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Review Paper

Promising potentials for the application of phytochemical synergies of oregano-cranberry extract mixture towards the growth inhibition of *Salmonella typhimurium*

Ajayi Adeyinka. O.

Natural Resources Institute, University of Greenwich, Medway Chatham Maritime, Kent, United Kingdom.

E-mail: checkyinka2010@yahoo.com

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Abstract. *Salmonella* species continue to present serious public health challenges. Salmonellosis is the second most common gastroenteritis infection after campylobacteriosis. Salmonellosis is a very serious and grave problem in third world countries where there are massive rates of mortality in thousands. *Salmonella typhimurium'*s potential to develop antibiotic resistance means that use of drugs may not be too effective against the pathogen. Oregano and cranberry are two plants gaining attention in the food industry due to the presence of bioactive compounds called phenolics which have antimicrobial and antioxidant properties. This study seeks to review recent advances in scientific literature on use of these medicinal herbs in inhibiting growth of pathogenic bacteria. Recent successes in the use of synergy of extract mixtures to greater effect against pathogens suggest such techniques could be employed against *S. typhimurium* in pork and pork products where they are prevalent.

Keywords: Plant extracts, synergy, phenolics, antimicrobial growth.

INTRODUCTION

Salmonella is a food borne, Gram negative and facultative anaerobe (Krieg and Holt, 1984). Salmonellosis (infection caused by *Salmonella* spp.) continue to present serious public health challenges as several million cases of human infection are reported globally every year (Oliveira et al., 2006). The antimicrobial resistance property of *Salmonella* is a major driver of the ongoing research interest in plant antimicrobial molecules. Also, increasing consumer demand for more clean-label products due to health concerns associated with chemical preservatives has necessitated the food industry's drive to look at possible use of natural preservatives in a range of food products (Sullivan et al., 2012; Dorman and Deans, 2000).

Studies have shown that the antimicrobial activity of the phytochemical synergies of extracts of oregano (*Origanum vulgare*) and cranberry (*Vaccinium macrocarpon*) are greater compared to individual extracts of each species (Lin et al., 2004; Lin et al., 2005). Oregano extracts have been found to have a moderately inhibitory effect on *Salmonella typhimurium* (Tayel et al., 2012). However, the applications of possible synergistic effects of oregano and cranberry combinations to control *Salmonella* growth are yet to be explored.

The objective of this study is to assess the potential antimicrobial effectiveness of the phytochemical synergies of oregano and cranberry extracts on *Salmonella typhimurium.*

LITERATURE REVIEW ON *SALMONELLA* SP.

Economic and health importance

The pathogen is the second most implicated cause of food-borne gastroenteritis worldwide after *Campylobacter.*

More than 2500 serotypes of the pathogen exist in nature although only about 500 of these are truly zoonotic (isolated from animals and capable of causing human infections) (EFSA, 2012). Salmonellosis continues to be a serious public health concern worldwide. Millions of human cases are reported yearly (Oliveira et al., 2006). EFSA/ECDC (2010) reported that in 2008, there were 1888 reported outbreaks in the European Union involving 133,543 confirmed cases, 2868 hospitalisations and 20 deaths. The importance of Salmonella as a food borne pathogen cannot be over-emphasized hence a knowledge of the foods associated with the pathogen is an effective public health strategy in combating salmonellosis.

Salmonella in foods

Most *Salmonella* species thrive well in chill to ambient temperature conditions (2 to 25°C). Some strains have adapted to temperatures as high as 54°C (Montville and Matthews, 2008). An interesting aspect of *Salmonella* infections is that the prevalence of pathogenic serotypes is determined by geographical location (Waite and Yousef, 2010). In the United States, the enteritidis, newport, javiana and typhimurium serotypes are dominant. The typhimurium serotype is common within the United Kingdom while the montevideo serotype is prevalent in South America. Symptoms of salmonellosis include diarrhoea, gastrointestinal pains, nausea and feverish chills which progress to fluid loss and dehydration and possible mortality if not quickly addressed (Richard et al., 2008). Recent studies have implicated *S. enteritidis* as the serotype most frequently associated with egg consumption, with about 95.9% of *S. enteritidis* infections being layer-associated cases (EFSA, 2010a; DTU/EFSA, 2011). EFSA (2010a) also reported the occurrence of egg-related *S*. *typhimurium* infections in humans while *S*. *kentucky* has also being isolated from poultry birds (EFSA, 2010c). The point of transmission of the infection to humans is usually through the consumption of contaminated products of animal origin including meats, eggs and dairy products, hence good hygienic practices and proper cooking methods are essential in mitigating *Salmonella* contamination of foods (Karns et al., 2005).

