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# The Antibacterial Effect of Some Medicinal Plant Extracts and their Synergistic Effect with Antibiotics

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**Abstract**
The aim of the study was to assess the antibacterial effect of some medicinal plant extracts and their synergistic antibiotics against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extract of medicinal plants were prepared using Soxhlet apparatus for alcoholic extract, and water reflux for aqueous extracts. The antibacterial activities of extracts were evaluated using the disk diffusion method as well as well diffusion method; the inhibitory zones were recorded in millimeters. The minimal inhibitory concentration (MIC) of the plant extracts against *E. coli*, *S. aureus* and *P. aeruginosa* were assessed using microdilution method. The synergistic effect between plants and extraction of antibiotics was assessed using disk diffusion method. The results of this study showed that ethanolic extracts used against *E. coli*, *S. aureus* and *P. aeruginosa* were showed antimicrobial and synergistic effect with most antibiotics better than methanolic and aquatic extracts. The results of this study showed that there is a decrease in MIC in case of methanolic extract of *E. camaldulensis* against *E. coli* (3.125 mg/ml), and the methanol and aquatic extract of F. sycomorus (leaves) against *S. aureus* varying from 6.25 to 3.125 mg/ml, and the ethanol extract of *E. camaldulensis* against *P. areuginosa* (6.25 mg/ml). Thereby, our results indicate the possibility of using these extracts in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects in vivo. These results was revealed the importance of plant extracts when associated with antibiotic and Non-antibiotic drugs in control of bacteria.
**Keywords:** Plant extracts, Synergistic effects, Antimicrobial, Microdilution method

## Introduction

The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind. Different antibiotics exercise their inhibitory activity on different pathogenic organisms **(Chanda and Rakholiya, 2011)**. But in recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents. but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs **(Abiramasundari *et al*, 2011)**. So be searched for natural sources to be used as antimicrobial. Where that there are many research in the use of plants as antimicrobial.

Plants a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Over 50% of all modern clinical drugs are of natural product origin **(Kirbag *et al,* 2009)**. And it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants'. Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications, it is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods **(Abdel Rahman *et al*, 2011)**.

Some Palestinian plants exhibit significant potency against human bacterial pathogens. However, at present, plant extracts are rarely used as antimicrobials or as a systemic antibiotics and this may be due to their low level of activity, especially against gram-negative bacteria **(Adwan and Mhanna, 2008)**.

In this research we used some medicinal plant including *Nerium oleander, Artemisia herba-alba, Withania somnifera*, *Lantana camara, Ficus sycomorus, Allium sativum* and *Eucalyptus camaldulensis* **(Table. 1)** and its influence on *S. aureus*, *P. aeruginosa, E. coli* and then was search for synergies between these plants and some antibiotic and non-antibiotic drugs and then determining the MIC of the plant extract .

 

**(Table. 1) Ethnobotanical data of the investigated plants in this study.**

| Scientific name | Plant origin | Solvent | Antimicrobial activity | References |
|---|---|---|---|---|
| *Withania somnifera* | Root and leaves | Ethyl Acetate, Methanol, Water | *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* | Owais *et al*, 2003 |
| | Root and leaves | Methanol, Hexane, Diethyl ether | *S. typhimurium and E. coli* | Arora *et al*, 2004 |
| *Nerium oleander* | Flowers | Hexane | *E. coli ,Pseudomonas aeruginosa, S. aureus* | Derwich *et al*,2010 |
| | Leaves | Chloroformic, ethnolic, methanolic. | *Bacillus pumillus, Bacillus subtilius, S. aureus, E.coli* | |
| | | Chloroformic | *E.coli* | Hussain. M and Gorsi. M, 2004 |
| | Roots bark | Ethnolic, methanolic. | *B. pumillus, B. subtilius, S. aureus, E.coli* | |
| *Lantana camara* | Leaf | Mixture of dichloromethane and methanol. | *P. aeruginosa, E. coli* | Kumar *et al*, 2006 |
| *Ficus sycomorus* | Leaves and Stem bark | 70% aqueous ethanol | *S. aureus, Salmonella typhi* | Olusesan *et al*, 2010 |
| *Eucalyptus camaldulensis* | Leaf | Methanol | *Klebsiella spp, S. typhi, Yersinia enterocolitica, P. aeruginosa, S. aureus, B. subtilis.* | Ayepola and Adeniyi, 2010. |
| | Leaf | Aqueous, acetone, chloramphenicol | *E. coli, K. pneumoniae, S. typhi, S. aureus* | El-Mahmood Muhammad Abubakar, 2010 |
| *Artemisia herba-alba* | Leaf | Methanol | *S. aureus* | Seddik *et al*, 2010 |
| *Allium sativum* | Bulbs | 70% ethanol | *Mycobacterium tuberculosis.* | Hannan *et al*, 2009. |
| | Bulbs | Water and methanol | *E.coli, K. Pneumoniae, S. typhi, B. cereus, S. mutans.* | Saravanan *et al*, 2010 |

