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# Incidence of Fungi Contaminating Some Medicinal Plants and Their Antimicrobial and Anticancer Properties at Qena Governorate, Egypt

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MEDICINAL plants contain big numbers of phytochemicals that make them widely used in folk medicine and food industry as aroma and flavor additives. Fungal contamination may occur at pre-and post-harvest. In the current investigation twenty-three fungal species related to 11 genera were isolated from 30 samples of medicinal plants by using the dilution plate method. The results revealed that fungi were abundance in marjoram samples followed by rosemary, and thyme. The most frequent species were *Aspergillus niger*, *A. flavus*, *Emericella nidulans*, *Penicillium chrysogenum*, and *Rhizopus stolonifer*. Rosemary methanol extract was the only effective one against *Escherichia coli* with the highest inhibition diameter (13 ± 0.5 mm). Thyme methanol extract achieved highly suppression of *Candida tropicalis* with the highest inhibition zone (31± 2 mm). N-butanol extract of the selected plants did not display any antifungal efficacy except marjoram extract slightly inhibited the tested pathogenic fungi. Interestingly, the tested plants significantly inhibited the proliferation of breast and lung tumor cells and rosemary extract showed the highest cytotoxicity against human causian breast adenocarcinoma (MCF-7) followed by marjoram and thyme was the lowest with $IC_{50}$: 8.9, 28.4, and 45.6 µg/mL, respectively. On the other hand, lung adenocarcinoma (A549) was highly inhibited by thyme extract then rosemary, and lastly, marjoram with ($IC_{50}$: 33.7, 36.3, and 40.4 µg/mL, respectively). Fourier Transform Infrared (FTIR) analysis of rosemary residue discovered the existence of a greater number of effective compounds than thyme and marjoram.

**Keywords:** Medicinal plants, Extract, Antifungal efficacy, Cytotoxicity, FTIR.

## Introduction

Recently, herbal medicine and using medicinal plant extracts as an alternative of chemicalshas gainedgreat attention to biocontrol microorganisms (Bidaki et al., 2015 and Raeisi et al., 2019). Developing countries depend mainly on herbal medicine as a cheap source to treat health problems (Nimri et al., 1999). *Thymus vulgaris* L. (Thyme) is a therapeutic plant belonging to the family Lamiaceae is used in traditional folk therapies such as analgesic, carminative, antiseptic, antioxidant, antifungal, antibacterial, and also isused in the food industry as a flavor and toraise their shelf-life (Ramezani et al., 2004; Shokri et al., 2006; Babaie et al., 2007; Assiri et al., 2016). Currently, thyme used in the treatment of cough,stomachache, flatulence, bronchitis, candidiasis, laryngitis, and trichomoniasis (Sajed et al., 2013). Rosemary (*Rosmarinus officinalis*, family Lamiaceae) is a small perennial shrub and occurs all over the world with long, sharp, and slightly rough leaves (Mirheydar, 2001 and Atti-Santos et al., 2005). It is used broadly in pharmaceutical products and traditional medicinal

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purposes. Additionally, rosemary is broadly used as a food preservative and additive due to antimicrobial and antioxidant activities, and desirable flavor (Mirheydar, 2001; Tavassoli et al., 2011; Azizkhani & Tooryan, 2015). *Origanum majorana* L. (marjoram) belonging to the family Lamiaceae. Commercially used as a spice and characterized by a wide range of volatile secondary metabolites. It is conventionally used to cure asthma, dizziness, headache, rheumatism, indigestion, gastrointestinal disorder, and migraine. It showed efficiency to inhibit food-borne bacteria and mycotoxigenic fungi and so, it is used widely in industrial applications (Busatta et al., 2008; Mohamed & Mansour, 2012; Abdel-Massih & Abraham, 2014).

Soil is the source of medicinal plant raw materials fungal contamination, and addition contaminationduring harvesting, handling and production practices (Abou-Arab et al., 1999). The most common fungal pollutants of medicinal plants and spices are *Aspergillus* and *Penicillium* species (Silliker et al., 1992).

Dietary plant products and their phytochemicals with antioxidant, anti-inflammatory, and immunomodulatory activity can reduce the growth and spread of cancer (Kapinova et al., 2017; Kapinova et al., 2018). Recently, the anticancer prospective of rosemary, thyme and marjoram and their major compounds has been discussed (Al Dhaheri et al., 2013; Moore et al., 2016; Benhalilou et al., 2019).

The Fourier transform infrared (FTIR) spectroscopy is a physic-chemical analytical methodthat has confirmed to be a beneficialway for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts (Surewicz et al., 1993; Maobe & Nyarango, 2013; Imad et al., 2015).

The present investigationdesigned to isolation and identification of the mycobiota contaminating 30 samples of medicinal plants. The antimicrobial efficacy of the selected plants extracts against pathogenic fungi and bacteria was evaluated. Antitumor potential of these plants was also examined against human breast and lung adenocarcinoma. Active compounds in tested plants were monitored by FTIR.

## Materials and Methods

*Materials*

Thirty samples of thyme, rosemary, and marjoram (10 samples of each plant) were purchased from different supermarkets inQena city, Egypt. All samples were kept in a refrigerator until mycological analysis.All chemicals and solvents from different suppliers for analytical grade.

