

## Synergistic effects of neem (*Azadirachta indica* L.) leaves extract with conventional antibiotic against gram positive and negative microorganism

Somnath D. Bhinge<sup>1\*</sup>, Dheeraj S. Randive<sup>1</sup>, Mangesh A Bhutkar<sup>1</sup>, Kiran P Shejwal<sup>1</sup>,  
Amit D. Jadhav<sup>1</sup>, Rahul P. Jadhav<sup>1</sup>

<sup>1</sup> Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, India.

### ABSTRACT

**Background:** Neem has been known to possess several complex phytoconstituent(s) and exhibits wide array of medicinal and household uses that are attributed to its active isolate(s) that possesses an ability to cure many chronic disease(s) and disorder(s). The current investigation aimed to study the combined antimicrobial effect of crude Neem extract and selected antibiotics namely Ciprofloxacin, Cefixime, Chloramphenicol, Ampicillin, Sulfamethoxazole, Tetracycline and Ofloxacin on selected Gram's positive and negative micro organism. Crude alcoholic Neem leaf extract was used for the study.

**Method:** Solution A containing Neem extract 5 mg mL<sup>-1</sup> alone, Solution B comprising of Standard antibiotics alone 5 mg mL<sup>-1</sup> and Solution C containing combination of 2.5 mg mL<sup>-1</sup> of Neem extract and selected standard antibiotic at a concentration of 2.5 mg mL<sup>-1</sup> were tested for their antibacterial potential against selected strain of micro-organisms namely *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Bacillus subtilis* using agar plate technique.

**Results:** The obtained results indicated the synergistic activity of the Neem extract and the selected antibiotics. It was observed that half concentration of antibiotic was sufficient to exert the antimicrobial effect when they were combined with Neem extract. Thus, the dose of standard antibiotics may be marginally reduced to almost half in concentration when combined with the Neem extract without compromising the efficacy. The zones of inhibition indicated that the combination of Neem extract and antibiotic exerted a synergistic effect which will facilitate to achieve reduction of the dose of standard antibiotics.

**Conclusions:** It can be concluded from the research that Neem extract when combined with the conventional antibiotic(s) can be used as a novel antimicrobial agent which exhibits a synergistic effect and also helpful to achieve a dose reduction of conventional antibiotic(s).

**Keywords:** Neem; Microorganism; Antibacterial; Synergistic effect; Antibiotics.

### 1. INTRODUCTION

*Azadirachta indica* L. (Family: Meliaceae) which is commonly known as Neem in India and South East,

comprises of several complex plant phytoconstituent(s); and traditionally well known for its numerous medicinal properties and uses. It is a commonly occurring plant known traditionally for its wide array of medicinal uses. It is fast-growing tree that usually attains a height of around 50–72 feet. It is deciduous tree and most of its leaves fall off during the winter season. The branches of the tree are usually broad and extend out with its crown rather thick and roundish, having a diameter of around 65–79 ft. The

---

\*Corresponding author: Somnath D. Bhinge

[somu1245@yahoo.co.in](mailto:somu1245@yahoo.co.in)

ORCID: <https://orcid.org/0000-0002-5959-5833>

Received on 7/9/2021 and Accepted for Publication on 27/12/2021.

neem tree resembles its close relative, the chinaberry (*Melia azedarach*). It possesses plentiful medicinal effects and therefore used widely for the treatment of several chronic diseases and disorders.<sup>1</sup>

Owing to its antibacterial, anthelmintic, antifungal properties it has been recommended for treatment and cure of multiple skin diseases.<sup>2</sup> A variety of plant compounds were isolated from the Neem extracts. Quercetin (polyphenolic flavonoid) and  $\beta$ -sitosterol (phytosteroids) are the prime phytoconstituents that were isolated from fresh Neem leaves which have been reported to exhibit prominent antifungal and antibacterial effects.<sup>3</sup> Studies on 21 isolates from Neem extracts demonstrated efficacy of these compounds against foodborne and spoilage microorganisms.<sup>4,5</sup> There are also reports about effectiveness of Neem against *Mycobacterium tuberculi*, *Vibrio cholerae* and against some Gram negative and positive bacteria.

Amongst Quinolone antibiotics, Ciprofloxacin and Ofloxacin are of major concern and used widely for the treatment of various bacterial infections, whereas, Cefixime is a Cephalosporin antibiotic of 3<sup>rd</sup> generation.<sup>6</sup> It exhibits broad and dominant actions against various pathogens, chiefly Gram-negative organisms.<sup>8,9</sup> Chloramphenicol and Ampicillin, the semi-synthetic moieties are available in the form of orally effective broad-spectrum antibiotics. Sulfamethoxazole is a Sulfonamide of prime importance, whereas, Tetracycline is a wide-spectrum antibiotic. Particularly, these antibiotics have verified superior pharmacokinetic properties and antibacterial actions. The aforesaid antibiotics are used in the treatment of Gram negative and occasionally gram-positive infections.<sup>10-16</sup> These antibiotics have been known to produce several side effects namely diarrhoea, abdominal pain, dizziness, sleepiness, headache, nausea, bad vision, nervousness, anxiety, agitation, insomnia or nightmares, and skin rash.<sup>17,18</sup> Alternative healthcare systems, such as Ayurveda, Siddha and Unani, have proved their prospective for complementing the healthcare

system in India and other developing countries.<sup>19</sup> Moreover, the recent times, have witnessed a greater inclination towards the use of medicinal plants and herbs, owing to their safety and lesser side effect(s) as compared to their allopathic counterparts.<sup>20,21</sup>

Certain researches have recommended use of antibiotic combinations that have demonstrated a synergistic effect above their individual inhibitory effects. Therefore, in many instances, antibiotics are combined to thwart development of resistance to the agents used alone and also to enjoy a synergistic effect.<sup>22</sup>

Synergistic combinations are often commercially used in the treatment of various infections in antimicrobial chemotherapy. Antibiotic resistance is amongst the supreme threat to the society.<sup>23</sup> Antão and Wagner-Ahlf have consequently reported that every year more than 700,000 people all over the world lose their lives owing to the drug-resistant infections.<sup>24</sup>

A number of antibiotics used for the treatment of a variety of infectious diseases in humans exhibit limited antimicrobial spectrum particularly due to the emergence of MRD bacterial strains.<sup>25,26</sup> Thus, it is justified to use two or more antimicrobial agent(s) in combination with intent to holdup or avoid the chances of drug resistant microbial strains. Moreover, in certain instances, the synergistic effect is also observed. Neem is a worldwide recognized natural antibiotic in the ancient Indian system of medicine. Hence, in the present investigation an attempt has been made to study the effect of use of Neem extract along with conventional antibiotics against the selected antibacterial strains.

