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1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-ones: Synthesis, antioxidant and antimicrobial activities

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ABSTRACT

A series of 1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-one derivatives were synthesized and evaluated for their antioxidant activity by ABTS radical scavenging assay. Compounds with –OH attached to C-2 of ringB (D6) and -OH and –OCH_3 attached to C-4 and C-3 respectively of the ringB(D4) were found to be more potent than the standard used. The antibacterial activity of synthesized compounds was investigated against the bacterial strains Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae and antifungal activity against Candida albicans using agar well diffusion method. All the compounds showed good inhibition against Klebsiella pneumoniae at 12.5μg /mL concentration.CompoundsD1 and D10displayed good activity against E.coli as well.

Key words: 1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-ones, Antioxidant, Antibacterial, ABTS

INTRODUCTION

Flavonoids are important to human health due to their activity as free radical acceptors. Flavonoids can protect against cancer by inhibiting the damage caused by oxidation processes and are distinguished by their capability to scavenge free radicals and active oxygen groups [1-3]. Flavonoids, which are antioxidants derived from natural sources, including vegetables and fruits, exhibited high reduction activity in the oxidation process, which confirmed the known antioxidant properties that make them successful anti-ageing substances

Dihydrochalcones are reduced forms of chalcones which are open chain flavonoids. They are formed by reduction of α,β -double bond in chalcones. Their structure consists of two benzene rings linked through a saturated three carbon chain. The simplest dihydrochalcone isolated from the stinkhorn (*Phallus impudicus*)had no substituent on ring A or ring B. [4].Their reported activities include antioxidant, anti-inflammatory, antiplasmodial, anticancer and insecticidal activity [5-11].Seven dihydrochalcones from the leaves of Malus crab apples were isolated and characterized. The *in vitro* anticancer activity evaluation showed that one of the isolated compounds showed significant activity against four cell lines. 2',6'-dihydroxy-4'-methoxydihydrochalcone,2',6'-dihydroxy-4,4'-dimethoxydihydrochalcone and phloretin (4,2',4',6'-tetrahydroxydihydrochalcone) markedly augmented TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) mediated apoptosis in LNCaP cells. Sensitization of prostate cancer cells to TRAIL mediated apoptosis by chalcones and dihydrochalcones suggest the potential role of these compounds in anticancer immune defense in which endogenous TRAIL takes part [12].

However in contrast to the synthetic, semi-synthetic and plant derived chalcones, only a few dihydrochalcones have been investigated for possible biological activities. Dihydrochalcones 10',6'-diacetoxy-4,4'-dimethoxydihydro chalcone,4,2',6'-trihydroxy-4'-methoxy dihydrochalcone, 2',6'-dihydroxy-4'-methoxydihydrochalcone and chalcone 2',4'-diacetoxy chalcone isolated from the leaves of Carthamusarborescens showed cytotoxic activity on cell lines P-388, A-549 and HT-29. Of these chalcones 10',6'-diacetoxy-4,4'-dimethoxy- dihydrochalcone was the most potent against human cell line tested [13].

Free radicals are constantly formed in human system either as accidental products during metabolism or deliberately during the process of phagocytosis or due to environmental pollutants, ionizing radiations, ozone, heavy metal poisoning, cigarette smoking and chronic alcohol intake. Free radicals being highly reactive can oxidize biomolecules leading to tissue injury and cell death [14].Antioxidants can be defined as "any substance which significantly delays or inhibits oxidative damage to a target molecule". Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health. The need for antioxidants becomes even more critical with increased exposure to free radicals.

MATERIALS AND METHODS

Melting points were determined by open capillary method .The IR spectra in KBr pellets were recorded using Shimadzu FTIR 8400S spectrophotometer. ^{1}H spectra were recorded by Bruker AV400 (400MHz) spectrometer in deuterated dimethyl sulphoxide using tetramethylsilane as internal standard. Mass spectra were taken using Shimadzu LCMS (ESI) 2010A spectrometer.

