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Synthesis and antibacterial evaluation of some novel hesperidin semisynthetic derivatives

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ABSTRACT

Flavonoids are one of the active principles isolated from plant sources. These compounds have found to have enormous pharmacological activity, Hesperidin, a molecule with a wide spectrum of pharmacological activity still has not been used for treatment of any diseases due to its lacking potency. The present study was focused to synthesize various derivatives of Hesperidin and to evaluate their activity. Among the various synthesized compounds, compound Hg, Hn and Hj have shown more potent activity even when compared with the compound. Whereas all other compounds were not potent as standard compound but were found to have increased in potency while comparing with the standard compound. Thus we conclude substitution of the oxygen moiety with the hydrazide to form hydrazones increases the antibacterial activity and presence of electron donating groups further increases the activity.

Keywords: Hydrazone derivatives, Hesperidine, Ampicillin, Antibacterial activity.

INTRODUCTION

The flavonoids are one of the active principles isolated from plant sources. These compounds have found to have enormous pharmacological activity. Even though these compounds possess pharmacological activity still no remarkable molecule has come out of these molecules. Thus in the present study Hesperidin a flavonoid found and isolated from grape peel extract was used for the study. Many studies have reported that Hesperidine has reported to contain many pharmacological activities like antidepressant activity[1], antioxidant activity[2], hepatoprotective activity[3], anti-inflammatory activity[4], hypoglycemic effects[5], hyaluronidase inhibitor activity[6], hypercholesterolemic activity[7], antibacterial[8,9] etc., Being a molecule with a wide spectrum of pharmacological activity still now these molecules have not been used for treatment of any diseases due to its lacking potency. Thus in the present study various derivatives of Hesperidine was synthesized by reacting with various hydrazine and semicarbazone derivatives to form their respective analogs.

Even though a wide variety of drugs are being used in the treatment of bacterial infections still there is a search for a safe and potent antibacterial agent. Since these antibacterial agents are supposed to be taken for more than three days to complete their doses, thus there is a real need in a safer drug for the treatment of antibacterial infections.

MATERIALS AND METHODS

All the chemicals used for the present study are of Laboratory grade reagents and the standard compounds and the solvents used were of analytical grade. All the chemicals and solvents were pure.

1. Synthesis of various semisynthetic derivatives of Hesperidin

1.1 General procedures for the synthesis of hydrazone and carbazone derivatives of Hesperidin[10]:-

In a 250ml conical flask, 1g of hydrazine or Semi carbazide hydrochloride analog, 1.5g of crystallized sodium acetate in 8-10 ml of water and 0.5g of the hesperidin was taken and shake for few minutes. Few ml of alcohol was added to form a clear solution. Then the reaction mixture was slightly heated on a water bath to increase the rate of the reaction and placed in ice bath to form crystals of semicarbazone / hydrazones. Then it was filtered and washed using little cold water & recrystallized from ethanol. The purity of the recrystallized compound was identified using TLC and the structure was confirmed using physical and spectral analysis.

1.2 General procedures for the synthesis of benzoic acid derivatives of Hesperidin[11]:-

In a 250ml round bottom flask, 1g of Benzoic Acid / substituted benzoic acid dissolved in 5ml of Methanol (absolute) and 2-3 drops of Conc.H2SO4 was taken and refluxed on a steam bath to form Methyl substituted benzoates. The methyl ester formed was refluxed on water bath with 1ml of hydrazine hydrate dissolve in methanol to form benzhydrazides. To the above reaction mixture 0.5g of hesperidin dissolved in methanol was added along with catalytic amount of acetic acid and stirred along with slight warming for few minutes and then placed in ice bath until crystals of carbazone are formed. The crystals were filtered and washed using little cold water & recrystallized from ethanol. The purity of the recrystallized compound was identified using TLC and the structure was confirmed using physical and spectral analysis.

