



## SCREENING OF SOME EGYPTIAN PLANT EXTRACTS FOR BIOLOGICAL ACTIVITY AGAINST SOME PATHOGENIC BACTERIA

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**Keywords:** Irradiation, Antibacterial Plant extracts,  
Pathogenic bacteria

### ABSTRACT

Plants and plant by-products are now gaining attractiveness in treatment of bacterial infections and food preservation. The objective of this study was to assess antibacterial activity of some Egyptian plant and plant by-products against the locally pathogenic isolates from patients having infectious diseases in our country. Screening of antibacterial activity of ethanol, methanol and hexane extracts of some plants: grape leaves (*Vitis vinifera*), mulberry leaves (*Morus alba*), mallow leaves (*Corchorus olitorius*) and lemon leaves (*Citrus limon*) toward *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* sp., were investigated. Antibacterial activity was performed by the agar disc diffusion method. The ethanol and methanol extract of tested plant leaves showed promising antibacterial activities against both Gram-positive and Gram-negative tested bacterial isolates due to its great ability to extract those polyphenolic and biological active compounds from natural sources which effectively act against broad spectrum bacteria. Ethanol followed by methanol were found to be the best solvents of choice to extract natural products to get maximum health and medicinal benefits. The results revealed that the extraction efficiency increase with polarity increasing of the solvents, hence the highest extraction done with ethanol and methanol and the lowest extraction with nonpolar solvent n-hexane did not exhibit any activity against all the tested bacteria. Irradiation at 5 and 10 kGy did not significantly affect the antibacterial activity of all tested

plant leaves. Results indicate the potential of these plants for further work on isolation and characterization of the active compounds responsible for antibacterial activity and its exploitation as therapeutic agents

### INTRODUCTION

Infectious diseases and microbial infections are considered as the major killing factors in the third world countries and the most important causes of premature death. The difficulty of controlling the sources of infection, the high cost of treatment/prevention, poor compliance, low efficacy, poor safety and drug resistance are the major factors that may retard the treatment of these diseases. The drug resistance has further complicated the treatment of infectious diseases in immune-compromised AIDS and cancer patients (Singh et al 2010 and Gawad et al 2015).

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times as reported by (Mariita et al 2011). Medicinal plants represent a rich source of antimicrobial agents. These plants possess a potent medicinal value that is due to the presence of a variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body (Thalwal et al 2013).

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. It was clear that the primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. A lot of supplementary

(Received 8 May, 2017)

(Revised 14 May, 2017)

(Accepted 16 May, 2017)

treatment strategies were tried. Current social trends in health care showed a definite movement towards the use of natural remedies like medicinal plants away from chemotherapeutic regimens (Selvamohan et al 2012).

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. Also, use of plant essential oils in both food and pharmaceutical industries has been developed interestingly; systematic examination of plant extracts for these properties has become increasingly important. The use of natural plant antimicrobial compounds is important not only in the preservation of food, but also in the control of microbial growth in the disease condition (Rasool, 2013). Antimicrobial agents are effective in curing diseases because of their selective toxicity against pathogenic microbes without causing any harm to the cells of the host (Thalwal et al 2013).

Plants contain innumerable constituents and are valuable sources of new and biologically active molecules possessing antimicrobial properties, such as phenolic acids, flavonoids, tannins, lignin and other small compounds. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic, antioxidant, antibiofilm and vasodilatory activities (Osugwu and Ihenwosu, 2014). In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Thoppil et al 2014). It is also important to mention that, plant by-products such as leaves, bark and peels may be an abundant source of polyphenols and tannins. Thus the use of the waste as a source of the antibacterial compound could provide health benefits to humans and may be employed in food preservation purpose (Balasundram et al 2006 and Wonghirundecha & Sumpavapol, 2012). Therefore, this study was conducted to evaluate the antimicrobial activities of four irradiated and nonirradiated plants and plant by-products (grape, mulberry, mallow and lemon) against local human pathogenic bacterial isolates.

