

## Artificial Intelligence and Machine Learning Applied Interpretation and Analysis of Formulated Curcumin Loaded Nanoparticles for Antibacterial Activity

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### Keywords

Nanoparticles, Curcumin, Piperine, Artificial Intelligence, Jupyter Notebook

### Abstract

Artificial Intelligence and Machine Learning studies of data collected during the research and review studies has been done using Jupyter Notebook (Anaconda). Pie chart along with exclusive graphs like tree map, donut graph and others are used to make a comprehensive study of the data. Curcumin-piperine loaded nanoparticles are prepared successfully using Fessi method. The antimicrobial activity of Curcumin-Piperine (Cu-Pi) loaded nanoparticles (NPs) is studied, focusing on the effect of nanonization with bioavailability enhancer (piperine) on the Minimum Inhibitory concentration and growth inhibition.

### 1. Introduction

Curcumin is one of the important source of *Curcuma longa* L. (Zingiberaceae family), also called turmeric. The yellow colour of the herb is due to curcumin. It is mostly cultivated in tropical and subtropical regions. It is also used to flavour food and dye clothes. Solvent extraction (preferably with ethanol) is used to extract curcumin from turmeric through various methods like Soxhlet ultrasonic microwave, and supercritical carbon dioxide followed by purification via column chromatography. It also enhances the effects of antibacterial, antifungal, antioxidant, and anticancer activities. Curcumin is less to nil toxic at active doses. Curcumin has a variety of specific characterizations which makes it an interest of study for scientists in recent years. Curcumin is also a hydrophobic polyphenol because of which it modulates multiple signaling pathways and has a wide range of pharmacological activities, such as anti-inflammatory, antioxidant, immunomodulatory, neuroprotective, antitumor, antibacterial, antidiabetic and anticarcinogenic

activities. It is also used as medicine in India and China Tradition for treating jaundice, hepatic disorder, fever, diabetic wounds etc. But the only problem is its poor bioavailability. To overcome this limitation piperine is used which is also known as an inhibitor of intestinal hepatic and hepatic glucuronidation. Curcumin has been "generally recognized as safe" by Food and Drug Administration of the United States.<sup>[1]</sup>

### Artificial Intelligence and Machine Learning

Artificial Intelligence is one of a wide field of computer science which emphasizes on organizing intelligent machines which are capable of completing various tasks, which requires human intelligence. Artificial intelligence is also considered as a technique or a technology that allows machines to replicate human behavior. Machine learning is a subspace of Artificial Intelligence, which allows the machine to learn from the past given data without any obvious planning accordingly. Artificial Intelligence has been used in the field of medicine for discovery and drug designing.<sup>[2,3]</sup>

## 2. Material And Methods

Procurement of curcumin ,piperine, Eudragit , Dimethyl sulphoxide (DMSO) & acetone and downloading the Artificial intelligence and machine learning tools like python 3 and Anaconda (Jupyter notebook) .

**Preparation and Characterization of Curcumin loaded Nanoparticles:-** Curcumin, Piperine and Eudragit E 100 were dissolved in 20 ml of acetone under sonication. In 50 mL of ultra pure water this organic phase was mixed containing sodium lauryl sulphate (SLS) under constant stirring at 500 or 1000 rpm for a time period of atleast 1 hour. Nanoparticles were spontaneously formed turning the solution slightly turbid. Overnight stirring was done to remove the organic solvent. Centrifugation was done to remove the free drug at 3000rpm for 10minutes at 4°C .The resultant formulation was used for further characterization.

### Formulation Optimization

Optimization of the Curcumin-Piperine nanoparticles was done using Design Expert® software after applying specific check on the chosen dependent variables. The main aim of the optimization was to maximize percent Entrapment Efficiency, minimize Particle Size .The EE% & PS of optimized Cu-Pi nanoparticles was determined.<sup>[4]</sup>

### Antibacterial study

**Methods of Determination of Minimum Inhibitory Concentration (MIC):-** Agar dilution method was used to test the minimum inhibitory concentration of curcumin and nanocurcumin. For this firstly a stock solution of curcumin nanoparticles was prepared by taking 2mg nanoparticles in 1mL of distilled water(DW).After which a nanodispersion showing an orange colour. The prepared stock solution was then diluted serially from 10- 100 µg/mL. Then, an another stock solution was formed by dissolving 2 mg of curcumin in 1 mL of Dimethyl sulphoxide since curcumin is insoluble in water. Then some flasks

were taken and 20 mL of melted agar, different concentrations of curcumin in Dimethyl sulphoxide and Cu-Pi nanoparticles in water were added separately. An equivalent amount of Dimethyl sulphoxide was used in the control plates, and after this solidifying process was done. Under controlled aseptic conditions 100 µL of culture was inoculated. The prepared plates were incubated for 24 hours at 37°C .

