Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790
On Line ISSN:2805-2528

FJARD VOL. 38, NO. 1. PP. 36-44 (2024)

Antimicrobial activity of two natural plants grown in Egyptian environment: *Ipomoea carnea* and *Tamarix nilotica*

Tarek Kamal El-Sawah[1,2*], Rabea Mohamed El-Shahawy[2], Ashraf Ibrahim Nageeb[1] and Khaled Mohamed Atalla[2]

[1] Horticultural Crops Research Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

[2] Department of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Egypt.

ABSTRACT:

Plants are the largest biochemical and pharmaceutical stores ever known on our planet. Some medicinal plants have not found wider application and sometimes are referred as "forgotten plants". Antimicrobial activity of *Ipomoea carnea* and *Tamarix nilotica* were evaluated by disk diffusion method and broth microdilution method. Extraction was performed by ethanol 50%. Results showed antibacterial activity of *Tamarix nilotica* extract with inhibition zones; 15, 14 and 19 mm against *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*, respectively. *Ipomoea carnea* extract revealed a good effect against *Staph. aureus* with 18 mm inhibition zone and MIC value against *Staph. aureus* was 8 mg/ml. Gram positive bacteria; *B. cereus* and *L. monocytogenes* showed MIC value at 16 mg/ml likewise *E. coli* was at 16 mg/ml. *Pseudomonas aeruginosa* and *Salmonella typhimurium* were inhibited at 64 and 32 mg/ml, respectively. Gram positive strains were also susceptible to *Tamarix nilotica* extract with MIC values from 8 to 32 mg/ml. Gram negative strains were more resistant as MIC value were at 64 and 128 mg/ml. Bactericidal activity (MLC) at 16 mg/ml for both extracts against *Staph. aureus* while *P. aeruginosa* showed MIC at 128 mg/ml with *Tamarix nilotica* extract. Fungal strains were more resistant to the tested plant extracts whereas *Ipomoea carnea* extract showed MIC value 16 mg/ml against *Penicillium italicum* while *Aspergillus flavus* and *Rhizopus stolonifera* not inhibited at the highest concentration 128 mg/ml the yeast strains: *Saccharomyces cerevisiae* and *Candida albicans* were inhibited at 64 mg/ml. *Tamarix nilotica* extract showed MIC values ranged from 64 to 128 mg/ml against all tested fungal strains. It is reasonable to assessing their applicability and benefits using modern scientific analysis methods.

KEYWORDS: Antimicrobial, plant extracts, MIC, MLC, disk diffusion method.

*Corresponding author Email: tk1128@fayoum.edu.eg
Received:13 /11/ 2023 - Accepted: 7/11/ 2023 - Published:18/2/2024

1. INTRODUCTION:

The contemporary trend in modern science is to explore the multiple advantages of scattered plants, in order to benefit from every part of it which seems has no value **(Elbossaty, 2020).** In recent years, one of the most active areas of research is the search for natural components of plants with potent biological activity and low toxicity **(Yili et al., 2014).** *Ipomoea carnea*, the pink morning glory, is a species of morning glory. *Ipomoea carnea,* belongs to Convolvulaceae family. *Ipomoea carnea* was recorded along canals and drains, road sides, railways, waste lands and fluid edges in the Nile Delta. Many *Ipomoea* species having antimicrobial activities were reported in literature (**Khatiwora et al., 2010 and Kamal et al., 2017).**

Tamarix nilotica is a native plant in Egypt with a long history known as Nile tamarisk belonging to the Tamaricaceae family. *Tamarix* name may have referred to the Tamaris river in Spain and *nilotica* referred to the valley of the Nile. *T. nilotica* was known in Egypt as Tarfa or Abal or Nile tamarisk, in Saudi Arabia and Palestine as Athel. *T. nilotica* widespread in Egypt growing in saline sandy soils on the edges of salt marshes coastal and inland sandy plains and Nile banks. Many compounds have been isolated from *T. nilotica* including carbohydrates, phenols, flavonoids, terpenoids, steroids, tannins, and cardiac glycosides. A significant antibacterial and antifungal activity was related to *Tamarix nilotica* extract. The pure compounds (flavone and dihydroflavonol) isolated from *Tamarix nilotica* extract also revealed significant antibacterial activity and antifungal activity **(Abdelgawad, 2017).**

