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ANTIMICROBIAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST PATHOGENIC BACTERIA

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Abstract

The aim of the study is to assess the antimicrobial effect of some edible plant extracts against pathogenic bacteria. The aqueous extract of *Brassica oleracea var. botrytis* (cauliflower), *Brassica oleracea var. capitata* (cabbage)and *Allium ampeloprasum* (leek) were investigated effect against on eight bacterial isolates *Staphylococcus aureas*, *Escherichia coli*, *Morganella morganii*, *Vibrio fluvialis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Enterococcus faecalis* and *Serratia marcescens* in vitro. The extract solution from leaves with solvents water at ratio 1:2 by leaves grinder with distill and sterilized water. The results of investigation showed the antibacterial activity of aqueous cabbage extract was effects on *Staphylococcus aureas*, *Escherichia coli*, *Morganella morganii* and *Enterococcus faecalis*. The result showed the cauliflower was effective in growth of *Vibrio fluvialis*, *Enterococcus faecalis* and *Escherichia coli* and also the growth of *Morganella morganii*, *Vibrio fluvialis, Pseudomonas aeruginosa*, *Enterococcus faecalis and Serratia marcescens* that affected by leek.

Key Words: Antimicrobial, Plant, Extract,Bacteria

Introduction

Recently, there are an enormous covenant of interest rewarded in remedial treatments to plant extracts with nature biological features since of the side effects and resistance that the pathogens microorganisms have exposed in the use of antibiotics. The public health on a global scale is threaten by the increase of microbial resistance to antibiotics because it reduces treatments quality and increases both morbidity and, mortality as well as health care costs (Abd El-Kalek and Mohamed, 2012). Plants employed in this case for traditional medicine contain a variety of substances which is able to treat various infectious diseases (Oskay *et al*., 2009).

The considerable examples of plant secondary metabolites in this study (phytoalexins and phytoanticipins) they constitute a vital mechanism to have the ability to stop spreading of phytopathogens *in planta* by behaving as antimicrobials and clinically-relevant pathogens as well as their usage as "antibiotic potentiators" or "virulence attenuators" for making the control of infectious diseases in humans possible (González-Lamothe *et al*., 2009). The possible replacement to antibiotic-mediated bacteria either killing or growth inhibition in this study is attenuation of bacterial virulence. This, in turn, is able to make the failure in the organism to embed a successful infection and is cleared by the host immune response (Namasivayam and Vivek, 2016).

Several studies have confirmed the plant can act antimicrobial efficacy. *In vitro* antibacterial activity of plant solution extracts against both Gram positive and negative pathogenic bacteria (Soniya *et al*., 2013). The extracts of *Lawsonia inermis*, *Embellia ribes* as well as *Santalum album* stated antibacterial behavior against the examined bacteria *Pasteurella multocida*, *Escherichia coli, Bacillus cereus*, *Staphylococcus aureus*, *Corynebacterium bovis* (Hussain *et al*., 2011). While, the plant extracts were used against bacteria *Staphylococcus aureus* (Gram positive) bacteria *Salmonella typhi*, *Shigella flexineri* and *Enterococcus faecalis* (Gram negative) by Kirby Bauer method (Rachuonyo *et al*., 2016). The extracts of *Torilis anthriscus* active and significant antibacterial properties into *Pseudomonas glycinea. Aegopodium podagraria*, *Daucus carota*, *Heracleum sphondylium* and *Pimpinella saxifraga*, the plant extracts encouraged using for control of selected phytopathogenic bacteria (Duško *et al*., 2006). Additionally, the high activity in plant seeds extract with inhibition of *S. aureus* and *P. aeruginosa* after the treated (Al-Zahrani *et al*., 2016). They are revealed that thirteen types of plant extracts exhibited an important broad-spectrum antibacterial behavior against both Gram-positive as well as negative bacteria. However, seven extracts concluded only a narrow spectrum behavior against Gram-positive bacteria (Abdel-Sattar *et al*., 2008). Moreover, *both Psidium cattleianum* and *Myracrodruon urundeuva* extracts concluded a noticeable inhibitory activity on all

bacterial strains tested. In addition to that, both alcoholic and aqueous solutions showed similar results (Gaetti-Jardim *et al*., 2011). The existence of a variety of alkaloids, phenols, terpenes derivatives compounds and other antimicrobial compounds mode action against the ample variety of pathogenic microorganisms (Akthar *et al*., 2014).