*Methods*

*Isolation of fungi*

Dilution plate method was performed for isolation of fungi associated with the selected medicinal plants as mentioned by Christensen (1963). A known weight of each sample was suspended in 100 mL sterilized distilled water. Serial dilutions were made to obtain the suitable one. One mL of the appropriate dilution was poured in a sterilized petri plate followed by 20 mL of potato dextrose agar medium (PDA) which was obtained by mixing 39 g of PDA powder in 1000 mL of distilled water. Triplicates were prepared for each sample and plates were incubated at 28 °C for one week. The developed fungal colonies were counted, examined,and identified (based on macro-and microscopic features) (Domsch et al., 2007).

*Preparation of extracts*

Ten grams of the testedmedicinal plants powder were sequentially extracted with 100 mL methanol and n-butanol at room temperature for 24 hr on a shaker incubator. The solvent concentrated under vacuum, weighted and kept in a refrigerator (4 °C) for antimicrobial assays.The tested extracts solutions were obtained by adding dimethylsulphoxide (DMSO) to the dried residues to obtain the used concentrations (Ozcan, 1998).

*Antibacterial efficacy of medicinal plants extracts*

Four human pathogenic bacterial strains{*Escherichia coli*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (MRSA)} were gained from the International Luxor Hospital in Luxor Governorate, Egypt. Disc diffusion method was performed to determine the antibacterial activity of the tested plant's extracts. Every strain of selected bacteria was grown on nutrient broth. The optical density ($OD_{595}$) was adjusted to 0.001. 100 µL of the adjusted bacterial culture weretransferred and spread on 20 mL of nutrient agar plate. Antibacterial efficiency of the fixed extracts was evaluated by using the agar diffusion technique. Sterile cork borer was used to make Cavities of 8 mm diameter in nutrient agar. 50 µL of the different used concentrations (50 mg/mL, 100 mg/mL, and 200 mg/mL) were added into the cavities on nutrient agar. 10% DMSO was used

as control (Kirkwood et al., 2018). cultures were incubated for 24 hr at 37 °C (Baydar et al., 2004). The inhibition diameter was estimated using a ruler.

*Antifungal activity of medicinalplants extracts*

The susceptibility of 2 fungi (*Fusarium solani*, *Alternaria alternata*) and one human pathogenic fungal strains (*Candida tropicalis*) obtained from Microbiology Laboratory, Faculty of Agriculture, South Valley University, Qena, Egypt, and *C. tropicalis* was isolated from the vagina of patients women at Qena to tested plants extracts was evaluated by using disc diffusion method. The selectedfungal isolates were grown on Potato Dextrose Agar (PDA) medium for 7 days. 10 mL of sterile water was added to the plate and gently joggling and dislodging spores into a 50 mL Erlenmeyer flask with glass beads. The Erlenmeyer flask was shaken for 1 hr and the spore suspension was filtered through two layers of sterile cheesecloth to remove mycelial remains. Spores concentration was set to $10^7$ spores/ mL using a hemocytometer. The antifungal activity was estimated by pouring 20 mL PDA on sterilized Petridish containing 1 mL of fungal spore suspension. After media solidifying 3 cavities were made in each plate and supplemented with 50 µl of the used concentrations (50 mg/mL, 100 mg/mL, and 200 mg/mL) then incubated at 28±2 °C for 5 days plates (Maria et al., 2005).

*Antitumor potential of tested medicinal plants*

This trial was performed by the Bioassay-Cell Culture Laboratory, Department of Pharmacognosy, National Research Centre, Dokki, Giza, Egypt.

*Cell lines*

The cytotoxic effect of tested medicinal plants methanol extracts was evaluated against human caucasian breast adenocarcinoma (MCF7) and lung adenocarcinoma (A549) cell lines

*MTT assay*

The method mentioned by Mosmann (1983) was used to determine cell viability by color change of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan due to mitochondrial dependent reduction. Cells were suspended in RPMI 1640 medium (for MCF7 and A549 cell lines) supplemented with 1% antibiotic-antimycotic mixture (10,000U/mL Potassium Penicillin, 10,000µg/mL Streptomycin Sulfate and 25µg/mL Amphotericin B) and 1% L-glutamine at 37 ºC underneath 5% CO2. Cells were groupcultivated for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h below 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was removed, fresh medium (without serum) was added.Cells without plant extract (negative control) andtreated cells with different concentrations of plant extract (100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/mL) were incubated. After 48 h of incubation, medium was aspirated, each well was supplemented with 40ul MTT salt (2.5µg/mL), and incubated for extra4 h at 37ºC beneath 5% CO2. 200µL of 10% Sodium dodecyl sulphate (SDS) was added to end the reaction and dissolving the made crystals, and incubated overnight at 37ºC. *Annona cherimolia* extract (100µg/mL) was used as a natural cytotoxic positive control thatkills 100% of the cells under the similar conditions (El-Menshawi et al., 2010). Multi-well reader microplate was used for measuring the absorbance at 595nm. A statistical significance was verified between samples and negative control using independent t-test by SPSS 11 program. Plant extracts residues were dissolved in DMSO with concentration fewer than 0.2%. The percentage of the alternation in cell viability was countedas follows: ((Reading of treatment / Reading of negative control) -1) x 100

*Determination of bioactive groups by Fourier transform infrared spectrometer (FTIR)*

The presence of bioactive functional groups in the residue of thyme, rosemary, and marjoram methanol extract was discovered by FTIR. Magna-FTIR 560 (USA) instrumentwas used at 2 $cm^{-1}$resolution ranged from 400 to 4,000 in KBr pellet using diffuse reflectance mode operated by Nicolet Omnic Software® as manufacturer›s instructions.

