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Research Article

Design, Synthesis, Characterization and Biological Evaluation of *Para*-Amino Salicylic Acid (PAS) Analogues: An Approach to Repurpose

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Abstract: The ever-emerging microbial resistance has imposed great challenges for the medicinal chemist in designing newer chemical entities, which is a lengthy and time-consuming process. An alternate approach of repurposing older drugs can be undertaken to accelerate the discovery of some newer derivatives with lesser side effects. The work reported in the present paper is an extension of the idea of repurposing older drugs. The study aimed to synthesize a series of ester and amide derivatives (P1-P28) of *Para*-amino salicylic acid. The antimicrobial evaluation of all the synthesized derivatives was performed *via* the serial dilution method. The Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* were used for the antibacterial activity, and *Candida albicans* were used for the antifungal activity. Ciprofloxacin (antibacterial) and Fluconazole (antifungal) were the standard drugs. The antioxidant evaluation (ester derivatives, P1-P19) was carried out by using the DPPH scavenging method and BHA (butylated hydroxy anisole) was used as the reference. The derivative 5-acetamido-2-(propyl carbamoyl) phenyl acetate (P22) was the most effective antibacterial agent, which may be attributed to the presence of 3-carbon aliphatic alkyl chain. At the same time, 4-methoxy phenyl-4-amino-2-hydroxy benzoate (P15) and 2-chloro phenyl-4-amino-2-hydroxy benzoate (P16) displayed effective antifungal activity. Ethyl-4-amino-2-hydroxy benzoate (P2) demonstrated the most potent free radical scavenging activity. Further, the spectroscopic characterization and physicochemical characterization of the synthesized derivatives have been carried out and were found to be in accord with the assigned structures.

Keywords: *Para*-amino salicylic acid, antimicrobial activity, antioxidant activity, minimum inhibitory concentration (MIC), free radical scavenging activity

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1. INTRODUCTION

The development of resistance to existing antibiotics has always been a great challenge for the medicinal world. Although continuous efforts are being made to develop newer antimicrobials, its lengthy and time-consuming process leads to the repurposing of old drugs as an alternative choice for treating bacterial infections.[1] Tuberculosis is one of the deadliest infectious diseases spreading worldwide, leading to approximately 1.3 million deaths annually and responsible for infecting almost one-quarter of the world's population.[2] By the 1990s, the treatment and the prevention of tuberculosis has become more difficult due to the emergence and spread of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB)[3,4]. *Para*-amino salicylic acid (PAS) was the second-line antitubercular agent after streptomycin, which also possesses antibiotic properties. It was introduced in 1946 based on salicylic acid's observed effects on *Mycobacterium tuberculosis*'s metabolism. PAS is related to sulphonamides in the mechanism of action and structurally. Both the sulphonamides and PAS are antifolates and inhibit folate biosynthesis in bacteria. Sulphonamides and PAS were the important components of the early TB chemotherapies[4,5]. But later on, due to the introduction of newer antibiotics with better tolerance and lesser side effects, PAS was limited as a second-line antitubercular agent [6]. Further, it has been observed that resistance to *para*-amino salicylic is slowly developing [6,7]. It increases the rate of cure and decreases the development of resistance to the drugs that are given along with it. The effects of streptomycin and isoniazid were enhanced by PAS[8,9]. Though the associated gastrointestinal disturbances have limited the use of PAS, with the development of improved formulations and the need of action against emerging resistant strains of MDR and XDR tuberculosis, the PAS has re-entered the antimicrobial drug regimens[10]. Reactive oxygen species (ROS) such as superoxide radical ($2O^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl ($HO^{\bullet}$), and hypochlorous acid (HOCl) are the species which were found to be involved in various pathogenic conditions. Various inflammatory diseases have been observed to be caused by the overproduction of these species. NSAIDs, including salicylates (Aspirin), were found to reduce these oxidants, especially the levels of superoxide ions and hydroxyl ions, significantly[11]. PAS, a salicylate, has also been reported to have antioxidant properties when chelated with different metals[12]. PAS has been reported to have antimicrobial[13], anti-inflammatory[14], antiviral[15], and antihyperlipidemic[16] activities. Various approaches have been followed, viz. Schiff base conjugates, phenol class azo derivatives for synthesizing more promising derivatives of PAS[17]. Therefore, it's critical to create innovative approaches to increase PAS potency, reduce side effects, and raise therapy success rates. In light of the above facts, the aim and the objective of the study were to synthesize some potential ester and amide derivatives of PAS and to carry out biological evaluation *via* antimicrobial activity and antioxidant activity, which may further prove to be useful in developing some potential biologically active agents with lesser side effects and wide therapeutic index.

2. MATERIALS AND METHODS

All chemicals used were of HiMedia Laboratories Pvt. Ltd., S D Fine Chemical Ltd., and Sisco Research Laboratories. Thin Layer Chromatography was done on glass plates with silica gel Gas adsorbent, and spots were visualized by exposure to iodine vapours. Using the ELICO Melting point apparatus, melting points were taken in open glass capillary tubes and were uncorrected. Perkin Elmer IR spectrophotometer was used to record compounds' Infrared (IR) spectra. The Bruker Avance II 400 NMR Spectrophotometer was used to record ^{1}H NMR by using tetramethylsilane (TMS) as an internal standard.

2.1. General procedure for the synthesis of ester derivatives (P1-P19)

0.013 moles of Para- aminosalicylic acid and 0.217 moles of corresponding alcohol were refluxed in sulphuric acid. After the completion of the reaction, 50 ml of ice-cold water was added to the reaction mixture. Next, 5 ml of saturated sodium bicarbonate solution was added to it. The mixture was then extracted with 50 ml of ether. After the extraction, all the ether layers were combined and evaporated, thus yielding the crude ester. The ester was then recrystallized from alcohol[18].

2.2. General procedure for the synthesis of amide derivatives (P20 – P28)

Step 1: Acetylation of hydroxyl and para-amino group
0.01mol (1 gm) of the compound was dissolved in 5 ml of 3M sodium hydroxide solution, and then 10 -20 gm of crushed ice was added to the solution. Then 1.5 ml of acetic anhydride was added. The resultant mixture was shaken for 30-60 s vigorously. The acetate was separated in pure conditions in the first step or separated by acidification by adding a mineral acid. The product was collected. The recrystallization was carried out by using hot water or dilute ethanol[19].