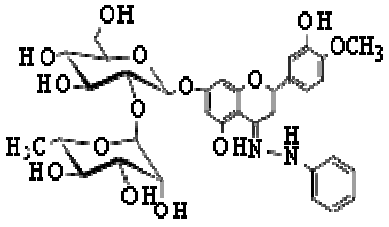
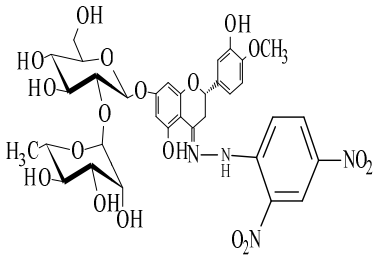
1.3 General procedures for the synthesis of benzaldehyde derivatives of Hesperidin[12]:-

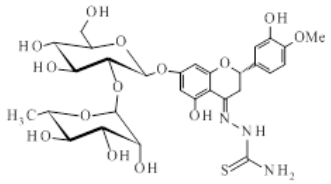
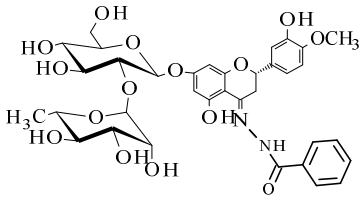
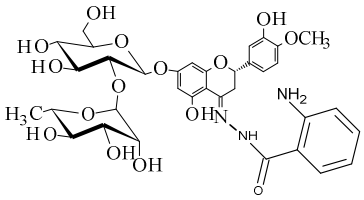
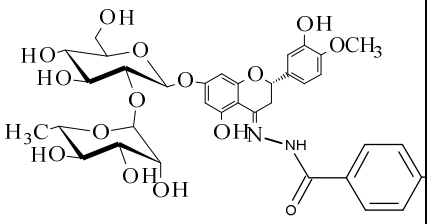
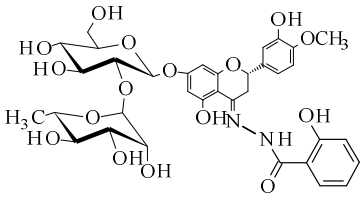
In a 250ml conical flask, 0.5g of hydrazine hydride, 0.8g of sodium acetate in 5ml of water and a solution of 0.5g of benzaldehydes / substituted benzaldehydes in a little ethanol were taken and shaken for few minutes. Few ml of alcohol was added to form a clear solution. Then the reaction mixture was slightly heated on a water bath to increase the rate of the reaction and placed in ice bath to form crystals of hydrazones. Filter and wash with cold water and ethanol was used for recrystallization. The recrystallized product along with 0.8g of sodium acetate in 5ml of water and add a solution of 0.2-0.4g of hesperidin in little amount of ethanol was taken in a 250ml conical flask and shaken for few minutes. Few ml of alcohol was added to form a clear solution. Then the reaction mixture was slightly heated on a water bath to increase the rate of the reaction and placed in ice bath to form crystals of hydrazones. Filter and wash with cold water and ethanol was used for recrystallization. The purity of the recrystallized compound was identified using TLC and the structure was confirmed using physical and spectral analysis.

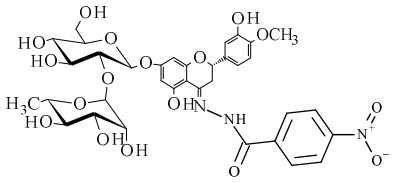
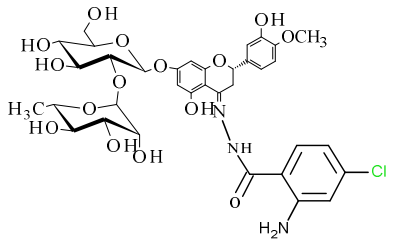
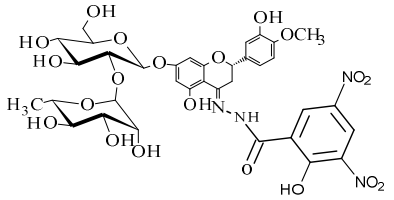
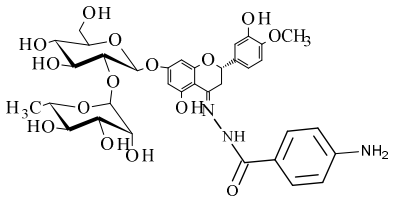
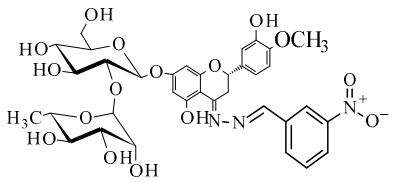
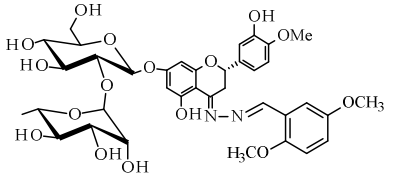
Table No. 1. Physical properties of the synthesized compounds