**Material and Methods:**

**Plant Materials**: The plant materials used in this study consisted of *Nerium oleander* (leaf), *Artemisia herba alba* (leaf), *Withania somnifera* (leaf), *Lantana camara* (leaf), *Ficus sycomorus* (leaf and bark), *Allium sativum* (bulb), *Eucalyptus camaldulensis* (Table.2) which are growing in Palestine. These plants collected from different areas in Gaza strip.

**Microorganism:** Pathogenic strains of *S. aureus*, *P. aeruginosa*, and *E. coli* were obtained from the biological science department at the Islamic University of Gaza (IUG) and microbiology department at Al-Shifa hospital, and were maintained on Brain Heart Infusion (BHI) agar medium at 4 ºC for further experiments.

**Preparation of plant extract:**

The powdered materials of plants (20 g) were extracted with water (150ml, 2h) by water reflux and methanol and ethanol (150 ml, 8h) by soxhlet apparatus. And then the extract was filtered and allowed to evaporate in oven at

 

45 ºC. Aquatic extract dissolved in distilled water, while alcoholic extract dissolved in 10% Dimethyl sulfoxide (To prepared 200mg/ml as a standard concentration) **(Parekh and Chanda, 2006; Shihabudeen *et al,* 2010 and Jameela *et al*, 2011)**.

**Preparation of stock solution of the Non-Antibiotic drugs**

Different concentrations of Non-antibiotic drugs were prepared using water as solvent for Vit. C and methanol for Loperamide HCl and Paracetamol solutions. Different working concentrations (100µM, 50µM and 10µM) were prepared using serial dilution of the prepared stock solution of 1mM concentration.

**Antibiotics activity assay**

The filter paper discs (antibiotics) were placed on the surface of a Mueller-Hinton agar that has been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone diameter of inhibition is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs **(Sockett, 2006)**.

**Plant extracts activity assay**

**Agar-Well Diffusion Methods:** According to **Obeidat *et al*** with few modification. An inoculum suspension was swabbed uniformly to solidified 20 ml Mueller-Hinton Agar (MHA). And the inoculums were allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using Glass Pasteur pipettes. Aliquot of 20 µl from each plant crude extract (200 mg $ml^{-1}$) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37ºC for 24 h. The resulting inhibition zones were measured in millimeters (mm).

**Paper Disk Diffusion Assay:** A suspension of testing microorganisms were spread on MHA medium. The filter paper discs (5mm in diameter) was placed on the agar plates which was inoculated with the test microorganisms and then impregnating with 20µl of plant extract (concentration 200 mg/ml). The plates were subsequently incubated at 37º C for 24 Hrs. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm **(Kumar *et al*, 2009)**.

**Determination of MIC of plant extract by Micro-dilution Method**

The 96-well plates were prepared by dispensing 50 µl of Mueller–Hinton broth, into each well. A 50 µl from the stock solution of tested extracts (concentration of 200 mg/ml) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a micropipette. The obtained concentration range was from 100 to 0.1953 mg/ml. And then added 10 µl of inocula to each well except a positive control (inocula were adjusted to contain approximately $1.5X10^8$ CFU/mL). Plant extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37 ºC for 18 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduce to red colored product by biological active microorganisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth **(Abu-Shanab *et al*, 2004 and Abou Elkhair *et al*, 2010 Radojević *et al*, 2012)**.

**The Synergistic Effec**t: Commercially available antimicrobial disks (Table. 3) were applied on the surface of inoculated MHA by pressing slightly, and then 20µl from the extracts and/or non-antibiotics was carefully and slowly dispensed on the antibiotic disk. The plates were incubated at 37ºC for 24 h. At the end of the period, the inhibition zone formed on the media was measured with a transparent ruler in mm. while combinations of plant extract & Non- antibiotics, 20 µl of Non- antibiotics and 20 µl of plant extracts were mixed and put together on a filter paper disk which was left for one hour to dry and then the inoculated plates were incubated at 37º C for 24 h. The diameters of inhibition zones were measured and compared with that of the plant extracts alone.