## Results and Discussion

*Mycobiota contaminating medicinal plants*

Twenty-three fungal species belonging to 11 genera were isolated from the collected 30 samples of medicinal plants by using the dilution plate method on PDA medium. Marjoram samples recorded the highest number of genera and species (19 fungal species comprising 7 genera). Rosemary samplescame in the second position with 13 fungal species and 5 genera. The lowest number of genera and species was observed in thyme samples (9 species including 6 genera) (Table 1). Our results are greatly similar with the previous data obtained by Wójcik-Stopczyńska et al. (2009) who found that the marjoram samples recorded the maximum number of molds ($10^3$

CFU/ g). Marjoram was highly contaminated with fungi than cinnamon and clove (Kocić-Tanackov et al., 2007). In contrast, mold and yeast count range in thyme leaves ($7.0 \times 10^3$–$1.6 \times 10^4$ CFU/ g) was higher than those obtained from marjoram leaves ($2.0 \times 10^3$–$1.8 \times 10^4$ CFU/ g) (Tournas & Katsoudas, 2008). *Aspergillus* was the most dominant genus as it was detected in 100% ofthe samples. *A. niger* was the most common species followed by *A. flavus*. *Emericella nidulans* and *Penicillium* were detected in the three tested medicinal plants with high occurrence in marjoram samples. *P. chrysogenum* was the most predominant species (Table 1). *Rhizopus stolonifer* was isolated from 80% of thyme, 60% of rosemary, and 40% of marjoram samples. In a past study by El Shafiae et al. (2002) confirmed that *A. flavus*, *A. niger*, *Penicillium*, *Rhizopus* were the dominant fungi contaminating herbs and spices.Tournas and Katsoudas (2008) mentioned that*A. niger*, *A. flavus* and *Penicillium* spp. were the most frequently fungi recovered from marjoram samples.

*Antibacterial efficacy of medicinal plants extracts*

The obtained results confirmed that the tested medicinal plant extracts displayedan assorted degrees of bacterial growth inhibition depending on the type of plant and extraction solvent and extractconcentration (Table 2). Methanol extracts of tested medicinal plants were more effective than n-butanol extracts and rosemary methanol extract was the most effective (Table 2). The 3 tested concentrations of rosemary methanol extract depressed the growth of the 4 selected human pathogens. *S. aureus* (MRSA) was highly sensitive to rosemary methanol extract with the highest inhibition diameter (27±1 mm) followed by *A. baumanii* (15± 2 mm) then *E. coli* (13± 0.5 mm) and *P. aeurognisa* showed the lowest sensitivity with the highest inhibition diameter (10± 0 mm) at the highest concentration (200 mg/mL) (Table 2). In contrast, methanol extract of thyme and marjoram did not exhibit any antibacterial efficacy against*E. coli*and*P. aeurognisa* (Table 2). Also, n-butanol extract of the tested plants did not show any inhibitory effect against *E. coli* and *P. aeurognisa* (Table 2). our results proved that methanol extract of the selected plants in general, was most effective than n-butanol extracts. The type and content of some compounds and the used concentrations can affect the antimicrobial efficiencies of extracts (Matsuzaki et al., 2013; Abdulaziz et al., 2015; Sabzikar et al., 2020). Rosemary methanol extract was the only extract that suppressed *E. coli*. The obtained results were in accordance with an earlier study by Moreno et al.(2006), who reported that rosemary methanol extract recorded the greatest inhibition against *E. coli* in comparison with acetone and aqueous extracts whereas methanol and acetone extracts showed fungistatic activity against tested yeasts. Abdel-Massih et al. (2010) proved that*Rosmarinus officinalis* extract showed higher antibacterial activity against resistant *E. coli* than *Origanum majorana*extract.

*Antifungal susceptibility*

Our results revealed that n-butanol extract of rosemary and thyme did not show any inhibitory effect against the tested fungi but marjoram n-butanol extract slightly inhibited *A. alternata* and *C. tropicalis* growth (Table 3). Rosemary methanol extract did not affect *A. alternata* growth.In contrast, *F. solani* and *C. tropicalis* growth were suppressed by rosemary methanol extract with inhibition diameter (19± 2.64 and 19± 1.32 mm, respectively) was observed at the highest concentration (200 mg/mL). Thyme methanol extract highly suppressed*C. tropicalis* and the inhibition diameter increase by increasing the concentration with maximum inhibition diameter (31±2 mm) . In contrast, *F. solani* was resistant to all the tested concentrations. *A. alternata* slightly affected and the maximum inhibition diameter (16±0 mm) at concentration 200 mg/mL. N-butanol marjoram extract was more effective than methanol extract and recorded the highest inhibition diameter (17± 3.12 mm and17±0.5 mm) for both *A. alternata* and *C. tropicalis*, respectively at concentration, 200 mg/mL. The lowest concentration of marjoram methanol extract did not exhibit any suppressive activity against the tested fungi and the highest concentrations showed a low inhibitory effect (Table 3). In harmony with our obtained dataSacchetti et al. (2005) showed that *Thymus vulgaris* essential oil was more effective than *Rosmarinus officinalis* essential oil against *Candida albicans*and yeasts. Also, Sabzikar et al. (2020) proved the same results that rosemary ethanol extract showed lower antifungal activity against *C. albicans* than thyme extract. Soil-borne and foliar pathogenic fungi were highly inhibited by *Thymus vulgaris*, *Majorana syriaca* and *Origanum syriacum* essential oils (Sokovic et al., 2002).

