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Microwave-assisted rapid and green synthesis of novel substituted trifluoro schiff bases and their biological evaluation

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Abstract
A novel approach for the synthesis of substituted (E)-2-(((3-(trifluoromethyl) phenyl)imino) methyl) phenol (3a-e) were synthesized by the reaction of Trifluoro aromatic aldehyde and aromatic amine using a catalytic amount of Cu-HAP as catalyst under microwave irradiation. This reaction is rapid, efficient, and solvent-free and involves the one-pot synthesis of Schiff bases under microwave irradiation. The catalyst was quantitatively recovered from reaction mixture by simple filtration and reused for three cycles with consistence activity. All these compounds have been characterized by modern spectral techniques such as IR, ^{1}H NMR, Mass etc. Evaluation of synthesized compounds for antimicrobial activity against specific bacterial strains like 1) *Escherichia coli* 2) *Klebsiella pneumoniae* 3) *Bacillus subtilus* 4) *Staphylococcus aureus*.

Keywords: Cu-HAP, Green chemistry, substituted (E)-2-(((3-(trifluoromethyl) phenyl) imino)methyl) phenol (3a-e) Antimicrobial activity

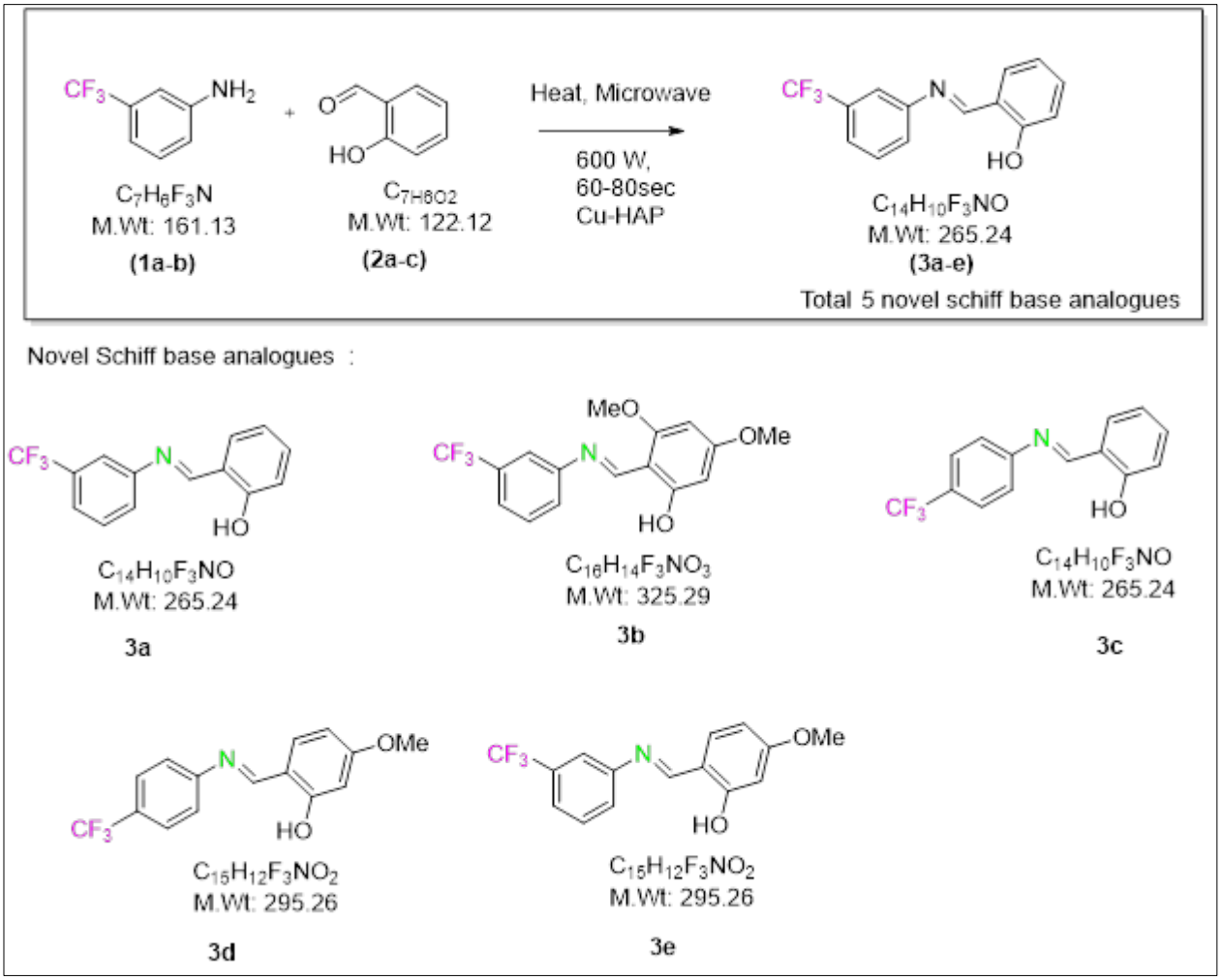
Introduction
In the past few decades, the synthesis of new heterocyclic compounds has been a topic of great attention due to their extensive applicability. Green chemistry is the global term for the development and modification of chemical processes [1-2]. The chemical products are prepared using conventional and nonconventional synthesis processes. However In conventional processes, it takes a longer time, resulting in a higher cost and requires some hazardous solvents and reagents. To overcome these drawbacks, the nonconventional processes are the best alternative to perform organic transformations. In nonconventional processes, microwave-assisted synthesis has become an interesting method for researchers in the past few decades. The synthesis of organic molecules using microwaves has proven to be economical, clean, and environmentally friendly with a shorter reaction time [3-4].