## MATERIALS AND METHODS

### Tested microorganisms

Nineteen bacterial isolates were collected from Clinical Microbiology Laboratories, Ahmed Maher,

El Helal and Abbasiya Fever Hospital, Cairo, Egypt and identified based on Bergey's Manual of Determinative Bacteriology (Holt et al 1994).

### Plant material

Four plants and plant by-products viz., (grape, mulberry, mallow and lemon) were bought from a commercial sources in Cairo, Egypt, and then divided into three groups; the first was left without irradiation and considered as control, while the second and the third groups were exposed to gamma irradiation at dose levels of 5 and 10 kGy. Irradiation was performed in the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt, at dose rate 3.49269 kGy/h using the "Indian Gamma Chamber 4000 A" with a  $^{60}\text{Co}$  source.

### Preparation of soluble plant extracts

The dried leaves were ground into fine powder with an electric blender and extracted by the cold-maceration method as described by Anowi et al (2012). Briefly, 50 g of each dried plant material was soaked in 250 ml of the solvents ethanol, methanol and hexane. The plant materials were macerated at room temperature. After three days, the supernatant was filtered through Whatman No.1 filter paper and the filtrate was concentrated by evaporating in a rotary evaporator (IKA, Germany) at 40°C. The residue was weighed, dissolved in 2.5% dimethyl sulfoxide (DMSO) and stored in the refrigerator at 4°C prior to use.

### Antibacterial activity

The antibacterial activity of the tested plant extracts was determined according to (CLSI, 2012). Briefly, Whatman filter paper no.1 discs were prepared (diameter 6 mm). Discs were loaded with 40 µl of 50 mg/ml of each extract (dissolved in DMSO 2.5%) per disc and dried at room temperature. 100 µl bacterial suspensions (Five ml of a fresh growth 24h old culture with  $10^6$  CFU/ml) at wavelength 620 nm were spread on Muller-Hinton agar plate (oxid). The discs impregnated with plant extract were placed on the agar surface. The plates were incubated at 37°C for 24 h and the inhibition zone was measured. Discs prepared with DMSO 2.5% served as negative control. Ciprofloxacin (5 µg) was used as a positive control. Three replicates were carried out. The clearance zones (inhibition zones mm) including the disc diameter were rec-

ordered, clearance zones having 6> mm were considered as positive results (Allam et al 2015).

### Statistical Analysis

Standard deviation ± was applied using the Microsoft Excel program (2010)

## RESULTS AND DISCUSSION

### Antibacterial activity assay of plant extracts

In the present investigation, methanol, ethanol and n-hexane extracts of the grape, mulberry, mallo and lemon leaves were evaluated for their antibacterial activity against 19 bacterial isolates which divided into; 5 isolates for each of *Staphylococcus aureus*. (*St*), *Escherichia coli* (*Ec*), *Pseudomonas aeruginosa*. (*Ps*), and 4 isolates of *Salmonella* sp., as shown in Figs. (1-4). These results indicated that both ethanol and methanol extracts have antibacterial activity against all of the tested pathogenic bacteria depending not only on the tested bacteria, but also on the polarity of the solvent (Padalia and Chanda, 2015). There are various reports that antibacterial activity depends on the solvent used, structure of the compound in the extracts and the strain under investigation (Nair et al 2006).

