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Hybrid systems through β-Lactam compounds and NEPA-NCA: An approach towards new molecular entities

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***Abstract**: Hybrid systems are constructs of different chemical entities to generate functional molecules in which the characteristics of productsmay get modulated, amplified or give rise to entirely new properties. The key attributes of such condensation of molecules led to the idea of generating novel molecular entities by rationally combining two classes of compounds with different therapeutic properties. An appealing feature of this approach is that it may provide possibilities for generating a diverse array of new types of molecules for application in medicinal chemistry.*

In this review, synthesis of novel β-Lactam derivative comprising of β-Lactam moieties and NEPA-NCA i.e. [(S)-ethyl 2-((S)-4-methyl-2,5-dioxooxazolidin-3-yl)-4-phenylbutanoate], is described. The synthesis of intended compound has been characterized and confirmed by 1H-NMR, 13C-NMR and Mass. The antibacterial activity of this compound was screened by assaying against Staphylococcus aureus, Escherichia coli and Bacillus subtilis. These compounds showed significant activity in vitro against the three tested bacteria at concentrations of 1 mg/mL, 1 mg/5mL and 1 mg/10mL.

***Key words:** β-Lactam, NEPA-NCA*

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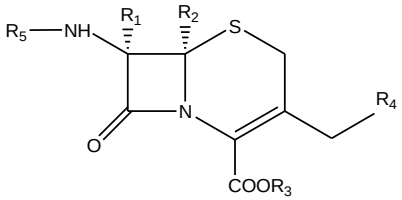
I. Introduction

Cephalosporinsare specific class of β-Lactam antibiotics, consisting of all antibiotic agents that contain a β-Lactam ring in their chemical structures. Most of the therapeutically useful β-Lactam antibiotics possess the structurally modified side chains that enhance the activity of the drug. Large number of such analogs has been in use till date. But, in recent years, bacterial resistance to these antibiotics is found to be increasing at an alarming rate due to the resistance of bacteria to these drugs. As a result, successful treatment to bacterial infection is getting affected. In order to overcome this challenge, there is an ever-growing need to synthesize new antibiotics with enhanced activity.

Cephalosporins are indicated for the prophylaxis and treatment of infections caused by bacteria susceptible to this particular form of antibiotic. The cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first cephalosporins were designated as first generation, whereas later, more extended spectrum cephalosporins were classified as second, third, fourth and fifth generation cephalosporins. Each newer generation of 6 has significantly greater Gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against Gram-positive organisms. Fourth generation cephalosporins; however, have true broad-spectrum activity. Fifth-generation cephalosporins are effective against MRSA (methicillin-resistant *Staphylococcus aureus*) too. The cephalosporin nucleus is basically consists of amino acid of formula (I).

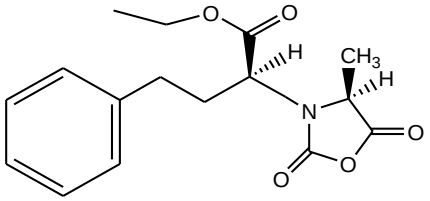


(I)

Where R1 is H or $-OCH_3$; R_2 is H; R_3 is H, a negative charge or together a with the COO^- group to which R_3 is attached is an ester or an alkali or alkaline earth metal; or is a silyl group; R_4 is H or is a substituent useful in Cephalosporin chemistry; and R_5 is H or a silyl group with the proviso that, when R_3 is H; R_5 is also H; when R_3 is a silyl group R_5 is also a silyl group; and when R_3 is an ester or an alkali or alkaline earth metal R_5 is H to give formula.

On the other hand, there is another class of compound [(S)-ethyl-2-((S)-4-methyl-2,5-dioxooxazolidin-3-yl)-4-phenylbutanoate] of following formula (II) (herein after referred to as "NEPA-NCA"). It is a well-known chemical intermediate in pharmaceutical industry, which plays an important role in synthesizing

Angiotensin-I converting enzyme (ACE) inhibitors such as delapril, enalapril, imidapril, indolapril, moexipril, quinapril, ramipriland trandolapril and used in treatment of hypertension and congestive heart failure. There are so many molecules available where different amino acids have been condensed with NEPA-NCA to yield product of medicinal use.



