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Research Article **BOTANY**

Antimicrobial activities of some essential oils and antibiotics on multidrug-resistant microbial vaginitis

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KEY WORDS

Essential oil, antimicrobial agents, vaginitis microorganisms and antibiotics.

ABSTRACT

The present study aimed to investigate the efficacy of some natural essential oils on microbial vaginitis as a new promising alternative remedy. fifty cases of microbial vaginitis collected and were identified biochemically using the VITEK 2 compact system, the results showed that, 41 isolates were referred to 4 normal microflora and 37 opportunistic bacterial isolates, 7 fungal isolates and 2 parasitic infections. The antibiotics sensitivity testing was performed using different antibiotics for pathogenic bacteria and yeast isolates. The results showed that nine bacterial and two yeast isolates out of the forty-four isolates exhibited multi-drug resistance (MDR). The antimicrobial activities of the used oils against the eleven pathogenic MDR isolates recorded that turmeric oil was the most potent essential oil against each microbe, which was confirmed by molecular identification. The MIC of turmeric oil against the pathogenic MDR *Klebsiella Pneumoniae* and *C. albicans* recorded that the MIC of turmeric oil was 0.781 mg/ml for both isolates. The combination between the most effective essential oils (turmeric and parsley oils separately) and the selected resisted antibiotics (levofloxacin and nystatin) on the most dominant MDR bacterial and yeast isolates showed increasing in the diameters of inhibition zones from 38 to 40 mm and from to 24 mm respectively. Cellular damage under SEM was conducted for the turmeric oil, which was analyzed by GC-MS to confirm its chemical composition with an abundance of Aromatic-turmerone. The present study concluded that using turmeric oil is an effective antimicrobial agent against most pathogenic MDR microbial vaginitis.

Introduction

Vaginitis is an inflammation of the vagina that can be caused by bacterial, fungal, parasites, viral infections, or chemical and physical irritation **(Gupta *et al.*, 2019).** The vagina is usually acidic and contains normal flora of microorganisms. Certain conditions such as menstrual cycle, pregnancy, and cosmetic/hygienic agents (shampoos or shaving creams) can interfere with the acidic environment or normal flora. They can cause severe inflammation of the vagina and discharge. There are six types of vaginitis: bacterial (most common), candidal, parasitical, viral, atrophic and cytolytic vaginosis. Bacterial vaginitis (BV) is an inflammation of the vagina caused by several bacterial species, including *Gardnerella vaginalis* and *Mobiluncus curtisii* **(Wilson and Wilson, 2021).**

Hundreds of microorganisms were commonly isolated from vaginal swabs worldwide, which can be classified into normal microflora, opportunistic pathogens and true pathogens **(Ravel *et al.*, 2011).** Bacterial vaginitis (BV) is an inflammation of the vagina caused by several bacterial species, including *Gardnerella vaginalis, Mobiluncus curtisii* and *Neisseria gonorrhaeae*. It is known to affect many women and is usually associated with other complications such as pelvic inflammatory disease (PID), preterm labor, and low birth weight, among others **(Muzny and Schwebke, 2016).**

The polymorphic opportunistic fungus *Candida albicans* is primarily responsible for vulvovaginal candidiasis (VVC), an incredibly prevalent mucosal infection of the lower female reproductive tract (FRT). *C. albicans*, a typical component of the human microbiota, frequently and asymptomatically colonies the vaginal lumen **(Owolabi *et al.*, 2018).**

Treating bacterial vaginosis is advised to alleviate symptoms and lower the risk of contracting *Herpes simplex* type 2 (HSV-2), *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis*, human immunodeficiency virus (HIV), and other diseases **(Ryden, 2020).**

The goal of treating a candida infection is to lessen symptoms. Along with many topical azole formulations and regimens, oral fluconazole (Diflucan) in a single 150-mg dose is also readily accessible. When deciding between topical and oral treatment, there are various factors to consider **(Roberts *et al.*, 2015)**. Due to the calming nature of the topical treatment, topical medicines may offer more immediate relief. On the other hand, they may trigger localized hypersensitivity reactions that cause itching or burning. Available over the counter, many patients use topical antifungals to treat suspected vulvovaginal candidiasis **(Paladine and Desai, 2018).**

Antibiotic misuse has led to microorganism resistance, another issue harming public health. The appearance of multi-drug resistant bacteria and fungi leads to searching for new substances with antimicrobial activities. Plant extracts and essential oils are frequently used in popular medicine as remedies for many infectious diseases. Recently, medical plants that have fewer side effects and are more cost-effective have been considered due to the side effects, expense, and complexity of producing therapeutic-chemical materials. *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and coagulase are part of pathogens acquired in the community and hospitals. *Staphylococcus* spp., *Shigella* spp, *Enterococcus* spp., *Escherichia coli* and *Klebsiella pneumonia* are some of the most common bacteria with multi-drug resistant organisms (MDROs). Consumers now have a high demand for novel antibiotics to combat diseases as a result of this **(Sepahvand *et al.*, 2017).**

Effective antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal and insecticidal activity has been observed for essential oils. The ability to partition with the lipids found in bacteria and mitochondrial cell membranes, making them more effective by

upsetting the cell structures, is a crucial property of essential oils and the substances that make them up. The significant loss of necessary chemicals and ions from the bacterial cell ultimately causes the bacterial cell's death. Some substances target the efflux pump systems in Gram-negative bacteria to regulate drug resistance **(Evans *et al.*, 2004).** With the limitations of conventional drugs, using a new treatment for these diseases seems necessary. So, the purpose of the present work was to investigate the efficacy of some essential oils on bacterial and yeast vaginitis as a new promising alternative remedy.

Materials and Methods

The Media used:

In this study different media for vaginitis bacterial isolates such as Nutrient agar (NA) and Nutrient broth (NB) media **(Lapage *et al.*, 1970).** Soft nutrient agar medium **(Wu *et al.*, 1987)**. Mueller-Hinton agar medium (MHA) (**Llácer *et al.*, 2009**). Trypticase soy broth (TSB) and for fungal isolates Sabouraud's dextrose agar (SDA) with chloramphenicol **(Scognamiglio *et al.*, 2010).**

The used essential oils:

Seventeen prepared medicinal plant essential oils, namely: Black seed, Onion, Parsley, Garlic, Green tea, Turmeric, Peppermint, Clove, Lavender, Marjoram, Anise, Fenugreek, Basil, Fennel, Eucalyptus, Olive, and Sage oils were purchased from El-Captin company for extracting natural oils, plants, and cosmetics, AL Obour city Cairo-Egypt.

The tested antibiotics:

Twenty-one different commercial microbial and broad-spectrum antibiotic discs (Antibacterial agents namely: Cefaclor, Cefotaxime, Cefoperazone, Cefepime, Clindamycin, Imipenem, Doxycycline, Levofloxacin, Ciprofloxacin, Amikacin, Sulphamesoxyzole/ Trimethoprim, Azithromycin, Ampicillin, Amoxicillin/Clavulanic acid, Piperacillin/ Tazobactam and Nitrofurantoin discs, and five antifungal agents namely: Metronidazole, Fluconazole, Itraconazole, Ketoconazole and Nystatin) 6mm in diameter belonging to different families were purchased from Al-Gomhoria company for pharmaceutical industries. All antibiotics were used for investigating their potency against the isolated microbial vaginitis isolates.

Isolation, purification and morphological identification of the isolated microbial vaginitis.

This study was conducted from February 2018 to December 2021 on 50 clinical cases of ten different groups (Sup1). Fifty clinical vaginal swabs of bacterial and fungal isolates were collected from various clinical laboratories. The swabs were placed separately in different media and transported within one hour to the bacteriology and mycology labs in the Botany Department, Faculty of Science, Tanta University, Egypt, for further investigations. In addition, more samples were stored in nutrient broth for delivery to El-Asafra hospital labs in Alexandria for identification of the isolated microbial vaginitis by automated VITEK 2 compact system **(Ling *et al.*, 2003),** which includes automated analytical culture/sensitivity testing, identification of isolates by biochemical reactions and determination the multi-drug resistance MDR isolates.

Study the antibiotics sensitivity of the isolated microbial vaginitis isolates

Sixteen different commercial and broad-spectrum antibiotic discs 6mm were selected for investigating their potency against the isolated bacterial isolates according to the standard Kirby- Bauer disk diffusion method **(Bauer, 1966).** Under sterilized conditions, four to five similar colonies from each bacterial isolate (overnight growth) were transferred separately into sterile distilled water and vigorously agitated to give turbidity that matches the 0.5 McFarland standard (approximately 10^6 CFU/ml) according to

D'Amato and Hochstein, (1982). Within 15 min, a sterile cotton swab was dipped into the culture suspension for inoculating the surface of solidified Mueller-Hinton agar plates **(Counts *et al.*, 2007).** Antibiotic discs were dispensed onto the inoculated plate's surfaces. After 15 minutes the plates were incubated at 37°C for 24h. The resulting diameters (mm) of inhibition zones around the antibiotic discs were measured. The results were interpreted according to protocols standardized for the assay of antibiotic compounds as guided by the National Committee for Clinical Laboratory Standards "NCCLS". The data were categorized as: R (resistant), I (intermediate sensitive), and S (sensitive) **(Rijal *et al.*, 2010).** For sensitivity testing of yeast isolates, five broad-spectrum antifungal discs (Oxoid USA) from different families were used. The zone size chart 2018 was used for interpreting the data. The plates were inoculated by dipping a sterile swab into the inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard (10^8 cells/ml) and streaking it across the surface of the SDA medium in all directions. Antibiotic discs were dispensed onto the inoculated plate surface. The plates were incubated at 28°C for 24h. The diameters (mm) of inhibition zones were measured **(Khan *et al.*, 2006).**