Data in Fig. (1) revealed that the antibacterial activity of grape leaves ethanol extract showed harbor antibacterial activity followed by methanol

extract against all tested pathogens, there was a statistical difference in inhibition zones at the tested concentration of both ethanol and methanol extracts of grape for all tested bacterial isolates and the largest diameter of inhibition zone was obtained with *Ec3* (29.7 and 24.2 mm), followed by *Ps1* (28.7 and 23.2 mm) and *St1* (27.5 and 22.7 mm), respectively. These results indicated that, the bacteria belonged genus *Staphylococcus*, *Escherichia*, and *Pseudomonas* were the most sensitivity to tested plant leaves extract, it could be due to these extracts contained high amount of Polyphenolic derivatives such as: anthocyanins, leucoanthocyanins, Flavonoids (rutin, quercitrin, isoquercitroside, luteolol), Gallic tannins and Catechins which have remarkable antibacterial activity have been suggested by (Alhamd et al 2015). On the other hand, bacterial isolates of *Sa2* and *Sa4* (11.7 and 12.5mm), were considered as the least sensitive against methanolic extract. Similar results have been found by Abed et al (2015) reported that, grape leaves exhibited high antibacterial activity against bacterial human pathogens, such as *Ps. aeruginosa* and *Staph. aureus*. The ethanol extracts of grape leaves showed broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria (Oskay and Sari, 2007). The antimicrobial activity of plant extracts depends on the type and amount of bioactive compounds in the plant tissue and the pathogen's inherent resistance (Martini et al 2004).

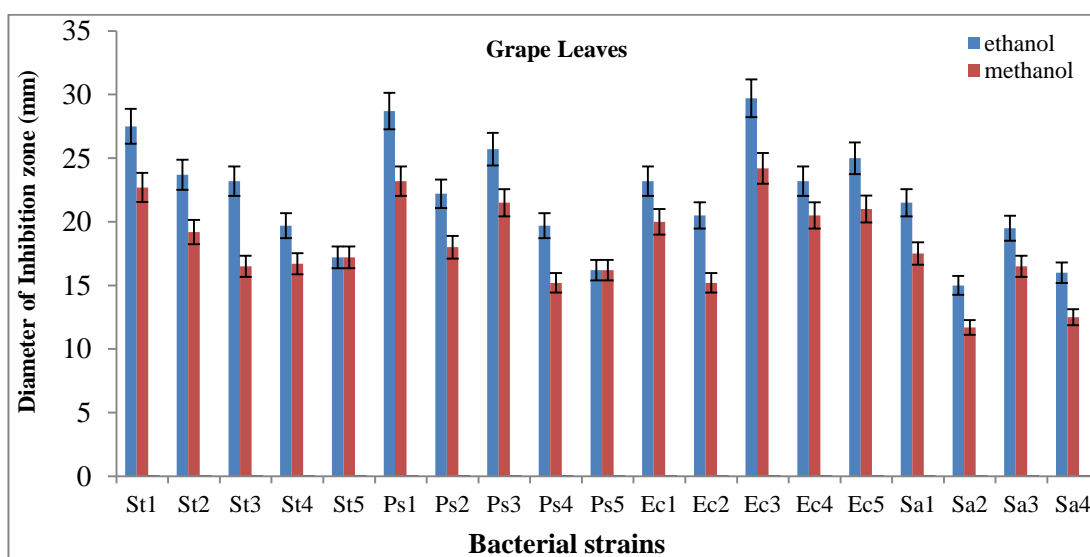
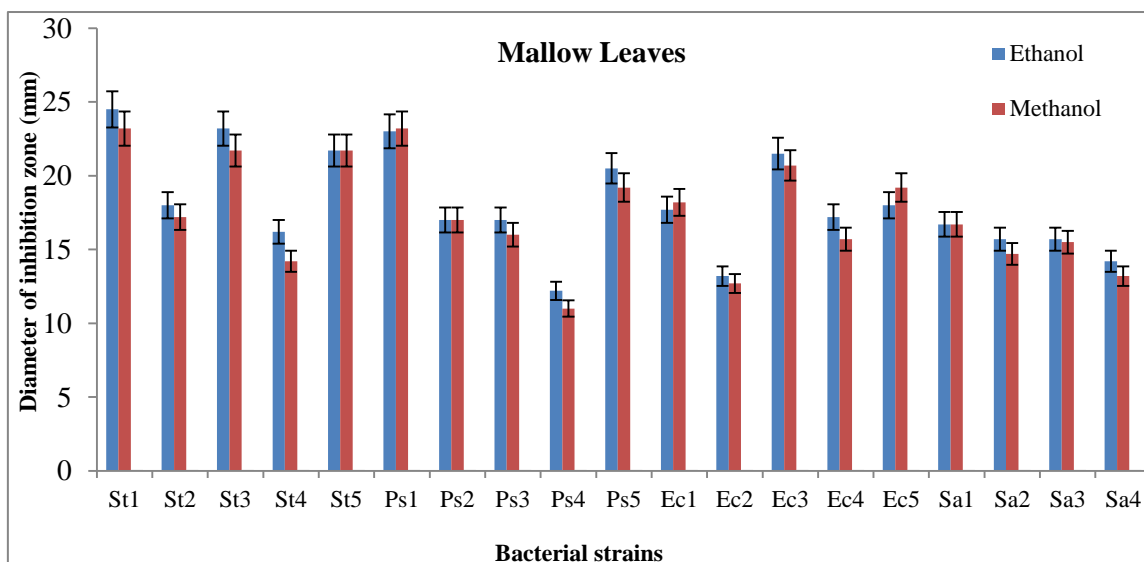