The micro-organisms diseases are still dangerous to human health. The multi drug resistant strains were sensitive to the antimicrobial behavior of *Acacia nilotica*, *Syzygium aromaticum* as well as *Cinnamum zeylanicum*, while they showed excess resistance to both *Terminalia arjuna* as well as *Eucalyptus globules* extracts (Khan *et al*., 2009). Antimicrobial resistance is the continuous usage of the misuse of antimicrobial medicines and in the same time develops when a microorganism mutates or acquires a resistance gene (Alwash *et al*., 2013).

In the other side, bacteria is able to undergo gene exchange in all cases by sequence specific mechanisms such as transposition. The resistance gene is flanked with genes encoding by insertion sequences of DNA (Stockert and Mahfouz, 2012). The increased antibiotic resistance may render the current antimicrobial agents insufficient to control, some bacterial infections (Elmanama *et al*., 2011). Thus, the discovered compounds from these plants can be used as an example to develop a new antibacterial agents (Abdulzahra and Mohammed, 2014). It is suggested that using natural products as therapeutic will perhaps not elicit resistance in microorganisms (Eltaweel *et al*., 2014). Therefore, this study was conducted to assess the antimicrobial activity of *Brassica oleracea var. botrytis*, *Brassica oleracea var. capitat*a and *Allium ampeloprasum* against bacterial isolates *Staphylococcus aureus*, *Escherichia coli*, *Morganella morganii*, *Vibrio fluvialis*, *Pseudomonas aeroginosa*, *Klebsiella pneumonia, Enterococcus faecalis and Serratia marcescens*

Material and Methods

Plants Extracts Preparation

Plants extracts were carried out in present study using three common plants, *Brassica oleracea var. botrytis* (Cauliflower*), Brassica oleracea var. capitata* (Cabbage) and *Allium ampeloprasum* (Leek) which were collected from local market of Hilla city-Iraq. The first step in this study was rinsing both the fresh white cauliflower head as well as green cabbage head leaves by tap distilled water to remove impurities such as dust. Then, it was grinded for about Five minutes with add sterilized distilled water at ratio 1:2 .The extracts were filtered by muslin cloth, then by filter paper (Whatman No 1) and was kept in sterile polyethylene bottle under refrigerated at 4ºC for further processing.

Antimicrobial activity

Plants extracts were added 5% into culture and Nutrient broth inoculated with isolates bacterial with incubator at 37 °C for 24 hours for investigated effect against on eight bacterial isolates in vitro. The antimicrobial activity of these extract were assay performed using optical density (OD) of bacterial growth culture at 600 nm.

The antibacterial activity of the aqueous plant extracts determined by employing positive controls for the antimicrobial activity. The experiment was run in triplicates and the mean values were reported.

Bacterial strains

The bacterial strain used in this study were *Staphylococcus aureus*, *Escherichia coli*, *Morganella morganii*, *Vibrio fluvialis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Enterococcus faecalis* and *Serratia marcescens*, which obtained from Department of biology, Science College, Babylon University

Standardization of Inoculum

The first step in the Standardization process was dispensing 0.2ml of 24/hours old culture of each organism into 20ml of sterile nutrient broth. Then, this was done by incubated for 3-5 hours to make the culture standardize to 10^6cfu/ml (Collins *et al*., 1995).

