); ^{1}H NMR (*$CDCl_3$*, ppm, 500 MHz): δ 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^+, 28); Elem. Anal.: Calculated for $C_{13}H_{12}N_2$ (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) ν $_{max}$ cm^{-1}: 3450 (NH_2), 3040(Aromatic-CH), 1674 (C=O), 1456 (C=N); ^{1}H-NMR (*DMSO-d_6*, ppm, 400 MHz): δ 9.50 (1H, s, CH=N), 8.10-7.50, (5H, m, C_6H_5), 5.70 (2H, s, NH_2, amide); EI-MS: *m/z* (relative abundance in %), 148(M^+, 34); Elem. Anal.: Calculated for $C_8H_8N_2O$ (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: 110-111ºC; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3247 (NH_2), 3032 (Aromatic-CH), 1615 (C=N), 1600,1450 (Aromatic, C=C), 1245 (C=S),1190 (C−N); ^{1}H NMR (*DMSO-d_6*, ppm, 400 MHz): δ 9.00 (1H, s, CH=CN), 8.00-7.50 (5H, m, C_6H_5), 2.20 (2H, s, S=C-NH_2); EI-MS: *m/z* (relative abundance in %), 164 (M^+, 32); Elem. Anal.: Calculated for $C_8H_8N_2S$ (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 ºC; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3240(NH_2), 3102(Aromatic-CH),1650(C=N), 1620, 1472(Aromatic, C=C), 1322(O=S=O), 823(C-S); ^{1}H NMR (*DMSO-d_6*, ppm, 400 MHz): δ 8.60 (1H, s, CH=N), 7.90-7.40 (4H, m, C_6H_4), 8.20-7.35 (5H, m, C_6H_5), 6.85 (2H, s, SO_2NH_2); EI-MS: *m/z* (relative abundance in %), 260(M^+, 27); Elem. Anal.: Calculated for $C_{13}H_{12}N_2O_2S$ (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) ν $_{max}$ cm^{-1}: 3449 (O-H), 1627(C=N), 1589, 1453 (C=C), 1377 (C-O); ^{1}H NMR (*DMSO-d_6*, ppm, 200 MHz): δ 9.55(1H, s, OH), 8.45(1H, s, CH=N), 7.45-7.87(5H, m, -C_6H_5), 7.16(2H, d, J= 8.8Hz, C_6H_4OH), 6.84(2H, d, J= 8.8Hz, -C_6H_4OH); EI-MS: *m/z* (relative abundance in %), 197(M^+, 36); Elem. Anal.: Calculated for $C_{13}H_{11}NO$ (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) ν $_{max}$ cm^{-1}: 3375 (O-H), 1625 (C=N), 1584, 1482 (C=C), 1380 (C-O); ^{1}H NMR (*DMSO-d_6*, ppm, 200 MHz): δ 9.05(1H, s, OH);8.69(1H, s, CH=N); 7.92-7.47(5H, m, -C_6H_5), 7.18-6.87(4H, m, -C_6H_4OH); EI-MS: *m/z* (relative abundance in %), 197 (M^+, 30); Elem. Anal.: Calculated for $C_{13}H_{11}NO$ (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) ν $_{max}$ cm^{-1}: 3030(Aromatic-CH), 1635(C=N),1600,1470 (Aromatic, C=C),1192 (C−N); ^{1}H NMR (*$CDCl_3$*, ppm, 400 MHz): δ 8.68 (1H, s, CH=N), 7.90-7.46 (5H, m, -C_6H_5), 7.30-7.00 (4H, m, -C_6H_4Cl); EI-MS: *m/z* (relative abundance in %), 216(M^+, 30); Elem. Anal.: Calculated for $C_{13}H_{11}N$ (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) ν $_{max}$ cm^{-1}: 3057 (Aromatic-CH), 1607–1597 (C=N), 1542, 1442(Aromatic, C=C); ^{1}H NMR (*$CDCl_3$*, ppm, 400 MHz): δ 9.06(1H, s, CH=N), 8.03-7.09(9H, m, -C_6H_5, benzothiazole-H); EI-MS: *m/z* (relative abundance in %), 238(M^+, 28); Elem. Anal.: Calculated for $C_{14}H_{10}N_2S$ (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) ν $_{max}$ cm^{-1}: 3052(Aromatic, CH), 1616(C=N), 1574, 1495(Aromatic, C=C); ^{1}H NMR (*$CDCl_3$*, ppm, 400 MHz): δ 8.50 (1H, d, CH=N), 7.91-7.46 (9H, m, -C_6H_5, -C_6H_4); EI-MS: *m/z* (relative abundance in %), 284 (M^+, 30); Elem. Anal.: Calculated for $C_{20}H_{16}N_2$ (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).

