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Comparative Studies on Phytochemical Components and Curd Probiotics against *Escherichia coli* and *Klebsiella sp.* in *In Vitro* Analysis

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Abstract
Comparative analysis stood for studying outcomes of medicinal and phytochemical effect of individual gut health. To find out the basic differentiation between curd probiotics and phytochemical components against isolated pathogens is manifested for studying on its fruitful outcomes in public health significance. Propagation of culture is assessed to assure the significant activities of antagonistic actions against gram negative characterized organisms and their functions as selective isolates on specified phytochemicals and dilution samples from homemade curd. Here Chowdhury AP and Sarma B., (2018) signified their outcomes on basis of future trends on medicinal implementation by comparative studying in *in vitro* analysis. **Aims:** Assessment of visible and non-visible findings of antagonistic action of phytochemical components and curd Probio against isolated infectious pathogens. **Objectives:** To carry out phytochemical analysis of leaf extract of plant *Nyctanthes arbor-tristis.* To determine antimicrobial activity of leaf extract of *Nyctanthes arbor-tristis* against *Escherichia coli*. Screening of probiotic from curd according to their isolation and identifications. Comparative analytical study on characteristics of phytochemicals and curd probiotics against the implemented and isolated pathogens. *(Escherichia coli. Klebsiella sp.)*

Keywords
Nyctanthes arbor- tristis, Escherichia coli., Klebsiella sp., Invitro Analysis.

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INTRODUCTION
Therapeutic properties of medicinal plants have been used traditionally to treat human diseases. Growing populations of developing countries use plant derived medicines to be a normal part of primary healthcare. Hundreds to thousands of

diverse secondary metabolites with different biological activities were found recorded in higher plants (Hamburger and Hostettmann, 1991). In recent years, multiple drug resistance (MDR) human pathogenic microorganisms has developed due to indiscriminate use of synthetic antimicrobial drugs commonly used in the treatment of infectious diseases (Singh *et al*., 2013). Natural antibiotics may have meager side effects and easy to treat human pathogenic microorganisms (Mitscher *et al*., 1987). Only 5-15% of 250k higher plants have been studied for a potential therapeutic value in the world and a large number remains to be investigated (Kinghorn, 1992). *Nictanthes arbor-tristis* L. (Night Jasmine) belongs to the family Oleaceae is found on rocky ground dry hills side and as under growth in dry deciduous forests [1]. It is native of South Asia, Nepal through Northern India to Southeast Thailand. Flowering usually occurs from July to October [3]. *Nictanthes arbor-tristis* L. stem bark of this plant is taken to cure dysentery, ulcer of palate and internal injuries (Ray and Gupta, 1980). The researchers used the juice of the leaves to treat chronic and bilious fever, rheumatism, as a laxative, diaphoretic and diuretic and the plant has been reported to be effective anti-leishmanial, antiviral and anti-amoebic infections (Sarema *et al*., 1980; Puri *et al*., 1994; Khatune *et al*., 2001). Use of *N. arbor-tristis* has long been known Ayurvedic system of medicine for the cure of snake bite, bites of wild animals, cancer, sores, ulcers, dysentery, and menorrhagia (Purushothaman et al., 1985). *Nyctanthes arbor-tristis* L. in its natural habitat. *Nictanthes arbor-tristis* flowers have shown promising results as potential sources for the isolation of bioactive compounds with anticancer activities (Timsina and Nadumane, 2016). Only very few reports have been published in *N. arbor-tristis* flower extracts and no systematic work has been done on the phytochemical and antioxidant potential, hence the present study has been taken with the aim of extraction, phytochemical characterization and evaluation of antimicrobial and antioxidant potential [4]. The most common means to incorporate probiotics to fermented milk include: (*i*) addition of probiotics with the starter cultures; (*ii*) the production of two batches separately, (one containing the probiotic microorganism in milk to achieve a high concentration of viable cells and another with starter cultures, when the fermentation stages are completed) and the batches are mixed; (*iii*) the use of a probiotic microorganism as a starter culture where as the time of fermentation is generally higher than traditional processes using non-probiotic starter cultures [2].

