

## Synthesis and Biological Activities of a Novel Naringin based Heterocyclic Derivatives

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### Abstract

A number of naringin based heterocyclic derivatives dioxolane and imidazolidine have been synthesized and evaluated for their antioxidant and biological activities. The chemical structures of the newly synthesized compounds were verified on the basis of spectral and elemental methods of analysis. Investigation of antibacterial activity of these compounds was determined by measuring MIC value using broth micro dilution method for Gram-positive and Gram-negative bacteria, among the various synthesized compounds. Dioxolane showed the highest antibacterial activity with minimal inhibitory concentration (MIC) of 0.125mg/ml, in addition this compound exhibit the best antioxidant activity with inhibition concentration (IC<sub>50</sub>) of 18.7 µg/mL, compared with other semi synthetic derivative.

**Keywords:** *antibacterial activity, antioxidant, naringin; dioxolan, imidazolidin.*

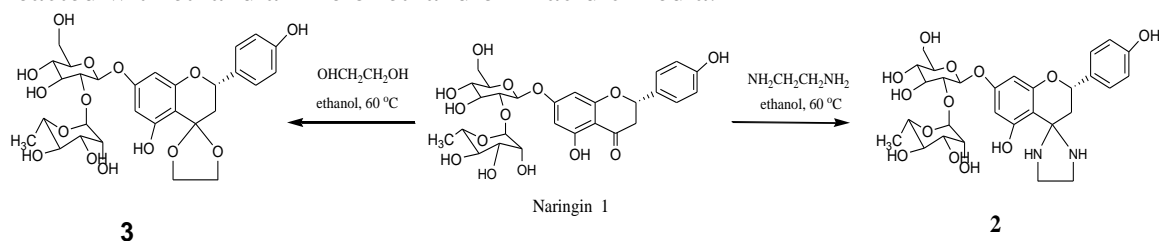
### 1. Introduction

Heterocyclic compounds are very important class of organic compounds with various bioactivities ranging from antibacterial to anticancer [1- 4]. For example, imidazolidine compounds shows significant bacterial effects against (Escherichia coli, Staphylococcus aureus and Mycobacterium tuberculosis) and Leishmania protozoa [5] Also, 1,3-dioxolane heterocycles have anticancer activity and they are effective modulators to overcome multidrug resistance [6]. At the present work, we have employed naringin as a naturally occurring skeleton for the synthesis of heterocyclic system such as imidazolidine and dioxolane which might exhibit promising biological activities.

Naringin; (S)-7-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one, is a flavonoid found and isolated from grape peel extracts. Flavonoids, in general are a group of polyphenolic compounds found in fruits and vegetables. This class of compounds has received much attention because of their pharmacological activities in the treatment of diseases such as allergy, diabetes mellitus, cancer, viral infections, bacterial inflammations and others [7 - 9]. Many studies have reported that naringin contains many pharmacological activities like antibacterial[10], antioxidant activity[11], lipid lowering effect [12], hypercholesterolemic activity[13], cytotoxic effect [14], anti-inflammatory effect [15], hepatoprotective activity [16], etc. These literature findings have led us to synthesize hybrid of naringin - based heterocyclic derivatives, such as dioxolane and imidazolidine then screen them against representative panel of Gram-positive and Gram – negative bacteria, and measure scavenging effect against free radicals compared with the corresponding free flavonoid, naringin

## 1. Experiment

All chemicals used in this study were pure and used without any further purification unless otherwise specified. The solvents and standard compounds used were of analytical grade. All prepared compounds were characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, IR spectroscopy, MS, and melting point. Nuclear Magnetic Resonance spectra were recorded on Varian Gemini 2000, 300 MHz instrument and on Bruker DPX-300 MHz instruments. Infrared spectra were recorded in KBr on a Shimadzu 820 PC FT-IR spectrometer. All  $^1\text{H}$  NMR experiments were reported in  $\delta$  units, parts per million (ppm) downfield from (DMSO). All  $^{13}\text{C}$  NMR were reported in ppm relative to (DMSO). All melting points were determined in an open capillary tube and are uncorrected. At least two measurements were carried out for each compound. TLC analysis was performed on silica gel plates pre-coated with Merck Kiesegel 60 F254 and visualization was done using UV lamp. Samples purifications were performed using flash chromatography with silica gel (100-200) mesh. Compounds used in this study were prepared following literature procedure with minor modification [17]. In this procedure, naringin (**1**) was reacted with ethandiamine or ethandiol in acidic media.

