RESEARCH ARTICLE

Bacterial Inhibition by Nanoparticles Treated Eucalyptus Extract

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ABSTRACT

This study aimed to test the efficiency of eucalyptus extracts (aqueous and alcoholic) supplemented with zinc nanoparticles in inhibiting gram-negative and gram-positive bacteria isolated in patients and their identification. The results showed the identification of two gram-negative bacteria: *Salmonella* and *Entamoeba coli*.

Three types of gram-positive bacteria are *Staphylococcus aureus*, *Staphylococcus albus*, *Staphylococcus epidermidis*

The effect of the aqueous extract of eucalyptus on the aforementioned species was tested, as it proved highly effective in influencing the growth of gram-positive bacteria than it is with gram-negative bacteria. While the effect of the alcoholic extract on the growth of negative bacteria was greater than its effect on the gram-positive bacteria and it proved highly effective on *E. coli* compared to *Salmonella*.

The results of the synergistic action of the above-mentioned extracts when mixed with nano-zinc, showed a significant increase in the inhibition of the growth of bacterial species. Whereas the turbulent effect of the alcoholic extract with nano-zinc showed a significant effect on the growth of dye-negative bacteria compared to the effect of the aqueous extract supplemented with nano-zinc, which gave the highest effect in inhibiting the growth of gram-positive bacteria.

Keywords: Bacteria, Eucalyptus extracts, Nano-zinc, Synergistic action.

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INTRODUCTION

Medicinal plants occupy an important place in traditional medicine and herbal treatment in various countries of the world. Total of 80% of the world's population still depends on them to provide ease of access, low costs, promising effectiveness, and to avoid the negative effects resulting from the use of chemical drugs. Most medicinal plants are non-toxic, but some are highly toxic to humans and animals. Scientists estimated the number of medicinal plants present on the surface of the earth at about 250,000–500,000 species of medicinal plants, and a small percentage of these plants are used as food for humans and animals, and a very small part are used as medical treatment. The active substances used in traditional medical treatment are obtained from whole plants or from their parts such as roots, leaves, bark or seeds. The extraction of biologically active compounds depends on the extraction solvent used and the temperature of extraction or mixing, with the presence of three techniques that are considered classic: saxolites, soaking and hydro-distillation.[1] Eucalyptus is an evergreen tree native to Australia, used to treat coughs, congestion, colds and relieve joint and vertebrae pain. Eucalyptus leaves are an important source of antioxidants that protect the body from free radical damage that causes cancer, dementia and heart disease. A large study of 38,180 men and 60,289 women found that a diet high in flavonoids reduced the risk of heart disease by 18%. Boiled leaves of this plant are a source of flavonoids that are safe for adults, but they can be toxic to children, so a healthcare professional should approve before giving them to children. Although it is not possible to eat it fresh, eucalyptus leaves can be boiled and drunk as tea in balanced quantities, as its excessive use may cause poisoning. It is used as a natural remedy for cough and cold cases. Research has shown that it expands the bronchi and airways and reduces phlegm secretion.It contains eucalyptol (also known as cineol), which relieves cold and cough symptoms, such as nasal congestion and headaches. It also relieves asthma symptoms. Treats dry skin.[2] Research has shown the effectiveness and benefits of eucalyptus oil in fending off mosquitoes and other insects for up to eight hours after topical application. It also poorly treats head lice. The United States is officially registered eucalyptus oil as an insecticide to kill mites and ticks in 1948. A body of research points to the pain-relieving properties

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of eucalyptus oil. One of these studies was published in the American Journal of Natural Medicine. The study revolves around the application of eucalyptus oil to the skin of ten people, which contributed to the treatment of muscle and joint pain associated with sprains, arthritis and back pain. The use of eucalyptus oil stimulates the response of the immune system. The researchers found that it boosts the immune system's response to pathogens in one of their experiments on mice. Eucalyptus also helps reduce damage to stuffy noses, wounds and sores, bladder diseases, diabetes and fever. Eucalyptus can be used in all its available forms. Eucalyptus tea bags can be boiled and drunk as regular tea. A few drops of its oil can be added to the steaming bowl. The whole leaf can be used in baths to increase the feeling of freshness and relieve stress. It is also used in topical products, such as oils, mouthwash, chewing gum, etc.[3] The use of this plant may cause a range of side effects. Some people consume eucalyptus in small amounts as a seasoning for certain foods. But it may cause side effects if you try to take more than a teaspoon of its pure oil. It causes nausea, vomiting and diarrhea. The condition may also progress to poisoning, which causes stomach pain, muscle weakness, and suffocation accompanied by coughing and coma, which may sometimes lead to death. It's probably not safe to apply eucalyptus oil directly to the skin. It can cause nervous system problems, also there is not enough documented information to be sure of its safety in this case.[3]