In this respect, it is necessary to consider the supplementation of the culture medium and the production conditions (*e.g*. incubation temperatures), **[5].** In addition, the probiotic strains must be compatible with starter cultures, since the latter could produce inhibitory substances that damage the probiotics **[6].**

Curd with high fat content showed inhibitory effects against probiotic cultures, particularly *B. bifidum* BBI **[7].** The supplementation with vitamins (*e.g.* ascorbic acid) has been reported to improve the viability of *L. acidophilus* in yoghurts **[10].** The addition of substances such as whey protein may also enhance the viability of some probiotics, probably due to their buffering property. In addition, the employment of prebiotic in yoghurt formulations could stimulate the growth and activity of probiotics **[8].** In this regard, fructose fermentation showed to be most effective in maintaining the probiotic viability **[9].**

Ecology and Distribution:

Nyctanthes arbor-tristis (Night-flowering Jasmine, Coral Jasmine) is a species of Nyctanthes, native to South Asia and Southeast Asia. It is a shrub or a small tree growing to 10 m tall, with flaky grey bark, large attractive rough and hairy leaves, and exotic highly perfumed small white flowers with an orange-red tube in the center. The tree is sometimes called the "Tree of sorrow", because the flowers lose their brightness during daytime; the scientific name *arbor-tristis* also means "sad tree". The flower is the official flower of the state of West Bengal, India, and for Kanchanaburi Province, Thailand. Night Jasmine blooms profusely, flowers are opening at night and casting off in the morning thus making a carpet of flowers, which are used in Buddhist temples, for worship. Parijat (*Nyctanthes arbor-tristis*), appears in several Hindu religious stories and is often related to the Kalpavriksha. According to mythology, this heavenly tree was brought to earth by Krishna. Satyabhama and Rukmini, Krishna's wives, had a quarrel over this tree. But Krishna planted the tree in Satyabhama's courtyard in a way that when the tree flowered, the flowers fell in Rukmini's courtyard.

Table 2.1

Taxonomical Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Oleaceae
Genus	Nyctanthes
Species	arbor-stritis

Table 2.2

Vernacular Name	
Language	Nyctanthes arbor-stritis
English	Night jasmine
Sanskrit	Parijatha
Assamese	Sewali

MATERIALS

Table 3.1 Material Required for Phytochemical studies:

Mechanical grinder	Chemical balance
Container	Water bath
Heating mantle	Hot air bath
Beaker	Autoclave
Conical flax	Laminar air flow
Test tube	Inoculation loop
Petri dishes	Spirit lamp
Filter paper	Cotton
Test tube	Aluminum foil
Inoculation loop	Paraffin

Table 3.2 Reagent required for Phytochemical studies:

Wagner's reagent	Molish's reagent
Conc. Sulphuric acid	Fehling's solution A and B
Benedict's reagent	Conc. Nitric acid
Acetic acid	Ferric chloride
Diluted sodium hydroxide	Potassium ferricyanide
Distilled water	Nutrient agar(NA)
Mayer's reagent	Molar Hilton agar(MHA)
Chloroform	Barfoed reagent
Sodium hydroxide	Dilute HCL

Methodology:

4.1 Plant Sample Collection:

This plant is found various regions of Assam. I have collected the plant material (leaves), from the Assam on 12th February 2018.

After collection the plant of *Nyctanthes arbor-tristis* and then shade dried. After drying the leaves of the plant is grinded in a mechanical grinder till it become powder form.

4.2 Extraction process

4.2.1. a Methanol extract

80 gm of air dried plant extracts was taken in 450ml of 96% methanol for 72hours in Soxhlet apparatus and then the extract was allowed to evaporate in hot plate magnetic stirrer at 450C. The dried extract is stored in refrigerator for further use.

4.2.1. b Petroleum ether extract

80 gm of air dried plant extracts was taken in 450ml of 96% petroleum ether for 72 hours in Soxhlet apparatus and then the extract was allowed to

evaporate in hot plate magnetic stirrer at 450C. The dried extract is stored in refrigerator for further use.

4.2.1. c Phytochemical Analysis

Chemical analysis for the screening and identification of bioactive chemical constituents in the medicinal plants under the study were carried out extracts using the standard procedures as describes by Software (1993) Trease and Evans (1989) and Harborne (1973).

4.2.2 Detection of Alkaloids

4.2.2. a Wagner's reagent: - 2ml of extract was taken in a test tube. Acidified the solution by adding 0.1 ml of hydrochloric acid then added o.1ml Wagner's reagent. Reddish brown colour precipitate was formed indicating the presence of alkaloids.

