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Study of antimicrobial effect for some extracts of *Anabasis aphylla* on Salmonella

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ABSTRACT

In this study, determine the inhibitory effect of Aqueous, Alcoholic, Flavonoid, Glycoside extracts of plant *Anabasis aphylla* on the salmonella, the microbial strain was exposed to the following four different concentrations of extracts 1, 10, 25 and 100 mg/ml. there has been an increasing effect on microbial growth inhibition with increasing concentration of extracts. The largest zone of inhibition was obtained with Flavonoid extract (24mm). The results For the study showed that the Aqueous extract of *Anabasis aphylla* has the inhibitory effect on the growth of bacteria which used in the study by inhibition diameter 20 mm, also gave Alcoholic extract inhibitory effect on bacterial growth by inhibition in diameter 15 mm, and gave aglycoside extract inhibitory effect on bacterial growth by inhibition in diameter 14 mm, the antimicrobial activity of extracts was compared with standard antibiotics like amoxicillin and ketoconazole (100µg/disk).

Keywords: extracts, antimicrobial activity, *Anabasis aphylla*

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INTRODUCTION

The development of microbial resistance towards antibiotics has heightened the importance of the search for new potential effective plants and plant constituents against pathogenic microorganisms (1).The outcome of infections with *Salmonella* is influence by the dose of organism ingested, the serotype of *Salmonella* and resistance of host [2].There are three division into which members of the genus may be placed according to their host preferences or adaptation, there are primarily adapted to man *(S. typhi)(S. paratyphi*)or primarily adapted to the animals and un adapted (those attack man and animals) [3].Typhoid Mary was the most famous carrier *Salmonella* who worked for eight families and was responsible for seven epidemics Salmonella including over 200 persons [4]. Chronic carrier are rarely observed in childhood but increased with increasing age. They are more among females than males in a ratio of 3: 1 [5]. In typhoid fever and non-typhoidal Salmonellosis, two other factors have epidemiologic significance. First, an asymptomatic human carrier state exists for the agents of either form of the disease. Approximately 3% of persons infected with S typhi and 0.1% of those infected with non-typhoidalsalmonellae become chronic carriers. The carrier state may last from many weeks to years. Thus, human as well as animal reservoirs exist. Interestingly, children rarely become chronic typhoid carriers. Second, use of antibiotics in animal feeds and indiscriminant use of antibiotics in humans increase antibiotic resistance in salmonellae by promoting transfer of R factors [6]. Every year, approximately 800,000 to 4 million cases of Salmonella result in 500 deaths in the United States. Children are the most likely to get Salmonella. Young children, the elderly, and the immunocompromised are the most likely to have severe infections[7].medicinal herbs That many and commonly used in folk medicine, has studied the inhibitory effect of some of them on the growth of germs (8), by many researchers in different parts of the world ,for its high capacity to inhibit the growth of some types 0f germs. The inhibitory effect of these plants is probably due to contain some active compounds such as phenols and derivatives, which are Strong disincentives for germs (9) and is the plant kingdom is essential for effective compounds antimicrobial effect(10).

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (11). Biologically active widespread from plant sources have always been of great interest to scientists working on infectious diseases. Over the past decade there has been an explosion of interest in the antimicrobial, particularly antibacterial and antifungal, activity of natural products (12). Since the current study, aims to study the effect of extracts of anabasis plant on salmonella , so necessary Provide a simplified description of the plant used in this study.

The plant:

Plant belongs to the family *Chenopodioideae*, plant contains a group of alkaloids are concentrated in the fruit with Percentage about 3.2%, mainly alkaloid *Anabasis aphylla* is a volatile liquid and has highly toxic effect , and other alkaloids amorphous such as, aphylline , aphyllidine and lupinine**.**

The aim of study:

Due to the fact that ***Anabasis aphylla*** is one of important medicinal plants, used in the treatment of many conditions Including bacterial cases. The study about it did not enjoy extensive, so the present study aimed to study the inhibitory effect of extracts prepared from these plants on salmonella.

MATERIAL AND METHODS

Collecting plant specimens

Collected *Anabasis aphylla* seeds from local markets, diagnosis was verified at the College of Veterinary Medicine - University of Qadisiya.

Preparation of plant extracts:

Aqueous extract:

Preparing an aqueous cold extract by method (13,), which included the mixed amount of dry powder of the plant under study with the amount of distilled water per 1 g: 2 ml of distilled water, using a blender and at room temperature, leaving the solution for 24 hours and then filtrated the resulting mixture by several layers of sterile medical gauze and the resulting sediment put in test tubes of centrifuge (3500 rpm \ minutes),Taken the filtrate for the purpose of drying.

