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Antibacterial properties of extracts of selected herbs and spices at different pH against *Bacillus cereus* isolates from retailed foods

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ABSTRACT Herbs and spices improve the taste of food and also have medicinal properties. This study determined the antimicrobial activity of selected spices against *Bacillus cereus* at different pH. The active ingredients of five herbs and spices; ginger (*Zingiber officinale*), garlic (*Allium sativa*), turmeric (*Curcuma longa*), thyme (*Thymus vulgaris*) and bay leaf (*Laurus nobilis*) were extracted with sterile water and ethanol; and reconstituted into different concentrations. The spice extracts were tested against thirty (30) *Bacillus cereus* isolates recovered from food using the Agar well diffusion method. Clindamycin (2 µg) was used as standard antibiotic. The ethanol extracts of the spices were more active than the aqueous extracts. Thyme extract showed the highest activity at 100 % concentration with mean zone of inhibition of 14mm followed by bay leaf with 12 mm. Thyme and turmeric had the highest activity at pH 6 with 11 mm as the mean zone of inhibition. Ginger and garlic were more active at pH 3, thyme and turmeric at pH 7 while bay leaf was at pH 8. Only five isolates were resistant to the antibiotics. Therefore, the active ingredients of spices can be extracted, concentrated, purified and used in the treatment of infections by these pathogens and preservation of foods since most of them are now resistant to common antibiotics.

KEYWORDS antibiotic, aqueous extract, *Bacillus cereus*, ethanol extract, herbs and spices

Introduction

Spices are used to improve the taste of foods and they are plant substances with strong taste. Though the main purpose of spices is to enhance flavor of food, they are important for their therapeutic, antimicrobial and antioxidant properties (Joe *et al.*, 2009; Das *et al.*, 2012). Spices like clove, garlic, cinnamon and ginger have been used conventionally for both cooking and therapeutic purposes. They have excellent antibacterial properties (Sah *et al.*, 2012), and have wide range activities against bacteria, yeasts and molds (Tajkarimi *et al*., 2010). World Health Organization (WHO) reported that more than 80% of the world populace depends on old-style remedy for their main health care needs (Diallo *et al.*, 1999).

According to Joe *et al.* (2009), garlic known to many as *Allium sativum*, is recognized for having a range of antifungal, antibacterial and antiviral properties. Along with its protective abilities, allicin is believed to be the natural chemical component responsible for the antimicrobial effects of garlic. There is lower prevalence of cancer in countries where garlic is consumed in higher quantities, through traditional cooking. *Allium sativum* has been found to decrease platelet accumulation and hyperlipidemia (Steiner and Lin, 1998; Rahman, 2011; Chan *et al.*, 2003).

It has been revealed that ginger is effective against the growth of both gram-positive and gram-negative bacteria. The core active ingredients in ginger are the phytochemicals which are paradols, shogoals and gingerols (Joe *et al*., 2009; Das *et al.*, 2012). The volatile oil gingerol in ginger aside giving ginger is spicy aroma, is medically potent since they prevent prostaglandin and leukotriene formation; products that impact blood flow and inflammation (Omoya and Akharaiyi, 2012).

Turmeric contains natural polyphenolic compound called curcuminoids (Hay *et al.*, 2019). *Staphylococcus aureus, Bacillus cereus*, *Bacillus coagulans, Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* were found to be susceptible to Turmeric oil obtained as a by-product from curcumin manufacture (Negi *et al*., 1999).

Bay leaf (*Laurus nobilis*) is used in adding flavors to soup, stew, meat and fishes. The leaves and its essential oil, offers antimicrobial, anti-inflammatory and antioxidant qualities (Oliver, 2020). Moreover, they have been used for skin diseases, rheumatism, urinary problems and stones (Ali-Shtayeh *et al.*, 2000). It has been found to inhibit different food spoilage bacteria and yeast (Yilmaz *et al.*, 2013)

Bacillus cereus is a gram-positive toxin-producing facultatively anaerobic bacterium (McDowell *et al.*, 2021). Food borne pathogens such as *Bacillus* spp. is ubiquitous in nature, causing considerable illness and death in the population (Bintsis, 2017; Horn and Bhunia, 2018). They are known for the production of numerous membranes damaging toxins which results in two types of food borne illnesses: emetic and diarrhea.