(1*E*, 2*E*)-N,3-diphenylprop-2-en-1-imine (sp-11)
M.P: 141-142 °C; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3010 (Aromatic-CH), 1599(C=C) 1610(C=N),1600,1455 (Aromatic, C=C); ^{1}H NMR (*$CDCl_3$*, ppm, 400 MHz): δ 8.25 (1H, d, J=10.0Hz, CH=N), 7.53-7.19(9H, m, -C_6H_5, -N-C_6H_5), 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).

(1*E*, 2E)-N-(3-chlorophenyl)-3-phenylprop-2-en-1-imine (sp-12)
M.P: 185-186 °C; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3030(Aromatic-CH), 1590(C=C) 1605(C=N), 1600,1450 (Aromatic, C=C); ^{1}H NMR (*CDCl$_3$*, ppm, 400 MHz): δ 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) ν $_{max}$ cm^{-1}: 3312 (N-H), 3040 (Aromatic, C-H), 1610 (C=N), 1600, 1455 (Aromatic, C=C); ^{1}H NMR (*DMSO-d$_6$*, ppm, 400 MHz): δ 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) ν $_{max}$ cm^{-1}: 3335 (O-H), 3082(Aromatic, C-H), 1600, 1450 (Aromatic, C=C), 1582 (C=C), 1610 (C=N); ^{1}H NMR (*DMSO-d$_6$*, ppm, 300 MHz): δ8.40 (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*)-[(2*E*)-{3-phenylprop-2-en-1-ylidene] amino} phenol (sp-15)
M.P: 125-126 °C; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3362(O-H), 3066(Aromatic, C-H), 1580, 1430 (Aromatic, C=C), 1582 (C=C), 1556 (C=N); ^{1}H NMR (*DMSO*, ppm, 500 MHz): δ 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) ν $_{max}$ cm^{-1}: 3480 (OH), 3062(Aromatic, C-H), 1622 (C=N), 1590, 1470 (Aromatic, C=C); ^{1}H NMR (*DMSO-d$_6$*, ppm, 400 MHz): δ 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% (Amane and Bouhdada, 2014).

1-[(E)-(2-hydroxyphenyl) methylidene] urea (sp-17)
M.P: 149-150°C; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3470 (O-H), 3060(Aromatic, C-H), 1632 (C=O), 1610 (C=N), 1590-1470 (Aromatic, C=C); ^{1}H NMR (DMSO-*d$_6$*, ppm, 500 MHz): δ 11.00(1H, s, 0H), 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) ν $_{max}$ 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); ^{1}H NMR (*CDCl$_3$*, ppm, 300 MHz): δ 12.00 (1H, s, OH), 10.2O (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) ν $_{max}$ cm^{-1}: 3450 (O-H), 3053 (Aromatic, C-H), 1622 (C=N), 1580, 1470 (Aromatic, C=C); ^{1}H NMR (*DMSO-d$_6$*, ppm, 400 MHz): δ7.90(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) ν $_{max}$ cm^{-1}: 3470 (O-H), 3025 (Aromatic-CH), 1590, 1470 (Aromatic**,** C=C), 1622 (C=N), 1242(C=S); ^{1}H NMR (*DMSO-d_6*, ppm, 300 MHz): δ 11.05 (lH, s, OH),9.08 (1H, s, CH=N); 7.98-6.90(4H, m, -C_6H_4OH), 2.2(2H, s, -$CSNH_2$); EI-MS: *m/z* (relative abundance in %), 180(M^+, 28); Elem. Anal.: Calculated for $C_8H_8N_2OS$ (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) ν $_{max}$ cm^{-1}: 3429 (O-H), 3062(C-H), 3040 (Aromatic, C-H), 1628 (C=N), 1540-1470 (Aromatic, C=C); ^{1}H NMR (*DMSO-d_6*, ppm, 500 MHz): δ 13.73 (2H, s, -OH), 8.90 (1H, s, CH=N), 7.65-6.82 (8H, m, -C_6H_4OH); EI-MS: *m/z* (relative abundance in %), 213. (M^+, 31); Elem. Anal.: Calculated for $C_{13}H_{11}NO_2$ (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) ν $_{max}$ cm^{-1}: 3425 (O-H), 3058(Aromatic, C-H), 1620 (C=N), 1590-1470 (Aromatic, C=C); ^{1}H NMR (*DMSO-d_6*, ppm, 400 MHz): δ 9.72 (1H, s, -OH), 8.65(1H, s, CH=N), 7.54-6.98(8H, m, -C_6H_4OH); EI-MS: *m/z* (relative abundance in %), 213(M^+, 30); Elem. Anal.: Calculated for $C_{13}H_{11}NO_2$ (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).