Nanoparticles

Nanoparticles are unique in that they can be produced with high surface area and unusual crystalline structures and sizes. The main advantages of using nanoparticles is their stability or long shelf life with antimicrobial agents. The range of nanoparticles was ZnO is an important field for biologists because of the distinctive antimicrobial properties possessed by nanoparticles and its outstanding activity, which opened new horizons for a biology of science, especially in its nano-form. At the al-Hussaini Holy Shrine using zinc oxide nanoparticles.[4]

Nano Zinc Oxide

Nanoparticles are characterized by being active and effective with biological systems due to their varying shapes, large surface area, highly charged surfaces, and high surface adsorption capacity. The inhibitory activity of zinc oxide against pathogens may come through its formation of free radicals ROS on the surface, leading to damage in the lipids of the cell membrane of microbes through the interaction of free radicals with the lipids, and then leads to the breakdown of the cell membrane of the microbial cells.[5]

Nano zinc is used in many fields, such as food, cosmetics, pharmaceuticals and biological applications. It is an antimicrobial agent, promotes growth, regulates immunity and indirectly prevents environmental pollution. Due to zinc nanoparticles' unique chemical, electrical and optical properties, they have achieved special interest in many fields, especially medicine. Zinc nanoparticles exhibit potent activity against a wide range of pathogenic fungi.[6] Scanning electron microscopy is used to analyze the phenotypic variations of microbes induced by nanoparticles in order to determine the different mechanisms, although the activity of antimicrobial nanoparticles induced by nanoparticles in order to determine the different mechanisms, although the activity of antimicrobial nanoparticles is still unclear and is still controversial because Antimicrobial efficacy requires a deep explanation of the mechanisms that use nanoparticles to eliminate microbes, but there are distinct mechanisms of nanoparticles that can be explained in the following form:

- Releasing antimicrobial ions, especially dual-charged zinc ions.
- Direct contact of zinc nanoparticles with the microbial cell wall leads to the destruction of this wall.
- Formation of reactive oxygen species or so-called free radicals.

In the field of biology, nano-zinc oxide has received great attention due to its antimicrobial activity, which opened new horizons for biological sciences. He explained that zinc oxide has a significant and clear effect as an antimicrobial, and therefore it has been exploited in industrial fields, including water treatment, dyes, and cosmetics. It was also used in parchment paste to protect against fungi.[7]

Various mechanisms have been proposed to discuss the effect of nanoparticles on fungal growth. The first is the formation of H_2O_2 on the surface of ZnO NP due to the possibility of heaping a hydrogen bond between the hydroxyl group of cellulose molecules with the oxygen atom of ZnO NP, which leads to the inhibition of the growth of fungi, and the second mechanism is the release of ZN + 2 ions that cause damage to the cell membrane and interact with the content inside. Zinc nanoparticles generate free radicals such as hydroxyl and single oxygen radicals that induce cell death. Zinc nanoparticles affect cells when they touch the outer part of the plasma membrane and interact with it, and this interaction changes the structure of the membrane and changes its permeability and leads to the breakdown of the plasma membrane and interacts with it. This interaction changes the membrane's structure and permeability, leading to a breakdown in the plasma membrane and accumulation in the cytoplasm. There is interference with the basic processes of cell growth, which leads to the inhibition of its growth.

