



ANTIMICROBIAL ACTIVITY OF DIFFERENT CONCENTRATION OF AQUEOUS PLANT EXTRACTS OF *ARTEMISIA VULGARIS* LEAVES ON MANY PATHOGENIC MICROORGANISM

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Abstract

The objective of the study was to determine the presence of antimicrobial activity and minimum inhibitory zone (MIZ) of many concentration (25 %, 50%, 75%, 100%) of aqueous plant extracts of *Artemisia vulgaris* leaves, Plant aqueous extract was tested on many selected gram-negative pathogenic bacteria (*Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*) and one fungus (*Candida albicans*), Agar disc diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations (MIZ) of aqueous plant extracts on these microorganisms and the results show no effect of these concentrations on the growth of bacterial isolates while there is a significance effect of the same extract on yeast *Candida albicans* growth and with inhibition zones ranging from (15-24 mm) according to the concentration of the plant extract.

Key words: Aqueous plant extracts, *Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Candida albicans*, antimicrobial sensitivity test

Introduction

Pathogens like (*Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*) are Members of these family referred as Enterobacteriaceae or “enteric bacteria” they are widely distributed in the environment, soil, water, plant as well as in the intestines of animals and human, It is a large family of Gram-negative bacteria, It was first proposed by Rahn in 1936, and now includes over 30 genera and more than 100 species, Enterobacteriaceae includes, along with many harmless, symbiotic, many of the more familiar pathogens, such as *Proteus*, *Salmonella*, *E. coli*, *Klebsiella* and *Shigella*. (Don *et al.*, 2005) Most members of Enterobacteriaceae are gram negative , facultative anaerobic, peritrichous arrangement of flagella if motile, fermenting sugars to produce lactic acid. Most also reduce nitrate to nitrite, although exceptions exist and Unlike most similar gram negative bacteria Enterobacteriaceae generally lack cytochrome c oxidase, catalase reactions vary among Enterobacteriaceae. (Markey *et al.*, 2013). While *Candida albicans* is a commensal yeast of the intestinal and genital tract of many animal species, and

humans, Most infections are endogenous and predisposing causes, such as pregnancy, Acquired Immune Deficiency Syndrome, Autoimmune disease, and malnutrition can induce infection (Brooks *et al.*, 2013) Because of the problem of antibiotic resistance and the emergence of, as well as the evolution of new strains of disease pathogens that cause great concern to the global health community, effective treatment of a disease require the development of new pharmaceuticals or some alternative source of novel drugs, commonly used of plants as a therapeutic uses in our community could be a good source of drugs to overcome this problem (Manandhar *et al.*, 2019) & (Aqil *et al.*, 2005) Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties and in recent years the use of and search for, drugs and dietary supplements derived from plants have accelerated. Ethnopharmacologists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals to develop treatments for infectious diseases. Traditional healers have long used plants to cure infectious diseases and the new medicine is trying to

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duplicate their successes (Cowan, 1999) & (Talib & Mahasneh, 2010) Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts, first it is very likely that these phytochemicals will find their way to use as antimicrobial drugs and several are already being tested in humans and It is reported that, on average each year just two or three antibiotics derived from microorganisms are launched also scientists realize that the effective life span of any antibiotic is limited which require worldwide spending on finding new anti-infective agents (Clark, 1996). Medicinal plants is an important source to cure a number of diseases among human beings and a large number of these plants around the world have been extracted, semi-purified to investigate individually their antimicrobial activity. However, not much, information is available on such activity of medicinal plants and out of the 400,000 plant species on earth, only a small amount has been systematically investigated for their antimicrobial activities (Varahalarao & Chandrashekhar, 2010) The large number of plant compounds is readily available over-the-counter from herbal suppliers and self-medication with these plant is customary and the use of plant extracts, as well as other alternative forms of medical treatments, is commonplace in the last decade and in a survey during the previous year in the United States approximately one-third of people used at least one unconventional therapy like herbal plant (Eisenberg *et al.*, 1993). Even today, plant materials still to play an important role in health care as therapeutic remedies in many developing countries and Many infectious diseases are known to be treated with

