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Research Article

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Antibacterial, Antifungal activities on derivatives of Tyrosine and Tryptophan

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Abstract

The Schiff bases of tyrosine and tryptophan (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4-hydroxyphenyl)ethyl)imino)methyl)-2-hydroxybenzoic acidwere prepared and characterized by physical and analytical data, FTIR , ^{1}H NMR, ^{13}C NMR spectra and were screened against gram positive bacteria *Staphylococcus aureus, Bacillus subtilis* and gram negative bacteria *Escherichia coli, Klebsiella aerogenes* for antibacterial activity and were screened against *Aspergillus niger* and *Candida albicans* for antifungal activity by disc diffusion method. Ciprofloxacin and Nystatin were used as standard for bacteria and fungi.

Keywords: Tyrosine, tryptophan, antibacterial activity*, antifungal activity*, 3,5-diiodosalicylaldehyde, 5-formylsalicylic acid, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella aerogenes, Aspergillus niger, Candida albicans.* Ciprofloxacin and Nystatin

Introduction

Nobel laureates Alexander Fleming warned of the dangerous of antimicrobial resistance[1-4]in his Nobel Prize speech in 1945. Klebsiella, a common bacteria and fungi can cause a wide range of conditions as the report on super bugs highlights. They fit into a wider group of bacteria and fungi with an acronym *E.coli, Candida albicans* owing to their ability to avoid the effects of the antimicrobials used against them. They stand for the name of the bacteria and fungi group members. These infections were increasingly being acquired in a small and big dispensary. Antibiotic resistance can be mitigated to some extent with good hand and body hygiene taking antimicrobials prescribed by a doctor and completing the full course if one has to take them. Medical expert believe this will help reduce the problem of resistance. Other area of key concern is to discourage dispensing antimicrobial of the counter. This may be small steps but they will have an impact and may avert a future where treatable disease become fatal. In recent years[5-7]derivatives of dapsone and amino acid were found have potential non antibiotic resistance antibacterial, antiviral and anticancer properties. Our study clearly focuses on the antibacterial antifungal activities of derivatives of tyrosine and tryptophan derived[8-19] from 3,5-diiodosalicylaldehyde, 5-formyl salicylic acid.

Materials and Methods

Materials

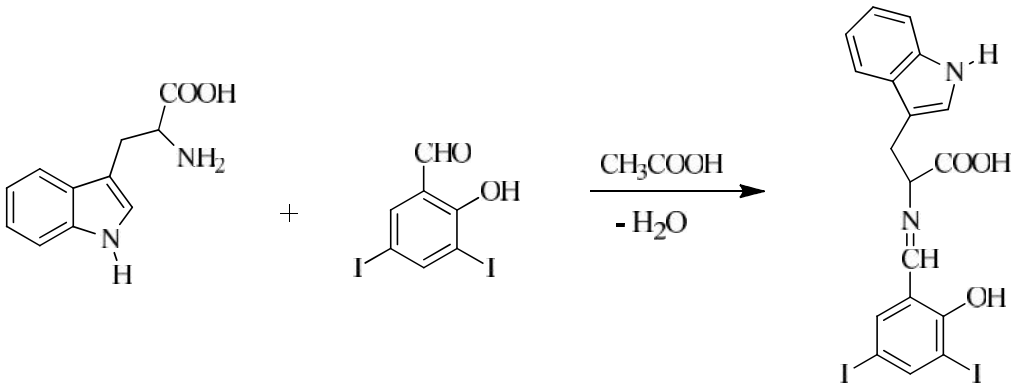
All the reagents used were of AR grade. Commercially available rectified spirit was dried over anhydrous quicklime for 24 hours, filtered and distilled before use (BP 78°C). Dimethylsulphoxide (sigma) and N, Ndimethylformamide (sigma) were used as such. Thyrosine, tryptophan, 3,5-diiodosalicylaldehyde and 5-formylsalicylic acid were purchased from Alfa Aesar.