One of his studies noted that the dry weight of the biomass of mushrooms *Aspergillus flavus* and *Aspergillus fumigatus* were very small when the fungus was treated with nano-zinc at a concentration of part per million (ppm) 100 with a size of 25–20 nm (Nano meter). The living cell generates oxidative stress towards glycine-gystine-glutamyl, which is responsible for inhibiting fungi and bacteria. Ahmad and his group (2020) that at a concentration of 100 ppm of nano-zinc led to an inhibition in the growth rate of fungi, where the rate of inhibition reached 76.6% for *Alternaria mali*, 65.4% for mushroom *Botryosphaeria dothidea*, and 55.2 for *Diplodia seriata*. It was observed by microscopic examination of the cells treated with nano-zinc that it breaks down in the wall

layers of the fungal cell, which reduces the number of fungal hyphae. Fungal and ensuring protection for fruit crops. The effect of antifungal nanoparticles is also related to its size and concentration in the medium, whereby the smaller the size, the higher the inhibition rate. A study conducted by Mehdi and his group (2018) showed that zinc oxide nanoparticles affected the fungus cell through the assembly of the protoplasmic substance, the separation of the plasma membrane from the cell wall, and the occurrence of anti-fungal nanoparticles. Deviation in the flow of nutrients and shrinkage and destruction of the fungal cell.[8]

One of the main advantages of using nanoparticles, including nanoparticles of zinc oxide, is the stability or long life of the organic antimicrobial agents.

MATERIALS AND WORKING METHODS

Isolation

The bacteria are isolated from patients. To keep bacterial colonies, we prepare nutrient agar. Before preparing the medium, read the amount of the dissolved substance in 1-L of water. Here we used 289 g per 1L of distilled water according to the information in the box. In this process we need 250 mL and to find out the amount of agar we use the formula

1000 : 28

250X = 7 g per 250 mL of distilled water.

Initially we weight the material amount in the flask material amount in the scale and put it flask then we weight the amount of the material in the inserted cylinder according to the required qunatity and put it on top of the material in flask and then put the cotton on the nozzle of the flask to close it and then move it over a morning of five burner we continue to move it until the material is completely thawed or put it in the mobile water bath device water bath The sterilize the agar cultural medium in the autoclave by placing distilled water in the device for an hour or half an hour at 121°C and keeping for 15 minutes after reaching the required temperature after the time expires we remove the flask form the device and cool to s temperature of 50–45°C then we start the process of pouring in the slant and put it in italicsto cool then put it in the incubator for 24 hours then we remove it form the incubator so if there is growth then it is contaminated and if there is no growth then We put it in the refrigerator unit the start of the transplant process.[9]

Diagnosis of Bacterial Species

In this step, we need two agricultural media blood agar types to prepare it. We read the can and how much melt is dissolved in one liter. The distilled water is 39.5 g work step: The amount of agar used to prepare 250 mL we extract the amount of agar form this equation: Total agar amount➞one liter of water The amount of agar required➞the amount of water required.

1000 **:** 39,5

250 X=9,875g per 250 mL[1]

We weigh the amount of lime in the scale and put it in a flask.[2] We measure the amount of distilled water using the required cylinder and put it above the amount of agar in the flask.[3] We put the cotton on the flask to close it move it over the burner lamp, keep moving unit the thaws have completely dissolved or put it in the water bath.[9]

Next, sterilize the culture medium in autoclave by placing distilled water in the device for a 1-hour with a temperature of 121°C and keep 15 minutes after the desired temperature has carried after time has passed. Remove viral form of the device and cool it unit reaches a temperature of 50–45°C. Then added 40 mL of blood in petri dish. A total of 250 mL of water then added 10 mL of blood and sterilized the syringe before use for fear of snow. The cotton was removed from the flask's nozzle and sterilized and blood was added to it in flask and under the lamp was constantly stirred. Put it in petri dish and leave until it hardens at room temperature after the petri dish is hardened. All the air bubbles were removed using a fire lamp and were kept in the incubator for 24 hours.[9]

McConkey agar was prepared using the agar dissolved in 1-L of distilled water. The amount of McConkey agar dissolved in 1-L was 51.5.

The amount needed to prepare 250 mL in water is was extracted from the following equation:

1000➞151.5 X 250 =12.875 g of distilled water 250 mL

The amount of McConkey was weighed in agar and was left to set in the flask. The amount of distilled water was measured using the interred cylinder according to the required quantity and poured over McConkey solution in the flask.