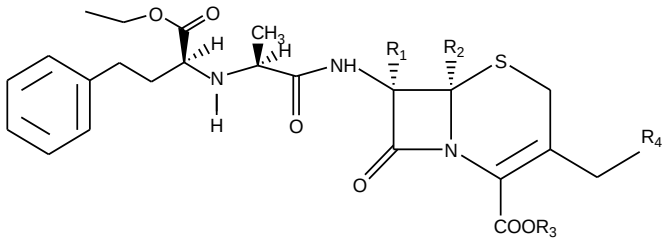
(II)

The role of NEPA-NCA has been well reported in literature justifying their application as antihypertensive role. Several compounds have been described where amino acids have been condensed with NEPA-NCA to get product of different ACE inhibitors, for example, Ramipril, Trandolapril, Delapril, Imidapril, and Quinapril.HCl.

Similarly,β-Lactam compounds have been also used as precursor of potential antibacterial drugs.Accordingly, it was designed to synthesize a hybrid molecule of these two chemical compounds to generate functional molecule in which the characteristics of hybrid molecule may get modulated, amplified or give rise to entirely new properties.

Therefore, a protocol was designed to synthesize the intended molecule (III)and this protocol involves the multi-step synthesis of β-Lactam derivatives by reaction of β-Lactam compounds (I) with NEPA-NCA (II) (Scheme-1). The process involves silylation of cephalosporin intermediates using Hexamethyldisilazane (HMDS) and Trimethylchlorosilane (TMCS) in methylene chloride as solvent. The next step involves the condensation of silylatedβ-Lactam compound with NEPA-NCA followed by desilylation and work-up step to afford the desired hybrid compound.

These chemical moieties upon condensation will lead to the formation of hybrid molecule of formula (III).



(III)

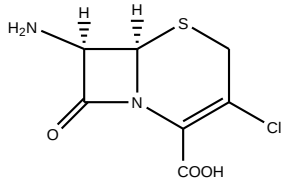
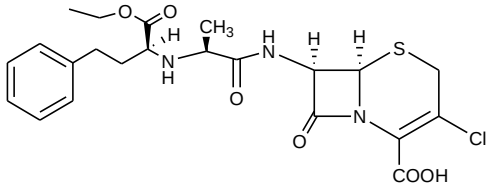
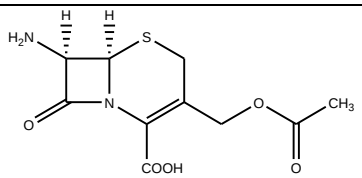
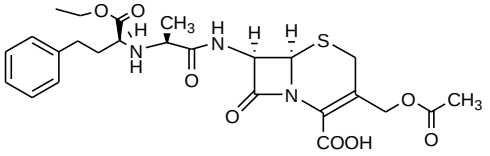
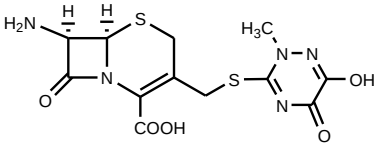
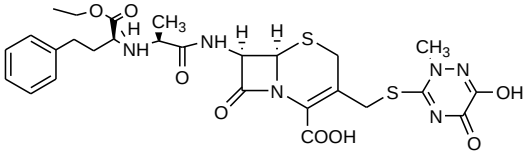
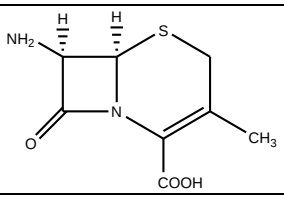
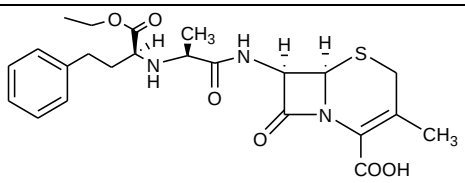
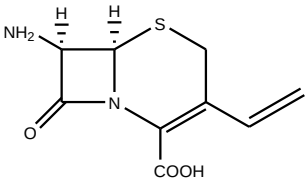
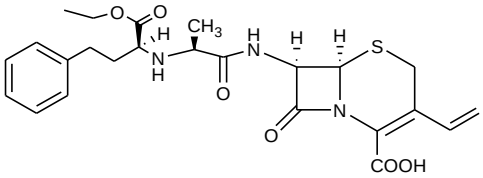
Wherein R_1, R_2 and R_4 have the same meanings as defined herein earlier and R_3 is H, a negative charge or together with the COO^- group to which R_3 is attached is an ester or an alkali or alkaline earth metal; R_4 is H or is a substituent useful in Cephalosporin chemistry.