2. MATERIALS AND METHODS:

2.1. Preparation of extracts

A portion of 10 grams of the dried ground plant was extracted with 100 ml of ethanol 50% by soaking for 48 hours **(Yusof** and **Saat, 2017).** Samples were filtered and the filtrates were evaporated at temperature below 50°C. The residues (crude extract) were weighed, and then reconstituted to prepare a stock solution with known concentrations for the following procedures.

2.2. Microbial strains

Microorganisms used in this study including: Three Gram positive bacterial strains: *Bacillus cereus* ATCC 33018, *Listeria monocytogenes* ATCC 19115 and *Staphylococcus aureus* ATCC 20231. Three Gram negative bacterial strains: *Escherichia coli* 0157:H7 ATCC 6933, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028. Six fungal strains including four molds: *Penicillium italicum* RCMB 03924, *Aspergillus flavus* ATCC 247, *Aspergillus niger* ATCC 16404, *Rhizopus stolonifer* ATCC 14037 and two yeast strains: *Saccharomyces cerevisiae* ATCC 4126 and *Candida albicans* RCMB 05031. All strains were obtained from Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, and Regional Center for Mycology and Biotechnology (RCMB), Al Azhar University, Cairo, Egypt.

2.3. Antimicrobial effect of plant extract by disk diffusion method

Disks were impregnated with 10 mg/disk of each plant crude extract. Mueller Hinton agar (MHA) medium was used with bacterial strains and potato dextrose agar PDA medium was used with fungal strains **(Owayss et al., 2020)**. Incubation for bacterial strains was at 37°C for 24 hours while was at 28 - 30°C for 2 - 3 days for fungal strains. Inoculation of plates was made by swabbing agar surface with fresh

pure cultures of tested bacterial and fungal strains **(Elbanna et al., 2014)**. Disks of examined plant extracts were directly put down in each plate of bacterial strain using clean and aseptic forceps. Clear zones of inhibition around disks were measured in mm after incubation.

2.4. Antimicrobial effect of plant extract by broth microdilution method (MIC & MLC)

2.4.1. Broth microdilution method for tested bacteria

The method of **EUCAST Discussion Document E. Dis 5.1, 2003** as described by European Committee for Antimicrobial Susceptibility Testing, were selected using microtiter plate 96 wells with some modification in stock solution preparation of examined extracts. The solvent ethanol 50% was selected to make several dilutions for MIC determination of each tested extract as they were 1, 2, 4, 8, 16, 32, 64, 128 mg/ml in the final concentration. Mueller Hinton broth medium was inoculated with examined bacterial strains, incubated at 37^{o}C for 24 hours. The bacterial count was determined visually through comparing the turbidity of growth to McFarland standards (that represents a known number of bacteria in suspension) by spectrophotometer and then diluted to the appropriate bacterial count in double strength Mueller Hinton broth MHB. Standardization of each bacterial strain according to **EUCAST Discussion Document E. Dis 5.1, 2003** method was performed to adjust the count to 1×10^{6} cfu/ml which will be 5×10^{5} in final concentration. 100 µl of each concentration of plant extracts were added to each well of the microtiter plate and 100 µl of adjusted bacterial strain count was inoculated to each well of all tested plant extract concentrations and moving the plate softly like number 8 several times for blending and homogenizing components. It is noteworthy that each microtiter plate inoculated with only one strain of tested microorganisms to avoid cross contamination. After 24 hours incubation, the microtiter plates were examined visually for turbidity, indicating the growth of the microorganism. The microorganism was grown in the control wells and in other wells that do not contain adequate concentration of extracts that required for growth inhibition. The lowest concentration of the agent that inhibits growth of the bacteria, as detected by absence of visual turbidity (matching the negative growth control), is considered as the minimum inhibitory concentration (MIC). An aliquot 100 µl from each of wells of broth that showed no visible turbidity after 24 hours incubation was sub-cultured to the surface of MHA plates. The count of grown colonies on this subculture after overnight incubation was compared to the number of cfu/ml in the original inocula. Within those wells that showed no turbidity, microorganisms were either still viable or they were killed by the antimicrobial agent. The lowest concentration of antimicrobial agent that allowed less than 0.1% of the original inocula count to survive was deemed minimum bactericidal concentration (MBC), also called the minimum lethal concentration (MLC). Some of the tested plant extracts caused turbidity in the broth medium which interferes with the determination of MIC. So, MLC was determined and in this case, the preceding concentration was considered as the MIC of the tested plant extract **(Atalla, 1998)**.