In this context, development of novel compounds with a simple approach holds lot of promise keeping in view of the alarming situation. Among the several chemical compounds with antimicrobial activity, Schiff base compounds are interesting candidates due to the ease with which they can be prepared. Chemically, Schiff bases are the compounds that have azomethine -CH=N functionality in their chemical structure. They are generally prepared using primary amines and compounds having carbonyl group by condensation reaction [5]. Schiff bases are play an important role in the field of agrochemical, medicinal and other disciplines as they exhibit many medicinal properties. Many heterocyclic compounds containing Schiff bases in their molecular structure have been synthesized as “privileged” scaffolds to produce active pharmaceutical ingredients. In this context, Schiff base derivatives show diversified applications in antimicrobial activity and antitumor activity and have excellent chelating ability with metals [6–8]. The Schiff base analogues shows no cytotoxic activity compared to its metal complexes derivatives [9]. The various organic conversions using a microwave mediated reaction are reported in the literature [10].
In this work, we report a microwave-assisted synthesis of novel Schiff bases by using Cu-HAP as a green catalyst. This methodology has a faster reaction rate, shorter reaction time, easy workup, and excellent to good yield. When one biologically active derivative is connected to another active moiety, the resultant molecule generally has increased potency. Hence in the present study the two pharmacophores, i.e. Trifluoro aromatic amines and aromatic aldehydes moieties are connected to may obtain effective, specific and less toxic antimicrobial agents.

The chemical and pharmaceutical industries are always under pressure to progress more environmentally friendly organic reaction methodologies. In this regard, the increasing demand for cleaner procedures supported by stringent environmental laws necessitates use of eco-friendly and discriminating catalysts. Hydroxyapatites (HAP) possess Ca^{2+} sites surrounded by PO_4^{3-} tetrahedra parallel to the hexagonal axis, which have attracted considerable interest in view of their potential usefulness as biomaterials, adsorbents, and ion exchangers [11]. Apatites are metal basic phosphates, various kinds of cations and anions can be readily introduced into their frame work due to their large ion-exchange ability and such exchanged apatites are already in use in several organic transformations.