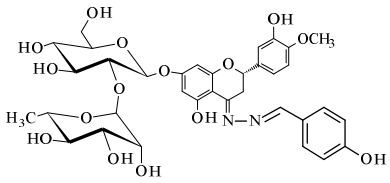
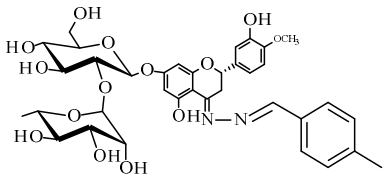
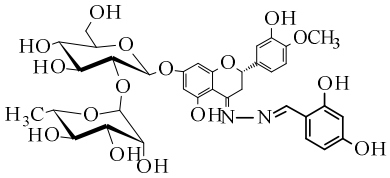
Compound Name	Molecular formula	Molecular Weight	Melting point (ºC)	% yield	R_f value
H.a	$C_{28}H_{36}N_2O_{13}$	608	185	81	0.67
H.b	$C_{34}H_{40}N_2O_{13}$	684	252	73	0.59
H.c	$C_{34}H_{38}N_4O_{17}$	774	249	59	0.78
H.d	$C_{28}H_{35}NO_{14}$	609	238	65	0.83
H.e	$C_{29}H_{37}N_3O_{14}$	652	235	65	0.90
H.f	$C_{29}H_{37}N_3O_{13}S$	668	210	94	0.75
H.g	$C_{35}H_{40}N_2O_{14}$	713	230	68	0.91
H.h	$C_{35}H_{41}N_3O_{14}$	728	210	54	0.6
H.i	$C_{35}H_{39}ClN_2O_{14}$	747	200	46	0.81
H.j	$C_{35}H_{40}N_2O_{15}$	729	219	55	0.73
H.k	$C_{35}H_{39}N_3O_{16}$	758	238	72	0.98
H.l	$C_{35}H_{40}ClN_3O_{14}$	762	220	58	0.83
H.m	$C_{37}H_{44}N_2O_{15}$	757	200	65	0.7
H.n	$C_{35}H_{41}N_3O_{14}$	728	198	86	0.64
H.o	$C_{35}H_{39}N_3O_{15}$	742	250	67	0.63
H.p	$C_{37}H_{44}N_2O_{16}$	773	252	76	0.66
H.q	$C_{35}H_{40}N_2O_{14}$	713	249	82	0.70
H.r	$C_{36}H_{42}N_2O_{13}$	711	255	58	0.74
H.s	$C_{35}H_{40}N_2O_{15}$	729	245	69	0.65

Table No. 2 IR and ^{1}HNMR Spectral Data's of all the Synthesized Compounds (Ha-s)

Compound Name	Molecular formula	Final structure	IR SPECTROSCOPY		^{1}HNMR δppm 300 MHz, DMSO- d6
			Spectral peaks cm^{-1}	Functional groups	
H.a	$C_{28}H_{36}N_2O_{13}$		3410 2915 2848 1644 1355 1277 1091	-NH_2 Stretching -C-H Stretching -C-H Stretching -C=C Stretching -C-N Stretching -C=N Stretching -O-C Stretching	9.10 (1H, s, 5-OH), 8.78 (1H, s, 3′- OH), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H,d, H-6), 7.5.46 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.b	$C_{34}H_{40}N_2O_{13}$		3641 3401 2886 1642 1603 1512 1359 1276 1130	-O-H Stretching -NH_2 Stretching -C-H Stretching -C=C Stretching -N-H Bending -C=C Stretching -C-N Stretching -C=N Stretching -O-C Stretching	9.10 (1H, s, 5-OH), 8.63 (1H, s, 3′- OH), 7.44 (4H, dd, 2,3,5,6), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.c	$C_{34}H_{38}N_4O_{17}$		3856 3417 2915 1644 1604 1515 1277 1092	-O-H Stretching -NH_2 Stretching -C-H Stretching -C=C Stretching -N-H Bending -C=C Stretching -C=N Stretching -O-C Stretching	9.80 (1H, s, 5-OH), 9.10 (1H, s, 3′- OH), 7.44 (4H, dd, 2,3,5,6), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.d	$C_{28}H_{35}NO_{14}$		3731 3413 2916 2848 1644 1514 1277 1091	-O-H Stretching -NH_2 Stretching -C-H Stretching -C-H Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	11.80 (1H, s, 5-OH), 9.80 (1H, s, N-OH), 9.10 (1H, s, 3′- OH), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.e	$C_{29}H_{37}N_3O_{14}$		3729 3537 3408 2916 1644 1514 1277 1091	-O-H Stretching -NH_2 Stretching -NH_2 Stretching -C-H Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	9.10 (1H, s, 5-OH), 7.67(1H, s, 3′- OH), 7.64 (2H, dd, NH2), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);