Chemical preservatives are used in food industry to slow down the microbial deterioration of processed foods, however, they cause serious side effects such as hypersensitivity, asthma, allergy, neurological damage, cancer and hyperactivity. There is search for natural preservatives from minerals, microorganisms, plants and animals (Anand and Sati, 2013). Spices are now being more exploited as an alternative source of preservative because of their antioxidant properties. Addition of spices into food can increase the shelf life of the food by retarding the bacteria growth (Oiye and Muroki, 2002).

Over the last few years, a number of antibiotics have lost their efficiency as a result of growth of resilient strains, which was brought about by the expression of resistance genes (Marchese and Schito, 2000). In addition to this problem, antibiotics are sometimes known to have side effects such as gastrointestinal and allergic reactions (Mohsen *et al*., 2020). Therefore, there is a need to get substitute antibacterial drugs from different sources such as medicinal plants for the treatment of infectious diseases.

Furthermore, it was reported that antibiotics that perform today may not be effective tomorrow and that it is necessary to explore novel drugs to which there is minor resistance. As resistance to old antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained (Sarkar *et al.,* 2003). According to World Health Organization (WHO) greater than 80% of the world populace depend on local medicine for their main health care needs (Diallo *et al.,* 1999).

Therefore, the aim of the study is to determine the antimicrobial activity of aqueous and ethanol extracts of bayleaf, turmeric, ginger, garlic and thyme against *Bacillus cereus* isolates from retailed foods to know if they are good alternative for antibiotics and chemical preservatives against the organism; also, to determine if the antibacterial properties of these spices are affected by changes in pH.

Materials and Methods

Collection of spice sample

The spices used in this study were: ginger (*Zingiber officinale*), garlic (*Allium sativum*), thyme (*Thymus vulgaris*), turmeric (*Curcuma longa*) and bayleaf (*Laurus nobilis*). They were purchased from Ago-Iwoye market in Ogun State, South-western, Nigeria. The spices and herbs were the fresh rhizomes of ginger and turmeric, fresh bulbs of garlic and dry leaves of bayleaf and thyme.

Test Organisms

Thirty (30) *Bacillus cereus* isolates recovered from retailed foods (Adesetan *et al*., 2019) were employed in this study. The bacteria were maintained on nutrient Agar slant at 4°C. They were also re-confirmed for their viability with Gram stain reaction, catalase, citrate and haemolysis tests.

Preparation of Spices for Extraction

Garlic, ginger and turmeric were rinsed with sterile water and allowed to dry so as to reduce the microbial load of the spices. The outer covering of ginger, garlic and turmeric were manually peeled off and were sliced into cutlets. The cutlets were sun dried for 4 days. Electrical blender was used to ground the spices into fine consistent powder, sifted and then stored in sterile air tight container at 4°C until required (Rafat *et al.*, 2010).

Extraction of Spices

One hundred grams (100g) of each spice powder was weighed and mixed with 200ml of absolute ethanol and water separately in respective flasks. The flasks were then allowed to stand for 72 hours at room temperature with occasional shaking. The mixture was filtered with Whatman no.1 filter paper; and then placed on water bath for evaporation. The extracts obtained were stored in air tight bottles in the refrigerator at 4°C (Ugoji *et al.,* 2000).

Preparation of Different Concentration of spice Extracts

Different concentrations (25, 50, 75 and 100 % w/v) of the extract were used. For 25 % w/v (25 g of extract + 100 ml of solvent), 50% w/v (50g of extract + 100 ml of the solvent), 75% w/v (75g of extract + 100 ml of solvent) and for 100% w/v (100 g of extract + 100 ml of solvent).

Preparation of Different pH of Spice Extracts

The 100% concentration of each extract were adjusted to pH 3, 6, 7, 8 and 11 using phosphate standard buffer of pH 4, 7 and 11. The buffer was added dropwise, stirred and checked for change in pH with a pH meter.

Preparation of Inoculum

Three (3) ml of saline water was pipetted into sterile clean test tubes and were covered using cotton plugs. Using a flamed inoculating loop, the bacterial culture was picked and dissolved in saline water with its turbidity compared with that of 0.5 McFarland standards.

Determination of Antimicrobial Activity of spices

A sterile cotton swab stick was dipped into the inoculum and pressed firmly on the wall of the tube to remove excess fluid. The swab stick was thereafter used to inoculate the dried surface of the solidified Mueller Hinton agar by streaking the swab three times over the entire agar surface; the petri plates were covered immediately. Five (5) equidistant well of 6 mm diameter were made with sterile borer in the agar for the four concentrations and distilled water as negative control in the fifth well. The plates were incubated in an upright position at 37ºC for 24 hours. The zones of inhibition were measured in mm and the results recorded. The experiment was repeated twice.