Detection of some Phytochemical Compounds

Phytochemical analysis were carried out within the procedures as described for presence of Flavones: (Jaffer *et al*., 1983), for phenolic compounds existence: (Harborne, 1973), for saponins presence: (Stahle, 1969), for tannins detected of (Al-Shami, 1982), for glycosides detected (Shihata, 1951).

Results and Discussion

Lately, there is been a concern of entering a post-antibiotic era with capability reduction of combat microbes. Thus, the many therapeutic development to the treatment infections constitutes a vital point of the researcher.

Three plant were investigated to evaluate their antibacterial activity against bacteria *in vitro* including six strains of Gram-negative bacteria (*Escherichia coli*, *Morganella morganii*, *Vibrio fluvialis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae and Serratia marcescens*) and two strains of Gram positive bacteria (*Staphylococcus aureas* and *Enterococcus faecalis*). Furthermore, plant solution extracts are still as a vital antibacterial against pathogens. For instance, the plant

extracts of *Ocimum tenuiforum* and *Syzygium aromaticum* against *B. subtilis* as well as *Cuminum cyminum* against *E. coli*. However, aqueous extracts of *Piper nigrum* produced high inhibition against *Proteus* sp. (Soniya *et al*., 2013).

The results in Figure 1 revealed that the antibacterial activity of aqueous *Allium ampeloprasum* extract according to growth at 600 nm that effect on *Enterococcus faecalis*, *Morganella morganii*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Vibrio fluvialis*. In addition to that, some extract solutions are able to be used in combination to improve the effectiveness in treating the diseases which is basically caused by the bacterial pathogens (Rachuonyo *et al*., 2016). The usage of 2% and 3% olive leaf extract had the beneficial effect in controlling the microbial load, total viable and coliform counts (Aytul, 2012). Several studies showed that plant extracts had against a broad antibacterial. The cold water extract of fresh leaf of *Moringa oleifera Lam* displayed a vital antibacterial withany of tested four Gram-negative bacteria: *Shigella shinga*, *Pseudomonas aeruginosa*, *Shigella sonnei* as well as *Pseudomonas* spp. and six Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus*-B- haemolytica, *Bacillus subtilis*, *Sarcina lutea* and *Bacillus megaterium* (Rahman *et al*., 2009). The results indicated that extract of leek were potentially effective in growth of some bacteria with variable potency. Guava leaf extracts solutions as well as essential oil are very active against *S. aureus*. Therfore, making up a vital sources of new antimicrobial compounds (Gonçalves *et al*., 2008). Olive leaf extract was found to be most active against *Salmonella typhimurium*, the olive leaves had the beneficial effect in controlling the microbial infections (Aliabadi *et al*., 2012).

The extract of *Brassica oleracea var. capitata* investigation the antibacterial activity have represented on Figure 2. According to the current results and assessments of cabbage extract that effect on *Escherichia coli*, *Staphylococcus aureas*, *Morganella morganii* and *Vibrio fluvialis*. The extracts from three plants had antimicrobial effects against *E coli* and *Staphylococcus aureus* isolates.

The water plant extract was more effective than the 60% ethanol plant extract (Ibrahim, 2015). Similarly, the evaluated the antibacterial activity in the leaf water extracts as well as essential oil of *Mentha piperita* L. against pathogenic bacteria like *Bacillus subtilis, Pseudomonas aureus, E. coli*, *Salmonella typhi* and *Streptococcus aureus* (Al-Taweil, 2014). The plant *Cnestis ferruginea* should be validated and be used to treat infections caused by *E. coli, Staphylococcus aureus* and *Salmonella* spp. (Enemor *et al*., 2015). The fine antibacterial activities of plant extracts could be basically employed as a proper treatments for infections caused by *E. coli* and *Pseudomonas aeruginosa* (Jahani *et al*., 2016).