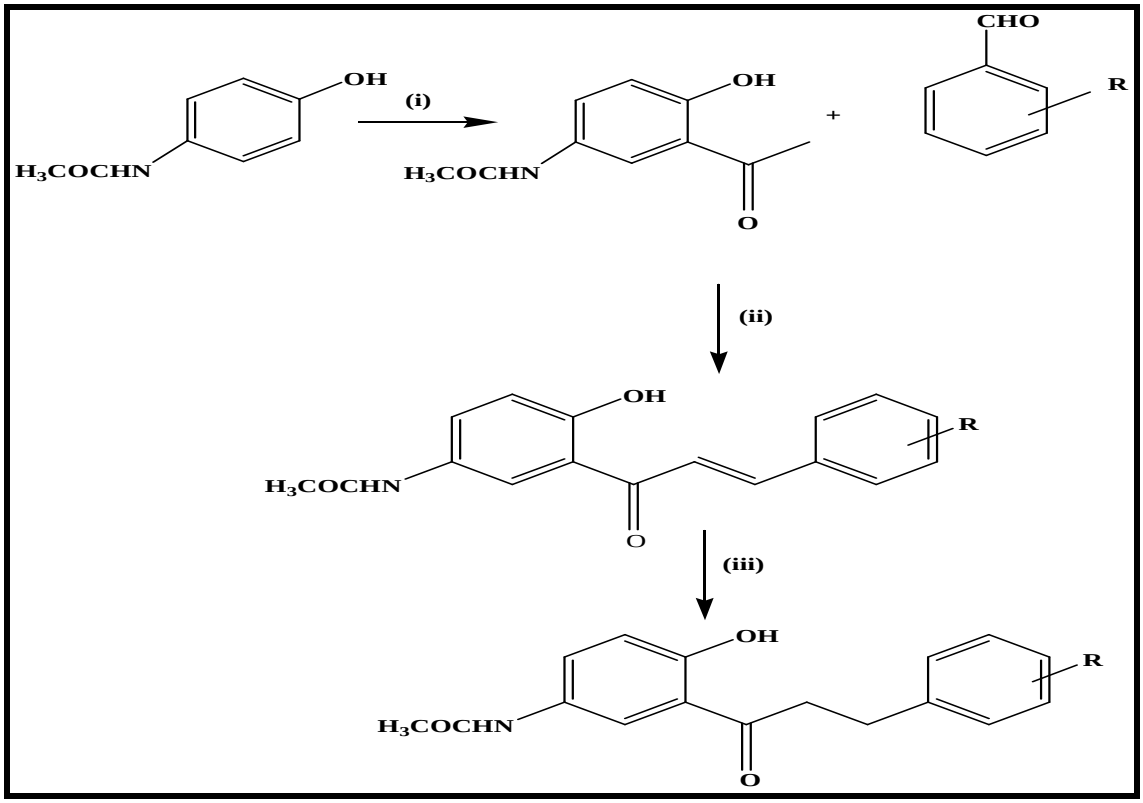
Antibiotic disc of ampicillin (10µg), amoxicillin/ clavulanic acid (20/10 µg), gentamycin (10 µg), amikacin (30 µg), levofloxacin (5 µg), imipenem (10 µg), cefazolin (30 µg), cefepime (30 µg) were purchased from HI-Media, Mumbai, India.

General method for the synthesis of 1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-ones (D1-D10)
1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-ones (5'-Acetamido-2'-hydroxydihydrochalcones) (D1-D10) were synthesized in three steps.

Step-1: Synthesis of 5'-acetamido-2'-Hydroxyacetophenone
Acetyl chloride (0.132mol) was gradually added to a mixture of 4-Acetamido phenol (0.132 mol) and anhydrous aluminium chloride (0.032 mol) in nitrobenzene (100mL) over a period of 30 minutes. The temperature was slowly raised to 130ºC over a period of 0.5 h and maintained for 3h.The reaction mixture was cooled slowly to 40 ºC, poured into a mixture of crushed ice and conc. hydrochloric acid with vigorous stirring and filtered. The crude product thus obtained was washed with water till free from acid followed by toluene and crystallized from isopropanol to yield light brown needle shaped crystals; m. p. 160-164 °C