Using the below formula the inhibitory effect was calculated:-

$$\% \text{ Inhibition} = (1 - T / C) \times 100$$

where T is cfu/mL for the prepared test sample &

C is cfu/mL for the control.

Again the experiment was performed twice and it was continued for 3 times. The lowest concentration of curcumin was considered as the MIC.

**Zone of Inhibition:-**Well-diffusion antibacterial susceptibility assay was conducted with few modification. In the saline solution emulsified colonies were inoculated using Nutrient Agar Medium in which wells were made using a sterilized cork borer. After absorbing the moisture, the sample; Curcumin and Cu-PiNPs (1mg/mL) were loaded into the well, and it was incubated at 37°C for 24hrs.

## 3. Results

### Formulation Optimization

Optimization of the formulation was done using Design Expert®. A formulation with a calculated desirability of 0.889 was selected on applying the criteria of Maximum entrapment efficiency and minimum particle size. The conclusion of the optimization was as follows: the concentration of curcumin , concentration of piperine and concentration of Surfactant are 0.13 % , 0.09% & 2.3% respectively.<sup>[5-8]</sup>

## Antibacterial study

**Table 1.** Zone of Inhibition (mm)

Organism	Curcumin	Cu-Pi NPs
<i>Staphylococcus aureus</i>	21	30
<i>Bacillus subtilis</i>	26	32
<i>E.coli</i>	24	31
<i>Pseudomonas aeruginosa</i>	22	25

**Table 2.** Minimum Inhibitory Concentration (MIC) ( $\mu\text{g/mL}$ )

Organism	Curcumin	Cu-Pi NPs
<i>Staphylococcus aureus</i>	64	21
<i>Bacillus subtilis</i>	32	16
<i>E.coli</i>	64	32
<i>Pseudomonas aeruginosa</i>	128	64

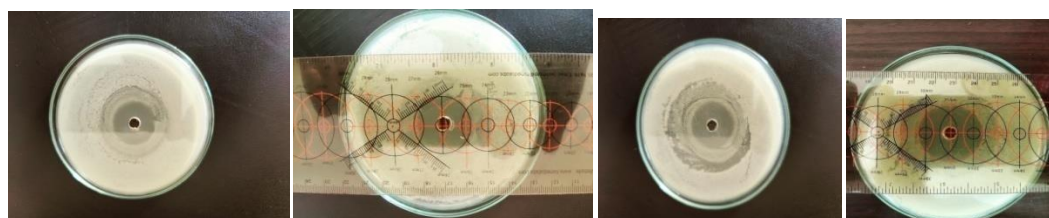


*Pseudomonas Aeruginosa* Cu-Pi NPs

*Pseudomonas Aeruginosa* Curcumin NPs

Fig 1

Fig.2



*Bacillus Subtilis* Curcumin NPs

*Bacillus Subtilis* Cu-Pi NPs

Fig.3

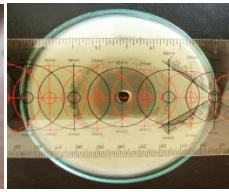
Fig.4





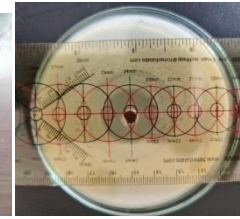
*E.coli Cu-Pi NPs*

Fig.5



*E.coli Curcumin NPs*

Fig.6



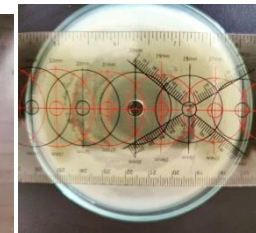
*Staphylococcus Aureus Curcumin NPs*

Fig.7



*Staphylococcus Aureus Cu-Pi NPs*

Fig.8



## Analysis of the results of antibacterial study obtained using Artificial Intelligence & Machine Learning

### Artificial Intelligence Analysis of Zone of Inhibition

```
In [2]: # preprocessing
import pandas as pd
import numpy as np
from sklearn.preprocessing import LabelEncoder
from sklearn.preprocessing import StandardScaler
import pandas as pd
import seaborn as sns
import matplotlib.pyplot as plt
import matplotlib.dates as mdates
import plotly.express as aware
```

```
In [3]: df = pd.read_csv('ZoI.csv')
df
```