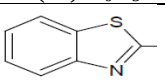
2-{(E)-[(3-chlorophenyl) imino] methyl} phenol (sp-23)
M.P: 179-180 °C; FT-IR (potassium bromide) ν $_{max}$ cm^{-1}: 3062(Aromatic, C-H), 1640 (C=N), 1590, 1470 (Aromatic, C=C); ^{1}H NMR (*DMSO-d_6*, ppm, 400 MHz): δ12.90 (1H, s, -OH), 8.66 (1H, s, CH=N), 7.72-6.91 (4H, m, -C_6H_4OH), 7.41-7.15 (4H, m, -C_6H_4Cl); EI-MS: *m/z* (relative abundance in %), 231 (M^+, 32); Elem. Anal.: Calculated for $C_{13}H_{10}ClNO$ (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) ν $_{max}$ cm^{-1}: 3430 (O-H), 3050 (Aromatic, C-H), 1605 (C=N), 1597, 1470 (Aromatic, C=C); ^{1}H NMR (*DMSO-d_6*, ppm, 500 MHz): δ 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^+, 36); Elem. Anal.: Calculated for $C_{14}H_{10}N_2OS$ (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).

$$R_1CHO + R_2NH_2 \xrightarrow[)))))\ ,\ -H_2O]{\text{Natural acid, } H^+} R_1HC = NR_2 \quad \text{(for sp-10, } R_1HC = NR_2\text{ -N = }CHR_1\text{)}$$

(a) If R_1 is C_6H_5-

Compounds code	Compound name	R_2
sp-1	***(E)*-1-benzylideneaniline**	C_6H_5-
sp-2	***(E)*-1-benzylidene-2-phenylhydrazine**	C_6H_5-NH-
sp-3	***(E)*-1-benzylidene urea**	NH_2CO-
sp-4	**(*E*)-1- benzylidene thiourea**	NH_2CS-
sp-5	**4-amino-*N*-[(*E*)-phenylmethylidene] benzene sulfonamide**	*p*-(NH_2) C_6H_5-SO_2-
sp-6	**4-[(E)-benzylideneamino] phenol**	*p*-(OH) C_6H_5-
sp-7	**2-[(E)-benzylideneamino] phenol**	*o*-(OH) C_6H_5-
sp-8	**(E)-*N*-(3-chlorophenyl)-1-phenylmethanimine (SP-08**	*m*-(Cl) C_6H_5-
sp-9	**(E)-*N*-(1,3-benzothiazol-2-yl)-1-phenylmethanimine**	
sp-10	**(*E, E*)-*N, N*'-benzene-1,4-diylbis(1-phenylmethanimine)**	C_6H_5-

(b) If R_1 is C_6H_5-HC=CH-

Compounds code	Compound name	R_2
sp-11	**(1*E*, 2*E*)-N,3-diphenylprop-2-en-1-imine**	C_6H_5-
sp-12	**(1*E*, 2E)-N-(3-chlorophenyl)-3-phenylprop-2-en-1-imine**	*m*-(Cl) C_6H_5-
sp-13	**(2E)-1-phenyl-2-[(2E)-3-phenylprop-2-en-1-ylidene] hydrazine**	C_6H_5-NH-
sp-14	**2-{(E)-[(2E)-3-phenylprop-2-en-1-ylidene] amino} phenol**	*o*-(OH) C_6H_5-
sp-15	**4-{(*E*)-[(2*E*)-{3-phenylprop-2-en-1-ylidene] amino} phenol**	*p*-(NH_2) C_6H_5-