Step 2: Synthesis of 5'-Acetamido -2'-Hydroxychalcones
A mixture of 5'-acetamido-2'-Hydroxy acetophenone(0.01mol) and aryl aldehyde (0.01 mol) was dissolved in ethanol (30 mL).To this, aqueous potassium hydroxide solution (0.03 mol) was added slowly and stirred for 24 h, at room temperature. After completion of the reaction, the reaction mixture was poured into crushed ice and acidified with 5N hydrochloric acid. The solid separated was filtered and crystallized from ethanol.

Step-3: Synthesis of 5'-Acetamido -2'-Hydroxydihydrochalcones
5'-Acetamido -2'-Hydroxy chalcones, saturated ammonium formate solution [methanol :THF(1:1)] and 10% Pd /C were refluxed for 1.5h.The reaction mixture was filtered to remove the carbon. The product which remained in the filtrate was extracted with ethyl acetate, and dried over anhydrous Na_2SO_4 to obtain 5'-Acetamido-2'-hydroxydihydrochalcones.



Reagents and condition: (i) Acetyl chloride, nitro benzene,anhyrous$AlCl_3$,130°C,3h (ii) alcoholic KOH, rt, 12-24 h; (iii) 10% Pd-C, $HCOONH_4$, MeOH-THF (1:1), reflux, 90 min;

Scheme for the synthesis of 1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-ones

Table 1: Physical data of compounds (D1-10)

Compound Code	R	Yield (%)	MP(°C)	R_f^*
D-1	H	78	138-140	0.51
D-2	4-F	71	167-169	0.56
D-3	3-NO_2	56	168-170	0.58
D-4	4-OH,3-OCH_3	68	152-154	0.63
D-5	3-OH,4-OCH_3	66	163-165	0.69
D-6	2-OH	65	185-187	0.65
D-7	4-OCH_3	75	190-192	0.54
D-8	3,4 -Cl	57	188-191	0.50
D-9	3,4-O-CH_2-O	60	178-180	0.52
D-10	3,4,5-OCH_3	61	189-190	0.50

Solvent system for TLC : petroleum ether: ethyl acetate 2:3

Antioxidant activity

ABTS radical scavenging assay

ABTS is chemically 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid). ABTS˙is generated by addition of potassium per-sulphate to the colourless solution of ABTS. The radical anion (ABTS•+) has ablue-green colour and characteristic long wavelength absorption spectrum λ max of 734 nm. The ability of the test compounds to scavenge ABTS•+ is measured by noting the fall in absorbance at 734 nm.

ABTS (2mM) and Potassium persulphate (17mM) were prepared in distilled water.0.3 mL of prepared potassium persulphate was added in 50 mL 0f ABTS solution.The mixture was covered with aluminium foil and stored overnight in dark at room temperature before use.Different concentration of test compounds were prepared in DMSO starting from 100µM to 0.78 µM and ascorbic acid from 100 µM to 0.8 µM by serial dilution method.To the test and standard well, 25 µL of each of the test and standard dilutions, 100 µL of distilled water and 50 µL ABTS solution was added (n=3).To the sample blank well, 25 µL of each of the test and standard sample and 175 µL of distilled water was added (n=2).To the Control well, 150 µL of distilled water and 50 µL ABTS solution was added (n=16).To the control blank well 150 µL of distilled water and 50 µL of DMSO was added(n=16). The plate was incubated at 37 °C for 30 minutes and absorbance of each well was read at 690 nm using ELISA plate reader.

%Inhibition =(Absorbance of control –Absorbance of test sample / Absorbance of control) X 100

Antimicrobial activity

For antibacterial activity, the bacteria used were Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922), clinical isolate of *Klebsiella pneumonia* (multi drug resistant strain) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and for the antifungal studies *Candida albicans*was used. The antibacterial and antifungal activity were determined using the agar well diffusion method.