The group R_4, which is a substituent useful in Cephalosporin chemistry includes inter alia those substituents which are conventional in Cephalosporin chemistry and which are useful in pharmaceutically active Cephalosporins and thus includes unsubstituted and substituted alkyl; unsubstituted and substituted alkenyl; alkyl; and an alkenyl substituted by alkoxy; heterocyclic-thio, heterocyclic carbonylthio, alkylcarbonyloxy and heterocycyl. Heterocycyl including a bicyclic ring system having 10 to 12 carbon atoms; a heterocycyl having 1 to 4 hetero atoms, selected from N, O or S.

Hybrid molecule of these moieties may provide new opportunities for drug discovery and material applications. This review article describes such approaches on the development of new hybrid compounds of NEPA-NCA & cephalosporin nucleus i.e. β-Lactam nucleus amino acids. Hence, a brief review on the preparation and developments of the relevant synthetic strategies of NEPA-NCA & cephalosporin is described to provide an overall research perspective of the area.

The antibacterial activity of the hybrid compound was screened by assaying against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. These compounds showed significant activity in vitro against the three tested bacteria at concentrations of 1 mg/mL, 1 mg/5mL and 1 mg/10mL. It is expected that screening of these hybrid compounds may further enhance the medicinal and therapeutic use of these β-Lactam derivatives.

Following β-Lactam derivatives (III) have been synthesized by condensing β-Lactam Compounds (I) with NEPA-NCA (II).

S. No.	β-Lactam Compound (I)	β-Lactam derivative (III)	Abbreviated Name
1.			Compound-X
2.			Compound-Y
3.			Compound-A
4.			Compound-B
5.			Compound-C

II. Experimentation

A suspension ofβ-Lactam Compound (I) in MDC (methylene chloride)was heated to reflux with hexamethyldisilazane (HMDS)and trimethylchlorosilane (TMCS) for 4-6 hours to silylate the β-Lactam compound. The solution containing silylatedβ-Lactam compound was slowly cooled to room temperature (20-30°C) and subsequently added NEPA-NCA (III). The above mixture was stirred 2-3 hours, added water andtetrahydrofuran or acetone over a period of 10-20 minutes. The mixture was stirred at the same temperature for 30-60 minutes to precipitate the product. Filtered the material and washed with methylene chloride followed by water to afford the wet product, which is subjected for drying at reduced pressure at 40-45°C for 5-6 hours (Yield 80-95%).

Antibacterial activity of these β-Lactam derivatives was analyzed by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Synthetic compound 1mg was immersed in 1mL, 5mL and 10mLof 1% Sodium bicarbonate solution and left at room temperature for one hour so that it dissolved completely. Antibacterial test of compounds were done *in-vitro*. The bacteria *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were inoculated into separate nutrient broths and incubated at 27°C for 24 hours. Broth culture of the test bacterium (0.2 mL) was evenly spread on a nutrient agar plate under sterile conditions with the help of sterile glass spreader. Sterile filter paper discs (What man No.1;7 mm) were soaked in test compound, allowed to dry it for a few minutes in a sterile Petri dish. Each disc was placed at the center of a nutrient agar plate, which were earlier inoculated with the respected bacterium. Filter paper discs having 50µm of distilled water and cephalosporin were used separately as controls. All experiments were maintained in triplicate. The Petri dishes were incubated at 27°C. After 24 hours, the diameter of any clear inhibition zone around the discs was measured.

III. Results& Discussion

The Compound, prepared by utilizing the β-Lactam moiety and NEPA-NCA was analysed by the spectral studies. Compounds were characterized by spectral analysis like 1H-NMR (400 MHz), 13C-NMR (300 MHz), and Mass. 1H-NMR, 13C-NMR and Mass exhibits their distinct characteristics and confirms the formation of desired product.

Antibacterial activity of these compounds was tested by assaying against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. These compounds showed biological activity *in-vitro* against the three tested bacteria at concentrations of 1g/1mL, 1g/5mL and 1g/10mL. To determine the antibacterial sensitivity of compound-A, it was compared with standard cephalosporin under the same conditions. The degreeof antibacterial activity of compounds was calculated.

