equipment, air, etc., and thereafter proliferates. Several human pathogenic bacteria like that of species of *Staphylococcus, Streptococcus, Escherichiacoli, Pseudomonas, Campylobacter,* etc. are reported in milk that are possible cause of diseases transmitted through raw milk and dairy products made from unpasteurized milk (Msalya, 2017).

Apart from this milk contains significant amount of antibiotic residues that enters milk either through its indiscriminate use as feed additives or via injudicious application in treatment of infectious animal diseases(Zhang et al., 2009). As per the report of (Aarestrup, 2012) published in Nature, global use of antimicrobials in animals is double as compared to humans. Presence of antibiotics guides resistance among microflora of milk, making increasing antibiotic resistance a global concern (Na lampang et al., 2007; Westh et al., 2004).

Medicinal plants are considered as an important element of traditional medicine being much effective in the treatment of bacterial infections (Rakib et al., 2012). Since time immemorial several workers have been investigating role of various plant and their products to restore the fatal diseases (Dogra et al., 2015). *Tagetes* species is one such medicinal plant belonging to Asteraceae family, having antibacterial activities. Leaves, flowers and seed of *Tagetes* are reservoir of active constituents viz., flavonoids, carotenoids etc. used for treatment of wound, muscle pain, piles and beneficial for eyesight, skin, heart diseases and sometimes even cancer (Janardanam,2016). Fruits plays an important role in human nutrition as it supplies us necessary growth factors like minerals and vitamins (Al-Hindi et al., 2011). *Citrus* fruits are acidic fruits are good source of natural bioactive compounds such as carotenoids, flavonoids, limonoids, vitamin C, acridone alkaloids, folic acid, potassium, selenium, dietary fibers and a wide range of phytochemicals. They have some antifungal and antiviral activities due to the presence of flavonoids. Activities of gram-negative bacteria are inhibited by the use of C. *bergamot* peel which is a natural antimicrobial. Limonin which is present in Citrus juice helps in lowering the level of cholesterol (Kurowska et al., 2000).

Keeping these facts in mind the aim of present is to isolate dairy contaminants and investigate antibacterial effect of plant extract of *Tagetes species* and *Citrus sinensis.*

Materials and Methods :

Sample collection : Different dairy products were collected from different places of Patna. The selected samples were raw cow milk from a cowbarn in Kankarbagh locality (25.5938°N, 85.1605°E), boiled cow milk from Phulwarisharif (25.5732°N, 85.0693°E), khoya from a shop located in Kankarbagh (25.5938°N, 85.1605°E), and lassi from Boring road (25.6151°N, 85.1384°E). Samples were collected aseptically in the sterilized vials and brought to the laboratory for experimental studies.

Isolation of bacteria : Bacterial colonies were isolated on nutrient agar (NA) plates by serial dilution (10-1 to 10-7) technique (Aneja, 2015). The inoculated NA plates were incubated at 37ºC for 48 hr for regular observation. Bacterial colonies were enumerated on colony counter to evaluate colony forming unit (CFU) (Brugger *et al.,* 2012). Morphologically different bacterial colonies were repeatedly sub cultured to get pure isolates. Each pure isolate was streaked on selective media viz., eosin methylene blue (EMB) media for *Escherichia* species and Mac Conkey agar for *Klebsiella* species, while *Staphylococcus* species was confirmed by performing morphological and biochemical studies.

CFU/ml = (number of colonies * dilution factor)/volume of culture plate.

Morphological characterization : The morphological characteristics of isolates were studied in terms of shape, colour, elevation, margin and Gram's staining (Aneja, 2015).

Biochemical characterization : Bacterial isolates were further characterized on the basis of different biochemical tests like, catalase test, coagulase test, indole test, mannitol motility test, methyl red test and citrate utilization test (Aneja, 2015).

Antibiotic sensitivity test : Antibiotic sensitivity test was studied followingKirby-Bauer disk diffusion method (Hudzicki, 2009). 24 hr old bacterial culture was inoculated on nutrient agar plate by a sterile swab to get lawn culture. Thereafter different antibiotic disks (*ofloxacin, ciprofloxacin, cephalexin, moxifloxacin, norfloxacin and levofloxacin*) were aseptically placed on inoculated agar plates and incubated at 37°C for 24 hr. They were observed for presence or absence of zone of inhibition by bacterial isolates. The measurement of zone of inhibition was done by millimetre scale according to the range provided by Clinical Laboratory Standard Institute (CLSI).