Study the antimicrobial activities of the used essential oils against the most dominant MDR microbial vaginitis

In this experiment, seventeen essential oils were used for testing the antimicrobial activities against the pathogenic MDR bacterial and yeast vaginitis isolates according to the method described by **Haller *et al.*, (2015).** A volume of 0.1 ml suspension of each of the selected pathogenic MDR bacterial and yeast isolates were inoculated separately on the surface of Mueller Hinton Agar plates and each was spread homogenously using a sterile glass rod and left to dry at 37°C for 15 min. Three discs made from sterilized Whatman No.1 filter paper discs (3 replicates) with a diameter of 6 mm were applied to the prepared plates using sterile forceps and pressed gently against the agar surface. The sterile and refined essential oils were dissolved in sterilized 10% aqueous dimethyl sulfoxide (DMSO) with Tween 80 (0.5% v/v for easy diffusion) and impregnated with 10µl of each of the tested essential oils separately by using sterile automated pipette tips. The plates were incubated at 37°C for 24 h. The antimicrobial activity of each of the used essential oil against the selected MDR bacterial isolates was estimated by measuring the diameters (mm) of the inhibition zones by caliper and compared with negative control (Sterile filter paper saturated with sterile DMSO) and recorded as the average of three replicates.

Determination of the minimum inhibitory concentration (MIC) of the most effective essential oil against the most dominant MDR bacteria and Yeast isolates using ELISA reader

The (MIC) were determined by using the broth microdilution method as approved by the guidelines of Clinical and Laboratory Standards Institute **(Mann and Markhon, 1998).** Different concentrations of turmeric oil were prepared by suspending 1 gm of oil in 10 ml of 5% dimethyl sulfoxide solution (DMSO) to get stock solution of 100 mg/ml oil. Then half fold serial dilution was made for this stock with sterile distilled water to get oil concentrations of 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195 and 0.097 mg/ml. The microtiter plates were prepared by adding 100µL of Mueller Hinton Broth (MHB) for bacteria and 100µL of Sabouraud Dextrose Broth (SDB) for yeast. Twenty microliters (20µL) of bacterial suspension (0.5 McFarland standards) and twenty microliters (20µL) of yeast suspension were added to each well except the control wells (control wells contained broth only and sterile distilled water only) **(Thabaut and Meyran, 1984).**

Effect of the combination between the most effective essential oils and the selected resisted antibiotics on the most dominant MDR bacterial and yeast isolates

The most effective essential oils Turmeric, Parsley, Garlic, and Black seed oils were tested in combination with the selected

four resisted antibiotics Cefaclor, Nitrofurantion, Amoxicillin/Clavulinic acid and Levofloxacin, related to four different antibiotic families' patterns (Cephalosporins 2nd generation, Nitrofurans, Penicillin combination and Quinolones/Floroquinolones families respectively) against the most dominant isolated MDR bacterial isolate. The selected bacterial isolate was cultured for study of the combination effects between the most effective essential oils and the four resisted antibiotics using disc diffusion method. A volume 0.1 ml of the bacterial suspension with final concentration 10^6 CFU/ml was dropped separately on the surface of the (MHA) and was spread homogenously using sterile glass rod and left to let cells to settled down at room temperature for 15 min. **(Knezevic *et al.*, 2016)**. Then the selected resisted antibiotics discs with known concentration were impregnated separately with fixed volume 10µl of the most potent essential oils and placed on the surface of MHA plates, the plates were incubated for 24 h at 37°C, and the diameters of inhibition zones were measured as the average of three replicates **(Van *et al.*, 2009).**

Also the effect of the most effective essential oils in combination with the three selected resisted antibiotics; Metronidazole, Nystatin and Itraconazole which related to three different antibiotic families patterns (Nitroimidazole, Polyenes and Triazole families respectively) were tested against the most dominant MDR yeast **(Van *et al.*, 2009)** using disc diffusion method as described above with the bacterial isolate but with inoculated the yeast isolate in sterile SDB and incubated for 48 h at 28°C.

Molecular identification of the most inhibited pathogenic (MDR) bacteria by 16S rRNA

The extraction was done using Gene Jet genomic DNA purification Kit (Thermo) protocol **(Tampieri *et al.*, 2005).** Amplification of the 16SRNA region was conducted in an automated thermal cycler (C1000™ Thermal Cycler, Bio-RAD) using F (5`- AGA GTT TGA TCC TGG CTC AG -3`) and R (5`- GGT TAC CTT GTT ACG ACTT-3`) primers **(Allen and Walter, 2016)**. Finally, sequencing was made to the PCR product on GATC Company using ABI 3730xl DNA sequencer using forward and reverse primers **(Brugha *et al.*, 2014).**

Molecular identification of the most inhibited pathogenic (MDR) fungal by 16S rRNA

The surface of Sabrouaud's dextrose agar plate was scratched to remove the pseudomycelium of the most prevalent MDR yeast. In liquid nitrogen, 50 mg of fresh pseudomycelium was pulverized with a mortar and pestle. Using the genomic plant DNA extraction Mini Kit (iNtRON Biotechnology, Inc, Cat. Non 17371) and following the manufacturer's instructions, DNA was extracted from the powdered tissue. At -20°C, the eluted DNA was kept. Internal transcribed spacer (ITS) region was conducted in an automated thermal cycler PCR machine. ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') primers were used in an automated thermal cycler (C1000TM Thermal Cycler, Bio-RAD) to amplify the ITS region **(Hassan, *et al.*, 2019)**. Thirty-five cycles of 94°C for 30 S, 51°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 3 min were employed as the parameters for every PCR (**Windels *et al.*, 2020**).

Study the antivirulance activity of the most effective essential oils on formation of biofilm by the selected MDR isolates

The turmeric oil was tested for its potential to overcome and prevent biofilm formation of the most dominant MDR bacteria and yeast isolates. Different concentrations of turmeric oil (0.097, 0.195, 0.390, 0.781, 1.562, 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml) were tested against the most dominant MDR bacterial and yeast isolates. To acquire the initial concentrations in 100µl for microorganisms, an aliquot of two-fold serial dilutions was made on a 96-well microtiter plate with trypticase soy broth with 2% of

glucose (TSBGlc). To get the initial concentrations in 100 µl for yeast, an aliquot of two-fold serial dilutions was made on a 96-well microtiter plate using Sabouraud's dextrose broth. Then, bacterial suspensions (50 µl; final concentration: $5x10^5$ CFU/ml) were poured into the plate. TSBGlc and Sabouraud's dextrose-containing distilled water were used as negative control. The positive control was inoculated TSBGlc and Sabouraud's dextrose without the essential oil (untreated cells) **(Sambanthamoorthy *et al.*, 2014).** The production of bacterial biofilms in the presence of the tested plant essential oil measure at 570 nm and the results were compared to the positive control **(Obied *et al.*, 2018).**

Detection the antimicrobial effect of turmeric oil on the structure of the most inhibited pathogenic MDR microbial vaginitis using Scanning Electron Microscope (SEM)

In this experiment, a scanning electron microscope (SEM) was applied to detect the effect of turmeric oil on the cellular structure of the pathogens compared with the control. The nutrient broth containing the growth of the most dominant MDR *K. pneumoniae* and *C. albicans* sensitive to Turmeric oil, control growth (without oil) and treated growth (with oil) were examined using SEM. It was analyzed using a scanning electron microscope (Model JEOL, JSM-5200 LV) and was applied in SEM Unit in the Faculty of Medicine, Tanta University. Treated cultures with Turmeric oil at (MIC) concentration of 0.781 mg/ml for the most dominant MDR bacterial and yeast isolates were incubated for 24h. At appropriate conditions for the type of bacteria and yeast. None treated bacterial, and yeast cultures from each bacterial type and yeast were included as a negative control, and then all of them were investigated by SEM **(Altemimi *et al.*, 2017).**

Determination of the active antimicrobial materials produced by the most effective essential oils using Gas chromatographic-mass spectrometry analysis (GC- MS)

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to determine the main compounds in turmeric essential oil, especially the antimicrobial agents. This analysis was carried out in the central lab of biochemistry, Tanta University **(George *et al.*, 2004).** Turmeric essential oil was examined by gas chromatography, Mass spectroscopy in Claurs 580/560S. Work was done with column 30.0 m x 250 µm, Rtx–5MS (crossband 5% diphenyl 95% dimethyl polysiloxane). The GC conditions were employed using helium as carrier gas (0.8 ml/min), and the temperature program was 60°C for 1 min, followed by an increase of 10°C /min to 180°C for the remainder of the run. Detector and injection point heaters were 260°C and 280°C, respectively and typically 0.1 or 1.0 µl was injected at a 20: 1 split **(Peruzza *et al.*, 2003).**

Results

Fifty clinical vaginal swabs from ten different groups (each containing several cases) were investigated for their load of isolated bacteria, fungi, and parasites. These groups were arranged from 1st to 10th as the following: normal healthy, microbial vaginosis, pre- and post-vaginal area, pregnant, nosocomial infection, unexplained infertility, hyperglycemia with obesity, a child with poor hygiene, dysfunctions of the immune system and parasitic vaginal infection cases, respectively as shown in table (1). Table (2) showed that 47 microbial isolates were identified using an automated VITEK2 compact system, the identified microbial isolates belonged to ten genera, nine genera of bacteria and one genus of *Candida*, one species only related to each genus of bacteria and two species related to genus *Candida*. The results recorded 20 *Klebsiella pneumoniae*, 6 *Escherichia coli*, 4 *Staphylococcus aureus*, 1 *Neisseria gonorrhoeae*, 2 *Pseudomonas aeruginosa*, 1 *Acinetobacter baumannii*, 5 *Lactobacillus plantarum*, 1 *Gardnerella vaginalis*, 6 *Candida albicans*, 1 *Candida lusitaniae* and 1 *Peptostreptococcus prevotii* case no.33 specially identified by ID32A kit.