Fig. 1. Antibacterial activity of different grape leaf extracts (ethanol and methanol) against *Staph. aureus*, *E. coli*, *Ps. aeruginosa* and *Salmonella* spp. for 24h at 37°C.

Results in **Fig. (2)** showed that, both ethanol and methanol extract gave similar results in their influence on antibacterial against tested bacteria, and the mean of inhibition diameters of these extracts were between 12.2 to 24.5 mm & 11.0 to 23.2 mm respectively. The ethanolic extract of mallow leaves recorded the inhibition zone diameter ranged from 16.2 to 24.5 mm, 12.2 to 23.0 mm, 13.2 to 21.5 mm and 14.2 to 16.7mm toward *Staph. aureus*, *Ps. aeruginosa*, *E. coli* and *Salmonella* spp, respectively. Whereas, the inhibition zones were recorded in methanol extract against *Staph. aureus* (ranged from 14.2 to 23.2mm), *Ps. aeruginosa* (ranged from 11.0 to 23.2 mm), *E. coli* (ranged from 12.7 to 20.7mm) and *Salmonella* spp (ranged from 13.2 to 16.7mm). The obtained re-

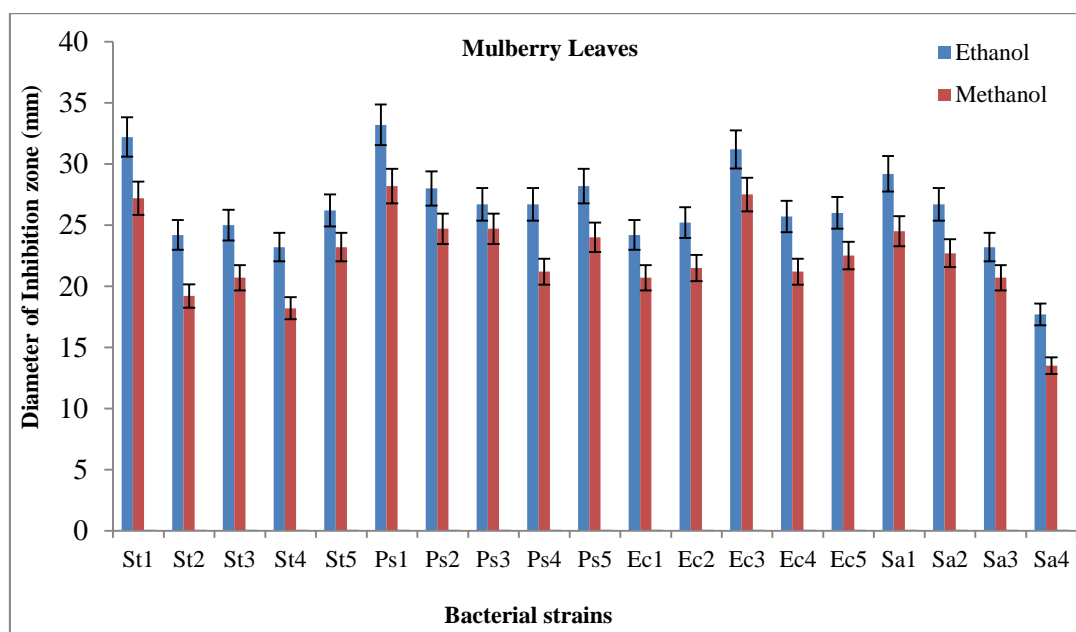
sults are in the same line with **Das et al (2010)** reported that, mallow leaves possess antimicrobial, antitumor, and anti-inflammatory activities. Also, **Hayyawi (2012) and Mohammed (2016)** reported that, the ethanolic 96% and methanolic extracts of mallow leaves exhibited significant antimicrobial activity. They also found some active compounds such as sterols, triterpenes, carotenoids, alkaloids, tannins, saponins, coumarins, and carbohydrates beside contains hydrocyanin and cardiac glycosides in large quantity and, appreciable quantities of flavonoids, and anthraquinones. The Phenolics and Flavones compounds found in mallow extracts inhibit the effect of the bacterial enzymes needed for essential metabolic reactions by interfering with the bacterial proteins.