Instruments

Melting points were determined using Elico melting point apparatus. Elemental analysis were performed using Elementar Vario EL III. IR spectra of the compounds were recorded with KBr pellets with carry630 FTIR Spectrometer in the 4000-400 cm^{-1} range. The 1HNMR and 13CNMRspectra were recorded on a Bruker 400 MHz FT- PMR Spectrometer.

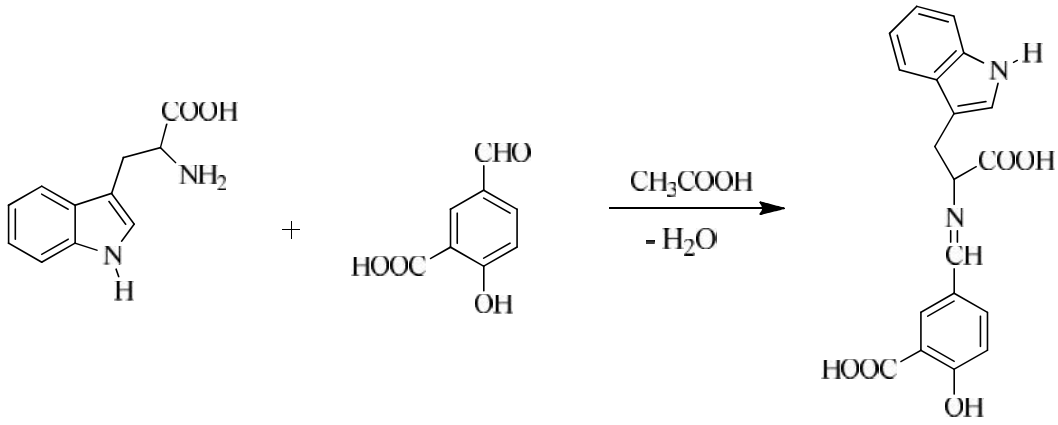
Preparation of (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl) ropanoic acid[8-19]

2- ((2- hydroxy- 3, 5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid was prepared from equimolar quantity of tryphtophan (2.04 g, 0.01 mol) and 3,5-diiodosalicylaldehyde (2.73 g, 0.01 mol) in 30 ml of methanol were heated at 70°C on water bath for 4-hrs in presence of few drops of glacial acetic acid. The crude product were obtained after removal of methanol under reduced pressure. The products were recrystallized from methanol and then dried over vacuum desiccator.



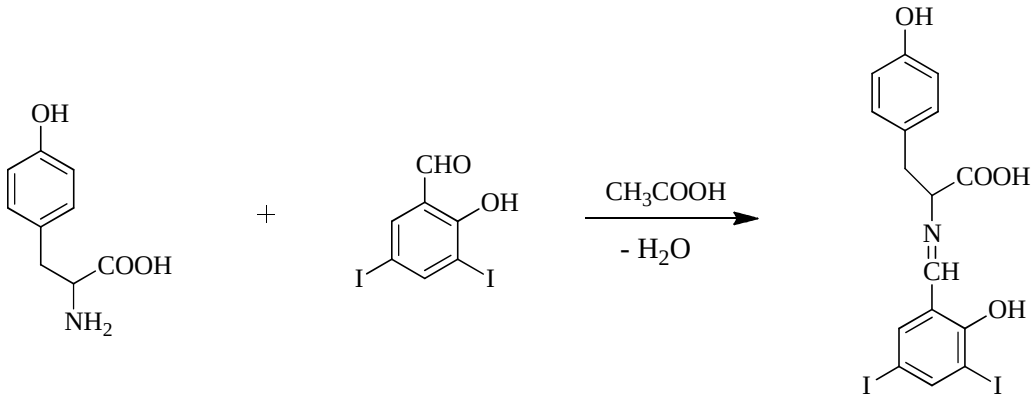
Preparation of (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid

A mixture of 5-formylsalicylic acid 1.66 g (0.01 mol) and tryphtophan 2.04 g (0.01 mol) were grained with a pestle in an open mortar at room temperature for 3 minutes. To this reaction mixture sulphuric acid 2 drops and 20 ml DMF were added and grained for 5 minutes. On completion of reaction as monitored by TLC, the light greenish-colored 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid was separated out. The obtained solid was isolated by simple Buchner filtration and was recrystallized from DMF and then dried over vacuum desiccator.