The bacterial species was transplanted in both mediums and left for 24 hours in the incubator for diagnosis. After the period ends, we make a swab on the slide and examine the spices in the microscope using the oil. The pink bacterial means that it is negative and violet means positive bacteria.[7]

Prepare Moller Hinton Agar

The guidelines were mentioned on the box containing substance and were added accordingly. The amount of muller used was 38 g and was dissolved in 1-L of distilled water to prepare a 1-L solution. The following steps were followed to prepare 500 mL of solution as required:

- We measure the amount of (Moller hinton agar and add it to the flask.
- Measure the amount of distilled water required (500 mL) graduated cylinder and add it to The Substance in the flask.
- we put cotton on the nozzle of the flask to Close it, Then We moved it over The burner and Keep Stirring untit the all amount of agar melts or use The water bath To dissolve the substance-than we put the flask to the autoclave in (12C) (15P) for 1:30 hours to sterilization. After sterilization We take out the flask and wait for it cool down after that We Put in number of petri dish and incubated it in incubator for 24 hours then we put them in the fridge for later use.[6]

Prepare of Eucalyptus Extract

We got a fresh eucalyptus leaves and dried at room temperature. Then it was grinded and turned into powder. A total of 30 gm eucalyptus in 100 mL ethanol and leave at room temperature 100 gm eucalptos in 500 mL DW then put it on a hot plate for boiling for 30 minutes then leave at room temperature. After

the preparation is complate, we filtered them. The next step was to spread the bacteria on the culture media and distribute the tablets in it, We have numbered cohol, DW, control. The process was as follows: swap: we put a drop of sterile water into the culture media, take a swap from the colony bacteria, mix it with steril water, and brush it on the medium by making zikzak. then we distribute the numbered descs to the media with writing of the dite and number of bacteria on the petridish.[4]

Diluted Eucalyptus

Eucalyptus is prepared with diluted concentrations through: 1-Take a touch of the bacterial colony and mix it with 1-mL of distilled water and take 5 tubes containing 0.9 mL of distilled water and take 0.1 mL of eucalyptus extract and mix it in the first tube. Then take 0.1 mL of the mixture and mix it with distilled water in the second tube and take the same amount Mix it with 3, 4 and 5 tubes and take the same amount from tube 5. Take a touch of the bacterial colony and mix it with 1-mL of ethanol alcohol and take 5 tubes containing 0.9 mL of alcohol and take 0.1 mL of eucalyptus extract and mix it in the first tube and take 0.1 mL of the mixture and mix it with alcohol in the second tube and take the same amount Mix it with 3, 4 and 5 tubes and take the same amount from tube 5.

After diluting the alcoholic and aqueous extracts to different concentrations, we planted different types of isolates used on the Muller Hinton medium and then the paper tablets were soaked in these diluted concentrations and then they were fixed to the culture media on which the bacteria were planted and then we left them for minutes to stick to the medium and then put them upside down in the incubator With a temperature of 37°Cand for 24 hours.[5]

Table 1: Diagnosis of bacteria isolates depending on gram stain.

Type of bacteria	*No.of isolates*	*Results of gram stain*
E. coli	4	Gram-negative
S. aureus	2	Gram-positive
S. albus	5	Gram-positive
Sallmonella	1	Gram-positive
S.epidermidis	3	Gram-positive
Sallmonella	2	Gram-negative

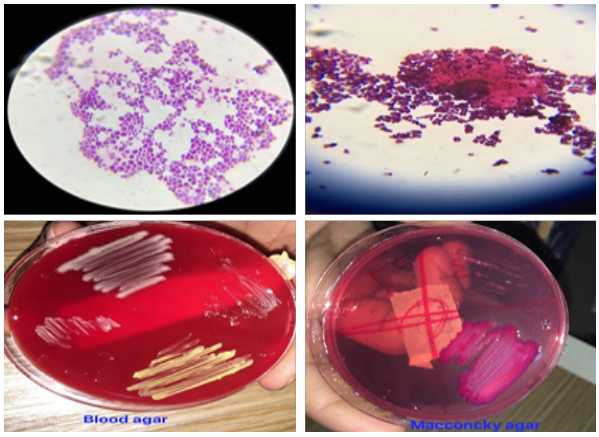

Figure 1: Growth type of bacteria.

RESULTS AND DISCUSSION

Results of Growth

Twelve bacterial isolates were collected from patients in the hospital and cultivated in nutritional media, all of which were positive for growth . depending on the visual examination, these types of bacteria were diagnosed and known as (4) *E. coli,* (5) *S. albus.,* (2) *S. aureus,* (1) *Salmonella* and *S. epidermidis.*

Results of Stain

After knowing the types of bacteria, we had to know their diagnosis in terms of gram stain, some isolates were stained with gram stain, while others were cultured on two mediums are blood agar and McConkey agar, so the total positive samples for the gram stain were eight isolates and four negative isolates for the gram stain as shown in Table 1 and Figure 1.