4.2.2. b Mayer's reagent: - 1.2ml of extract was taken in a test tube. 0.2ml of dilute hydrochloric acid 0.1ml of Mayer's reagent was added formation of yellowish buff colour precipitate indicates the presence of alkaloids.

4.2.3 Detection of Carbohydrate

a) **Molish's reagent**: - Aquous or alcoholic solution of substance and 10% alcoholic solution on of alpha-nephtol, shake well then added conc. Sulphuric acid along the side of the tube. A violet ring at the junction of two liquids of two liquid confirmed the presence of carbohydrate.

b) **Fehling's solution**: - 2ml of Fehling's solution A+ 2ml of Fehling's solution B+ 2ml of extract. Boiled, brick red precipitate appeared indicating reducing sugar present.

c) **Benedict's solution**: - 5ml of Benedict's reagent+ 3ml of test solution mixed well then boil in water bath. If appearance of brick red precipitation. At the bottom of test. (0.5% carbohydrate present).

4.2.4 Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid solution. Formation of yellow colour indicates the presence of protein.

4.2.5 Detection of Glycoside

a) **Liebermann's test**: - 2ml of the organic extract was dissolved in 2ml of chloroform and 2ml of Acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of steroid nucleus.

b) **Salkowaski's test**: - 2ml of each extract was dissolved in 2ml of chloroform. 2ml of Sulfuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of steroid ring.

4.2.6 Detection of Tannins

a) **Tannin test**: - About 0.5gm of dried powered sample was boiled in 2ml of water in test tube and then filtered. A few drops of 0.1% ferric chloride solution was added and observed for brownish green or blue black colouration but there is no colouration.

b) **Phlobatannis test**: - Deposition of red precipitate when an aquous extract of each plant sample was boiled with 1% aqueous extract of each plant sample was boiled with 1% aquous hydrochloric acid was taken an evidence for the presence of Phlobatannins. No any extract showed red coloration.

4.2.7 Detection of Saponins

a) 5ml of aquous extract was shaken vigorously with 5ml of distilled water in a test tube and warmed. The formation of stable form was taken as an indication for the presence of Saponins.

b) Foam test: - Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of Saponin.

4.2.8 Detection of Flavinoids:

a) 2ml of dilute sodium hydroxide was added to 2ml of extract. The appearance of low-colour indicates the presence of flavonoids.

b) flavonoids given a dull green/reddish brown colour conc. Treatment with ferric chloride solution.

4.2.9 Detection of steroids:

a) Salkowaski's test: - 2ml of each extract was dissolved in 2ml of chloroform. 2ml of Sulfuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of steroid ring.

b) Liebermann's test: - 2ml of the organic extract was dissolved in 2ml of chloroform and 2ml of Acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A color change from violet to green indicates the presence of steroid nucleus.

4.3 Detection of Phenolic compound

The extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicates the presence of phenolic compounds.

4.4 Antibacterial Test

The dried plant leaf methanol, petroleum ether leaf extract was taken to see the antimicrobial activity. Antimicrobial test carried out by well diffusion method *Escherichia coli*, which was used as inoculum.

Muller Hinton Agar media was prepared and poured it in sterile petriplates.

When the media was solidified, then the indicator microorganism was swab in tree dimension to ensure complete plate coverage. Then a well was made which filled with 0.2g crude methanol, petroleum ether extract and incubated at 37C for 24 hours.

The zone of inhibition was measured with antibiotic zone scale in mm. the extract that showed antimicrobial activity.

4.5.1 Isolation and Purification of the Microorganisms

4.5.2. Stock Cultures: For the production of different dairy products it is required to have appropriate culture of microorganisms. The pure culture or stock culture are available in lypolized or freeze dried form. Stock cultures of desired organisms may be maintained in the dairy cultures of lactic *Streptococci sp, Bacillus sp.*, and *Lactobacillus sp.* were used. On the other hand, Starter culture was produced by mixing 1% pure culture in 500 ml distilled water. This is heated in 121°C for 15 minutes and then cooled to room temperature and incubated to give starter culture.