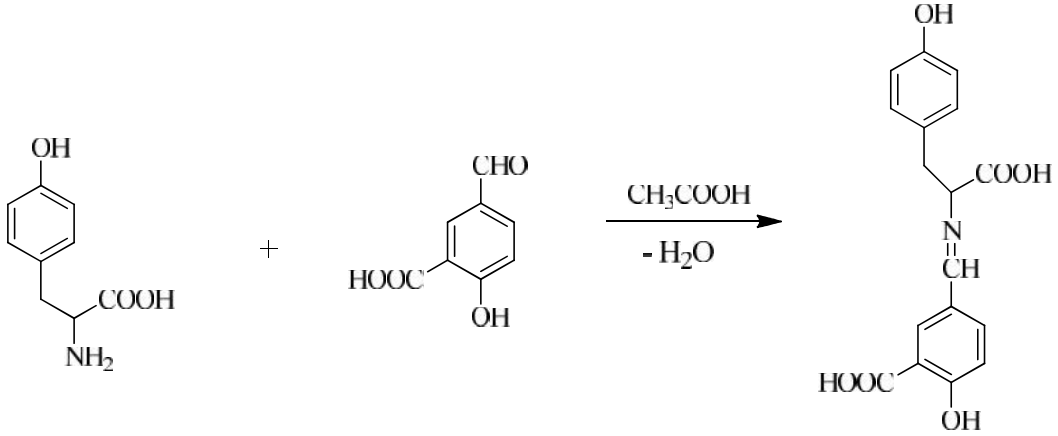
Preparation of (III)2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl) propanoic acid

3.6 grams of tyrosine (0.02mol) was mixed with 3.3 g of 3,5-diiodosalicylaldehyde 0.02mol) and was grained well in acidic acid medium at room temperature. The mixture was transferred into hundred milliliter Round Bottom flask and was refluxed for six hours in oil bath. The solid product 2-((2-hydroxy-3,5-diiodobenzylidene) amino) -3 - (4-hydroxyphenyl) propanoic acid was filtered and washed with ethanol and recrystallized in DMSO and then dried over vacuum desiccator.



Preparation of (IV) 5-(((1-carboxy-2-(4-hydroxy phenyl)ethyl)imino)methyl)-2-hydroxybenzoic acid

A mixture of 5-formylsalicylic acid (1.66g, 0.01mol) and tyrosine (1.81 g, 0.01mol) was grained in a mortar with a pestle made of porcelain for 10 minutes. The mixture turned pasty after few minutes of grainding. It was grained yellow colour product appears. The mixture was left overnight. The resultant product 5-(((1-carboxy-2- (4-hydroxyphenyl) ethyl) imino) methyl)-2-hydroxybenzoic acid was recrystallized using ethanol and then dried over vacuum desiccator.



Antimicrobial susceptibility test by Disc diffusion Technique

Principle

Disc impregnated with known concentration of **antibacterial** drug are placed on an agar plate that has been inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18 to 24 hours at 37^{o}C. During this period, the **antibacterial** agent diffuses through the agar and may prevent the growth of the organism. Effectiveness of susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Procedure

The plate was labeled with the name of the culture, sample and standard at the bottom of the plate. Then sterile cotton swab on a wooden applicator stick was dipped into the bacterial suspension. Excess fluid was removed by rotating the swab and rubbed gently over the plate to obtain uniform distribution of the inoculums. The sterile disc was held on the inoculated plate with the help of micropipette. The sample was leveled in the sterile disc and incubated at 37^{o}C in an incubator. After incubation, the diameter of the zone of inhibition of growth was measured.

Observation Report

Table 1.

Inhibition zone > 15mm	**Highly active**
Inhibition zone > 10mm	**Moderatively active**
Inhibition zone > 5mm	**Slightly active**
Inhibition zone ≤ 5mm	**Inactive**

Results and Discussion

The physical and analytical data of the derivatives tyrosine and tryptophan(I) 2-((2-hydroxy 3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl) imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxy phenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4-hydroxyphenyl)ethyl)imino)methyl)-2-hydroxybenzoic acid are given in Table2.