Results and Discussion

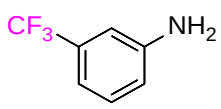
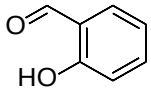
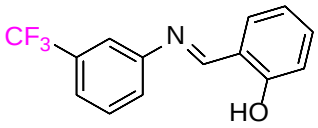
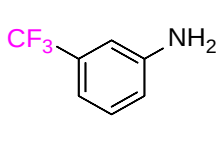
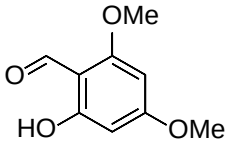
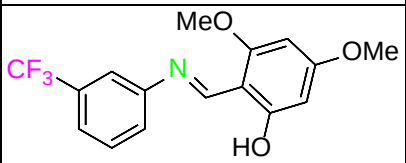
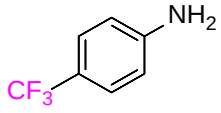
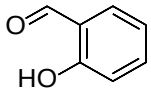
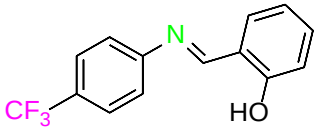
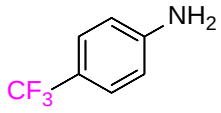
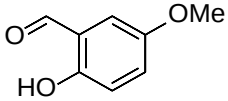
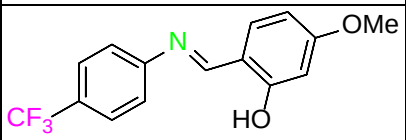
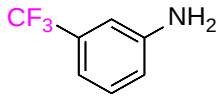
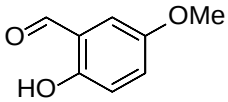
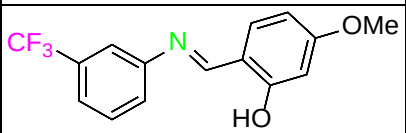
Synthesis of (E)-2-(((3-(trifluoromethyl) phenyl) imino) methyl) phenol (3a-e): The reaction of Trifluoro anilines **(**1a-b) with Substituted salicyaldehydes (2a-c) in the presence of Cu-HAP and The reaction mixture was kept in a microwave at 600 W. The time required to prepare Schiff bases under microwave irradiation varies between 60-80 seconds for all entries and the title (3a-e) compounds afford in almost good to excellent yields.

1. Scheme



In the IR spectrum 3a, peaks were observed at 1682 and 1626 cm^{-1}(-N=CH-), 2884 cm^{-1} for aromatic stretching (Ar-H), 3075 cm^{-1} for aromatic –OH strecthing. In the ^{1}H-NMR of spectrum 3a, the newly formed (E)-2-(((3-(trifluoromethyl)phenyl)imino)methyl)phenol protons of Ar-OH appeared as a singlet at δ 12.81and characteristic proton of imine (-N=CH-), appeared as singlet at δ 8.61. In the ^{13}C-NMR spectrum of 3a, the carbon signal assignments are as follows 164.5, 161.2, 151.6, 133.9, 132.7, 126.6, 122.7, 119.4, 118.8, and 117.4. In the mass spectrum of 3a, molecular ion peak was observed at m/z 266.18 [M+H].

Table 1: Microwave-Assisted Synthesis of Schiff Bases in the Presence of Cu-HAP catalyst.

Entry	Aromatic Amines	Aromatic Aldehydes	Schiff bases	% Yield	Time (Secons)
1				88	60
2				82	80
3				81	60
4				83	80
5				89	78

Antibacterial activity:

"All the synthesized substituted (E)-2-(((3-(trifluoromethyl)phenyl)imino)methyl)phenol (Table 1, Figure 1) 3a-e were screened for their antibacterial activity against different types of bacterial strains" they are "Gram positive bacterial strains of *Bacillus subtillis and Staphylococcus aureus* Gram negative bacterial strains of *Escherichia coli and Klebsiella pneumoniae"* at a concentration of 30 µg/mL and 50 µg/mL [11-14]. Some of the synthesized compounds showed high activity and some showed moderate activity compared to standard drug *Ampicillin* at a concentration of 30 µg/mL and 50 µg/mL.

