**Benha Journal of Applied Sciences (BJAS)** **print: ISSN 2356–9751**
**Vol. (9) Issue (3) (2024), (219-225)** **online: ISSN 2356–976x**
**http://bjas.bu.edu.eg**

# Antibacterial activity of some plant extracts on human pathogenic bacteria

**Yasmin M. Elsayed[1], M.O. Abdel-Monem[1], Amr A. Elwaseif[2], Hamed M. Elshora[3] and Mervat G. Hassan[1]**
[1]Botany, Dept., Faculty of Science, Benha Univ., Benha, Egypt
[2]Botany and Microbiology Dept., Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt
[3]Botany Department, Faculty of Science, Mansoura University,Mansoura, Dakahlia, Egypt
**E-mail:** yasmenbasha886@gmail.com

**Abstract**

Traditional medicine across all traditions has relied on plant remedies since ancient times. Traditional medicine relies on a wide variety of herbs and antibacterial compounds found in plants for dental care. Antimicrobial activity against three pathogenic bacteria—Staphlococcus aureus, Acinetobacter baumannii complex, and Klebsiella pneumoniae—was evaluated by assaying extracts of Thymus Vulgaris and Cinnamomum camphora. The bacteria were detected biochemically and using the VITEK2 system. Antibiotic susceptibility testing revealed that all three isolates were resistant to the twenty-plus antibiotics tested. In vitro investigations of five Egyptian plant extracts of Anastatica hierochuntica L , Thymus vulgaris , Olea europaea leaf and costus sasurrea, Cinnamomum camphora showed that aqueous Thymus Vulgaris and aqueous Cinnamomum camphora extract could exhibit an antibacterial activity against human pathogenic isolates and inhibition zones of 20.0±1.5 ,3.0±0and 20.0 ±1 mm were observed when Thymus Vulgaris extract applied against the above-mentioned bacteria, while the inhibition zones of 15.0±0.577,2±0.577 and 18.0±0.577 mm were observed by Cinnamomum camphora extract while aqueous plant extracts of Anastatica hierochuntica L, Olea europaea leaf and Costus sasurrea don´t have any effect on the bacterial isolates .Thymus vulgaris, Cinnamomum camphora, Staphylococcus aureus, Acinetobacter baumannii complex, Klebsiella pneumoniae

**Keywords:** Staphylococcus aureus, Acinetobacter baumannii complex, Klebsiella pneumoniae, Thymus vulgaris, Cinnamomum camphora.

## 1. Introduction

Growing health care costs and rising rates of morbidity and death are persistent issues (1). Although there may be much to be learned by focusing on resistance mechanisms themselves, the quest for new therapeutic targets is driven by the need to find new antimicrobials to combat resistant bacteria (2). The wide range of secondary chemicals found in plants has piqued the interest of researchers looking for new antibacterial agents (3). The primary sources of natural medicinal agents are the native bioactive chemicals found in these plants (4). Mycobacterium spp. a. Staphylococci are well known as bacteria that belong to the family Micrococcaceae. Their propensity to promote abscess development has traditionally classified them as extracellular pyogenic infections. Because they lack motility, the bacteria typically colonize in groups. They have remarkable resilience and may remain on surfaces exposed to the elements for lengthy durations. Staphylococcus aureus, epidermidis, and saprophyticus are all members of the same family (5). Being strictly aerobic, oxidase-negative, and catalase-positive, Acinetobacter is a non-motile coccobacillus bacterium (6). Patients with ventilator-associated pneumonias (VAPs) were most likely to have Pseudomonas aeruginosa or Acinetobacter baumannii. Several risk factors for antibiotic-resistant Pseudomonas aeruginosa or Acinetobacter baumannii include late onset pneumonia and using carbapenems within 72 hours before symptoms appeared (7). The rod-shaped, nonmotile, encapsulated, lactose-fermenting, facultatively anaerobic Klebsiella pneumoniae (8) is gram-negative. The presence of Klebsiella pneumoniae may lead to a variety of illnesses, such as pneumonia, UTIs, bacteremia, and liver abscesses. In addition to susceptible clinical isolates that cause nosocomial infections, there are other strains that have evolved to be hypervirulent (hvKP) or multidrug-resistant (MDR) (9). A member of the Lamiaceae family, Thymus vulgaris (T. vulgaris) is widely grown throughout the Mediterranean, the Middle East, southern Europe, and central Africa under the name "Zaatar" in Algeria (10). Its antibacterial characteristics have made it a popular choice in traditional medicine[11], [12].Not only that, but its antifungal, analgesic, anti-inflammatory, diuretic, sedative, antispasmodic, and antiseptic characteristics have made it useful in a variety of medical contexts [13]. Camphor is a popular Chinese herbal remedy for inflammatory skin conditions, arthritis, and diabetes-related inflammation [14]. It is also used topically to alleviate pain and other symptoms associated with inflammation. Also, reducing alkenes, polyols, and ketones are abundant in camphor, according to our analysis of the tree's extract [15].