 

**Table 3** list of antibiotic potency

| Antibiotics | Antibiotics potency | Manufactured by |
|---|---|---|
| **Vancomycin** | 30 µg | Himedia, Indian |
| **Cefotaxime** | 30 µg | Bioanalyse, Turkey |
| **Ofloxacin** | 5 µg | Himedia, Indian |
| **Ceftriaxone** | 30 µg | Himedia, Indian |
| **Ceftazidime** | 30 µg | Himedia, Indian |
| **Tetracyclines** | 30 µg | Bioanalyse, Turkey |
| **Amikacin** | 30 µg | Bioanalyse, Turkey |
| **Chloramphenicol** | 30 µg | Bioanalyse, Turkey |
| **Gentamicin** | 10 µg | Bioanalyse, Turkey |
| **Ampicillin** | 10 µg | Bioanalyse, Turkey |
| **Erythromycin** | 15 µg | Liofilchem, Italy |
| **Rifampicin** | 30 µg | Liofilchem, Italy |
| **Neomycin** | 30 µg | Himedia, Indian |
| **Co-trimoxazole** | 25 µg | Liofilchem, Italy |
| **Pencillin G** | 10 IU | Liofilchem, Italy |
| **Cefazolin** | 30 µg | Liofilchem, Italy |
| **Ceflexin** | 30 µg | Himedia, Indian |
| **Nalidixic acid** | 30 µg | Liofilchem, Italy |

**Result and Discussion**

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest. The main objective of the present study was to evaluate the ability of the plants extract to inhibit the growth of pathogenic bacteria with and without antibiotics and non-antibiotics drugs and to determine their ability to enhance the activity of antibiotics or non-antibiotics drugs. Antimicrobial activity was recorded when the zone of inhibition is greater than 5 mm.

**Antibacterial Activity of the Plant Extracts**

Most tested plant extracts showed antibacterial activity against *E. coli*, *S. aureus* and *P. aurgenosa* which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth.

In our experiments, *Artemisia herba-alba* (leaves) ( extracted by methanol for 8 h) and *Ficus sycomorus* (bark) (extracted by methanol and also ethanol for 8 h) were showed the highest effect against *E. coli* with a zone of inhibition = 9 mm. While, no antibacterial activity of most plant extracts (extracted by water for 2 h) was found against *E. coli* except with *Artemisia herba-alba* which showed low antimicrobial activity with a zone of inhibition = 6 mm, While against *S. aureus* the *Artemisia herba-alba* extract was showed the highest inhibition zone by Well diffusion method in comparison with another method with a zone of inhibition = 19 and 20mm ( extracted by methanol and ethanol for 8 h, respectively), and *L. camara* was showed the highest effect with a zone of inhibition = 14 and 10mm and also *F. sycomorus* (bark) extract with a zone of inhibition =15mm ( extracted by methanol and ethanol for 8 h, respectively) by disk diffusion method, probably the reason that the paper disc retains the active component and does not allow it to diffuse into the Muller Hinton Agar, because some compounds does not diffuse in the agar especially non polar compounds. As for well diffusion method may be the reason is the proliferation of extract bottom agar away from the growth of bacteria. The best antibacterial activity of methanol and ethanol extracts of *Ficus sycomorus* bark with a zone of inhibition 12 and 11mm, respectively and *Eucalyptus camaldulensis* leaves with a zone of inhibition 11mm and 10mm, respectively were recorded against *P. aeruginosa*.

It was noted that alcoholic extract has greater effect in the inhibition from aqueous extract, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts **(Al-Saimary et al)**. The difference in antibacterial activity of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process **(Okigbo and Mmeka)**.

As for absence of effectiveness to *A. sativum* on *E. coli* and *P. aeruginosa*, even they have a very strong synergistic effect which may probably due to overuse of garlic by human that may lead to increase bacterial resistance to it even it has an effective antibacterial ingradiants. In addition, the therapeutic effect of garlic was very weakly when it was exposed to heat (during drying), which may be explained by the fact that heat is working to break down the enzyme alliinase, thus preventing the conversion of a compound alliin to allicin (active compound) **(Ilić et al.,)**.

 

**MIC of plant extracts**

Microdilution method was used to determine the lowest plant extracts concentration that inhibiting the growth of the bacteria and found effective in the evaluation of MIC.

The MIC value of *E. camaldulensis* was found as the lowest (3.125mg/ml) against *E. coli* and the methanol extracts of *E. camaldulensis* gave the best antibacterial activity against *E. coli*.

The methanol and aquatic extract of *F. sycomorus* (leaves) was significantly active exhibiting the highest potency with MIC from 6.25-3.125 mg/mL against *S. aureu*s. This activity may be attributed to the rich plant contents of active components such as tannins, saponins, alkaloids and flavone aglycones **(Zaku et al)**. The MIC for *A. sativum* extracts against *S .aureus* particularly was found to be significantly active exhibiting the little potency with all solvents used (50 mg/ml), and this confirms of the need for a high concentration of garlic until affect of the bacteria.