2.4.2. Broth microdilution method for tested fungi

The method of **EUCAST Definitive Document E. Def 9.3.2, 2020** as described by European Committee for Antimicrobial Susceptibility Testing, were selected using microtiter plate for testing molds and the method of **EUCAST Definitive Document**

E. Def 7.3.2, 2020 for testing yeasts with some modification following the previously mentioned procedure with bacteria in addition to use PDB as growth medium. Potato dextrose broth medium was used with examined fungal strains. The fungal count was adjusted using spore suspension method for molds as the fungal strain sub cultured on PDA slant and incubate at 35ºC for 2-5 days for adequate sporulation. 5 ml of sterilized distilled water were added to the slant to cover the growth, then rubbing the colonies of mold carefully using a sterile cotton swab and transfer the solution of spores to a sterile tube with micropipette and by a vortex mixer for 15 seconds homogenization of suspension was performed. Hemocytometer slide used to count the spores to adjust the count at 2 - 5 × 10^5 cfu/ml by dilution of the suspension with double strength PDB. For yeast strains, fresh culture was prepared with 24-48 hours incubation time. The count was adjusted by hemocytometer count of original suspension and diluted with double strength PDB. Standardization of each fungal strain was performed to adjust the count to 2 - 5 ×10^5 cfu/ml which will be 1-2.5×10^5 in final concentration for both molds and yeasts after inoculation of microtiter plate wells. The previously mentioned procedure with bacteria was used with fungal strains to inoculate wells with different concentrations of tested plant extracts starting with 8 mg/ml till 128 mg/ml. After 24-48 hours incubation for yeasts and 2-5 days for molds, the microtiter plates were examined visually for growth or turbidity, indicating growth of the microorganism. The lowest concentration of the agent that inhibits growth of the fungal strains, as detected by absence of visual turbidity (matching the negative growth control), is considered as the minimum inhibitory concentration (MIC). Minimum lethal (fungicidal) concentration was determined by subculture from clear wells with no fungal growth or non-turbid wells.

3. RESULTS AND DISCUSSION:

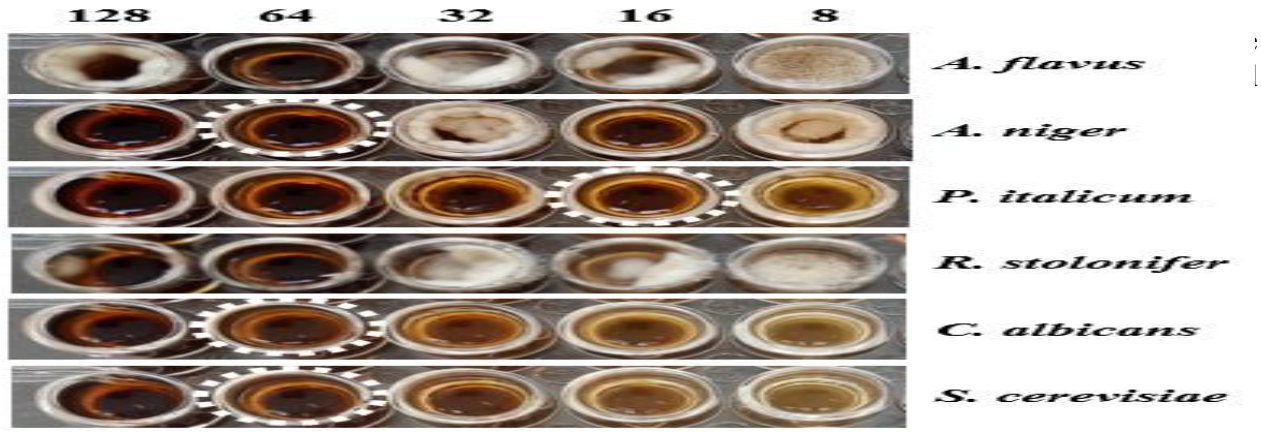
3.1. Antimicrobial activity by disk diffusion method