## 2. Materials and methods

### 2.1 Chemicals

Analytical grade chemicals and solvents were used during the entire course of experimental work. The required media and other microbiology accessories were procured from Himedia. The antibiotics used in the experimental work were procured from Research laboratories, Mumbai, Maharashtra.

## 2.2 Microorganism used

*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were used in the study. The cultures of the aforesaid microorganisms were collected from Yashwantrao Chavan (Y.C) College of Science, Saidapur, Karad (MS) INDIA - 415110.

## 2.3 Preparation of the crude extracts

In the month of September 2019, the fresh leaves were collected from Kasegaon region, Near National Highway No. 4 (Tal – Walwa, Dist – Sangli, MS – 415404). The taxonomical recognition of the plant material was carried out from Y.C. College Saidapur, Tal – Karad, Dist – Satara, MS – 415110 with (voucher number YC-5). The collected leaves were subjected to drying in a shade for 2 weeks. 250 gm of the dried samples were ground to obtain a coarse powder. Extraction was executed in hot continuous process through Soxhlet apparatus for 48 hours using ethanol (500 mL) as a solvent. Thereafter, the contents of the round bottom flask were filtered through Whatman filter paper and the extract was allowed to evaporate in warm water and dried at room temperature. 22.30 g of the recovered crude neem extract were obtained and the yield was noted to be 8.92% w/w. Condensed extract was weighed and stored in air tight container for 4 °C till further investigation.

## 2.4 Preparation of Dilution

Preparation of Antibiotic solution – **Solution A:** Ciprofloxacin (50 mg), Cefixime (50 mg), Chloramphenicol (50 mg), Ampicillin (50 mg), Sulfamethoxazole (50 mg), Tetracycline (50 mg) and Ofloxacin (50 mg) were accurately weighed and transferred separately to the labeled test tubes. Thereafter 10 mL of DMSO solution was added to each test tube to obtain a concentration of 5 mg mL<sup>-1</sup>.

Preparation of the Neem extract solution – **Solution B** was prepared to yield a final concentration of 5 mg mL<sup>-1</sup> by adding 50 mg of dry Neem extract diluted with 10 mL of DMSO solution.

Preparation of combination (Neem extract and antibiotic solution) (**Solution C**) – It was prepared by adding equal volume of Solution A and Solution B (1 mL each) to get a Solution C containing 2.5 mg mL<sup>-1</sup> of antibiotic and 2.5 mg mL<sup>-1</sup> of neem extract separately for each antibiotic.

## 2.5 Preparation of Agar plate

Nutrient agar is a general-purpose medium that promote the growth of a wide range of micro organisms. It comprises of 0.5% Peptone, 0.3% of beef extract/yeast extract, 1.5% of agar, 0.5% Sodium Chloride and distilled water. All of the above ingredients were combined and boiled for about a minute to facilitate proper mixing and then subjected to sterilization. The contents were then cooled at about 50 °C (122 °F). The resultant liquid was then transferred into labeled petri plates and the cap was immediately secured. Once the dishes grip solidified agar, they were stored upside down and were refrigerated until used.

## 2.6 Preparation of test organism

Normal agar plates were inoculated with respective bacteria namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, thereafter incubated at 37° C for overnight. Each time, a fresh bacterial culture was prepared.

## 2.7 Antimicrobial activity

Antimicrobial activity (*In-vitro*) was determined using the Agar well diffusion technique.<sup>21, 27, 28</sup> Sterile agar was inoculated with the bacterial culture of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* for 48 h, at 37°C. Antimicrobial activities of solution A, B and C were tested on nutrient medium against *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* which are representative types of Gram-positive and Gram-negative microorganisms. Sterile borer was used to make wells in the poured culture containing microorganisms and 50 µL of solution A, B and C were added into it. Thereafter, the

plates were kept for 2 hours in a refrigerator to enable pre-diffusion of the solution A, B and C. Finally, the plates containing solution A, B and C were incubated overnight (24 hours, at 37°C). The antimicrobial activity was assessed by measurement of the diameter of zone of inhibition.

### 3. Results

Test results revealed that the combination of Neem extract with the antibiotic exerts a synergistic effect thereby reducing the dose of the conventional antibiotics (Figure 1, 2, 3, 4 and 5). Antimicrobial activity was assessed by measuring the diameter of zone of inhibition. The outcomes in the assessment of the antimicrobial activity of Solution A, Solution B and Solution C are noted in Table 1. Solution C which comprised of Neem extract along with Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline (1:1) showed zone of inhibition values as 21.00±1.5000, 16.90±1.8520, 13.10±0.3605, 39.83±0.7637, 39.83±0.7637, 26.43±0.9073 and 23.13±1.1060 respectively against the *Klebsiella pneumoniae*. Whereas, solution A which comprised of standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline at a concentration of 5 mg mL<sup>-1</sup> exhibited zone of inhibition as 21.91±0.6291, 20.00±1.0000, 18.06±0.4041, 40.03±1.1503, 40.03±1.1503, 25.43±1.2096 and 25.16±0.6658, respectively. For *Staphylococcus aureus* the zone of inhibition was observed to be 38.10±0.3605, 45.13±0.7094, 15.93±0.5033, 42.23±0.3214, 37.76±0.8504, 35.30±1.3114 and 42.50±0.9165 for solution C which comprised of Neem extract along with standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline respectively. Solution A which comprised of standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline exhibited the zones of inhibition to be 42.00±0.5011, 41.93±1.9008, 15.86±0.5131, 47.46±1.5502,