Results of Inhibition

After preparing the alcoholic and aqueous plant extract, alcoholic at a concentration of 30 gm/100 mL and aqueous at a concentration of 100 gm/500 mL, and the preparation of culture medium is Muller Hinton to detect and experiment the effect of extracts on the growth of bacteria. After completing the experiment, gram-positive and gram-negative bacteria growth was clearly inhibited. While there are some types of bacteria didn't appear to inhibit their growth, due to difference in the strain of bacteria.

The results showed that gram-positive bacteria are more sensitive to plant extract compared to gram-negative bacteria. The aqueous extract had the best inhibiting efficacy on the growth of multiple types of bacteria isolated depending on the measurement of inhibition diameters as the inhibitory diameter reached 20 mm for *E. coli* as a maximum limit for inhibition and reached 12 mm for *S. albus* as a minimum, as shown in Table 2.

Also, the alcoholic extract had a clear inhibiting activity for the growth of types of bacteria, as inhibition diameter reached to 20 mm for bacteria *E. coli* as a maximum for inhibition

Table 2: Diameter (mm) of microbial free zone area of aqueous eucalyptus leaves extract for many types of bacteria

No.	*Bacteria*	*Diameter of inhibition (mm) for aqueous extract*
200	*E. coli*	-
201	*S. epidermidis*	14
202	*S. aureus*	14
203	*S. epidermidis*	13
204	*S. albus*	12
205	*S. epidermidis*	11
206	*E. coli*	9
207	*S. aureus*	10
208	*E. coli*	8
209	*S. albus*	12
210	*E. coli*	7
211	*Sallmonella*	+9

and as a minimum it reached 7 mm for bacteria *S. aureus*, as shown in Table 3.

After the inhibition of the concentrated plant extract, results appeared, The extract was diluted for the purpose of knowing less concentrated than it could inhibit the growth of the bacterial isolated used and it was actually diluted and the results are shown in Table 4.

In Figure 2, a comparison is made between the results of Table 2, 5, and Figure 3 shows the difference of results between Table 3 and Table 4.

DISCUSSION

The increasing use of antimicrobial agents in recent years has also led to the development of resistance to these drugs, so interest has increased in plants with antimicrobial properties, as a result of the current problems associated with the use of antibiotics and because they represent a rich source of antimicrobial agents.[10] The activity of plant extracts on microorganisms has been studied before. Too many researchers, In this researcher this inhibition, is due to the eucalyptus leaves containing flavonoids as well as containing phenolic compounds, which have an important role in inhibiting the growth of bacteria that inhibit the enzymes responsible for basic metabolic reactions by their non-specialized interaction with proteins, which leads to protein transformation and thus the inhibit to continue. While he explained the effectiveness of this plant because it contains tanin, which is effective in inhibiting bacteria and viruses because of it's ability to stimulate phagocytic cells and it has the potential to destroy proteins and other structures present on the bacterial cell wall that bacteria use for adhesion. The

Table 3: Diameter (mm) of microbial free zone area of alcoholic eucalyptus leaves extract for many types of bacteria.

No.	*Bacteria*	*Diameter of inhibition (mm) of alcoholic extract*
200	*E. coli*	20
201	*S. epidermidis*	11
202	*S. aureus*	7
203	*S. epidermidis*	8
204	*S. aureus*	7
205	*S. epidermidis*	9
206	*E. coli*	15
207	*S. aureus*	8
208	*E. coli*	12
209	*S. albus*	9
210	*E. coli*	14
211	*Sallmonella*	16

Table 4: Diameter (mm) of microbial free zone area of alcoholic eucalyptus leaves extract Zinc nanoparticles added for many types of bacteria

No.	*Bacteria*	*Diameter of inhibition (mm) of alcoholic extract +nano-zinc*
200	*E. coli*	25
201	*S. epidermidis*	15
202	*S. aureus*	16
203	*S. epidermidis*	10
204	*S. aureus*	17
205	*S. epidermidis*	19
206	*E. coli*	22
207	*S. aureus*	18
208	*E. coli*	12
209	*S.albus*	19
210	*E. coli*	24
211	*Sallmonella*	20