**TABLE 1. Average total count (ATC), percentage (% C), frequency (F%) and number of cases of isolation (NCI) of mycobiota contaminating 30 samples of *Thymus vulgaris, Rosmarinus officinalis*, and *Origanum majorana* medicinal plants (10 samples for each type) on potato dextrose agar medium (PDA) at 28 °C for 7 days.**

| Fungal genera and species | *Thymus vulgaris* (Thyme) | | | | *Rosmarinus officinalis* (Rosemary) | | | | *Origanum majorana* (Marjoram) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ATC | %C | F% | NCI | ATC | %C | F% | NCI | ATC | %C | F% | NCI |
| *Alternaria alternata* | - | - | - | - | 200 | 1.39% | 10 | 1 | - | - | - | - |
| *Aspergillus* | 9300 | 56.7% | 100 | 10 | 8100 | 56.25% | 100 | 10 | 23900 | 69.27% | 100 | 10 |
| *A. candidus* | - | - | - | - | 200 | 1.39% | 10 | 1 | 700 | 2.03% | 30 | 3 |
| *A. carenus* | 200 | 1.2% | 10 | 1 | - | - | - | - | 400 | 1.16% | | |
| *A. fumigatus* | - | - | - | - | 300 | 2.08% | 30 | 3 | 1000 | 2.9% | 50 | 5 |
| *A. flavus* | 1800 | 10.98% | 40 | 4 | 500 | 3.47% | 30 | 3 | 3300 | 9.6% | 70 | 7 |
| *A. niger* | 7100 | 43.29% | 100 | 10 | 6300 | 43.75% | 100 | 10 | 15700 | 45.5% | 100 | 10 |
| *A. ochraceus* | - | - | - | - | 400 | 2.8% | 10 | 1 | 700 | 2.03% | 30 | 3 |
| *A. sydowii* | - | - | - | - | - | - | - | | 1000 | 2.9% | 20 | 2 |
| *A. terreus* | 200 | 1.2% | 10 | 1 | 400 | 2.8% | 10 | 1 | 1100 | 3.19% | 10 | 4 |
| *Chaetomium globosum* | - | - | - | - | 700 | 4.86% | 20 | 2 | - | - | - | - |
| *Circinella muscae* | - | - | - | - | - | - | - | - | 200 | 0.58% | 10 | 1 |
| *Emericella nidulans* | 800 | 4.88% | 10 | 1 | 500 | 3.47% | 20 | 2 | 4400 | 12.75% | 80 | 8 |
| *Fusarium moniliforme* | 400 | 2.44% | 20 | 2 | - | - | - | - | - | - | - | - |
| *F. oxysporum* | - | - | - | - | - | - | - | - | 200 | 0.58% | 10 | 1 |
| *F. solani* | - | - | | | - | - | - | - | 600 | 1.74% | 30 | 3 |
| *Mucor circinelloides* | - | - | - | - | - | - | - | - | 200 | 0.58% | 10 | 1 |
| *M. racemosus* | | - | - | - | - | - | - | - | 200 | 0.58% | 10 | 1 |
| *Paecilomyces variotti* | 200 | 1.2% | 10 | 1 | - | - | - | - | - | - | - | - |
| *Penicillium* | 1200 | 7.32% | 20 | 2 | 900 | 6.25% | 40 | 4 | 3400 | 9.86% | 80 | 8 |
| *P. chrysogenum* | 1200 | 7.32% | 20 | 2 | 700 | 4.86% | 40 | 4 | 1800 | 5.22% | 70 | 7 |
| *P. citrinum* | - | - | - | - | - | - | - | - | 200 | 0.58% | 10 | 1 |
| *P. corylophilum* | - | - | - | - | 200 | 1.39% | 10 | 1 | 800 | 2.32% | 30 | 3 |
| *P. oxalicum* | - | - | - | - | - | - | - | - | 600 | 1.74% | 30 | 3 |
| *Rhizopus stolonifer* | 4500 | 27.44% | 80 | 8 | 1800 | 12.5% | 60 | 6 | 1400 | 4.06% | 40 | 4 |
| *Sterile mycelia* | - | - | - | - | 1700 | 11.8% | 60 | 6 | - | - | - | - |
| *Ulocladium atrum* | - | - | - | - | 500 | 3.47% | 20 | 2 | - | - | - | - |
| Total | 16400 | 100% | | | 14400 | 100% | | | 34500 | 100% | | |
| Number of genera = 11 | 6 | | | | 5 | | | | 7 | | | |
| Number of species = 23 | 9 | | | | 13 | | | | 19 | | | |

**TABLE 2. Antibacterial efficacy of Medicinal plants organic extracts**

| Medicinal plant | | Tested bacteria | *E. coli* | *A. baumanii* | *P. aeurognisa* | *S. aureus* **(MRSA)** |
|---|---|---|---|---|---|---|
| Thyme | methanol | 50 mg/mL | NI | 11±0.5 mm | NI | NI |
| | | 100 mg/mL | NI | 12±1.32 mm | NI | 111.04± mm |
| | | 200 mg/mL | NI | 14±1 mm | NI | 252.5± mm |
| | n-butanol | 50 mg/mL | NI | 12±0.5 mm | NI | NI |
| | | 100 mg/mL | NI | 14±0.5 mm | NI | 151.32± mm |
| | | 200 mg/mL | NI | 16±1 mm | NI | 170± mm |
| Rosemary | methanol | 50 mg/mL | 80± mm | 11±0.5 mm | NI | 152.5± mm |
| | | 100 mg/mL | 102± mm | 13±1.04 mm | 80± mm | 151.5± mm |
| | | 200 mg/mL | 130.5± mm | 15±2 mm | 100± mm | 271± mm |
| | n-butanol | 50 mg/mL | NI | 12±0 mm | NI | NI |
| | | 100 mg/mL | NI | 14±2.5 mm | NI | 150± mm |
| | | 200 mg/mL | NI | 16±1 mm | NI | 170.5± mm |
| Marjoram | methanol | 50 mg/mL | NI | 11±1.73 mm | NI | 101± mm |
| | | 100 mg/mL | NI | 13±2.5 mm | NI | 130± mm |
| | | 200 mg/mL | NI | 15±2.5 mm | NI | 140.5± mm |
| | n-butanol | 50 mg/mL | NI | 10±0 mm | NI | NI |
| | | 100 mg/mL | NI | 12±1.5 mm | NI | 100.5± mm |
| | | 200 mg/mL | NI | 13±0 mm | NI | 190.5± mm |