Antibiotics sensitivity testing for clinical vaginal bacterial and yeast isolates

The antibiotics sensitivity of the tested bacterial isolates showed different susceptibilities ranging from sensitive (S), intermediate (I) and resistant (R) reactions against 16 tested antibiotics as shown in table (2) Cefaclor (CEC30), Cefotaxime (CTX30), Cefoperazone (CFP75), Cefepime (FEP30), Clindamycin (DA10), Imipenem (IPM10), Doxycycline (DO30), Levofloxacin (LEV5), Ciprofloxacin (CIP5), Amikacin (AK30), Sulphamethoxazole/ Trimethoprim (SXT25), Azithromycin (AZM15), Ampicillin (AMP25), Amoxicillin/ Clavulinic acid (AMC30), Pipracillin/ Tazobactam (TZP110) and Nitrofurantion (F300).Table (3) showed that out of the thirty-seven pathogenic bacterial isolates, nine bacterial isolates exhibited multidrug resistance (MDR) against the used antibiotics, theses bacterial isolates were *Latobacillus plantarum*, *Acinetobacter baumannii*, *Nieseeria gonorrhoeae*, *Gardenerrella vaginalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Peptostreptococcus prevotii* where the numbers of these isolates were 4, 9, 13, 14, 15, 25 28, 32, and 33 respectively and the numbers of antibiotics that were resisted by each MDR isolate were 2, 13, 9, 11,7, 12, 9, 3 and 8 respectively where the inhibition zones ranged between 6 to 10 mm. in diameters. The remaining isolates exhibited various sensitive reactions as shown in table (3). The yeast isolates showed different susceptibilities ranging from sensitive (S), intermediate (I) and resistant (R) reactions against the five tested antibiotics, Metronidazole (MTZ50), Fluconazole (FCA25), Itraconazole (IT30), Ketokenazole (KT10) and Nystatin (NS100) as illustrated in Table (3). Two isolates out of the seven isolates of yeasts, *Candida albicans* no. 48 and *Candida lusitaniae* no. 49 showed MDR against 4 and 3 of the used antibiotics respectively.

S (1): Groups of infected female patients based on their clinical signs and symptoms

Group 1: Normal healthy cases.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years**
From isolate No. 1 to 5	**Four cases with normal vaginal odour, normal exudate quantity, consistency and no symptoms or signs for infections, exceptcase no. 4.**	**From 13 to 40**
Group 2: Microbial vaginosis cases.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years**
From isolate No. 6 to 15	**BIS**	**From 20 to 51**
From isolate No. 42 and 43	**YIS**	**27, 32**
Group 3: Pre and post vaginal area cases such as clitoritis, vulval infections, Bartholnitis (Bartholin`s gland abscess) and cervicitis microbial vaginosis.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/ years**
From isolate No. 16 to 17	**BIS**	**29, 39**
Group 4: Three stages of pregnant cases. Pregnant at 1st, 2nd, and 3rd trimester microbial vaginosis.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/ years and gestational stages**
From isolate No. 18 to 21	**BISDG**	**From 22 to 37, 1st trimester**
From isolate No. 22 to 24	**BISDG**	**From 21 to 29, 2nd trimester**
From isolate No. 25 to 27	**BISDG**	**From 20 to 35, 3rd trimester**
From isolate No. 44 to 46	**YISDG**	**From 20 to 34, 1st trimester**
Group 5: Nosocomial infection cases or the hospital-acquired infections (HAIs).“Post- operative infections to the vaginal area”.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years andoperation types**
From isolate No. 28 to 33	**BIS**	**40, PCOs (Polycystic ovary syndromes) withrecurrent abortion**
		42, Hysterectomy with chocolate cyst
		35, Endometriosis
		39, Bartholin’s cystabscess
		33, ICSI intra cytoplasmic sperm injection, IMSI intracytoplasmic morphologically selected sperm injection, IVF in-vitro fertilization, IUI intrauterine insemination
		36, Postpartum fever with ectopic pregnancy
Isolate No. 47	**YIS**	**21,Vaginal adhesions with curettage**
Group 6: Unexplained infertility cases (sterility) with microbial vaginosis.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years**
Isolate No. 34 and 35	**BIS**	**27, 34**
Group 7: Hyperglycemia with obesity cases (obesity with diabetes mellitus type I and II).		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years and body weight**
From isolate No. 36 to 38	**BIS**	**Aged from 29 to 40, Weight from 80 to 99 kg**
Isolate No. 48	**YIS**	**Aged from 28 to 41, Weight from 88 to 115 kg**
Group 8: Pre-pubertal and pre-adolescent cases due to fecal contamination and poor hygiene,especially when using the toilet.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/ years**
Isolate No. 39	**BIS**	**7**
Isolate No. 49	**PIS**	**4**
Group 9: Dysfunctions and disorders of the immune system in immunocompromised patient cases.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years, and causes**
Isolate No. 40 and 41	**BIS**	**50, Radiotherapy and chemotherapy**
		46, +HIV with solid organ transplant
Group 10: Parasitic vaginal infection cases.		
Clinical cases No.	**Clinical symptoms & signs presentation**	**Ages/years and Diagnostic stages**
Isolate No. 50	**TIS**	**29, Trophozoite**

BIS: Bacterial infection symptoms, **YIS**: Yeast infection symptoms, **GIS**: Gonorrheal infection symptoms, **TIS**: *Trichomonas vaginalis* (TV), **BISDG**: Bacterial infection symptoms during gestation, **YISDG**: Yeast infection symptoms during gestation and **PIS:** Parasitical infection symptoms

Table (1): Clinical cases and types of microbial isolates detected in fifty collected clinical vaginal swabs

No. of groups	Type of groups	No. of swab specimens	% Out of total swab specimens	No. of isolates	No. of yeast vaginosis	No. of isolate parasitical vaginosis
Group 1 (5 cases)	**Normal healthy cases**	**5**	**10%**	**5**	**0**	**0**
Group 2 (12 cases)	**Microbial vaginosis cases**	**12**	**24%**	**12**	**Isolate No. 42 Isolate 43**	**0**
Group 3 (2 cases)	**Pre and post vaginal area cases**	**2**	**4%**	**2**	**0**	**0**
Group 4 (13 cases)	**Pregnant cases**	**13**	**26%**	**13**	**Isolate No. 44 Isolate No. 45 Isolate No. 46**	**0**
Group 5 (7 cases)	**Nosocomial infections cases**	**7**	**14%**	**7**	**Isolate No. 47**	**0**
Group 6 (2 cases)	**Unexplained infertility cases**	**2**	**4%**	**2**	**0**	**0**
Group 7 (4 cases)	**Hyperglycemia with obesity cases**	**4**	**8%**	**4**	**Isolate No. 48**	**0**
Group 8 (2 cases)	**Child with poor hygiene cases**	**2**	**4%**	**2**	**0**	**Isolate No. 49**
Group 9 (2 cases)	**Cancer disease cases**	**2**	**4%**	**2**	**0**	**0**
Group 10 (1 cases)	**Parasitic vaginal infections cases**	**1**	**2%**	**1**	**0**	**Isolate No. 50**
Total		**50**	**100 %**	**50**	**7 isolates**	**2 isolates**

Table (2): Frequency of microbial isolates incidence in clinical vaginitis.

Group No.	Bacterial isolates									Fungal isolates	Parasitical isolates
	K. pneumonia	***S. aureus***	***E. coli***	***P. aeruginosa***	***L. plantarum***	***N. gonorrhea***	***G. vaginalis***	***A. baumannii***	***P. prevotii***	***Candida* spp**	***Parasite isolates***
1	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**0**	**0**
2	**4**	**1**	**2**	**0**	**0**	**1**	**1**	**1**	**0**	**2**	**0**
3	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
4	**6**	**1**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**3**	**0**
5	**3**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**
6	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
7	**2**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
8	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
9	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
10	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
Total	**20**	**4**	**6**	**2**	**5**	**1**	**1**	**1**	**1**	**7**	**2**

Table (3): Antibiotics sensitivity testing for isolated bacteria and yeast by Kirby-Bauer disk diffusion method.

