Health Organization (WHO) has created a list of 20,000 plants that are used for plant therapy in herbal systems of medicine all over the planet (Manavalan and Manian 2001). The essential oils are defined as complex volatile substances, synthesized naturally in different plant parts during the process of metabolism (most probably secondary metabolism). Essential oils have great potential in the field of biomedical research as they effectively destroy several bacterial, fungal, and viral pathogens. Presence of different types antimicrobial compounds found in thisoilmeans that the essential oils are effective against a diverse range of pathogens.

The high and effective; reactive property of essential oil depends upon the nature, composition, and orientation of its functional groups. The sole purpose of our research is to review the antimicrobial potential of the common market essential oil mechanism of action against pathogens like bacteria *E. coli.* Our comprehensive investigation and review will benefit scientists; to find out the potential of essential oils in the development of mechanism against a broad range of drug-resistant pathogenic microbes.

The grave struggle of mankind against infectious diseases is well known. Therefore, the discovery of antibiotics led to a vast range of application in science that helped infections' control and prevention. We have used several antibiotics in our work to study the antibacterial profile.

Panipuri or golgappa is very famous in all cities of India. They are consumed by huge population and frequently associated with food borne illness due to their improper handling and serving practices. The spicy-sour like water of Panipuri is found to be contaminated with different bacterial pathogens like *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and yeast. Many an often, food-borne and diarrheal diseases are caused by many bacterial pathogens including *Enterobacter* sp. Specially, (Tambekar et al., 2011). Street food which is exposed to the outer environment makes a proper habitat for bacterial growth.

Materials and Methods:

Sample Collection: The street food stall was opened at 12 pm. Samples were collected at 5 pm. A total of 10 Panipurisamples were collected from the local street food stall of Patna, Bihar. The samples were separately collected. *2 puris,* 30 gm of stuff and 25 ml of the *khattapani,* and placed in containers.

Determination of pH of sample: pH of the samples (khattapani, puri,stuff) were determined with help of pH meter.

Determination of bacterial load: The collected samples were serially diluted (up to10–7) and plated on nutrient agar plates with help of pipettes and incubated at 37°C for 24 hours. (Cruickshank et al. 1975)

Isolation of bacteria: After incubation, selected colonies were inoculated on NA media plates by streak plate method. The inoculated plates were incubated overnight.

Detection of Coliforms: Pure culture was prepared on EMB Agar Media which showed result of coliform growth after incubation.

Identification of isolates: Identification and characterization of the isolates were done by standard microbiological methods. (Collins and Lyne 1970)

Antibiotic sensitivity test: A total of 6 antibiotics (Ciprofloxacin [5 µg], Erythromycin [15µg], Penicillin [10µg], Gentamicin [10 µg], Azithromycin [15 µg] and Doxycycline [30 µg]), were purchased to test against the isolates of bacterial culture by disc diffusion method (Bauer et al., 1966).

Study of antibacterial effect by essential oils: A total of 5 essential oils were purchased (*Kalonji,* Clove, Tea Tree, Cinnamon, and Eucalyptus) and studied against isolates by disc

diffusion method.(Bauer et al 1966) Freshly grown culture was swabbed on solid agar plates. Whatman Paper 1 were cut in small pieces with diameter 5mm and sterilized. The sterilized paper was dipped in oil, and dried for a while. The papers were kept on the spread plates.

Results and Discussion:

The pH of the liquid sample (khattapani) was observed to be highly acidic (pH 4.0-5.5). The pH of stuff ranged between 6.5-7.0 and that of puri was between 8.0-8.5.The CFU/mL was determined for the samples determining the bacterial count of NA and EMB.EMB plates contained 72 colonies at 7 dilution factor, with a CFU/ml of 7.6×105. Table 1 & 2 shows the CFU of both NA and EMB, respectively. Graphical data including Figure 1 shows the bar graph depicting growth on NA and EMB agar media.