The above mentioned bacterial and fungal strains were revived by plating on nutrient agar and Sabouraud dextrose agar (SDA) respectively. After overnight incubation at 37°C, isolated colonies were selected. Standard procedure was used for the identification of the organisms. Isolated bacterial colonies were then transferred to sterile Muller-Hinton Broth (MHB) and Candida albicans was transferred to Sabouraud Dextrose Broth (SDB) and incubated overnight. 0.5 McFarland's turbidity standard was used to adjust the concentration of growth of microorganisms to10^5CFU/mL. Drugs used as positive control were imipenum 10µg, ampicillin 10µg and ketaconazole 10µg. DMSO was used as a negative control.

Determination of Antibacterial Activity: Muller-Hinton Agar (MHA) measuring 20 mL each was poured into petri dishes. The bacterial culture was spread over the surface of the MHA plate. 4mm diameter wells were punched into the agar and filled with 20µl solution of test compounds in various concentrations (200 µg/mL, 100 µg /mL, 50 µg /mL, 25µg/mL and 12.5 µg/mL).The inoculated plates were then kept in the incubator for 18 hrs at 37°C. Tests were done in triplicates and the average of the three was considered for the study.

Determination of antifungal activity: Sabouraud's Dextrose Agar measuring 20 mL each was poured into petridishes. Culture of the Candida albicans was spread over the surface of the SDA plate. Wells were punched into the agar plate measuring 4mm in diameter and filled with 20µl solution of test compounds in various concentrations (200 µg/mL, 100 µg /mL, 50 µg /mL, 25 µg/mL and 12.5 µg/mL). The plates were then kept in the incubator for 18 hrs at 37°C. Tests were done in triplicates and the average of the three was considered for the study.

RESULTS AND DISCUSSION

The reduction of chalcones to dihydrochalcones was done using ammonium formate and 10% Pd-C. ^{1}H NMR showed a multiplet in the range of 3.20-3.25 ppm corresponding to H-α (2H) and a triplet corresponding to H-β (2H) in the range of 2.79- 2.83 ppm characteristic of dihydrochalcones.

1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-one (D1)

IR (KBr) cm^{-1}:3565(OH), 3334(NH), 3010(=CH), 1648 (-NH-C=O), 1685(C=O), 1270(C-O),1178(C-N); ^{1}HNMR (400MHz, DMSO-d6): 2.4 (s,3H,CH_3), 2.79(t,2H,H-β), 3.21-3.25(m,2H,H-α), 6.81-7.51(6H,Ar-H), 9.90 (s,1H,NH),11.20(s,Ar-OH); LC-MSm/z 283.17 (M^+)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(4-fluorophenyl)-propan-1-one (D2)

IR (KBr) cm^{-1}:3534(OH), 3308(NH), 3010(=CH), 1672 (-NH-C=O), 1641 (C=O), 1276(C-O),1201(C-N); ^{1}HNMR(400MHz,DMSO-d6): 2.20 (s,3H,CH_3), 2.80(t,2H,H-β), 3.25-3.28(m,2H,H-α), 6.60-7.21(7H,Ar-H), 9.82 (s,1H,NH),10.90(s,Ar-OH); LC-MSm/z 302.20 (M+1)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(4-nitrophenyl)-propan-1-one (D3)

IR (KBr) cm^{-1}:3524(OH), 3312(NH), 3010(=CH), 1654 (-NH-C=O), 1664 (C=O),1263(C-O),1178(C-N); ^{1}HNMR(400MHz,DMSO-d6): 2.23 (s,3H,CH_3), 2.85(t,2H,H-β),3.18-3.36 (m,2H,H-α), 6.72-7.12 (7H,Ar-H),9.79(s,1H,NH),11.21(s,Ar-OH); LC-MS m/z 329.08 (M+1)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-propan-1-one (D4)