H.f	$C_{29}H_{37}N_3O_{13}S$		3737 3540 3416 2919 1643 1515 1277 1091	-O-H Stretching -NH_2 Stretching -NH_2 Stretching -C-H Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.09 (1H, s, 3′- OH), 8.63 (2H, s, NH2), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.g	$C_{35}H_{40}N_2O_{14}$		3712 3396 2919 1728 1643 1511 1275 1057	O-H Stretching -NH_2 Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.09 (1H, s, 3′- OH), 7.44 (2H, s, NH2), 6.91(3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.h	$C_{35}H_{41}N_3O_{14}$		2918 1645 1508 1278 1132	-C-H Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 8.38 (1H, s, 3′- OH), 7.68 (4H, dd, 2,3 ,5,6), 7.44 (2H, s, NH2), 6.91 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.i	$C_{35}H_{39}ClN_2O_{14}$		3542 3469 3417 2921 2853 1734 1643 1513 1274 1127	O-H Stretching -NH_2 Stretching -NH_2 Stretching -C-H Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.09 (1H, s, 3′- OH), 8.63 (1H, s, N-H), 7.56 (4H, dd, 2,3 ,5,6), 7.44 (2H, s, NH2), 6.91 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.j	$C_{35}H_{40}N_2O_{15}$		3737 3540 3416 2919 1643 1515 1277 1091	-O-H Stretching -NH_2 Stretching -NH_2 Stretching -C-H Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.86(1H, s, 2-OH), 9.09 (1H, s, 3′- OH), 8.63 (1H, s, N-H), 7.56 (4H, dd, 2,3 ,5,6), 6.91 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);

H.k	$C_{35}H_{39}N_3O_{16}$		3422 2950 1714 1645 1520 1276 1097	-NH_2 Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.09 (1H, s, 3′- OH), 7.59 (4H, dd, 2,3 ,5,6), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.l	$C_{35}H_{40}ClN_3O_{14}$		3422 2950 2852 1714 1645 1520 1276 1097	-NH_2 Stretching -C-H Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.09 (1H, s, 3′- OH), 7.59 (4H, dd, 2,3 ,5,6), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.m	$C_{37}H_{44}N_2O_{15}$		3624 3387 3076 2925 1675 1645 1529 1265 1063	-O-H stretching -NH_2 Stretching -C-H Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 9.09 (1H, s, 3′- OH), 7.59 (4H, dd, 2,3 ,5,6), 7.44 (2H, s, NH2), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.n	$C_{35}H_{41}N_3O_{14}$		3712 3396 2919 1728 1643 1511 1275 1057	O-H Stretching -NH_2 Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH), 8.77 (1H, s, 3′- OH), 8.76 (4H, dd, 2,3 ,5,6), 8.55 (2H, s, NH2), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.12 (1H, d, H-6), 5.46 (1H, dd, H-2), 5.3 (1H, s NH), 4.97 (1H, d, H-1″), 4.53 (1H, s, H-1‴), 3.77 (3H, s, 4′-OCH_3), 3.25 (1H, m, H-3b), 2.78 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.o	$C_{35}H_{39}N_3O_{15}$		3672 3470 3084 2925 1744 1644 1518 1273 1066	O-H Stretching -NH_2 Stretching -C-H Stretching -C-H Stretching -C=O Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.01 (1H, s, 5-OH), 9.01 (1H, s, 3′- OH), 8.92 (4H, dd, 2,3 ,5,6), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.10 (1H, d, H-6), 5.51 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.50 (1H, s, H-1‴), 3.75 (3H, s, 4′-OCH_3), 3.22 (1H, m, H-3b), 2.72 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.p	$C_{37}H_{44}N_2O_{16}$		3856 3417 2915 1644 1604 1515 1277 1092	-O-H Stretching -NH_2 Stretching -C-H Stretching -C=C Stretching -N-H Bending -C=C Stretching -C=N Stretching -O-C Stretching	12.01 (1H, s, 5-OH), 9.10 (1H, s, 3′- OH), 8.90 (4H, dd, 2,3 ,5,6), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.10 (1H, d, H-6), 5.51 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.50 (1H, s, H-1‴), 3.75 (3H, s, 4′-OCH_3), 2.49(6H, s, 2-OCH_3), 3.22 (1H, m, H-3b), 2.72 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);