Step 2: Formation of acid chloride
The acetylated derivative was refluxed with distilled thionyl chloride for 2 to 3 h to prepare the acid chloride. The excess of thionyl chloride was then distilled off. [19]

Step 3: Reaction of the acid chloride with the corresponding amine
0.1 ml of the corresponding amine in ether (50 ml) was added dropwise to 0.1 ml of acid chloride in ether (50 ml). While in addition, the temperature was maintained at 0-10^{0}C. The stirring was performed for 30min, and the precipitated amide was separated by filtration. The recrystallization of crude amide was done from alcohol[20].

2.3. Antimicrobial activity

2.3.1. Evaluation of antibacterial activity

The evaluation of antimicrobial activity was performed by tube dilution method. This method leads to the inhibition of the growth of a microbial culture in a uniform solution in a fluid medium that is favourable for its rapid growth. Still, the presence of antibiotics leads to the inhibition of microbes. Antibacterial agents are substances that kill or prevent the growth of various bacteria. MIC is the minimum inhibitory concentration of the antimicrobial agent, which is required to inhibit the growth of microorganisms *in-vitro.* The Gram-positive bacteria: *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 16) and Gram-negative bacteria: *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 424) were used for the activity. Nutrient broth IP (Indian Pharmacopoeia, 2007) was used as the media to grow the respective bacterial strains. Each microorganism was suspended by transferring the organism from the culture to 10 mL of sterile normal saline

solution. The incubation was carried out at 37±1^{0}C for 24 h for the different bacterial strains used, and the minimum inhibitory concentrations were recorded[21]. 1 mL of sterilized media was poured into sterilized test tubes. Then, 1mL of 100 µg/mL test solution was transferred in one tube and serially diluted to give a concentration of 25, 12.5, 6.25, 3.12, 1.56 µg/mL. To all the test tubes, 1mL of suspension of bacteria in saline was added, and the tubes were incubated at 37±1^{0}C for 24 h. The growth in the tubes was observed visually for turbidity and the absence of growth-determined inhibition. The standard drug taken for the antibacterial activity was Ciprofloxacin. DMSO was used to prepare different dilutions for standard drugs and test compounds. MIC (µg/ml) was determined by the lowest concentration of the sample that prevented the development of turbidity and the results have been recorded as a negative logarithm of MIC (pMIC, (µM/ml)) [21].

2.3.2. Evaluation of antifungal activity

The antifungal activity of the synthesized compounds was evaluated against *Candida albicans* (MTCC 183). The nutrient media used was Sabouraud dextrose broth IP (Indian Pharmacopeia, 2007). Fluconazole was used as the standard drug, and the activity of the synthesized compounds was compared with the standard drug. The standard drug and the test compounds were dissolved in DMSO to obtain a concentration of 100 µg/ml[21]. One ml of sterilized media was poured into sterilized test tubes. Next, 1 ml of 100 µg/ml test solution was transferred in one tube and serially diluted to give concentrations of 25, 12.5, 6.25, 3.12, and 1.56 µg/mL. The microorganisms were suspended by transferring the microorganism from the culture to 10mL of sterile normal saline solution. To all the tubes, 1mL of fungi suspension in saline was added, and the tubes were incubated at 37±1^{0}C for 24 h to 48 h for *C.albicans*. The growth in the tube was observed visually by turbidity and the absence of growth-determined inhibition. MIC (µg/ml) was determined by the lowest sample concentration that prevented turbidity development.

2.4. Antioxidant activity

2.4.1. *Evaluation of antioxidant activity*

Antioxidants are included in pharmaceutical products to prevent deterioration from oxidation. The antioxidant activity of all the ester-synthesized derivatives was evaluated *in-vitro* by DPPH (1,1-diphenyl-2-picryl-hydroxyl) free radical scavenging method. BHA (Butylated Hydroxy Anisole) was used as the standard antioxidant. First, 3 ml of different concentrations (2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml) of the test compounds were mixed with 3ml of methanolic DPPH solution. The mixture was then incubated at 35±1^{0}C for 30 min. The absorbance was measured at 517 nm immediately after incubation. The scavenging capacity was determined by calculating the decrease in the absorbance value and was expressed as the percentage inhibition/reduction. The DPPH Scavenging Activity of BHA at various concentrations was also measured and compared with those of the newly synthesized compounds. The formula for calculating the percentage inhibition of DPPH

$$\textbf{DPPH Q} = 100(A_0 - A_C)/A_0$$

Q = percentage reduction of DPPH.
A_0 = initial absorbance.
A_C = absorbance of the sample concentration

The standard curve was obtained by plotting the graph between different concentrations of DPPH and its absorbance value. The linear regression plot between % inhibition of DPPH versus conc. BHA and PAS have been plotted, and the IC_{50} value was calculated graphically. The linear regression plots between percentage inhibition of DPPH action versus different concentrations of the test compounds were plotted, and IC_{50} was determined[22].