[I] 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid

FTIR (cm^{-1}): 3253 & 778 cm^{-1} (Ar–O–H), 3082 & 877 cm^{-1} (–N(H)–), 1687 cm^{-1} (R–C(=O)–), 1660 cm^{-1} (–N=CH–), 1381 cm^{-1} (R–C(=O)–OH), 1264 cm^{-1} (Ar–O–H) & 607 cm^{-1} (Ar–I)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 10.1 (s, 1H), 8.65 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 7.60 (d, 1H), 7.32 (d, 1H), 7.18 (s, 1H), 7.11 (t, 2H), 5.35 (s, 1H), 4.39 (t, 1H) & 3.15; 2.90 (d, 2H)

^{13}CNMR δ (ppm): 177.5 (s), 160.8 (s), 159.1 (s), 147.6 (s), 136.9 (s), 127.7 (d), 123.4 (s), 121.7 (s), 119.8 (s), 118.8 (s), 111.8 (s), 111.1 (s), 86.6 (s), 83.8 (s), 77.9 (s) & 30.8 (s)

[II] 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid

FTIR (cm^{-1}): 3271 & 841 cm^{-1} (–N(H)–), 3181 & 661 cm^{-1} (Ar–O–H), 1741 cm^{-1} (R–C(=O)–), 1705 cm^{-1} (Ar–C(=O)–), 1660 cm^{-1} (–N=CH–), 1183 cm^{-1} (R–C(=O)–OH) & 1147 cm^{-1} (Ar–C(=O)–OH)

^{1}HNMR δ (ppm): 11.0 (s, 2H), 10.1 (s, 1H), 8.65 (s, 1H), 8.32 (s, 1H), 8.12 (d, 1H), 7.60 (d, 1H), 7.32 (d, 1H), 7.23 (d, 1H), 7.18 (s, 1H), 7.11 (t, 2H), 5.35 (s, 1H), 4.39 (t, 1H) & 3.15; 2.90 (d, 2H)

^{13}CNMR δ (ppm): 177.5 (s), 171.8 (s), 164.5 (s), 160.8 (s), 136.5 (s), 135.8 (s), 132.3 (s), 130.9 (s), 127.7 (s), 123.4 (s), 121.7 (s), 119.8 (s), 118.8 (s), 112.1 (s), 111.8 (s), 77.9 (s) & 30.8 (s)

[III] 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl)propanoic acid

FTIR (cm^{-1}): 3451 & 607 cm^{-1} (Ar–O–H), 1696 cm^{-1} (R–C(=O)–), 1660 cm^{-1} (–N=CH–), 1480 cm^{-1} (R–C(=O)–OH) & 517 cm^{-1} (Ar–I)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 8.11 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 7.12 (d, 2H), 6.70 (d, 2H), 5.35 (s, 1H), 4.39 (t 1H) & 3.27; 3.02 (d, 2H)

^{13}CNMR δ (ppm): 177.5 (s), 160.8 (s), 159.1 (s), 155.7 (s), 147.6 (s), 136.9 (s), 130.5 (s), 130.2 (s), 127.8 (s) 115.8 (s), 88.6 (s), 83.8 (s), 70.8 (s) & 38.1 (s)

[IV] 5-(((1-carboxy-2-(4-hydroxyphenyl) ethyl)imino)methyl)-2-hydroxybenzoic acid

FTIR (cm^{-1}): 3208 & 661 cm^{-1} (Ar–O–H), 1705 cm^{-1} (R–C(=O)–), 1696 cm^{-1} (Ar–C(=O)–), 1660 cm^{-1} (–N=CH–), 1345 cm^{-1} (Ar–C(=O)–OH), 1246 cm^{-1} (R–C(=O)–OH) & 1183 cm^{-1} (Ar–OH)

^{1}HNMR δ (ppm): 11.0 (s, 2H), 8.32 (s, 1H), 8.12 (d, 1H), 811 (s, 1H), 7.23 (d, 1H), 7.12 (d, 2H), 6.70 (d, 2H), 5.35 (s, 2H), 4.39 (t, 1H) & 3.27; 3.02 (d, 2H)