H.q	$C_{35}H_{40}N_2O_{14}$		3672 3470 3084 2925 1644 1518 1273 1066	O-H Stretching -NH_2 Stretching -C-H Stretching -C-H Stretching -C=C Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.00 (1H, s, 5-OH),9.15 (1H, s, 3′- OH), 8.90 (1H, s, 4’-OH), 7.45 (4H, dd, 2,3 ,5,6), 6.94 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.10 (1H, d, H-6), 5.51 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.50 (1H, s, H-1‴), 3.75 (3H, s, 4′-OCH_3), 2.49(6H, s, 2-OCH_3), 3.22 (1H, m, H-3b), 2.72 (1H, dd, H-3a), 1.08 (3H, d, H-6‴);
H.r	$C_{36}H_{42}N_2O_{13}$		3733 3542 3471 2926 2840 1645 1296 1095	O-H Stretching -NH_2 Stretching -NH_2 Stretching -C-H Stretching -C-H Stretching -C=C Stretching -C=N Stretching -O-C Stretching	12.01 (1H, s, 5-OH),9.10(1H, s, 3′- OH), 8.62 (1H, s, 4’-OH), 7.81 (4H, dd, 2,3 ,5,6), 6.92 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.10 (1H, d, H-6), 5.50 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.50 (1H, s, H-1‴), 3.75 (3H, s, 4′-OCH_3), 3.22 (1H, m, H-3b), 2.72 (1H, dd, H-3a), 1.08 (3H, d, H-6‴), 1.06 (3H, d, H-6‴).
H.s	$C_{35}H_{40}N_2O_{15}$		3650 3453 1609 1511 1249	O-H Stretching -NH_2 Stretching -C=C Stretching -C=C Stretching -C=N Stretching	12.01 (1H, s, 5-OH),11.39 (2H,s, 2,4-OH), 9.09(1H, s, 3′- OH), 8.76 (1H, s, 4’-OH), 7.42 (4H, dd, 2,3 ,5,6), 6.92 (3H, m, H-2′,5′,6′), 6.12 (1H, d, H-8), 6.10 (1H, d, H-6), 5.50 (1H, dd, H-2), 4.97 (1H, d, H-1″), 4.50 (1H, s, H-1‴), 3.33 (3H, s, 4′-OCH_3), 3.22 (1H, m, H-3b), 2.49 (1H, dd, H-3a), 1.08 (3H, d, H-6‴).

The compounds synthesized and their structures, their physical data and their spectral data’s are represented in table no, 1 and 2 respectively.

2. Antibacterial Assay:

The compounds synthesized using the above mentioned procedures were evaluated for antibacterial activity as per the reported methods [13, 14].

The antibacterial activity of synthesized compounds was performed against gram positive bacteria viz., M.luteus (MTCC NO 1538), B.subtilis (MTCC NO 441) and S.aureus (MTCC NO 3160) and three gram negative bacteria viz., E.coli (MTCC NO 443), P.fluorescens (MTCC NO 2421) and P. aeroginosa (MTCC NO 441) by using cup plate method. Ampicillin sodium was employed as standard to compare the results.