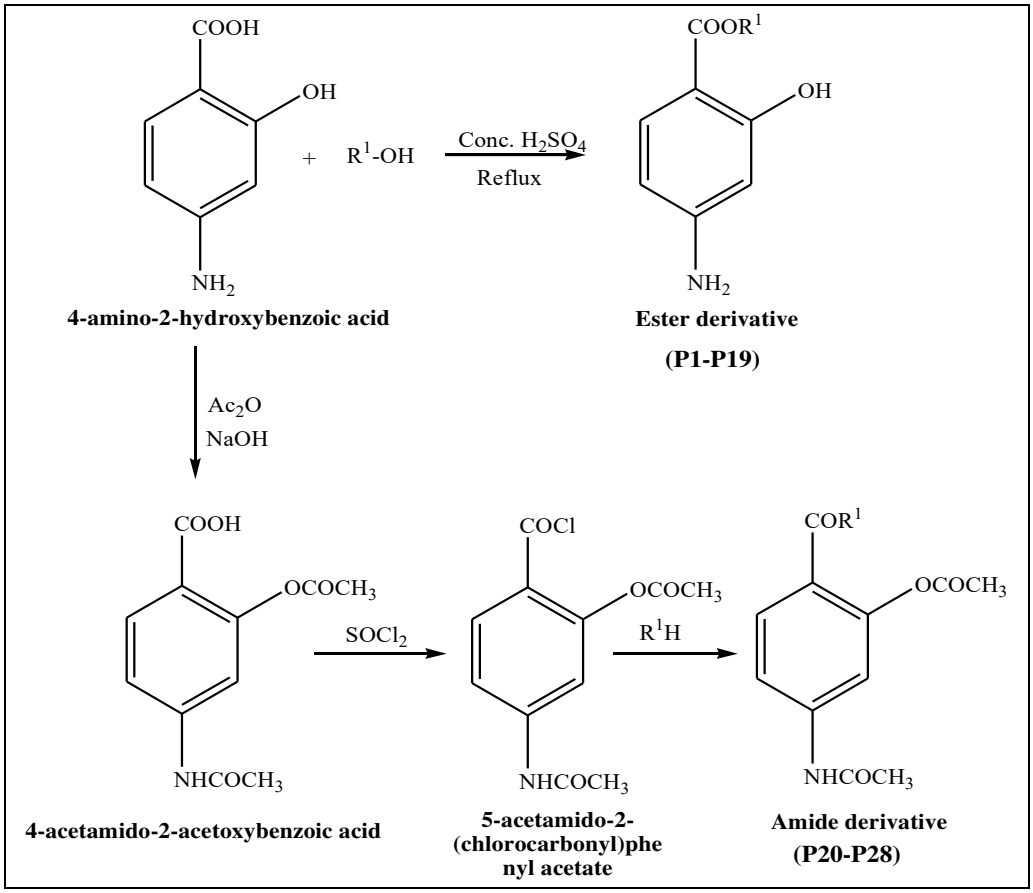
2.5. Determination of the inhibitory concentration (IC_{50})

IC_{50} is the effective concentration that expresses the number of test compounds necessary to decrease the absorbance (activity/colour) of DPPH by 50%. For the interpretation of free radical scavenging effects of the ester derivatives, IC_{5O} was determined graphically by plotting a linear regression plot between the percentage inhibition values of the synthesized derivatives at different concentrations against DPPH.

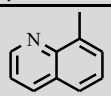
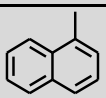
3. RESULTS AND DISCUSSION

3.1. Chemistry

Synthesis of esters and amide derivatives of *para*-amino salicylic acid has been displayed in Scheme 1. The esters (P1-P19) were synthesized by reacting *p*-amino salicylic acid with different alcohols (aliphatic and aromatic) catalyzed by H_2SO_4. Next, the amides were synthesized by protecting the hydroxyl and amino groups, followed by the acylation of the carboxyl group of the parent compound. Finally, the acid chloride was formed to react with the corresponding amine. The derivatives of *p*-amino salicylic acid were obtained in appreciable yield and purified by recrystallization from alcohol. The determination of various physicochemical characters was done by the parameters shown in Table 1.1.



Scheme 1. Synthesis of ester and amide derivatives (P1 – P28).

S.no	R^1 substituent	S.no	R^1 substituent	S.no	R^1 substituent	S.no	R^1 substituent
P1	CH_3	**P8**	$CH_3(CH_2)_3CH_2$	**P15**	*p*-$CH_3OC_6H_4$	**P22**	$CH_3CH_2CH_2N$
P2	CH_3CH_2	**P9**	$CH_3(CH_2)_4CH_2$	**P16**	*o*-ClC_6H_4	**P23**	$HOCH_2CH_2N$
P3	$CH_3CH_2CH_2$	**P10**	*p*-$CH_3C_6H_4$	**P17**	*p*-ClC_6H_4	**P24**	$(CH_3)_2N$
P4	$(CH_3)_2CH$	**P11**	$CH_3(CH_2)_3(CH_3)CH$	**P18**		**P25**	$CH_3CH_2(CH_3)CHN$
P5	$CH_3(CH_2)_2CH_2$	**P12**	C_6H_5	**P19**	*o*-$CH_3OC_6H_4$	**P26**	CH_3N
P6	$CH_3CH_2(CH_3)CH$	**P13**	*o*-$CH_3C_6H_4$	**P20**	$(CH_3CH_2)_2N$	**P27**	$(HOCH_2CH_2)_2N$
P7	$(CH_3)_3C$	**P14**		**P21**	$(CH_3CH_2)_2N$	**P28**	$C_6H_4CH_2N$

3.2. Spectral analysis

The molecular structures of the synthesized derivatives were found to be according to the spectral data obtained, and the characterization was carried out by IR and ^{1}H NMR spectral characteristics. The presence of peaks between the 3200cm-1- 3364 cm^{-1} region corresponds to the NH str. of the synthesized derivatives. The compounds P1, P2, P3, and P6 showed the characteristic peak for NH str. at 3378 cm^{-1}, 3354 cm^{-1},3427 cm^{-1},3362 cm^{-1}, respectively. The peak for the –OH group was observed in the range of 3000 cm^{-1}-3450 cm^{-1}. The appearance of IR peaks in the region of 1720 cm^{-1} - 1750 cm^{-1} indicates the presence of COO str. P1, P2, P3, P6 showed the peak for carbonyl group at 1720 cm^{-1}, 1606 cm^{-1}, 1629 cm^{-1}, 1727 cm^{-1} indicating the conversion of acid to ester derivatives. C=C str. of aromatic ring was observed between 1550 cm^{-1} – 1650 cm^{-1} in all the synthesized derivatives. The compounds P20, P21, and P24 gave the characteristic peak for amide at 1605 cm^{-1} and 1680 cm^{-1}. IR peak value of 1155 cm^{-1} in P16 indicated chloro substitution on the aromatic ring. A signal for the hydrogen attached to the aromatic (benzenoid) ring at 6.5–8.0 ppm was shown by the ^{1}H NMR spectra of the synthesized compounds, thus confirming its synthesis. The compounds P1 and P2 showed a singlet at 4.5 and 4.6, indicating aromatic NH_2. The peak at 8.0 ppm confirmed the presence of a proton of NH in $NHCOCH_3$. A broad peak around 5.8 ppm indicated the presence of aromatic OH[23].Compound P1: M.pt:115^{0}C, yield: 56%, IR(KBr Pellets) cm^{-1}: 3471(OH, aromatic), 3378 (NH str., aromatic), 3261(=C-H str.), 1720 (aromatic ester), 1600 (aromatic C=C str.), 1465 (CH_2, methylene group), 1382 (CH_3 str., methyl), 1219 (C-O-H), 1181(C-N str.), 1098 (C-O str.). 1H-NMR (DMSO)ppm: 4.5(s,1H, NH), 6.0(s, 1H, C_3 of aromatic ring), 5.80(s,1H, OH aromatic), 3.88(s,3H, CH_3 of $COOCH_3$), 6.10-6.15(d, 1H, C_5 of aromatic ring), 6.7-6.8(d,1H, C_6 of aromatic ring). Compound P2: M.pt: 115^{0}C, yield: 43%, IR (KBr Pellets) cm^{-1}: 3471(OH, aromatic), 3354 (NH str., aromatic), 3034(=C-H str..), 1606(aromatic ester), 1478 (CH_2 methylene group), 1394(CH_3 methyl group), 1270 (C- O-H), 1163 (C-N). NMR (DMSO)ppm: 4.6(s,1H, NH),5.8(s,1H, OH aromatic),1.2(s,1H,