Table 1. CFU/ml calculated for the colonies on NA plates

Dilution Factor	Colony	CFU/mL
6	52	5.2×10^{4}
7	59	5.9×10^{5}
8	63	6.3×10^{6}

Table 2. CFU/ml calculated for the colonies on EMB plates

Dilution Factor	Colony	CFU/mL
5	64	6.4×10^{3}
6	72	7.2×10^{4}
7	76	7.6×10^{5}

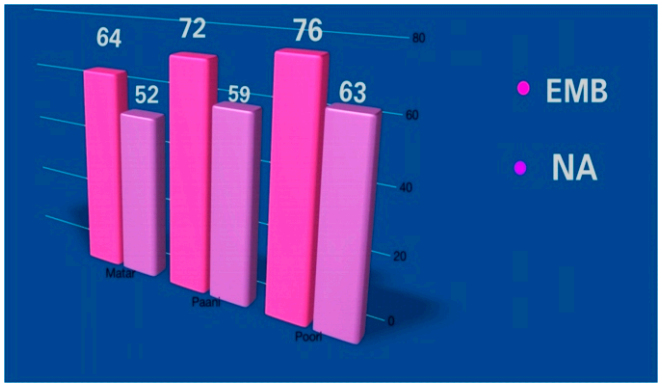

Fig. 1. Bargraph depicting growth on NA and EMB after colony forming unit's calculation

Standard methods were applied for identification and characterization of growth on NA and EMB plates (Table 3, 4). Isolated strains after Gram's staining were detected with presence of Salmonella spp., *E.coli.,* followed by other coliforms and species of *Staphylococcus.* There was a major occurrence of *Enterobacter* sp. EMB plates contained Gram negative enteric bacterium. NA plates had a vast variety of both Gram positive and Gram negative. Mucoid and slimy colonies were observed with smooth margin, which were white or cream colored, on NA. In contrast, the growth on EMB had pink-colored colonies.

Table 3. Colony and Morphological Characteristics on NA Media

Food Sample	Color	Config-uration	Margin	Eleva-tion	Texture	Dilution	Morpho-logical Struct-ure	Gram Stain
Pani	White	Round	Smooth	Convex	Mucoid	10^{-7}	Rod	+ve
Stuff	Cream	Oval	Smooth	Flat	Slimy	10^{-7}	Rod	-ve
Puri	Cream	Round	Smooth	Flat	Slimy	10^{-7}	Rod	+ve

Table 4. Colony and Morphological Characteristics on EMB Media

Food Sample	Color	Config-uration	Margin	Eleva-tion	Texture	Dilution	Morpho-logical Struct-ure	Gram Stain
Pani	Pink	Round	Smooth	Convex	Mucoid	10^{-7}	Rod	-ve
Stuff	Pink	Round	Smooth	Convex	Mucoid	10^{-7}	Rod	-ve
Puri	Pink	Round	Smooth	Flat	Slimy	10^{-7}	Rod	-ve

A number of Coliformson *khattapani,* like *E.coli, Salmonellaspp, Klebsiellasppwere* detected on EMB Agar plate (Table 5), with shiny green colony appearance of *E.coli.* Pink-red colonies *Salmonella* were observed on EMB Agar. Cream-white coloured colonies of *Streptococusand Staphylococcus* was observed on NA. Different isolates like *Bacillus spp, Streptococcus, Staphylococcus,* on different samples were (Table 6) observed where results proved that khattapaniwas the most contaminated sample, containing large number of Coliformsfollowed by

stuff. Streptococcal count on Pani, Puri and stuff was 12, 21, 7, respectively showing varying distribution in different samples. It can be observed that the contamination in Panipuri is high because of the conditions under which it is prepared, vended and the area that is open air. (Tambekar et al 2008). Colony count revealed presence of high mesophilic colonies.