The MIC values obtained showed that ethanol extract of *E. camaldulensis* has the most potent effect against *P. aeruginosa***.**

**Synergistic activity of Plants Extracts and Antibiotics**

In our study, the plant extracts had different synergistic ability to inhibit the growth of microorganism depending on the method of extraction. Plants antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (Rakholiya and Chanda, 2012).

It has been known that one of the effective approaches to overcome bacterial resistance is restoration of antibiotic activity through the synergistic action of antibacterial materials from natural and synthesized agents (Stefanovic *et al*., 2011).

Drug synergism between known antibiotics and bioactive plant extracts is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) (Adwan and Mhanna, 2008).

Despite the abundant literature about the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been used for clinical use as antibiotics (Adwan and Mhanna, 2008).

**Against *Escherichia coli***

The protein synthesis inhibitors such as (Amikacin and Chloramphenicol) were showed the strongest synergistic effect with most of methanol plant extracts. The better synergistic effect was found with *Artemisia herba-alba* and *Allium sativum*. Only, amikacin was showed synergistic effect with all methanol plant extracts. Whereas folic acid, bacterial cell wall synthesis and nucleic acid synthesis inhibitors (such as Co-trimoxazole, Cefotaxime and Nalidixic acid, respectively) were showed weak synergism with methanol extracts. The ethanolic extract of *Nerium oleander* and *Artemisia herba-alba* were showed synergistic effect with all tested antibiotics except Ceftazidime that showed antagonistic effect with all ethanolic plant extracts and also protein synthesis inhibitors were showed stronger synergistic effect with most ethanol plant extracts compared with the rest of the antibiotics used. For the aqueous extract, a combination between most plant extracts and the antibiotics protein synthesis inhibitors showed synergistic activity against *E. coli* better than other antibiotics that works as inhibitors of cell wall synthesis (such as Cefazolin, Cefotaxime and Ampicillin). However,folic acid and nucleic acid synthesis inhibitors of antibiotics have a weak or no synergistically activity against *E. coli* (Table 4, 5 and 6).

 

**Table 4. Antibacterial and synergistic effect of plant extracts, extracted by methanol for 8h. on *E. coli* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| VA | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| CTX | 8 | | - | | 8 | | 10 | | 9 | | 12 | | 14 | | 8 | | 10 |
| OF | 0 | | - | | 8 | | - | | - | | 20 | | 18 | | 9 | | 7 |
| CTR | 9 | | - | | 13 | | 16 | | 14 | | - | | - | | 9 | | 9 |
| CTZ | 11 | | - | | - | | 8 | | 7 | | 7 | | - | | 7 | | 7 |
| TE | 0 | | 7 | | 9 | | - | | 8 | | - | | - | | 8 | | 9 |
| AK | 10 | | 19 | | 18 | | 18 | | 18 | | 20 | | 20 | | 20 | | 19 |
| CL | 24 | | 26 | | 25 | | 23 | | 26 | | 28 | | 28 | | 26 | | 28 |
| ER | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| GN | 7 | | - | **9** | 9 | **7** | 9 | **0** | 9 | **0** | - | **9** | - | **0** | 13 | **7** | 7 |
| AMP | 0 | | 7 | | 9 | | - | | - | | - | | - | | 7 | | 8 |
| RF | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| N | 14 | **7** | 17 | | 15 | | 19 | | 17 | | 15 | | 15 | | 18 | | 13 |
| SXT | 0 | | - | | - | | - | | 7 | | - | | - | | 7 | | 8 |
| P. G | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| KZ | 0 | | - | | - | | - | | - | | - | | - | | 7 | | - |
| CN | 7 | | 7 | | 7 | | - | | - | | - | | - | | - | | - |
| N.A | 0 | | - | | 7 | | - | | 7 | | - | | - | | 7 | | 9 |

* extracted assay by Disc diffusion method.
** Have not been tested.
- No synergism