**Fig. 2.** Antibacterial activity of different mallow leaf extracts (ethanol and methanol) against *Staph. aureus*, *E. coli*, *Ps. aeruginosa* and *Salmonella* spp. for 24h at 37°C

With regard to mulberry leaves, a broad spectrum of antibacterial activity (ranged from 18.2 to 33.2 mm) against all tested pathogenic bacteria have been illustrated by **Fig. (3)**. These results demonstrated that ethanolic extract harbor significant antibacterial activity against all tested isolates and the maximum growth inhibition was observed against *Ps. aeruginosa* (ranged from 26.7 to 33.2mm) and *Staph. aureus* (ranged from 23.2 to 32.2mm), followed by *E. coli* (ranged from 24.2 to 31.2mm) and *Salmonella* spp (ranged from 17.7 to 29.2mm), respectively. Furthermore, antibacterial potential was also noticed in methanol extracts against *Ps. aeruginosa* (21.2 - 28.2mm) followed by *Staph. aureus* (ranged from 18.2 to 27.2mm), *E.*

*coli* (ranged from 20.7 to 27.5mm) and *Salmonella* spp. (ranged from 13.5 to 24.5mm). While hexane extracts showed no activity against all tested isolates. Different organic solvent extracts have different phytoconstituents in different amounts and this explains why there is differential inhibition of the bacteria (**Padalia and Chanda, 2015**). Several investigators have been reported the antimicrobial activity of mulberry leaves methanol extracts and different species of mulberry leaves exhibited antibacterial activity against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, *Salmonella typhi*, *Shigella flexneri* and *Candida albicans* (**Aditya rao et al 2012 and Emniyet et al 2014**). In addition, **Emniyet et al (2014)** identified bioactive



**Fig. 3.** Antibacterial activity of different mulberry leaf extracts (ethanol and methanol) against *Staph. aureus*, *E. coli*, *Ps. aeruginosa* and *Salmonella* spp. for 24h at 37°C

compounds which extracted from mulberry leaves by ethanol. These compounds, namely, gibberellic acid and 9,12,15-octadecatrienoic. So, antimicrobial activity can be related to these molecules. The observed antimicrobial activities in mulberry leaves could possibly be due to the presence of tannins, triterpenes, sterols, bioflavonoids, coumarins, volatile oil, alkaloids, organic acids and amino acids, glycosides, and saponins (Emniyet et al 2014 and Gunjal et al 2015).