Means of inhibition diameter ±SD. NI: No inhibition

**TABLE 3. Antifungal activity of Medicinal plants organic extracts**

| Medicinal plant | | Tested fungi | *F. solani* | *A alternata* | *C. tropicalis* |
|---|---|---|---|---|---|
| Thyme | methanol | 50 mg/mL | NI | NI | 27.5 ± 0 mm |
| | | 100 mg/mL | NI | 13.5±3.5 mm | 29.5±1 mm |
| | | 200 mg/mL | NI | 16±0 mm | 31±2 mm |
| | n-butanol | 50 mg/mL | NI | NI | NI |
| | | 100 mg/mL | NI | NI | NI |
| | | 200 mg/mL | NI | NI | NI |
| Rosemary | methanol | 50 mg/mL | NI | NI | NI |
| | | 100 mg/mL | 100± mm | NI | 11±1 mm |
| | | 200 mg/mL | 192.64± mm | NI | 19±1.32 mm |
| | n-butanol | 50 mg/mL | NI | NI | NI |
| | | 100 mg/mL | NI | NI | NI |
| | | 200 mg/mL | NI | NI | NI |
| Marjoram | methanol | 50 mg/mL | NI | NI | NI |
| | | 100 mg/mL | 102.29± mm | NI | 10±0.76 mm |
| | | 200 mg/mL | 11.54.44± mm | 13±1.80mm | 14±0 mm |
| | n-butanol | 50 mg/mL | NI | 10±0.5 mm | 12±1 mm |
| | | 100 mg/mL | NI | 14±0.76 mm | 15±1.80 mm |
| | | 200 mg/mL | 100± mm | 17±3.12 mm | 17±0.5 mm |

Means of inhibition diameter ±SD.NI: No inhibition

*Cytotoxicity of the tested medicinal plants*

The cytotoxic potential of the selected medicinal plants methanol extract against human caucasian breast adenocarcinoma (MCF7) and lung carcinoma (A549) cell lines was evaluated (Table 4; Fig. 1, 2). The obtained results revealed that rosemaryextract showed the highest anti-proliferative potential towards MCF7 with $IC_{50}$= 8.9 µg/mL and $IC_{90}$= 15.8 µg/mL. Followed by marjoram with $IC_{50}$= 28.4 µg/mL and $IC_{90}$= 49.0 µg/mL. Thyme showed the lowest cytotoxic activity with $IC_{50}$= 45.6 µg/mL and $IC_{90}$= 67.9 µg/mL (Table 4; Fig 1). Thyme was the most effective against lung adenocarcinoma (A549) followed by rosemary and marjoram with $IC_{50}$= 33.7, 36.3, 40.4 µg/mL and $IC_{90}$= 57.0, 58.4, 64.8 µg/mL, respectively (Table 4; Fig 2). Cancer is the most critical disease confronted by the mankind. Various methods are used to treat cancer but using herbal medicine remains the safest method due to their availability in nature containing a variety in chemical compounds with greaterefficiency against cancer and minor side effects (Lachenmayer et al., 2010 and Rafieian-Kopaie & Nasri, 2015). Rosemary methanol extract showed the highest antitumor efficacy against breast adenocarcinoma MCF-7 cell lines in comparison with the other 16 tested plants (Badisa et al., 2003). Hussain et al. (2010), reported that *Rosmarinus officinalis* showed antiproliferative activity against human breast cancer and prostate carcinoma cell lines. Rosemary extract showed antitumor potential towards A549 lung cell line and enhanced apoptosis through decreasing phosphorylated/ activated Akt, mTOR and p70S6K levels (Zhu et al., 2016). Also, Moore et al. (2016) reported that rosemary extract showed anti-tumorgenic activity against A549 human lung carcinoma cell line. A previous study by Abaza et al. (2015) proved that naringenin compound purified from *Thymus vulgaris* suppressed the proliferation of human breast cancer cells. Hussain et al. (2013) found that the essential oils of *T. linearis* and *T. serpyllum* showed antitumor activity against human breast adenocarcinoma (MCF-7). *Thymus serpyllum* hexane extract showed high anticancer activity against two breast tumor cell lines; MCF7 and MDA-MB-231, and A549 lung carcinoma cell line (Baig et al., 2014). *T. serpyllum* rosmarinic acid oppressed lung metastasis through activation AMP-activated protein kinase enzyme (Han et al., 2018). *Origanum majorana* is rich in phytochemicals with antitumor efficacy such as luteolin, ß-caryophyllene, quercetin, and rosmarinic acid (Hossain et al., 2014; Bina & Rahimi, 2017; Benhalilou et al., 2019). Luteolin, is the major flavonoid present in *O. majorana* extract with potent anticancer activity against breast, lung, colon, and liver cancer cells (Attoub et al., 2011). Al Dhaheri et al. (2013) reported that the noncytotoxic concentrations of *O. majorana* showed effective anti-metastatic efficiencies against MDA-MB-231breast cancer cell line.