## 2.Materials and Methods

This research used five therapeutic herbs. After rinsing with distilled water, the newly harvested plant components (leaves and stems) were left to dry in the dark for six days at room temperature. Using a grinding machine (Siemens-blender) to reduce the dried medicinal plant ingredients to a powder. Afterwards, they were left at room temperature in dry bags until extraction.

Making the extract from water: We conducted the experiment in accordance with the method described in [16], which included shaking constantly a mixture of 100 ml of distilled water and 10 grams of dried powdered plant material for 48 hours. Next, he filtered the mixture through muslin cloth and centrifuged it at 5000 rpm for 10 minutes. After that, he collected the supernatant and concentrated it under vacuum using a rotary evaporator at temperatures below 40°C. He then kept the concentrated liquid in sterile bottles in a freezer at 4 oC until another time. certain types of microbes: From pus, sputum, and urine samples collected at Benha University Hospital's Microbiology and Immunology Department, three bacterial isolates—Staphylococcus aureus, Acinetobacter baumannii complex, and Klebsiella pneumoniae—were identified. Isolates were isolated using the VITEK2 system version: 07.01 apparatus and nutritional agar medium. The medium was chosen based on cultural, morphological, and biochemical examination.

**Evaluation of antibiotic resistance**

The antibiotics used for testing the sensitivity of the isolated strains using disk diffusion method were Amikacin (AK)(30µg), cefoxitin(fox)(30µg), Azithromycin (AZM)(15µg), Erythromycin (E)(15µg), Tobramycin (TOB)(10µg),levofloxacin (LEV)(5µg),cefuroxime (CXM)(30µg), ciprofloxacin(cip)(5 µg), trimethoprim +sulfamethoxazole (SXT) (25µg) , Chloramphenicol (C)(30µg), Rifampin (RA)(5µg), Amoxicillin(AX) (30 µg) , Clindamycin (DA) (2 µg),Vancomyin (VA) (30µg), Meropenem (MRP)(30 µg),Imepenm (IPM)(30µg), Cefotaxime(CTX)(30µg), ceftazidime-clavulonic acid (CAL)(10µg) ,colistin(CL)(10µg),piperacillin(PRl) (5µg) cited as [18].

**Assay for the activity of plant extracts using the well diffusion method:**

Each sterile petridish was filled with one milliliter of inoculum suspension, followed by about twenty milliliters of autoclaved nutritional agar, and then allowed to set. A non-hazardous metal polish was used. For the seeded agar, holes were drilled with a diameter of 6 mm. Each seeded medium well was seeded with 50 cc of crude plant extract. In order to determine the inhibition zones in millimeters (mm), the plates were incubated at 37 degrees Celsius for 24 hours [19].

## 3: Discussion and Results

From samples of pus, sputum, and urine collected at Benha University Hospital's Microbiology and Immunology Department, three distinct bacterial strains (1P; 2S; 3U) were identified. The growth of these isolates on nutrient agar medium allowed them to be purified. A facultative an aerobic, non-motile, catalase-positive, coagulase-positive gram-positive cocci population was isolated. The colonies of the No(2S) isolate are smooth to pitted, dome-shaped, and lack pigmentation. The gram-negative rods and nonmotile, large, grayish, and very mucoid colonies were seen in isolate (3U).

**Features based on culture and anatomy:**

On nutritional agar medium, the cultural traits of three different bacterial isolates were examined. If one looks at table (1)

**Table (1)** Bacterial isolates' cultural traits and morphological features.

| **Basic characteristic** | **Bacterial isolates** | | |
|---|---|---|---|
| | ***1P*** | ***2S*** | ***3U*** |
| **source** | pus | sputum | Urine |
| **Culture character** | They appear around cocci and form in grape like clusters under microscop | Colonies non pigmented, domed and mucoid with smooth to pitted surface | Large, greyish and highly mucoid colonies |
| **shape** | cocci | coccobacillus | Rods |
| **Gram staining** | (+) | (-) | (-) |
| **Motility** | Non-Motile | Non-Motile | Non-Motile |

Table(1)Clusters of isolate No(1p) resembling grapes emerge under a microscope as they surround cocci. Nonpigmented, dome-shaped, mucoid colonies with a smooth to pitted surface are seen in Isolate No. (2S). Colonize three unlabeled U-shaped colonies that are big, mucoid, and grayish.