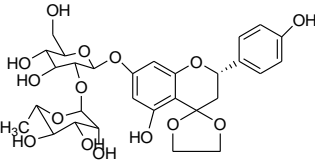


**Figure 1.** Naringin based heterocyclic derivatives

Acid used for this purpose was dilute hydrochloric acid, and ethanol used as a solvent, and the reaction was carried under water bath at 60 °C. The progress of the reaction was monitored by thin layer chromatography(TLC). Purification of the products was performed using flash chromatography. Purified products were analyzed by various analytical and spectroscopic techniques, such as; melting point, IR, LC/MS,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR. In all cases results are consistent with the expected structures. Both compounds were

obtained in acceptable yield. The structures and the characterization data for the prepared compounds are summarized in. **Fig 1** and in table 1. The reaction mechanism occurs by nucleophilic attack on the carbonyl group leading to a heterocycle formation

**Table 1:** Naringin Based derivatives Prepared From Reacting naringin with ethanediamine and ethanediol

Compound	Compound structure	Elemental Analysis	Mp (°C)	Yield %
2		Anal. Calcd for C <sub>29</sub> H <sub>38</sub> N <sub>2</sub> O <sub>13</sub> : C 55.94, H 6.15, N 4.50, O 33.41.	<b>60-62</b>	<b>52.6</b>
3		Anal. Calcd for C <sub>29</sub> H <sub>36</sub> O <sub>15</sub> : C 55.77, H 5.81, O 38.42.	<b>223-225</b>	<b>46.8</b>

### 1.1. General Procedure for preparation of naringin based compounds [17]

The general experimental procedure for the preparation of compounds reported in table 1 was as follows: In a round bottom flask, the desired amine (1.5 mmole) or diol was dissolved in 8 – 10 mL ethanol. This mixture was acidified with few drops of 3M HCL solution. The mixture shaken and warmed in water bath ( about 60 °C) for 10 min. In a separate flask naringin (1.5 mmole, 0.87 g) was dissolved in ethanol (10 ml) and the solution was shaken and slightly warmed in water bath until clear mixture is obtained. The produced solution was slightly heated on a water bath for another 10 -15 min to increase the rate of the reaction. The progress of the reaction was monitored by TLC. After complete conversion, the mixture was cooled in ice bath to form crystals. Then it was filtered and washed using little cold ethanol and recrystallized from ethanol. The produced solid was collected by suction filtration and purified by flash chromatography (ethanol:EtOAc 6:4).

### 1.2. (2S,3R,4R,5R,6S)-2-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-(((S)-5-hydroxy-2-(4-hydroxyphenyl)spiro[chroman-4,2'-imidazolidin]-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (2)

ethylene diamine (1.5 mmole, 0.1 ml) was dissolved in 8-10 mL ethanol. This mixture was acidified with few drops of 3M HCL solution. The mixture shaken and warmed in water bath ( about 60 °C) for 10 min. In a separate flask naringin (1.5 mmole, 0.87 g) was dissolved in ethanol (10 ml). This solution was shaken and slightly warmed in water bath until clear mixture is obtained then added to the hot solution of amine. The produced solution was slightly heated on a water bath for another 10 -15 min to increase the rate of the reaction. The progress of the reaction was monitored by TLC. After complete conversion, the mixture was cooled in ice bath to form crystals. Then it was filtered and washed using little cold ethanol and recrystallized from ethanol.

The produced solid was collected by suction filtration and purified by flash chromatography (ethanol:EtOAc 6:4).