Group No.	Isolate No.	CEC	CTX	CFP	FEP	DA	IPM	DO	LEV	CIP	AK	SXT	AZM	AMP	AMC	TZP	F	Resistance level (MDR)
1	1	Normal commensal microbiota																
	2																	
	3																	
	4	20 (S)	20 (I)	24 (S)	21 (S)	7 (R)	25 (S)	12 (I)	19 (S)	25 (S)	20 (S)	14 (I)	6 (R)	14 (I)	21 (S)	22 (S)	19 (S)	2
	5	Normal commensal microbiota																
2	6	7 (R)	19 (I)	21 (S)	25 (S)	15 (I)	28 (S)	15 (S)	23 (S)	18 (I)	24 (S)	14 (I)	18 (S)	17 (S)	19 (S)	24 (S)	17 (S)	1
	7	22 (S)	17 (I)	23 (S)	29 (S)	20 (S)	33 (S)	19 (S)	9 (R)	12 (R)	18 (S)	21 (S)	15 (I)	17 (S)	20 (S)	24 (S)	14 (I)	2
	8	17 (I)	9 (R)	16 (I)	24 (S)	22 (S)	23 (S)	20 (S)	18 (S)	17 (I)	12 (R)	14 (I)	16 (I)	19 (S)	21 (S)	21 (S)	18 (S)	2
	9	22 (S)	10 (R)	22 (S)	9 (R)	8 (R)	9 (R)	9 (R)	6 (R)	10 (R)	19 (S)	6 (R)	7 (R)	10 (R)	8 (R)	8 (R)	10 (R)	13
	10	18 (S)	14 (R)	20 (I)	29 (S)	20 (S)	30 (S)	14 (S)	22 (S)	16 (I)	27 (S)	8 (R)	10 (R)	10 (R)	17 (I)	20 (I)	12 (R)	5
	11	19 (S)	23 (S)	21 (S)	20 (S)	19 (S)	25 (S)	11 (I)	18 (S)	10 (R)	20 (S)	19 (S)	19 (S)	9 (R)	8 (R)	24 (S)	18 (S)	3
	12	18 (S)	23 (S)	21 (S)	20 (S)	18 (I)	24 (S)	12 (I)	16 (I)	25 (S)	18 (S)	10 (R)	12 (I)	11 (R)	13 (I)	22 (S)	12 (R)	3
	13	15 (I)	10 (R)	18 (I)	16 (I)	10 (R)	24 (S)	9 (R)	11 (R)	20 (I)	16 (I)	8 (R)	9 (R)	7 (R)	7 (R)	21 (S)	7 (R)	9
	14	10 (R)	25 (S)	12 (R)	10 (R)	9 (R)	8 (R)	6 (R)	20 (S)	8 (R)	8 (R)	15 (I)	10 (R)	8 (R)	9 (R)	25 (S)	15 (I)	11
	15	10 (R)	14 (R)	12 (R)	9 (R)	10 (R)	10 (R)	8 (R)	21 (S)	27 (S)	20 (S)	13 (I)	14 (I)	15 (I)	20 (S)	22 (S)	17 (S)	7
3	16	17 (I)	22 (I)	21 (S)	14 (R)	12 (R)	18 (I)	12 (I)	17 (I)	26 (S)	11 (R)	19 (S)	19 (S)	9 (R)	10 (R)	19 (I)	20 (S)	5
	17	19 (S)	13 (I)	22 (S)	16 (I)	21 (S)	17 (I)	16 (S)	10 (R)	25 (S)	10 (R)	20 (S)	17 (I)	19 (S)	17 (I)	21 (S)	9 (R)	3
4	18	22 (S)	23 (S)	19 (I)	22 (S)	19 (I)	30 (S)	18 (S)	19 (S)	10 (R)	19 (S)	20 (S)	9 (R)	10 (R)	20 (S)	29 (S)	17 (S)	3
	19	10 (R)	14 (R)	20 (I)	25 (S)	19 (I)	25 (S)	16 (S)	20 (S)	18 (I)	20 (S)	21 (S)	17 (I)	16 (I)	21 (S)	21 (S)	20 (S)	2
	20	17 (I)	26 (S)	15 (I)	19 (I)	22 (S)	23 (S)	18 (S)	17 (I)	29 (S)	27 (S)	9 (R)	8 (R)	16 (I)	23 (S)	17 (I)	12 (I)	2
	21	25 (S)	24 (S)	20 (I)	14 (R)	19 (I)	23 (S)	11 (I)	18 (S)	11 (R)	16 (I)	9 (R)	20 (S)	20 (S)	21 (S)	26 (S)	18 (S)	4
	22	18 (S)	10 (R)	19 (I)	21 (S)	19 (I)	24 (S)	16 (S)	16 (I)	9 (R)	19 (S)	19 (S)	17 (I)	10 (R)	16 (I)	25 (S)	19 (S)	3
	23	15 (I)	22 (I)	27 (S)	21 (S)	22 (S)	26 (S)	17 (S)	12 (R)	14 (R)	19 (S)	19 (S)	22 (S)	9 (R)	20 (S)	21 (S)	8 (R)	4
	24	22 (S)	20 (S)	20 (I)	14 (R)	16 (I)	26 (S)	13 (I)	9 (R)	20 (I)	17 (S)	21 (S)	20 (S)	18 (S)	21 (S)	20 (I)	23 (S)	2
	25	9 (R)	8 (R)	16 (I)	21 (S)	11 (R)	26 (S)	7 (R)	15 (I)	13 (R)	10 (R)	7 (R)	8 (R)	6 (R)	10 (R)	6 (R)	6 (R)	12
	26	19 (S)	23 (S)	21 (S)	20 (S)	19 (S)	25 (S)	11 (I)	18 (S)	10 (R)	20 (S)	19 (S)	19 (S)	9 (R)	8 (R)	24 (S)	18 (S)	3
	27	22 (S)	25 (S)	20 (I)	14 (R)	16 (I)	26 (S)	13 (I)	9 (R)	20 (I)	17 (S)	21 (S)	20 (S)	18 (S)	21 (S)	20 (I)	23 (S)	2
5	28	7 (R)	9 (R)	12 (R)	14 (R)	10 (R)	24 (S)	12 (I)	11 (R)	16 (I)	19 (S)	13 (I)	7 (R)	12 (I)	7 (R)	21 (S)	6 (R)	9
	29	22 (S)	23 (S)	15 (I)	22 (S)	18 (I)	17 (I)	14 (S)	13 (R)	24 (I)	18 (S)	15 (I)	9 (R)	11 (R)	14 (I)	22 (S)	17 (S)	3
	30	16 (I)	17 (I)	22 (S)	17 (I)	15 (I)	25 (S)	9 (R)	18 (S)	19 (I)	14 (I)	15 (I)	9 (R)	17 (S)	21 (S)	26 (S)	19 (S)	3
	31	14 (I)	25 (S)	14 (I)	10 (R)	9 (R)	23 (S)	9 (I)	11 (I)	18 (I)	19 (S)	21 (S)	10 (R)	9 (R)	21 (S)	24 (S)	18 (S)	4

	Table continue:																	
	32	20 (S)	24 (S)	23 (S)	15 (I)	16 (I)	23 (S)	8 (R)	19 (S)	26 (S)	16 (I)	21 (S)	6 (R)	8 (R)	14 (I)	23 (S)	18 (S)	3
	33	15 (I)	21 (I)	11 (R)	6 (R)	11 (R)	26 (S)	11 (I)	23 (S)	9 (R)	6 (R)	6 (R)	17 (I)	13 (I)	22 (S)	9 (R)	12 (R)	8
6	34	15 (I)	22 (I)	27 (S)	21 (S)	22 (S)	26 (S)	17 (S)	12 (R)	14 (R)	19 (S)	19 (S)	22 (S)	9 (R)	20 (S)	21 (S)	8 (R)	4
	35	22 (S)	17 (I)	23 (S)	29 (S)	20 (S)	33 (S)	19 (S)	9 (R)	12 (R)	18 (S)	21 (S)	15 (I)	17 (S)	20 (S)	24 (S)	14 (I)	2
7	36	20 (S)	24 (S)	20 (I)	20 (S)	19 (S)	14 (R)	14 (S)	22 (S)	25 (S)	18 (S)	17 (I)	9 (R)	8 (R)	18 (S)	21 (S)	17 (S)	3
	37	18 (S)	10 (R)	19 (I)	21 (S)	19 (I)	24 (S)	16 (S)	16 (I)	9 (R)	19 (S)	19 (S)	17 (I)	10 (R)	16 (I)	25 (S)	19 (S)	3
	38	16 (I)	15 (I)	21 (S)	22 (I)	16 (I)	24 (S)	14 (S)	16 (I)	14 (R)	16 (I)	9 (R)	10 (R)	13 (I)	19 (S)	24 (S)	10 (R)	4
8	39	14 (I)	25 (S)	14 (I)	10 (R)	9 (R)	23 (S)	9 (I)	20 (S)	18 (I)	19 (S)	21 (S)	10 (R)	9 (R)	21 (S)	24 (S)	18 (S)	4
	40	Parasite (No antibiotics sensitivity tests)																
9	41	9 (R)	16 (I)	23 (S)	16 (I)	10 (R)	17 (I)	16 (S)	16 (I)	12 (R)	23 (S)	17 (I)	17 (I)	19 (S)	20 (S)	23 (S)	19 (S)	3
	42	24 (S)	24 (S)	22 (S)	21 (S)	20 (S)	25 (S)	11 (I)	18 (S)	10 (R)	20 (S)	19 (S)	20 (S)	9 (R)	8 (R)	25 (S)	20 (S)	3
10	43	Parasite (No antibiotics sensitivity tests)																

Antifungal sensitivity testing (AST)						
Group No.	**Isolate No.**	**Metronidazole (MTZ)**	**Fluconazole (FCA)**	**Itraconazole (IT)**	**Ketokenazole (KT)**	**Nystatin (NS)**	**Resistance level (MDR)**
2	44	18 (S)	22 (S)	17 (I)	25 (S)	28 (S)	0
	45	9(R)	20(S)	16(I)	24(S)	27(S)	1
4	46	9(R)	18(I)	22(S)	22(S)	25(S)	1
	47	11(I)	24(S)	19(S)	23(S)	24(S)	0
	48	10(I)	8(R)	8(R)	10(R)	22(R)	4
5	49	8(R)	22(S)	9(R)	18(I)	13(R)	3
7	50	9(R)	18(I)	18(S)	19(I)	25(S)	1

Efficacy of the tested essential oils on the MDR bacterial and yeast isolates

Seventeen antibacterial plant essential oils were tested against nine MDR bacterial isolates (*L. plantarum, A. baumannii, N. gonorrhoeae, G. vaginalis, E. coli, P. aeruginosa, K. pneumoniae, S. aureus* and *P. prevotii*) and two MDR yeast isolates (*C. lusitaniae* and *C. albicans*). The results in Table (4) showed that turmeric, garlic, black seed and parsley oils exhibited the highest antibacterial and antifungal activities against the MDR bacteria and yeast isolates. Inhibition zones recorded were 38, 32, 21, 10 mm for the *K. pneumoniae* isolate, and 33, 27, 11, and 10 mm for the *C. albicans* isolate, respectively. However, the same bacterium and yeast isolates showed resistance to onion, green tea, peppermint, clove, lavender, marjoram, anise, fenugreek, basil, fennel, eucalypts, olive and sage oils without any inhibition zones.