Out[3]:

	Organism	Curcumin	NPs
0	S. aureus	21	30
1	B.subtilis	26	31
2	E.coli	24	32
3	P.aeruginosa	22	25

### EDA

```
In [4]: df.head(2)
```

Out[4]:

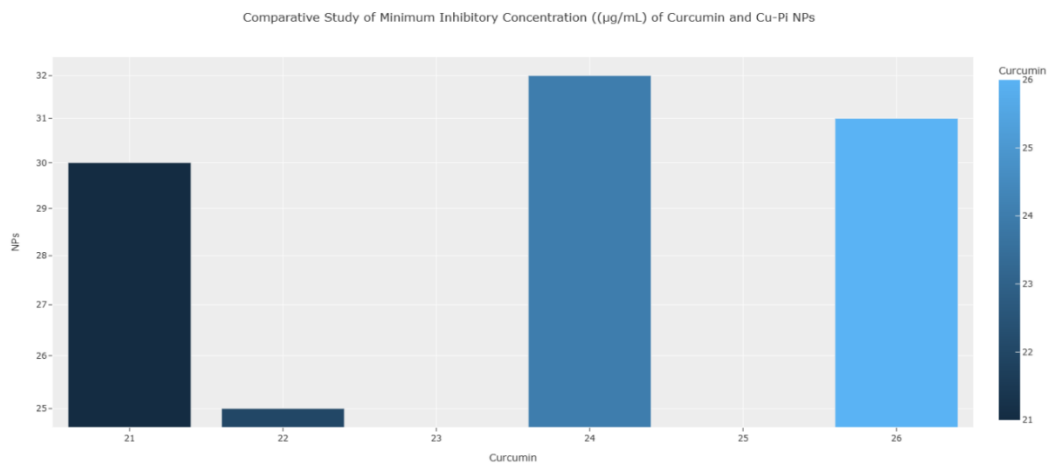
	Organism	Curcumin	NPs
0	S. aureus	21	30
1	B.subtilis	26	31

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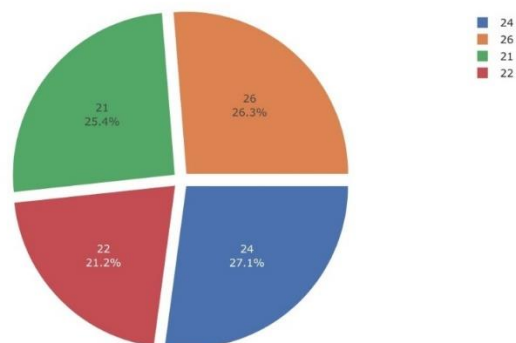
```
In [5]: df.info()
```

```
<class 'pandas.core.frame.DataFrame'>  
RangeIndex: 4 entries, 0 to 3  
Data columns (total 3 columns):  
#   Column      Non-Null Count  Dtype  
---  ---  
0   Organism    4 non-null      object  
1   Curcumin    4 non-null      int64  
2   NPs         4 non-null      int64  
dtypes: int64(2), object(1)  
memory usage: 224.0+ bytes
```

```
In [6]: fig = aware.bar(df[['Curcumin', 'NPs']].sort_values('NPs', ascending=False),  
y="NPs", x="Curcumin", color='Curcumin', width=1500, height=700,  
log_y=True, template='ggplot2',  
title='Comparative Study of Minimum Inhibitory Concentration ((µg/mL) of Curcumin and Cu-Pi NPs')  
fig.show()
```

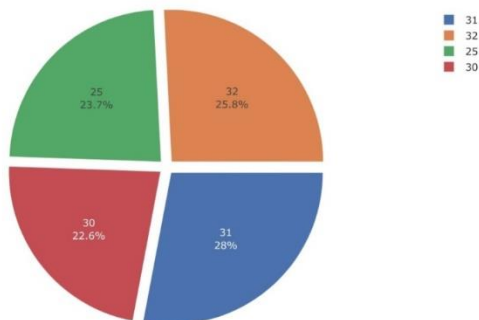


```
In [30]: fig = aware.pie(df,  
values="NPs",  
names="Curcumin",  
template="seaborn")  
fig.update_traces(rotation=90, pull=0.05, textinfo="percent+label")  
fig.show()
```