**features of the body's structure:**

physical features (such as cell size, shape, and organization, as well as gram stain response)

Under the microscope, bacterial isolates 1P, 2S, and 3U were examined using an oil immersion objective.found in the table (1)

Clusters of gram-positive, nonmotile cocci made up Isolate No(1P). Bacterial isolate No. (2S) was a nonmotile, gram-negative, coccobacillus organized in pairs or long chains. Isolate No. (3U) consisted of gram-negative rods grouped in pairs, short chains, or clusters.

Biochemical and practical features of bacterial strains.

The bacterial isolates' physiological and biochemical characteristics are shown in table (2). Here are the results for Isolate No. 1P: it showed positive results for catalase, citrate, coagulase, Indol, MR, VP, oxidase, Urease, TSI, glucose, lactose, sucrose, mannose, and mannite.

Isolate 2S tested positive for catalase, citrate, coagulase, Indol, MR, VP, oxidase, Urease, TSI, glucose, lactose, sucrose, mannose, and mannite. Purified Isolate No. (3U) tested positive for catalase, citrate, coagulase (not detected), Indol, MR, VP, oxidase, Urease, TSI, glucose, lactose, sucrose, mannose, and mannite.

**Table (2)** Biochemical properties of bacterial isolates.

| **Biochemical characteristic** | **Bacterial isolates** | | |
|---|---|---|---|
| | ***1p*** | ***2s*** | ***3U*** |
| **Catalase** | **(+)** | **(+)** | **(+)** |
| **Citrate** | **(+)** | **(+)** | **(+)** |
| **Coagulase** | **(+)** | (-) | ND |
| **Indol** | (-) | (-) | (-) |
| **MR(Methylred)** | **(+)** | (-) | **(+)** |
| **VP(Voges proskauer)** | **(+)** | (-) | **(+)** |
| **Oxidase** | (-) | (-) | (-) |
| **Urease** | **(+)** | (-) | **(+)** |
| **TSI(triple sugar iron agar)** | **(+)** | (-) | **(+)** |
| **Glucose** | **(+)** | **(+)** | **(+)** |
| **Lactose** | **(+)** | **(+)** | **(+)** |
| **Sucrose** | **(+)** | **(+)** | **(+)** |
| **Mannose** | **(+)** | **(+)** | **(+)** |
| **Mannite** | **(+)** | **(+)** | **(+)** |

**(+) Positive result – (-) Negative result – ND:not detected**

**Biochemical identification using automated identification system (VITEK2):**

The traditional biochemical identification of the four bacterial isolates, as shown in tables 1P, 2S, and 3U, was confirmed using the VITEK2 system version :07.01 equipment. Isolates 1P, 2S, and 3U were determined to be Staphylococcus aureus, Acinetobacter baumannii complex, and Klebsiella pneumonia, respectively, based on morphological, cultural, and conventional biochemical features and confirmation of identification by the VITEK2 system.

**Table (3)** Staphylococcus aureus biochemical findings (1p)

| **Biochemical Details** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2** | **AMY** | **-** | **4** | **PIPLC** | **-** | **5** | **dxyl** | **-** | **8** | **ADH1** | **+** | **9** | **BGAL** | **-** | **11** | **AGLU** | **-** |
| **13** | **APPA** | **-** | **14** | **CDEX** | **-** | **15** | **AspA** | **-** | **16** | **BGAR** | **-** | **17** | **AMAN** | **-** | **19** | **PHOS** | **+** |
| **20** | **LeuA** | **-** | **23** | **ProA** | **-** | **24** | **BGURr** | **-** | **25** | **AGAL** | **-** | **26** | **PyrA** | **+** | **22** | **BGUR** | **-** |
| **28** | **AlaA** | **-** | **29** | **TyrA** | **-** | **30** | **dSOR** | **-** | **31** | **URE** | **-** | **32** | **POLYB** | **+** | **32** | **dGAL** | **+** |
| **38** | **dRIB** | **-** | **39** | **ILATK** | **-** | **42** | **LAC** | **-** | **44** | **NAG** | **+** | **45** | **dMAL** | **+** | **39** | **BACl** | **-** |
| **47** | **NOVO** | **-** | **50** | **NC6 5** | **+** | **52** | **dMAN** | **+** | **53** | **dMNE** | **+** | **54** | **MBdG** | **+** | **45** | **PUL** | **-** |
| **57** | **dRAF** | **-** | **58** | **O129R** | **-** | **59** | **SAL** | **-** | **60** | **SAC** | **-** | **62** | **dTRE** | **+** | **57** | **ADH2s** | **-** |
| **64** | **OPTO** | **+** | | | | | | | | | | | | | | | |

