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Antimicrobial Effect of Garlic, (*Allium sativum*) Extract and Apple Cider Vinegar on Some Species of *Salmonella* Isolated from Raw and Processed Meat Products

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ABSTRACT

With the efflorescence of antibiotic-resistant bacteria, it is judicious to survey new sources of natural compounds with antimicrobial activity. Edible plants have been upheld and verified to be harmless and economical. In this study, a marked inhibitory effect of aqueous garlic extract and apple cider vinegar was 5% against 7 *Salmonella* serovars isolated from raw and processed meat products. The efficacy of aqueous garlic extract and apple cider vinegar was measured. The antibacterial potential of both substances was checked by the agar well diffusion method and minimum inhibitory concentrations (MIC). The MIC values of garlic extract against *Salmonella* serovars were (4-64 µg/ml) while the MIC values of apple cider vinegar were (2-16 µg/ml).

INTRODUCTION

Meat and meat products are idealistic cultural media for being attacked by organisms (Gracey, 1986). It is because of the various intrinsic factors such as high moisture level, high nitrogenous compounds, proteins and other growth factors (Fratmico *et al.*, 2005). The majority of the spoilage organisms are contaminants that may arise from external sources during unhygienic and improper slaughtering, handling, and processing. Currently, the most important pathogens associated with meat products are some members of *Enterobacteriaceae* especially *Salmonella* (Harakeh *et al.*, 2005).

The genus *Salmonella* is considered one of the most common causes of food-borne infectious diseases in the world. A characteristic feature of this organism is its wide host range, which comprises most animal species including mammals, cold-blooded animals, and humans as well (Baird-Parker, 1990). During the past decades, the limit of *Salmonella* infections has been overrun dramatically and this pathogen has become an obstacle in the food industry because of its widespread distribution all over the world. *Salmonella* is not only caused mild to severe infections but also may be responsible for fatal injuries threatening human life. *Salmonella* bacteria are zoonotic in nature and lower the food quality severely in addition to being hazardous to humanity (Vivek *et al.*, 2012).

Salmonella enterica serovar *typhimurium* and *Salmonella enterica* serovar *enteritidis* are the most frequently isolated serovars from foodborne outbreaks throughout the world. Salmonellosis usually induces self-limiting gastroenteritis or an asymptomatic carrier state in a wide array of animal species (Uzzau *et al.*, 2000).

Recently, the ascending concern about the safety of food and the global escalation in antibiotic resistance has led to the need to develop alternative natural antimicrobials so as to rule foodborne pathogens. Natural products contain natural antimicrobial compounds that can play role in the stabilization and prevention of food deterioration by microorganisms. Moreover, these natural ingredients impart characteristic taste, aroma, or tastiness and color to foods (Abdulrahman *et al.*, 2020). Although hundreds of researchers approved the antimicrobial activity of natural compounds against pathogenic or spoilage biota in the last two decades, few of them have been used industrially (Roller, 2003).

The first ancient cultivated vegetable all over the centuries is the *Allium* family that is an amazing group that contains over 500 species that vary in shape, color, and taste but they are close in their biochemical components. One of the most common *Allium* family members is *Allium sativum* (i.e., garlic) (Ahmed *et al.*, 2016). Garlic has been used for a long time to resist infectious diseases by various societies all over the world. The French scientist Louis Pasteur firstly demonstrated the antibacterial effect of garlic against all bacterial species and many published studies have clarified the effective role of garlic juice against common pathogenic bacteria and against multidrug-resistant bacteria (El-Azzouny *et al.*, 2018). The characteristic activity of garlic against these pathogenic bacteria may be attributed to an organosulfur compound known as allicin which has multiple inhibitory effects on the microbial cell (Ankri and Mirelman, 1999).

Furthermore, various studies have proved the great antibacterial effect of organic

acids on different species of pathogenic and foodborne microflora. Apple cider vinegar (ACV) is a natural product extracted from apple fermentation, contains organic acids as 3-9% of acetic acid, it is known to be the main constituent of apple vinegar. It also contains minerals and vitamins (Nasiroglu *et al.*, 2014). Organic acids are weak acids meaning the antibacterial property is contributed to its undissociated forms (Malicki *et al.*, 2004). They passively diffuse via the bacterial cell wall and internalize into neutral pH dissociating into anions and protons. The pumping of protons decreases the internal pH which exerts an antibacterial effect on microorganisms (Ricke, 2003). With the effect of external chemical alteration and change in pH, the structure of cell membrane units as proteins and phospholipids alter. These alterations also affect the permeability of the cell membrane and result in leakage of the internal cell metabolites (Langworthy, 1978). Apple cider vinegar acts on the bacteria by penetrating the cell wall of the bacteria and destroying their DNA retarding their reproduction and protein synthesis (Yasothai and Giriprasad, 2015). Eventually, this process ends up with cell death. Therefore, the objective of this study was to investigate the antimicrobial capacity of aqueous garlic extract (AGE), oily plant extracts such (Thyme, Curcuma, Cinnamon, and Mustard), Pimenton spices, and apple cider vinegar (ACV) against foodborne *Salmonella* species.