terminal CH_3 of CH_2CH_3), 5.96-5.98(s,1H,CH of CH_2 of CH_2CH_3),6.01(s,1H,C_3 of aromatic ring),6.01-6.12(1H,d,C_5 of aromatic ring). Compound P3: M.pt: 118^{0}C, yield: 45%, IR (KBr Pellets)cm^{-1}:3427 (NH str.., aromatic), 1629 (aromatic ester), 1539 (C=C, aromatic),1461 (CH_2 methylene group), 1396(C-O- H str..), 1155(C-O-H), 1094(C-O), 1008(C-N), 783(=C-H oop). Compound P6: M.pt: 117^{0}C, yield: 47%, IR (KBr Pellets) cm^{-1} : 3362(OH, aromatic), 3309 (NH str..,aromatic), 1727(aromatic ester), 1666(C=C str..,aromatic), 1441(CH_2,methylene group), 1368(CH_3,methyl group), 1258(C-O-H, alcohol), 1017(C-O),1090(C-N). Compound P7: M.pt: 128^{0}C, yield: 53%, IR (KBr Pellets) cm -^{1}:1608(aromatic ester), 1502 (C=C str.., aromatic), 1437(CH_2, methylene group), 1376(CH_3, methyl group), 1302(C-O-H, alcohol), 1167(C-N), 1016(C-O). Compound P8: M.pt: 117^{0}C, yield:39.5%, IR (KBr Pellets) cm^{-1}: 3284 (NH str..,aromatic), 1727(aromatic ester), 1650(C=C str..,aromatic), 1499(CH_2,methylene group), 1319(CH_3,methyl group), 1258(C-O-H), 1180(C-N), 1029(C-O), 767(alkyl chain). Compound P12: M.pt: 175$\pm$ 5^{0}C, yield:37.8%, IR (KBr Pellets)cm^{-1}:3362 (OH, aromatic), 1605(aromatic ester), 1507(C=C str..,aromatic), 1466(CH_2, methylene group), 1388(CH_3, methyl group), 1303(C-O-H), 1258(C-O), 1180(C-N). Compound P16: M.pt: 179$\pm$ 5^{0}C,yield:34.5%IR (KBr Pellets) :3460(OH str.. aromatic), 3149 (NH str..,aromatic), 1715(COO, aromatic ester), 1642(C=C), 1490(CH_2 methyl group), 1396(CH_3, methyl), 1237(C-O-H), 1155 (Chloro, aromatic), 1098(C-O). Compound P20: M.pt:131^{0}C,yield:63% IR (KBr Pellets) : 3364(secondary amide, NH str..), 1744(aromatic ester), 1605(C=O, amide), 1450(CH_2 , methylene group), 1396(CH_3, methyl group), 1281(C-N). Compound P21: M.pt:142^{0}C, yield:56% IR (KBr Pellets) cm^{-1}: 3564 (secondary amide,NH str..), 1744(aromatic ester),1605(C=O, amide), 1450(CH_2,methyl group),1396(CH_3,methylene group), 1281(C-N). NMR (DMSO)ppm: 8.0(s,1H, NH of $NHCOCH_3$), 7.34-7.36ppm (d,1H,C_5 of aromatic ring), 2.1 (s,1H,CH_3 of $NHCOCH_3$), 2.55(s,IH,CH of NC_1H_3), 2.56(s,1H,CH of NC_2H_3), 6.7(s,1H,C_6 of aromatic ring), 7.75-7.78(d,1H,C_6 of aromatic ring). Compound P24: M.pt:125^{0}C, % yield:32% IR (KBr Pellets): 3148(secondary amide, NH str..), 1680(aromatic ester),1602(C=O, amide), 1572(C=C str.., aromatic), 1489(CH_2, methyl group), 1356(CH_3, methylene group), 1285(C-N). NMR (DMSO)ppm: 8(s,IH,NH of $NHCOCH_3$), 7.91-7.94(d,1H,C_5 of aromatic ring), 7.5(s,1H,C_3 of aromatic ring), 3.1-3.2(d,1H,CH_2 of $CONCH_2H_3$), 2.7(s,1H,CH_3 of $OCOCH_3$), 1.29-1.39(d,IH,terminal CH_3 of $CONCH_2CH_3$).

Table 1.1: Physicochemical characterization of synthesized derivatives of *para*-amino Salicylic Acid.

