

## Synergistic Interaction of Cannabis and Garlic with Commercial Antibiotics

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### ABSTRACT

Plants are known to be the source of many bioactive chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. For current study we have selected four traditional medicinal plants, *Calendula officinalis* (Marigold), *Cannabis sativa* (cannabis), *Allium sativum* (Garlic) and *Ocimum sanctum* (Tulsi). Among these four plants, cannabis and garlic has shown clear zone of inhibition and they have also show the synergistic combination with commercial antibiotics, gentamicin and vancomycin. As a result the dose of commercial drugs and so the side effects caused by these commercial drugs can be reduced with the use of combination with phytochemicals. Pharmacodynamics studies of the combinations need to be performed to decide on the effective dosage.

**Key words:** synergy, plants, antibiotics, side effect, resistance

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### INTRODUCTION

Medicinal plants, particularly their active components, have been a dependable source of therapeutics for the treatment of various ailments since time immemorial<sup>1</sup>. The rapid propagation of antibiotic resistance has urged the human kind to use plants as a reliable source for the discovery of active antimicrobial agents and possibly, even novel classes of antibiotics. Alternative approach to the use of new antibacterial compounds is the use of antibiotic synergists. Synergism in antimicrobial therapy is well known and is used to describe supra-additive activity of antibiotics when used in combinations with other compounds. Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs<sup>1</sup>.

In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods<sup>2</sup>. The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steroids, phenols glycosides and tannins<sup>3</sup>.

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such

as anticancer drugs<sup>4</sup>, antimicrobial drugs<sup>5</sup>, antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized.

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens<sup>6</sup>. Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity<sup>7-8</sup>.

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances<sup>9-10</sup>. Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms. The worldwide emergence of multi drug

Table 1: Antimicrobial properties of medicinal plants

Test organism	<i>Ocimum sanctum</i>	<i>Calendula officinalis</i>	<i>Cannabis sativa</i>	<i>Allium sativum</i>
<i>Staphylococcus aureus</i>	No	No	9 mm	No
<i>Klebsiella pneumoniae</i>	No	No	No	7 mm

Table 2: MIC for the water extract of medicinal plants and antibiotics

Antibiotics/ Plant extract (mg/ml)	Test organism	
	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumonia</i>
Gentamycin	5	7.5
Vancomycin	10	10
Cannabis water extract	1000	500
Garlic water extract	500	250

resistant *Escherichia coli* and many other  $\beta$ -lactamase producers has become a major therapeutic problem<sup>11</sup>. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy<sup>12</sup>. For our study we used four traditional medicinal plants which were used as medicine traditionally and they are *Calendula officinalis* (Marigold), *Cannabis sativa* (cannabis), *Allium sativum* (Garlic) and *Ocimum sanctum* (Tulsi).

## MATERIAL AND METHODS

### Bacterial strains

The strains used in this study were *Staphylococcus aureus* and *Klebsiella pneumoniae*. They are specifically grown in Ca supplemented Muller Hinton Broth (Himedia, India) at an optimal growth temperature of 37°C with aeration for 16–18h. The Minimum Inhibitory Concentration (MIC) of antibiotics and plant extracts were determined with 10<sup>5</sup> CFU/ml of colonies by adjusting the optical density at 600 nm and growing them on appropriate agar plates.

### Antimicrobial compounds

The antibiotics selected for the study were gentamycin and vancomycin which were obtained from Himedia (India).

### Preparation of extracts

Plants leaf, bud of *Ocimum sanctum* and *Cannabis sativa* is first collected from the local Jalandhar area. Fresh leaf and bud is collected and it was dried under shade so that no direct sun light come in contact to the leaf and bud. They were allowed to stay in proper stable condition minimum for 15 to 20 days and need to check them at regular interval. When these leaf and bud were at the crunchy condition that means they were ready for the extraction.

5 gm of the coarsely powdered sample was macerated with 100 ml of water in a closed flask for 24 hour, shaking frequently during six hours and allow to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. First dried over water bath and then at 105°C in hot air oven, to constant weight and it was noted down. From the weight of the residue, the percentage of water soluble extract was calculated.