4.5.3 Isolation of Bacteria:

a) Preparation of the dilution

The microbial colonies were isolated by serial dilution plate procedure **(Foster et al. 1958).** For this purpose, 5gm of the sample was taken in 90ml of the sterile distilled water in a sterile conical flask. It is used as the 10^{-1} dilution. Further dilutions were prepared up to 10^{-6} from the 10^{-1} dilution in sterile distilled water.

b) Media

For the isolation and enumeration of microorganisms particularly bacteria, nutrient agar media, MacConkey, Muller Hinton and CLED agar media were used although the study.

c) Plating

Pour plating in duplicate plates were made for each diluted sample in the following. One ml of each of the dilution was added to about 20 ml of sterile melted agar (40^0C) medium in sterilized Petri plates and mixed thoroughly by rotating the plates both clockwise and anticlockwise. In this way, all plating's were carried out from all serial diluted samples.

d) Incubation

After solidification of the medium, the plated were incubated (Binder Incubator) at inverted position at 37± 1^0C for 24 to 72 hours.

4.5.4 Enumeration of Bacteria

After 24 to 48 hours of incubation, the plates having 30 to 300 colonies were selected for counting. The selected plates were placed on a colony counter **(Stuart Scientific, UK)** for counting is normally done by visual observation.

The colonies or viable aerobic bacterial count per ml or gm were calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution **(Collins and Lyne 1984).** The calculated results would be as colony forming units (cfu) per ml or gm of sample.

4.5.5 Isolation of the Discrete Bacterial colonies

Isolation of the discrete bacterial colonies was carried out immediate after counting of the colonies. On the basis of their morphology, several different colonies were selected for the purpose. The selected colonies were marked and their characteristics were studied depending on the various points' viz. form, elevation, margin, surface, color etc. (Eklund and Lankford 1967; Bryan 1950). Then the marked and observed bacterial colonies were transferred to nutrient agar slant aseptically for purification.

4.5.6 Purification of the Isolates

The isolated organisms were purified through repeated plating method. Both pour plate and streak plate methods were used for the purpose. Media used for this purpose were nutrient agar (NA). When a plate yielded only one type of colony the organism was considered to be pure. The purification of the isolates was also confirmed under microscopic observation. The pure bacterial isolates were preserved on the nutrient agar slant aseptically for further studies.

4.5.7 Maintenance and Preservation of the Isolates

The purified bacterial isolates were maintained on nutrient agar slants during the course of investigation. The culture tubes (slant tube) were kept in polythene bags. The bags were tied up and preserved in a refrigerator at 4^0C as stock culture. These isolates were transferred to fresh medium periodically.

4.5.8 Final Selection of the Isolates

Final selection was made on the basis of their colonial morphology on the agar plate, on agar slants and their microscopic features under the microscope.

4.5.9 Condign of the Isolates

The pure culture of the isolates was coded according to the number of colonies and the serial of the sample used. The code numbers were maintained and followed till identification of the isolates after through characterization.

4.6 Morphological and Cultural studies of Selected Isolates

Identification of the microorganisms was a sequential process, which included a series of different types of experiments. In the present study

the following sequence of experiments were carried out for the presumptive identification of the isolates. Antibacterial activity was performed for methanol extract and petroleum ether against the bacteria by agar well diffusion method. The extracts were effective against the isolated bacterial.

RESULT

5.1.1 Antibacterial activity of Methanol extract and Petrolium Ethar extract of *Nyctanthes arbor-tristis* leaves.

The methanol extract was effective against bacterial strain but petroleum extract was little effective against *Escherichia coli*. The result of the extracts of methanol and petroleum ether of the plant using 500mg/ml crude extract.

It is shown that methanol extracts of *Nyctanthes arbor- tristis* have effect on *Escherichia coli* 25 mm and petroleum ether 3mm.