*Determination of bioactive groups in tested medicinal plants by FTIR*

The FTIR spectrum was used for recognizing the functional groups in the selected plant's extracts.The infra-red spectra of various extracts of the tested plants were recorded by a Magna-FTIR 560 (USA) and execute under the Infra-red region between the ranges of 400-4000 $cm^{-1}$. Dominant peaks intensities and the wavenumber ($cm^{-1}$)were recorded from absorption spectra obtained for medicinal plant powders. FTIR analysis of rosemary powder showed absorption bands at 3365, 2929, 1614, 1517, 1455, 1037, 718 $cm^{-1}$. The peak position at 3365 $cm^{-1}$ is due to OH-group stretch, hydrogen-bonded (Alcohol). The peak at 2929 $cm^{-1}$ reflects the presence of C-H stretch of alkanes and carboxylic acid (O-H) and the band at 1614 $cm^{-1}$ correspond to C=C stretching conjugated alkenes. The peak at 1517 $cm^{-1}$ reflects the presence of N-O asymmetric stretch. Band at wave number 1455 $cm^{-1}$ corresponding to alkanes C-H bend. The peak at 1037 $cm^{-1}$reveals the presence of C-O stretch and C-N (amine). The peak position at 718 $cm^{-1}$ corresponding to C-H bend alkene and alkyl halide (C-CL) (Fig. 3A).

**TABLE 4. Antitumor potential of Medicinal plants methanol extract against MCF7 and A549 cell lines**

| Medicinal plant scientific name | $IC_{50}$ µg/mL | | $IC_{90}$ µg/mL | |
|---|---|---|---|---|
| | MCF7 | A549 | MCF7 | A549 |
| *Thymus vulgaris* L. | 45.6 | 33.7 | 67.9 | 57.0 |
| *Origanum majorana* L. | 28.4 | 40.4 | 49.0 | 64.8 |
| *Rosmarinus officinalis* L. | 8.9 | 36.3 | 15.8 | 58.4 |

The FTIR spectra of the thyme plant showed the following absorption bands, band peaking at 3431cm$^{-1}$ corresponding to OH stretching bands of alcohols/phenol vibrations. Band at 2932 cm$^{-1}$ reflects the presence of C-H of symmetric and asymmetric stretching alkanes. The spectra observed at 1635 cm$^{-1}$, 1435 cm$^{-1}$, 1258 cm$^{-1}$, 1038 cm$^{-1}$ and 525 cm$^{-1}$ corresponding to C=C stretching conjugated alkenes, C=C stretching vibration of the Medicinal ring, C-O stretching Medicinal esters, C-N stretching amines and alkyl halide (C-Br), respectively (Fig. 3B). The FTIR spectra of marjoram powder are presented in (Fig. 3C). The obtained peaks at 3381 correspond to N-H (Amines). The wave number 2929 cm$^{-1}$ corresponding to C-H stretching of alkanes. Bands at 1636 cm$^{-1}$, 1384 cm$^{-1}$, 1068 cm$^{-1}$, and 719 cm$^{-1}$ relate to C=C conjugated alkenes and amide I, C-N stretching amines, stretching vibration absorption peaks of C-O and C-O-C, and C=C bending alkenes, respectively. The highest efficiency of the selected medicinal plants may be due to the occurrence of a large number of bioactive compounds confirmed by FTIR analysis. Highest numberfounded in rosemary followed by thyme and marjoram was the lowest and this indicate the greatest antimicrobial and antitumor activity of rosemary methanol extract (Fig. 3 A, B, C). Aldehydes, Alkanes, Alkenes, Alcohols, Carboxylic acids, Nitro compounds, Aliphatic fluoro compounds, Ethers, Esters, ketones compounds were obtained by FTIR analysis of rosemary leaves (Hameed et al., 2015). The main constituents of rosemary oils are 1,8-cineole, α-pinene, camphor, p-cymene-7-ol, and borneol (Bendeddouche et al., 2011; Jiang et al., 2011; Sienkiewicz et al., 2013). In a previous study by Valderrama and Rojas De (2017), who reported the presence of peak bands at 807, 945, 1087, 1153 and 1289 cm$^{-1}$ in *Thymus vulgaris* essential oil confirming that thymol is the main component by ATR-FTIR analysis. The presence of thymol and carvacrol as the main constituents of thyme essential oil reflects their antimicrobial efficacy (Marino et al., 1999; Mehdizadeh et al., 2012). FTIR analysis of *T. linearis* methanol extract discovered the incidence of alcohols, carboxylic acids, amides, ketones, ethers (Naz et al., 2015). Mohammadian et al.(2018)confirmed the incidence of many compounds in marjoram extract used in green synthesis of ZnO-NPs. Marjoram essential oil main components are terpinene, terpinen-4-ol and cis-sabinene hydrate (Baranska et al., 2005).