**Table (4)** Analysis of the Acinetobacter baumannii complex (2S) biochemically.

| Biochemical Details | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | + | 9 | BGAL | - | | | |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | + | 13 | dGLU | + | 14 | GGT | - | 15 | OFF | - | | | |
| 17 | BGLU | - | 18 | dMAL | - | 19 | dMAN | - | 20 | dMNE | + | 21 | BXYL | - | 22 | BAIap | - | | | |
| 23 | ProA | - | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | + | 31 | URE | - | 32 | dSOR | - | | | |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTRE | - | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | - | | | |
| 40 | ILATK | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - | | | |
| 46 | GlyA | - | 47 | ODC | - | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | + | 57 | BGUR | - | | | |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | + | | | | | | |

**Table (5)** Evaluation of Klebsiella pneumoniae biochemistry (3U)

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | + | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | + | 9 | BGAL | + |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | + |
| 17 | BGLU | + | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | + | 22 | BAIap | - |
| 23 | ProA | - | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | - | 31 | URE | + | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | - |
| 40 | ILATK | + | 41 | AGLU | - | 42 | SUCT | - | 43 | NAGA | - | 44 | AGAL | + | 45 | PHOS | + |
| 46 | GlyA | - | 47 | ODC | - | 48 | LDC | + | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

**Antibiotic susceptibility testing**

Qualitative The three bacterial isolates were resistant to a minimum of 10 antibiotics, according to the findings of the antibiograms (Table 6). These antibiotics include many common ones such as Amikacin (AK), cefoxitin, cefotaxime, ceftazidime-clavulonic acid, tobramycin, levofloxacin, cefuroxime, ciprofloxacin, trimethoprim+ sulfamethoxazole, and chloramphenicol. But Meropenem and Rifampin worked on all three strains. Generally speaking, resistance to antibiotics (AB) has arisen swiftly, often even within the first years of introduction (20), since their discovery and widespread use in the early 20th century. Consequently, a large number of bacteria, particularly Gram-negative ones, have evolved resistance to the majority of the antibiotic classes [21]. Consequently, the search for novel antimicrobial drugs to combat bacterial infections is gaining momentum. When it came to clinical bacterial isolates, amikacin was the antibiotic of choice [22]. Antibiotic rotation and restricted use of ceftazidime and ciprofloxacin reduced the number of cases of VAP associated with resistant gram-negative bacilli and increased the number of methicillin-sensitive S. aureus, suggesting that this strategy may be useful in reducing the frequency of resistant organisms [23].

**Antibacterial activity of some plant extracts against the human pathogenic bacteria.**

Tables 7, 8, and 9 summarize the results of an examination of the antibacterial activity of five medicinal plant extracts against the isolated bacteria. The human pathogenic bacteria were only effectively targeted by extracts of Thymus vulgaris and camphor.

**Table (6)** Bacterial isolates' sensitivity to fourteen different antibiotics.

| Bacteria | Ax | PRL | IPM | MRP | Fox | cal | cxm | sxt | cxt | VA | DA | Cl | RA | AK | Tob | Cip | Lev | Azm | E | c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Staphylococcus aureus(1p) | R | R | R | R | R | R | R | R | R | S | R | R | S | R | R | R | R | S | S | R |
| Acinetobacter baumannii complex(2S) | R | R | R | S | R | R | R | R | R | R | R | S | R | R | R | R | R | R | R | R |
| Klebsiella pneumonia(3U) | R | R | R | S | R | R | R | R | R | R | R | S | R | R | R | R | R | R | R | R |