Table 5. Table showing different coliforms isolated on EMB Agar

Food Sample	Coliforms Isolated
Pani	*Salmonella spp., E.coli, Klebsiella*
Stuff	*Salmonella spp., Enterobacter*
Puri	*Klebsiella, E.coli*

Table 6. Table showing distribution of different isolates on different sample of Panipuri

Isolates	Pani	Puri	Stuff
Coliforms	28	6	13
Bacillus spp	13	18	6
Staphylococcus	9	-	3
Streptococcus	12	21	7

From the antibiogram pattern studies it was observedthat all the isolates including *Enterobacter, Streptococcus, Staphylococus, Salmonella spp,* were susceptible to Penicillin, Ciprofloxacin, Gentamicin, and Doxycycline. *E.coli* was moderately sensitive to Azithromycin and Erythromycin. Salmonella spp. was resistant to Azithromycin. Antibiogram profile was carried out for separate isolates on NA and EMB plates. (Table 7, 8, 9, 10) Distinct zone of inhibition was observed on EMB for *E.coli* and *Salmonella spp.* Antibiogram profile suggests the presence of antibiotic resistant strains of *Salmonella spp.* Antibiotic sensitivity was tested and measured by CLSI Standards. Zones of inhibition on NA were clearly visible.

Table 7. Effect of various antibiotics like Penicillin, Ciprofloxacin, Azithromycin, Erythromycin, Doxycyline, and Gentamicin on *E.coli*

Antibiotics	Susceptible (mm.)	Moderately Sensitive (mm.)	Resistant (mm.)	Inhibition zone (mm.)	Effect
Penicillin	≥ 29	--	≤ 28	31 mm	Susceptible
Ciprofloxacin	≥ 21	16-20	≤ 15	35 mm	Susceptible
Azithromycin	≥ 18	14-17	≤ 13	16 mm	Moderately Sensitive
Erythromycin	≥ 23	14-22	≤ 13	22 mm	Moderately Sensitive
Doxycycline	≥ 14	11-13	≤ 10	21 mm	Susceptible
Gentamicin	≥ 15	13-14	≤ 12	30 mm	Susceptible

Table 8. Effect of various antibiotics like Penicillin, Ciprofloxacin, Azithromycin, Erythromycin, Doxycyline, and Gentamicin on *Salmonella*

Antibiotics	Susceptible (mm.)	Moderately Sensitive (mm.)	Resistant (mm.)	Inhibition zone (mm.)	Effect
Penicillin	≥ 29	--	≤ 28	32 mm	Susceptible
Ciprofloxacin	≥ 21	16-20	≤ 15	35 mm	Susceptible
Azithromycin	≥ 18	14-17	≤ 13	2 mm	Resistant
Erythromycin	≥ 23	14-22	≤ 13	29 mm	Susceptible
Doxycycline	≥ 14	11-13	≤ 10	27 mm	Susceptible
Gentamicin	≥ 15	13-14	≤ 12	25 mm	Susceptible

Table 9. Effect of various antibiotics like Penicillin, Ciprofloxacin, Azithromycin, Erythromycin, Doxycyline, and Gentamicin on *Streptococcus*

Antibiotics	Susceptible (mm.)	Moderately Sensitive (mm.)	Resistant (mm.)	Inhibition zone (mm.)	Effect
Penicillin	≥ 29	--	≤ 28	32 mm	Susceptible
Ciprofloxacin	≥ 21	16-20	≤ 15	30 mm	Susceptible
Azithromycin	≥ 18	14-17	≤ 13	25 mm	Susceptible
Erythromycin	≥ 23	14-22	≤ 13	31 mm	Susceptible
Doxycycline	≥ 14	11-13	≤ 10	25 mm	Susceptible
Gentamicin	≥ 15	13-14	≤ 12	23 mm	Susceptible

Table 10. Effect of various antibioticslike Penicillin, Ciprofloxacin, Azithromycin, Erythromycin, Doxycyline, and Gentamicin on *Staphylococcus*