The antibacterial activity of compound 3b, (R2 = -OCH_3, R3 = -OCH_3) 3d (R2 = -OCH_3, R3 = -OCH_3), 3e (R3 = -OCH_3) showed good zone of inhibition against *Echerichia coli, Klebsiella pneumoniae , Bacillus subtillis and Staphylococcus aureus* compared to the standard drug at a concentration of 30 µg/mL and 50 µg/mL. Whereas the compounds 3a, 3c, and 3e were showing moderate activity against all the bacterial strains when compared to standard drug. It leads us to conclude that from Table 1 and Figure 1 mono methoxy, dimethoxy, and m-CF3 substituted compounds showed higher zone of inhibition could be attributed the presence of an electron donating groups when compared with other compounds. Furthermore, substitutions like other groups like -H, did not provide any significant change in the levels of activity against bacterial strains.

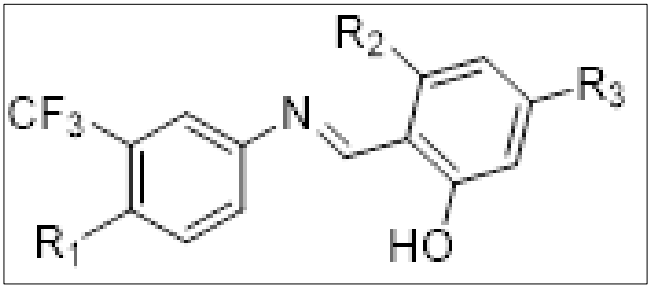


Table 2: Evaluation of anti-bacterial activity of synthesized (E)-2-(((3- (trifluoromethyl)phenyl)imino)methyl)phenol (3a-e)

Comp.	R	R1	R2	R3	Gram Negative				Gram Positive			
					E. coli		*K. pneumonia*		*B. subtilus*		*S. aureus*	
					30 µl	50 µl	30 µl	50 µl	30 µl	50 µl	30 µl	50 µl
3a	H	H	H	H	3	-	4	2	4	2	-	2
3b	H	H	OMe	OMe	4	7	8	10	4	5	4	8
3c	H	CF3	H	H	3	3	6	4	6	3	4	3
3d	H	CF3	OMe	OMe	2	8	4	8	4	2	6	7
3e	H	H	H	OMe	4	8	2	6	-	6	4	3
Ampicillin					8	12	7	12	9	11	8	14

In vitro Antibacterial activity of Compounds (Concentration used 30 µg/30 µl and 50 µg/50 µl)