As shown by the results in the Figure 3 indicates that the of *Brassica oleracea var. botrytis* extract affect *Escherichia coli*, *Enterococcus faecalis*, *Vibrio fluvialis* and *Morganella morganii*. The *E. coli* isolates noticed to be more resistance against the most extracts with different concentrations comparing with other isolates (Akrayi *et al*., 2012). Thus, the primary advantages of using plant extracts as medicines are safer than synthetic alternatives, which is basically offering a tremendous therapeutic benefits as well as more affordable treatment (Ibrahim and Abu-Salem, 2014). It was noticed that cloves are the highest antibacterial activity due to the presence of terpenoids, flavonoids and phenolics, followed by cherry then rosemary, whereas mint possessed the least (Shehadi *et al*., 2014). Antimicrobial activity was tested by disc diffusion method. It was found out the less effective extracts were from thyme and lavender, whiles, basil, chive and parsley extracts showed higher potential to inhibit bacterial growth (Dostalova *et al*., 2014). More research is needed to isolate bioactive compounds which may be highly recommended as possible antimicrobials for therapeutic usage as an alternative medicine (Rizwana *et al*., 2016)

Results of detection of some phytochemical compounds of plant extract showed that present flavones, tannins and phenolic compounds (Table 1). Plant extracts solutions are representing a vital source of bioactive compounds which are able to provide countless opportunities for new antibacterial agents. The relationship between the microbial activity and the chemical structures of the abundant compounds in the extracts of plant. They observed antimicrobial properties can attribute to the existance of bioactive materials. These include tannins, flavonoids, saponin, alkaloids and phenolic compounds (Okwulehie and Akanwa, 2013). Both the leaves of Eucalyptus plant aqueous as well as alcohol extracts contained resins, tannins and finally phenols. It tested the effectiveness of inhibitory extract plant against a group of 5 isolates bacterial as well as 5 isolates of molds (Al-Manhel *et al*., 2015). Despite of a number of natural-synthetic antimicrobial agents have isolated and developed to kill some pathogenic microorganisms, globally the resistance of antimicrobial is basically still an increasing public health problem. Moreover, many plants have continued to be an important therapeutic aid for eliminating the ailments of mankinds (Okwulehie and Akanwa, 2013).

Recently, the use of antibiotics in uncontrolled manner may have ability to cause emergence of microbial resistance with pathogenic agents. Thus, the usage of totally new synthetic as well as natural antimicrobial compounds is still inevitable. One source of natural compounds in this respect comes from plants (Naziri *et al*., 2012). In 2011, Elmanama and his colleague demonstrated the importance of plant extracts in to control the bacterial resistance, which are clearly becoming a threat to human health. Plants are good sources of pharmaceutical compounds, these plant extracts can be used as a possible alternative or supportive alternative to different oral care products (El-Maghraby *et al*., 2014).

The aqueous extract of *R. coriaria* in this study stated an important role to inhibition the growth of tested isolate *in vitro* and *in vivo*. The phytochemical screening showed that *R. coriaria* contains tannin, phenol, anthraquinon and saponin (Akrayi & Ahmed, 2013). Aqueous of leaf extracts (*Pistacia vera*, *Pistacia atlantica*, *Schinus terebenthifolius* and *Schinus molle)* exhibited a high level antibacterial activity (Rhouma *et al*., 2009).

Antibacterial behavior of a solution extracts of nominated mint in this work were basically examined with multi drug resistant bacteria. This stated that their extracts could be employed against multi drug resistance bacteria that able to cause both nosocomial as well as community acquired infections (Al-Sum *et al*., 2013). Both *Lagerstroemia indica* as well as *Annon areticulata* leaf extracts against human bacterial pathogens viz*., Klebsiella pneumoniae, Staphylococcus auerus, Salmonella typhi*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (Chandra, 2013). Additionally, *Ephedra pachyclada* extract has effective antimicrobial ingredients which are cheap and readily available. It can be used for medicinal purposes in the production of antimicrobial drug (Dosari *et al*., 2016). It is recommended to develop investigations on utilized of this plant against some pathogenic bacteria that appear resistance to most widely used antibiotics and some isolates to all tested antibiotic, Plant extracts have the possibility to antimicrobial compounds with microorganisms. Therefore, they can be employed in the treatment of infectious diseases which is simply caused by resistant microbes (Salih *et al*., 2014). The extract of plants has significant to decrease growth of some bacteria studied.