(c) If R_1 is *O*-(OH) C_6H_5-

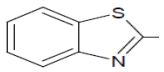
Compounds code	Compound name	R_2
sp-16	**2-[(E)-(phenylimino) methyl] phenol**	C_6H_5-
sp-17	**1-[(E)-(2-hydroxyphenyl) methylidene] urea**	NH_2CO-
sp-18	**4-{[(*E*)-(2-hydroxyphenyl) methylidene] amino} benzene sulfonamide**	*p*-(NH_2) C_6H_5-SO_2-
sp-19	**2-[(E)-(2-phenylhydrazinylidene) methyl] phenol**	C_6H_5-NH-
sp-20	**1-[(E)-(2-hydroxyphenyl) methylidene] thiourea**	NH_2CS-
sp-21	**2-[(E)-(2-hydroxybenzylidene) amino] phenol**	*o*-(OH) C_6H_5-
sp-22	**2-{(E)-[(4-hydroxyphenyl) imino] methyl} phenol**	*p*-(NH_2) C_6H_5-
sp-23	**2-{(E)-[(3-chlorophenyl) imino] methyl} phenol**	*m*-(Cl) C_6H_5-
sp-24	**2-[(E)-(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

Table 2. In vitro antibacterial activity of compounds **(sp-1 to sp-24).**

Microorganism	**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**
Gram positive bacteria													
Bacillus cereus	-	-	16	-	-	16	13	-	-	26	-	29	-
Bacillus subtilis	-	-	20	-	-	14	13	-	-	34	-	20	13
Bacillus thuringiensis	-	-	10	-	-	19	10	-	-	22	-	19	-
Corynebacterium diphtheria	-	10	-	-	-	-	21	-	-	25	10	12	-
Corynebacterium hoffmanii	-	-	-	-	-	-	16	-	-	29	-	-	-
Corynebacterium xerosis	-	26	-	-	-	-	15	-	-	27	-	09	-
Staphylococcus aureus	-	18	-	-	-	-	-	-	-	-	11	07	-
Staphylococcus aureus (MRSA)	-	-	-	-	-	-	-	-	-	-	-	09	-
Staphylococcus epidermidis	-	-	-	-	-	14	-	-	18	20	-	21	-
Staphylococcus saprophyticus	-	20	-	-	-	-	-	-	-	-	-	-	-
Streptococcus feacalis	-	19	25	-	-	-	20	-	16	-	09	-	-
Streptococcus pyogenes	-	24	21	-	-	-	13	-	21	-	-	-	-
Streptococcus saprophyticus	-	-	-	-	-	19	-	-	20	17	-	15	-
M.smegmatis	-	-	-	-	-	-	-	-	-	-	-	-	-

Microorganism	**sp-14**	**sp-15**	**sp-16**	**sp-17**	**sp-18**	**sp-19**	**sp-20**	**sp-21**	**sp-22**	**sp-23**	**sp-24**	**Gentamycin**
Bacillus cereus	-	-	12	-	-	-	-	-	-	-	-	18
Bacillus subtilis	24	15	10	10	31	-	-	-	-	-	-	18
Bacillus thuringiensis	-	-	14	-	-	-	-	-	-	-	-	22
Corynebacterium diphtheria	-	-	-	-	32	-	13	25	18	16	-	18
Corynebacterium hoffmanii	-	-	-	-	-	-	-	-	-	-	-	25
Corynebacterium xerosis	-	-	-	09	20	-	-	-	24	18	-	18
Staphylococcus aureus	-	-	-	-	35	-	08	10	22	10	-	25
Staphylococcus aureus (MRSA)	-	-	-	-	35	-	-	-	-	-	-	18
Staphylococcus epidermidis	15	25	21	-	29	07	10	-	-	20	-	25
Staphylococcus saprophyticus	-	06	-	-	-	-	-	-	15	15	-	18
Streptococcus feacalis	-	-	18	12	20	-	-	-	10	10	-	18
Streptococcus pyogenes	-	-	-	-	-	-	-	-	20	20	-	18
Streptococcus saprophyticus	-	-	22	-	-	-	09	-	-	-	-	22
M.smegmatis	-	-	-	-	-	-	-	-	-	-	-	22