The contemporary trend for pharmaceutics, cosmetics, and food industries are interested in utilization of medicinal plants **(Sakkas and Papadopoulou, 2017)**. Results in Table (1) showed that the plant extracts were active against only Gram positive bacterial strains at concentration 10 mg/disk whereas better antibacterial activity of *Tamarix nilotica* extract was obtained with inhibition zones; 15, 14 and 19 mm against *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*, respectively. *Ipomoea carnea* extract revealed a good effect against *Staphylococcus aureus* with 18 mm inhibition zone and 11 mm against *Listeria monocytogenes* while other bacterial and fungal strains resist the effect of the tested plant extracts.

Table 1. The inhibition zone diameter (mm) of ethanol 50% extracts of studied plants against tested bacteria.

Plant Extract	Inhibition zone diameter (mm)					
	B.c	***E.c***	***L.m***	***P.a***	***Sa.t***	***St.a***
Ipomoea carnea	0.0	0.0	11.0	0.0	0.0	18.0
Tamarix nilotica	15.0	0.0	14.0	0.0	0.0	19.0

B.c=Bacillus cereus, E.c= Escherichia coli, L.m=Listeria monocytogenes, P.a=Pseudomonas aeruginosa, Sa.t=Salmonella typhimurium, St.a=Staphylococcus aureus



Escherichia coli	16	64
Listeria monocytogenes	16	64
Pseudomonas aeruginosa	64	128
Salmonella typhimurium	32	64
Staphylococcus aureus	8	16

Data in Table (2) showed that *Staph. aureus* was the most susceptible strain to the plant extract since it was inhibited at 8 mg/ml. Both Gram positive bacteria; *B. cereus* and *L. monocytogenes* were suppressed at 16 mg/ml. Likewise *E. coli* was also inhibited at 16 mg/ml while *P. aeruginosa* and *S. typhimurium* were inhibited at 64 and 32 mg/ml, respectively. The plant extract showed bactericidal activity at 32 mg/ml for *B. cereus* and 16 mg/ml for *Staph. aureus*. The rest of studied strains were responded at 64 mg/ml as bactericidal effect while *P. aeruginosa* at 128 mg/ml. Similar findings by **Hasan et al., 2015** who evaluated the antimicrobial activities of *Ipomoea carnea* spp*. fistulosa* which was carried out against *Staph. aureus*, *E. coli*, *Candida albicans* and *Penicillium chrysogenum*. The methanolic extract from the leaves and its n-butanol fraction exhibited higher indicative antibacterial activity. 2-C-methyl-D-erythritol and quercetin were isolated for the first time, in addition to *β*-sitosterol, umbelliferon, kaempferol-3-*O*-glucoside (astragalin) and swainsonine alkaloid.

Fig 1. MIC values of *Ipomoea carnea* extract against tested bacteria by broth microdilution method.

Table 3. MIC and MLC values of *Ipomoea carnea* extract against tested fungal strains.

Fungal strain	MIC	MLC
Aspergillus flavus	NI	NI
Aspergillus niger	64	>128
Penicillium italicum	16	128
Rhizopus stolonifer	NI	NI
Candida albicans	64	>128
Saccharomyces cerevisiae	64	>128

Results in Table (3) indicated that *P. italicum* was inhibited at 16 mg/ml while *A. niger* was inhibited with more concentration at 64 mg/ml. *A. flavus* and *R. stolonifer* resist all examined concentrations with no inhibition effect. The yeast strains *Candida albicans* and *S. cerevisiae* were inhibited at 64 mg/ml. With the exception of *P. italicum*, all tested fungal strains weren't affected as fungicidal effect by the studied concentrations.