38.23±0.7094, 35.66±0.7371, and 39.66±1.4294 respectively against *Staphylococcus aureus*. Further, Solution C which comprised of Neem extract along with Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline separately (1:1) showed zone of inhibition values to be 19.10±0.6557, 13.46±0.568624, 7.20±0.2645, 32.63±1.0692, 42.23±0.3214, 34.87±0.9073, and 22.93±0.5131 respectively against *Pseudomonas aeruginosa*. While solution A which contained standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline at a concentration of 5 mg mL<sup>-1</sup> exhibited zone of inhibition to be 17.73±0.4041, 15.00±0.4001, 7.10±0.3605, 33.76±0.8736, 41.87±0.7094, 35.00±0.9005 and 30.26±0.9609. Whereas the zone of inhibition for Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline for solution C (Antibiotics and Neem extract) was observed to be 25.46±0.8962, 13.00±0.4001, 11.96±0.3511, 33.30±1.0816, 33.30±1.0816, 23.10±1.2489, and 28.50±0.5567 against *Bacillus subtilis*. While solution A (Standard antibiotic) containing Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline separately noted zone of inhibition to be 27.16±0.5686, 10.86±0.4163, 14.90±0.5567, 33.93±0.7023, 33.93±0.7023, 23.37±0.7023 and 30.63±0.4725, respectively.

In case of *E. coli*, the zone of inhibition was observed to be 32.46±0.8962, 23.43±0.5033, 15.70±0.8001, 33.13±0.9073, 35.13±1.1239, 20.33±0.7681, and 26.85±0.5766 for solution C containing neem extract along with standard antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline respectively. Solution A containing plain antibiotics namely Chloramphenicol, Ampicillin, Sulfamethoxazole, Ofloxacin, Ciprofloxacin, Cefixime and Tetracycline showed the zone of inhibition as 35.90±0.9539, 25.97±0.7371, 20.43±0.7571, 33.63±1.2055, 38.60±0.8185, 25.73±1.0692 and 34.60±1.4525, respectively against the *E. coli*.

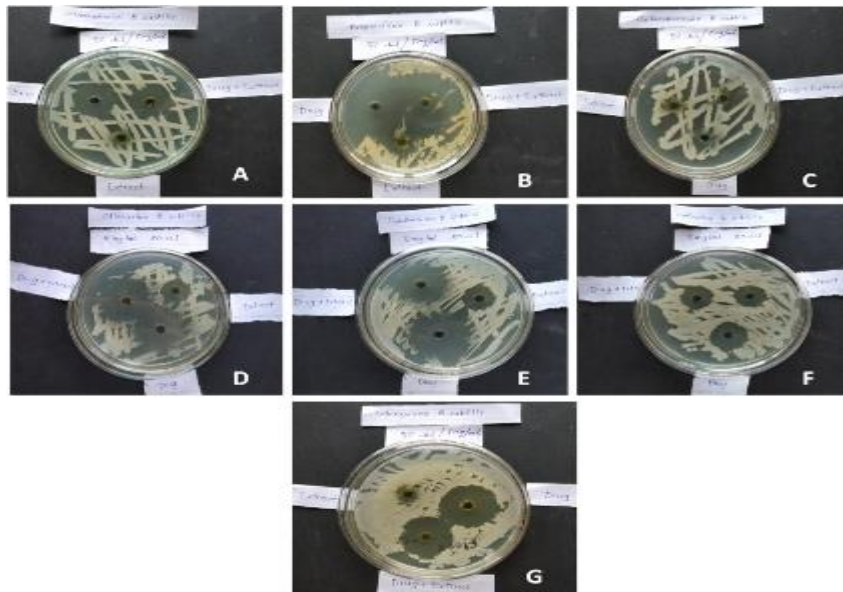


Figure 1. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Bacillus subtilis*

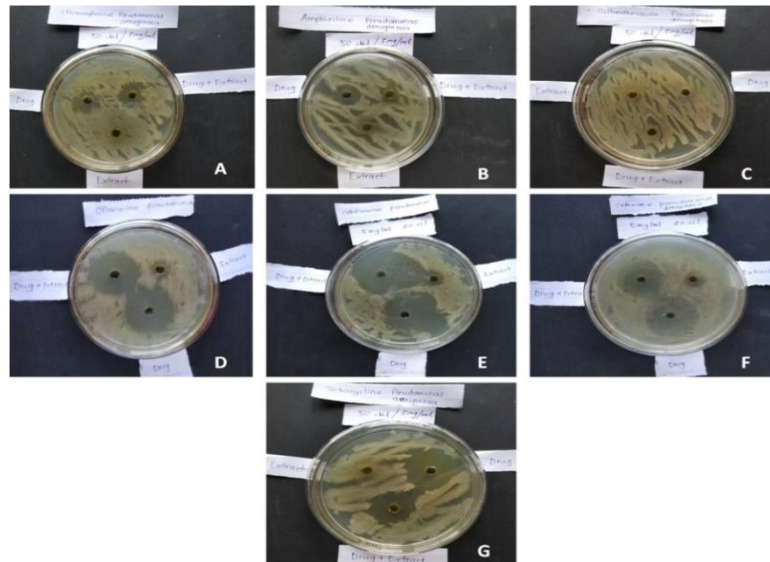


Figure 2. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Pseudomonas aeruginosa*