S.No	Mol. form.	Mol. wt.	R_f value	MP.	% yield
P1	$C_8H_9O_3N$	167	0.50	115^{0}C	56.0
P2	$C_9H_{11}O_3N$	181	0.35	115^{0}C	43.0
P3	$C_{10}H_{13}O_3N$	195	0.60	118^{0}C	45.0
P4	$C_{10}H_{13}O_3N$	195	0.30	140^{0}C	40.0
P5	$C_{11}H_{15}O_3N$	209	0.75	120^{0}C	32.5
P6	$C_{11}H_{15}O_3N$	209	0.45	117^{0}C	47.0
P7	$C_{11}H_{15}O_3N$	209	0.40	128^{0}C	53.0
P8	$C_{12}H_{17}O_3N$	223	0.26	117^{0}C	39.5
P9	$C_{13}H_{19}O_3N$	237	0.55	167^{0}C	48.0
P10	$C_{14}H_{13}O_3N$	243	0.15	165$\pm$5^{0}C	42.0
P11	$C_{13}H_{19}O_3N$	237	0.33	183^{0}C	55.0
P12	$C_{13}H_{11}O_3N$	229	0.31	175$\pm$5^{0}C	37.8
P13	$C_{14}H_{13}O_3N$	243	0.25	165^{0}C	33.0
P14	$C_{17}H_{13}O_3N$	279	0.32	185^{0}C	48.0
P15	$C_{14}H_{13}O_4N$	259	0.33	168^{0}C	53.0
P16	$C_{13}H_{10}O_3NCl$	263	0.46	179$\pm$5^{0}C	34.5
P17	$C_{13}H_{10}O_3NCl$	263	0.90	185^{0}C	37.0
P18	$C_{16}H_{12}O_3N_2$	280	0.38	>250$\pm$5^{0}C	38.0
P19	$C_{14}H_{13}NO_4$	299	0.75	>250$\pm$5^{0}C	33.0
P20	$C_{15}H_{20}N_2O_4$	292	0.39	131^{0}C	63.0
P21	$C_{15}H_{20}N_2O_4$	292	0.47	142^{0}C	56.0
P22	$C_{14}H_{18}N_2O_4$	278	0.57	137$\pm$5^{0}C	48.0
P23	$C_{13}H_{16}N_2O_5$	280	0.74	145^{0}C	67.0
P24	$C_{13}H_{16}N_2O_4$	264	0.64	125^{0}C	32.0
P25	$C_{15}H_{20}N_2O_4$	292	0.38	140^{0}C	54.0
P26	$C_{12}H_{14}N_2O_4$	250	0.77	135^{0}C	77.0
P27	$C_{15}H_{20}N_2O_6$	324	0.56	155^{0}C	76.0
P28	$C_{18}H_{18}N_2O_4$	326	0.56	158$\pm$5^{0}C	64.0

The table illustrates the physicochemical properties of the synthesized derivatives *viz.,* Molecular formula, molecular weight, R_f value, melting point and the percentage yield. The solvent system used for calculating the R_f value is Hexane: Ethylacetate (1:2)

3.3. Antimicrobial activity

The *in vitro* serial dilution method was followed for the antimicrobial evaluation for the synthesized derivatives was carried out against Gram-positive *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 16) and Gram-negative *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC

424) and antifungal activity against *Candida albicans* (MTCC 183). Table 1.2 displays the results obtained by antimicrobial evaluation. Compounds P17, P20, and P22 showed effective antibacterial activity against *S. aureus*. P5 and P20 had remarkable activity against *B. subtilis*. The compounds which were effective against *E. coli* include P9, P11, P13, P17, P21 and P22. The derivative P19 had considerable activity against *E. coli* and *P. aeruginosa*. The P22 had a significant antibacterial activity of all the derivatives synthesized. The compounds P15 and P16 showed moderate antifungal activity. The varying degree of sensitivity for the microorganisms of the synthesized derivatives may be because of the intrinsic tolerance of the bacterial strains. The pMIC values of the derivatives synthesized are given in Table 1.2

Table 1.2: pMIC values of different compounds synthesized.

Compound	$pMIC_{Sa}$	$pMIC_{Bs}$	$pMIC_{Ec}$	$pMIC_{Pa}$	$pMIC_{Ca}$	$pMIC_{Ab}$	$pMIC_{Af}$	$pMIC_{Am}$
P1	1.13	1.13	1.43	1.13	0.82	1.20	0.82	1.13
P2	1.46	1.16	1.46	1.16	1.16	1.31	1.16	1.28
P3	1.49	1.19	1.19	1.49	1.49	1.34	1.49	1.37
P4	1.80	1.19	1.80	1.80	1.49	1.65	1.49	1.61
P5	1.83	2.13	1.81	1.83	1.52	1.9	1.52	1.82
P6	1.22	1.81	1.52	1.52	1.22	1.52	1.22	1.46
P7	1.22	1.83	1.52	1.52	0.92	1.52	0.92	1.40
P8	1.22	1.52	1.52	1.52	0.92	1.45	0.92	1.34
P9	1.58	1.88	2.18	1.58	1.28	1.80	1.28	1.70
P10	1.59	1.29	1.89	1.29	1.29	1.51	1.29	1.47
P11	1.27	1.88	2.18	1.27	1.58	1.65	1.58	1.64
P12	1.26	1.56	1.85	1.56	1.26	1.56	1.26	1.5
P13	1.59	2.19	2.19	1.87	1.59	1.96	1.59	1.89
P14	1.65	1.65	1.65	2.25	1.65	1.80	1.65	1.77
P15	1.92	1.62	1.92	1.32	1.92	1.69	1.92	1.74
P16	1.62	1.62	1.93	1.62	1.93	1.70	1.93	1.74
P17	2.23	1.93	2.23	1.93	1.62	2.08	1.62	1.99
P18	1.95	1.65	1.65	2.25	1.35	1.88	1.35	1.77
P19	1.98	1.38	2.28	2.28	1.38	1.98	1.38	1.86
P20	2.27	2.27	1.97	1.37	1.67	1.97	1.67	1.91
P21	1.67	1.37	2.27	1.67	1.07	1.74	1.07	1.61
P22	2.25	2.25	2.25	1.93	1.65	2.17	1.65	2.07
P23	1.65	1.35	1.65	1.65	1.65	1.58	1.65	1.59
P24	1.32	1.63	2.23	1.32	1.02	1.63	1.02	1.51
P25	1.67	1.67	2.27	1.67	1.67	1.82	1.67	1.79
P26	1.90	1.60	1.60	1.90	1.00	1.75	1.00	1.60
P27	1.71	2.32	2.32	2.02	1.41	2.09	1.41	1.96
P28	2.02	2.32	1.72	2.02	1.42	2.02	1.42	1.90
SD	0.33	0.37	0.32	0.33	0.30	0.25	0.30	0.23
Std.	2.33*	2.33*	2.33*	2.33*	2.03**			

***Ciprofloxacin** ****Fluconazole** **SD: Standard deviation**

The table shows the log values of minimum inhibitory concentrations (MIC) of the synthesized derivatives when evaluated against the bacterial strains *viz.,* two Gram-positive *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 16), two Gram-negative bacteria: *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424) and fungal strain *viz., Candida albicans* (MTCC 183)). Ciprofloxacin and Fluconazole were used as reference drugs for antibacterial and antifungal activities.