MATERIALS AND METHODS

This study was carried out at Animal Health Research Institute in Zagazig, Agriculture Research Center in the period from August 2020 till May 2021.

Sampling: A total of 120 samples of raw and processed meat products (sausage, burger, and kofta), 30 samples for each type, were purchased from different markets in Zagazig and Faqous, Sharkia province, Egypt.

Isolation and Identification:

Pre-enrichment. A pre-enrichment stage using buffered peptone water was employed throughout this investigation. 25 g of minced

meat or finely cut pork sausage, burger or kofta were added to 225 ml of buffered peptone water (BPW). The BPW was then incubated at 37°C for 18-24h (Edel and Kampelmacher, 1973).

Enrichment and Selective Media. The Rappaport Vassiliadis (RV) medium used in this study was prepared as described previously by Vassiliadis, 1983 while the selective and differential plating medium was xylose-lysine deoxycholate agar (XLD) (Rambach, 1990).

Inoculations and Methods:

In a series of experiments, 0.1 ml of inoculum of the pre-enrichment culture was introduced to 10 ml of RV medium in test tubes and incubated at 43°C for 24 h, and subcultures were made at 24 and 48 h to XLD agar medium (9 cm plates). The plates were incubated at 37°C for 24h and examined for the presence of *salmonellas* and for the degree of growth of competing organisms. The suspicious colonies on the XLD plates were cultured onto triple sugar iron agar (TSI) slants, Citrate agar, urea agar, and lysine-iron agar (LIA) then incubated at 37°C for 24h. Isolates were serotyped in the Serology Unit Animal Health Research Institute, Dokki, Giza, Egypt using commercial antisera (Difco, Detroit, MIUSA) according to the manufacturer's instructions.

Extracts Preparation:

Aqueous garlic extract (AGE) was prepared according to (Onyeagba, 2004), Oily plant extracts were prepared according to (Guenther, 1961). Pimenton extract was prepared as mentioned by (Rivillas and Soriano, 2007).

Commercially available apple cider vinegar 5% was obtained from a local market in Faqous, Sharkia Governorate.

Determination of the Antibacterial Activity of Garlic Extract and Acid Cider Vinegar on *Salmonella* spp.:

Agar Diffusion Assay:

The tested isolates were inoculated into 10 ml of sterile nutrient broth and incubated at 37°C for 8 hours. The cultures with the optical density of 1.5×10^8 CFU were swabbed on the surface of sterile nutrient agar

plates using a sterile cotton swab. Agar wells were prepared by a sterile cork borer with a 10 ml diameter (CLSI, 2013). By a micropipette, 100 µl of different concentrations of each tested extract was added to wells in the plate. In the case of plant oil extracts, 100 µl of 5% dimethylsulphoxide (DMSO) was added to the oil extract in order to solubilize it.

The plates were incubated in an upright position at 37°C for 24h. The diameter of inhibition zones was measured in mm and the results were recorded. The inhibition zones with a diameter of less than 12 mm were neglected and considered as having no antibacterial activity (Durairaj *et al.*, 2009).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assessment:

The broth microdilution method was carried out using 96 well plates (TPP Switzerland). Both garlic extract and ACV were diluted twofold in LB broth® (Acumedia, Michigan, USA), and the wells were inoculated with 1×10^6 CFU of bacteria (in a 0.2 ml final volume). The incubation period was 24 h at 37°C. The MIC testing was performed according to the recommendations of the CLSI, 2013.

The MIC value is defined as the lowest antimicrobial concentration that inhibits microorganism growth while the subinhibitory concentration (SIC) value is an antimicrobial concentration that is below one capable of inhibiting the detectable growth and replication of a microorganism. The minimum bactericidal value (MBC) was determined according to (Khosravi and Malekan, 2004).

RESULTS

The Recovery Rate of Isolates and Identification:

Of all the 120 samples tested, only 7 (5.83%) *salmonella* serovars were obtained from raw meat and processed sausage. Serotyping of these serovars revealed that *Salmonella birkenhead* and *S. typhimurium* predominated (28.56%) followed by *S. paratyphi*, *S. montevideo* and *S. kentucky* (14.28%).

Table 1. *Salmonella* serovars characteristics.

Serovars	Number of isolates	Percentage (%)	Source
<i>S. paratyphi</i>	1	14.28%	Sausage
<i>S. birkenhead</i>	2	28.56%	Brazilian raw meat
<i>S. montevideo</i>	1	14.28%	Sausage
<i>S. kentucky</i>	1	14.28%	Kofta
<i>S. typhimurium</i>	2	28.56%	Burger

Antibacterial Activity:

All tested isolates were treated with aqueous garlic extract, oil extracts of Thyme, Curcuma, Cinnamon and Mustard, Pimenton spices and apple cider vinegar 5% by disc agar diffusion method; both aqueous garlic extract and apple cider vinegar were the most highly antibacterial activity against the 7

salmonella serovars and the *salmonella* inhibition zones range was (20-40mm). Moreover, The MIC values of aqueous garlic extract (4-64µg/ml) against *Salmonella* isolates while the MIC values of apple cider vinegar were (2-16µg/ml) ACV was more effective antibacterial agent against *Salmonella* (MBCs of 4-32µg/ml) than AGE (MBCs of 8-128µg/ml).