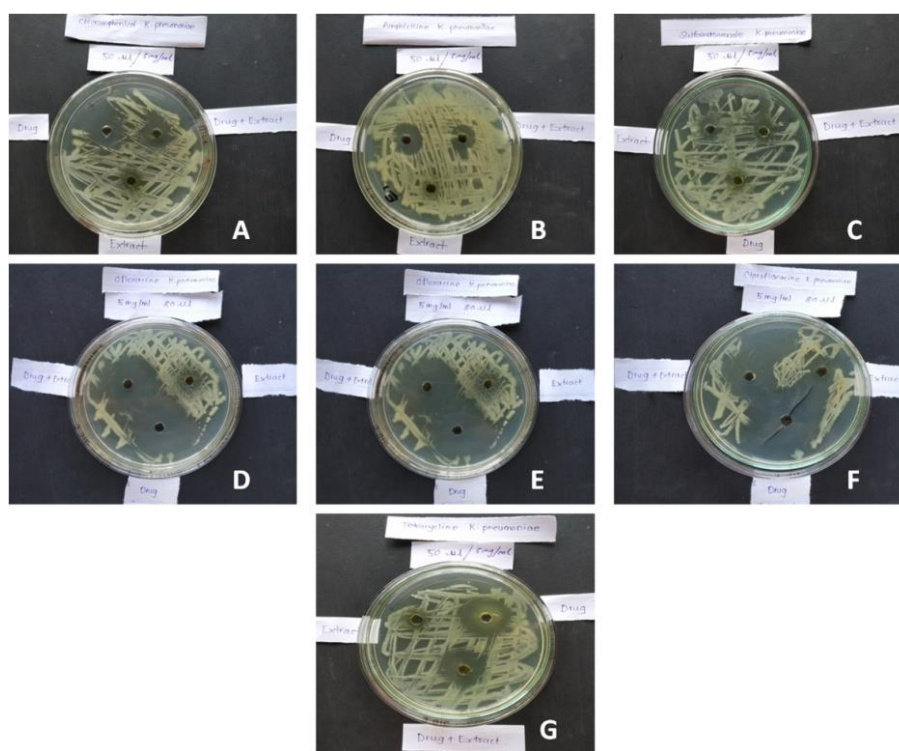


Figure 3. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Klebsiella Pneumoniae*

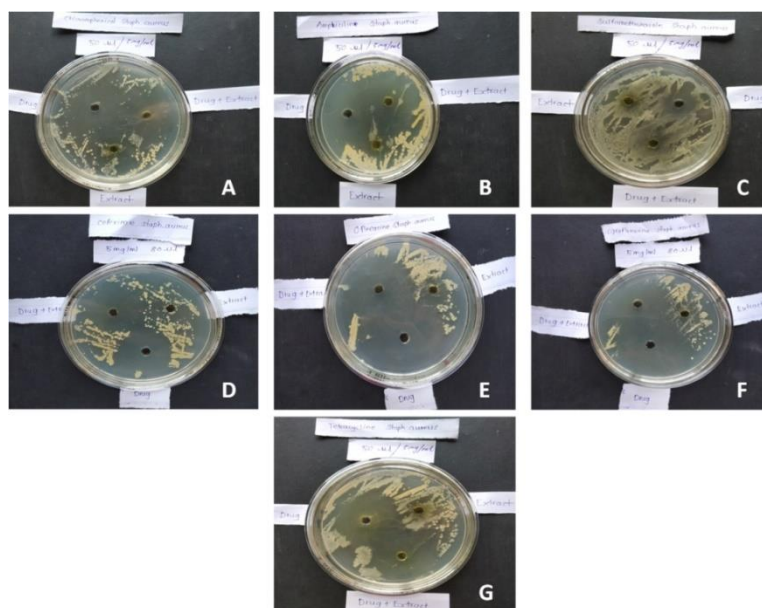


Figure 4. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *Staphylococcus aureus*



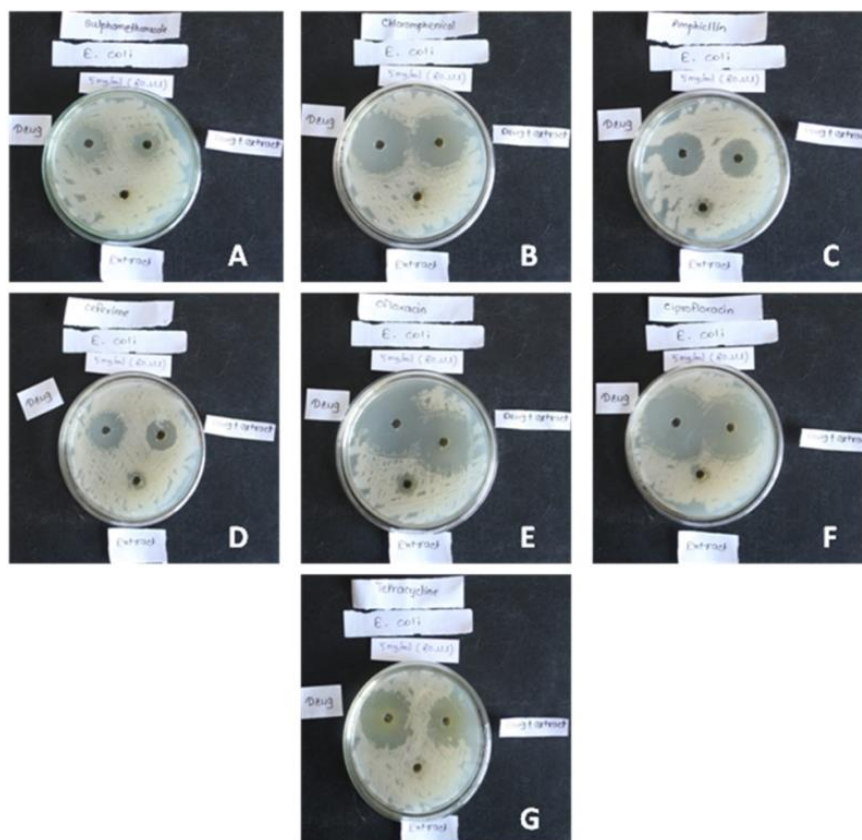


Figure 5. Photograph of Antimicrobial activity of A) Chloramphenicol B) Ampicillin C) Sulfamethoxazole D) Ofloxacin E) Ciprofloxacin F) Cefixime G) Tetracycline against *E-coli*