**Table 5. Antibacterial and synergistic effect of plant extracts, extracted by ethanol for 8h. on *E. coli* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| VA | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| CTX | 8 | | 10 | | 9 | | 8 | | - | | 15 | | 13 | | 10 | | 10 |
| OF | 0 | | 7 | | 8 | | - | | - | | 20 | | 18 | | 7 | | 8 |
| CTR | 9 | | 14 | | 16 | | 17 | | 14 | | 15 | | - | | 7 | | 7 |
| CTZ | 11 | | 10 | | 9 | | 9 | | - | | 9 | | 7 | | 7 | | 7 |
| TE | 0 | | 6 | | 7 | | 7 | | 8 | | - | | - | | 8 | | 9 |
| AK | 10 | | 19 | | 17 | | 19 | | 18 | | 19 | | 20 | | 20 | | 21 |
| CL | 24 | | 26 | | 25 | | 26 | | 23 | | 29 | | 29 | | 26 | | 28 |
| ER | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| GN | 7 | | 8 | | 8 | | 8 | | 9 | | - | | - | | 12 | | 9 |
| AMP | 0 | **7** | 8 | **7** | 9 | **8** | 8 | **0** | - | **8** | - | **8** | - | **0** | 8 | **8** | 9 |
| RF | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| N | 14 | | 16 | | 15 | | 19 | | 19 | | 14 | | 13 | | 18 | | 14 |
| SXT | 0 | | 8 | | 7 | | 8 | | 8 | | - | | - | | - | | 8 |
| P. G | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| KZ | 0 | | 7 | | 7 | | 7 | | - | | - | | - | | - | | - |
| CN | 7 | | 8 | | 10 | | - | | - | | 7 | | - | | - | | - |
| N.A | 0 | | 7 | | 9 | | 8 | | 9 | | - | | - | | 7 | | 7 |

* extracted assay by Disc diffusion method.
** Have not been tested.
- No synergism

 

**Table 6. Antibacterial and synergistic effect of plant extracts, extracted by water reflux for 2h. on *E. coli* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| VA | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| CTX | 8 | | 9 | | - | | 9 | | 10 | | 12 | | 14 | | 9 | | - |
| OF | 0 | | - | | - | | - | | - | | 19 | | 17 | | - | | - |
| CTR | 9 | | 13 | | 13 | | 15 | | 14 | | 16 | | - | | - | | - |
| CTZ | 11 | | - | | 7 | | 10 | | 7 | | - | | 7 | | - | | - |
| TE | 0 | | - | | 8 | | - | | - | | - | | - | | - | | - |
| AK | 10 | | 17 | | 17 | | 17 | | 15 | | 18 | | 18 | | 18 | | 17 |
| CL | 24 | | 28 | | 25 | | 25 | | 22 | | 24 | | 26 | | 24 | | - |
| ER | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| GN | 7 | | - | | - | | - | | - | | - | | - | | 10 | | - |
| AMP | 0 | | 7 | **6** | 9 | | - | | - | | - | | - | | 8 | | - |
| RF | ** | | ** | | ** | | ** | **0** | ** | **0** | ** | **0** | ** | **0** | ** | **0** | ** |
| N | 14 | **0** | 15 | | 17 | **0** | 17 | | 19 | | 16 | | 15 | | 17 | | 13 |
| SXT | 0 | | - | | 8 | | - | | 7 | | - | | - | | - | | - |
| P. G | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** | | ** |
| KZ | 0 | | - | | - | | - | | - | | - | | - | | - | | - |
| CN | 7 | | 7 | | - | | - | | - | | - | | - | | - | | - |
| N.A | 0 | | - | | 7 | | - | | - | | - | | - | | - | | - |

* extracted assay by Disc diffusion method.
** Have not been tested.
- No synergism

**Some of synergistic effects between Antibacterial drugs and plant extracts on *E. coli***

| Antibiotic * /plant extract | Inhibition zone (mm) | | |
|---|---|---|---|
| | Methanol extract | Ethanol extract | Water extract |
| ***F. sycomorus* (leaves)** | **0** | **8** | **0** |
| ***F. sycomorus* (bark)** | **9** | **8** | **0** |
| ***A. sativum*** | **0** | **0** | **0** |
| *** Amikacin (10mm)** | | | |
| **AK+ *F. sycomorus* (leaves)** | **19** | **19** | **18** |
| **AK+ *F. sycomorus* (bark)** | **18** | **18** | **17** |
| **AK+ *A. sativum*** | **20** | **20** | **18** |
| *** Ofloxacin (0mm)** | | | |
| **OF+ *F. sycomorus* (leaves)** | **20** | **20** | **19** |
| **OF+ *F. sycomorus* (bark)** | **18** | **18** | **17** |
| **OF+ *A. sativum*** | **0** | **0** | **0** |
| *** Co-trimoxazole (0mm)** | | | |
| **STX+ *F. sycomorus* (leaves)** | **0** | **0** | **0** |
| **STX+ *F. sycomorus* (bark)** | **0** | **0** | **0** |
| **STX+ *A. sativum*** | **7** | **8** | **0** |
| *** Cephalexin (7mm)** | | | |
| **CN + *F. sycomorus* (leaves)** | **0** | **7** | **0** |
| **CN + *F. sycomorus* (bark)** | **0** | **0** | **0** |
| **CN + *A. sativum*** | **0** | **0** | **0** |

**Against *Staphylococcus aureus***

The protein synthesis inhibitors were showed synergistic effect with most plant extracts better than cell wall synthesis inhibitors. The strongest synergistic effect was with methanolic extract of *Artemisia herba-alba* and ethanolic extracts of *Ficus sycomorus* (leaves) and *Allium sativum* with Tetracycline.