Antibiotic sensitivity test

The Kirby Bauer method was used to determine the susceptibility of the bacterial isolates to Clindamycin (10 µg) antibiotic. The test organism was inoculated onto Muller Hinton plates with sterile cotton swab stick after the inoculum was standardized using 0.5 McFarland solutions. Thereafter, the antibiotic disc was laid on the agar plates. The plates were incubated at 37ºC for 24 hours. Appearance of clear zones around the disc was checked for and interpreted using CLSI (2013) guidelines.

Statistical analysis

Data were analyzed using Microsoft excel 2010. ANOVA and Turkey-Kramer test were performed using JMP software (version 3 for windows; SAS Institute).

Results and Discussion

Confirmation of B. cereus

The biochemical test re-confirmed the isolates as *B. cereus*. They were Gram positive rods, catalase and citrate positive; and they were all β-hemolytic (Chessborough, 2000).

Antimicrobial assay

The antimicrobial activity of the aqueous and ethanol extracts of the selected herbs and spices against *Bacillus cereus* is presented in figures 1-9. All the spices showed a remarkable antimicrobial activity but with varied zone of inhibition. The ethanol extracts of the spices were more effective than the aqueous extracts.

At 25% concentration, ethanol extracts of thyme showed the highest inhibition with mean inhibition zone of 12 mm followed by turmeric with 11mm while ethanol extract of bayleaf had a mean inhibition zone of 5mm (fig 1). At 50% concentration, ethanol extract of thyme was more effective with mean inhibition zones of 13mm, followed by bayleaf and turmeric (fig 2). Both aqueous and ethanol extracts of thyme inhibited the test organisms at 75% concentration with mean zone of inhibition of 9 mm and 13 mm respectively. The ethanol extracts of the other spices also showed a significant effect with mean inhibition zone in the 7 – 12 mm (fig 3). At 100%, all the ethanol extracts of the spices inhibited the bacteria with the least inhibition zone displayed by ginger and turmeric. Both aqueous and ethanol extract of thyme showed remarkable activity against the organism with 14mm as the mean zone of inhibition (fig 4).

There was an increase in the susceptibility of *B. cereus* to ethanol extract of the spices, as the concentration increases, so also their susceptibility to the aqueous extract. It has been reported by Bhalodia and Shukla (2011) that increase in the extract's concentration have direct relation to the antimicrobial activities. All the spices showed a notable activity against the test organisms, though thyme was the most effective.

B. cereus were susceptible to the ethanolic extracts of bay leaf compared to the aqueous extract. Mukherjee *et al.* (2015) also carried out an *in vitro* study of the therapeutic properties of *Laurus nobilis* (bay leaf) against *Bacillus* species and reported that the organism was susceptible to bay leaf extract.

Ethanolic extract of ginger has more inhibitory effects against the test organisms than the aqueous extract. Dhiman *et al*. (2016) and Mostafa *et al.* (2018) also reported the susceptibility of *B. cereus* to the ethanolic extract of ginger.

Both aqueous and ethanolic extracts of thyme inhibited the test organism. This differs from the findings of Mostafa *et al.* (2018) who stated that *B. cereus* was resistant to ethanolic extract of thyme. Only two *B. cereus* isolates were resistant to the ethanolic extract of the spice in this research.

Most of the *B. cereus* isolates in this research were not inhibited by both aqueous and ethanolic extracts of garlic. This is in contrast to the findings of Khusro *et al.* (2013) who stated that *Bacillus* spp. strains were susceptible to ethanolic extracts of garlic.

i. Ethanolic extract of turmeric was more effective in inhibiting the organism. This compares well to the findings of Sana and Ifra (2012) who reported that ethanolic extracts of turmeric inhibited *Bacillus* spp. with 14mm as zone of inhibition. Chandarana *et al.* (2005) reported that turmeric contains curcuminoid, a phenolic compound which makes it active against *Bacillus* spp.

Turmeric contains curcumins, essential oil, turmerol, veleric acid along with alkaloid which are responsible for its antibacterial activity. Odhav *et al.* (2002) suggested that the mode of operation of antibacterial activity of spices involve membrane disruption, cell wall disruption and destruction of electron transport systems.

The ethanolic extracts of all spices used in this study inhibited the organisms because organic compounds result in the liberation of substantial amount of active antimicrobial compounds such as saponins, tannins, flavonoids, anthraquinones etc. (Cowan, 1999).