Continue **Table -2**

Microorganism	**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**
Gram negative bacteria													
Acinetobacter baumanii	-	-	11	-	-	18	-	-	-	-	-	-	-
Aeromonas hydrophila	-	-	13	-	-	13	-	-	-	-	-	-	-
Campylobacter jejuni	-	-	-	-	-	-	15	-	-	14	-	-	-
Campylobacter coli	-	-	-	-	-	-	15	-	-	17	-	-	-
Enterobacter aerogenes	-	-	18	-	-	-	27	-	19	27	-	35	-
Escherichia coli	-	-	-	-	-	-	-	-	18	-	-	34	-
Escherichia coli ATCC 8739	-	-	-	-	-	-	-	-	20	-	-	31	-
E. coli MDR	-	-	-	-	-	-	-	-	17	-	-	31	-
Enteropathogenic Escherichia coli III	-	-	-	-	-	-	-	-	-	-	-	-	24
Helicobacter pylori	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemophilus influenza	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	34	-	-	10
Salmonella paratyphi A	-	-	-	-	-	-	-	-	-	32	-	-	25
Salmonella paratyphi B	-	-	-	-	-	-	-	-	-	34	10	-	27
Shigella flexeneri	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella dysenteriae	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella sonnei	-	-	-	-	-	-	-	-	-	-	10	15	25
Serratia marcesens	-	-	-	-	-	-	-	-	-	-	-	15	-
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	28	10	-	15
Vibrio cholera	-	-	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**
Acinetobacter baumanii	12	-	21	-	30	-	-	-	-	-	-	22
Aeromonas hydrophila	-	-	17	-	-	-	-	-	-	-	-	10
Campylobacter jejuni	-	-	15	-	-	-	-	-	-	-	-	18
Campylobacter coli	-	-	13	-	-	-	-	-	-	-	-	15
Enterobacter aerogenes	-	-	-	-	-	-	-	-	-	-	-	16
Escherichia coli	14	-	-	-	09	-	-	-	-	-	-	15
Escherichia coli ATCC 8739	-	-	-	-	-	-	-	-	-	-	-	16
E. coli MDR	-	08	-	-	-	-	-	-	-	-	-	17
Enteropathogenic Escherichia coli III	-	-	-	26	25	-	-	-	-	-	-	20
Helicobacter pylori	-	-	-	-	-	-	-	-	-	-	-	10
Hemophilus influenza	-	-	-	-	-	-	-	-	-	-	-	10
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	12
Proteus mirabilis	25	10	-	-	-	-	-	-	-	-	-	18
Salmonella typhi	12	11	-	-	32	11	-	-	-	-	-	18
Salmonella paratyphi A	16	14	-	25	20	10	-	-	-	-	-	18
Salmonella paratyphi B	14	25	-	-	25	10	-	-	-	-	-	20
Shigella flexeneri	-	-	-	-	-	-	-	-	-	-	-	10
Shigella dysenteriae	-	-	-	-	-	-	-	-	-	-	-	10
Shigella sonnei	13	23	-	20	26	10	-	-	-	-	-	17
Serratia marcesens	-	-	-	-	-	-	-	-	-	-	-	12
Klebsiella pneumoniae	20	25	25	07	25	17	-	-	-	-	-	10
Vibrio cholerae	25	-	-	-	-	15	-	-	-	-	-	18