Table 1. Effect of selected antibiotics (Solution A), neem extract (Solution B) and selected antibiotics+neem extract (Solution C) against the *Klebsiella Pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*

Sr. No.	Microorganism	Solution A (zone inhibition in mm ±SEM) <sup>x</sup>	Solution B (zone inhibition in mm ±SEM) <sup>y</sup>	Solution C (zone inhibition in mm ±SEM) <sup>x</sup>
<i>Klebsiella Pneumoniae</i> <sup>b</sup>				
1.	Chloramphenicol	21.91±0.6291		21.00±1.5000
2.	Ampicillin	20.00±1.0000		16.90±1.8520
3.	Sulfamethoxazole	18.06±0.4041		13.10±0.3605
4.	Ofloxacin	40.03±1.1503	13.53±1.6772	39.83±0.7637
5.	Ciprofloxacin	40.03±1.1503		39.83±0.7637
6.	Cefixime	25.43±1.2096		26.43±0.9073
7.	Tetracycline	25.16±0.6658		23.13±1.1060

Sr. No.	Microorganism	Solution A (zone inhibition in mm $\pm$ SEM) <sup>x</sup>	Solution B (zone inhibition in mm $\pm$ SEM) <sup>y</sup>	Solution C (zone inhibition in mm $\pm$ SEM) <sup>x</sup>
<b><i>Staphylococcus aureus</i><sup>a</sup></b>				
1.	Chloramphenicol	42.00 $\pm$ 0.5011		38.10 $\pm$ 0.3605
2.	Ampicillin	41.93 $\pm$ 1.9008		45.13 $\pm$ 0.7094
3.	Sulfamethoxazole	15.86 $\pm$ 0.5131		15.93 $\pm$ 0.5033
4.	Ofloxacin	47.46 $\pm$ 1.5502	12.36 $\pm$ 2.6674	42.23 $\pm$ 0.3214
5.	Ciprofloxacin	38.23 $\pm$ 0.7094		37.76 $\pm$ 0.8504
6.	Cefixime	35.66 $\pm$ 0.7371		35.30 $\pm$ 1.3114
7.	Tetracycline	39.66 $\pm$ 1.4294		42.50 $\pm$ 0.9165
<b><i>Pseudomonas aeruginosa</i><sup>b</sup></b>				
1.	Chloramphenicol	17.73 $\pm$ 0.4041		19.10 $\pm$ 0.6557
2.	Ampicillin	15.00 $\pm$ 0.4001		13.46 $\pm$ 0.5686
3.	Sulfamethoxazole	7.10 $\pm$ 0.3605		7.20 $\pm$ 0.2645
4.	Ofloxacin	33.76 $\pm$ 0.8736	9.53 $\pm$ 0.9890	32.63 $\pm$ 1.0692
5.	Ciprofloxacin	41.87 $\pm$ 0.7094		42.23 $\pm$ 0.3214
6.	Cefixime	35.00 $\pm$ 0.9005		34.87 $\pm$ 0.9073
7.	Tetracycline	30.26 $\pm$ 0.9609		22.93 $\pm$ 0.5131
<b><i>Bacillus subtilis</i><sup>a</sup></b>				
1.	Chloramphenicol	27.16 $\pm$ 0.5686		25.46 $\pm$ 0.8962
2.	Ampicillin	10.86 $\pm$ 0.4163		13.00 $\pm$ 0.4001
3.	Sulfamethoxazole	14.90 $\pm$ 0.5567		11.96 $\pm$ 0.3511
4.	Ofloxacin	33.93 $\pm$ 0.7023	13.75 $\pm$ 1.3871	33.30 $\pm$ 1.0816
5.	Ciprofloxacin	33.93 $\pm$ 0.7023		33.30 $\pm$ 1.0816
6.	Cefixime	23.37 $\pm$ 0.7023		23.10 $\pm$ 1.2489
7.	Tetracycline	30.63 $\pm$ 0.4725		28.50 $\pm$ 0.5567
<b><i>E-coli</i><sup>b</sup></b>				
1.	Chloramphenicol	35.90 $\pm$ 0.9539		32.46 $\pm$ 0.8962
2.	Ampicillin	25.97 $\pm$ 0.7371		23.43 $\pm$ 0.5033
3.	Sulfamethoxazole	20.43 $\pm$ 0.7571		15.70 $\pm$ 0.8001
4.	Ofloxacin	33.63 $\pm$ 1.2055	8.72 $\pm$ 1.1537	33.13 $\pm$ 0.9073
5.	Ciprofloxacin	38.60 $\pm$ 0.8185		35.13 $\pm$ 1.1239



Sr. No.	Microorganism	Solution A (zone inhibition in mm $\pm$ SEM) <sup>x</sup>	Solution B (zone inhibition in mm $\pm$ SEM) <sup>y</sup>	Solution C (zone inhibition in mm $\pm$ SEM) <sup>x</sup>
6.	Cefixime	25.73 $\pm$ 1.0692		20.33 $\pm$ 0.7681
7.	Tetracycline	34.60 $\pm$ 1.4525		26.85 $\pm$ 0.5766

Note – a indicate Gram Positive Bacteria, b – indicate Gram Negative Bacteria, x – indicate mean of 3 readings, y – indicate mean of 21 readings

#### 4. Discussion

In the present era, the use of herbal medicines is ever increasing owing to their minimal side effect(s) as compared to their allopathic counterparts.