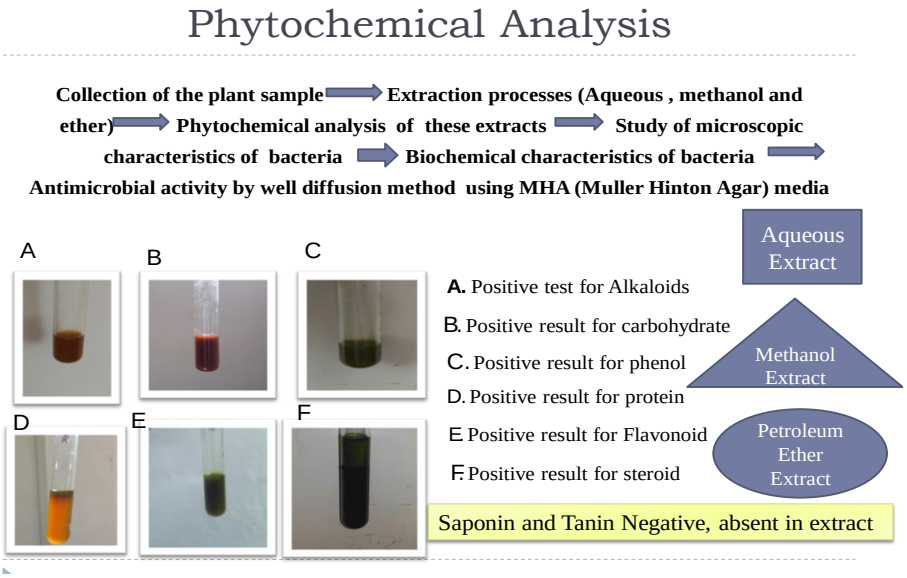


Fig. 5.1.1: Phytochemical Analysis on Biochemical studies.

Table 5.1.1.a: Screening of curd samples 5 fold dilutions

Samples	Dilution	No. of Colonies (McFarland scale)	Optical Density (496-500 nm)	No. of Colonies (1 loop *K. sp.*)	Optical Density (496-500 nm)
C1	5^{-1}	< 7.5 mcf	1.013	< 7.5 mcf	1.080
C2	5^{-2}	5.625 mcf	0.844	< 7.5 mcf	1.0125
C3	5^{-3}	2.325 mcf	0.272	3.4	0.460
C4	5^{-4}	0.6 mcf	0.079	1.05	0.141
C5	5^{-5}	0.235 mcf	0.20	1.02	0.1377
C6	5^{-6}	0.225 mcf	0.006	0.4	0.054
	Ref.	0 mcf	.		

Table 5.1.1.b: Screening of curd samples 10 fold dilutions

Samples	Dilution	No. of Colonies (McFarland scale)	Optical Density (496-500 nm)	No. of Colonies (1 loop *K. sp.*)	Optical Density (496-500 nm)
C7	10^{-1}	< 7.5 mcf	1.816 OD.	< 7.5 mcf	2.179
C8	10^{-2}	< 7.5 mcf	0.121 OD	< 7.5 mcf	1.937
C9	10^{-3}	6	0.060 OD.	< 7.5 mcf	1.816
C10	10^{-4}	4	0.045 OD.	6.4	1.549
C11	10^{-5}	2	0.042 OD.	3.26	0.789
C12	10^{-6}	2	0.041 OD.	2	0.484
	Ref.		0.00 OD.		

After completion of optical density screening, the protocols were followed by standard optimized readings on contrary propagation and culture techniques. The developing criteria in low amount of concentration showed very constituent efficacy as we required for analysis. On the basis of sequencing on sampling technique, isolation and identification applied for specific criteria developing against gram negative infectious pathogens.

So in case of comparative developmental analysis, researcher introduced first time dilution with plant ethanol to observe antibiotic susceptibility compared with curd probiotic and its toxin. This methodology was applied first time by Chowdhury AP[1] *et. al.* and showed antagonistic effect observing the result on each petriplates of plant extract and heat labile toxin of probiotic.

Table 5.1.1.c: Cultural, Morphological and Biochemical Characteristics of the Bacterial Isolate.

Place of collection	:	Homemade curd.
Vegetative Cells	:	Spherical or ovoid shape, Cells formed short chain
Cell Size	:	0.5-1.2 × 0.5-1.5µm
Spores	:	Endospores not formed
Gram stain	:	Gram positive
Acid-fast stain	:	Nonacid fast
Flagella stain	:	Non motile
Motility test	:	Non motile
Agar colonies	:	Circular colonies
Agar slant	:	Echinulate growth
Nutrient broth	:	Flocculent growth
Glucose broth	:	Flocculent growth with turbidity
Catalase activity	:	Negative
Oxidase test	:	Negative
Deep glucose agar	:	Moderate surface growth within the media
Oxygen relation	:	Facultative anaerobic
Indole test	:	Negative
Nitrate reduction test	:	Negative
Inorganic medium	:	No growth
Citrate medium	:	Negative
Gelatin liquefaction	:	Not liquefied
Hydrogen sulphide	:	Produced
Urease test	:	Negative
Milk agar plate	:	Not hydrolyzed
Starch agar plate	:	Negative
Methyl red	:	Positive
V. P. test	:	Negative
Growth at	:	0^0C 10^0C 20^0C 30^0C 37^0C 40^0C 45^0C - ++ ++ +++ +++ ++ +
Fermentation	:	**Acid and no gas form:** Glucose, Maltose, Lactose Xylose, Arabinose, Sucrose and Mannitol. **No change:** Raffinose, Inulin and Glycerol.

