

## Introduction :

Anti-bacterial properties are those types of properties which destroy or suppress the bacterial growth and their reproduction/multiplication ability. It can be shown by many herbs and spices we use in our daily life.

Over the past few decades there has been a significant increase in record of bacterial and fungal infection with pathogenic bacteria such as *Enterococcus* species, *E. coli*, *Shigella* species, *Staphylococcus* mutants, *Streptococcus* species etc. (Nabavi et al.,2015). The World health organisation (WHO) reported that 55 million people died world-wide in 2011, with one-third of the deaths due to infectious diseases. The major reason is development of tremendous increase in resistance of pathogenic bacteria species against several antibiotics. (Marchese et al.,2016).

Due to advancement in life and modern way of living many of us are ignoring the importance of these spices which had has been a part of our existence as a food supplement, herbal remedy, immunity enhancer, antimicrobial activity and many more (Singab and Eld-Ahshan, 2015 and Khumalo et al., 2018). Now a day we are depending on lots of antibiotics more than needed, but their excess consumptions have caused lots of problems to us and also poses serious threat to the environment by their removal through faecal matter. Chemical drugs on which we are depending too much are harmful to both the body and the environment because of their disposal and degradation which lead to many of the pathogenic bacteria to become resistant to the assigned drugs causing their growth other than suppression (Cycon et al.,2019).

There are lots of herbs found in our nature but we came across to know about cinnamon (*Cinnamomum verum*) which have anti-oxidant, anti-inflammatory, antimicrobial and many more properties and have bioactive phytochemicals – cinnamaldehyde and eugenol and are regarded as alternative to synthetic antibiotics, especially for multi-antibiotic resistant bacterial infections (Rao

and Gan, 2014 and Nabavi et al., 2015) which perfectly fit on our multi-drug resistant bacterial strains *Enterococcus faecalis* and *Shigella*.

Other herbs like carom seeds (*Trachyspermum ammi*) is known to act as herbal remedy in treating stomach related problems and have antioxidant, antimicrobial, cytotoxic and many more such properties due to bioactive chemicals thymol, g- terpinene and others volatile oils (Bairwa et al.,2012) which also fits on our bacterial strains *Shigella* and *Enterococcus faecalis*.

*Enterococcus faecalis* causes about 80% of human infection like bacteraemia, septicaemia (blood poisoning), oral infections with root canal, endocarditis (infection in lining of heart), prostatitis, urinary tract infection and many nosocomial diseases is one of multi-drug resistant bacteria around us. (Golob et al.,2019 and Farman et al.,2019).

*Shigella* species causes dysentery/diarrhoea, indigestion, inflammation and death of epithelial lining of colon and reactive arthritis (Taneja and Mewara, 2016 and Baker and The, 2018).

Pathogenic bacteria are becoming resistant to commonly used antibiotics due to excessive use of drugs (Nathan, 2004). Due to this there is a downfall of conventional medicines and search for anti-bacterial agents has become necessary. Since ancient times spices has been used as medicine and food preservatives. The secondary metabolites present in spices are known for anti-microbial agents (Billing and Sherman, 1998). So, we aimed at evaluating the anti-bacterial activities of spices against *Enterococcus faecalis* and *Shigella* which can be transmitted even in low doses through direct or indirect human communal bathrooms and faecal contamination due to poor hygiene maintenance.

The objectives of our research were preparation of aqueous extracts of cinnamon and carom seeds , collection of *E. faecalis* and *Shigella* species from pathological lab , sub-culturing of both

the strains in laboratory in control condition and to perform biochemical test to confirm the species, to test the efficacy of the extract on bacterial inhibition by serial dilution and to calculate the inhibitory zone and to test the antibacterial activities of the spices extracts on both the bacterial strains sub-cultured in the laboratory.

#### Materials and Methods :

The spices cinnamon and carom seeds were purchased from super market of Patna and were sun dried for 24 hours.

**Preparation of extracts:** 10g of spices were crushed and grinded in mortar and pestle separately and aqueous extract of both the spices were prepared by adding 50ml of distilled water with the help of rota vapour.

Serial dilution of cinnamon and carom seeds extract in concentration - was prepared by dissolving 100µl of distilled water from which again 100µl diluted extract was transferred in 900µl of distilled water. This process was repeated up to dilution.

**Test micro-organism:** Two bacterial strains of *E. faecalis* and *Shigella* were collected from SRL Diagnostics, Patna. The bacterial culture was maintained in CRL (Central Research Laboratory), Patna Women's College by sub-culturing it every week.

HiCrome UTI agar was prepared for sub-culturing *E. faecalis* and Mac Conkey and XLD agar for *Shigella*.