Since ancient times, these medicinal herbs have been used for the treatment of various diseases and disorders because of their effectiveness and nominal side effect(s). The Indian traditional medicine system, Ayurveda presents a classic example which describes herbal medicines for the prevention as well as treatment of several forms of human illnesses.

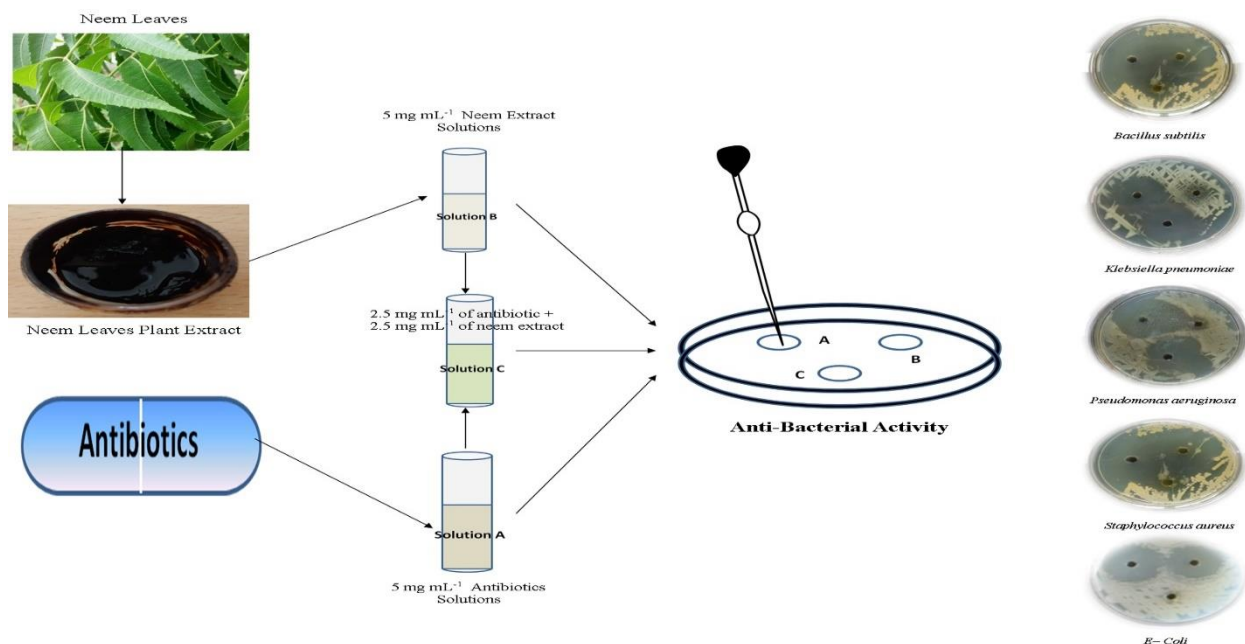
A range of human infections are treated by the several antibiotics and one of the approach for prevention or delaying of development of resistant microbial strains is to employ combination therapy of antimicrobials. Another prominent reason is that in some cases antibiotic combinations may lead to synergism, particularly in other-resistant cases, which prove to be of help for the treatment of infections. The combined administration of herbs / herbal extracts has also been shown to be beneficial (synergistic or additive) or deleterious in patient antibiotic therapy and that implies a possible herb-drug interaction (antagonistic or toxic outcome). Several side effect(s) previously discussed have been reported by antibiotics. Effective antibiotics with no or minimal side-effect(s) are therefore urgently needed. Neem is known worldwide for its excellent antimicrobial properties. Therefore, we have verified the antimicrobial activity of a combination of some conventional antibiotics with Neem extract.

We have tried to find out effect of use of Neem extract with standard antibiotics with intent to decrease the dose

of standard antibiotic. The standard antibiotic dose was reduced to half when combined with Neem extract, without affecting its effectiveness, as demonstrated in the study.

Neem extract alone exhibited zone of inhibition of 13.53 $\pm$ 1.6772, 12.36 $\pm$ 2.6674, 9.53 $\pm$ 0.9890, 13.75 $\pm$ 1.3871 and 8.72 $\pm$ 1.1537 against selected microbial strains namely, *Klebsiella Pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *E.coli* respectively. In case of *Klebsiella Pneumoniae*, the combination of Cefixime and neem exhibited zone of inhibition of 26.43 $\pm$ 0.9073, as compared to Cefixime alone which showed zone of inhibition of 25.43 $\pm$ 1.2096. For *Staphylococcus aureus*, 45.13 $\pm$ 0.7094 was observed to be the zone of inhibition in case of combination of Ampicillin and Neem, which was relatively better than Ampicillin alone which showed 41.93 $\pm$ 1.9008 as zone of inhibition. Similarly, combination of Tetracycline and Neem offered better inhibition of 42.50 $\pm$ 0.9165, than Tetracycline alone against *S.aureus*. Also, the combination of Neem and Chloramphenicol showed better zone of inhibition of 19.10 $\pm$ 0.6557 as compared to Chloramphenicol alone against *Pseudomonas aeruginosa*. In addition, the combination of Ciprofloxacin and Neem showed better efficacy than

Ciprofloxacin alone against the said organism. For *Bacillus subtilis*, 13.00 $\pm$ 0.4001 was noted to be the zone of inhibition for combination of Neem and Ampicillin than Ampicillin alone which showed zone of inhibition of 10.86 $\pm$ 0.4163. However, in case of *E. coli* the values of zones of inhibition of the selected antibiotics were



comparable with that of their combination with Neem extract.

The aforementioned results of solution A and solution C clearly demonstrate the synergistic activity of Neem extracts when combined standard antibiotics as observed in the values of zone of inhibition. Thus, Neem extract and antibiotic combination significantly minimize the standard dose of antibiotics.