The obtained results in Fig. (4) showed that, among the three extracts of lemon leaves, the ethanol extract had the highest activity against Gram-negative and Gram-positive bacteria but with variable degrees of inhibition growth zones. All of the bacterial isolates are sensitive to both ethanol and methanol extracts as well, while hexane extract had a weak or no antibacterial activity. The ethanol extract of lemon leaves showed a better inhibition zone than methanol extract. Maximum inhibition was shown by ethanol extract against *Ps. aeruginosa* (ranged from 17.2 to 28.0mm) and *Staph. aureus* (ranged from 12.0 to 28.0mm) followed by *E. coli* (ranged from 13.7 to 23.5 mm) and *Salmonella* spp (ranged from 13.0 to 18.0 mm), Also, the inhibition zones were recorded in methanol extracts against *Staph. aureus* (ranged from 9.2 to 24.5mm) and *Ps. aeruginosa* (ranged from 13.2 to 23.7mm) followed by *E. coli* (ranged from 10.2 to

19.2mm) and *Salmonella* spp (ranged from 12.5 to 15.2mm). The present results are in agreement with findings on antibacterial activities of lemon leaves, **Ewansiha et al (2016)** reported that, ethanol is a good solvent for the extraction of citrus leaf crude extract; ethanol gave the highest percentage yield of 10.93%, followed by ethyl acetate with 9.42% while water extract gave the lowest yield of 3.67%. The antibacterial activity of lemon leaves extracts exhibited by bioactive compounds such as alkaloids, flavonoids, saponins, anthraquinones, cardiac glycosides, tannins, steroids, terpenes, resins, phenols and volatile oils

The present results (Figs. 1-4) are in agreement with **Motavalizadehkakhky et al (2013)**, **Moglad et al (2012)** and **Entezari et al (2009)** with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, soil nature, other environmental factors, storage conditions, the part of plant used, method of extraction, method of screening, solvent used, concentration of extract and different sensitivity of the tested strains (**EL-Zawahry et al 2013**). The mode of action of plant extracts and their natural components is related to: degradation of the cell wall; damage to cytoplasmic membrane and membrane proteins; leakage of intracellular contents; coagulation of cytoplasm; interference with active transport or metabolic enzymes; dissipate