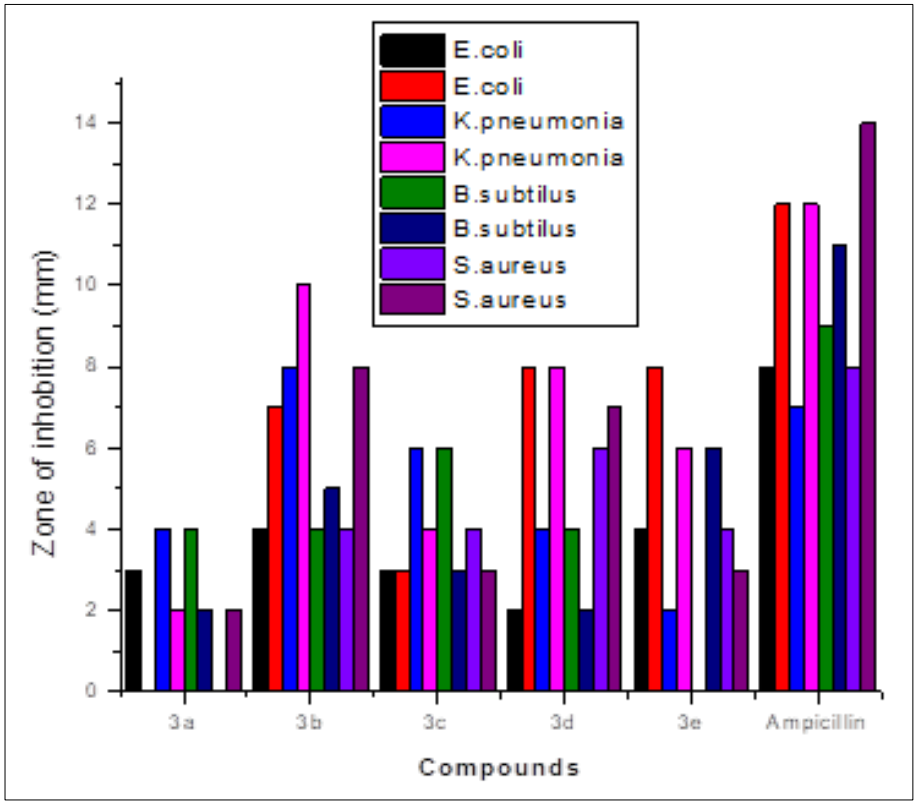


Fig 1: Antibacterial activity of compounds 3a-e against *Bacillus subtillis*, *Staphylococcus aureus Escherichia coli* and *Klebsiella pneumonia*.

Conclusion

In conclusion, we have developed a new and efficient a green methodology for the synthesis of Schiff base derivatives i.e substituted (E)-2-(((3- (trifluoromethyl) phenyl) imino) methyl) phenol in excellent yields using Cu-HAP as catalyst at microwave oven. The reaction was carried out without solvent in a conical flask.

Compared to other synthetic methods, this new method has the lead of good yields, inexpensive reagents, easily available, easy workup, mild reaction conditions, environmentally friendly reaction conditions, reusable catalyst makes this method simple, clean, practical, and economically viable. The *in vitro* antibacterial evaluation showed that most of the synthesized substituted novel derivatives exhibited moderate to good zone of inhibition. From the results of antibacterial activity of compounds it is interesting to note that substituent's like methoxy and trifluoro substituent's shows better antibacterial activity compared to other substituted compounds. Noticeably, compound 3b, 3d, and 3e were most potent compound *in vitro* activity against bacterial and fungal strains.

Experimental Section

Chemistry

All reactions were carried out under nitrogen atmosphere in oven-dried glassware with magnetic stirring**.** All the chemicals and solvents were purchased from Sd fine chemicals, Bombay, India. Solvents were purified and dried according to the standard procedures. Silica gel (60–120 mesh) for column chromatography was purchased from M/s Acme Synthetic Chemicals (Mumbai, India) and pre-coated TLC plates (Silica gel 60F254) were purchased from Merck (Darmstadt, Germany). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 and 100 MHz, respectively, and TMS was used as an internal standard. Chemical shifts relative to TMS as internal standards were given as δ values in ppm. Mass spectra were recorded using electron spray ionization on Waters e2695 Separators module (Waters, Milford, MA, USA) mass spectrometer. IR spectra were recorded on a Fourier transform (FT-IR), USA (Perkin-Elmer model 337) instrument. The melting points were determined on a Barnstead Electro Thermal 9200 Instrument.