The biological activity of compound-A was compared as against standard cephalosporin at 1 mg/ 1mL, 1 mg/ 5mL and at 1 mg/ 10mL. The inhibitory zone diameters of compounds against *Staphylococcus aureus*, *E. coli* and *Bacillus subtilis* were calculated

Acknowledgement

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Scheme-1:

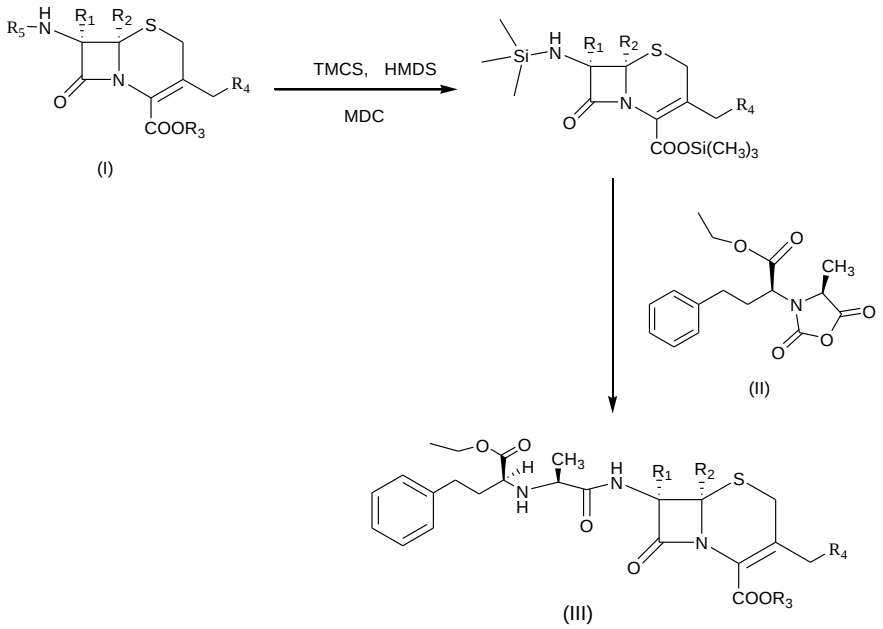

Table-1: Biological Activity of Compound-X

Name of Bacteria	Compound-X			Standard cephalosporin		
	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc
Staphylococcus aureus	10.13	6.6	1.8	11.0	7.5	7.0
Escherichia coli	9.25	6.0	0.9	9.5	7.0	6.3
Bacillus subtilis	10.80	5.8	1.1	11.0	7.0	6.5