Identification: Cultural, morphological and biochemical characteristics of the isolate **C12** was compared with the standard description given in Bergey's Manual of Determinative Bacteriology - 8th ed. (Buchanan and Gibbons, 1974) and 9th ed. (Holt *et al.,* 1984) and it was found closely related with the genus ***Streptococcus*** described by Rosenbach (1884). The isolate was provisionally identified as ***Streptococcus lactis*** (Lister) LÖhnis (1909).

On the contrarily the *Klebsiella sp.* showed similar effect along with other antibiotic discs. That significance augmented our research on basic principle of developing probiotic against *Klebsiella sp.* in urine samples. Nine urine samples from patient of different ages were selected from several category only basis on isolating *Klebsiella sp.* and *E. coli* (Trevisan 1887) in SRL Diagnostics.

Table 5.1.1.d: Morphological, Biochemical and Cultural Characteristics of the Isolates from urine sample.

Place of collection	:	Urine sample, SRL Diagnostic
Vegetative Cells	:	Cells straight rods.
Cell Size	:	1.1-1.5×2.0-6.0µm
Spores	:	Spores not formed
Gram stain	:	Gram negative rod shaped
Acid-fast stain	:	Nonacid fast
Flagella stain	:	Not done
Motility test	:	Non motile
Agar colonies	:	Colonies are found in dome shaped pinkish red.
Agar slant	:	Mucoid growth
Nutrient broth	:	Diffused cloudiness with diffuse growth
Glucose broth	:	Flocculent growth with heavy sediment.
Catalase activity	:	Positive
Oxidase test	:	Negative
Capsule stains	:	Encapsulated
Oxygen relation	:	Facultative anaerobic
Indole test	:	Positive
Nitrate reduction test	:	Positive
Inorganic medium	:	No growth
Citrate medium	:	Positive
Gelatin liquefaction	:	Not liquefied
Hydrogen sulphide	:	Negative
Urease test	:	Positive
Milk agar plate	:	Not hydrolyzed
Starch agar plate	:	Non Hydrolyzed
Methyl red	:	Negative
V.P. test	:	Positive
Growth at	:	0^0C 10^0C 20^0C 30^0C 37^0C 40^0C 45^0C - ++ ++ +++ ++ + +
Fermentation	:	**Acid and Gas form:** Glucose, Lactose, Sucrose, Arabinose, Mannitol and Xylose. **No Change:** Starch, Rhamnose and Raffinose.

Identification: Cultural, morphological and biochemical characteristics of the isolate **U_{10}** was compared with the standard description given in Bergey's Manual of Determinative Bacteriology - 8th ed. (Buchanan and Gibbons, 1974) and 9th ed. (Holt *et al.,* 1984) and it was found closely related with the genus ***Klebsiella*** (described by Schroeter 1886). The isolate was provisionally identified as ***Klebsiella sp.*** (Trevisan 1887).

Antibiotic associated diarrhea (AAD) is an acute inflammation of the intestinal mucosa caused by the administration of antibiotics and resulting in the disruption of the intestinal microflora. *Clostridium difficile* infection is caused 10-20% of AAD and 95% of pseudo membranous colitis (Katz *et al.,* 1996; Kelly *et al.,* 1994). Other infectious organisms causing AAD include *Clostridium perfringens*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Candida sp.* and *Salmonella sp.* (Hogenauer *et al.,* 1998).

5.1.1. e. Morphological, Biochemical and Cultural Characteristics of the Isolates U_7:

Place of collection	:	Urine sample, SRL Diagnostic
Vegetative Cells	:	Cells formed in medium sized rods.
Size	:	Colonies 2-5 mm in dm.
Spores	:	Spores not formed
Gram stain	:	Gram negative
Acid-fast stain	:	Nonacid fast
Flagella stain	:	Not done