### Antimicrobial activity

On the Mueller Hinton Agar plate the lawn culture were grown for the selected microorganisms. Then the filter paper disk which have specific concentration of plant extract were placed on the plate containing lawn culture. Cultured plates were incubated for 24 to 48 hours at 37°C.

### Minimum inhibitory concentration (MIC)

The determination of the MIC of the crude extracts was carried out using the test tube dilution methods<sup>13</sup>. Each of the extracts was dissolving as their concentrates in 10 ml of nutrient broth, making the concentration as for example to be 40 mg/ml. Five tubes of 5 ml of nutrient broth were set up, and 5 ml of the 40 mg/ml of the extracts were taken and used for two-fold dilution into the five tubes of the nutrient broth, making the concentration to be 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml.

Table 3: Synergistic combination of Cannabis and Gentamycin (- = no growth; 0\* 0\*=synergistic combination; + =light growth; ++=moderate growth)

Test organism	Cannabis water extract (mg/ml)				
	1000	500	250	125	62.5
<i>Staphylococcus aureus</i>	-	-	0*	+	++
	-	-	0*	+	++
	5	2.5	1.25	0.625	0.312

### Gentamycin(mg/ml)

Table 4: Synergistic combination of Garlic and vancomycin (- = no growth; 0\* 0\*=synergistic combination; + =light growth; ++=moderate growth)

Test organism	Garlic water extract (mg/ml)				
	250	125	62.5	31.25	15.62
<i>Klebsiella pneumoniae</i>	-	-	0*	+	++
	-	-	0*	+	++
	10	5	2.5	1.25	0.625

### Vancomycin(mg/ml)

**Interaction of phytochemicals with commercial antibiotics**  
As the MIC is determine of the drug and the plant extract then the further interaction is done to see which mixture concentration of the drug and the plant extract show the inhibition of the bacterial growth. For this 5 tube is taken

then Each of the extracts was dissolving as their MIC concentrates in 10 ml of nutrient broth, making the concentration as for example to be 100 mg/ml. Five tubes of 5 ml of nutrient broth were set up, and 5 ml of the 100 mg/ml of the extracts were taken and used for two-fold dilution into the five tubes of the nutrient broth, making the concentration to be 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. Then as the same way commercial drug is also added to those tube respectively starting with their MIC here also the two-fold dilution is used.

## RESULTS AND DISCUSSIONS

### Antimicrobial activity

This test was performed by disc diffusion techniques and the zone of inhibition was measured in all the cases and compared with the standard commercial antibiotics (Table 1). As *O. sanctum* and Marigold didn't show zone of inhibition so only cannabis and garlic has been used for further studies.

### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the all water extract of the plants different from the each other and is estimated by tube dilution method which shows the minimum concentration where the growth of the bacteria inhibit (Table 2).

### Synergistic combination of Cannabis and Gentamicin

The synergistic interaction between cannabis and gentamicin was done in the nutrient broth by two-fold dilution technique is use. Cannabis and Gentamicin were taken as their MIC value (Table 3).

### Synergistic combination of Garlic and Vancomycin

The synergistic interaction between garlic and vancomycin was done in the nutrient broth by two-fold dilution technique is use. Garlic and vancomycin is taken as their MIC value (Table 4).

## CONCLUSIONS

Here we have used four different medicinal plants and regarding these plants many other study shows their medicinal property. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the largest most ubiquitous groups of plant metabolites<sup>14</sup>. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities<sup>15</sup>. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds<sup>16</sup>. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall<sup>17</sup>. They also are effective antioxidant and show strong anticancer activities<sup>18</sup>. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation<sup>19</sup>. The results obtained in this study that suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an

increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

After that the combination of the plant phytochemicals and the commercial antibiotics are use at their MIC value so by the help of the two-fold dilution method the below MIC strength of the both plant phytochemicals and the commercial antibiotics can inhibit the bacteria at the mixture concentration. That mixture concentration is below MIC for the both plant phytochemicals and the commercial antibiotics. That below MIC combination of plant phytochemicals and the commercial antibiotics is the synergistic combination of the plant phytochemicals and the commercial antibiotic.

## CONFLICT OF INTEREST

Conflict of interest declared none.

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