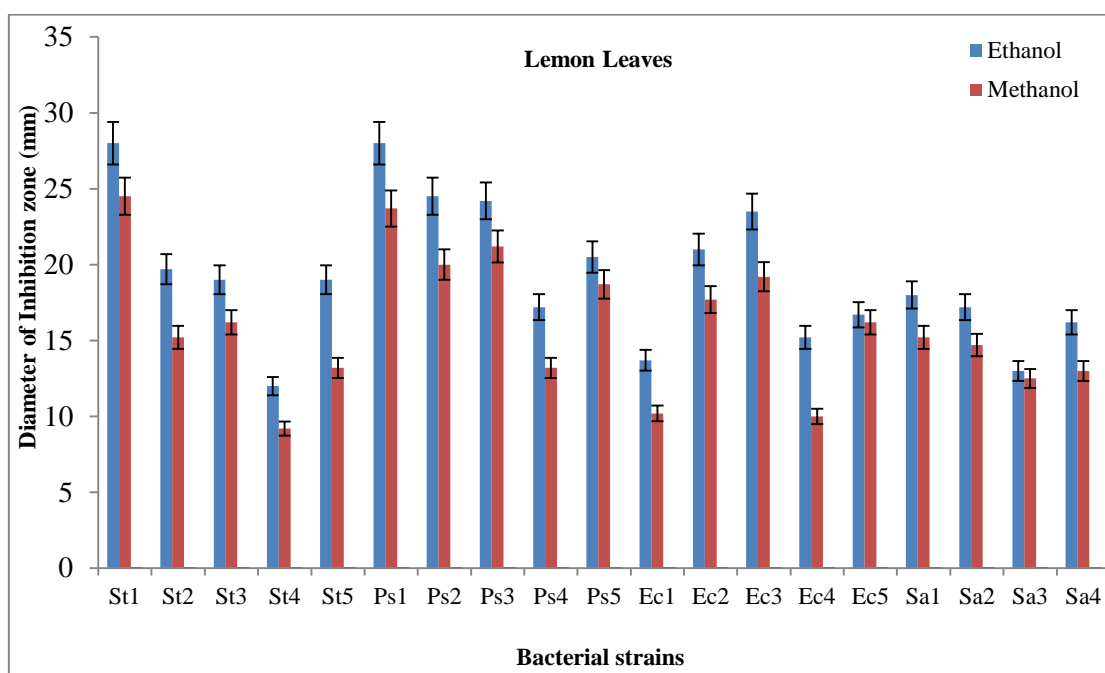


Fig. 4. Antibacterial activity of different lemon leaf extracts (ethanol and methanol) against *Staph. aureus*, *E. coli*, *Ps. aeruginosa* and *Salmonella* spp. for 24h at 37°C

cellular energy in ATP form and depletion of proton motive force (PMF) and electron flow, which can cause cell death (Negi, 2012 and Allam et al 2015).

From previous results, it could be demonstrated that plants and plant by products play a vital role in inhibited pathogenic bacteria when extracted these plants by ethanol gave a higher antibacterial activity than methanol and n-hexane because this solvent is able to dissolve both polar and non-polar substances, including a variety of plant-derived compounds, on the other side ethanol is a low toxicity compared with methanol (Allam et al 2015). So, ethanol plant extracts were selected for further studies.

#### Influence of gamma irradiation on antibacterial activity of plant materials

Gamma-irradiation has been widely used as a first choice sterilization method of raw medicinal plants to be used in the phytotherapeutic industry worldwide. In order to study the effect of gamma irradiation on antibacterial activity of different plants grape, mulberry, mallow and lemon leaves were exposed to dose levels of 5 and 10 kGy then the plants extracted with ethanol, and data were

recorded in Table (1). The results indicated that, irradiation at dose levels of 5 and 10 kGy did not affect the antibacterial activity of all tested plant materials, it might be due to the content of phenolic compounds which responsible for antibacterial activity were stable and were not decomposed by the irradiation (Ercisli et al 2008). The obtained results were very close to other plants where Ercisli et al (2008) showed that no significant changes in total phenolic contents of *Glycyrrhiza glabra* roots were observed following gamma-radiation treatment at dose levels of 5, 10 and 15 kGy, also Ibrahim et al (2016) reported that, irradiation at dose 10 and 30 kGy did not significantly affect the antibacterial activity of aniseeds and star anise waste residue extract. On the contrary, Zhu et al (2010) reported that the doses of 2-10 kGy significantly decreased three phenolic acids (p-coumaric acid, Ferulic acid and sinapinic acid). The difference in the effect of radiation on total phenolic content may be due to plant type, geographical and environmental condition, state of the sample (solid or dry), phenolic content composition, extraction solvent, extraction procedures, temperature, and dose of gamma irradiation, etc (Ibrahim et al., 2016).



### CONCLUSION

In the pursuit of searching for new antibiotics and natural alternatives to chemical preservative materials in food the role of grape, mulberry, mallow and lemon leave extracts cannot be negated as that is evident with the present results. It can be generally concluded that, the obtained results indicated that, the ethanol and methanol extracts of mulberry, mallow and lemon leaves possess significant antibacterial capacity and a good source of various phytoconstituents which recommends further research needed for isolation of bioactive principles. The obtained results indicated that, the tested plant and plant by-product extracts may be a new source of alternatives to conventional antibiotics and as natural sources and their extracts were recommended to be used as natural preservatives in food against the screened bacterial species which cause food-borne diseases and food spoilage, also this study proved partly support and justify the traditional use of these plant extracts for treating infections in traditional medicine.

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