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ANTIBACTERIAL EFFICACY OF ETHANOLIC EXTRACT OF GARLIC (*Allium sativum*) AND SUMAC (*Rhus coriaria*) AND ANTIBIOTIC ON *Salmonella typhimurium* ISOLATED FROM CHICKENS

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ABSTRACT

Antibacterial drug resistance is an increasingly worldwide occurred health problem presented by bacterial-originated defectiveness to the work of a wide-range of antibacterial drugs. Uncovering the antibacterial effects of ethanolic extract of garlic (*Allium sativum*) (Glc) and sumac (*Rhus coriaria*) (Smc) on *Salmonella typhimurium* isolated from chickens was the main goal of the present study.Fifty samples of intestinal contents of chickens were collected randomly from various farms located in Al-Diwaniyah province,All specimens inoculated into on macconky agar, Salmonella-Shigella agar at 37c for 24-48-hr, also examined on XLD agar and Salmonella CHROME agar *Allium sativum*(Glc) or *Rhus coriaria* (Smc)extract, at different concentrations, or antibacterial drugs (control), 10mcg ciprofloxacin (Cip), 30mcg amoxicillin/clavulanic acid (Amc), 10mcg neomycin (N), were employed to test their antibacterial activities (AAs) against *S. typhimurium* using agar-gel diffusion tests, The experiment included an investigation about of one isolates from origin 6 isolates of S. typhimurium , 6 out of the 50 chicken samples (12%) were culture positive for *salmonella* *typhimurium* , Significant ($p<0.05$) increases in the AAs against *S. typhimurium* were shown by Glc or Smc extracts when compared to those from the antibiotics. Moreover, these AA increases were revealed to be incremented as the concentrations of those extracts were elevated. No significant ($p>0.05$) differences were demonstrated between the AAs of both extracts. In addition, Cip, Amc, and N showed AAs against *S. typhimurium*; however, Cip revealed the strongest AAs followed by Amc.

INTRODUCTION

Resistance to antibiotics is a historic and a diverse health issue. Overcrowding, elevated world moving of people, leveled up of utilization of antibiotics in hospitals and food animal industries, selection pressure, low levels of hygiene, non-domestic life spreading, and improper waste disposal are some of the main factors that play important roles in dissemination of this problem. The medical intervention using antibiotics is one of the most important approaches in modern medicine to the fight against infectious disease (1, 2, 3). The "golden age" of the discovery of antibiotics was between the nineteen and sixties of the previous century resulted in finding numerous novel antibiotics. Unfortunately, that age came to an end, since scientists could not catch up with antibiotic discovery challenged by the presence of evolved pathogenic microorganisms with antibacterial resistance properties. The predisposing factors connected to the development of antibiotic resistance are the constant inability to create or find novel antibiotics and misuse of those drugs (4).

Staphylococcus aureus, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are currently among the most famous antibiotic-resistant bacteria. Multidrug Resistant (MDR) microorganisms are categorized as three distinct (i.e. urgent, serious and concerning) levels in the United States by the Centers for Disease Control and Prevention (CDC). Resistance against a particular antibiotic is comparative to the examined microorganisms and their prior exposures to antibiotics. Intrinsic and obtained are two different kinds of antibiotic resistance. As a consequence of vertically transmitted genetic materials, microorganisms may be intrinsically insensitive to some antibiotics. A specific antibiotic, for example, can not completely enter the outside layer of some microorganisms, or efflux pumps can remove the antibiotic which enters the membrane (5, 6, 7, 8).

The modern integrative healthcare (IHC) facilities are becoming progressively included with complementary and alternative medicine (CAM). Scholars have progressively reported that CAM is common and / or efficient in the integration of healthcare literature to treat and manage chronic illnesses, and particularly discomfort and stress-related diseases. CAM may offer possibly more efficient multidisciplinary solutions to the treatment of complicated chronic illnesses like HIV, acute ache and addiction. CAM may include the use of certain protocols or substances such as natural products, plants, and/or plant extracts (3, 9, 10, 11, 12).

Uncovering the antibacterial effects of ethanolic extract of garlic (*Allium sativum*) (Glc) and sumac (*Rhus coriaria*) (Smc) on *Salmonella typhimurium* isolated from poultry was the main goal of the present study.

MATERIALS AND METHODS

Intestinal samples and bacterial isolation

Fifty samples of intestinal contents of chickens were collected randomly from various farms located in Al-Diwaniyah province, Iraq. Samples were processed in the laboratory of Poultry Disease College of Veterinary Medicine, University of Al-Qadisiyah for bacteriological assay. Each sample was inoculated in 5ml of nutrient broth and incubated at 37°C for 24-48hrs. Then, by using a sterile loop, some of the cultivation from the broth was collected and streaked on MacConky agar. Visual examination of the bacterial colonies and Gram staining were produced. The samples were subculture and examined on xylose lysine deoxycholate (XLD) agar, then incubated for 24 hours at 37˚C. The isolates were activated by inoculating some of the colonies onto Salmonella Chrome agar (SCA) and incubated at 37˚C overnight. In addition, Vitek2system was employed for rapid identification of the isolates.