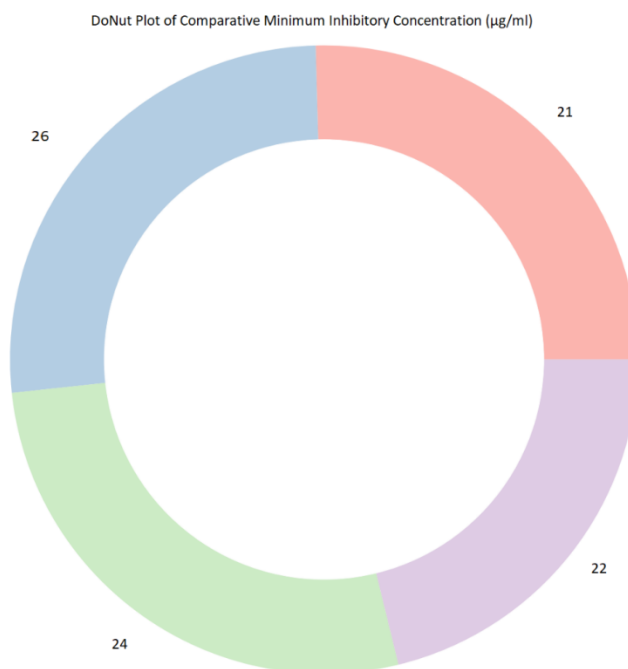


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```
In [31]: fig = aware.pie(df,
  values="Curcumin",
  names="NPs",
  template="seaborn")
fig.update_traces(rotation=90, pull=0.05, textinfo="percent+label")
fig.show()
```



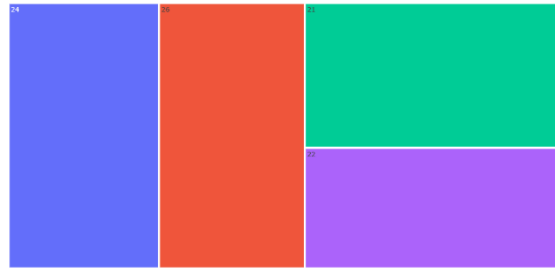
```
In [32]: from palettable.colorbrewer.qualitative import Pastel1_7
plt.figure(figsize=(25,25))
my_circle=plt.Circle((0,0), 0.7, color='white')
plt.pie(df['NPs'], labels=df.Curcumin, colors=Pastel1_7.hex_colors)
p=plt.gcf()
p.gca().add_artist(my_circle)
plt.title('DoNut Plot Of Compartive MINIMUM Inhibiotry Concentration ((µg/mL)')
plt.show()
```



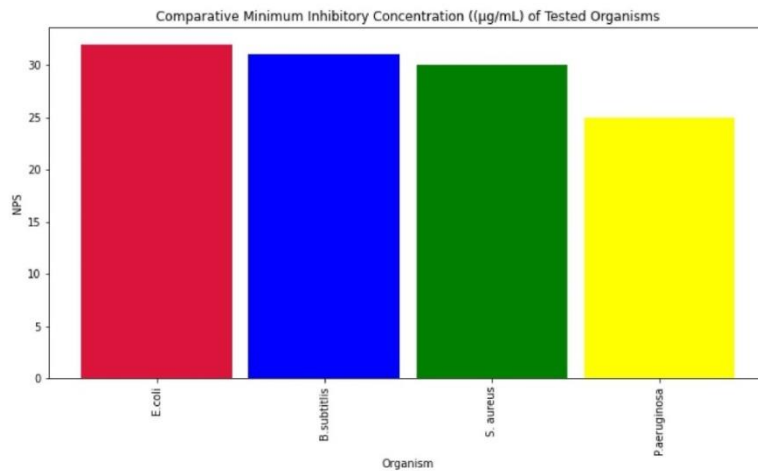
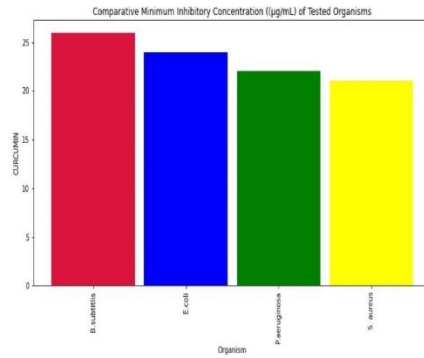
```
In [7]: fig = aware.treemap(df, path=['Curcumin'], values='NPs',
  title='Tree of Zone of Inhibition',width=1200, height=700)
fig.show()
```

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Tree of Zone of Inhibition