Alcoholic extract:

Attended alcoholic extract, to plant seeds. According to the method (14).add (50)g. of plant powder to (400)ml of solvent ethanol (70%) in conical flask, left on Magnetic stirrer At room temperature. For two (24) hours, Nominated Abstract. Using the nomination papers (Whatman No.1), filtrate focused Using (Rotary Vacuum Evaporator) dry the abstract at room temperature and collection of Abstract in sterile bottles.

Flavonoid extract

Attended flavonoid abstract of Anabasis aphylla seeds according to the method (14).added(50)g of vegetable powder to 500ml of ethanol concentration (80%), then left on Magnetic stirrer for a period of 24 hrs. nominated mixture by using (Whatman No.1), added to it 50 ml Lead acetate 1%, and leave for 15 min filtrated the mixture and treated the preciptated with (50 ml) acetone and 60 ml. HCL after that precipitated and drying the preciptated at room temprature and collection of abstract in sterile bottles.

Glycoside extract:

Attended glycoside Abstract of Anabasis aphylla seeds as indicated in (15). added (50) g Of vegetable powder to 400 ml of acetic acid (2%), put the mixture in a water bath for a period of 8 hours, then Filtrated the mixture and mixed the Filtrated with (n – BaoH)saturated by(NaCl)(3×50)ml. and dry the precipitated At room temperature and collection of Abstract in sterile bottles.

Sterilize prepared extracts:

Sterilized prepared extracts by passing through the membrane filters with diameter 0.2 micro liter type Whatman, U.K.

Bacterial suspension:

Bacterial suspension which prepare with concentration (1×106)bacterial cell\ ml. by McFarland method as referred in (16), add 0.05 ml. of BaCl 2 (1%) to 9.95 ml. of H2SO4 . compare the density of bacterial suspension with McFarland tube.

Preliminary screening of prepared extracts:

Evaluated the susceptibility prepared extracts on the inhibition of bacterial growth (bacteria Salmonella) according to the method (17), as the method is used Agar – well diffusion method , and included the preparation of Muller- Hinton agar , as instructed by the company processed, pour in Petri dishes, then vaccinated agar with (0.1) ml of airborne bacterial with concentration (1×106) bacteria cell \ ml, publish shake the publisher glass, left the dishes for a period of (15 - 30 minutes), (3-4) diameter hole (8 ml) \ hole ,and the added (100) MicroLiters of extract concentration of (1,10,25,100)mg \ mL into each hole using, dishes were incubated at 37 ° C was measured inhibition zone diameter.

RESULTS

Our results showed that the flavonoid extracts was the most active, followed by the Aqueous, alcoholic and glycoside extracts. Previous study on this plant showed the presence antibacterial phenolic compounds in ethyl acetate fraction (18).

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The mean zone diameter inhibitions by flavonoid, Aqueous, alcoholic and glycoside extracts were found to be 0-24, 0-20, 0-15 and 0-14 mm for bacteria with increasing concentrations, respectively. Table (1)

The rational for this effect may be based on the ability of polar extract permeability through the plasma membrane. It seems hydrophobicity is not the sole determinant factor for the active stability of a membrane structure. Many other molecular mechanisms other than hydrophobicity are involved such as protein flexibility and solubility(19).

Table 1: Antimicrobial activity of *Anabasis aphylla* extracts:

Micro.	Inhibition zone (mm)																		
	watery				alcohol				Flavonoid				Glycoside				**Standards**		control
	1	10	25	100	1	10	25	100	1	10	25	100	1	10	25	100	Amoxicillin	Ketoconazol	DMSO
	0	0	4	20	0	0	2	15	0	0	7	24	0	0	2	14	25	-	-

Diameter of Inhibition zone (millimeter)

-The flavonoid extracts at 100 mg/ml showed the maximum antibacterial activity.
-DMSO also had no effect on the growth of the microorganism. Standard disc inhibited the growth of the test microorganism.