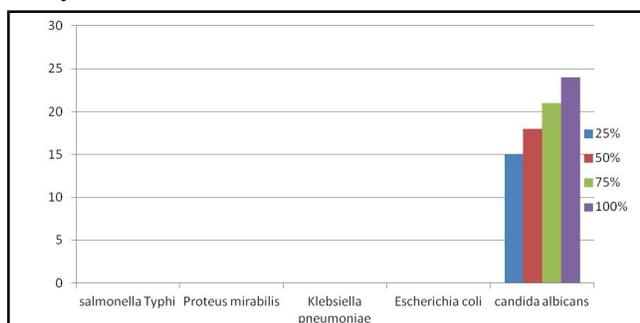


Fig. 1: MIZ of the different concentration of aqueous plant extracts against four types of bacteria and one yeast.

Table 1: The diameter of zone in mm obtained from bacterial strains and yeast against different concentrations of extract.

concentration of the extract	Diameter of (MIZ) in mm				
	<i>Salmonella typhi</i>	<i>Proteus mirabilis</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
25 %	-	-	-	-	15
50%	-	-	-	-	18
75 %	-	-	-	-	21
100 %	-	-	-	-	24

herbal remedies, throughout the history of, humaneness (Zakaria, 1991).

Materials and Methods

Microbial Culture

Four types of gram negative bacteria from family enterobacteriaceae (*Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*) isolates and one yeast (*Candida albicans*) isolate were obtained from the Bacteriology Unit of Al Mahmoudiyah General Hospital in Baghdad each of the bacterial strains were sub cultured on selective media for Enterobacteriaceae (MacConkey and XLD Agar) then biochemical test was done by using Tri sugar iron Agar to confirm bacterial strain while *Candida albicans* was sub cultured on Sabourauds Dextrose agar and all of isolates were incubated at room temperature (37°C) or 24 hours. (Brooks *et al.*, 2013).

Plants grind

Artemisia vulgaris leaves were carefully washed using tap water to remove the dusts and then dried in an oven at (60°C) for 4 hours. The dried leaves were milled separately in a small electric mill (High-Speed Grinder), The powdered leaves of these plants were transferred to a glass sealed cans and placed in the refrigerator before the extraction process. (Al-Manhel & Niamah, 2017).

Extract preparation

The aqueous extract of dried plant leaves was made in the distilled water. About 100 grams of plant leaves powder (*Artemisia vulgaris*) were taken and mixed in 100 ml of distilled water. The mixture was taken into 250 ml sterile conical flasks, plugged with sterile cotton and leave it for 48 hrs and the mixture was handy shaking from time to time then the solution was filtered through muslin cloth, This process was repeated three times after which a clear aqueous extract of the plant was taken and four concentration was prepared (25%, 50%, 75%, 100%) from this extract. (AL Othmani, 1997) & (Sarmamy, 2001)

Preparation of immersed Disc

The discs were made from filter paper by using hand small drill then the paper discs was placed in a beaker containing ethanol 95% for 2 hours to sterilize them after that the discs put on petri dish by sterile forceps and incubated for 5 hours at 45-50°C to get rid from ethanol after that discs immersed in the concentrations of the aqueous extract (25%, 50%, 75%, 100%) for 12 hours

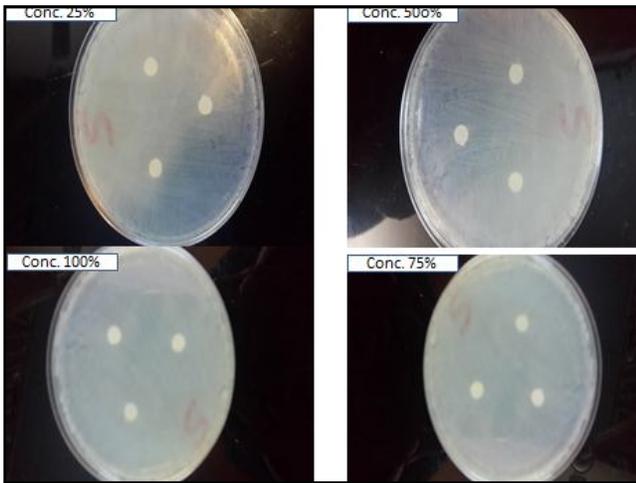


Fig. 2: Shows no antimicrobial activity of different plant extract against *S. typhi*.