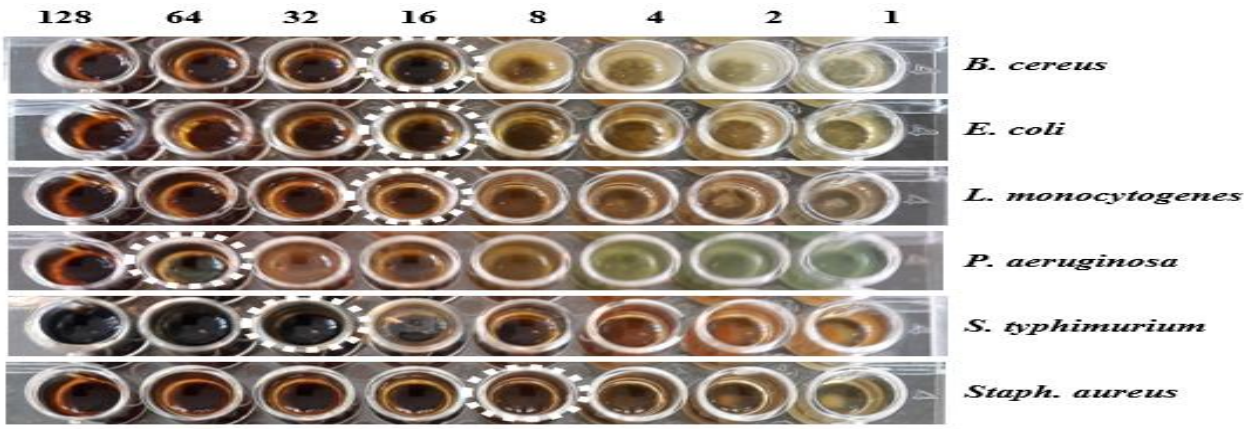


Fig 2. MIC values of *Ipomoea carnea* extract against tested fungal strains by broth microdilution method.

3.2.2. *Tamarix nilotica*

Table 4. MIC and MLC values of *Tamarix nilotica* extract against tested bacterial strains.

Bacterial strain	MIC	MLC
Bacillus cereus	32	64
Escherichia coli	64	128
Listeria monocytogenes	16	128
Pseudomonas aeruginosa	128	>128
Salmonella typhimurium	64	128
Staphylococcus aureus	8	16

Results in Table (4) indicated that *Staph. aureus*, *L. monocytogenes* and *B. cereus* were more susceptible to tested plant extract with inhibition zones; 8, 16 and 32 mg/ml, respectively. Gram negative strains were more resistant as MIC value were at 64 and 128 mg/ml. Bactericidal activity appeared at relatively high concentrations for all tested strains except *Staph. aureus* which obtained at 16 mg/ml while *P. aeruginosa* was inhibited only at the highest concentration 128 mg/ml with no lethal effect of all tested concentrations. These results in agreement with **Mustafa et al., 2018** who revealed that the antibacterial activity of *Tamarix nilotica* was found at MIC value 7.5 mg/ml, belonged to *Staph. aureus* and *P. aeruginosa*. In contrary they added that most susceptible Gram-negative bacteria were *P. aeruginosa* and least susceptible were *E. coli* and *S. typhi*. Moreover, they found that most and least susceptible Gram-positive bacteria were *Staph. aureus* and *B. cereus*, respectively. They added that phytochemical analysis of the ethanol extract of the leaves were Steroids, phenolic compounds, saponins, tannins, flavonoids and sugars.