Plants and extraction

Bulbs of garlic and sumac seeds were bought from local stores in Al-Diwaniyah City. The Bulbs were washed, sun-dried out, and sliced. Then, the sumac seeds and the dry garlic slices were grinded separately to produce a powder-like form and stored in nylon bags until use. Ethanolic-based extractions were conducted according to (13). Briefly, 50gm of each powder was mixed up with 250ml of 96% ethanol. The combination was maintained at room temperature for 2-5 days in closed bottles and rocked many times per a day. A filtering paper was used. The rest was extracted 3 to 5 times until a definite supernatant had been acquired. 40oC rotary evaporator was used. Weighing and storing the finished products at-20oC until use. The yield percentages of sumac (*Rhus coriaria*) 14.12% while to allium sativum was 9.25% (13).

Antibacterial susceptibility test

A serial dilution of diluting 2gm of each extract with 30% ethanol at 5ml resulting in a 400mg/ml stock solution. Working concentrations of 200, 100, 50, and 25mg/ml (each was done by mixing 1ml of 30% ethanol with 1ml of the extract solution) were generated.

Muller Hinton media (MHM) were used for the well diffusion method (14). In the test, the suspension of bacterial growth was moved over the entire surface of each plate by using cotton swabs excluding six uniform wells of 5mm diameters (15). A sterile cotton swab was employed dipping into the suspension of bacterial growth and inoculated onto the MHM surface by streaking swabs which was used for the antibiotic study, After drying the inoculums, 0.1ml each extract's concentration was placed into the wells besides 0.1ml of 30% ethanol, a negative control, on the same extract plate. Then, the petri-dishes were left for 4-6hrs for the plant extract to spread over in the cultivating medium. Antibacterial drugs (control), 10mcg ciprofloxacin (Cip), 30mcg amoxicillin/clavulanic acid (Amc), 10mcg neomycin (N), were employed to test their antibacterial activities (AAs) against *S. typhimurium* using agar-gel diffusion tests on different plates. These experiments were done in triplicates for each extract and for each antibacterial drug. Then, all treatments were placed in an incubator at 37ºC for 24hrs. Following that, the zone of inhibition's diameter was measured in millimeters by using a ruler (16).

Statistical analysis

Mean ±SE was considered, ANOVA test, least significant differences (LSD) were employed, and $p<0.05$ was followed. SPSS (Version 10) was utilized in the present tests (14).

RESULTS

Bacterial isolation: Out of 50 intestinal samples , 8 were suspect *Salmonella spp* isolates, All the suspect ***S spp*** isolates were colorless and transparent colonies on MacConkey agar media (figure: 1) *S. spp* circular colonies of pale color and a black centers on the XLD agar (figure: 2) while characterized by rounded pink colonies on salmonella CHROME agar media (figure: 3)

Figure 1; suspect *S. spp* colorless and transparent colonies on MacConkey agar.

Figure 2; suspect *S. spp circular* and transparent colonies and black centers on

Figure 3; suspect *S. spp* rounded pink colonies on the salmonella CHROM

From 8 suspected isolates Six has been confirmed as typhimurium isolates were confirmed by vitek 2 ,according to vitek2 Technique result (64 biochemical tests) ,out 50 sample were typhimurium prevalence rate in intestinal samples was (12%) .

Antibacterial activity: Significant ($p<0.05$) increases in the AAs against *S. typhimurium* were shown by Glc or Smc extracts when compared to those from the control drugs. Moreover, these AA increases were revealed to be incremented as the concentrations of those extracts were elevated, table 1 and 2 and figures 4-8. No significant ($p>0.05$) differences were demonstrated between the AAs of both extracts. In addition, Cip, Amc, and N showed AAs against *S. typhimurium*; however, Cip revealed the strongest AAs followed by Amc. The results showed there are significant difference($p<0.05$) between the used antibiotic & Garlic extract which give the highest zone of inhibition (30.3±0.68) mm.

Also, the Sumac extract showed significant increasing ($p<0.05$) in inhibition zone (29.76±0.959) mm as compared with other used antibiotics.

Table (1): Inhibition zones (mm) of *S. typhimurium* growth by extracts of sumac and garlic in culture media.

Type of plant	Extract concentration (mg/ml)				
	25	50	100	200	400
Sumac	17.9±1.20 Aa	20.4±0.29 Ab	23.2±0.75 Ac	26.4±0.86 Ad	29.7±0.95 Ae
Garlic	18.6±0.77 Aa	22.5±0.66 Ab	22.7±1.27 Ab	24.5±2.05 Ab	30.3±0.68 Ac

Different lowercase letters mean significance for horizontal comparisons.
Different uppercase letters mean significance for vertical comparisons.

Table (2): Inhibition zones (mm) of *S. typhimurium* growth by antibacterial drugs, control, in culture media

Positive and negative control	**Inhibition zone (mm)**
Ciprofloxacin (Cip)	24.8±0.13a
Amoxicillin/Clavulanic acid (Amc)	20.9±0.66b
Neomycin(N)	11.3±0.33c

Different lowercase letters mean significance difference (P<0.05)

Table (3): Inhibition zones (mm) of *S. typhimurium* growth by extracts of garlic, sumac and antibacterial drugs in culture media.