Preparation of plant extracts : Fresh fruit *Citrus sinensis* and fresh flower and leaf of *Tagetes* were collected from local market of Patna and garden of Chhapra respectively. For preparing different plant extract, the peel of *Citrus,* flower and leaf of *Tagetes* were separated, and dried under shade for about 15 days and powdered in a blender. Then 2.5 g of the powder was weighed and mixed in a conical flask containing 10 ml of ethanol. The extract was left for 48 hours with occasional stirring. Then the extract was filtered by using sterile What man No. 1 filter paper and the filtered part was stored in a sterile bottle (Madhuri et al., 2014). For preparation of orange pulp extract, the pulps were separated and squeezed aseptically by hands to obtain its juice then the collected juice was stored in a sterile glass vial and kept in refrigerator for further study.

Antibacterial assay of plant extracts : Antibacterial assay of extracts of different plant parts was performed by agar well diffusion method on nutrient agar plates. The test organisms were inoculated in nutrient broth and incubated at 37° C for 24 hours. The different bacterial culture broth was taken and swabbing was done on NA plates. Four wells were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different plant extract with the help of sterile pipette and allowed to diffuse for about 30 minutes at room temperature. Plates were incubated for 24 hours at 37° C.After the incubation has done, the plates were observed for the formation of clear zone around the wells. The zone of inhibition was measured with the help of millimetre scale. (Manandhar, 2019).

Results and Discussion :

Isolation of bacteria : A total of 17 morphologically different bacterial colonies appeared on NA plates at 37ºC (Table 1). Analysis of the total viable count of the different dairy product was done by calculating CFU (colony forming unit) at 10^{-4} dilution as presented in Table 1. Sample 1 (raw cowmilk) was having maximum number of visible colonies i.e., 7.2×10^3 cfu/ml, while sample 4 (lassi) was found to have least number of visible colonies i.e., 5.9×10^2 cfu/ml. Significant microbial load in milk is indicative that milk is highly prone to contamination and can serve as an efficient vehicle for human transmission of foodborne pathogens. Highest bacterial load in raw milk signifies that consumption of pasteurized milk should be practiced over raw milk.

Table 1. Total viable count of dairy products

Sample	Cfu/ml	No. of bacterial isolates
Sample 1 (Raw cowmilk)	7.2x103	7
Sample 2 (Boiled cowmilk)	4.6x103	3
Sample 3 (Khoya)	6.3x103	5
Sample 4 (Lassi)	5.9x102	2
Total No. of isolates	17	

Morphological characterization : Morphological features of the bacterial isolates are presented in Table 2. The bacteria isolated from sample 1 and sample 4 was Gram negative bacteria

that were rod in shape while bacteria isolated from sample 2 and sample 3 was Gram positive and coccus in shape.

Table 2. Morphological characteristics of isolated bacteria from different sample

Dairy Sample	Morphological characteristics of all the isolates					
	Colony characteristics					
	Colour	Texture	Elevation	Margin	Gram stain	Shape under microscope
Isolate 1	Greyish white	Shiny	Convex	Dome-shaped	-ve	Rods
Isolate 2	White	Creamy	Flat	Circular	+ve	Coccus
Isolate 3	White	Creamy	Concave	Circular	+ve	Coccus
Isolate 4	Greyish white	Shiny	Raised	Circular Fixed	-ve	Rods

Biochemical Analysis : Biochemical properties of bacterial isolates is presented in Table 3. Isolate 1 identified as *Escherichia* spp. as they are Gram negative rod and have shown positive result for catalase test, indole test, methyl red test and motility test and were sub-cultured on selective media (EMB), Isolate 2 was identified as *Klebsiella* spp. due to the positive result of citrate utilization test, catalase test and were further sub-cultured on selective media(MacConkey agar) and Isolate 3 as *Staphylococcus* spp. as they appear as a bunch of grapes and show positive result for coagulase test.

Table 3. Biochemical characteristics of bacterial isolates of different samples

Biochemical test	Isolate 1	Isolate 2	Isolate 3
Motility test	+ve	-ve	+ve
Citrate utilization test	-ve	+ve	+ve
Catalase test	+ve	+ve	+ve
Coagulase test	_	_	+ve
Indole test	+ve	-ve	-ve
Methyl Red test	+ve	-ve	+ve

Antibiotic Sensitivity test : All the bacterial isolates subjected to antibiotic sensitivity test. The CLSI (Clinical and Laboratory Standard Institute) standards had been used to detect the sensitivity pattern of bacterial isolates. According to the observations, isolate 1 and isolate 2 were found resistant to all the antibiotics used in the test while isolate 3 was found resistant to ofloxacin, ciprofloxacin, cephalexin, moxifloxacin, norfloxacin and intermediate to levofloxacin. The result of antibiotic sensitivity test was as follows