The product weight was 0.34g ( 52.6 %), m.p 60-62 °C, IR (KBr ), **Fig[2]**: 3265(–C–OH), 1597 (C=C aromatic), 1247 (C–N) , 1369 (C–N) and 1169 (C–O ether)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO)  $\delta$  ppm: 1.13 – 1.15 (t, 4H,  $\text{CH}_2\text{CH}_2$ ) , 2.5 (d, 2H, NH), 2.8 (d, 2H, NH), 2.9 – 3.3 (m, 6H, OH of sugar), 3.6 (t, 1H, OH of sugar), 6.8 (m, 2H, Ar–H), 7.3 (m, 2H, Ar –H).  $^{13}\text{C}$  NMR (DMSO), **Fig[3]**: 38.984, 39.121, 39.262, 39.400, 39.541, 39.820, 41.824, 48.027, 60.381, 68.211, 69.604, 70.502, 71.838, 76.121, 67.327, 67.751, 77.243, 98.336, 100.584, 109.555, 109.586, 115.194, 128.365, 129.220, 157.775, 159.577, 159.680, 162.944, 171.866. MS: TOF – MS( $m/z$ ) = 623.421  $\text{M}^+$ , theoretical MS = 622. Anal. Calcd for  $\text{C}_{29}\text{H}_{38}\text{N}_2\text{O}_{13}$  : C 55.94, H 6.15, N 4.50, O 33.41.**Fig[4]**.

**1.3. (2S,3R,4R,5R,6S)-2-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-(((S)-5-hydroxy-2-(4-hydroxyphenyl)spiro[chroman-4,2'-[1,3]dioxolan]-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (3)**

Ethane diol (1.5 mmole, 0.1 ml) was dissolved in 8-10 mL ethanol this mixture was acidified with few drops of 3M HCL solution, the mixture shaken and warmed in water bath ( about 60 °C) for 10 min. In a separate flask naringin (1.5 mmole, 0.87 g) was dissolved in ethanol (10 ml). This solution was shaken and slightly warmed in water bath until clear mixture is obtained then added to the hot solution of diol. The produced solution was slightly heated on a water bath for another 10 -15 min to increase the rate of the reaction. The progress of the reaction was monitored by TLC. After complete conversion, the mixture was cooled in ice bath to form crystals. Then it was filtered and washed using little cold ethanol and recrystallized from ethanol. The produced solid was collected by suction filtration and purified by flash chromatography (ethanol:EtOAc 6:4).

The product weight was 0.351g (46.8 %), m.p 223-225 °C, IR (KBr ): 3316 (–C–OH), 2971 (=CH aromatic), 1378 (C=C aromatic), 1087 (C–O) of ring and 1045 (C–O ether)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO)  $\delta$  ppm, **Fig [4]**: 2.5 (d, 3H,  $\text{CH}_3$ ), 2.6 – 2.7 (d, 2H, OH of sugar 3.2 – 3.3 (m, 6H, OH of sugar), 3.4 (t, 1H, OH of sugar), 3.7 (s, 1H, O–CH) , 5.4 (d, 4H ,  $\text{CH}_2\text{CH}_2$ ), 6.7 (d, 25H, Ar–H), 6.8 (d, 2H, Ar –H), 7.3 (d, 2H, Ar –H), 7.33 (d, 2H, Ar –H) , 9.5 (s, 1H, Ph–OH), 10.7(s, 1H, Ph – OH).  $^{13}\text{C}$  NMR (DMSO): 39.512, 39.653, 39.791, 39.932, 40.069, 40.211, 40.348, 42.429, 42.471, 78.910, 78.932, 95.413, 95.497, 96.245, 96.325, 102.246, 110.989, 115.639, 128.798, 128.859, 129.328, 158.216, 163.420, 163.977, 167.123, 196.865. MS: TOF – MS ( $m/z$ ), **Fig[5]** = 624.346  $\text{M}^+$  (then 607.358 [ $\text{M}^+$  - OH]), theoretical MS = 624. Anal. Calcd for  $\text{C}_{29}\text{H}_{36}\text{O}_{15}$  : C 55.77, H 5.81, O 38.42.

**1.4. Antioxidant activity**

**1.4.1. Chemical Reagents for antioxidant evaluation**

(DPPH) 2, 2-Diphenyl-1-picrylhydrazyl was ordered from Sigma-Aldrich (Germany). Trolox (6-hydroxy- 2, 5, 7, 8 –tetramethylchroman-2 carboxylic acid) was purchased from Sigma-Aldrich (Denmark).

**1.4.2. Trolox standard and plant working solutions**

A stock solution of a concentration of 1mg/ml in methanol was firstly prepared for each Naringin semi synthetic derivative and Trolox (the standard reference) the working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 µg/ml) were prepared by serial dilution with methanol from the stock solution.

#### *1.4.3. Spectrophotometric measurements*

DPPH was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above prepared working concentration in a ratio of 1:1:1 respectively. The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 minute at room temperature before the absorbance readings were recorded at 517nm.