Motility test	:	Motile
Agar colonies (EMB)	:	Colonies are found in metallic sheen.
Agar slant	:	Mucoid growth
Nutrient broth	:	Diffuse cloudiness with diffuse growth
Glucose broth	:	Flocculent growth with heavy sediment.
Catalase activity	:	Catalase positive
Oxidase test	:	Oxidase negative
Deep glucose agar	:	Moderate surface growth within the media
Oxygen relation	:	Facultative anaerobic
Indole test	:	Negative
Nitrate reduction test	:	Negative
Inorganic medium	:	No growth
Citrate medium	:	Negative
Gelatin liquefaction	:	Negative
Hydrogen sulphide (TSI)	:	Negative
Urease test	:	Negative
Milk agar plate	:	Not hydrolyzed
Starch agar plate	:	Hydrolyzed
Methyl red	:	Positive
V.P. test	:	Negative
Growth at	:	0^0C 10^0C 20^0C 30^0C 37^0C 40^0C 45^0C
		- + ++ ++ +++ +++ +
Fermentation	:	Gas production is occurred by fermentation.
	:	Glucose, Arabinose, Mannitol, Xylose.
		No change: Lactose, Sucrose, Starch, Rhamnose, Cellulose.

Identification: The morphological, cultural and biochemical characteristics of isolate **U_7** was compared with standard description of Bergey's Manual of Determinative Bacteriology'-8th Ed. (Buchanan and Gibbons, 1974) and found closely related with the genus Escherichia. The isolate was provisionally identified as *Escherichia coli. Escherichia coli* described by Escherich *et al.,* in 1885.

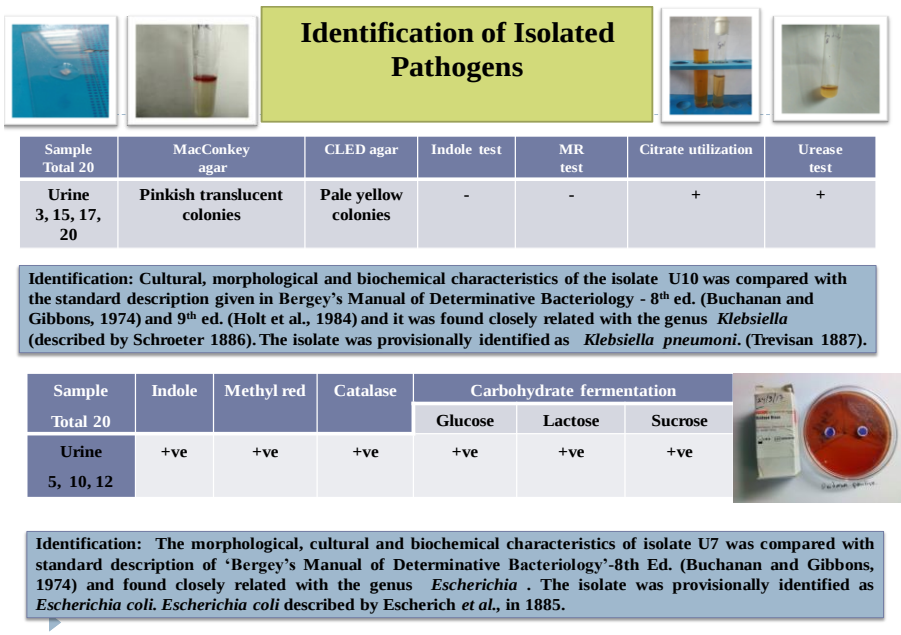


Figure 5.1.2: Identification of isolated pathogen.

Table: 5.1.2 Toxin preparation: (Heat labile)

Probiotic samples	Volume in solution	Autoclaved			Chemical indicator	Biological indicator
		1	2	3		
Escherichia coli.	One loop full colonies in three ml distilled water+ 50 ml MacConkey	done	done	done	+	+
Curd	10^{-1} diluted sample in 50 ml MacConkey	done	done	done	+	+
Distilled water (control)	10^{-1} diluted sample in 50 ml MacConkey	done	done	done	+	+
Klebsiella sp.	One loop full colonies in three ml distilled water+ 50 ml MacConkey	done	done	done	+	+

Table 5.1.3: Curd samples (C5, C12)

Anti-microbial agent	Disc content	Symbol	Interpretative Criteria			Inference
			Result	Intermediate	Resistant	
Ampicillin	10	AMP	0	14-16	13	R
Amikacin	30	AK	12	15-16	14	R
Amoxicillin/ Clavulanic acid	30	AMC	13	14-17	13	I
CotTrimoxazole	25	COT	0	11-15	10	R
Cefepime	30	CPM	14	15-17	14	R
Cefotaxime	30	CTX	20	23-25	22	R
Erythromycin	15	E	25	14-22	13	S
Gatifloxacin	5	GAT	22	15-17	14	S
Imipenem	10	IPM	32	20-22	19	S
Ofloxacin	5	OF	20	13-15	12	S
Piperacillin/ Tazobactam	100/10	PIT	8	18-20	17	R
Tobramycin	10	TOB	18	13-14	12	S
Vancomycin	30	VA	10	15-16	14	R

Previous study was done by meta-analysis based on five randomized-controlled trials (619 participants) displayed this effect (Szajewska *et al.,* 2007). Combined data showed that *S. boulardii* reduces significantly diarrhea duration and the risk of prolonged diarrhea compared with control. A recent study conducted in 100 hospitalized children.