**Bio-chemical testing of bacterial strains:** To confirm the bacterial strains, they were subjected to gram staining and necessary biochemical tests by Bergey's Manual of Determinative Bacteriology.

**Determination of anti-bacterial activities:**

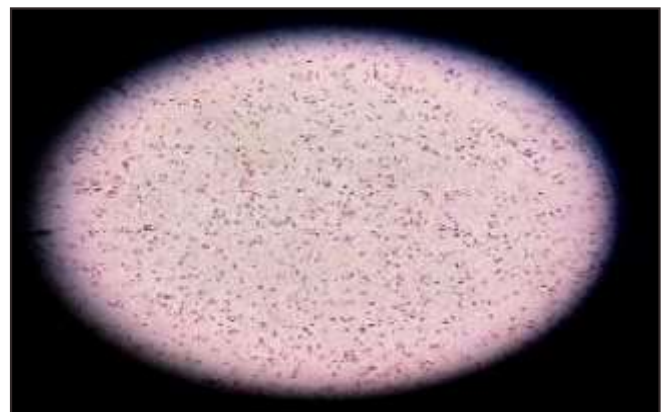
Anti-bacterial activities of cinnamon and carom seeds were assayed through Disk Diffusion Method (KIRBY-BAUER METHOD). 100µl of bacterial cultures were uniformly spread onto the surface of MHA plate with sterile glass spreader. Sterile discs were dipped in the prepared aqueous extracts for 20 seconds then disks were placed onto the surface of MHA plate and incubated for 24 hours at 37°C.

The zones of inhibitions obtained were measured by Digital Vernier Calliper in 5 different plates to calculate mean and standard deviation.

#### Results and Discussion :



A



B

Fig 1. A. Gram negative rods of *Shigella*,

B. Gram Positive coccus of *E. faecalis*

**Table 1. Summary of biochemical tests of *E faecalis* and *Shigella* species**

Parameters/Tests	<i>E. faecalis</i>	<i>Shigella</i>
Grams Stain	+	-
Shape	Coccus	Rods
Motility	-	-
Indole Test	-	+
Methyl Red Test	-	+
Voge's Proskauer Test	+	-
Bile esculin Test	+	-
Catalase Test	-	+
Citrate Utilization Test	-	-
Oxidase Test	-	-
Salt Tolerance Test	+	-
Triple Sugar Iron Test	-	+
Nitrate Test	-	+
Urease Test	-	-

**Table 2. Zone of inhibition (in mm) shown by *Enterococcus faecalis* on cinnamon extract**

No. of replicates	Crude	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$
Plate 1	23	19	16	10	6	5
Plate 2	22.5	18	14	8	7	5
Plate 3	22	19	15	9	6	5
Plate 4	23	18	15	9	6	5
Plate 5	22	18	14	8	6	5
<b>Mean ± SD</b>	<b>22.5±0.5</b>	<b>18.5±0.5</b>	<b>14.8±0.8</b>	<b>8.8±0.8</b>	<b>6.2±0.4</b>	<b>5±0.0</b>