## 5. Conclusions

As compared with their combination with the antibiotics, the individual plant extract have been less efficient. According to our study a synergistic effect have been observed with the use of combination of Neem extract and antibiotic against the selected microbial strains which is an important finding of this work. This synergism may be due to complex formation that is more effective in destruction of microbial cell, either through inhibition of

cell wall synthesis or its lysis. Furthermore, the research showed that Neem extract can be used with conventional antibiotics as this combination exhibited a synergistic effect that may lead to reduction in dose of the antibiotics.

## Abbreviations

MDR - Multidrug-resistant; *K. pneumonia* - *Klebsiella pneumonia*; *P. aeruginosa* - *Pseudomonas aeruginosa*; *S. aureus* - *Staphylococcus aureus*; *E. coli* - *Escherichia coli*; *B. subtilis* - *Bacillus subtilis*; DMSO – Dimethylsulfoxide; °C – Degree Celsius; µL – micro liter; mL – mille liter

## Acknowledgements

Authors are thankful to Dr. C. S. Magdum, Principal, Rajarambapu College of Pharmacy, Kasegaon (MS), INDIA for providing necessary facilities to carry out the research work.

## REFERENCES

- Margaret A.A., Investigation of the Synergistic Effects of the Extract of Neem Tree and Caffeine on the Promoter Activity of Cyp6a8 in *Drosophila melanogaster*, University of Tennessee Honors Thesis Projects. [https://trace.tennessee.edu/utk\\_chanhonoproj/1041](https://trace.tennessee.edu/utk_chanhonoproj/1041), 2007.
- Asimuddin M., Shaik M.R., Adil S.F., Siddiqui M.R.H., Alwarthan A., Jamil K. and Khan M. *Azadirachta Indica* Based Biosynthesis of Silver Nanoparticles and Evaluation of their Antibacterial and Cytotoxic Effects. *J King Saud Univ Sci* 2020;32:648-656. <https://doi.org/10.1016/j.jksus.2018.09.014>
- Govindachari T.R., Suresh G., Gopalakrishnan G., Banumathy B. and Masilamani S. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. *Phytoparasitica* 1998;26:109–116.
- Alzohairy M.A. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evid Based Complement Alternat Med* 2016;7382506. <https://doi.org/10.1155/2016/7382506>
- Mahfuzul H.M.D., Bari M.L., Inatsu Y., Juneja V.K. and Kawamoto S. Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathog Dis* 2007;4:481–488. DOI: 10.1089/fpd.2007.0040
- Bhinge S.D., Malipatil S.M., Sonawane L.V. and Hariprasanna R.C. Simultaneous Estimation of Cefixime And Cloxacillin In Bulk And Tablet Formulation By RP-HPLC Method. *Indian Drugs* 2012;49:23-29.
- Bhinge S.D., Malipatil S.M. and Sonawane L.V. Bioanalytical Method Development and Validation for Simultaneous Estimation of Cefixime and Dicloxacillin by RP-HPLC in Human Plasma. *Acta Chim Slov* 2014;61:580–586.
- Bhinge S.D., Malipatil S.M., Sonawane L.V. and Hariprasanna R.C. Simultaneous Estimation of Cefixime and Dicloxacillin in Bulk and Tablet Formulation by RP-HPLC Method. *FABAD J Pharm Sci* 2012;37:63-71.
- Bhinge S.D., Malipatil S.M. and Sonawane L.V. Simultaneous estimation of cefixime and cloxacillin in human plasma by reversed phase-HPLC with UV detection. *Thai J Pharm Sci* 2012;36:63-71.
- Chopra I. and Roberts M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiol Mol Biol Rev* 2001;65:232–260.
- Drusano G.L., Standiford H.C. and Plaisance K. Absolute oral bioavailability of ciprofloxacin. *Agents Chemother* 1986;30:444–446.
- Maestrelli F., Jug M., Cirri M., Kosalec I. and Mura P. Characterization and microbiological evaluation of chitosan-alginate microspheres for cefixime vaginal administration. *Carbohydr Polym* 2018;192:176-183. doi: 10.1016/j.carbpol.2018.03.054
- Ozcan K., Uzelb A. and Bedir E. Anti-Microbial Activity of Chloramphenicol from *Streptomyces* sp.10CM9. *Procedia - Social and Behavioral Sciences* 2015;195:1736–1739.
- Sato K., Inoue Y., Fujii T., Aoyama H. and Mitsuhashi S. Antibacterial activity of ofloxacin and its mode of action. *Infection* 1986;14:S226-230.
- Shib A.M. Antibacterial activity of ampicillin alone and in combination with sulbactam: Correlation with beta-lactamase production. *Curr Ther Res* 1994;55:1304-1309.
- Zandera J., Besiera S., Faetkea S., Saumb S.H., Müller V. and Wichelhaus T.A. Antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2'-deoxyuridine and rifampicin against *Staphylococcus aureus*. *Int J Antimicrob Ag* 2010;36:562-565.
- Koppen B.C., Mulder P.P.G., Boer L.D., Riool M., Drijfhout J.W. and Zaat S.A.J. Synergistic Microbicidal Effect of Cationic Antimicrobial Pep-tides and Teicoplanin against Planktonic and BioPlm-Encased *Staphylococcus aureus*. *Int J Antimicrob Ag* 2019;53:143-151. doi: 10.1016/j.ijantimicag.2018.10.002
- Richard P. 2015 EAHP survey: managers urged to support systems to reduce medication errors. *Eur J Hosp Pharm* 2015;23:245–247. doi: 10.1136/ejhp-2016-001014
- Randive D.S., Bhinge S.D., Sayyad S.F. and Wadkar G.H.