General procedure for the synthesis of (E)-2-(((3-(trifluoromethyl)phenyl)imino)methyl)phenol under microwave irradiation (3a-e)

Aromatic aldehyde(1 mmol), Trifluoro aromatic amine (1 mmol), and Cu-HAP (10 mol%) were added to a 100 mL conical flask and flask covered with aluminum foil. The reaction mixture was kept in a microwave at 600 W for 60-80s for synthesis of Schiff bases. This reaction was carried out without any solvent. The progress of the reaction monitored by TLC. After the completion of reaction, the reaction mass was diluted with using ethyl acetate (15 mL). the reaction mixture was cooled to rt and the catalyst was filtered. Then, this organic layer was dried using Na2SO4.This organic layer was evaporated on a rotatory evaporator to obtain the crude product. This crude product was purified by column chromatography on silica gel (60−120 mesh) as astationary phase and a mobile phase of 0−5% n-hexane/ethyl acetate. The purified Schiff base derivative was characterized by FTIR, 1H NMR, 13C NMR, and GC−MS.

i) **(*E*)-2-(((3-(trifluoromethyl)phenyl)imino)methyl)phenol (3a): Off** White solid, m.p. 174^{0}C. Yield 88%. IR (KBr) cm^{-1}: 3064, 2970, 2880, 1688, 1626, 1570, 1508, 1080, 1042. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 12.81 (s, 1H), 8.61 (s, =CH, 1H), 7.81 (d, 2H), 7.52-7.42 (m,4H), 7.11(d, 1H), 6.98(d, 1H) . ^{13}C-NMR ($CDCl_3$, 400MHz): δ 164.5, 161.2, 151.6, 133.9, 132.7, 126.6, 122.7, 119.4, 118.8, 117.4. MASS (ESIMS): 266.18 [M+H].

ii) **(*E*)-3,5-dimethoxy-2-(((3-(trifluoromethyl)phenyl)imino)methyl)pheno (3b): Off**

White solid, m.p. 183 °C. Yield 82%. IR (KBr) cm^{-1}: 3054, 2973, 2881, 1686, 1628, 1572, 1509, 1082, 1044. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 14.41 (s,1H), 8.96 (s, =CH, 1H), 7.52-7.41 (m, 4H), 6.10 (s,1H), 5.92(s, 1H), 3.84(s, 6H) . ^{13}C-NMR ($CDCl_3$, 400MHz): δ 166.78, 165.9, 161.2, 158.2, 148.7, 132.2, 125.2, 124.3, 122.5, 117.7, 103.1, 93.6, 90.1, 55.67. MASS (ESIMS): 326.24 [M+H].

iii) **(*E*)-2-(((4-(trifluoromethyl) phenyl)imino) methyl)phenol) phenol (3c):** Off White solid, m.p. 178^{0}C. Yield 81%. IR (KBr) cm^{-1}: 3066, 2971, 2883, 1684, 1626, 1572, 1509, 1084, 1044. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 12.82 (s,1H), 8.62 (s, =CH, 1H), 7.54-7.49 (m, 3H), 7.43-7.38 (m,3H), 7.02(d, 1H), 6.98(d, 1H) . ^{13}C-NMR ($CDCl_3$, 400 MHz): δ 164.2, 161.1, 149.1, 133.8, 132.8, 131.7, 125.2, 123.3, 119.3, 118.9, 118.0, 117.4, MASS (ESIMS): 266.18 [M+H].

iv) **(*E*)-3,5-dimethoxy-2-(((3-(trifluoromethyl) phenyl)imino) methyl)pheno (3d):** Off White solid, m.p. 182^{0}C. Yield 81%. IR (KBr) cm^{-1}: 3056, 2972, 2882, 1684, 1626, 1569, 1509, 1081, 1042. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 13.31 (s,1H), 8.52 (s, =CH, 1H), 7.66-7.64 (m, 1H), 7.32-7.25 (m,3H), 6.52(s, 1H), 3.85(s, 3H) . ^{13}C-NMR ($CDCl_3$, 400MHz): δ 164.5, 163.8, 163.2, 151.6, 134.0, 128.3, 126.5, 112.8, 107.6, 101.0, 55.5. MASS (ESIMS): 296.21 [M+H].