**Table 3. Zone of inhibition (in mm) shown by *Enterococcus faecalis* on carom seeds extract**

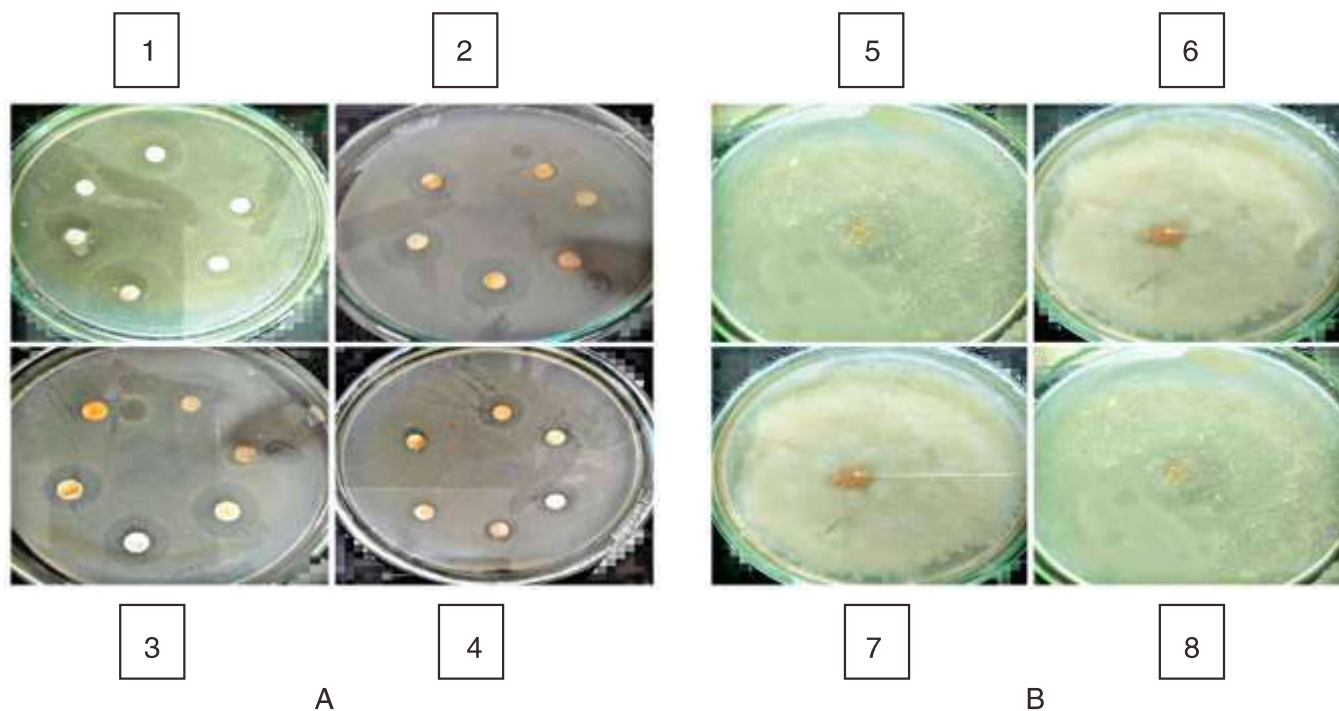
No. of replicates	Crude	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$
Plate 1	20	16	13	10	7	5
Plate 2	20	16	13	9	6	5
Plate 3	19	14	13	10	6	5
Plate 4	19	14.5	12	10	6	5
Plate 5	20	14.5	12	9	7	5
<b>Mean±SD</b>	<b>19.6±0.5</b>	<b>15±0.9</b>	<b>12.6±0.5</b>	<b>9.6±0.5</b>	<b>6.4±0.5</b>	<b>5.0±0.0</b>

**Table 4. Zone of inhibition (in mm) shown by *Shigella* on cinnamon extract**

No. of replicates	Crude	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>
Plate 1	21	17	13	10	7	5
Plate 2	20	16	13	9	7	5
Plate 3	20	16	13	9	7	5
Plate 4	21	17	12	10	6	5
Plate 5	21	16	12	10	6	5
<b>Mean± SD</b>	<b>20.6±0.5</b>	<b>16±0.5</b>	<b>12.6±0.5</b>	<b>9.6±0.5</b>	<b>6.6±0.5</b>	<b>5±0</b>

**Table 5. Zone of inhibition (in mm) shown by *Shigella* on carom seeds extract**

No. of replicates	Crude	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>
Plate 1	23	20.5	18	15	9	5
Plate 2	23	20.5	18	14	9	5
Plate 3	22.5	20	17	14	9	5
Plate 4	22	20	17	14	8.6	5
Plate 5	22	20	17.8	15	8.6	5
<b>Mean± SD</b>	<b>22.5±0.5</b>	<b>20.2±0.2</b>	<b>17.5±0.5</b>	<b>14.4±0.5</b>	<b>8.84±0.2</b>	<b>5.0±0.0</b>



**Fig. 4. Zone of Inhibition (ZOI) of aqueous extracts (A) and powder (B) of Cinnamon and Carom seeds.**

- A. 1. ZOI of Carom seeds in *Shigella*,  
 2. ZOI of Carom seeds in *E. faecalis*  
 3. ZOI of Cinnamon in *Shigella*,  
 4. ZOI of Cinnamon in *E. faecalis*

- B. 5. ZOI of Carom seeds in *Shigella*,  
 6. ZOI of Cinnamon *Shigella*  
 7. ZOI of Cinnamon in *E. faecalis*,  
 8. ZOI of Carom seeds in *E. faecalis*