IR (KBr) cm^{-1}:3546(OH), 3317(NH), 3001(=CH), 1658 (-NH-C=O), 1647 (C=O), 1260(C-O),1169(C-N);^{1}H NMR(400MHz,DMSO-d6): 2.3 (s,3H,CH_3), 2.82(t,2H,H-β), 3.426(m,2H,H-α), 3.74(s,3H-Ar-OCH_3, 6.65-8.89 (6H,Ar-H), 9.34(s, Ar-OH), 9.92(s,1H,NH), 11.24(s, Ar-OH);LC-MSm/z 330.17 (M+1)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-propan-1-one(D5)

IR (KBr) cm^{-1}:3580(OH), 3303(NH),3012(=CH), 1672 (-NH-C=O), 1643 (C=O), 1269(C-O),1178(C-N);^{1}HNMR(400MHz,DMSO-d6): 2.4 (s,3H,CH_3), 2.79(t,2H,H-β), 3.51(m,2H,H-α), 3.79(s,3H-Ar-OCH_3, 6.5-8.81 (6H,Ar-H), 9.24(s, Ar-OH), 9.80(s,1H,NH), 11.11(s, Ar-OH); LC-MSm/z 330.17 (M+1)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(2-hydroxyphenyl)-propan-1-one (D6)
IR (KBr) cm^{-1}:3529(OH), 3300(NH), 2998(=CH), 1648 (-NH-C=O), 1668 (-NH-C=O), 1659 (C=O),1260(C-O), 1154(C-N); ^{1}HNMR (400MHz,DMSO-d6):2.41 (s,3H,CH_3),2.85(t,2H,H-β), 3.23 (m,2H,H-α), 6.72-7.12(7H,Ar-H), 9.41(s, Ar-OH), 9.54(s,1H,NH), 11.25(s, Ar-OH; LC-MS m/z 299.08(M^+)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(4-methoxyphenyl)-propan-1-one (D7)
IR (KBr) cm^{-1}:3571(OH), 3310(NH), 3009(=CH),1661 (-NH-C=O),1659 (C=O), 1263(C-O),1178(C-N); ^{1}HNMR (400MHz,DMSO-d6): 2.34 (s,3H,CH_3), 2.83 (t,2H,H-β), 3.20-3.25(m,2H,H-α), 3.81(s,3H,OCH_3), 6.74-7.05 (7H,Ar-H), 9.80(s,1H,NH),10.50(s,Ar-OH); LC- MS *m/z*:314.17 (M+1)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(3,4-dichlorophenyl)-propan-1-one(D8)
IR (KBr) cm^{-1}:3530(OH), 3300(NH), 3010(=CH), 1675 (-NH-C=O), 1644 (C=O), 1276(C-O),1201(C-N);^{1}HNMR(400MHz,DMSO-d6): 2.23 (s,3H,CH_3), 2.81(t,2H,H-β), 3.25-3.29(m,2H,H-α), 6.60-7.10 (6H,Ar-H), 9.80 (s,1H,NH),10.91(s,Ar-OH); LC-MSm/z 353.17(M+1)

1-(5'-acetamido-2'-hydroxyphenyl)-3-(3,4-methylenedioxyphenyl)-propan-1-one(D9)
IR (KBr) cm^{-1}:3582(OH), 3328(NH),3020(=CH), 1654 (-NH-C=O), 1641 (C=O), 1263(C-O),1178(C-N);^{1}H NMR (400 MHz, (DMSO-d_6) δ: 2.38 (s,3H,CH_3),2.80(t,2H,H-β),3.25-3.28(m,2H,H-α), 5.91(s, 2H, O-CH_2),6.51-7.02 (6H,Ar-H),9.90 (s,1H,NH),11.0(s,Ar-OH);LC-MS *m/z*: 327.05 $(M)^+$

1-(5'-acetamido-2'-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)-propan-1-one (D10)
IR (KBr) cm^{-1}:3433(OH), 3315(NH_2), 2939(=CH), 1646 (-NH-C=O), 1670 (C=O),1267(C-O),1180(C-N);^{1}HNMR(400MHz,DMSO-d6): 2.42 (s,3H,CH_3), 2.83(t,2H,H-β), 3.20-3.25(m,2H,H-α), 3.74(s,6H,2xOCH_3), 3.793(s,3H,OCH_3), 6.71-7.09(5H,Ar-H), 9.84(s,1H,NH),11.20(s,Ar-OH); LC-MSm/z 373.08 (M^+)