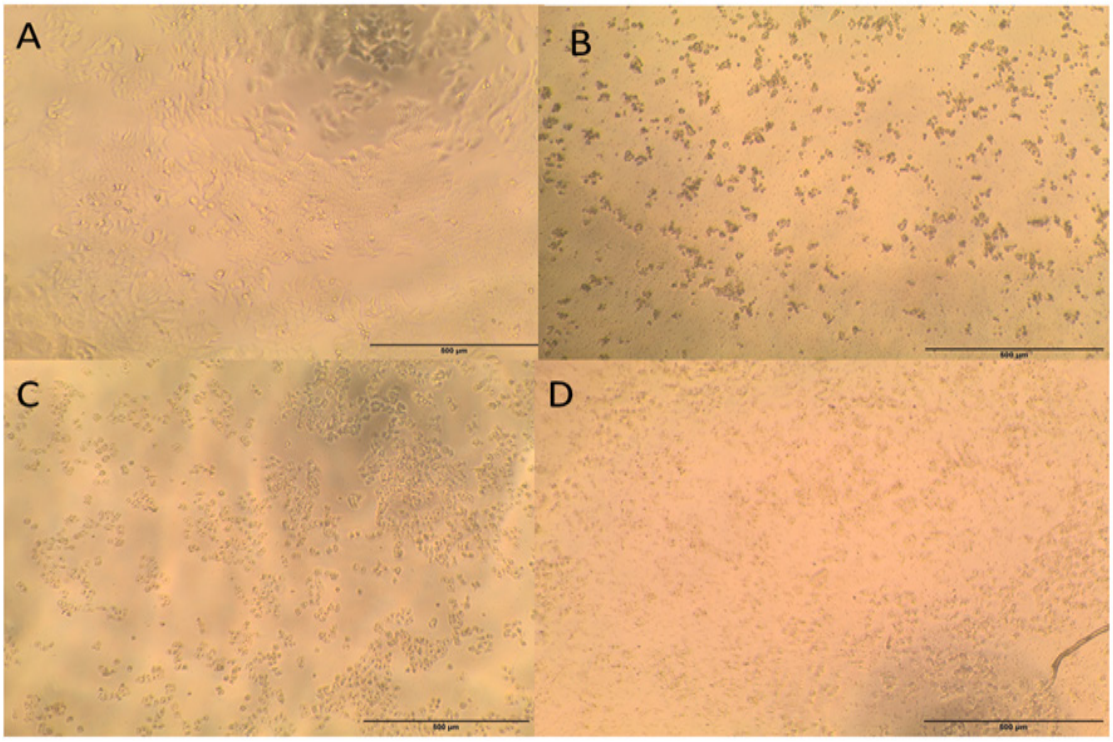


**Fig. 1. Antitumor efficacy of medicinal plants methanol extracts against MCF-7 cell line; A: control, B: thyme, C: marjoram, D: rosemary at 100 µg/mL.**

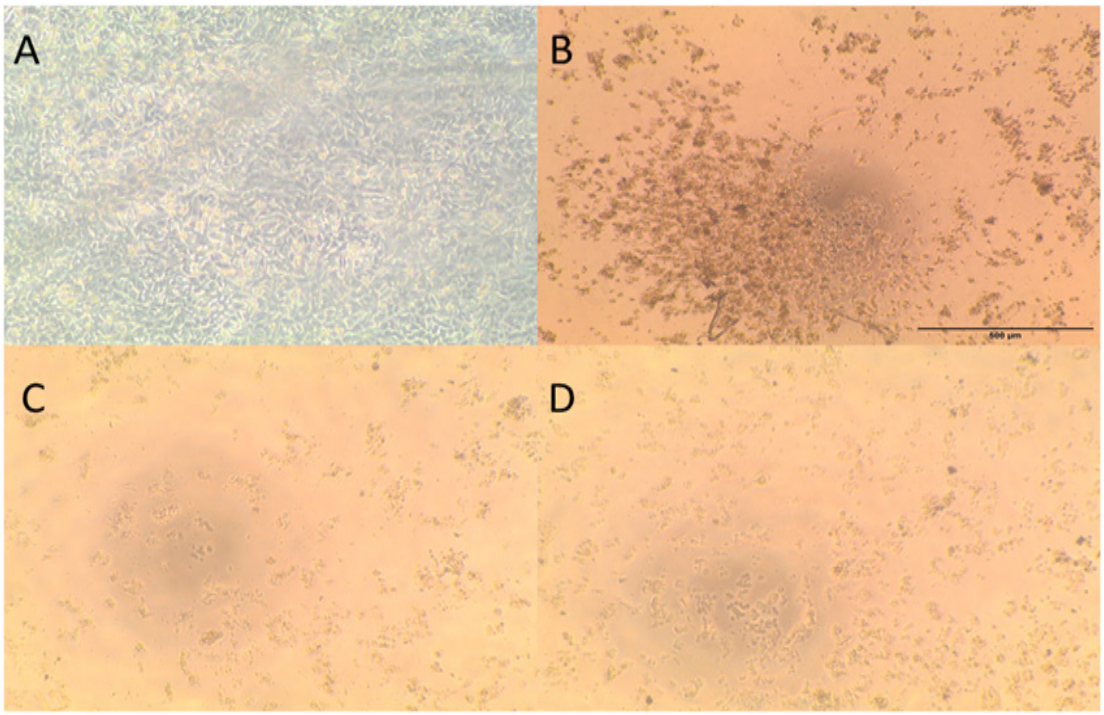


**Fig. 2. Antitumor efficacy of medicinal plants methanol extracts against A549 cell line; A: control, B: thyme, C: marjoram, D: rosemary at 100 µg/mL**