However in recent times, *S*. *typhimurium* has attracted the attention of the meat industry and public health sector due to the increase in the number of cases caused by the serotype (EFSA/ECDC, 2010) and its prevalence with meat consumption. *S*. *typhimurium* has been isolated from pigs, cattle and poultry animals. Research carried out by Boyen et al. (2008) and Perugini et al. (2010) has shown that *S*. *typhimurium* is the most common non-typhoid serotype isolated from pork and also humans. It is estimated that more than 63.1% of *S*. *typhimurium* infections in humans are due to pork-associated cases (DTU/EFSA, 2011). This makes it the most important serotype from a food safety perspective. The risk of cross contamination is always highest during further processing and preparation of meat. During the meat processing stage, *S*. *typhimurium* from contaminated meat could spread to other pork preparation (Gonzales-Barron et al., 2010a). In Denmark and the Netherlands, pork-related human *Salmonella* infections have been estimated at around 9 to15% and 21% respectively (EFSA, 2008). Data from the Health Protection Agency (2008 to July 2009) showed that *S*. *typhimurium* was responsible for 2,690 (around 18.8%) of *Salmonella* infections from humans in England and Wales (Peters et al., 2010).

Pork and pork products are the most common sources of food borne outbreak of *S*. *typhimurium* (EFSA/ECDC, 2010) hence reduction of *Salmonella* risks associated with these products can appreciably contribute to protection of human health. However, poor hygienic practices and sanitary conditions in pork production, processing and distribution chains have led to instances of pork-related salmonellosis outbreaks.

Salmonellosis (*S. typhimurium*) outbreaks

Within the EU, the two most common *Salmonella* serotypes are *S*. *enteritidis* and *S*. *typhimurium*. While the number of cases associated with *S*. *enteritidis* (prevalent with egg consumption) is decreasing, cases caused by S. *typhimurium* (prevalent in pork consumption) are actually on the rise (EFSA/ECDC, 2010). In 2008, 26,423 cases of *S*. *typhimurium* were reported (EFSA/ECDC, 2010). Percentage estimate of reported cases of human salmonellosis caused by *S*. *typhimurium* increased from 21.9 to 23.3% in 2009 (EFSA, 2010a). In 2006, two outbreaks caused by *S*. *typhimurium* involving 133 persons in Luxembourg resulted in 24 hospitalizations and one death. Statistics showed that a significant proportion of the cases were from institutions for the elderly and day care centres. Locally-produced pork was the vehicle of transmission (Mossong et al., 2007).

Bone et al. (2010) reported an outbreak in France that took place in 2010. There were 90 cases of illness associated with *S*. *typhimurium* in which 37% of 54 persons interviewed had been hospitalised. The incident was linked to the consumption of a dried pork sausage sold in France and Belgium, although no cases were reported in Belgium. It is important to note that dried sausages are high-risk products because they are made with minced meat hence they are subject to contamination during preparation.

In 2008, 49 cases of *S*. *typhimurium* U288 infections were associated with contaminated pork products were recorded in Scandinavia, there were 37 cases with 4 fatalities in Denmark, 10 cases in Norway and 2 in Sweden (Gideon, 2010). Two outbreaks of a multi-drug resistant strain of *S*. *typhimurium* (DT 120) occurred in

England, United Kingdom in 2011. 51 cases were confirmed. Epidemiological investigations suggested that pork consumption was the most likely cause of the outbreaks. Both outbreaks were linked to a butcher's shop and a pig farm in the midlands (Paranthaman et al., 2013)

The majority of *S*. *typhimurium* outbreaks were attributed to contaminated pork meat. This agrees with the work of Boyen et al. (2008) and Perugini et al. (2010) that identified *S*. *typhimurium* as the most common isolated non-typhoid serotype from pigs. The outbreak of the multi-drug resistant *S*. *typhimurium* (DT 120) in England brings to fore the antimicrobial resistance potential of serotype typhimurium.