 

Ofloxacin which exhibit nucleic acid synthesis inhibitor showed stronger synergistic effect with *Allium sativum*. Whereas folic acid synthesis inhibitors (Co-trimoxazole) showed stronger synergistic activity with methanolic and ethanolic extracts of *Ficus sycomorus* (Bark) (Table 7, 8 and 9).

**Table 7. Antibacterial and synergistic effect of plant extracts, extracted by methanol for 8h. on *S. aureus* (all value in mm).**

| **Anti.** | **Anti. alone** | ***N. oleander* (leaves)** | | ***A. herba-alba* (leaves)** | | ***W. somnifera* (leaves)** | | ***L. camara* (leaves)** | | ***F. sycomorus* (leaves)** | | ***F. sycomorus* (bark)** | | ***A. sativum* (bulbs)** | | ***E. camaldulensis* (leaves)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| VA | 15 | | 17 | | 16 | | 15 | | 16 | | 16 | | 17 | | 18 | | 21 |
| CTX | 11 | | 14 | | 16 | | 15 | | 16 | | 17 | | 18 | | 18 | | 22 |
| OF | 20 | | 20 | | 23 | | 24 | | 25 | | 28 | | 22 | | 29 | | 26 |
| CTR | 12 | | 8 | | 9 | | 16 | | 14 | | 15 | | 16 | | 11 | | 15 |
| CTZ | 0 | | 0 | | 8 | | 10 | | 9 | | 13 | | 14 | | 7 | | 14 |
| TE | 21 | | 27 | | 30 | | 22 | | 24 | | 27 | | 25 | | 27 | | 26 |
| AK | 20 | | 24 | | 24 | | 24 | | 22 | | 22 | | 24 | | 25 | | 26 |
| CL | 21 | | 22 | | 22 | | 21 | | 24 | | 22 | | 22 | | 21 | | 25 |
| ER | 17 | | 20 | | 18 | | 21 | | 22 | | 16 | | 17 | | 21 | | 19 |
| GN | 21 | **7** | 24 | **8** | 23 | **7** | 22 | **14** | 23 | **11** | 24 | **15** | 28 | **7** | 22 | **11** | 19 |
| AMP | 0 | | 11 | | 12 | | 10 | | 8 | | 12 | | 14 | | 11 | | 13 |
| RF | 19 | | 21 | | 22 | | 20 | | 19 | | 18 | | 20 | | 21 | | 23 |
| N | 20 | | 23 | | 21 | | 23 | | 23 | | 18 | | 20 | | 18 | | 20 |
| SXT | 10 | | 9 | | 10 | | 11 | | 10 | | 14 | | 18 | | 10 | | 14 |
| P. G | 0 | | 9 | | 10 | | 11 | | 8 | | 0 | | 17 | | 10 | | 17 |
| KZ | 0 | | 11 | | 12 | | 12 | | 10 | | 14 | | 16 | | 10 | | 16 |
| CN | 10 | | 15 | | 17 | | 15 | | 14 | | 22 | | 27 | | 15 | | 26 |

**Table 8. Antibacterial and synergistic effect of plant extracts, extracted by ethanol for 8h. on *S. aureus* (all value in mm).**

| **Anti.** | **Anti. alone** | ***N. oleander* (leaves)** | | ***A. herba-alba* (leaves)** | | ***W. somnifera* (leaves)** | | ***L. camara* (leaves)** | | ***F. sycomorus* (leaves)** | | ***F. sycomorus* (bark)** | | ***A. sativum* (bulbs)** | | ***E. camaldulensis* (leaves)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| VA | 15 | | 15 | | 17 | 8 | 15 | 10 | 14 | 12 | 18 | 15 | 15 | 7 | 18 | 13 | 19 |
| CTX | 11 | | 15 | | 12 | | 15 | | 15 | | 15 | | 20 | | 18 | | 17 |
| OF | 20 | | 23 | | 21 | | 22 | | 24 | | 25 | | 19 | | 29 | | 24 |
| CTR | 12 | | 9 | | 11 | | 15 | | 17 | | 13 | | 19 | | 8 | | 16 |
| CTZ | 0 | | 7 | | 9 | | 11 | | 12 | | 14 | | 16 | | 7 | | 13 |
| TE | 21 | | 27 | | 27 | | 25 | | 25 | | 29 | | 25 | | 30 | | 24 |
| AK | 20 | | 25 | | 24 | | 22 | | 24 | | 24 | | 24 | | 27 | | 24 |
| CL | 21 | | 24 | | 23 | | 23 | | 21 | | 24 | | 21 | | 23 | | 23 |
| ER | 17 | | 18 | | 18 | | 23 | | 22 | | 17 | | 15 | | 23 | | 21 |
| GN | 21 | | 22 | | 23 | | 24 | | 22 | | 28 | | 25 | | 24 | | 21 |
| AMP | 0 | **6** | 9 | **9** | 11 | | 11 | | 11 | | 12 | | 16 | | 9 | | 14 |
| RF | 19 | | 20 | | 19 | | 22 | | 21 | | 21 | | 18 | | 24 | | 23 |
| N | 20 | | 22 | | 23 | | 21 | | 22 | | 19 | | 20 | | 21 | | 20 |
| SXT | 10 | | 9 | | 11 | | 13 | | 10 | | 12 | | 17 | | 12 | | 13 |
| P. G | 0 | | 9 | | 11 | | 13 | | 11 | | 11 | | 13 | | 10 | | 15 |
| KZ | 0 | | 8 | | 10 | | 12 | | 8 | | 15 | | 17 | | 9 | | 13 |
| CN | 10 | | 12 | | 22 | | 25 | | 15 | | 23 | | 24 | | 12 | | 26 |