```
In [8]: MIC = ['Curcumin', 'NPs']
for x in MIC:
    if #[x].dtypes != 'string':
        #print(x)
        data = df.groupby(['Organism'])[x].sum().sort_values(ascending=False)[:10]
        plt.figure(figsize=(12,6))
        plt.bar(data=data, x = data.index, height = data.values, width=0.9,
               color = ['crimson', 'blue', 'green', 'yellow', 'magenta'])
        plt.xticks(rotation='vertical')
        plt.xlabel('Organism', size = 10)
        plt.ylabel(x.upper())
        plt.title('Comparative Minimum Inhibitory Concentration ((µg/mL) of Tested Organisms')
```

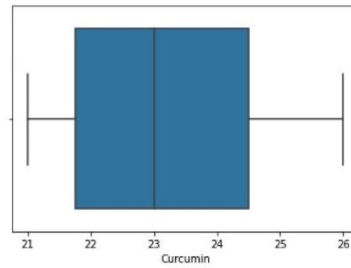




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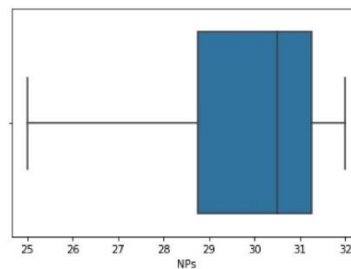
```
In [35]: sns.boxplot(df['Curcumin'])
```

```
Out[35]: <AxesSubplot:xlabel='Curcumin'>
```

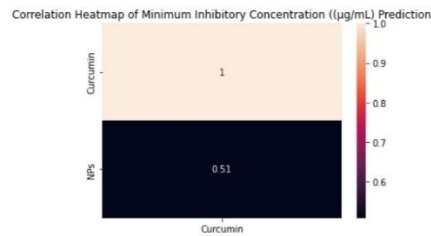


```
In [36]: sns.boxplot(df['NPs'])
```

```
Out[36]: <AxesSubplot:xlabel='NPs'>
```

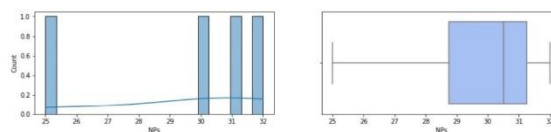
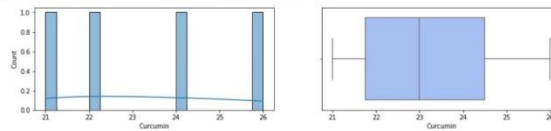


```
In [37]: sns.heatmap(df.corr()[['Curcumin']].sort_values(by='Curcumin', ascending=False), annot=True)  
plt.title('Correlation Heatmap of Minimum Inhibitory Concentration ((µg/mL) Prediction)')  
plt.show()
```



```
In [38]: num_col = ['Curcumin', 'NPs']
```

```
for i in num_col:  
    fig, axs = plt.subplots(1, 2, figsize=(15, 3))  
    sns.histplot(df[i], bins=20, kde=True, ax=axs[0]);  
    sns.boxplot(df[i], ax = axs[1], color='#99b6fd', fliersize=1);
```





## 4. Discussion

Artificial Intelligence and Machine Learning studies of data collected during the research and review studies has been done using Jupyter Notebook (Anaconda). Different graphical representations like bar diagram, Pie chart along with exclusive graphs like tree map, donut graph and others allow to make a comprehensive study of the same data.

Curcumin-piperine loaded nanoparticles were prepared successfully using Fessi method. The antimicrobial activity of Cu-Pi loaded NPs was studied, focusing on the influence the main focus of which was on the nanonization with bioavailability enhancer (piperine) on the Minimum Inhibitory Concentration and growth inhibition. Lower MIC is obtained by the use of polar solvents like DMSO and acetone.

Minimum Inhibitory Concentration of curcumin and Cu-Pi nanoparticles was tested against two Gram-positive bacteria namely *S. aureus* and *B. subtilis* and two Gram-negative bacteria namely *E. coli* and *P. aeruginosa*. The antibacterial activity of nanoparticle against these bacteria's informed that it has a wide spectrum inhibitory effect. Minimum Inhibitory Concentration of nanoparticle for *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* was 21, 16, 32, and 64 µg/mL, respectively, compared to 64, 32, 64, and 128 µg/mL for curcumin. The extent of inhibition of different bacteria of Cu-Pi nanoparticle in water and curcumin in DMSO solutions followed the order: *B. subtilis* ≤ *S. aureus* ≤ *E. coli* ≤ *P. aeruginosa*. Further, maximum efficiency of the diameter of inhibition was seen for *B. Subtilis*.

As a result it was found that the sensitivity of the selected Gram-positive bacteria's is higher than the selected Gram-negative bacteria's.

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