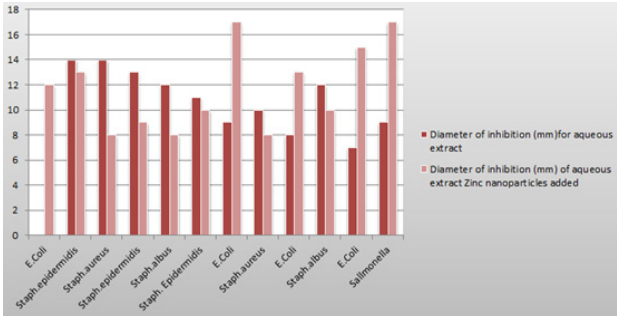

Figure 2: comparison between results of Table 2 and 4.

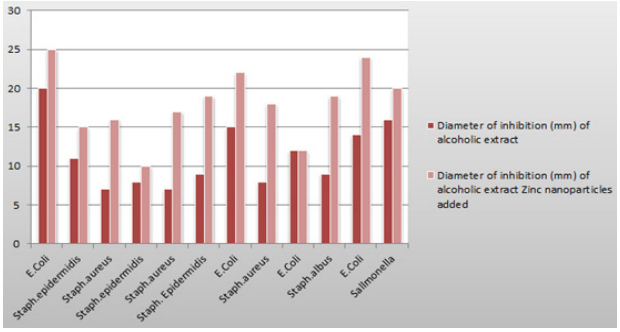

Figure 3: comparison between results of Table 3 and 5.

Table 5: Diameter (mm) of microbial free zone area of aqueous extract Zinc nanoparticles added for many types of bacteria.

No.	*Bacteria*	*Diameter of inhibition (mm) of aqueous extract+ nano-zinc*
200	*E. coli*	12
201	*S. epidermidis*	13
202	*S. aureus*	8
203	*S. epidermidis*	9
204	*S. aureus*	8
205	*S. epidermidis*	10
206	*E. coli*	17
207	*S. aureus*	8
208	*E. coli*	13
209	*S. albus*	10
210	*E. coli*	15
211	*Sallmonella*	17

results confirm that the inhibitory efficacy of the plant extract increases with increasing it's concentration and that the inhibiting diameters vary depending on the type of extract and the type of bacteria.Boland (2018) mentions that the compound 1,8 cineoled is essential in oils for must eucalyptus extracts. This compound affects the cytoplasmic membrane in the target bacteria and returns the eucalyptus extract's antimicrobial activity. Singh (2000) stated that the effect of the inhibiting eucalyptus leaves extract is due to this plant rich in essential oils that have antimicrobial properties against a wide range of bacteria that show high resistance to types of antibiotics used.[11]

This study's results are consistent with the findings of (Takahashi andothers, 2004) regarding the eucalyptus plant and the effective effect on gram-negative bacteria ex. *E. coli* and gram-positive ex. *S. aureus* due to the presence of carvacroland thymol phenol in it's volatile oils.[12] This study's results are consistent with his findings (Sundus Adil Naji and Saeed H. Mahammed, 2018) and consistent with the results of (Jammoul Maya W and Nawas Tariq E, 2019) . But the results of this study don't agree with results of (Fatima Ahmadi MotaMayel, Soraya Hassanpur, Mohammad Yousef Alikhani, Jalalpoorolajal, JavadSalehi, 2013), this incompatibility may be due to different bacterial isolates that were used.[13] The results showed that the synergistic effect of alcoholic extract with zinc nanoparticles was more effective on gram-negative bacteria compared with the aqueous extract supplemented with zinc nanoparticles, while there was a clear inhibition on gram-positive bacteria compared with gram-positive bacteria.[14-20]

CONCLUSION

Results of this study have shown that the aqueous and alcoholic supported by nanoparticles of eucalyptus leaves extract have great potential as the best antimicrobial agents in the treatment of bacterial infection.