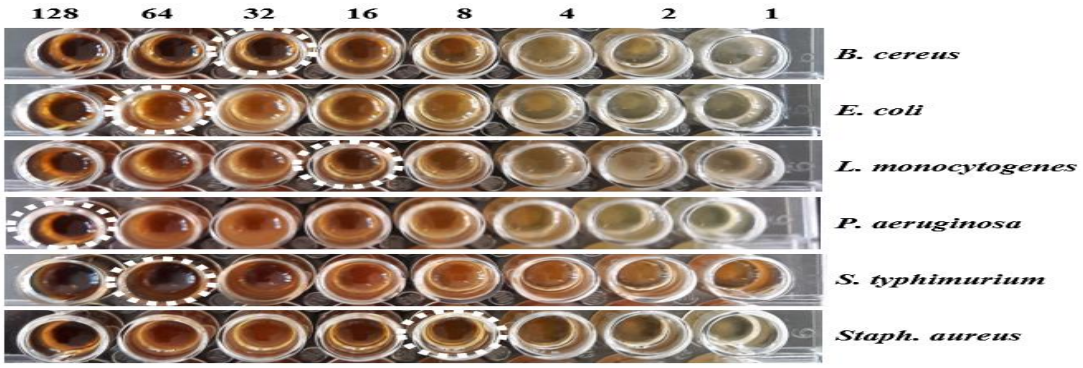


Fig 3. MIC values of *Tamarix nilotica* extract against tested bacteria by broth microdilution method.

Table 5: MIC and MLC values of *Tamarix nilotica* extract against tested fungal strains.

Fungal strain	MIC	MLC
Aspergillus flavus	128	>128
Aspergillus niger	64	>128
Penicillium italicum	64	>128
Rhizopus stolonifer	128	>128
Candida albicans	64	128
Saccharomyces cerevisiae	64	128

Data in Table (5) exhibited the antifungal activity of *Tamarix nilotica* extract against studied mold and yeast strains whereas all strains were inhibited at concentrations 64 and 128 mg/ml. *A. flavus* and *R. stolonifer* were inhibited at 128 mg/ml while other strains inhibited at 64 mg/ml. Fungicidal activity was observed against yeast strains: *Candida albicans* and *S. cerevisiae* at 128 mg/ml.

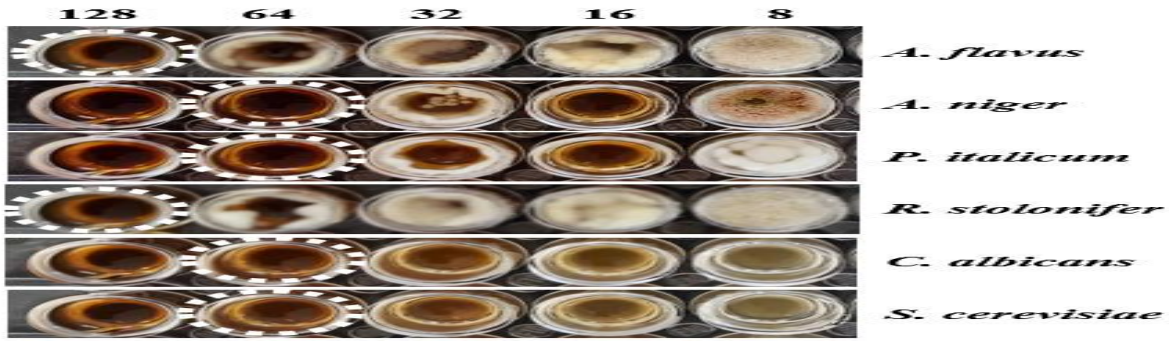


Fig 4. MIC values of *Tamarix nilotica* extract against tested fungal strains by broth microdilution method.

CONCLUSION :

Ipomoea carnea and *Tamarix nilotica* ethanolic extracts showed good antimicrobial activity against all tested microbial strains with various degrees of sensitivity as fungal strains were more resistant to the plant extracts. Furthermore, Gram negative bacterial strains were sensitive to the extracts at relatively high concentrations than Gram positive bacteria.

In view of the obtained results , it could be recommended that to study these plants furtherly to detect additional values and benefits to human health.

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