^{13}CNMR δ (ppm): 177.5 (s), 171.8 (s), 164.5 (s), 160.8 (s), 155.7 (s), 135.8 (s), 132.3 (s), 130.5 (s), 130.2 (s), 118.0 (s), 115.8 (s), 112.1 (s), 70.8 (s) & 38.1 (s)

Table. 2 -The physical and analytical data of the derivatives of tyrosine and tryptophan

Derivatives of Amino acids	Molecular Weight	Nature of Appearance	% of yield	Elemental Analysis (in %)				
				C	H	O	N	I
[I] $C_{18}H_{14}I_2N_2O_3$	560.12	Yellow Crystalline Solid	88	38.60	2.52	8.57	5.00	45.31
[II] $C_{19}H_{16}N_2O_5$	352.34	Yellow Crystalline Solid	81	64.77	4.58	22.70	7.95	-
[III] $C_{16}H_{13}I_2NO_4$	537.08	Yellow Crystalline Solid	76	35.78	2.44	11.92	2.61	47.26
[IV] $C_{17}H_{15}NO_6$	329.30	Yellow Crystalline Solid	69	62.00	4.59	29.15	4.25	-

Antibacterial bioassay

Antibacterial activities[20,21] of derivatives of tyrosine and tryptophan were screened against bacterial gram positive bacteria *Staphylococcus aureus,* and gram negative bacteria *Escherichia coli, Klebsiella aerogenes* and *Bacillus subtilis* by disc diffusion method and the results obtained were formulated in Table.3 and Fig. 1-8. The experiments were carried out in DMSO solution at a concentration of 100ppm using Muller Hinton agar media. Ciprofloxacin was used as a standard drug.

Antifungal bioassay

Antifungal[22,23] screening of derivatives of tyrosine and tryptophan were carried out against *Aspergillus niger* and *Candida albicans* by disc diffusion method and the results obtained were formulated in Table.3 and Fig. 9-12. The test was carried out in DMSO solution at a concentration of 100 ppm. Results were compared with standard drug Nystatin at the same concentration.

Table. 3. Antibacterial and antifungal activity of derivatives of tyrosine and tryptophan

S.No	Zone of inhibition						
	Name of organism	A	B	C	D	S.Control	Standard
1	*E.coli*	25	24	23	21	-	38
2	*Klebsiella aerogenes*	25	23	22	19	-	30
3	*Bacillus subtilis*	26	25	24	20	-	40
4	*Staphylococcus aureus*	30	28	26	24	-	35
5	*Aspergillus niger*	22	21	19	17	-	35
6	*Candida albicans*	26	20	18	16	-	32