v) **(*E*)-5-methoxy-2-(((3-(trifluoromethyl) phenyl)imino) methyl)phenol (3e):** Light cream solid, m.p. 186 °C. Yield 89%. IR (KBr) cm^{-1}: 3059, 2971, 2878, 1688, 1629, 1569, 1519, 1086, 1046. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 13.31 (s,1H), 8.52 (s, =CH, 1H), 7.53-7.41 (m, 3H), 7.42 (m,1H), 7.29 (d, 1H), 6.52(m, 1H), 3.84(s, 3H) . ^{13}C-NMR ($CDCl_3$, 400MHz): δ 164.3, 163.7, 163.0, 149.2, 133.9, 1316, 125.2, 122.5, 118.0, 112.8, 107.5, 101.0, 55.51 MASS (ESIMS): 296.22 [M+H].

1. Antibacterial activity by disc diffusion method

The antibacterial activity of synthesized compounds was conducted against two gram positive bacteria *viz*., *Bacillus subtillis and Staphylococcus aureus* Gram negative bacterial strains of *Escherichia coli and Klebsiella pneumoniae"* by using disc diffusion method. Ampicillin sodium was employed as standard to compare the results.

[A] Preparation of Mueller-Hinton agar

1) Beef extract: 300 g
2) Acid hydrolysate of casein: 17.5 g
3) Starch: 1.5 g
4) Agar: 17 g
5) Distilled water: 1 Lit.

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar was dissolved. This was then sterilized in an autoclave at 15 lbs pressure and 121 ^{o}C for fifteen minutes. The sterilized medium (20 mL) was poured in sterilized Petri dishes under aseptic condition, allowing them to solidify on a plane table.

[B] Preparation of Anti-bacterial Solution

All the compounds were dissolved in DMSO and proper drug controls were used. Compound was taken at concentration of 1mg/ml for testing anti-bacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the zones of inhibition were measured in mm. The tabulated results represent the actual readings against the control.

[C] Test cultures

Following common standard strains were used for screening the antibacterial activities:

- Escherichia coli [Gram negative] MTCC – 443
- Klebsiella pneumoniae [Gram negative] MTCC – 424
- Bacillus subtilis, [Gram positive] MTCC – 96
- Staphylococcus aureus, [Gram positive] MTCC – 442

[D] Inoculum's preparation

The inoculum was standardized at 1× 106 CFU/ml comparing with turbidity standard (0.5 MacFarland tube)

2. Antifungal activity by paper disc method

All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were *Aspergillus niger*, *Aspergillus foetidus*, *Candida albicans* and *Candida rogosa*. Antifungal activity was tested at the following concentrations 50 µg/ µl and Fluconozole was employed as standard to compare the results.

[A] Medium Composition

Potato infusion	200 gm
Dextrose	20 gm
Agar	20 gm
Distilled water	1 liter

The medium was sterilized in the autoclave at 121 °C (15 lbs) pressure for 15 min. The medium was cooled to 45-50 °C and poured in 20ml volume in each Petridish and allowed to solidify. The antifungal activity screening is done by the paper disc method.

Testing equipment's

Tubes of uniform size, paper discs and petridishes were employed.

Maintenance of sterility

All required apparatus were sterilized before use and necessary precautions were taken to avoid contamination.

Preparation of sample solutions

The testing compounds 1mg was dissolved in 1 ml of DMSO. This gives the concentration of the sample compounds as 1 µg/1 µl. Two Different dilutions such as 30 µg /30 µl and 50 µg/ 50 µl were prepared from the sample solution.

Anti-fungal testing

Anti-fungal activity the synthesized compounds were screened for their anti-fungal activity against four fungi. They are *Aspergillus niger*, *Aspergillus foetidus*, *candida albicans* and *Candida rogosa*. Potato Dextrose Agar (PDA) medium was prepared and about 15 ml of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of seven day old culture of the test fungi was placed at the center of the petriplate and incubated at 26 °C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Fluconazole was used as the standard. All the synthesized compounds were tested (at the dosage of 50 µl of the novel compounds/ petriplate, where concentration was 1 mg/ml) by poisoned food technique.

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