Table (4): Efficacy of the tested essential oils on the MDR bacterial and yeast isolates

Diameter of inhibition zones from different essential oils for clinical isolated bacteria and yeast (mm)											
Essential oil	***K. pneumoniae***	***C. albicans***	***C. lusitaniae***	***E. coli***	***S. aureus***	***N. gonorrhoeae***	***P. aeruginosa***	***A. baumannii***	***L. plantarum***	***G. vaginalis***	***P. prevotii***
Black seed oil	**21**	**11**	**25**	**10**	**8**	**8**	**-**	**8**	**9**	**7**	**11**
Onion oil	**9**	**9**	**10**	**8**	**9**	**20**	**8**	**7**	**15**	**-**	**-**
Parsley oil	**10**	**10**	**12**	**-**	**11**	**17**	**9**	**10**	**-**	**8**	**8**
Garlic oil	**32**	**27**	**27**	**12**	**12**	**12**	**13**	**7**	**20**	**10**	**10**
Green tea oil	**-**	**8**	**10**	**-**	**-**	**-**	**10**	**10**	**18**	**7**	**13**
Turmeric oil	**38**	**33**	**38**	**11**	**14**	**8**	**8**	**8**	**9**	**9**	**10**
Peppermint oil	**-**	**-**	**7**	**8**	**9**	**12**	**8**	**7**	**-**	**7**	**7**
Clove oil	**-**	**-**	**7**	**-**	**8**	**11**	**-**	**8**	**8**	**8**	**-**
Lavender oil	**-**	**-**	**-**	**7**	**10**	**-**	**-**	**9**	**-**	**-**	**8**
Marjoram oil	**-**	**-**	**-**	**8**	**-**	**-**	**7**	**10**	**8**	**-**	**9**
Anise oil	**-**	**-**	**-**	**-**	**-**	**11**	**8**	**12**	**7**	**7**	**10**
Fenugreek oil	**-**	**-**	**-**	**-**	**-**	**10**	**9**	**9**	**10**	**8**	**13**
Basil oil	**-**	**-**	**7**	**7**	**8**	**-**	**8**	**15**	**-**	**10**	**10**
Fennel oil	**-**	**-**	**8**	**-**	**7**	**-**	**-**	**8**	**8**	**8**	**-**
oil Eucalyptus	**-**	**-**	**-**	**10**	**-**	**-**	**-**	**-**	**10**	**9**	**9**
Olive oil	**-**	**-**	**-**	**8**	**8**	**-**	**8**	**14**	**7**	**-**	**9**
Sage oil	**-**	**-**	**8**	**-**	**10**	**7**	**13**	**9**	**-**	**14**	**-**

Determination of the minimum inhibitory concentration (MIC) of the most effective essential oil against the most dominant MDR bacteria and yeast isolates using ELISA reader

From Fig. (1) the activity of turmeric oil against *Klebsiella pneumonia* was confirmed by recording the values of both minimum inhibitory concentration (MIC) at (0.781) mg/ml giving a stable reading for the obtained microbial dry weight, that confirmed by recording the same value of MIC against *Candida albicans* as shown in Fig. (2) minimum bactericidal concentration (MBC) at (0.781) mg/ml accompanying the complete death (CFU= 0.0) of the re-cultured treated organisms, and minimum fungicidal concentration (MFC) for *Candida albicans* was recorded at a higher value at (1.562) mg/ml.

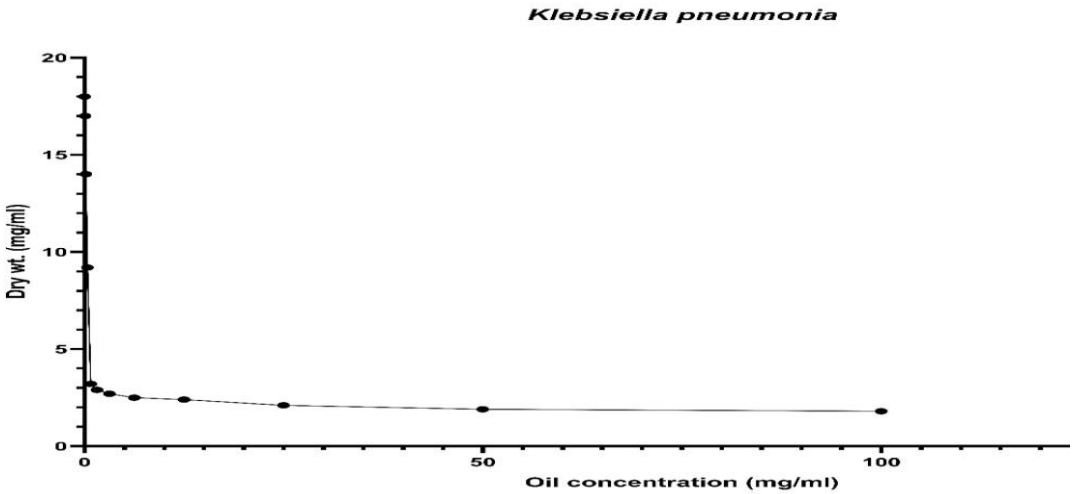


Fig. (1): Determination of the minimum inhibitory concentration (MIC) of the most effective essential oil (Turmeric oil) against the most dominant MDR bacterium (*K. pneumonia*)

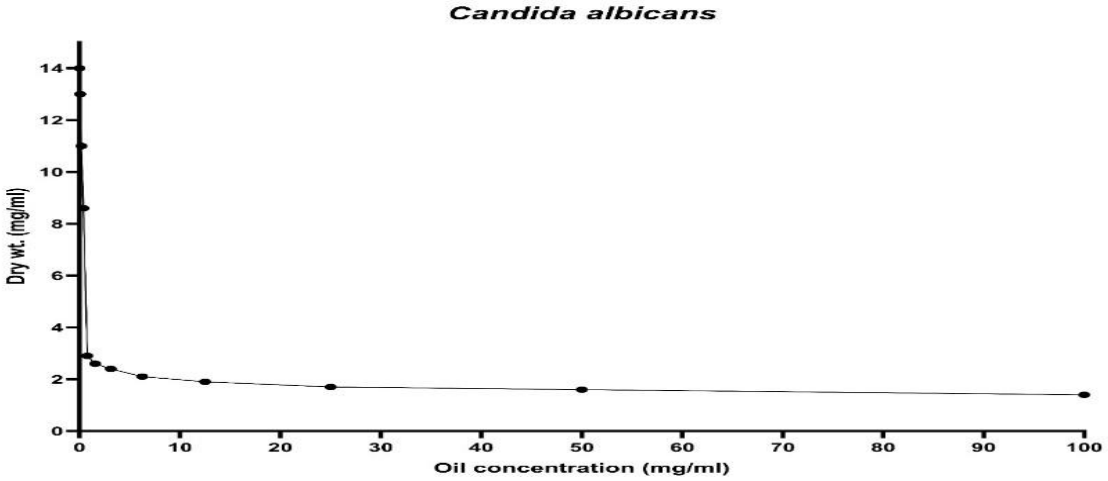


Fig. (2): Determination of the minimum inhibitory concentration (MIC) of the most effective essential oil (Turmeric oil) against the most dominant MDR yeast (*C. albicans*).

Effect of the combination between the most effective essential oils and the selected resisted antibiotics on the most dominant MDR bacterial and yeast isolates

The combination of the four selected resistant antibiotics with each of the most potent essential oils (turmeric, parsley, garlic, and black seed oils) against the pathogenic MDR *K. pneumoniae* was illustrated in Table (5). The results showed the levofloxacin antibiotic with all essential oils (garlic, parsley, black seed, and turmeric oils) had the highest inhibition activity against the pathogenic MDR *K. pneumoniae*, according to the results, the combination of Cefaclor, Nitrofurantoin and Amoxicillin/Clavulanic acid antibiotics had no effect on the most dominant MDR bacterium. When compared to other garlic, black seed, and turmeric oils, the combination of levofloxacin antibiotic and parsley oil demonstrated the greatest impact against *K. pneumoniae* (the diameter of the inhibitory zone increases from 10 to 22 mm) (Table 5). This result suggested that parsley oil and levofloxacin could be used to reduce *K. pneumoniae*'s resistance to the most efficient antimicrobial antibiotics. The results in table (6) demonstrated that the inhibitory effect of nystatin antibiotic appeared to increase when combined with all essential oils (garlic, parsley,

black seed, and turmeric oils), while the combination with parsley oils and black seed showed the best effect against *C. albicans* (the diameter of the inhibition zone increases from 10 and 11 to 24 and 25 mm for parsley oils and black seed, respectively). Additionally, the combination of Metronidazole and Itraconazole antibiotics showed no effect on the most dominant MDR fungus (*C. albicans*). From the previous results the most effective combination between levofloxacin and nystatin antibiotics with parsley oils showed the highest effect against both MDR *K. pneumoniae* and *C. albicans*.