Antimicrobial resistance

As a result of widespread and extensive use of antibiotics to treat infections, antibiotic resistance has developed in *Salmonella* species (Oliveira et al., 2006). This makes it more difficult to cure infections caused by antimicrobial-resistant *Salmonella* (Oliveira et al., 2006). The 1960s saw the first emergence of reports on resistant strains and these were mainly associated with mono-resistant strains (Helminth, 2000). During the late 1980s, an isolate of *S*. *typhimurium* (DT 104) was found to show multiple drug resistance against ampicillin, chloramphenicol, tetracycline, sulphonamides and streptomycin (Montville and Matthews, 2005). This exhibited resistance could have been as a result of factors such as drug inactivation, alteration of metabolic pathway and target site (Barbosa and Levy, 2000).

The ability of the pathogen to develop antibiotic resistance means the use of drugs for the treatment of salmonellosis (caused by antibiotic resistant *S*. *typhimurium*) may not be effective. However, a study by Lin et al. (2004) revealed that the addition of plant antimicrobials to meat systems can effectively inhibited microbial growth and also significantly decimated the population of *L. monocytogenes*. Tayel et al. (2012) reported that the application of plant antimicrobials to meat systems inhibited growth of *S*. *typhimurium* and *Staphylococcus aureus* and also significantly reduced bacterial cell population. A study by Sullivan et al. (2012) on the consumer perception of antimicrobial-treated meat showed that there was no significant difference in taste between antimicrobial-treated and untreated meats. Tayel et al. (2012) also stated that the treatment of meat steaks with plant extracts resulted in improved quality and good organoleptic attributes of the product.

The application of plant antimicrobials to control *S. typhimurium* in broth and potential introduction in meat systems is a novel approach in food preservation and this could provide a more practical and proactive way to mitigate pathogenic contamination in meats as there is also yet to be any scientific evidence of *Salmonella* resistance to plant antimicrobials.

Plant extracts as antimicrobials

Growing concerns over use of chemical preservatives

The FDA (2009) defines chemical preservatives as any chemical introduced to foods to prevent or retard deterioration. Common salts, sugars, spices and oils extracted from spices, direct exposure to wood smoke and chemicals used for their insecticidal properties are not included in the definition. The European Commission Regulation on food additives (EC) 1335/2008 contains a list of permitted additives together with their approval number (E). These additives are only permitted to be used in certain foods and are subject to specific quantitative limits. Additives such as preservatives and antioxidants are useful for their antimicrobial, antioxidant and sometimes flavour-enhancing properties. Examples of permitted preservatives include benzoic acid (E210), sorbic acid (E200) and sulphur dioxide (E220).

However in recent years, there has been an increased scepticism and consumer-led concerns about the use of artificial preservatives in food. Associated with this, there has been increased demand for naturally preserved foods without the presence of artificial additives. Health concerns (about the possible carcinogenic properties of some additives) are usually overwhelming reasons for this. The food industry is focusing its attention on the development of clean label products, that is, no chemical additives were added (Sullivan et al., 2012). These emergent issues have led to an increasing focus on the use of natural antimicrobials in preserving food, inhibiting microbial growth and enhancing flavour instead of the continued use of chemical preservatives (Theron and Lues, 2007).

Introduction to plant extracts

Plant extracts (and essential oils) have been used in various ways for thousands of years (Jones, 1996). In fact, Reynolds (1996) reported that the antimicrobial activity of plant extracts (and oils) has been the cornerstone of various scientific applications in food processing, industries, pharmaceuticals, alternative medicine and some natural health therapies. Plant extracts are crude mixtures of compounds from different parts of plants and are attested to have inhibitory effects on the growth and survival of both spoilage and pathogenic microorganisms (Lin et al., 2004). The plant parts from which extracts are prepared could be the leaves, stems, flowers, fruits, roots and bark.