#### *1.4.4. Percentage of inhibition of DPPH activity*

The percentage of antioxidant activity of Naringin semi synthetic derivatives and the Trolox standard were calculated using the following formula:

Percentage of inhibition of DPPH activity (%) =  $(A-B)/A \times 100\%$

Where: A = optical density of the blank and B = optical density of the sample.

The antioxidant half maximal inhibitory concentration (IC<sub>50</sub>) for the derivatives and the standard were calculated using BioDataFit edition 1.02 (data fit for biologist).

### *1.5. Antibacterial Activity:*

#### *1.5.1. Materials:*

Micro broth plates (Greiner bio-one, North America), Muller Hilton broth culture media.

#### *1.5.2. Microorganisms used:*

Bacterial strains used in the study were obtained from the American Type Culture Collection (ATCC), which were Staphylococcus aureus (ATCC 25923), Staphylococcus aureus (MRSA positive), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853).

#### *1.5.3. Procedure*

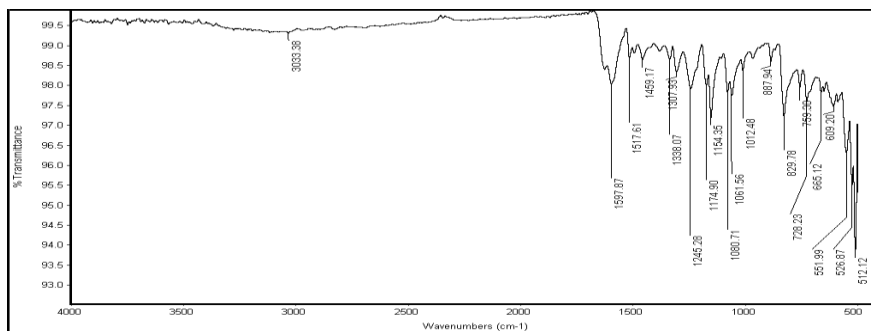
These bacterial strains were tested for their susceptibility to the prepared Naringin synthetic derivatives as follows:

Solutions of these derivatives were prepared at concentration of 4 mg per 1 ml of dimethyl sulfoxide (100% DMSO) solvent, and then incubated for 24 hours at 37 °C.

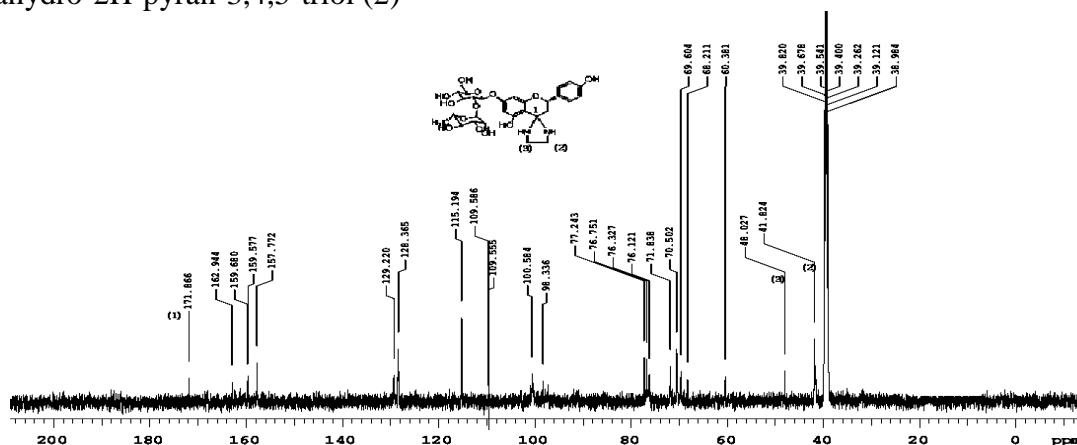
#### *1.5.4. Determination of Minimum Inhibitory Concentration (MIC):*

The antibacterial activities of the prepared derivatives were screened using micro-broth dilution method reported in the literature [20], 4mg/ml of each derivative was dissolved in 100% Dimethyl Sulfoxide (DMSO). Syringe filters with 0.45 mm pore size were used to sterilize the resulting solutions. Then solutions were serially diluted (2-fold) for 11 times with nutrient broth. Well # 11 was considered negative control of microorganism