Ethanol extract of bayleaf was more effective at pH 8 while it was least effective at acidic pH. The aqueous extracts were least effective (fig 5). Ethanol extract of ginger was more effective at all the pH especially at pH 3 with 10mm as the zone of inhibition while the aqueous extracts were also least effective (fig. 6). In figure 7, ethanolic extracts of thyme was effective at all the pH especially at pH 6 while aqueous extract of thyme did not inhibit the test organisms at pH 3. Ethanol extract of garlic was not all that effective at all the pH while aqueous extract was only effective at pH 6 with mean zone of inhibition of 2.3 (fig. 8). Ethanol extracts of turmeric was more effective at pH 7 with 11mm as the mean zone of inhibition while the aqueous extract was more effective at pH 11 with 6.5 mm as mean zone of inhibition. Antibacterial activity of the different spices was fluctuating with increase in pH except for turmeric in which the antibacterial activity of the aqueous extract increased with the rise in pH. The research has showed that the spices were effective at pH around neutral towards alkaline pH except for ginger and garlic that showed higher antimicrobial activity at acidic pH. Anees *et al*. (2015) reported that the antibacterial effect of the spices used in their research decreased with an increase in pH but that they still retained their antibacterial property. The effectiveness of spice extracts on bacteria depends on the concentration of the spices, the solvent used for extraction and condition of the environment.

The resistant of *B. cereus* isolates to some of the spices especially garlic might probably be as a result of mutation and development of new variants caused by continuous periodic transfer of the isolates. Also, drying of the garlic in the sun for 4 days might have had a negative effect on the active ingredients.

Overall, ethanol and aqueous extract of thyme was the most effective followed by ethanol extract of turmeric. At the various pH, ethanol extracts of the different spices proved to be effective at neutral pH towards the alkaline pH.

The result of the antibiotic susceptibility test using clindamycin is presented in figure 10. Twenty-five (25) of the isolates were sensitive to clindamycin while five (5) showed either intermediate resistance or total resistance to the antibiotics. For the antibiotic sensitivity test. Adesetan *et al.* (2019) also reported the susceptibility of *B. cereus* to the same antibiotic. Godic Torkar and Seme (2009) reported that 90% of their *B. cereus* isolates were susceptible to clindamycin.

Conclusion

This research has revealed that spices like thyme, turmeric, ginger and bay leaf can be used to inhibit the growth and replication of food poisoning bacteria such as *Bacillus cereus* in food. Also, their active ingredients can be extracted, purified and used as antimicrobial against this organism. Therefore, the use of these spices in foods should be encouraged and promoted.

Recommendation

Further research can be done on these spice extracts to identify, concentrate and purify the active ingredients for use as preservatives in food instead of chemical preservatives which has various side effects on human. This study confirms the efficiency of the different spices as natural antimicrobials and recommend the possibility of their uses which requires that further studies be carried out on them.

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Conflict of Interest

Not Applicable

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Appendix

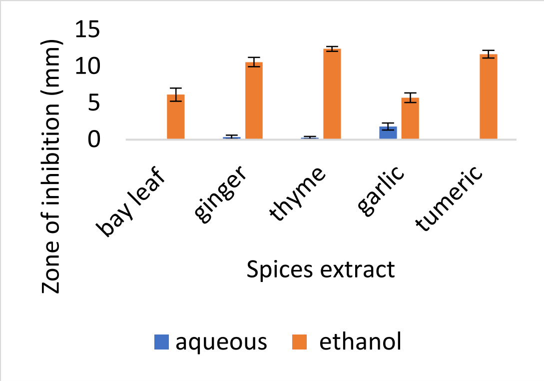

Fig 1: Mean zones of inhibition of aqueous and ethanol extracts of spices at 25% concentration against forty-eight *B. cereus* isolates

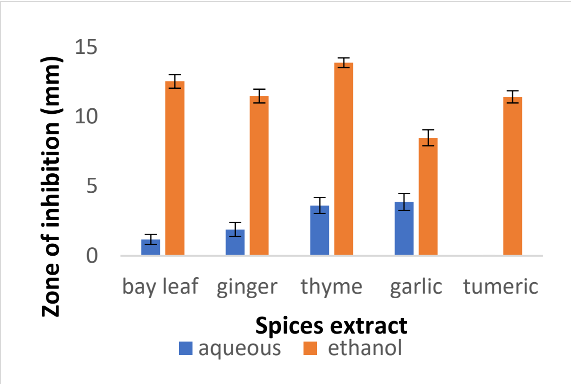

Fig 2: Mean zones of inhibition of aqueous and ethanol extracts of spices at 50% concentration