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

Table-3: MIC values of compounds **(sp-1 to sp-24).**

Microorganism	**MIC values of compounds (µg/mL)**																			
Gram positive bacteria	**sp-2**	**sp-3**	**sp-6**	**sp-7**	**sp-9**	**sp-10**	**sp-11**	**sp-12**	**sp-13**	**sp-14**	**sp-15**	**sp-16**	**sp-17**	**sp-18**	**sp-19**	**sp-20**	**sp-21**	**sp-22**	**sp-23**	**Gentamycin (µg/Disc)**
Bacillus cereus	-	40	40	178	-	26	-	28	-	-	-	94	-	-	-	-	-	-	-	0.8
Bacillus subtilis	-	84	84	124	-	25	-	92	40	25	40	80	84	20	-	-	-	-	-	0.8
Bacillus thuringiensis	-	64	45	190	-	76	-	100	-	-	-	78	-	-	-	-	-	-	-	0.4
Corynebacterium diphtheriae	75	-	-	64	-	24	75	100	-	-	-	-	-	20	-	50	20	30	30	0.8
Corynebacterium hoffmanii	-	-	-	166	-	22	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2
Corynebacterium xerosis	25	-	-	110	-	80	-	-	-	-	-	-	-	20	-	-	-	18	30	0.8
Staphylococcus aureus	45	-	-	-	-	-	70	-	-	-	-	-	-	18	-	-	50	20	45	0.2
Staphylococcus aureus (MRSA)	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-	0.8
Staphylococcus epidermidis	-	-	40	-	178	88	-	26	-	45	25	24	-	22	-	-	-	-	25	0.2
Staphylococcus saprophyticus	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	30	0.8
Streptococcus feacalis	45	25	-	100	56	-	-	-	-	-	-	82	70	20	-	-	-	70	65	0.8
Streptococcus pyogenes	25	28	-	90	24	-	-	-	-	-	-	-	-	-	-	-	-	25	25	0.8
Streptococcus saprophyticus	-	-	80	-	100	26	-	100	-	-	-	14	-	-	-	-	-	-	-	0.4
M.smegmatis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4
Gram negative bacteria																				
Acinetobacter baumanii	-	88	64	-	-	-	-	-	-	78	-	60	-	25	-	-	-	-	-	0.4
Aeromonas hydrophila	-	34	44	-	-	-	-	-	-	-	-	92	-	-	-	-	-	-	-	12.5

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

key: values are in zone of inhibition **(µg/mL)**, **(-)** indicates inactivity.

Table – 4: *In vitro* anti-fungal activity of compounds **(sp-1 to sp-24).**

Microorganism	**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**
Aspergillus flavis	12	09	-	-	21	-	-	-	-	-	16	14	-
Aspergillus nigar	17	-	-	-	15	-	-	-	-	-	15	15	15
Penicillium species	-	-	-	-	13	-	-	-	-	-	13	24	-
Rhizopus species	-	-	-	-	-	-	-	-	-	-	16	21	-
Candida albicans	14	08	-	-	16	-	33	-	21	23	-	14	-
Candida albicans ATCC	-	-	-	-	17	-	19	-	24	28	-	16	-
Candida galbrata	-	-	-	-	-	-	11	-	16	20	-	12	-
Candida tropicalis	-	-	-	-	-	-	25	-	21	29	-	16	15
Candida kruzei	-	-	-	-	-	-	21	-	32	28	-	16	-
Sachromyces cerevisiae	-	09	-	-	-	-	-	-	-	-	-	-	-
Fusarium species	-	-	-	-	-	-	-	-	-	-	-	-	-
Helminthosporum	-	-	-	-	-	-	-	-	-	-	-	-	-
Microsporum canis	-	-	-	-	14	-	-	-	27	18	15	20	-
Microsporum cereus	-	-	-	-	-	-	-	-	-	-	-	-	20
Microsporum gypsium	15	-	-	-	-	-	-	-	-	-	10	10	-
Neurospora	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichophyton rubrum	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichophyton tonsurans	-	-	-	-	18	-	-	-	-	-	25	-	25
Trichophyton mentagrophytes	-	-	-	-	-	-	-	-	-	-	-	-	-