Antimicrobial and antioxidant properties

Plant extracts contain compounds called phytochemicals which are usually either primary or secondary metabolites. These are naturally occurring biochemical compounds that impart on the plant its characteristic colour, flavour, smell or texture. While primary metabolites are the macromolecules: carbohydrates, proteins, fats and nucleic acids (Bako and Aguh, 2007), some secondary metabolites differ in that they are said to have a scientifically proven impact on human health. They are the constituents of plants that are said to be bioactive and include alkaloids, tannins, flavonoids, anthraquinones, phenols and polyphenols. These compounds are attested to have anti-pathogenic and anti-spoilage properties that inhibit the growth of viruses, bacteria, fungi and some insect parasites (FDA, 2009).

Phenolic compounds also possess strong and effective antioxidant properties, hence helping to prevent rancidity and spoilage in high fat and lipid-based foods (Lin et al., 2004).

Phenolic compounds, mechanism of action, synthesis and extraction

Phenolics and phenolics-based compounds are one of the most diverse groups of secondary metabolites in plants. They are found in fruits, vegetables, seeds, nuts, stems and flowers. Phenolics have been found to be one of the main bio-active compounds in plant extracts. (Lin et al., 2004). Rosmarinic acid, flavonoids, carvacrol and thymol are examples of phenolic compounds found in medicinal herbs such as oregano, rosemary, basil and sage. Proanthocyanidins and flavonoids are prominent phenolic compounds in fruits such as cranberry, raspberry and blueberry. According to Lin et al. (2004), phenolic compounds are a major determinant of inhibitory potency of plant extracts and oils. The synergy of antimicrobial mixtures increases with the diversity of phenolic compounds hence the combination of phenolic and antioxidant compounds of varying antimicrobial potencies could significantly affect inhibitory activity of these extract mixtures (Lin et al., 2004).

The synthesis of phenolic compounds involves three main pathways, the pentose phosphate pathway, the glycolysis pathway and the shikimate pathway. Glucose is the main precursor, the metabolism of glucose leading to the production of erythrose-4-phosphate and phosphoenolpyruvate (PEP) which enter the shikimate pathway where a series of enzyme-controlled reactions lead to production of phenolics and deposition of lignin.

Understanding the dynamics of how an antimicrobial works through the active compound is important in determining against which pathogen it is effective. Active compounds of antimicrobials work in different and diverse ways to stop microbial growth and improve the shelf-life of the food products or cause cell death. Unfortunately, the specific mode of action of plant antimicrobials is poorly defined, but there have been suggestions of a possible depletion of cellular energy (Conner et al., 1984). Wendakoon and Sakaguchi (1995) suggested that the hydroxyl group of certain phenolic compounds, e.g. eugenol has the ability to adhere strongly to proteins, making them inert and thus preventing enzymatic functions and reactions from taking place which sometimes results in cell damage and lysis. Moreno et al. (2006) reported the inactivation of cellular enzymes by action of phenolic compounds. Thymol, eugenol and carvacrol have been reported to be able to cause cell membrane disruption and inhibition of ATPase activity in micro-organisms such as *Escherichia coli*, *L. monocytogenes* and *Salmonella* spp. (Lambert et al., 2001). However, Gill and Holley (2006) stated that cinnamaldehyde inhibited ATPase activity in *E. coli* 0157:H7 without leading to changes on cell membrane; this has been attributed to the interaction between cinnamaldehyde and the cell membrane whereby disruption does not lead to ATP leakage. Rosmarinic acid is also an important phenolic compound reported to possess strong scavenging activity against free lipid radicals (Moreno et al., 2006). It also plays an important role in maintaining stability of the lipid membrane and preventing damage from oxidation (Perez-Fons et al., 2010).