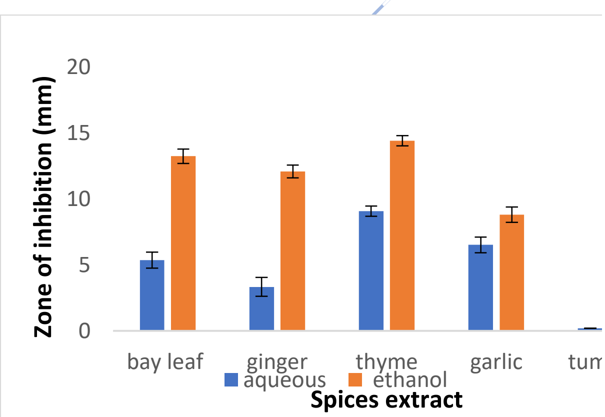

Fig 3: Mean zones of inhibition of aqueous and ethanol extracts of spices at 75% concentration

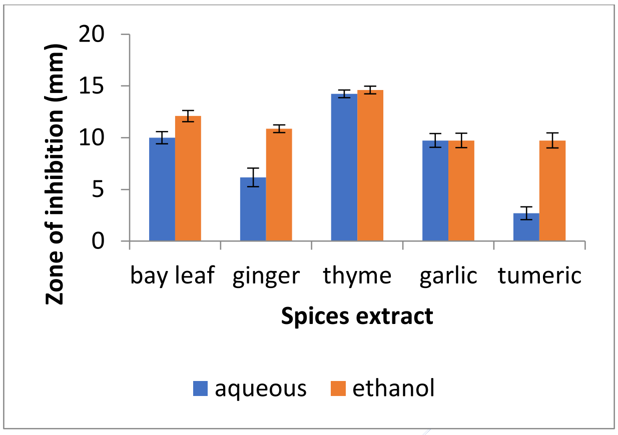

Fig 4: Mean zones of inhibition of aqueous and ethanol extracts of spices at 100% concentration

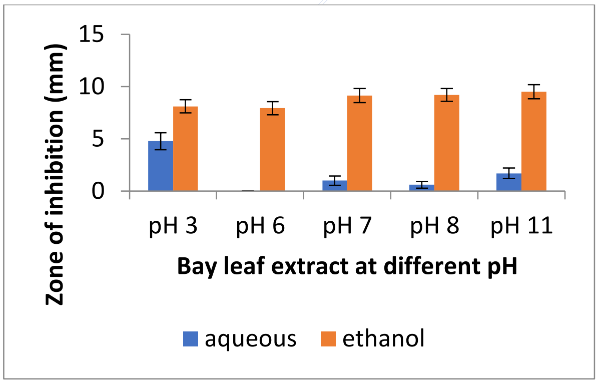

Fig 5: Mean zones of inhibition of aqueous and ethanol extracts of bay leaf at different pH

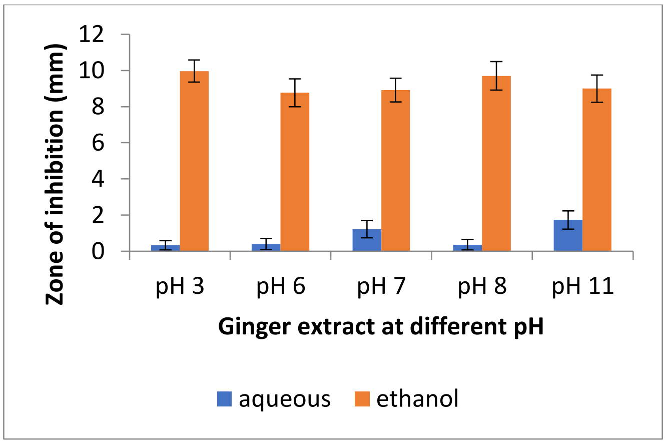

Fig 6: Mean zones of inhibition of aqueous and ethanol extracts of ginger at different pH