Table (5): Effect of the combination between the selected antibiotics and the most effective essential oils against the most dominant pathogenic MDR *K. Pneumonia*.

The resisted antibiotics by *K. pneumoniae*	The most effective essential oils	Inhibition zone diameters (mm) of *K. pneumonia* using		
		Antibiotics	Essential oils	Combination
Negative control	DMSO -ve control	00	00	00
Cefaclor	Turmeric oil	7	38	38
	Parsley oil		10	10
	Garlic oil		32	32
	Black seed oil		21	21
Nitrofurantion	Turmeric oil	6	38	38
	Parsley oil		10	10
	Garlic oil		32	32
	Black seed oil		21	21
Amoxicillin/Clavulinic acid	Turmeric oil	7	38	38
	Parsley oil		10	10
	Garlic oil		32	32
	Black seed oil		21	21
Levofloxacin	Turmeric oil	11	38	40
	Parsley oil		10	22
	Garlic oil		32	34
	Black seed oil		21	23

Table (6): Effect of the combination between the selected antifungal and the most effective essential oils against the most dominant pathogenic MDR yeast isolate *C. albicans*

The resisted antifungals by *C. albicans*	The most effective essential oils	Inhibition zone diameters (mm) of *C. albicans* using		
		Antibiotics	Essential oils	Combination
Negative control	DMSO -ve control	00	00	00
Metronidazole	Turmeric oil	8	33	33
	Parsley oil		10	10
	Garlic oil		27	27
	Black seed oil		11	11
Nystatin	Turmeric oil	13	33	40
	Parsley oil		10	24
	Garlic oil		27	35
	Black seed oil		11	25
Itraconazole	Turmeric oil	9	33	33
	Parsley oil		10	10
	Garlic oil		27	27
	Black seed oil		11	11

Molecular identification of the most inhibited MDR bacteria and yeast isolates.

Molecular identification of the most inhibited MDR bacterium (*Klebsiella pneumoniae*) by 16S rRNA

Klebsiella pneumoniae was the most dominant highly multidrug- resistant isolate from the nine MDR bacterial isolates and was the highly inhibited isolate by four of the tested essential oils. The identification of *K. pneumoniae* by VITEK 2 compact system was confirmed by molecular identification of 16S rRNA gene sequence using PCR. The results of identification were as shown in Fig. (3). By applying the biosystem 16S ribosomal RNA sequence, the tested Gram-negative bacterium isolate exhibited a similarity of 98% to the 16S ribosomal RNA sequence of *Klebsiella pneumoniae* subsp *pneumoniae* SUB11820947 *Klebsiella*-1 OP020451. The partial nucleotide sequence of 1021 bp 16s rDNA gene for *K. pneumoniae* isolate was done to determine the relationship with other recommended 16s DNA gene *K. pneumoniae* strains registered in GenBank. The sequencing was done from the forward direction at Mocrogen 3730X l6-1518-009 Korea Fig. (3).

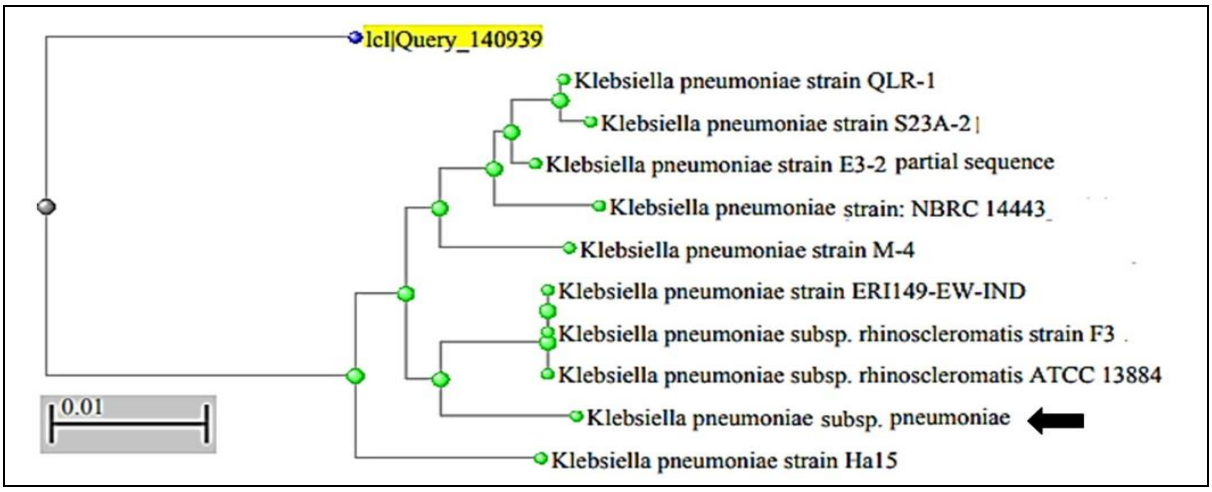


Fig. (3): Phylogenetic tree according to 16s rRNA gene for *K. pneumoniae* based on the nucleotide sequences.

Molecular identification of the most inhibited MDR yeast (*Candida albicans*) by 16S rRNA:

The pure band resulting from 18s rDNA required primer was partially sequenced. Sequences were then compared to the public database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). The comparison gave 98% identity to *Candida albicans* SUB11826412 Candida -1 OP023836. The partial nucleotide sequence of 361 bp 18s rDNA gene for *C. albicans* isolate was done to determine the relationship with other recommended 16s DNA gene *C. albicans* strains registered in GenBank. The sequencing was done from the forward direction at Mocrogen 3730X l6-1518-009 Korea Fig. (4).

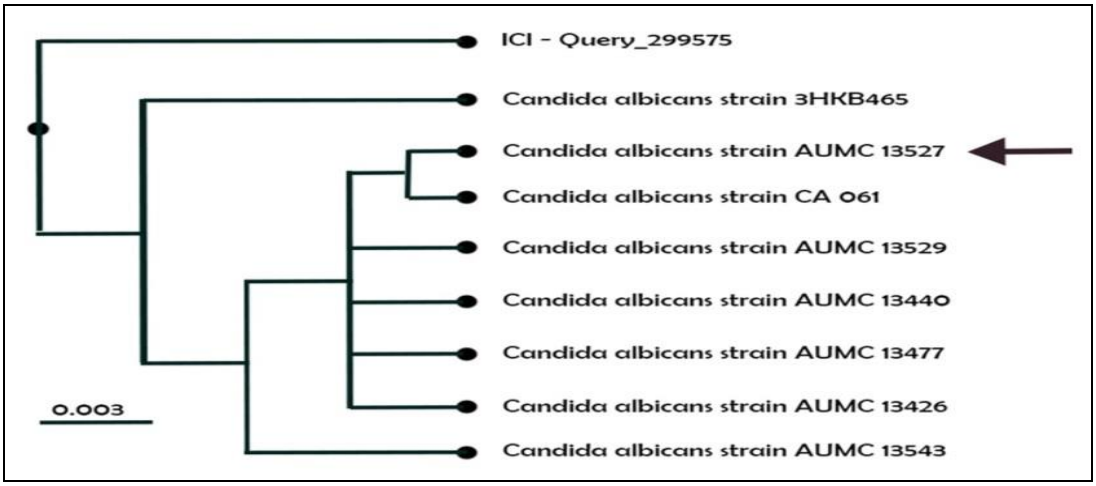


Fig. (4): Phylogenetic tree according to 16s rRNA gene for *C. albicans* based on the nucleotide sequences

Detection the antimicrobial effect of turmeric oil on the structure of the most inhibited pathogenic MDR microbial vaginitis using Scanning Electron Microscope (SEM)

The scanned images illustrated in (Fig. 5) showed severe damage to the tested microbe and lead to irregular cell shape with destroyed cell wall and shrinking of cells. Some of the cells were vacant, while others were flimsy. Additionally, most of them appeared to be melted and jammed together. *Klebsiella* that had been treated had enormous cells and appendages on their surface. Images of *Candida* (SEM) showed structures resembling pseudomycellium. The untreated (control) cells were whole and had a smooth surface. Generally, SEM images observations demonstrated physical damage and considerable morphological alteration to tested pathogenic microbial treated with turmeric oil extract.