Solution of the test compounds were prepared by dissolving 10mg each in dimethyl sulphoxide (10 ml, AR grade). A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of ampicillin sodium in sterile distilled water, separately.

About 27ml of Sterilized molten nutrient nutrient agar medium was taken in sterilized petriplate (10 cm diameter), and inoculated with the respective strain of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) and were left at room temperature for solidification. After solidification make three cups of 6 mm diameter to each plate and fill with 0.1 ml of the test solution aseptically and label, accordingly and placed in a refrigerator for 2 hours without disturbing to allow diffusion of the solution in the medium. Then incubated at $37^o \pm 1^oC$ for 24 hours, then the diameter of zone of inhibition was measured using antibiotic zone reader. All the experiments were performed in triplicate. 0.1 ml of dimethyl sulphoxide was used as control to observe the solvent effects. The results are presented in Tables No.3.

Table No.3 Antibacterial Activity of the Hesperidin derivatives (Ha-Hs)

Compound	Antibacterial Activity (Zone of inhibition in mm)					
	B. subtillis	*S. aureus*	*M.luteus*	*E. coli*	*P. aeruginosa*	*P. fluorescens*
H.a	18	17	19	16	13	14
H.b	14	15	21	14	11	12
H.c	14	16	12	13	15	13
H.d	13	17	18	13	16	11
H.e	14	14	22	14	12	12
H.f	17	13	16	15	15	13
H.g	15	14	18	14	12	11
H.h	16	15	17	15	14	13
H.i	19	18	21	23	19	16
H.j	22	28	24	21	20	25
H.k	14	16	18	20	21	14
H.l	15	15	17	18	19	15
H.m	16	17	20	19	21	12
H.n	22	23	20	25	23	28
H.o	17	12	22	13	16	11
H.p	15	11	17	14	13	10
H.q	16	12	18	13	15	11
H.r	15	13	19	12	16	13
H.s	16	11	18	14	12	12
DMSO (BLANK)`	-	-	-	-	-	-
STANDARD	24	20	24	20	20	19
Hesperidine	10	09	05	03	08	11
**Concentration of Test Compound:100 µg/cup*						

RESULTS AND DISCUSSION

The R_f value of the synthesized compounds differ from their parent compounds and all the TLC has shown a single spot which clearly proves the purity of the synthesized compounds. The IR Spectrum of the compounds shows the absence of C=O peak at 1700cm^{-1} clearly indicates that oxygen present in the carbonyl carbon is replaced by the hydrazide/ semicarbazide moieties to form respective hydrazone and semicarbazone derivatives. The ^{1}HNMR data's also clearly suggests the formation of the final compounds with the presence of doublet at around 4.97 and 4.53 clearly indicates that the sugar moieties are not cleaved and presence of doublet at 1.08 clearly indicates the presence of methyl group attached to the sugar moiety, presence of a singlet at the down field range of 9 to 12 indicates the presence of 5-OH group. The presence of singlet around 3.99 indicates the presence of methoxy group at the aromatic ring attached to the flavanone moiety. These data's clearly confirms the structures of the synthesized compounds.

Antibacterial Activity

The results of the antibacterial activity have shown that the modification of carbonyl compound to hydrazide and semicarbazone derivative have increased the antibacterial activity. The hydrazone moiety were found to increase the activity whereas while comparing the activity of aliphatic and aromatic hydrazones the compound with aromatic rings has shown good antibacterial activity, whereas while comparing the activity of the acyl and aryl hydrazone and carbazone derivatives the acyl derivatives have proven to have better antibacterial activity. While comparing the presence of substituents in the ring of the aromatic ring the presence of electron donating group has good activity whereas the presence of electron withdrawing group has significantly reduced the activity.

CONCLUSION

The present study was focused to synthesize various derivatives of Hesperidin and to evaluate their activity. Among the various synthesized compounds, compound Hg, Hn and Hj have shown more potent activity even when compared with the compound. Whereas all other compounds were not potent as standard compound but were found to have increased in potency while comparing with the standard compound. Thus we conclude substitution of the oxygen moiety with the hydrazide to form hydrazones increases the antibacterial activity and presence of electron donating groups further increases the activity.

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