**Stands for Immune-Defying, Intermediate, and Vulnerable.**

The antibiotics used for testing the sensitivity of the isolated strains using disk diffusion method were Amikacin (AK)(30µg), cefoxitin(fox)(30µg), Azithromycin (AZM)(15µg), Erythromycin (E)(15µg), Tobramycin (TOB)(10µg), levofloxacin (LEV)(5µg), cefuroxime (CXM)(30µg), ciprofloxacin(cip)(5 µg), trimethoprim+ sulfamethoxazole (SXT)(25µg), Chloramphenicol (C)(30µg), Rifampin (RA)(5µg), Amoxicillin(AX)(30 µg), Clindamycin (DA)(2 µg), Vancomyin (VA)(30µg), Meropenem(MRP)(30 µg),Imepenm (IPM)(30µg), Cefotaxime(CTX) )(30µg), ceftazidime-clavulonic acid (CAL(10µg)), colistin(CL)(10µg), piperacillin(PRl) (5µg)

**Table (7)** The antimicrobial efficacy of medicinal plant extracts against the pathogenic Staphylococcus aureus(1p) bacterium.

| Plant extract | Mean diameter of inhibition zone(mm), original diameter (5mm) *Staphylococcus aureus*(1p) |
|---|---|
| ***Anastatica hierochuntica L*** | R |
| ***Thymus vulgaris*** | 20.0±1.5 |
| ***Olea europaea leaf*** | R |
| ***Costus sasurrea*** | R |
| ***Cinnamomum camphora*** | 15.0±0.577 |

The mean of three measurements (mm) plus or minus the standard deviation (SD) equals the value of each.

**Table (8)** Antibacterial activity of medicinal plant extracts against the Acinetobacter baumannii complex(2S) bacterium.

| Plant extract | Mean diameter of inhibition zone(mm), original diameter (5mm) *Acinetobacter baumannii* complex(2S) |
|---|---|
| ***Anastatica hierochuntica L*** | R |
| ***Thymus vulgaris*** | 3.0±0 |
| ***Olea europaea leaf*** | R |
| ***Costus sasurrea*** | R |
| ***Cinnamomum camphora*** | 2.0±0.577 |

The mean of three measurements (mm) plus or minus the standard deviation (SD) equals the value of each.

**Table (9)** Antimicrobial action of medicinal plant extracts against the harmful Klebsiella pneumoniae (3U) bacteria.

| Plant extract | Mean diameter of inhibition zone(mm), original diameter (5mm) *Klebsiella pneumonia(3U)* |
|---|---|
| ***Anastatica hierochuntica L*** | R |
| ***Thymus vulgaris*** | 20.0±1 |
| ***Olea europaea leaf*** | R |
| ***Costus sasurrea*** | R |
| ***Cinnamomum camphora*** | 18.0±0.577 |

The mean of three measurements (mm) plus or minus the standard deviation (SD) equals the value of each.

**4.Conclusion**

The three human pathogenic bacteria—Klebsiella pneumoniae (3U), Acinetobacte rbaumannii complex (2S), and Staphylococcus aureus (1p)—isolated from Egypt are detailed in this paper. Thymus vulgaris and camphor extracts, in particular, were shown to be abundant sources of important chemicals with several medical uses when evaluated for antibacterial characteristics against isolated bacteria. When it comes to treating nosocomial pathogenic bacteria, both are seen as good alternatives to antibiotics. We are now investigating the effectiveness of phage treatment and certain nanoparticles as potential novel methods for treating germs that cause illness in humans.

**Reference**


[1] W.C.Hellinger. Confronting the problem of increasing antibiotic resistance. South. Med.vol. J. 93,pp.842–848,2000.

[2] K.Coleman, M.Athalye, and A. Clancey. Bacterial resistance mechanisms as therapeutic targets. J. Antimicrob. Chemother.vol.33,pp. 1091–1116,1994.

[3] Y.A.Mahmoud, M.EBRAHIM, and M.M.Aly. Influence of some plant extracts and microbioagents on some physiological traits of faba bean infected with Botrytis fabae. Turkish J of Botany.vol.28,pp.519-528 ,2004.

[4] M.M.Cowan. Plant products as antimicrobial agents. Clinical microbiology reviews.vol.12,pp.564-582,1999.