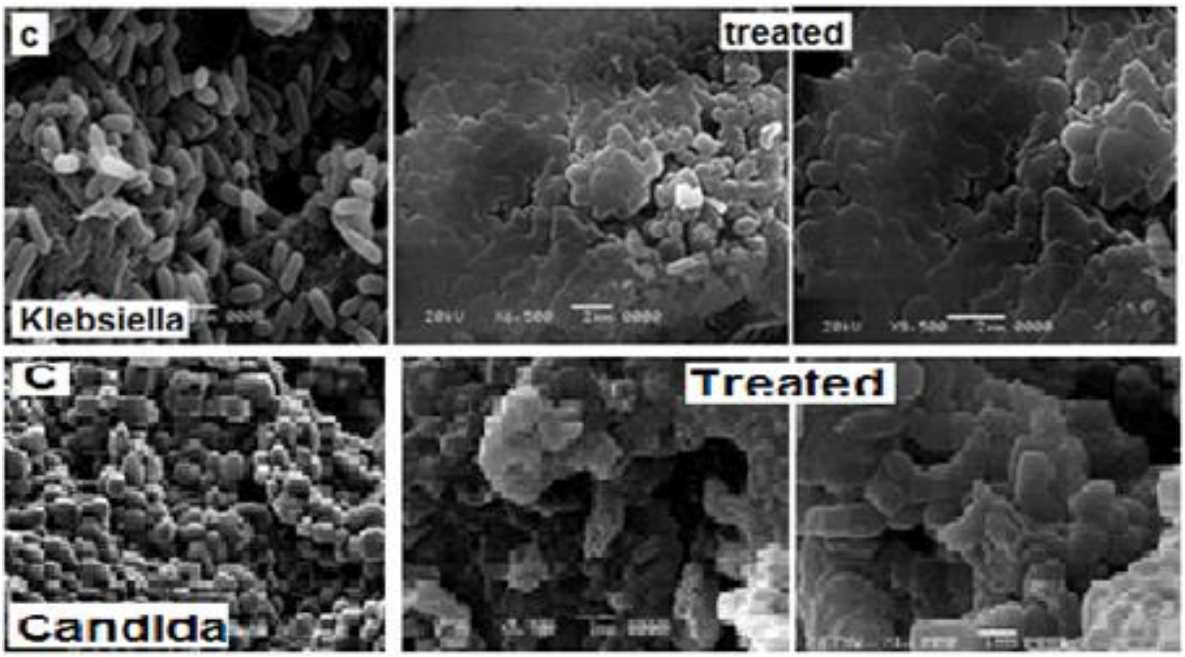


Fig. (5): Scanning Electron micrographs (SEM) showing morphological changes on *K. pneumonia* and *C. albicans* treated with turmeric oil extract. C: control cells.

Determination of the active antimicrobial materials produced by the most effective essenteial oils using Gas chromatographic-mass spectrometry analysis (GC- MS)

For confirmation of the antimicrobial activity of the turmeric oil against the tested vaginal pathogens GC-MS analysis was performed to detect the active ingredients and their concentrations in this extract, from table (7) and Fig. (6). The GC-MS determines the percentage and structure of major fragmentation ions component, Ar-turmerone as major ingredient 58.033%. The rest ingredients represented differential amounts as Curlone 14.000%, aromatic Ar-curcumene 7.025%, Phenol 4.515%, Zingiberene 3.480%, 1-Ethyl-4-isobutylbenzene 2.810%, α – Sesquiphellandrene 2.522%, 1,2,3,5-tetramethyl-Benzene 1.761%, Benzene 1.381%, Benzaldehyde 1.330%, α –Bisabolene 1.207%, 4-Methyl-carbanilonitrile 1.094% and saline 0.842%. Ar-turmerone, Curlone, and Ar-curcumene were represented with 58.033%, 14.000% and 7.025% respectively. These and other components in the analyzed oil were expected to be responsible for the previously recorded antimicrobial activity of turmeric oil against vaginal pathogens depending.

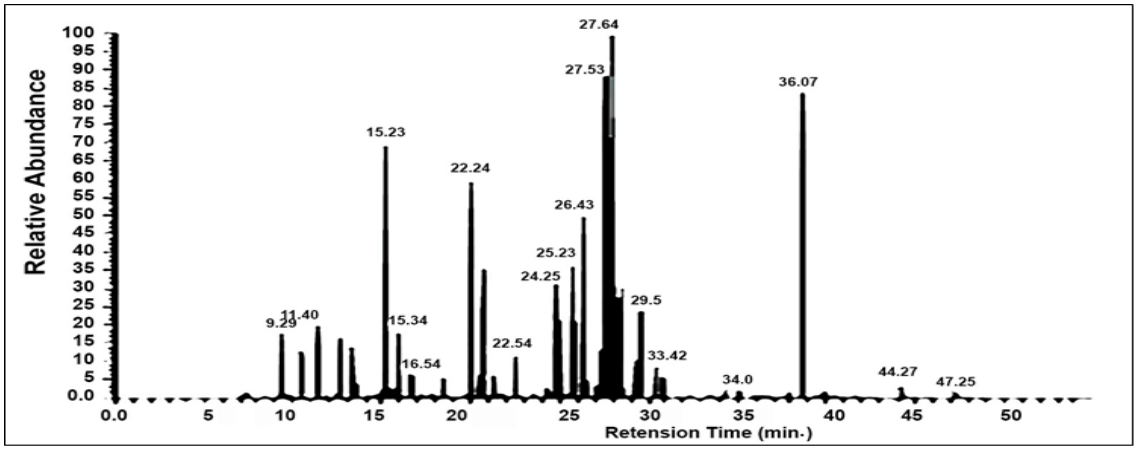


Fig. (6): GC-MS analysis profile of turmeric essential oil.

Table (7): The molecular formula of active components of turmeric oil (*Curcuma longa*)

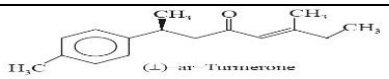
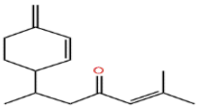
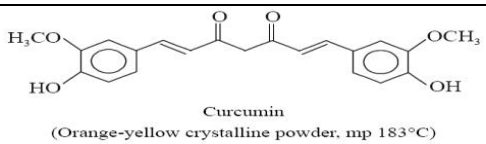
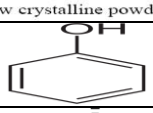
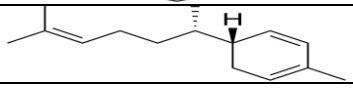
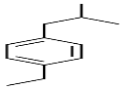
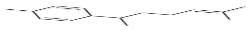
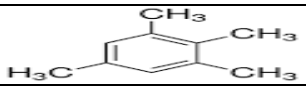
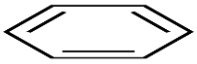
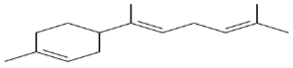
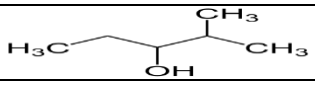
Major ingredient components		Percentage %
Ar-turmerone		**58.033**
Curlone		**14.000**
Ar-curcumene	 Curcumin (Orange-yellow crystalline powder, mp 183°C)	**7.025**
Phenol		**4.515**
Zingiberene		**3.480**
1-Ethyl-4-isobutyl benzene		**2.810**

Table continue:		
α - Sesquiphellandrene		**2.520**
1,2,3,5-tetramethyl-Benzene		**1.761**
Benzene		**1.381**
Benzaldehyde		**1.330**
α -Bisabolene		**1.207**
4-Methyl-carbanilonitrile		**1.094**
Saline	**NaCl**	**0.842**

Discussion

The present study aimed to investigate the efficacy of some natural essential oils on the microbial vaginitis collected from different clinical labs. Fifty clinical vaginal microbes (each containing several cases) were investigated for their load of isolated bacteria, fungi, and parasites. The results showed that 47 microbial isolates were identified using an automated VITEK2 compact system, the identified microbial isolates as nine genera of bacteria and one genus of *Candida*, one species only related to each genus of bacteria and two species related to genus *Candida*. The results recorded 20 *Klebsiella pneumoniae*, 6 *Escherichia coli*, 4 *Staphylococcus aureus*, 1 *Neisseria gonorrhoeae*, 2 *Pseudomonas aeruginosa*, 1 *Acinetobacter baumannii*, 5 *Lactobacillus plantarum*, 1 *Gardnerella vaginalis*, 6 *Candida albicans*, 1 *Candida lusitaniae* and 1 *Peptostreptococcus prevotii*. The study by **(Gokiladevi, 2019)** bacterial vaginosis was diagnosed in 48%, candidiasis in 24%, and Trichomoniasis in 3.3% of the cases by clinical examination. Microbiological diagnosis indicated pathological organisms in 62% of cases, whereas in 38% of the cases, the discharge was physiological **(Gokiladevi, 2019).** Among the pathological organisms the common isolates were bacterial vaginosis 82%, candidiasis 14% and parasites 4% **(Farhan *et al.*, 2017).**

The antibiotics sensitivity of the tested bacterial isolates showed different susceptibilities ranging from sensitive (S), intermediate (I) and resistant (R) reactions against 16 tested antibiotics. In this investigation, results showed that out of the thirty-seven pathogenic bacterial isolates, nine bacterial isolates exhibited multidrug resistance (MDR), theses bacterial isolates were *L. plantarum*, *A. baumannii*, *N. gonorrhoeae*, *G. vaginalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *P. prevotii*. The remaining isolates exhibited various sensitive reactions. The antibiotic sensitivity of the most predominant vaginal isolates *Klebsiella pneumonia* isolated from the fifth group was designated as a multidrug resistance organism because it showed resistance to 9 different antibiotics and it possessed intermediate sensitivity to 4 antibiotics with sensitivity to only 3 ones **(Barrett *et al.*, 1999).** *Neisseria gonorrhoeae* and *Peptostreptococcus prevotii* showed resistance same as *K. pneumonia*, however, they were not dominant isolates. This was coherent with studies in (**Delgado *et al.*, 2007)**, where they showed resistance of *N. gonorrhoeae* 37.5– 42.5%, 50– 94%, 25% and 50% resistance to ciprofloxacin, tetracycline, ampicillin, and erythromycin, respectively. In contrast, *Staphylococcus aureus* exhibited low levels of resistance to most of the tested

antibiotics as well as *Lactobacillus* **(Mwape *et al*., 2021).** The yeast isolates showed different susceptibilities ranging from sensitive (S) to resistant (R) reactions against the five tested antibiotics, Metronidazole (MTZ50), Fluconazole (FCA25), Itraconazole (IT30), Ketokenazole (KT10) and Nystatin (NS100). Two isolates out of the seven isolates of yeasts, *C. albicans* and *C. lusitaniae* showed MDR against Ketokenazole (KT10) and Itraconazole (IT30) of the used antibiotics respectively. The proportion of disease to be caused by *Candida lusitaniae* was about 2%. This proportion is like what was found in the most recent Centers for Disease Control and Prevention surveillance study **(Barrett *et al*., 1999)**. Fluconazole still tends to be quite active against most isolates of *Candida* spp. **(Tsega and mekonnen, 2019)** showed a relatively stable *C. albicans* sensitivity to fluconazole this indicates that there is no ongoing decrease in the rate of fluconazole susceptibility.