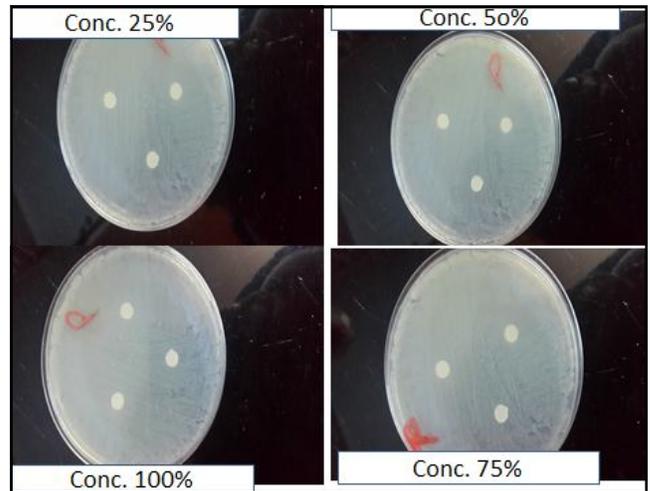


Fig. 4: Shows no antimicrobial activity of different plant extract against *E. coli*

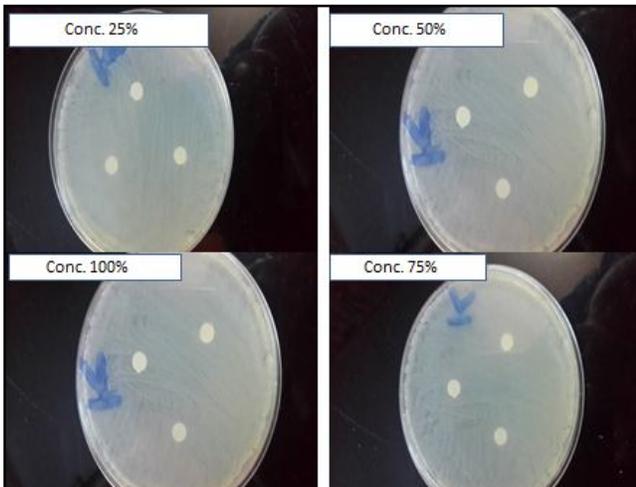


Fig. 3: Shows no antimicrobial activity of different plant extract against *K. pneumoniae* pneumoniae.

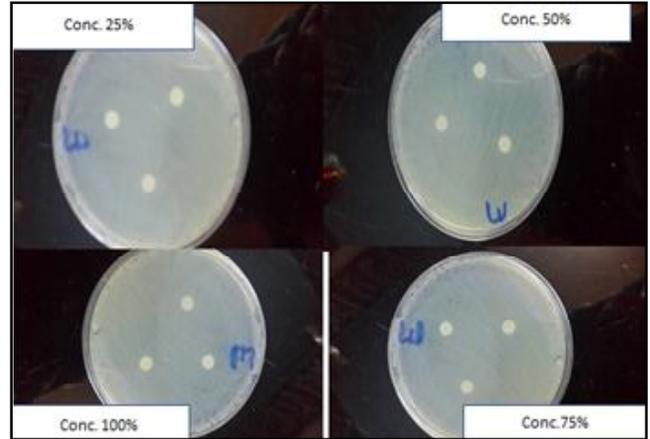


Fig. 5: Shows no antimicrobial activity of different plant extract against *P. mirabilis*.

and then the discs put on petri dish by sterile forceps and incubated for 5 hours at 40°C then placed in a closed container until using. (Sarmamy, 2001).