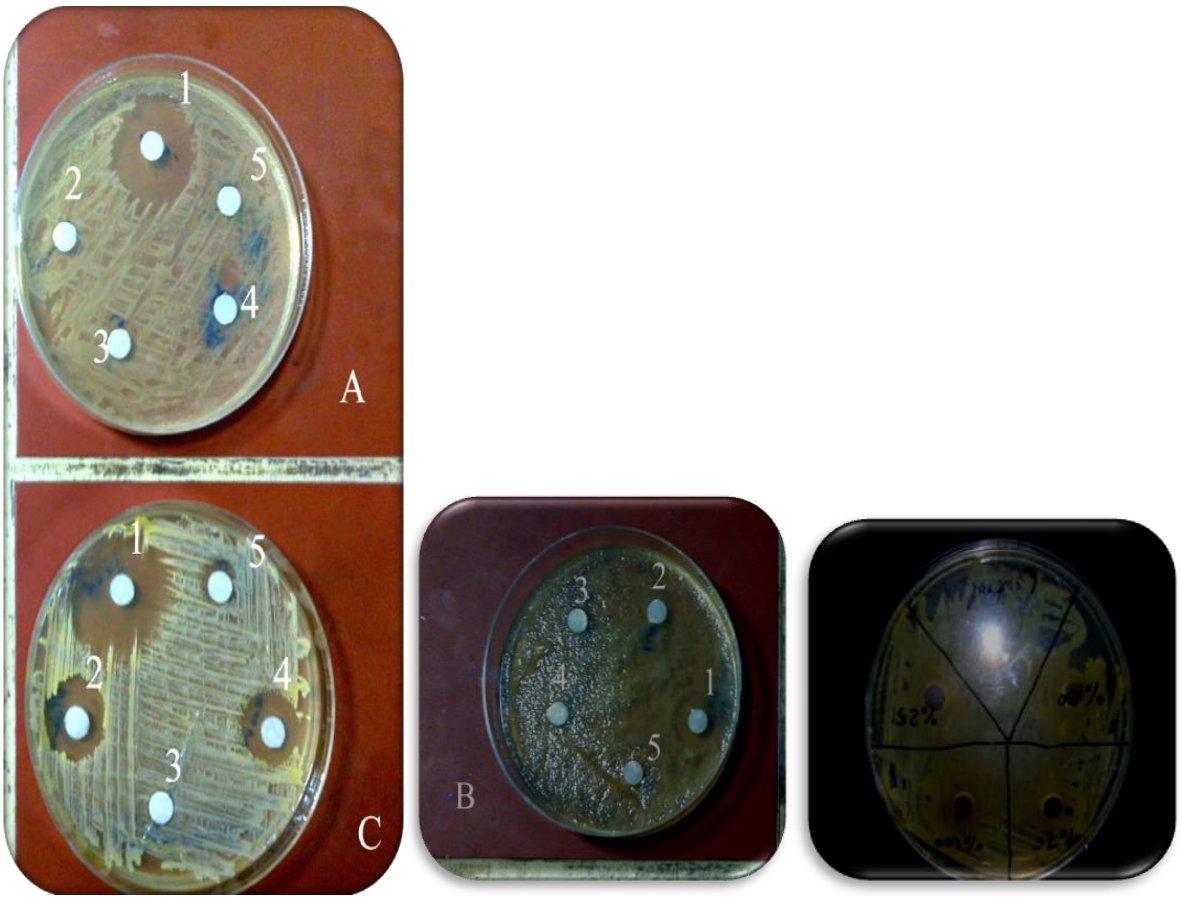

Figure 1: Describes theinfluence ofAbstract(aqueous,alcohol,flavonoid and glycoside of plant (*Anabasisaphylla*) inthe inhibition ofthe growth ofSalmonella

a-aqueous extract; b-alcoholicextract; c- flavonoid extract; d-extract glycoside

We note from the table that watery , alcoholic, flavonoid and glycoside extracts of plant (Anabasis aphylla) Has shown good results in terms of inhibition of Salmonella showed that the aqueous extract showed

20 mm , 25mm alcoholic extract , extract flavonoid 50 mm and glycoside 75 mm, due to the reason that Anabasis aphylla is alkaline substance which effective and this goes back to these extracts contain substances that have the ability to inhibit the growth of some microorganisms such as glycoside as well as the impact of other effective aggregates such as alkaloids, resins, phenolic compounds and tannins and essential –oils ,and, aphylline , aphyllidine and lupinine So this is the vehicle of the most important components of Anabasis aphylla, which contributed to restrain the growth of Salmonella [20].Figure (1)

CONCLUSION

In this study, the antimicrobial activities of the extracts of *A. aphylla*were evaluated. Results for the antimicrobial activity were relatively close to the standards(amoxicillin and ketoconazole).

The demonstration of broad spectrum of antimicrobial activity by *Anabasis aphylla* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

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