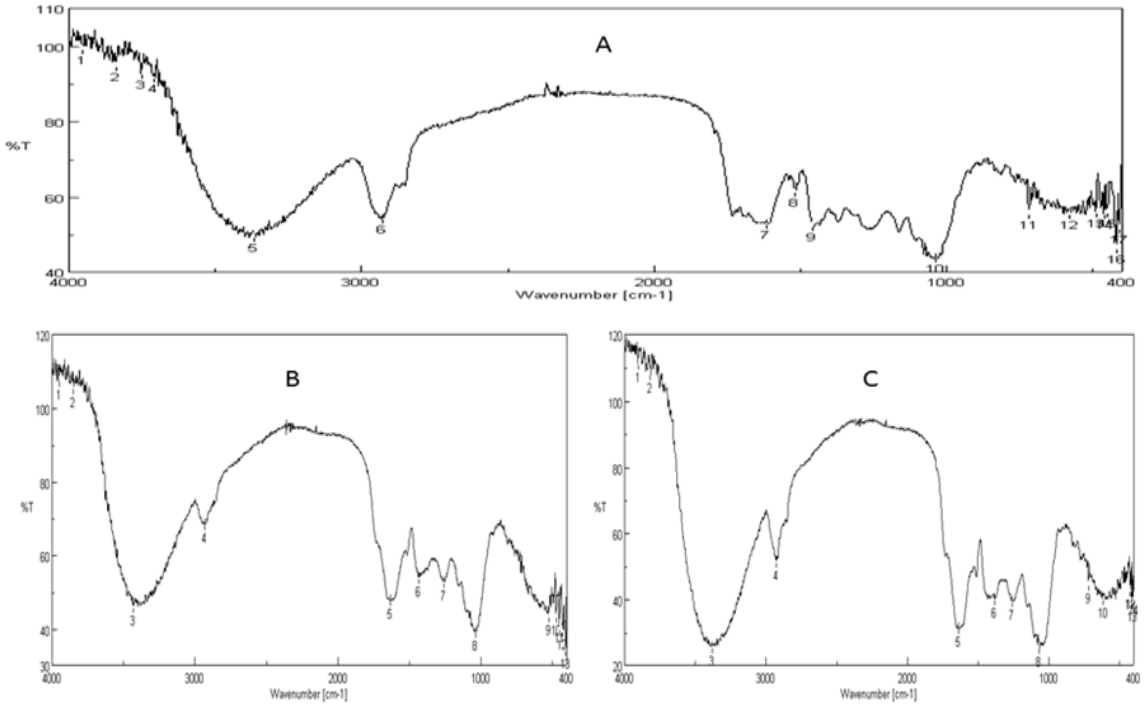


**Fig. 3. Fourier Transform infrared (FTIR) spectroscopy of tested medicinal plants. (A): rosemary, (B): thyme and (C): marjoram.**

## Conclusions

The current study confirmed that marjoram was heavily contaminated with fungi than rosemary and thyme. Methanol extracts showed higher antimicrobial efficiency than n-butanol and rosemary recorded the highest efficacy. All the tested plants showed antitumor activity against breast adenocarcinoma (MCF-7) and lung adenocarcinoma (A549) cell lines. Rosemary showed the highest antitumorigenic activity against MCF-7 cellline but thyme was the most effective against A549 cell line. FTIR analysis revealed the incidence of a large number of active compounds in rosemary that reflects their highest antimicrobial and antitumor efficacy.

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# انتشار الفطريات الملوثة لبعض النباتات الطبية وخصائصهاالمضادة للميكروبات والسرطان بمحافظة قنا, مصر

**أسماء صبري يسين**

قسم النبات والميكروبيولوجي - كلية العلوم - جامعة جنوب الوادي- قنا ٨٣٥٢٣ - مصر

تستهدف تلك الدراسة عزل وتعريف الفلورا الفطرية الملوثة ل ٣٠ عينة من النباتات الطبية حيث تم عزل ٢٣ نوعا فطريا ينتمى الى ١١ جنسا باستخدام طريقة التخفيف. كانت الفطريات اكثر عددا في عينات البردقوش ثم يليه الروزمارى والزعتر. وجد ان اكثر الفطريات شيوعا هى: اسبرجيللس نيجر, اسبرجيللس فلافس, ايميرسيلا نيدولانس, بنسيلليوم كريزوجينم و ريزوبس استولينيفر. مستخلص الميثانول لنبات الروزمارى هو المستخلص الوحيد الذى اظهر فعالية ضد الايشيرشيا كولاى بأقصى قطر تثبيط (١٣ مم). اظهر مستخلص الميثانول لنبات الزعتر اعلى تثبط لنمو الكانديدا تروبيكلس بأقصى قطر تثبيط (٣١ مم). مستخلصات الان-بيوتانول لتلك النباتات لم تظهر أي فعالية باستثناء مستخلص نبات البردقوش اظهر تثبيط بسيط لنمو الفطريات الممرضة. ومن المثير للاهتمام, مقدرة تلك النباتات على منع تكاثر خلايا اورام الثدي والرئة واظهر نبات الروزمارى اعلى نسبة سمية خلوية ضد سرطان الثدي البشرى المسبب (ام سي اف-٧) يليه البردقوش والزعتر كان الاقل وكانت التركيزات المثبطة لنمو ٥٠٪ من تلك الخلايا (٨,٩, ٢٨,٤ و ٤٥,٦ ميكروجرام/مل, على التوالي). على الجانب الاخر, تم تثبيط سرطان الرئة بشكل كبير بواسطة مستخلص الزعتر ثم الروزمارى واخيرا البردقوش وكانت التركيزات المثبطة لنمو ٥٠٪ من تلك الخلايا (٣٣,٧, ٣٦,٣ و ٤٠,٤ ميكروجرام/مل, على التوالي). اكتشف تحليل فوريه لتحويل الاشعة تحت الحمراء (اف تي اى ار) لنبات الروزمارى وجود عدد اكبر من المركبات الفعالة من الزعتر والبردقوش.