The antioxidant potential of the synthesized compounds expressed as IC_{50} values is presented in Table 2. All the compounds displayed good antioxidant potential. The compound D6 with IC_{50}=18.8 µMand D4 (IC_{50}=19.6 µM) showed better activity than the standard used.D6 has a hydroxyl group attached to C-2 of ring B and D4 has an hydroxyl group at C-4 and OCH_3at C-3 of ring B. D5 having hydroxyl substituent at C-3 and OCH_3 at C-4of ring B and D9 having a methylenedioxy substitution in the 3rd and 4th position of ring B of the dihydrochalconealso exhibited good antioxidant potential (IC_{50}=28.9 µM , IC_{50}=29.3 µM).

The antibacterial activity of synthesized compounds was investigated against the bacterial strains *Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumoniae* and antifungal activity against *Candida albicans* using agar well diffusion method. The effect of synthesized compounds on bacterial and fungal strains are summarized in Table 3. The diameter of the clear zone of inhibition surrounding the well was measured in mm. The results indicate that the test compounds displayed promising anti-bacterial activity against multi drug resistant, clinical strain *Klebsiella pneumoniae* and few of them showed sensitivity towards *E.coli* and *P.Arugenosa*. D 8 and D 9 showed sensitivity towards *C albicans*.

Table 2: IC_{50} of 1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-onesusingABTS scavenging assay

Compound Code	IC_{50} (µM)
D1	58.9
D2	63.3
D3	77.1
D4	19.6
D5	28.9
D6	18.8
D 7	40.1
D8	58.7
D9	29.3
D10	35.8
Ascorbic acid	20.81

Table 3: Antimicrobial activity of the synthesized compound: Zone of inhibition (mm) of synthesized compounds

Compound code	Anti-bacterial activity												Anti-fungal activity
	Gram negative						Gram positive						
	K. pneumoniae			*E coli*			*P.Arugenosa*			*S.aureus*			*C.albicans*
	50µg /mL	25µg /mL	12.5µg/ mL	50µg /mL	25µg /mL	12.5 µg/mL	50µg / mL	25µg / mL	12.5µg / mL	50µg /mL	25µg /mL	12.5 µg/mL	12.5 µg /mL
D1	14	13	13	13	13	13	8	8	-	-	-	-	-
D2	13	13	13	-	-	-	11	10	10	-	-	-	-
D3	13	13	13	-	-	-	8	8	8	-	-	-	-
D4	15	12	12	-	-	-	12	11	11	-	-	-	-
D5	13	13	12	-	-	-	-	-	-	-	-	-	-
D6	11	11	10	-	-	-	-	-	-	-	-	-	-
D7	12	12	10	-	-	-	-	-	-	-	-	-	-
D8	11	11	11	-	-	-	-	-	-		-	-	10
D9	11	11	10	-	-	-	-	-	-	--	-	-	-
D10	13	13	12	12	11	11	-	-	-	-	-	-	-
Imipenem (10µg)			17mm										
Ampicillin (10µg)						30mm			30mm				
Ketoconazole (10µg)													14mm
Klebsiella pneumoniae was resistant to ampicillin(10µg), amoxicillin/ clavulanic acid (20/10 µg), gentamycin (10 µg), amikacin (30 µg), levofloxacin (5 µg), imipenem (10 µg), cefazolin (30 µg) and cefepime (30 µg).													

(-) refers to no activity

CONCLUSION

The antioxidant potential of a series of 1-(5'-acetamido-2'-hydroxyphenyl)-3-propan-1-one derivatives were evaluated using ABTS radical scavenging assay. Compounds having -OH group attached to ortho or para position of ring B of dihydrochalcone showed very good antioxidant activity.Regardless of the nature of substituents present in ring B of synthesized dihydrochalcones , all the compounds exhibited significant antibacterial activity against multi drug resistant (MDR) strain of *Klebsiella pneumoniae.*

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