Extraction represents the first and essential step for the isolation and purification of bioactive components of plant materials. Different techniques of extraction and choice of solvents exist for obtaining phytochemicals of antimicrobial and antioxidant importance. There are several extraction techniques available, the most common of which are maceration, solvent extraction, hot continuous extraction, supercritical fluid extraction, steam distillation and solvent extraction (Kaufmann and Christen, 2002). Solvent extraction is the most widely used technique; the use of polar solvents (water) solvents of moderate polarity (hexane) and solvents of low polarity (ethanol) can be employed to extract phytochemicals from plant extracts. Extracts obtained from the identical plant parts with different solvent characteristics can possess different physical and biological properties. Also as reported by Lapornik et al. (2005), ethanol and methanol extracts of spices e.g. currants contained more proanthocyanidins and polyphenols than water extracts. It is therefore important that in solvent extraction of phytochemicals the ideal solvent that will give maximal yield of phytochemicals from a particular plant extract is employed (Allen et al., 2012)

Studies have shown that water extracts of oregano and cranberry contained more phenolics and gave enhanced inhibition of *L. monocytogenes* in broth and food systems (Lin et al., 2004).

Oregano and cranberry

Oregano (*Origanum vulgare*) is one of the most studied herbs; its name is derived from the Greek word “oros” meaning mountain and “ganos” meaning joy. It is an aromatic herb used for health and medicinal benefits, a member of the Lamiaceae family and a native of the

Mediterranean and southern Asia regions. This herb has been shown to contain high levels of phenolic compounds in its leaves and volatile oils (Zheng and Wang, 2001) and application of oregano phenolic compounds in food systems have been shown to be effective in preserving food and extending the shelf-life of products containing lipids (Vichi et al., 2001; Capecka et al., 2005).

Cranberry (*Vaccinium macrocarpon*) is a fruit bearing evergreen shrub that has erect branches and speckled leaves. Cranberry fruits are rich in antioxidant phenolic compounds including proanthocyanidins, which are responsible for the vibrant colour. The fruits also possess strong radical scavenging properties (Lin et al., 2004). There have been recent concerns over the low bioavailability of active ingredients present in cranberry fruits and possible use of large quantities of fruit extracts to achieve the desired objective (Hisano et al., 2012).

Oregano and cranberry are useful botanicals which are generally recognised as safe for food flavouring (GRAS) (Lin et al., 2005). Hence, this property coupled with increasing consumer perception that the addition of chemical preservatives to foods could have increased toxicological consequences has attracted the attention of the food industry to the use of these plant extracts.

Antimicrobial effects of oregano and cranberry extracts

The antimicrobial properties of oregano and cranberry have been investigated and assessed. Elgayyar (2001) found that oregano extracts (including the essential oils) showed complete growth inhibition against a wide array of Gram-positive and Gram-negative bacteria including *S*. *typhimurium*, *E. coli*, *S. aureus*, *L. monocytogenes* and *Lactobacillus plantarum*. Lin et al. (2004) observed the efficacy of an oregano/cranberry mixture against *L. monocytogenes* in broth and food systems where greater inhibition was shown by the combination of oregano and cranberry aqueous extracts (3.8 fold reduction) as against the effect obtained with the use of oregano (2.9 fold reduction) or cranberry (1.7 fold reduction) alone. A study conducted by Lin et al. (2005) showed the inhibitory action in a cranberry/oregano/lactic acid mixture against the growth of *Helicobacter pylori* in broth system. It has also been shown that lactic acid significantly enhanced the antimicrobial efficacy of oregano and cranberry synergies bringing about more than 3.8 log reduction in the bacterial cell count (Lin et al., 2004).

Seaberg et al. (2003) reported on the inhibition of an array of Gram-positive organisms including *L. monocytogenes* by extracts (in ethanol) of elite oregano clones in broth and food systems. It must be noted however that the antimicrobial efficacy of these extracts is usually related to the total phenolic content. This means that extracts with higher phenolic contents (in mg/ml) would likely possess higher antimicrobial potency. The antioxidant properties of cranberry extracts has also been reported by Lin et al. (2004), the presence of strong phenolic antioxidants in cranberry extracts helps to prevent spoilage and rancidity when applied to foods.

An important factor in antimicrobial activities of extracts is the Minimum Inhibitory Concentration (MIC) of the antimicrobial. The MIC (usually expressed in parts per million) is the smallest amount of an antimicrobial or antimicrobial mixture that will completely inhibit microbial growth after overnight incubation. MIC values are important in determining the strength or potency of an antimicrobial or antimicrobial mixture. The smaller the MIC, the more potent is the antimicrobial (Tayel et al., 2012).