3.4. SAR

The antimicrobial studies lead to the following depiction of structural activity relationship:

1. The amide derivative P22 was active against all the tested strains, showing that the aliphatic alkyl side chain up to C-3 was the prime requirement for antimicrobial activity. With branched alkyl side chains, the activity of P20 is restricted to Gram +ve bacteria only, which shows that 2^0 carbon has more consistent antibacterial activity than 3^0 carbon.
2. In the case of *E.coli*, the compounds P21, P24 and P27, having diethyl, dimethyl and diethyl alcohol substituents, showed better antibacterial activity than compounds having ethyl alcohol and dimethyl substituents, which showed that the branched amine substituents increase the antibacterial activity.
3. In the case of *S. aureus* and *E .coli*, compound P17 showed that *p*-chloro substituted aromatic ring increased the antibacterial activity, which proves the point of P. Sharma *et al.* that the presence of electron-withdrawing groups[24] increases antimicrobial activity[17].
4. In the case of *B. subtilis*, compounds P13 (*o*-methyl) and *E.coli*, compound P19 (*o*- methoxy) showed that electron donating groups contribute towards the antibacterial activity, which proves that different structural requirements are required for activity against the different microorganisms. The same results have been reported in our earlier studies[24].
5. 2-benzyl amide derivative (P28) and 8-quinoline ester (P18) derivative show good antibacterial activity against *B. subtilis* and *P. aeruginosa,* which again supports the fact that

different structural requirements are required for activity against the different microorganisms.

6. In the case *of C. Albicans*, the compounds P15 and P16 show antifungal activity, and this shows that the ester derivatives with the *p*-substituted electron donating group and *o*-substituted electron withdrawing group support the antifungal activity.

Table 1.3: Absorbance of DPPH solution in methanol at varying concentrations.

Conc. (µg/mL)	Absorbance value
2	0.095
4	0.134
6	0.168
8	0.194
10	0.245

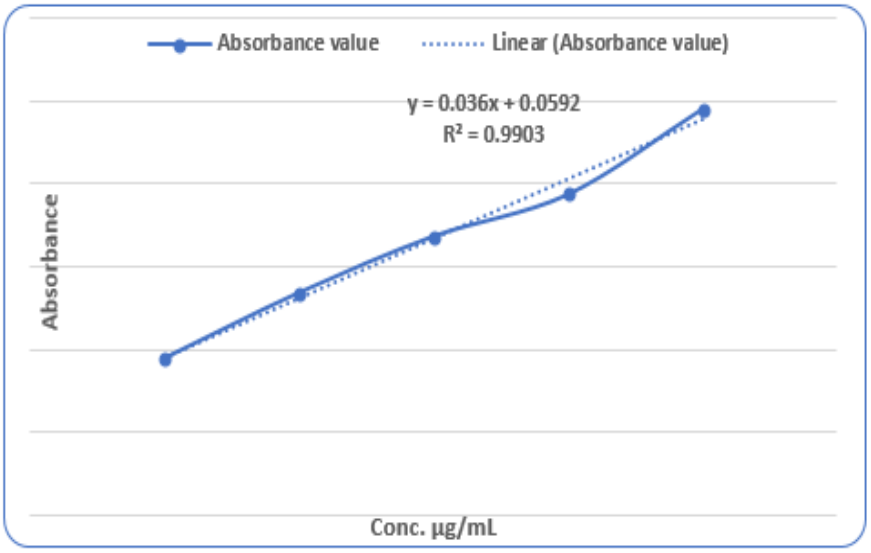


Fig. 1.1 The linear regression plot (standard curve) of DPPH at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml).

3.5. Antioxidant activity

With the help of DPPH free radical scavenging method, the scavenging ability of the synthesized derivatives was checked against the reference drug BHA. The bleaching of the purple colour of the methanol solution of DPPH indicated the hydrogen atom or electron-donating abilities of the compounds[25]. The data obtained by *in vitro* model establish the antioxidant potency of compounds. The standard curve was obtained for DPPH in methanol (Fig 1.1). The standard curve was obtained by plotting the graph between different concentrations of DPPH and its absorbance value. The linear regression plot between % inhibition of DPPH versus conc. of BHA and PAS (Fig 1.2 – 1.3) has been plotted, and the IC_{50} value was calculated graphically. The linear regression plots were plotted (Fig 1.4-1.7), and IC_{50} was determined and is given in Table 1.4.

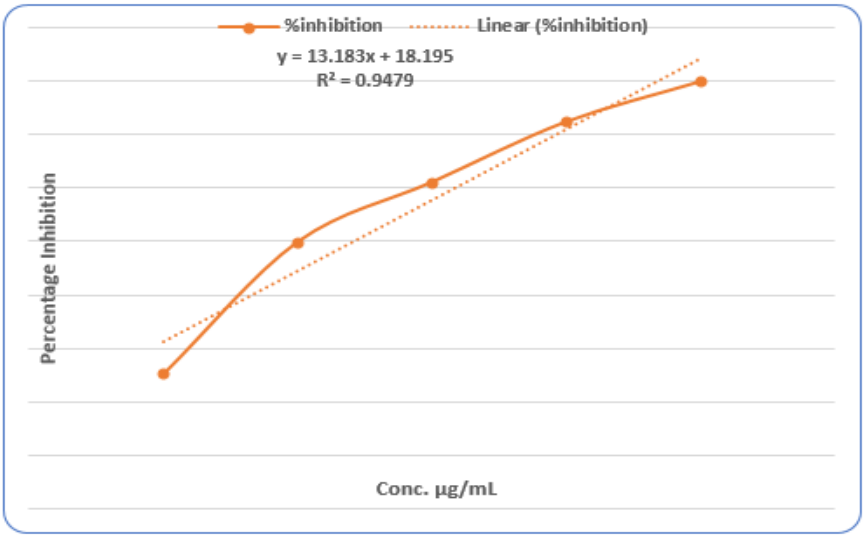


Fig. 1.2: Percent inhibition of DPPH by Butylated Hydroxy Anisole at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml).

Antioxidant activity of the ester derivatives of PAS reveals that ethyl-4-amino-2- hydroxybenzoate (P2) is the most potent antioxidant. The compounds propyl-4-amino-2- hydroxybenzoate (P4) and benzyl-4-amino-2-hydroxy benzoate (P10) have comparable antioxidant activity.