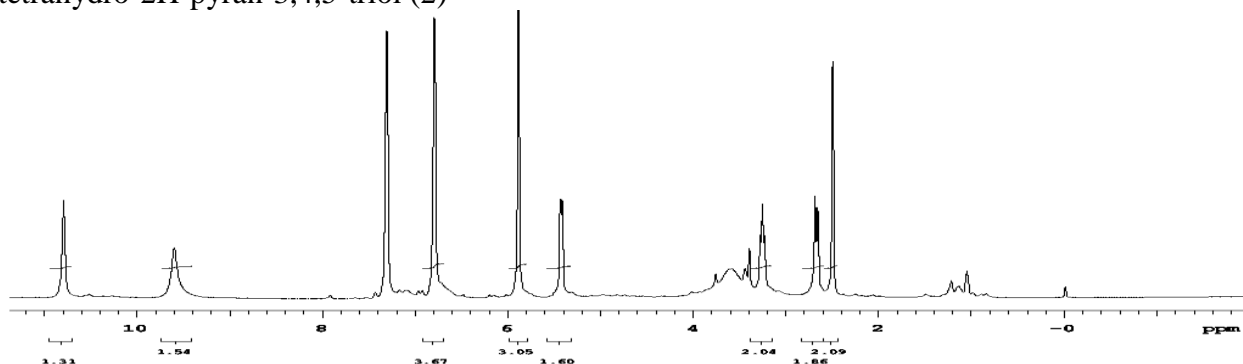
growth, while well # 12 contained nutrient broth only and was used as positive control of bacterial growth. The final bacterial concentration in each well (except negative control) was adjusted to  $5 \times 10^5$  CFU/ml. after inoculation of microorganisms, the plates were covered and incubated for 24 hours at 37 °C. Each microorganism isolate was examined in duplicate. The lowest concentration of derivative solution that did not allow any visible microorganism growth in the test broth was considered as minimal inhibitory concentration (MIC).



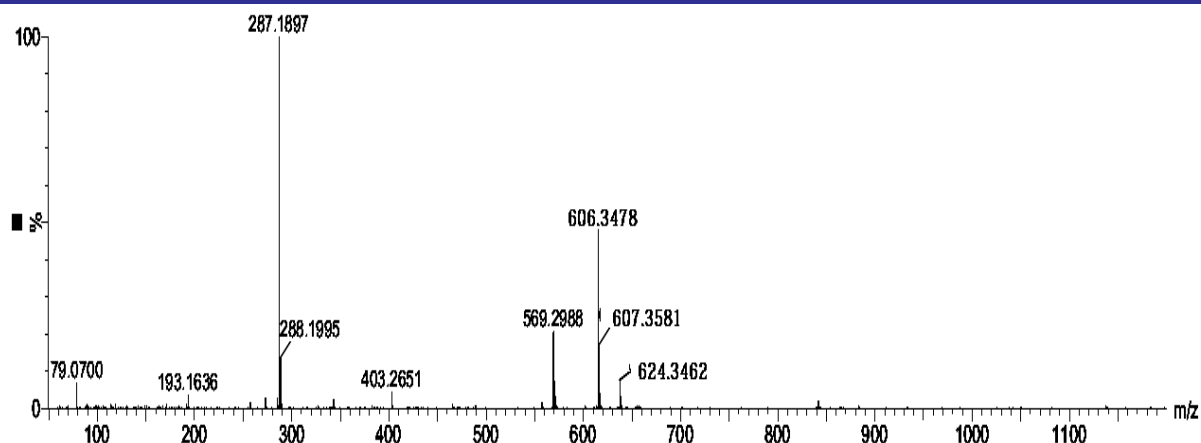
**Fig. 2.** FT-IR for (2S,3R,4R,5R,6S)-2-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-(((S)-5-hydroxy-2-(4-hydroxyphenyl)spiro[chroman-4,2'-imidazolidin]-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (2)



**Fig. 3.**  $^{13}\text{C}$  NMR (2S,3R,4R,5R,6S)-2-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-(((S)-5-hydroxy-2-(4-hydroxyphenyl)spiro[chroman-4,2'-[1,3]dioxolan]-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (2)



**Fig. 4.**  $^1\text{H}$  NMR for (2S,3R,4R,5R,6S)-2-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-(((S)-5-hydroxy-2-(4-hydroxyphenyl)spiro[chroman-4,2'-[1,3]dioxolan]-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (3)