- Comparative Standardization of Marketed Formulations of Fermented Biomedicine – Arjunaristha. *Indonesian J Pharm* 2016;27:220-225.
20. Rajesh T., Roy A.K., Erumalla V.N.R., Goli D. and Basha S.J. Development and evaluation of antimicrobial ointment formulation contain-ing extracts of *Ocimum sanctum*, *Anthocephalus cadamba*, *Allium sativum* and *Origanum vulgare*. *World J Pharm Res* 2014;3:398-422.
21. Bhinge S.D., Bhutkar M.A., Randive D.S., Wadkar G.H. and Todkar S.S. Development and evaluation of antimicrobial polyherbal gel. *Annal Pharm Fr* 2017;75:349-358. doi: 10.1016/j.pharma.2017.04.006.
22. Brooks B.D. and Brooks A.E. Therapeutic strategies to combat antibiotic resistance. *Adv Drug Deliv Rev* 2014;78:14–27. doi: 10.1016/j.addr.2014.10.027
23. Ababneh M. A., Issa N. and Alkhatatbeh M. Evaluation of Core Elements of Antimicrobial Stewardship Programs in Jordanian Hospitals, *Jordan Journal of Pharmaceutical Sciences*, 2017;10(2), 127-134.
24. Antão E.M., Wagner-Ahlf, C. Antibiotic resistance: A challenge for society. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2018; 61: 499-506. doi: 10.1007/s00103-018-2726-y
25. Bhinge S., Bhutkar M., Randive D., Wadkar G. and Todkar S. Investigation of the synergistic effects of the extract of neem with conventional antibiotic against gram positive and gram negative microorganism. *Int J Infect Dis* 2020;101:16. <https://doi.org/10.1016/j.ijid.2020.09.080>.
26. Bhinge S., Bhutkar M., Randive D., Wadkar G. and Todkar S. Synergistic effects of synthesized iron nanoparticles of neem extract with conventional antibiotic against gram positive negative microorganism. *Int J Infect Dis* 2020;101:16. <https://doi.org/10.1016/j.ijid.2020.09.158>
27. Ali H., Alkowni R., Jaradat N. and Masri M. Evaluation of phytochemical and pharmacological activities of *Taraxacum syriacum* and *Alchemilla arvensis*, *Jordan Journal of Pharmaceutical Sciences*, Volume 14, No. 4, 2021, 457-472,
28. Ismed F., Putra H. E., Arifa N. and Putra D. P. Phytochemical profiling and antibacterial activities of extracts from five species of Sumatran lichen genus *Stereocaulon*. *Jordan Journal of Pharmaceutical Sciences*, 2021;14(2):189-202.

## التأثيرات التآزرية لمستخلص أوراق النيم مع المضاد الحيوي التقليدي ضد الكائنات الدقيقة الإيجابية والسلبية للجرام

سومناث دز<sup>1\*</sup>، ديراج س. رانديب<sup>1</sup>، مانجيش أ بوتكارب<sup>1</sup>، كيران ب شجولب<sup>1</sup>،  
أميت د. جادافا<sup>1</sup>، راهول ب. جادافا<sup>1</sup>

<sup>1</sup> قسم الكيمياء الصيدلانية، كلية راجارامبابو للصيدلة، الهند.

### ملخص

**الخلفية:** من المعروف أن النيم يمتلك العديد من المكونات النباتية المعقدة ويعرض مجموعة واسعة من الاستخدامات الطبية والمنزلية التي تُعزى إلى العزلة (العزلات) النشطة التي تمتلك القدرة على علاج العديد من الأمراض المزمنة والاضطرابات (الاضطرابات). هدفت الدراسة الحالية إلى دراسة التأثير المشترك المضاد للميكروبات لمستخلص النيم الخام والمضادات الحيوية المختارة مثل سيبروفلوكساسين، سيفيكسيم، كلورامفينيكول، أمبيسلين، سلفاميثوكسازول، نتراتريكين وأوفلوكساسين على الكائنات الدقيقة الإيجابية والسلبية للجرام المختار. تم استخدام مستخلص أوراق النيم الكحولي الخام للدراسة.

**الطريقة:** المحلول أ يحتوي على مستخلص النيم 5 مجم مل 1 وحده، المحلول ب يتكون من المضادات الحيوية القياسية وحدها 5 مجم مل 1 ومحلول ج الذي يحتوي على توليفة من 2.5 مجم مل من مستخلص النيم ومضاد حيوي معياري محدد بتركيز 2.5 مجم تم اختبار mL-1 من حيث قدرتها المضادة للبكتيريا ضد سلالة مختارة من الكائنات الدقيقة مثل Klebsiella pneumoniae و Staphylococcus aureus و Pseudomonas aeruginosa و E. coli و Bacillus subtilis باستخدام تقنية لوحة أجار.

**النتائج:** أشارت النتائج المتحصل عليها إلى الفعالية التآزرية لمستخلص النيم والمضادات الحيوية المختارة. لوحظ أن نصف تركيز المضاد الحيوي كان كافياً لممارسة التأثير المضاد للميكروبات عند مزجها مع مستخلص النيم. وبالتالي، قد يتم تقليل جرعة المضادات الحيوية القياسية بشكل هامشي إلى النصف تقريباً في التركيز عند دمجها مع مستخلص النيم دون المساس بالفعالية. أشارت مناطق التثبيط إلى أن الجمع بين مستخلص النيم والمضاد الحيوي لهما تأثير تآزري يسهل تحقيق خفض جرعة المضادات الحيوية القياسية.

**الاستنتاجات:** يمكن الاستنتاج من البحث أن مستخلص النيم عند دمجها مع المضادات الحيوية التقليدية يمكن استخدامه كعامل مضاد للميكروبات جديد له تأثير تآزري ومفيد أيضاً في تحقيق تقليل جرعة المضادات الحيوية التقليدية.

**الكلمات الدالة:** نيم؛ الكائنات الحية الدقيقة. مضاد للجراثيم. تأثير تآزري مضادات حيوية.

\* المؤلف المراسل: سومناث دز

[somu1245@yahoo.co.in](mailto:somu1245@yahoo.co.in)

تاريخ استلام البحث 2021/9/7 وتاريخ قبوله للنشر 2021/12/27.