Conclusion

In conclusion, aqueous extracts of cabbage, cauliflower and leek exhibit relatively good bacteriostatic and bactericidal effects on some isolates of bacteria Gram-negative and Gram-positive. In this work, the results clearly indicated antimicrobial activity of aqueous extracts *was* effects on growth of *Staphylococcus aureas*, *Escherichia coli*, *Morganella morganii* and *Enterococcus faecalisvibrio fluvialis*, *Morganella morganii*, *Pseudomonas aeruginosa* and *Serratia marcescens*.

Hence, the risk of infections induced by these bacteria can be reduced and treatment of these infections can be increased through heating cabbage, cauliflower and leek. Generally, there is one approach to treat infectious diseases which is the use of plant extracts individually. This study could provide a new basis on the using some plant. However, further investigation upon research and development work should be undertaken on the active components of these plant extracts for their better economic and therapeutic utilization because the extracts of plants represent a rich source of antimicrobial agents.

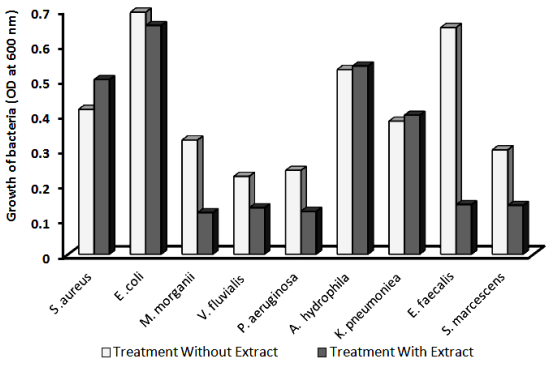

Fig. 1: Effect of *Allium ampeloprasum* extract on pathogenic bacteria growth

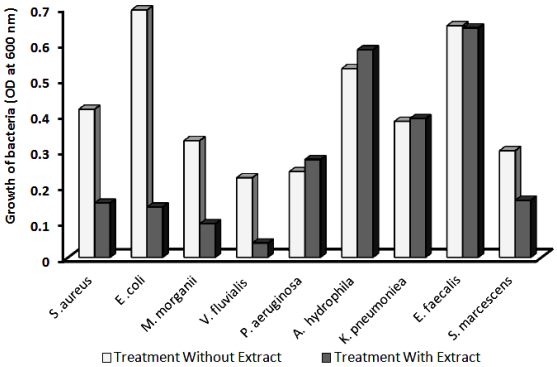

Fig. 2:Effect of *Brassica oleracea var. capitata* extract on pathogenic bacteria growth

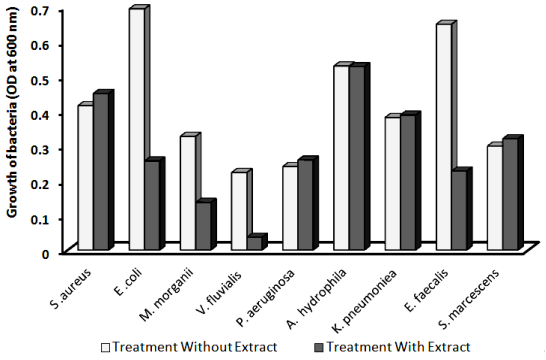

Fig. 3: Effect of *Brassica oleracea var. botrytis* extract on pathogenic bacteria growth

Table 1 : Detection of Some Phytochemical Compounds on plant extract

	Cabbage *Brassica oleracea var. capitata*	Cauliflower *Brassica oleracea var. botrytis*	Leek *Allium ampeloprasum*
Flavones	++	+	+
Tannins	+	+	+
Phenolic Compounds	++	+	+
Saponins	-	-	++
Glycosides	+	+	-

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