Physical parameters and Biochemical tests are presented in Table 1. *E.faecalis* was gram positive coccus and *Shigella* was gram negative rods Fig.1.Voge'sProskauer, Bile esculin and Salt tolerance test was found positive in *E.fecalis* whereas Indole, Methyl red, Catalase Triple sugar iron and Nitrate test were found positive in *Shigella*. Zone of inhibition of cinnamon extract in *E.faecalis* are presented in Table 2. Maximum zone of inhibition  $22.5 \pm 0.5$  mm was measured in crude extract and minimum inhibition was observed  $5.0 \pm 0.0$  mm in  $10^5$ . Zone of inhibition of carom seeds extract in *E.faecalis* are presented in Table 3. Maximum zone of inhibition  $19.6 \pm 0.5$  mm was measured in crude extract and minimum inhibition was observed  $5.0 \pm 0.0$  mm in  $10^5$ . Zone of inhibition of cinnamon extract in *Shigella* are presented in Table 4. Maximum zone of inhibition  $20.6 \pm 0.5$  mm was measured in crude extract and minimum inhibition was observed  $5.0 \pm 0.0$  mm in  $10^5$ . Zone of inhibition of carom seeds extract in *Shigella* are presented in Table 5. Maximum zone of inhibition  $22.5 \pm 0.5$  mm was measured in crude extract and minimum inhibition was observed  $5.0 \pm 0.0$  mm in  $10^5$  (Fig. 4).

Based on the results presented in Table 2-5, it showed that cinnamon and carom seeds crude extracts were able to check the growth of *Enterococcus* and *Shigella* species of microbes. T test performed in *Shigella* showed results were statistically significant ( $p$  value = 0.16). *Shigella* species were also found to be inhibited by thyme essential oil, thymol, cloves, oregano, basil, rosemary and carvacrol (Bagamboula et al., 2004). Similar results were reported by Vaishnavi et al in 2007, where she has suggested antimicrobial properties of cloves against *Shigella* species. According to Gull et al., (2012) aqueous extract of

garlic is found to be more susceptible than ginger aqueous extract.

Similar trend was also observed in *E.faecalis* where  $p$  value was 0.36 suggesting the statistical significance. According to Chow (2000), *Enterococcus* causes serious therapeutic problems in serious diseases. Cinnamon and carom seeds showed excellent antimicrobial activity. The present study has selected and analysed zone of inhibition through Disk Diffusion method as a parameter to show antimicrobial effect. According to Mandal et al., (2011) disk diffusion method shows the best sensitivity trends. Cinnamon contains cinamaldehyde and are effective against many gram positive and gram negative bacteria (Chow, 2000 and Mandal et al., 2011). According to Ali et al. in 2005 cinamaldehyde is an active agent to inhibit the growth of both antibiotic resistant and antibiotic sensitive pathogenic microbes.

As per Anonymous (1995), in Indian system of medicine carom seeds are administered as house hold remedy against stomach disorder and asthma. Carom seeds constitutes fibres (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing Ca, P, Fe and nicotinic acid (Chauhan et al., 2014). According to Sengar and Verma (2016), carom seeds can be used for clinical applications. Significant antibacterial activity of carom seeds was seen against *Pseudomonas aeruginosa*, *Bacillus primilus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia* and *Bordetella bronchiseptica* (Shahidi, 2004). Carom seeds not only shows antimicrobial activity but it also shows antifungal properties on various fungus (Ansari, 1995).

According to his study report, he showed carom seeds extract is an antifungal agent against *Rhizoctonia solani* which causes sheath blight disease in rice. According to Syed et al (1986), oil of carom seeds works against the growth *Staphylococcus aureus*, *E. coli*, *Salmonella*, *Vibrio*, cholera species. Similar findings were also reported by Singh et al (2002) and Mayaud et al (2008). The antimicrobial activity of aqueous extract of carom seeds was proved by Kaur and Arora (2009), where they have reported the carom seeds can cure gastrointestinal disorders. The spices may also be considered as a great tool in combating infectious disease caused by resistant bacteria.

#### **Conclusion :**

Spices are multi-beneficial naturally occurring substance which provide aroma and taste when used in food, anti-oxidants, have healing properties and other beneficial properties in curing diseases with antimicrobial properties. With these properties they are already paving their path for many herbal remedy and medicines with lesser or no harmful effect on both the biotic and abiotic factors of the ecosystem. This study concluded that the cinnamon (*Cinnamomum verum*) has better effect on *Enterococcus faecalis* than carom seed (*Trachyspermum ammi*) and on *Shigella* carom seeds have better effects than cinnamon. As the aqueous extracts of the spices get diluted, their efficiency in suppressing the colonisation of bacteria decreases. The concentrate extract of cinnamon and carom seed extract showed maximum zone of inhibition and in both the bacterial strains selected to study. Herbs and spices return the state of body with natural balance to heal itself, stimulate the immune system and aid the body in fighting infections. Many herbs are used as medicines to treat disease and enhance general

health. This study can provide a scope and potential material for others to study on other wonderful spices and herbs and observe their antimicrobial properties on other pathological and antibiotic resistant bacteria.

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