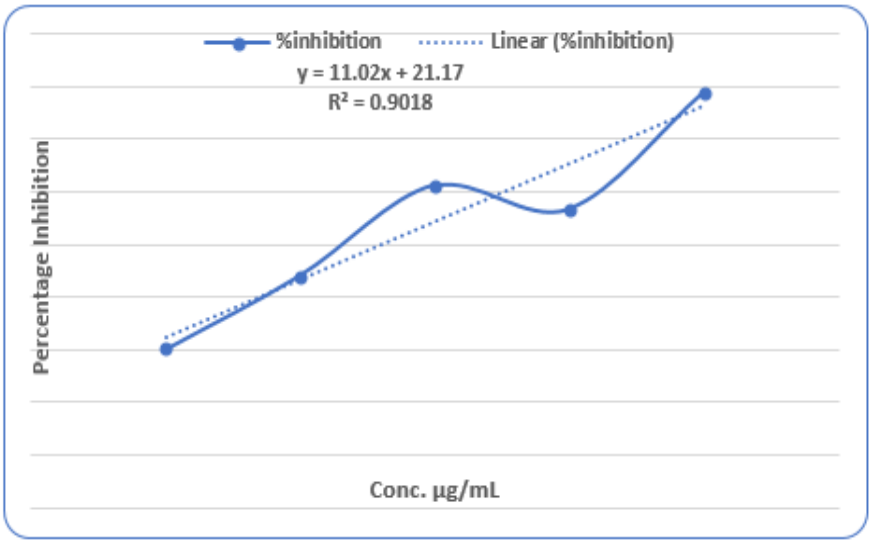


Fig. 1.3: Percent inhibition of DPPH by Para-amino Salicylic Acid at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml).

Table 1.4: The percentage inhibition values and IC_{50} values of the test compounds (ester derivatives of Para aminosalicylic acid).

S. no.	2µg/mL	4µg/mL	6µg/mL	8µg/mL	10µg/mL	IC_{50} value
P1.	30.23	40.23	56.34	58.25	68.19	5.72
P2.	23.31	38.34	43.28	51.72	54.26	8.24
P3.	21.12	28.21	53.31	57.34	69.46	6.38
P4.	29.41	33.52	47.08	56.63	67.58	7.86
P5.	34.40	43.83	56.36	64.42	75.65	5.54
P6.	30.01	40.28	56.09	58.08	68.28	5.91
P7.	40.25	56.29	61.43	58.31	63.34	3.74
P8.	35.32	41.37	57.73	62.32	67.25	5.90
P9.	40.36	51.12	63.21	72.56	79.27	4.00
P10.	38.61	42.27	49.05	53.41	61.06	7.82
P11.	42.21	51.35	60.23	48.71	37.18	5.83
P12.	42.36	56.61	64.46	75.26	87.37	5.57
P13.	30.32	41.23	53.08	59.32	63.32	6.00
P14.	35.23	41.56	57.47	64.48	71.05	6.81
P15.	33.21	42.38	59.91	64.76	75.53	5.42
P16.	30.32	40.01	56.32	65.50	79.05	5.94
P17.	30.01	40.23	56.46	58.08	68.26	2.93
P18.	23.32	43.32	57.71	64.46	79.48	2.72
P19.	43.44	59.14	66.39	62.21	50.17	1.91
BHA	25.32	49.92	61.10	72.37	80.01	6.41
PAS	30.23	43.98	61.23	56.78	78.93	5.83

The table shows the percentage inhibition values of the synthesized ester derivatives of Para-amino salicylic acid, Standard (BHA) and PAS against DPPH (1,1-diphenyl-2-picryl-hydroxyl) at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml) and IC_{50} values calculated graphically by following linear regression plots.

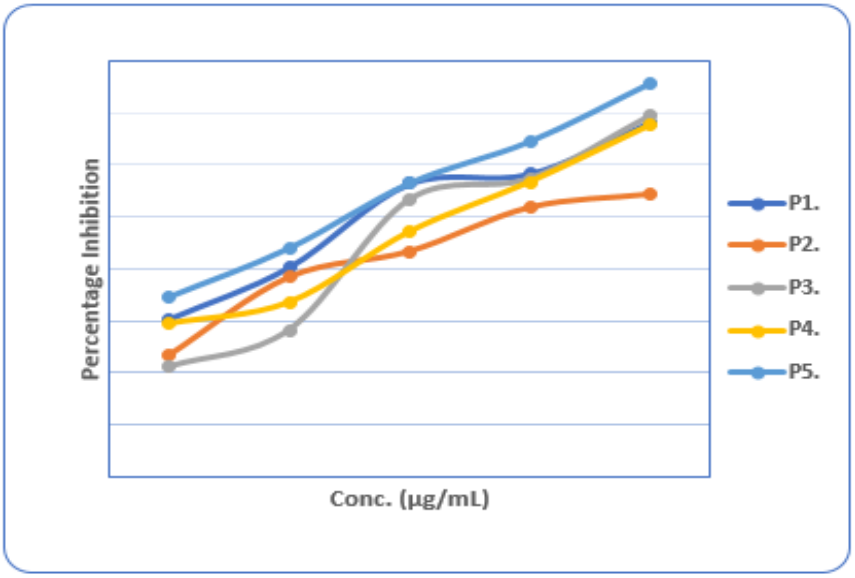


Fig. 1.4: Percent inhibition of DPPH by synthesized derivatives of Para-amino Salicylic Acid (P1, P2, P3, P4, P5) at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml).