Plant extract and antibiotic	**Inhibition zone(mm)**
Garlic	30.3±0.68a
sumac	29.76±0.959a
Neomycin(N)	11.33±0.333b
Amoxicillin/Clavulanic acid (Amc)	20.96±0.66c
Ciprofloxacin (Cip)	24.86±0.133d
Diluted ethanol (30%)	0±0e

Different lowercase letters mean significance difference (P<0.05)

Figure 4; Inhibition zones produced by sumac (*Rhus coraria*) against *S. typhimurium*.

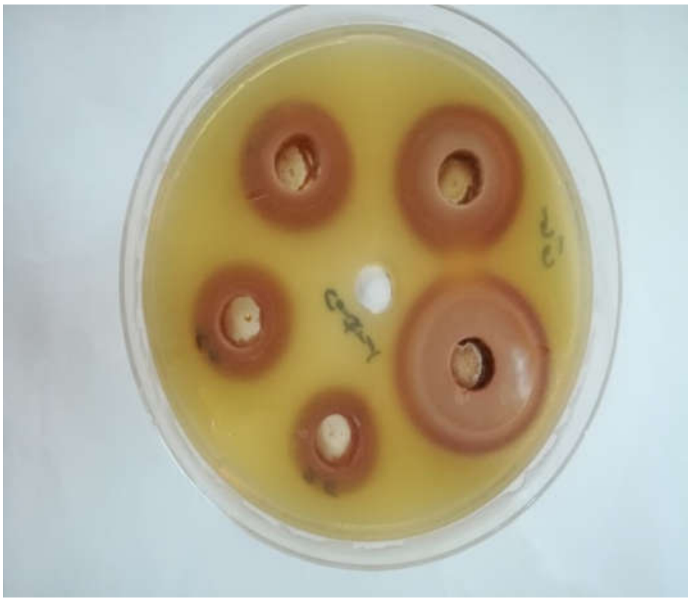

Figure 5; Inhibition zones produced by sumac (*Rhus coraria*) against *S. typhimurium*.

Figure 6; Inhibition zones produced by garlic (*Allium sativum*) against *S. typhimurium*.

Figure 7; Inhibition zones produced by garlic (*Allium sativum*) against *S. typhimurium*.

Figure 8; Inhibition zones produced by the antibacterial drugs, 10mcg ciprofloxacin (Cip), 30mcg amoxicillin/clavulanic acid (Amc), 10mcg neomycin (N), against *S. typhimurium*.

DISCUSSION

Since the clinical implementation in the 1940s, problems linked to the use of antibiotics have been recognized. The use and often improper use of those agents have since increased. Antibiotics are strong medicines used to fight deadly infectious illnesses. Antibiotics have a broad variety of harmful impacts like all strong medicines. An elevated consuming impact that neglects the danger of this use leads to diminish the beneficial effects of those agents. However, when antibiotics are appropriately used, there provide advantages for patients as long as they adhere to the consuming rules. In addition, the structure of the pathogenic microorganism is destructed by those drugs, contributing to bacterial adjustment or mutations, encouraging the production of new resistant strains to those antibiotics (19).

The present work unveiled the antibacterial effects of sumac on *S. typhimurium*. The actions of herbal extracts, like thyme, garlic, rosemary, sumac, spice, ginger and mustard, have been earlier explored as antibacterial and antioxidizing inhibitors. The antimicrobial impacts on foodborne bacteria of Smc have been demonstrated. Tannins and other compounds are the main reason for the antibacterial activity of sumac. Moreover, the elevated concentrations of carvacrol and thymol in Smc are associated with antioxidant and AAs in Smc (20-23)The AAs of Smc against *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria, and yeast-mold of meat were tested and revealed to be strong in the low concentration, negative correlation with concentration of Smc, (24) however, this disagrees with the present study data that showed a positive correlation with concentration.

The current results demonstrated the antibacterial effects of garlic on *S. typhimurium*. Alcohol extract was discovered to be much more durable and efficient than aqueous extract. Tests have shown, however, that the AA of garlic depends entirely on the allicin molecule, which is extra efficient on gram-positive bacteria. Although more lipid materials in the membranes of *S. typhimurium* are present making allicin unable to achieve this objective, as it is caught in this lipid material (25).

The present study data showed strong effects of garlic against the growth of this bacterium. Antimicrobial characteristics for garlic have been shown to have a range of anions such as nitrate, sulfide, chloride, and organosulfur substances. Furthermore, many study findings have shown that allicin is the most significant antibacterial drug, reducing RNA production and capturing RNA (25). The data of the present study show strong AAs of the Glc or Smc against *S. typhimurium*. The present work also recorded the significant ($P<0.05$) elevation of antibacterial activity of the both plant extract (garlic and sumac) versus the activity of tested antibiotics (neomycin, amoxicillin, ciprofloxacin) and this results may be attributed to the resistance of the isolated bacteria toward the above antibiotics due to misuse of these drugs in clinical fields which lead to restriction in the antibiotic activity against tested bacteria (26).

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