Disc Diffusion Method

For antimicrobial sensitivity test disc diffusion method testing was carried out according to the standard method by Bauer *et al.*, (1966) to investigate the presence of antibacterial activities of the plant extracts, A bacterial culture (which has been adjusted to 0.5 McFarland

standard), was used to lawn Muller Hinton agar plates by using a sterile swab, The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of aqueous plant extracts concentration (25, 50, 75, 100%) were placed on the Mueller-Hinton agar surface by sterile forceps . Each test plate include three discs also there was one negative control plate, all plate was then incubated at 37°C for 18 to 24 hours after the incubation, the plates were examined for inhibition zone, The inhibition

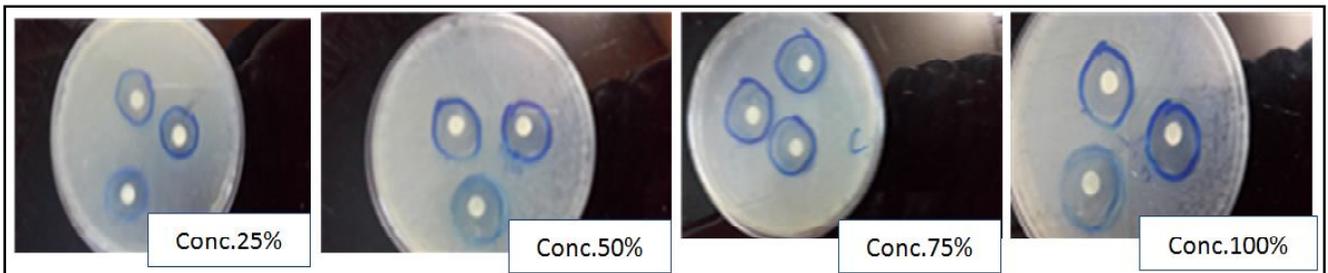


Fig. 6: Shows inhibitory zone to *Candida albicans* growth according to different conc. of plant extract.

zone were then measured using calipers and recorded (Zaidan *et al.*, 2005).

Results and Discussion

According to the result of this study aqueous plant extracts of *Artemisia vulgaris* leaves shows no inhibitory zone to the growth of (*Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*) isolates on the cultural media while the study recorded inhibitory zone on the growth culture of (*Candida albicans*) ranging from (15 - 24 mm) as in table below:

In spite of Iwu *et al.*, (1999) pointed that there is a large number of plants that have been recorded as valuable resources of natural antimicrobial compounds and considered as alternative that can be effectively used in the treatment of many bacterial infections However Varahalarao & Chandrashekar (2010) pointed that very little information is known about their activity as medicinal plants and their antimicrobial properties, (*Artemisia vulgaris* L.) is a weed entered into Iraq through the last few years and the new weed belong to composite family and identified as Mugwort (*Artemisia vulgaris* L.), This species is not common in Iraq, It was found that its seeds entered to Iraq with, impure shipment (polluted) of garden flower's seeds (AL-Juboory *et al.*, 2013) in this study the plant leaves extract antimicrobial effective only on the yeast but not on gram negative bacteria which agree with Manandhar *et al.*, (2019) whose study shows that *A. vulgaris* were effective only against gram positive bacteria *Staphylococcus aureus* but not obviously effective on gram negative bacteria like *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* but there is antifungal activity of the same extract. Also the result of the antimicrobial efficiency of (*Artemisia vulgaris* L.) extract is resemble to study done by Tuchilu *et al.*, (2009) which showed that plants alcoholic extracts have moderate or no activity against Gram-negative bacteria. On another study by Singh *et al.*, (2011) revealed that members of enterobacteria including *Escherichia coli*, *Salmonella enterica*, *Klebsiella* spp., strains growth were resistant to plant extract of *Artemisia vulgaris* L. when use as oil while (*Candida albicans*) growth were sensitive to same extract also Ahameethunisa & Hopper (2010) concluded that alcoholic extract of *Artemisia* spp. showed inhibitory activity for gram-negative bacteria except for *Klebsiella pneumoniae*. According to our result about the effect of aquatic extracts of *Artemisia vulgaris* leaves on some pathogens We suggest Further study, however, it is still not clear the effectiveness of plant extract in inhibiting the growth of some pathogenic microorganisms, Another possibility for the limited

antibacterial efficiency of some plants may be due to the cold percolation extraction method and the use of crude extract.

Conclusion

According to the results of this study, it can be concluded that using of aqueous leave extraction effective only on the yeast but not against bacterial strains tested so further investigations are necessary to evaluate antibacterial activity by using alcoholic extract or other parts of the plants to evaluate the studied plant as a potential antimicrobial agent.

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