Work done by Arjoon et al. (2012) revealed that ethanol extracts of oregano effectively inhibited growth of *E. coli* (MIC - 0.033 mg/ml), *Bacillus subtilis* (MIC - 0.00033 mg/ml) and *Mycoplasma mycoides*(an antimicrobial-resistant pathogen) (MIC-0.033mg/ml). The significantly lower MIC value for *B. subtilis* suggested a greater sensitivity of the pathogen to the antimicrobial than that of *M. mycoides* and *E. coli*. Arjoon et al. (2012) also reported that ethanol extracts of oregano had lower MICs against the three pathogens compared to the oil emulsions suggesting the possible differential solubility of antibacterial compounds in oils opposed to ethanol. Antibacterial activity of cranberry extracts has been assessed. Rahbar and Diba (2010) revealed the inhibitory activity of cranberry (in methanol) extracts in uropathogens (etiological agents of urinary tract infections) *in vitro*. MIC values for *Enterobacteraerogenes* and *S. aureus* were 0.39 mg/ml while that for *E. coli* and *Klebsiella pneumoniae* was 1.25 and 0.0195 mg/ml, respectively. The lower MIC value obtained for *K. pneumoniae* showed a greater vulnerability of the pathogen to cranberry extracts.

However, little work has been done on the effect of oregano and cranberry extracts on *S*. *typhimurium*. Tayel et al. (2012) worked on the antimicrobial activity of plant extracts on *S*. *typhimurium* and found that oregano extracts had a moderate inhibitory effect on the pathogen (MIC value of 825 ppm) compared to pomegranate peel extracts which were more effective having an MIC value of 250 ppm. There is therefore an increasing focus on exploring possible synergies of antimicrobials to broaden the antibacterial spectrum of plant extracts (Lin et al., 2004).

Synergistic effects of oregano/cranberry mixtures

Work has been carried out on the possible synergy of combining two or more antimicrobials. Mixtures of spice and herbs extracts have been discovered to be more effective in inhibiting microbial activity in ready to eat foods than when they are used alone (Weerakody et al., 2011). It is noted that the use of antimicrobial mixtures (when used at the right concentration and combined appropriately) do not significantly alter the taste and consumer acceptance of products (Sullivan et al., 2012). The extension of shelf-life of products ensures prolongation of viability and improved safety (Weerakody et al., 2011). Enhanced effects of phytochemical synergies of oregano and cranberry extracts have been observed against *L. monocytogenes* and *H. pylori* in broth system (Lin et al., 2004; Lin et al., 2005)

While oregano and cranberry extracts were able to control bacterial growth and increase lag phase, the phytochemical synergies of their combination in the presence of lactic acid was found to be significantly stronger than that of an individual extract. The antimicrobial potency of the synergy was greater at pH 6.0 than 7.0 suggesting that a lower pH increased the susceptibility of the pathogen. Acidification was done with lactic acid solution (Lin et al., 2004). It is important to understand the effect of the antimicrobial extracts at different concentrations and identify ideal solvents (Allen et al., 2012). Another important issue has to do with combining these extracts in the right proportions to produce an optimum concentration of active compounds in the extracts, that is, optimum concentration of phenolic compounds that must be reached to effectively curtail microbial activity. This is a very important detail to consider when considering synergistic combinations of two or more antimicrobials (Stasiewicz et al., 2010).

CONCLUSION AND RECOMMENDATION

Phytochemical synergies of oregano and cranberry extracts have been employed successfully in controlling growth of food borne pathogens including *L. monocytogenes* and *H. pylori* in broth and food systems and are more effective in the inhibition of pathogenic growth when compared to the use of individual extracts of the same species (Lin et al., 2004; Lin et al., 2005). Work done by Tayel et al., (2012) revealed the moderate inhibitory effect of oregano extracts on *S. typhimurium* growth (MIC of 825 ppm). However, the antimicrobial efficacy of the phytochemical synergy of oregano and cranberry have not been tested against *S. typhimurium*, therefore additional research is needed in this area to determine the antimicrobial potency of these extract mixtures against *S. typhimurium* growth.

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