Antibiotics	Susceptible (mm.)	Moderately Sensitive (mm.)	Resistant (mm.)	Inhibition zone (mm.)	Effect
Penicillin	≥ 29	--	≤ 28	35 mm	Susceptible
Ciprofloxacin	≥ 21	16-20	≤ 15	33 mm	Susceptible
Azithromycin	≥ 18	14-17	≤ 13	23 mm	Susceptible
Erythromycin	≥ 23	14-22	≤ 13	33 mm	Susceptible
Doxycycline	≥ 14	11-13	≤ 10	25 mm	Susceptible
Gentamicin	≥ 15	13-14	≤ 12	28 mm	Susceptible

While observing the antibacterial activity of the essential oils during the screening, primarily by disc diffusion method it was observed (Table 11, 12) that Cinnamon oil was effective, followed by Clove and *Kalonji.* The isolates were less affected by any oil.

Table 11. Antibacterial effect of essential oils like Kalonji,Eucalyptus, Tea Tree, Clove and Cinnamon on *Streptococcus*

Oil	Inhibition zone (mm.)	Effect
Kalonji (Nigella sativa)	1 mm	Moderately Sensitive
Eucalyptus (Eucalyptus citriodora)	-	None
Tea Tree (Melaleuca alternifolia)	-	None
Clove (Syzygiumaromaticum)	2.3 mm	Sensitive
Cinnamon (Cinnamomum verum)	13 mm	Sensitive

Table 12. Antibacterial effect of essential oils like Kalonji,Eucalyptus, Tea Tree, Clove and Cinnamon on *Staphylococcus*

Oil	Inhibition zone (mm.)	Effect
Kalonji (Nigella sativa)	2 mm	Moderately Sensitive
Eucalyptus (Eucalyptus citriodora)	-	None
Tea Tree (Melaleuca alternifolia)	1 mm	Moderately Sensitive
Clove (Syzygiumaromaticum)	6 mm	Moderately Sensitive
Cinnamon (Cinnamomum verum)	8 mm	Sensitive

Food acts as a substrate for all living organisms to grow and perform metabolic activities. Microbial food contamination in any case occurs, it is because of the manhandling and improper preparation of food and storage in poor sanitary conditions. (Saxena et al., 2013). In similar type of work from Baripada, Orissa, India *Shigella* spp. was found to be the main contaminant of Panipuri. (Das et al., 2012). The open environment can act as a medium for contamination. (Levine and Levine, 1991).

In the present work the most frequent contaminating microorganism in street foodstuffs like Panipuriwas *E. coli.* About 30.0% of *panisample* was contaminated by *E.coli.* Puri was contaminated by a large number of *Bacillus spp.*(15%).The stuff contained Coliforms as the most abundant bacteria. The main reason for contamination by *Staphylococcus* was because of the vendor's hygiene and the condition in which the food was prepared and served. The antibiogram study suggested that Ciprofloxacin was the most effective antibiotic for Gram negative whereas Penicillin was the most effective for Gram positive bacteria. *Salmonella spp.* was resistant to Azithromycin while *E. coli* was moderately sensitive towards it. Both *Staphylococcus* and *Streptococcus* were highly susceptible to Penicillin. On, *Staphylococcus* and *Streptococcus,* Cinnamon oil was effective, followed by Clove and *Kalonji.* So, it is important to maintain proper hygiene while serving or consuming street food.

Conclusion :

In the present investigation it can be concluded that some antibiotics like Ciprofloxacin, Penicillin showed positive impact on some isolates. The *Kalonji* and cinnamon oil had some effect on the bacterial culture. The antibacterial effect by essential oil (*Kalonji,* Clove, Tea Tree, Cinnamon, and Eucalyptus) was less effective compared to antibiotics. However, Tea tree and Eucalyptus were the aromatic essential oil exhibiting antibiotic

property. It can be said after the inference drawn from our work that these essential oils could be used in food items as antibacterial agents as well as natural food preservatives due to their non-poisonous, availability and non-pathogenic nature with antioxidant and potent antibacterial characteristics. The antibiotics proved to be of great sensitivity *in-vitro.*

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