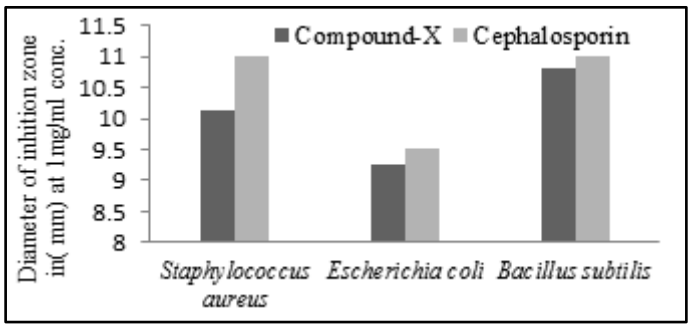

Fig. 1a.Antibacterial activity of compound-X and Cephalosporin at 1mg/Ml

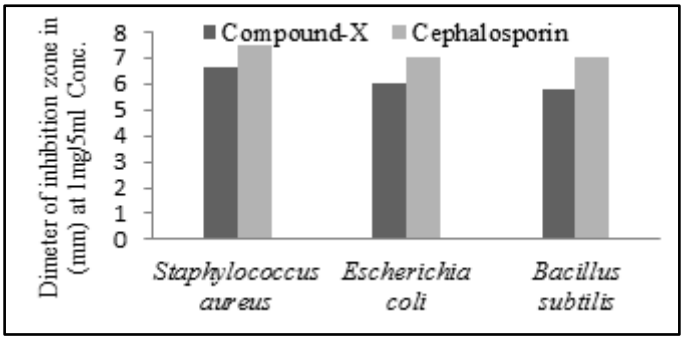

Fig. 1b. Antibacterial activity of compound X and Cephalosporin at 1mg/5mL

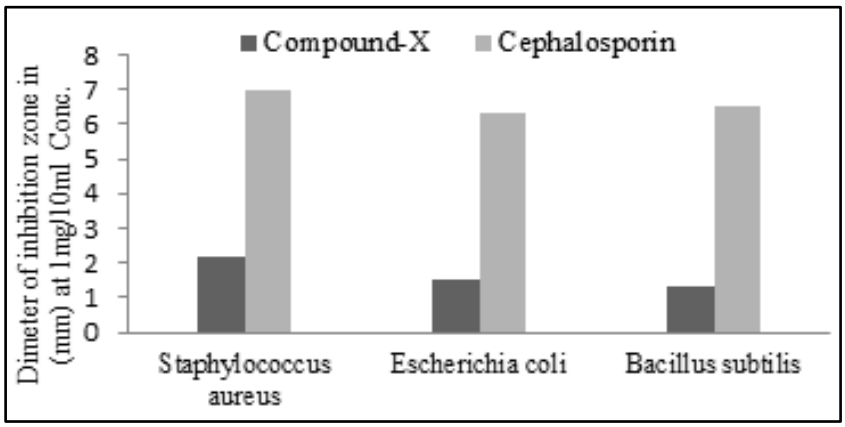

Fig. 1c. Antibacterial activity of compound X and Cephalosporin at 1mg/10mL

Table-2: Biological Activity of Compound-Y

Name of Bacteria	Compound-Y			Standard cephalosporin		
	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc
Staphylococcus aureus	9.5	7.2	4.4	11.1	7.4	6.9
Escherichia coli	8.0	6.5	5.0	9.3	6.0	6.2
Bacillus subtilis	8.6	6.2	5.1	10.9	7.1	6.4