 

**Table 9. Antibacterial and synergistic effect of plant extracts, extracted by water reflux for 2h. on *S. aureus* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| VA | 15 | 0 | 13 | 0 | 13` | 0 | 10 | 8 | 11 | 15 | 15 | 12 | 14 | 0 | 16 | 8 | 16 |
| CTX | 11 | | 12 | | 15 | | 14 | | 13 | | 15 | | 19 | | 15 | | 10 |
| OF | 20 | | 22 | | 21 | | 23 | | 20 | | 25 | | 20 | | 28 | | 20 |
| CTR | 12 | | 8 | | 8 | | 12 | | 13 | | 11 | | 14 | | 7 | | 13 |
| CTZ | 0 | | 0 | | 0 | | 7 | | 7 | | 13 | | 14 | | 0 | | 13 |
| TE | 21 | | 26 | | 26 | | 24 | | 23 | | 26 | | 24 | | 27 | | 12 |
| AK | 20 | | 25 | | 25 | | 23 | | 21 | | 24 | | 26 | | 25 | | 24 |
| CL | 21 | | 22 | | 21 | | 21 | | 21 | | 22 | | 21 | | 24 | | 21 |
| ER | 17 | | 17 | | 18 | | 21 | | 21 | | 17 | | 18 | | 20 | | 20 |
| GN | 21 | | 22 | | 24 | | 19 | | 19 | | 20 | | 24 | | 24 | | 15 |
| AMP | 0 | | 8 | | 8 | | 7 | | 0 | | 10 | | 7 | | 0 | | 11 |
| RF | 19 | | 20 | | 18 | | 20 | | 20 | | 19 | | 19 | | 21 | | 21 |
| N | 20 | | 22 | | 22 | | 20 | | 19 | | 18 | | 16 | | 18 | | 19 |
| SXT | 10 | | 10 | | 8 | | 0 | | 7 | | 16 | | 9 | | 16 | | 11 |
| P. G | 0 | | 7 | | 0 | | 7 | | 0 | | 13 | | 0 | | 9 | | 0 |
| KZ | 0 | | 0 | | 8 | | 8 | | 0 | | 10 | | 0 | | 0 | | 7 |
| CN | 10 | | 9 | | 11 | | 12 | | 10 | | 15 | | 24 | | 12 | | 24 |

**Against *Pseudomonas aeruginosa***

Protein synthesis inhibitors (such as Amikacin and Gentamicin) were showed strong synergistic effect with most plant extract using methanol, ethanol and water as a solvent ,followed by nucleic acid synthesis inhibitors such as Ofloxacin.

Cell wall synthesis inhibitors such as Ceftriaxone showed weak or no synergistic activity against *P. aeruginosa*, except Ceftazidime which showed significant synergistic activity (Table 10, 11and 12).