Fig -1

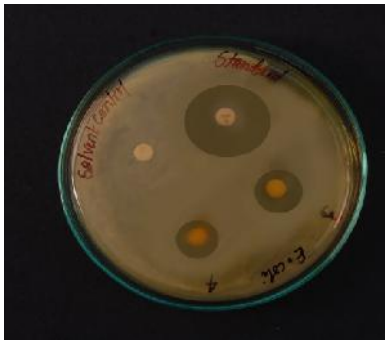


Fig -2

Fig -3

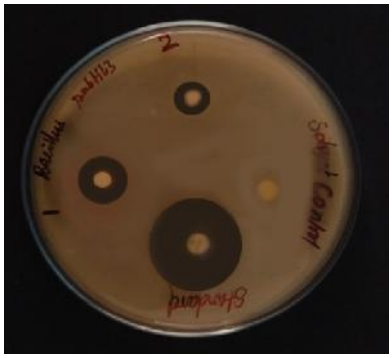


Fig -4

Fig -5

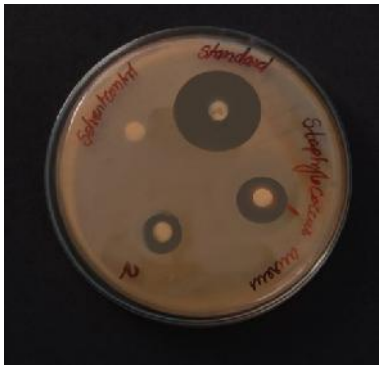


Fig -6

Fig -7

Fig -8

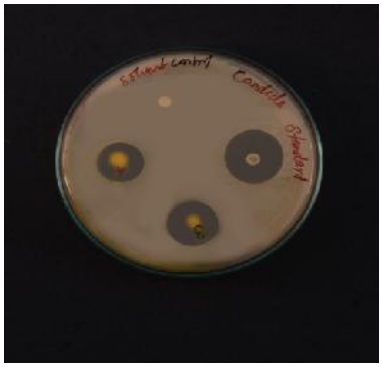


Fig -9

Fig -10

Fig -11

Fig -12

The antibacterial and antifungal activity of azomethine compounds I,II,III and IV (table. 3 and figure 1-12) clearly indicate that they inhibit the growth of tested bacteria and fungi in the decreasing order I>II>III>IV. Azomethine compounds I,II,III and IV prevent bacterial reproduction by acting as an antimetabolite to paraaminobenzoic acid (PABA), where PABA is a vital component in the biosynthesis of tetrahydrofolic acid. Competitive inhibition of PABA processing enzymes by I, II, III and IV ultimately blocks the action of dihydrofolic acid synthetase, and therefore prevents dihydrofolic acid formation. As bacteria are unable to take up tetrahydrofolic acid from their surroundings, inhibition of dihydrofolic acid synthetase will starve the bacteria of thymidine and uridine. These two nucleosides are required for DNA replication and transcription, therefore cell growth and division is disrupted, and thus provides enough time for the body's own immune system to eliminate the bacterial threat[26]

The nature of bonding and structure of azomethine organic compounds were elucidated by the elemental analysis, UV-Vis, FTIR, Melting Point, NMR, Chromatography and Molar ratio methods Gomathi et.al were prepared 4-(3-ethoxy-2-hydroxy benxzelideneaminno- N- (thiazole-2-yl-) benzene sulfonamide[24], Mohamed et.al and were prepared 4-(-phenyl-propylideneamino)-benzenesulfonamide[25]. In accordance with the data obtained from antibacterial activities of 4-((2-hydroxy-3,5-diiodobenzylidene) amino)benzenesulfonamide and 4-((2-hydroxy-3,5-diiodobenzylidene)amino)-N-(thiazole-2-yl-) benzene sulfonamide, were moderately inhibited the growth of tested bacteria but our derivatives of tyrosine and tryptophan(I) 2-((2-hydroxy-3,5-diiodobenzylidene) amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxy benzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene) amino)-3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4-hydroxyphenyl)ethyl)imino) methyl) -2-hydroxybenzoic acid were highly inhibited the growth of tested bacteria.

In accordance with the data obtained from antifungal activities of 4-(3-ethoxy-2-hydroxybenxzelideneamino-N-(thiazole-2-yl-)benzenesulfonamide (Gomathi et.al) were moderately inhibited the growth of tested fungi but our derivatives of tyrosine and tryptophan (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino) - 3- (1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy- 3,5- diiodobenzylidene) amino) -3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4-hydroxyphenyl)ethyl) imino) methyl) -2-hydroxy benzoic acid were highly inhibited the growth of tested fungi. From the results and previous work, antibacterial and antifungal activity studies were indicated that iodine substituted derivatives of tyrosine and tryptophan were moreactive against bacteria and fungi than other derivatives tyrosine and tryptophan.

Conclusion

The derivatives of tyrosine and tryptophan (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino) -3- (1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2- hydroxy - 3,5-diiodobenzylidene) amino)-3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4-hydroxyphenyl) ethyl) imino) methyl)-2-hydroxy benzoic acid were prepared and bio-assay were tested against important bacteria and fungi.It was shown that the growth of bacteria and fungi were highly inhibited by the derivatives of tyrosine and tryptophan (I) 2-((2-hydroxy-3,5-diiodobenzylidene) amino)- 3- (1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indSol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4-hydroxyphenyl)ethyl)imino)methyl)-2-hydroxybenzoic acid (I to IV)

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