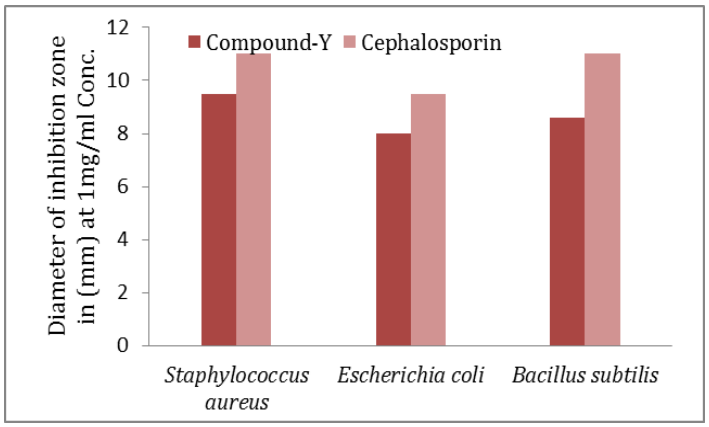

Fig.2a. Antibacterial activity of compound Y and Cephalosporin at 1mg/mL

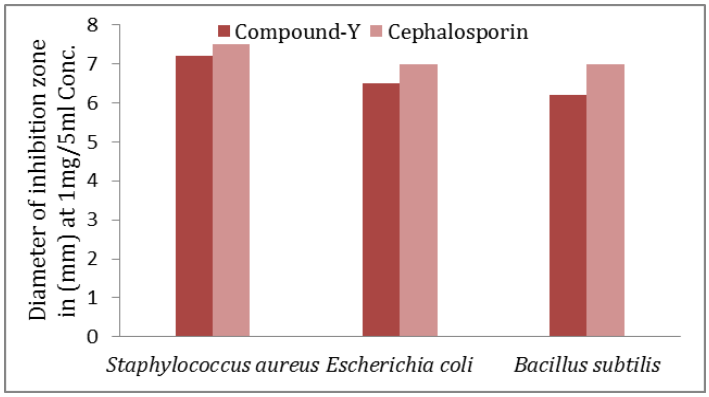

Fig.2b. Antibacterial activity of compound Y and Cephalosporin at 1mg/5mL

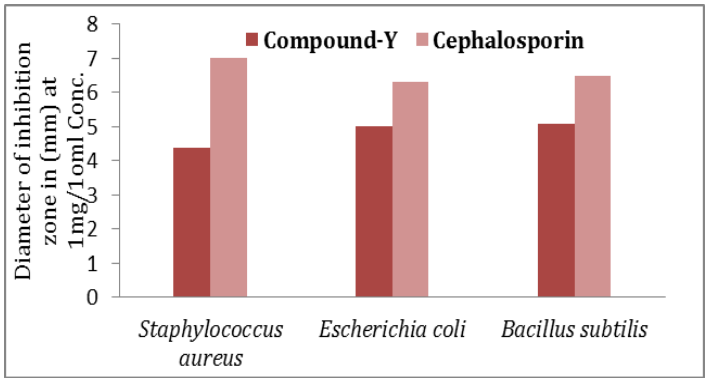

Fig.2c. Antibacterial activity of compound -Y and Cephalosporin at 1mg/10mL

Table-3: Biological Activity of Compound-A

Name of Bacteria	Compound-A			Standard cephalosporin		
	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc	Inhibition activity in mm at 1mg/1mL conc.	Inhibition activity in mm at 1mg/5mL conc	Inhibition activity in mm at 1mg/10mL conc
Staphylococcus aureus	5.5	4.2	1.4	11.0	7.5	7.0
Escherichia coli	5.1	4.0	1.0	9.5	7.0	6.3
Bacillus subtilis	6.3	4.8	1.1	11.0	7.0	6.5

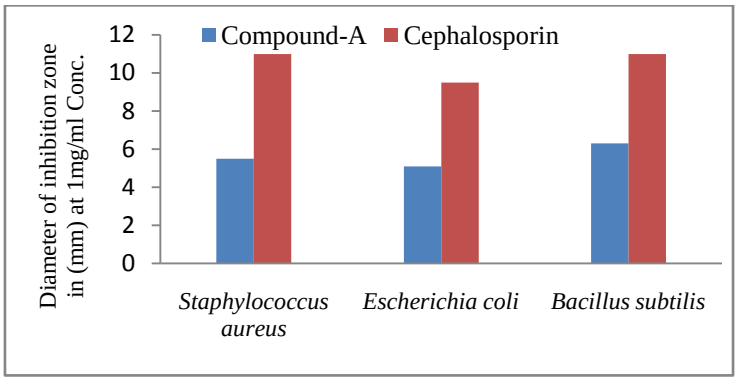

Fig.3a. Antibacterial activity of compound-A and Cephalosporin at 1mg/mL

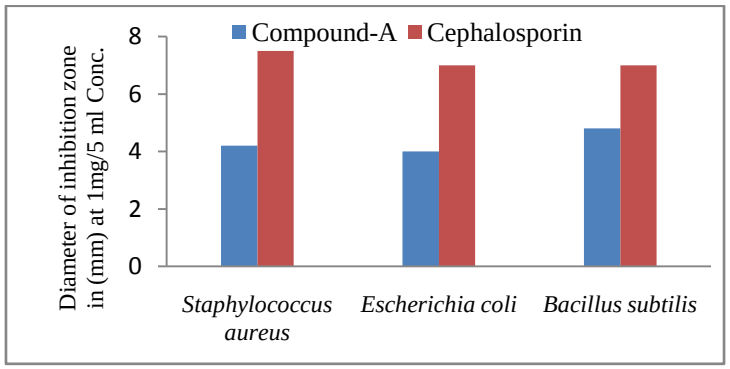

Fig.3b. Antibacterial activity of compound-A and Cephalosporin at 1mg/5mL

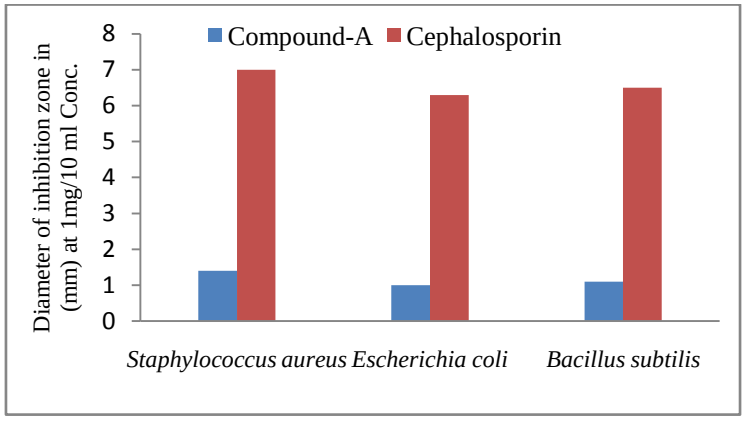

Fig.3c. Antibacterial activity of compound-A and Cephalosporin at 1mg/10mL

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