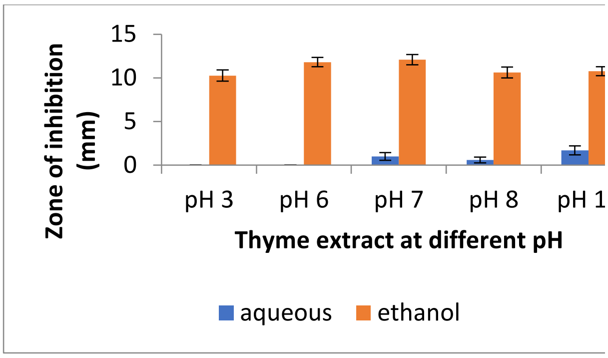

Fig 7: Mean zones of inhibition of aqueous and ethanol extracts of thyme at different pH

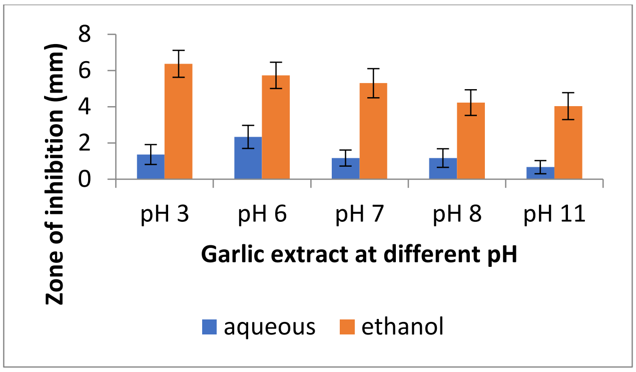

Fig 8: Mean zones of inhibition of aqueous and ethanol extracts of garlic at different pH

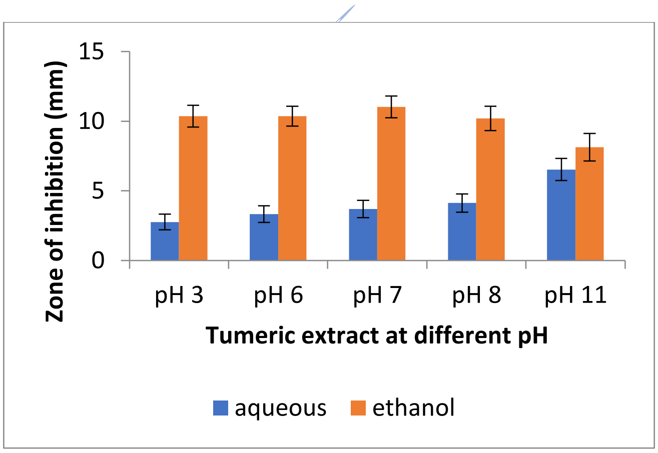

Fig 9: Mean zones of inhibition of aqueous and ethanol extracts of turmeric at different pH

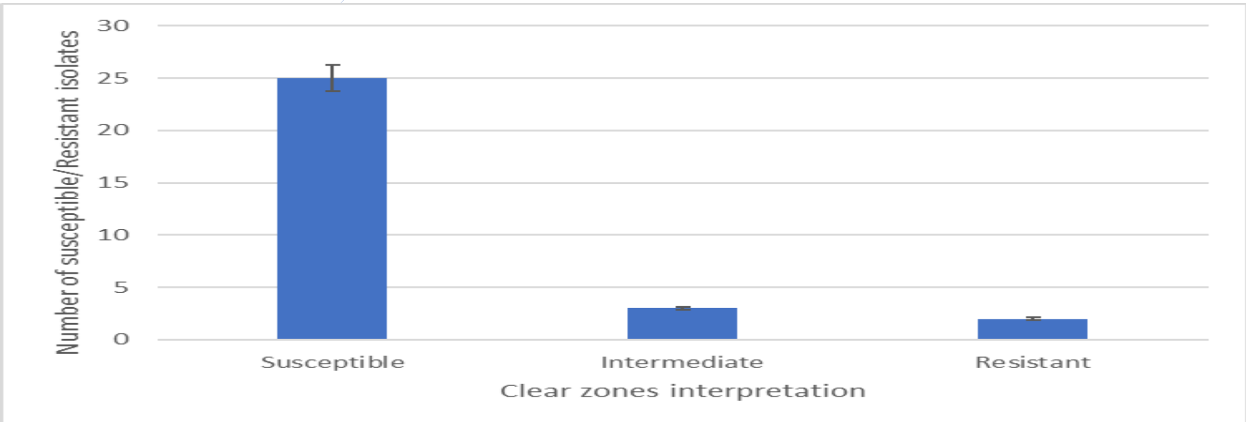

Fig 10: Antibiotic Susceptibility of *Bacillus cereus* with Clindamyci