[5] Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. and Pfaller, M.A. (2007). Manual of Clinical Microbiology, 9th Ed., ASM Press, Washington, D.C. 1-2310

[6] Nemec,A., Radolfova-Krizova,L. & Maixnerova,M.(2017), Acinetobacter colistiniresistens sp. nov. (formerly genomic species 13 sensu Bouvet and Jeanjean and genomic species 14 sensu Tjernberg and Ursing), isolated from human infections and characterized by intrinsic resistance to polymyxins. International Journal Of Systematic And Evolutionary Microbiology .67(7) https://doi.org/10.1099/ijsem.0.001903

[7] Peerawong Werarak ,M.D., Pattarachai Kiratisin, M.D.& Visanu Thamlikitkul ,M.D.(2010), Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia in Adults at Siriraj Hospital :Etiology, Clinical Outcomes, and Impact of Antimicrobial Resistance. J Med Assoc Thai .93 (Suppl. 1): S126-138

[8] Y.Guo, Z. Cen, and X.Fang. Whole-genome sequence of Klebsiella pneumonia strain LCT-KP214. J Bacteriol.vol. 194(12),pp.3281. doi: 10.1128/JB.00531-12,2012.

[9] W.C.Hellinger. Confronting the problem of increasing antibiotic resistance. South. Med.vol. J. 93,pp.842–848,2000.

[10] Aldosary S, El-Rahman S, Al-Jameel S, Alromihi N. Antioxidant and antimicrobial activities of Thymus vulgaris essential oil contained and synthesis thymus (Vulgaris) silver nanoparticles. Braz J Biol. 2021;3:83.

[11] Khalilnezhad F, Torabi S, Larijany K, Khosrowshahli M. Nano silver particle synthesis using leaf extract of pharmaceutical plant Thymus vulgaris. Int J Biosci. 2015;6(4):192–196

[12] Mohammadi M, Shahisaraee SA, Tavajjohi A, et al. Green synthesis of silver nanoparticles using Zingiber officinale and Thymus vulgaris extracts: characterisation, cell cytotoxicity, and its antifungal activity against Candida albicans in comparison to fluconazole. IET Nanobiotechnol. 2019;13 (2):114–119. doi:10.1049/iet-nbt.2018.5146

[13] Odemis O, Ozdemir S, Gonca S, Arslantas A, Agirtas MS. The study on biological activities of silver nanoparticles produced via green synthesis method using Salvia officinalis and Thymus vulgaris. Turk J Chem. 2022;46(5):1417–1428. doi:10.55730/1300-0527.3448

[14] P. Drikvandi, S. Bahramikia, M. Alirezaei, Modulation of the antioxidant defense system in liver, kidney, and pancreas tissues of alloxan-induced diabetic rats by camphor, J. Food Biochem. 00 (2020), e13527

[15] A. Shang, R.Y. Gan, J.R. Zhang, X.Y. Xu, M. Luo, H.Y. Liu, et al., Optimization and characterization of microwave-assisted hydro-distillation extraction of essential oils from Cinnamomum camphora leaf and recovery of polyphenols from extract fluid, Molecules 25 (14) (2020) 3213–3240.

[16] P.D.Lokhande, KR.Gawai, and K.M.Kodam. Antibacterial activity of extracts of Piper longum. J Pharmacol Toxicol.vol. 2,pp.574-9,2007.

[17] K.R.Aneja, R.Joshi, and C.Sharma. Antimicrobial efficacy of fruit extracts of two Piper species against selected bacterial and oral fungal pathogens. Braz J Oral Sci.vol. (4)9,pp.p 422-426,2009.

[18] V.Navarro, M.L.Villarreal, and G.Rojas. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of ethnopharmacology.vol. 53(3),pp.143-147,1996.

[19] M.M.Cowan. Plant products as antimicrobial agents. Clinical microbiology reviews.vol.12,pp.564-582,1999.

[20] Marston,H.D.,Dixon,D.M.,Knisely,J.M.,Palmore,T.N.,andFauci,A.S.Antimicrobialresistance.JAMA316,1193–1204.doi:10.1001/jama.2016.11764

[21] Centers for Disease Control and Prevention (2018). Antibiotic / Antimicrobial Resistance. Available online at: https://www.cdc.gov/drugresistance/index.html (accessedMarch20,2019).

[22] Y.A.El-Zawahry, F.M.Reda, and W.M.Azazy. Synergistic effect of combination treatment by certain plant extracts and some antibiotics on the resistance of pathogenic bacteria to some common antibiotics. Life Science Journal.vol.10(4),pp. 3477- 3489,2013

[23] D.Gruson, G.Hilbert,and F.Vargas. Impact of colony-stimulating factor therapy on clinical outcome and frequency rate of nosocomial infections in intensive care unit neutropenic patients. Crit Care Med .vol. 28,pp. 3155–60,2000.