**Table 10. Antibacterial and synergistic effect of plant extracts, extracted by methanol for 8h. on *P. aeruginosa* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| CTX | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 13 |
| OF | 0 | | 11 | | 10 | | 10 | | 10 | | 0 | | 0 | | 11 | | 12 |
| CTR | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 |
| CTZ | 9 | | 11 | | 13 | | 10 | | 12 | | 14 | | 13 | | 10 | | 12 |
| AK | 17 | | 26 | | 25 | | 25 | | 23 | | 20 | | 20 | | 22 | | 22 |
| GN | 8 | | 10 | | 12 | | 13 | | 10 | | 11 | | 0 | | 0 | | 15 |
| N | 0 | | 8 | | 8 | | 20 | | 20 | | 10 | | 0 | | 11 | | 10 |
| CN | 0 | | 9 | | 10 | | 0 | | 0 | | 0 | | 0 | | 0 | | 8 |

 

**Table 11. Antibacterial and synergistic effect of plant extracts, extracted by ethanol for 8h. on *P. aeruginosa* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| CTX | 0 | 0 | 0 | 8 | 8 | 0 | 7 | 0 | 0 | 7 | 0 | 10 | 0 | 0 | 0 | 8 | 12 |
| OF | 0 | | 9 | | 13 | | 11 | | 15 | | 0 | | 0 | | 12 | | 10 |
| CTR | 0 | | 7 | | 7 | | 0 | | 0 | | 7 | | 0 | | 0 | | 8 |
| CTZ | 9 | | 13 | | 13 | | 14 | | 13 | | 14 | | 14 | | 13 | | 13 |
| AK | 17 | | 25 | | 25 | | 27 | | 21 | | 18 | | 20 | | 21 | | 23 |
| GN | 8 | | 11 | | 10 | | 10 | | 11 | | 0 | | 0 | | 9 | | 9 |
| N | 0 | | 9 | | 9 | | 19 | | 22 | | 8 | | 0 | | 10 | | 10 |
| CN | 0 | | 8 | | 13 | | 0 | | 12 | | 0 | | 0 | | 0 | | 10 |

**Table 12. Antibacterial and synergistic effect of plant extracts, extracted by water reflux for 2h. on *P. aeruginosa* (all value in mm).**

| Anti. | Anti. alone | *N. oleander* (leaves) | | *A. herba-alba* (leaves) | | *W. somnifera* (leaves) | | *L. camara* (leaves) | | *F. sycomorus* (leaves) | | *F. sycomorus* (bark) | | *A. sativum* (bulbs) | | *E. camaldulensis* (leaves) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ex. alone* | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti | Ex. alone | Ex.+ Anti |
| CTX | 0 | 0 | 0 | 7 | 0 | 0 | 70 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 8 | 10 |
| OF | 0 | | 0 | | 0 | | 11 | | 9 | | 0 | | 9 | | 13 | | 12 |
| CTR | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 |
| CTZ | 9 | | 10 | | 11 | | 11 | | 8 | | 13 | | 14 | | 13 | | 11 |
| AK | 17 | | 23 | | 24 | | 19 | | 22 | | 20 | | 19 | | 20 | | 20 |
| GN | 8 | | 10 | | 9 | | 8 | | 9 | | 0 | | 7 | | 7 | | 0 |
| N | 0 | | 0 | | 0 | | 15 | | 16 | | 0 | | 8 | | 7 | | 8 |
| CN | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 7 |

**Conclusion**

On the basis of the antibacterial assay of this study *S. aureus* was found the more (susceptible to the employed plant extracts) than *E. coli* and *P. aeruginosa*.

All plant extracts were evaluted for their MIC against *E. coli*, *S.aureus* and *P. areuginosa*, The MIC value for each of methanolic extract of *E. camaldulensis* against *E. coli* was 3.125 mg/ml. And the methanol and aquatic extract of *F. sycomorus* (leaves) against *S.aureus* was from 6.25-3.125 mg/ml. And the ethanol extract of *E. camaldulensis* against *P. areuginosa* was 6.25 mg/ml . Suggesting that very small amount of the extracts are required to inhibit the growth of the bacteria thus *E. camaldulensis* (methanol extract), leaf extract of *F. sycomorus* (methanol and aquatic extract) and *E. camaldulensis* (ethanol extract) had very potent activity against *E. coli*, *S.aureus* and *P. areuginosa*, Respectively.

Ethanolic plant extracts were showed antimicrobial and synergistic activity with antibiotics better than methanolic and aquatic extracts.

The strongest effect agaist *E. coli* was recorded when *F. sycomorus* (leaves and bark) were mixed with Ofloxacin. And the strongest effect on *S. aureus* was observed when *A. sativum* was combined with Ofloxacin and Tetracyclin. The strongest effect against *P. areuginosa* was observed when Ceftazidime was combined with most plant extracts, especially with *F. sycomorus* (leaves and bark); when the extracts of *N. oleander*, *A. herba-alba* and *W. somnifera* were combined with Amikacin and also when the extract of *W. somnifera* and *L. camara* were mixed with Neomycin.

The results of this research work have revealed the importance of plant extracts when associated with antibiotics to bacteria control, which enables the use of a mixture of antibiotics and plant extracts against bacterial infections, when it is no longer effective by itself against bacterial infections during therapeutic treatment.

 

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