Table 4. Antibiotic sensitivity test result of isolate 1 *(Escherichia spp.)*

Antibiotic	Disc amount (microgram)	Zone of resistant (mm)	Zone of intermediate (mm)	Zone of susceptibility (mm)	Diameter Recorded (mm)	Inference
Ofloxacin (OF)	10	< or = 14	15-17	>or = 18	0	Resistant
Ciprofloxacin (CF)	5	< or = 15	16-20	>or = 21	0	Resistant
Cephalexin (Cp)	30	< or = 22	23-25	>or = 26	0	Resistant
Levofloxacin (Lv)	5	< or = 15	16-18	>or = 19	0	Resistant
Moxifloxacin (MF)	5	< or = 20	21-23	>or = 24	7	Resistant
Norfloxacin (NF)	10	< or = 12	13-16	>or = 17	0	Resistant

Table 5. Antibiotic sensitivity test result of isolate 2 *(Klebsiella spp.)*

Antibiotic	Disc amount (microgram)	Zone of resistant (mm)	Zone of intermediate (mm)	Zone of susceptibility (mm)	Diameter Recorded (mm)	Inference
Ofloxacin (OF)	10	< or = 15	16-21	>or = 22	0	Resistant
Ciprofloxacin (CF)	5	< or = 14	15-20	>or = 21	0	Resistant
Cephalexin (Cp)	30	< or = 22	23-25	>or = 26	6	Resistant
Levofloxacin (Lv)	5	< or = 15	16-18	>or = 19	0	Resistant
Moxifloxacin (MF)	5	< or = 20	21-23	>or = 24	0	Resistant
Norfloxacin (NF)	10	< or = 12	13-16	>or = 17	5	Resistant

Table 6. Antibiotic sensitivity test result of isolate 3 *(Staphylococcus spp.)*

Antibiotic	Disc amount (micro-gram)	Zone of resistant (mm)	Zone of interme-diate (mm)	Zone of suscep-tibility (mm)	Diameter Recor-ded (mm)	Inference
Ofloxacin (OF)	10	< or = 14	15-17	>or = 18	13	Resistant
Ciproflo-xacin (CF)	5	< or = 15	16-20	>or = 21	12	Resistant
Cephal-exin (Cp)	30	< or = 22	23-25	>or = 26	16	Resistant
Levoflox-acin (Lv)	5	< or = 15	16-18	>or = 19	17	Interme-diate
Moxiflox-acin (MF)	5	< or = 20	21-23	>or = 24	15	Resistant
Norflox-acin (NF)	10	< or = 12	13-16	>or = 17	10	Resistant

Antibacterial assay of plant extracts : Antibacterial assay of extracts of different plant parts was performed by agar well diffusion method on Nutrient Agar plates. The zone of inhibition was measured with the help of millimetre scale. According to the observations, plant extracts had shown highest antibacterial activity against isolate 3 (*Staphylococcus* spp.). Whereas least antibacterial effect had been shown against isolate 1 (*Escherichia* spp.). Among these bacterial isolates the isolate similar to *Staphylococcus* spp. was found susceptible to *Citrus* peel and juice extract as in Hindi and Chabuck,2013 and is also susceptible to *Tagetes* flower and leaf extract. The leaf, flower extract of *Tagetes* and the peel extract of *Citrus* had shown the higher antibacterial activity while the juice extract had shown the least antibacterial activity. This suggests that extract of *Tagetes* and *Citrus* sinensis can be beneficial in developing a natural antibacterial agent which can be used in the treatment of many infectious diseases. And they can be used as a substitute of antibiotics that has been used in medicines for treating different diseases.

Table 7. Zone of inhibition formed by bacterial isolates against plant extract

Plant extract	Zone of inhibition recorded in millimetre		
	Isolate 1 *(Escherichia spp.)*	Isolate 2 *(Klebsiella spp.)*	Isolate 3 *(Staphylococ-cus spp.)*
Citrus peel extract	9	8	22
Tagetes flower extract	0	15	18
Tagetes leaf extract	0	0	20
Orange pulp extract	0	0	16

Conclusion :

Milk is an excellent nutrient medium for the growth of pathogenic bacteria that contaminate milk from various sources.In the present study bacterial isolates *Escherichia* spp., *Klebsiella* spp. and *Staphylococcus* spp. were isolated from dairy samples. Considering dairy contamination with these pathogenic bacteria, sanitary practice during collection and transportation is recommended. All the three isolates showed resistance against all the test antibiotics signifying the presence of antibiotic resistant microflora in milk, which now has become a global concern. Antibacterial property of herbal extract suggested that *Tagetes* and *Citrus* are a good source of antimicrobial agents, particularly their flower and peels, respectively. In today's world where most of the population relies on the chemical formulates, herbal extract have proved to be more potent than that of chemical formulates.

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