Seventeen antibacterial plant essential oils were tested against nine MDR bacterial isolates. The results showed that turmeric, garlic, black seed and parsley oils exhibited the highest antibacterial and antifungal activities against the MDR bacteria and yeast isolates. However, the same bacterium and yeast isolates showed resistance to onion, green tea, peppermint, clove, lavender, marjoram, anise, fenugreek, basil, fennel, eucalypts, olive, and sage oils without any inhibition zones. **(Enioutina *et al*., 2017). Azimi *et al*., (2011)** studied the effect of anti-candida and anti-bacterial by different medical plants. The results showed that turmeric, parsley, garlic and black seed oils exhibited the highest antibacterial and antifungal activities. The antimicrobial activity of *Nigella sativa* oil (black seed oil) is attributed mainly to its phenolic constituents of the essential oil **(Emeka *et al*., 2015).** Other constituents, oleoresins, linoleic acid, and oleic acid may also have minor antimicrobial activity (**Mohammed *et al*., 2019**).

Klebsiella pneumoniae was the most dominant highly multidrug- resistant isolate from the nine MDR bacterial isolates and was the highly inhibited isolate by four of the tested essential oils. The identification of *K. pneumoniae* by VITEK 2 compact system was confirmed by molecular identification of 16S rRNA gene sequence using PCR. By applying the biosystem 16S ribosomal RNA sequence, the tested Gram-negative bacterium isolate exhibited a similarity of 98% to the 16S ribosomal RNA sequence of *Klebsiella pneumoniae* subsp. *pneumoniae* SUB11820947 *Klebsiella*-1 OP020451. In the case of fungal isolation by using 18s rDNA gene the comparison gave 98% identity to *Candida albicans* SUB11826412 Candida -1 OP023836.

The combination of the four selected resisted antibiotics with each of the most potent essential oils (turmeric, parsley, garlic, and black seed oils) against the pathogenic MDR *K. pneumoniae*. The results showed that Levofloxacin antibiotic with all essential oils (garlic, parsley, black seed, and turmeric oils) had the highest inhibition activity against the pathogenic MDR K. pneumoniae, the combination of levofloxacin antibiotic and parsley oil demonstrated the greatest impact against *K. pneumoniae*. This result suggested that parsley oil and levofloxacin could be used to reduce *K. pneumoniae*'s resistance to the most efficient antimicrobial antibiotics **(Ribeiro *et al*., 2017).** On the other hand, the combination with parsley oils and black seed showed the best effect against *C. albicans.* The previous results showed the most effective combination between levofloxacin and nystatin antibiotics with parsley oils showed the highest effect against both MDR *K. pneumoniae* and *C. albicans.* A value of combined essential oil situated between additive and antagonistic tendencies signifies an indifferent effect **(El Atki *et al*., 2019; Sharma *et al*., 2020).** The results of the current study show synergistic effects of situated essential oil and antibiotic mixtures against the tested bacterial and yeast isolates.

The antimicrobial effect of turmeric oil on the structure of the most inhibited pathogenic MDR microbial vaginitis using Scanning Electron Microscope (SEM) showed

severe damage to the tested microbe and lead to irregular cell shape with destroyed cell wall and shrinking of cells. Some of the cells were vacant, while others were flimsy. Additionally, most of them appeared to be melted and jammed together. *Klebsiella* that had been treated had enormous cells and appendages on their surface **(Elhamy, *et al*., 2021).** Images of Candida (SEM) showed structures resembling pseudomycellium. The untreated (control) cells were whole and had a smooth surface. Generally, SEM images observations demonstrated physical damage and considerable morphological alteration to tested pathogenic microbial treated with turmeric oil with turmeric oil at concentration 0.781 mg/ml.

For confirmation of the antimicrobial activity of the turmeric oil against the tested vaginal pathogens GC-MS analysis was performed to detect the active ingredients and their concentrations in this extract. It could be seen that curcumins and essential oils make up the majority of the extracts' ingredients. The essential oils contain Ar-turmerone (58.033%), Curlone (14%) and Ar-curcumene (7.025%) **(Crowley *et al*., 2012).** Zingiberene (3.480%), phenol (4.51%), 1-Ethyl-4-isobutyl benzene (2.81%) and α – Sesquiphellandrene (2.52%) are the possibly active elements in essential oils that can cause apoptosis and have the potential to be used as new functional food ingredients for the treatment and prevention of non-small-cell lung cancer **(Ma and Hovy, 2016)**. Both in vivo and in vitro, the ar-tumerone inhibits the proliferation of Hep-2 laryngeal carcinoma cells. **(Candes, Romberg and Tao, 2006).** 1,2,3,5-tetramethyl-Benzene (1.76%), Benzene (1.38%), Benzaldehyde (1.33%), α -Bisabolene (1.2%) and 4-Methyl-carbanilonitrile (1.09%) in essential oil can protect against carbon tetrachloride (CCl_4)-induced liver fibrosis in rats by downregulating the expression levels of plasma endotoxin and serum tumor necrosis factor-α (TNF-α) (Qin *et al.*, 2011). Thirteen compounds have been identified in essential oils, and the oils display remarkable antibacterial activity against *S. aureus*, *L. monocytogenes*, *B. subtilis*, *P. aeruginosa*, *S. typhimurium* and *E. coli*, **(Rahman, Al-Reza and Kang, 2011).** Essential oil's recognized constituents are frequently utilized as quality control indicators. Only a few of the purified single ingredients have reasonably high pharmacological effects, according to the pharmacological examination of those ingredients. Additionally, several studies show that the chemicals found in many Chinese medicine formulae and plants combine synergistically **(Chen, *et al*., 2021).** However, as secondary metabolites, essential oils vary greatly depending on the originating species, planting environment, and planting method. The quality and production of components are greatly influenced by the agroclimatic elements of growing conditions, such as rainfall, temperature, humidity, and soil nutrients **(Yue *et al.*, 2016).**

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الأنشطة المضادة للميكروبات لبعض الزيوت الأساسية والمضادات الحيوية في التهاب المهبل الجرثومي المقاوم للأدوية المتعددة

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قسم النبات، كلية العلوم، جامعة طنطا، ٣١٥٢٧، مصر

هدف الدراسة الحالية هو التحقق من فاعلية بعض الزيوت العطرية الطبيعية على التهاب المهبل الميكروبي كعلاج بديل واعد. تم جمع خمسين حالة من التهاب المهبل الميكروبي من مختبرات إكلينيكية مختلفة وتم التعرف عليها كيميائياً باستخدام نظام VITEK 2 المدمج، وأظهرت النتائج أنه تم إحالة ٤١ عزلة بكتيرية

عبارة عن٣٧ عزلة بكتيرية انتهازية و ٤ عزلت بكتيريا متعايشة طبيعية، ٧ عزلات فطرية و ٢ عدوى طفيلية. تم إجراء اختبار حساسية المضادات الحيوية باستخدام ستة عشرمن المضادات الحيوية واسعة الطيف للعزلات البكتيرية المعزولة الممرضة و ٥ من عزلات الخميرة، وأظهرت النتائج أن تسع عزلات بكتيرية واثنين من الخميرة من أصل أربعة وأربعين عزلة أظهرت مقاومة للأدوية المتعددة (MDR). أظهرت نتائج الأنشطة المضادة للميكروبات للسبع عشر زيوت الأساسية المستخدمة ضد عزلات MDR الممرضة الأحد عشر. زيت الكركم كان أقوى الزيوت الأساسية ضد أكثر مسببات الأمراض انتشاراً MDR *Klebsiella pneumoniae* و *Candida albicans* ، والتي تم تأكيدها من خلال التعريف الجزيئي. أظهر الجمع بين الزيوت الأساسية الأكثر فاعلية (الكركم وزيوت البقدونس) والمضادات الحيوية المقاومة (الليفوفلوكساسين والنيستاتين) على عزلات MDR البكتيرية والخميرة السائدة زيادة في أقطار مناطق تثبيط *K. Pneumoniae* و *C. albicans* (زيادة من ٣٨ إلى ٤٠ مم لـبكتيريا كلبسيلا نيومونيي و ١٠ إلى ٢٤ مم لـخميرة كانديدا ألبيكانس) على التوالي. تم إجراء دراسة التأثيرالخلوي تحت المجهر الإلكتروني الماسح (SEM) لزيت الكركم، والذي تم تحليله بواسطة مقياس الطيف الكتلي الغازي (GC-MS) لتأكيد تركيبته الكيميائية من Aromatic-turmerone. خلصت الدراسة الحالية إلى أن استخدام زيت الكركم هو علاج بديل فعال كعامل مضاد للميكروبات عديدة المقاومة للمضادات الحيوية MDR المسببة لإلتهاب المهبل الميكروبي.