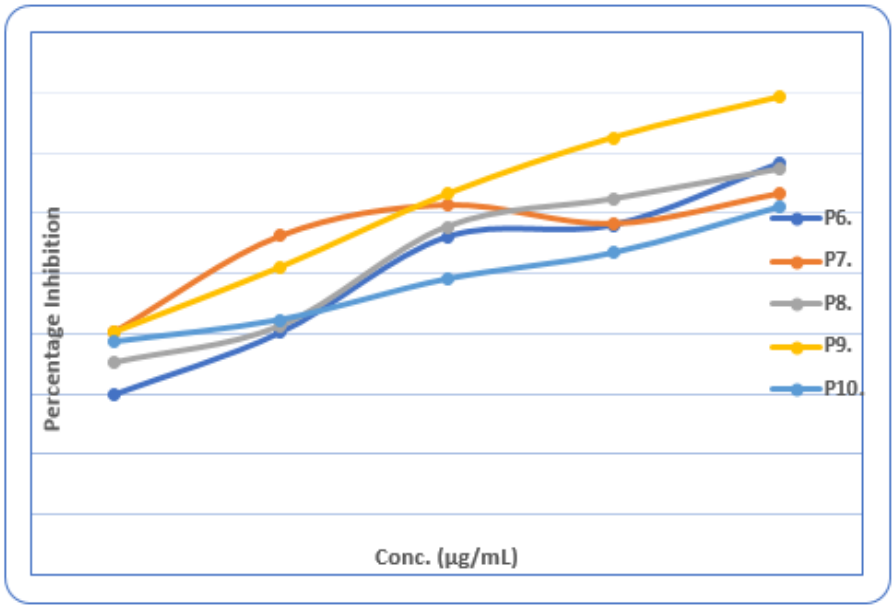


Fig. 1.5: Percentage inhibition of DPPH by Synthesized derivatives of Para-amino Salicylic Acid (P6, P7, P8, P9, P10) at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml).

3.6. Determination of the inhibitory concentration (IC_{50})

IC_{50} is the concentration of the test compounds necessary to decrease the absorbance (activity/colour) of DPPH by 50%. For the interpretation of free radical scavenging effects of the synthesized derivatives. IC_{5O} was determined graphically by plotting the absorbance against the different concentrations of the test compounds or by calculating the slope of the linear regression plot. The values of IC_{50} are given in Table 1.4.

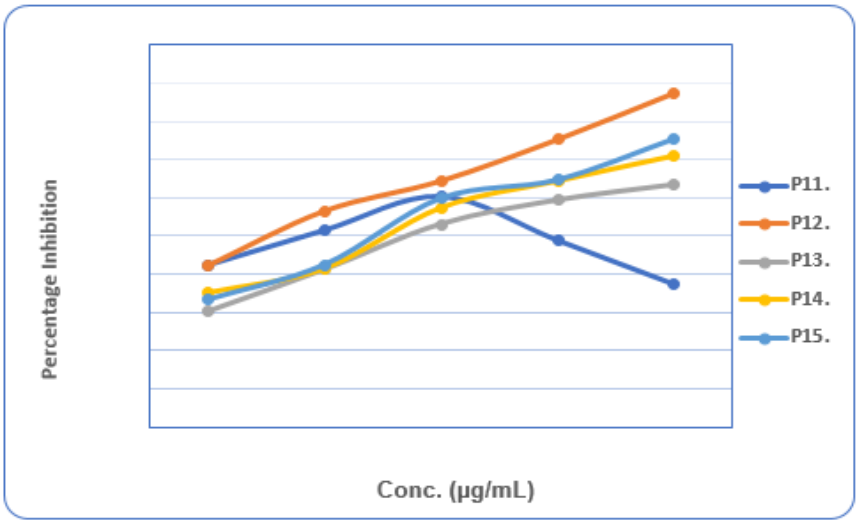


Fig. 1.6: Percentage inhibition of DPPH by Synthesized derivatives of Para-amino Salicylic Acid (P11, P12, P13, P14, P15) at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml)

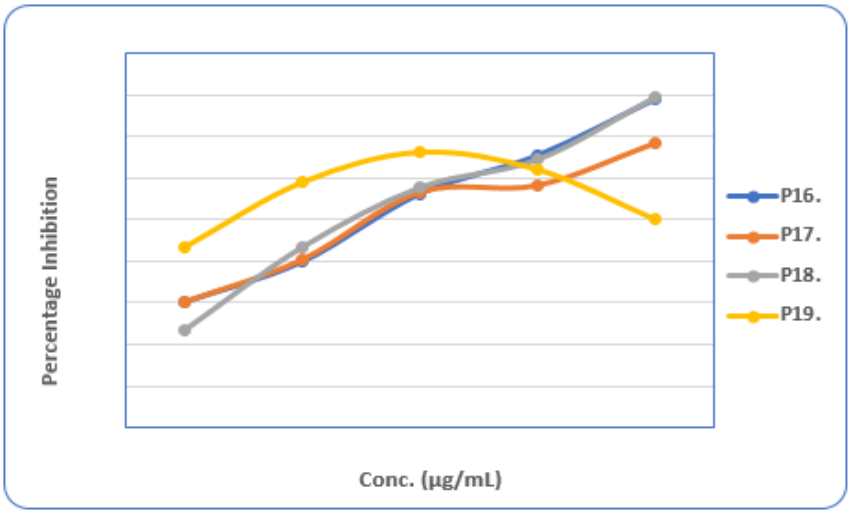


Fig. 1.7: Percentage inhibition of DPPH by synthesized derivatives of Para-amino Salicylic Acid (P16, P17, P18, P19) at different concentrations (2µg/ml, 4µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml)

4. CONCLUSION

In the present study, *para*-amino, salicylic acid derivatives (P1-P28) were synthesized and evaluated for them *in vitro* antimicrobial activity against *S. aureus, B. subtilis, P. aeruginosa, E. coli* and *C. Albicans*. Given all the results obtained, the conclusion can be derived that the ester 5-acetamido-2(propyl carbamoyl) phenyl acetate (P22) is the most effective antibacterial agent among the synthesized derivatives of *para*-aminosalicylic acid. The synthesized derivatives have shown significant activity for *E. coli*. The amide derivatives have shown antibacterial activity, and the ester derivatives 4-methoxy phenyl-4-amino-2-hydroxybenzoate (P15), and 2-chloro phenyl-4-amino-2- hydroxybenzoate (P16) are found to be the effective antifungal agents. Antioxidant activity of the ester derivatives of PAS reveals that ethyl-4-amino-2-hydroxybenzoate (P2) is the most potent antioxidant. The compounds propyl-4-amino-2- hydroxybenzoate (P4) and benzyl-4-amino-2-hydroxy benzoate (P10) have comparable antioxidant activity.

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6. AUTHORS CONTRIBUTIONS STATEMENT

Dr Archana Kapoor has conceptualized, designed and guided the project at all stages. Ms Bharti Thaiya has conducted the experimental part, analysis & biological evaluation of the synthesized derivatives. Bharti Thaiya drafted the manuscript as well. The reviewing and editing of the manuscript were taken care of by Dr Archana Kapoor. All authors have approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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