Microorganism	**sp-14**	**sp-15**	**sp-16**	**sp-17**	**sp-18**	**sp-19**	**sp-20**	**sp-21**	**sp-22**	**sp-23**	**sp-24**	**Gresiofulvin**
Aspergillus flavis	16	-	-	18	-	12	-	-	-	08	-	18
Aspergillus nigar	17	-	15	20	-	12	-	-	08	08	-	18
Penicillium species	15	10	18	18	-	09	-	-	-	-	-	22
Rhizopus species	-	-	-	15	-	-	-	-	-	-	-	22
Candida albicans	-	-	13	-	-	-	-	-	10	10	-	18
Candida albicans ATCC	-	-	15	-	-	-	-	-	-	-	-	22
Candida galbrata	-	-	-	-	-	-	-	-	-	-	-	22
Candida tropicalis	20	12	-	-	-	14	-	-	-	-	-	22
Candida kruzei	-	-	-	-	-	-	-	-	-	-	-	22
Sachromyces cerevisiae	-	-	-	-	-	-	-	-	16	08	-	22
Fusarium species	-	-	-	-	-	-	-	-	-	-	-	18
Helminthosporum	-	-	-	-	-	-	-	-	-	-	-	18
Microsporum canis	-	-	-	-	-	-	-	-	-	-	-	18
Microsporum cereus	16	-	-	15	-	20	-	-	-	-	-	22
Microsporum gypsium	-	-	-	-	-	-	-	-	-	-	-	22
Neurospora	-	-	-	-	-	-	-	-	-	-	-	22
Trichophyton rubrum	-	-	-	-	-	-	-	-	-	-	-	10
Trichophyton tonsurans	-	-	-	-	-	25	-	-	-	-	-	10
Trichophyton mentagrophytes	-	-	-	-	-	-	-	-	-	-	-	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 (µg/ml)**															
	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**	**Gresiofulvin (µg/Disc)**
Aspergillus flavis	40	62	-	-	-	100	120	-	200	-	-	200	78	-	-	0.8
Aspergillus nigar	44	60	-	-	-	54	200	32	120		60	166	78	-	-	0.8
Penicillium species	-	66	-	-	-	60	44	-	200	180	94	18	-	-	-	0.4
Rhizopus species	-	-	-	-	-	94	50	-	-	-	-	24	-	-	-	0.4
Candida albicans	78	88	14	120	94	-	242	-	-	-	72	-	-	180	200	0.8
Candida albicans ATCC	-	84	16	88	48	-	320	-	-	-	40	-	-	-	-	0.4
Candida galbrata	-	-	52	90	180	-	214	-	-	-	-	-	-	-	-	0.4
Candida tropicalis	-	-	44	210	200	-	204	30	80	70	-	-	50	-	-	0.4
Candida kruzei	-	-	40	22	80	-	144	-	-	-	-	-	-	-	-	0.4
Sachromyces cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	55	-	0.4
Fusarium species	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8
Helminthosporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8
Microsporum canis	-	32	-	140	12	220	10	-	-	-	-	-	-	-	-	0.8
Microsporum cereus	-	-	-	-	-	-	-	22	180	-	-	30	40	-	-	0.4
Microsporum gypsium	98	-	-	-	-	55	200	-	-	-	-	-	-	-	-	0.4
Neurospora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4
Trichophyton rubrum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5
Trichophyton tonsurans	-	98	-	-	-	20	-	20	-	-	-	-	23	-	-	12.5
Trichophyton mentagrophytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4

key: values are in zone of inhibition **(µg/mL)**, **(-)** indicates inactive.

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

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

Key: SD= standard deviation, EC_{50}= effective concentration of compounds that scavange 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 oftyrosinase IC_{50} ±SEM (µM)	**Inhibition ofurease IC_{50} ±SEM (µM)**		**Inhibition of tyrosinase IC_{50} ±SEM (µM)**	**Inhibition ofurease IC_{50} ±SEM (µM)**
sp-1	100.0±0.14	NS	**sp-14**	NS	NS
sp-2	95.2±0.59	135.1±0.69	**sp-15**	NS	NS
sp-3	231.0±0.12	NS	**sp-16**	NA	NS
sp-4	NA	42.7±0.56	**sp-17**	NA	NS
sp-5	NA	NS	**sp-18**	98.2±0.53	100.0±0.94
sp-6	NA	98.0±0.36	**sp-19**	97.2±0.55	NS
sp-7	100.0±0.22	38.6±0.86	**sp-20**	98.2±0.09	NS
sp-8	NA	NS	**sp-21**	NS	68.7±0.62
sp-9	NA	NS	**sp-22**	NS	NS
sp-10	NA	49.3±0.38	**sp-23**	NS	55.5±0.31
sp-11	NA	NS	**sp-24**	122.2±0.31	NS
sp-12	NA	NS	Kojic acid	16.67± 0.06	-
sp-13	NS	NS	Thiourea	-	21.6±0.12

Key: SEM= standard error of the mean, IC_{50}= 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 synthesized 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 **Table1**.

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 s**p-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_{50} 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_{50} = 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_{50} 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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