Table 2. SIC, MIC and MBC of aqueous garlic extract and apple cider vinegar against *Salmonella* spp. Isolates.

<i>Salmonella</i> spp.	Concentration of SIC, MIC, MBC (µg/ml)					
	Aqueous garlic extract			Apple cider vinegar		
	SIC	MIC	MBC	SIC	MIC	MBC
<i>S. paratyphi</i>	16	32	64	4	8	16
<i>S. birkenhead</i>	4	8	16	8	16	32
<i>S. montevideo</i>	16	32	64	1	2	4
<i>S. kentucky</i>	2	4	8	4	8	16
<i>S. birkenhead</i>	32	64	128	4	8	16
<i>S. typhimurium</i>	16	32	64	8	16	32
<i>S. typhimurium</i>	32	64	128	8	16	32

DISCUSSION

Salmonella bacteria have become the major reason for foodborne diseases which has raised a great safety concern to public sanitation. Meat is considered a requisite diet for most persons because of its palatability and its highly nutritious ingredients by protein and vitamins. For this reason, meat is considered a favorable media for the growth of pathogenic microorganisms (Sharp and Walker, 2003). Lack of personal hygiene, improper storage, impurity of food area preparation, and filthy utensils are considered the cause of contamination of raw and processed meat. In this study, the presence of

Salmonella was established to be nearly 7% in retail meat products.

In our work, bacteriological analysis of fresh meat and meat products revealed that *Salmonella* species were recorded with a percentage of 5.83%. The most commonly recovered serovars from different retail meat and meat products were *S. Typhimurium* and *S. birkenhead* with a percentage (28.56%) for each, followed by *S. paratyphi*, *S. Montevideo*, and *S. Kentucky* with a percentage of 14.28% for each. Detection of seven *Salmonella* serovars in this study reveals the likelihood of cross-contamination from various sources in slaughterhouses and

poor sanitation during the butchering and processing of meat (El-Azzouny *et al.*, 2018).

The global petition for foods deprived of any chemical additives or preservers has become a necessity because synthetic preservatives could be toxic to humans. Searching for alternative or natural food preservatives for extending the shelf life of foods is crucial to dismiss the chemical substances as food preservatives. Plant extracts may be a suitable solution because of their reputable antimicrobial activities over and above their relatively lower toxicity and reduced number of side effects (Kalemba and Kunicka, 2003). Moreover, plant extracts have a multi-component nature, so it is more difficult for bacteria to advance resistance than many commonly used antibiotics, which have a single target (Smith-Palmer, 2001).

Of interest, all isolated *Salmonella spp.* were inhibited by aqueous garlic extract (AGE) and apple cider vinegar 5% (ACV). It has been reported that the mechanism of herbal antimicrobial effects involves the inhibition of various cellular processes and increasing the plasma membrane permeability and ultimately ion leakage out of bacterial cells (Walsh *et al.*, 2003). In a similar study by (Ross *et al.*, 2001), they analyzed the antimicrobial properties of garlic products, the active ingredient found to be responsible for inhibition of antimicrobials was allicin (allyl 2-propene thiosulfinate). Our study revealed that the MIC of aqueous garlic extract against *Salmonella spp.* was (4-64 μ g/ml), while in a similar study, the MIC of aqueous garlic extract on *Salmonella* serovars was (0.125-1 μ g/ml) (10). Some studies have proved that garlic consumption increases the production of cytokines, the activity of macrophages and lymphocytes, and ultimately improves and stimulates the immune system (khodadadi *et al.*, 2013). Allicin was also proved to inhibit the development of the organism as it obstructs the acetyl CoA forming system, consequently, inhibition of DNA and protein synthesis then inhibits RNA synthesis as a primary target (Cutler and Wilson, 2004).

With respect to apple cider vinegar, numerous studies previously documented the constituents and health benefits of vinegar (Budak *et al.*, 2014). It is known that apple cider vinegar contains gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, and p-coumaric acid. Thanks to these bioactive substances, vinegar has several functional therapeutic properties such as antibacterial and antioxidant activities (Budak *et al.*, 2011).

In this study, apple cider vinegar had influenced greatly on *Salmonella spp.* with MIC (2-16 μ g/ml). According to (Malicki *et al.*, 2004), organic acids are considered weak acids meaning the antimicrobial effect of organic acids is mainly caused by their undissociated form. They passively diffuse through the bacterial cell wall, internalizing into neutral pH dissociating into anions and protons. The release of the protons decreases the internal pH which exerts an inhibitory effect on the bacteria (Ricke, 2003). Many previous types of research have proved the antibacterial effect of organic acids on different types of pathogenic bacteria. Tartaric, citric, malic, lactic, propionic, and acetic acids have been used for long periods to decontaminate bacteria from beef, pork, and poultry (Lopez *et al.*, 2011).

Authors' Contribution:

All authors are in agreement with the content of the manuscript and were involved in all steps of its preparation.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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