REFERENCES

1. Arredondo-García JL, Amábile-Cuevas CF. High resistance prevalence towards ampicillin, co-trimoxazole and ciprofloxacin, among uropathogenic Escherichia coli isolates in Mexico City. The Journal of Infection in Developing Countries. 2008 Oct 1;2(05):350-3.
2. Oulia P, Saderi H, Rasouli I, Sefidkon F. Antimicrobial characteristics of some herbal oils on Pseudomonas aeruginosa with special reference to their chemical compositions. Indian J Pharmacol Res. 107–114.
3. Chambers HF, DeLeo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Reviews Microbiology. 2009 Sep;7(9):629-41.
4. Mubita C, Syakalima M, Chisenga C, Munyeme. "Activity and composition of the essential oils of Eucalyptus camaldulensis Dehn and Myths communist L. growing in Northern Cyprus". Afr J Biotechnol, 2010, ppn. 531–535.
5. Boulekbache-Makhlouf L, Meudec E, Chibane M, Mazauric JP, Slimani S, Henry M, Cheynier V, Madani K. Analysis by high-performance liquid chromatography diode array detection mass spectrometry of phenolic compounds in fruit of Eucalyptus globulus cultivated in Algeria. Journal of agricultural and food chemistry. 2010 Dec 22;58(24):12615-24.
6. Jose B, Reddy LJ. Evaluation of antibacterial activity of the leaf and flower essential oils of Gliricidia sepium from south India. International Journal of Applied Pharmaceutics. 2010;2(2):20-2.
7. Hummelen R, Fernandes AD, Macklaim JM, Dickson RJ, Changalucha J, Gloor GB, Reid G. Deep sequencing of the vaginal microbiota of women with HIV. PloS one. 2010 Aug 12;5(8):e12078.
8. Ling Z, Kong J, Liu F, Zhu H, Chen X, Wang Y, Li L, Nelson KE, Xia Y, Xiang C. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC genomics. 2010 Dec;11:1-6.
9. Ali N, Ahmed G, Ali Shah SW, Shah I, Ghias M, Khan I. Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of callistemon citrinus curtis. BMC complementary and alternative medicine. 2011 Dec;11:1-8.
10. Schellenberg JJ, Links MG, Hill JE, Dumonceaux TJ, Kimani J, Jaoko W, Wachihi C, Mungai JN, Peters GA, Tyler S, Graham M. Molecular definition of vaginal microbiota in East African commercial sex workers. Applied and environmental microbiology. 2011 Jun 15;77(12):4066-74.
11. Alsuwayfee Ki, Ahmed M, Mohammed Qn. Congenital heart disease and pulmonary hypertension among Down syndrome pediatric patients. Annals of the College of Medicine, Mosul. 2020 Jul 28;42(1):50-6.
12. Liu MB, Xu SR, He Y, Deng GH, Sheng HF, Huang XM, Ouyang CY, Zhou HW. Diverse vaginal microbiomes in reproductive-age women with vulvovaginal candidiasis. PloS one. 2013 Nov 12;8(11):e79812.
13. Hussein K, Marianne H. "Eucalyptus", Asian Pacific Journal of Tropical Biomedicine, 2017, ppn. 157-160.
14. Joseph N. The health benefits of eucalyptus. 2018. www.medicalnewstoday.com.
15. Sabo VA, Knezevic P. Antimicrobial activity of Eucalyptus camaldulensis Dehn. plant extracts and essential oils: A review. Industrial crops and products. 2019 Jun 1;132:413-29.
16. Bush LM, Charles E. Schmidt College of Medicine, Florida Atlantic University. Последний полный пересмотр/исправление июнь. 2019.
17. Mohammmed GF, Sultan SM, Sadullah YQ. The relationship between Creatinine and patients with Renal Failure associated with anemia. Research Journal of Pharmacy and Technology. 2020;13(4):1633-5.
18. Sultan SM, Hasan RS, Sadullah YQ, Saadi AM. Effect of Radiation System on the blood variables of people working in some hospitals. Research Journal of Pharmacy and Technology. 2020;13(1):74-6.
19. Jarjees N, Irzogy M, Abdallah O, Sultan S. Testing effect some plant extracts on reducing growth of candida albicans which isolated from vaginal canal of pregnant woman. 2022. Jarjees, ppn. 1001-1007.
20. Gaddawi FY, Jarjees NA, Sultan SM, Irzoqy ME. Detection Effect Toxins Produced by Some Types of Fungi Isolated from Medicinal Plants. International journal of health science. 2022 ppn. 969-986.