**Fig. 5.** MS spectra for (2S,3R,4R,5R,6S)-2-(((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-(((S)-5-hydroxy-2-(4-hydroxyphenyl)spiro[chroman-4,2'-[1,3]dioxolan]-7-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (3)

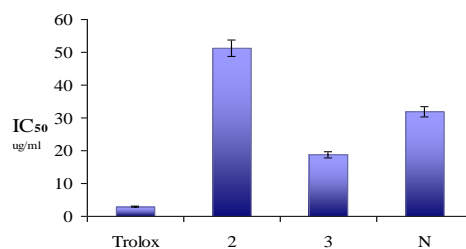
## 2. Results and Discussion.

### 2.1. Antioxidant Activity:

The antioxidant activity of the derivatives was done and compared with that of the original compound (naringin) and with trolox. The percentage of antioxidant activity of Naringin semi synthetic derivatives and the Trolox standard were calculated and the antioxidant half maximal inhibitory concentration ( $IC_{50}$ ) was reported in table 2 and **Fig. 6**. Results indicated that compound (3) has the lowest  $IC_{50}$  of 18.7  $\mu\text{g/mL}$ , which means that it has a good scavenging activity toward free radicals. This means that it could be a powerful antioxidant substance. The other derivative showed mild antioxidant activity with  $IC_{50}$  of 51.3  $\mu\text{g/mL}$  even less than original compound naringin. ( $\mu\text{g/mL}$ ) of naringin and synthesized naringin based derivative

**Table 2.**  $IC_{50\text{value}}$

Naringin semi synthetic derivatives	$IC_{50\text{value}}$ ( $\mu\text{g/mL}$ )
2	51.3
3	18.7
Naringin(N)	31.8
Standard(Trolox)	2.9



**Fig. 6.**  $IC_{50\text{val}}$  ( $\mu\text{g/mL}$ ) of naringin and synthesized naringin based derivative



## 2.2. Antibacterial Activity

Naringin showed mild antibacterial activity with MIC value ranging between (2-3 mg/mL) against different bacterial strains including *Staphylococcus aureus*, *Escherichia coli* as tested previously [18]. Also other researchers confirmed this mild antibacterial activity of Naringin against several bacterial strains and reported that Naringin showed MIC values more than 1 (mg/mL) [19]. In this research we will try to investigate the antibacterial activity of Naringin synthetic derivatives to show if they inhibit bacterial growth under 0.25mg/mL concentration to be considered as effective antibacterial agents.

Results for MIC test on four bacterial strains were summarized in Table 3. For dioxolane (3) the MIC was equal 0.125 mg/mL against all types of bacterial strains except *E.coli*. Also it seems to be effective antibacterial derivatives against *Staphylococcus aureus* (MRSA) with MIC value 0.25mg/mL. Imidazolidine derivative (2) did not show any bacterial inhibition under 0.25 mg/mL except against *Pseudomonas aeruginosa*. However, it can be considered that dioxolane derivatives of Naringin were the most effective antibacterial agents

**Table 3.** MIC of Naringin semi synthesized derivatives (mg/mL).

Microorganism	2	3
<i>Staphylococcus aureus</i> (ATCC 25923)	IN	0.125
<i>Escherichia coli</i> (ATCC 25922)	NI	IN
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	0.25	0.25
<i>Staphylococcus aureus</i> (MRSA Positive)	IN	0.125

NI: NO Inhibition of bacterial growth observed at 0.25 mg/mL \*

In conclusion, we have synthesized a couple of naringin heterocyclic derivatives and evaluated their antioxidant and antibacterial activities. Dioxolane (3) showed good antioxidant activity with lowest IC<sub>50</sub> of 18.7 µg/mL, and both derivatives showed antibacterial activity against *Staphylococcus aureus* (MRSA) with MIC value of 0.25mg/mL. More extensive study is needed to optimize the effectiveness of this type of compounds and to determine their mode of action.

## 3. Conclusion

Different compounds of naringin based heterocyclic derivatives dioxolane and imidazolidine have been synthesized and evaluated for their antioxidant and biological activities. The chemical structures of the newly synthesized compounds were verified on the basis of spectral and elemental methods of analysis. Investigation of antibacterial activity of these compounds was determined by measuring MIC value using broth micro dilution method for Gram-positive and Gram-negative bacteria, among the various synthesized compounds.



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