Ethar extract of *Nyctanthes arbor-tristis* leaf.

The dried plant leaf methanol, petroleum ether leaf extract was taken to see the antimicrobial activity. Antimicrobial test carried out by well diffusion method *Escherichia coli*, which was used as inoculum.

Muller Hinton Agar media was prepared and poured it in sterile petriplates.

When the media was solidified, then the indicator microorganism was swab in tree dimension to ensure complete plate coverage. Then a well was made which filled with 0.2g crude methanol, petroleum ether extract and incubated at 37C for 24 hours.

The zone of inhibition was measured with antibiotic zone scale in mm. the extract that showed antimicrobial activity. The methanol extract was effective against bacterial strain but petroleum extract was little effective against *Escherichia coli.* As shown in table and figure

The result came out using the extracts of methanol and petroleum ether of the plant in dose of 500mg/ml crude extract.

It is shown that methanol extracts of *Nyctanthes arbor- tristis* have effect on *Escherichia coli* 25 mm and petroleum ether 3mm.

Table 5.1.4.a: Category 1: Control

Extract	Zone of Inhibition
Methanol extract (Series 1)	0 mm
Petrolium Ethar Extract (Series 2)	0 mm
Aquous (Series 3)	0 mm

Table 5.1.4.b: Category 2:

Extract	Zone of Inhibition
Methanol extract (Series 1)	25mm
Petrolium Ethar Extract (Series 2)	3mm
Aquous (Series 3)	15mm

Table 5.1.4.c: Category 3:

Extract	Zone of Inhibition
Methanol extract (Series 1)	20 mm
Petrolium Ethar Extract (Series 2)	7 mm
Aquous (Series 3)	16 mm

Table 5.1.4.d: Category 4:

Extract	Zone of Inhibition
Methanol extract (Series 1)	4.5 mm
Petrolium Ethar Extract (Series 2)	2.5 mm
Aquous (Series 3)	4 mm

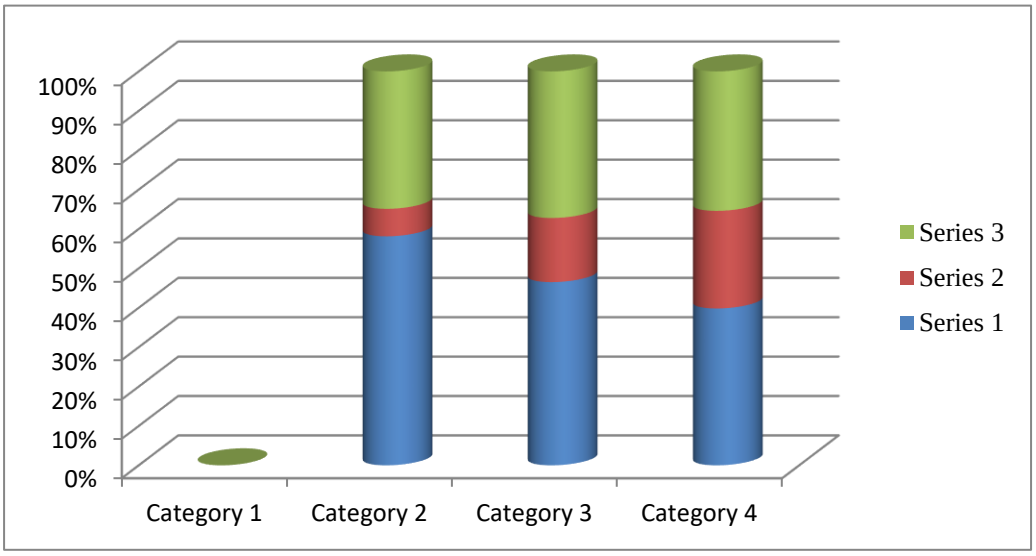
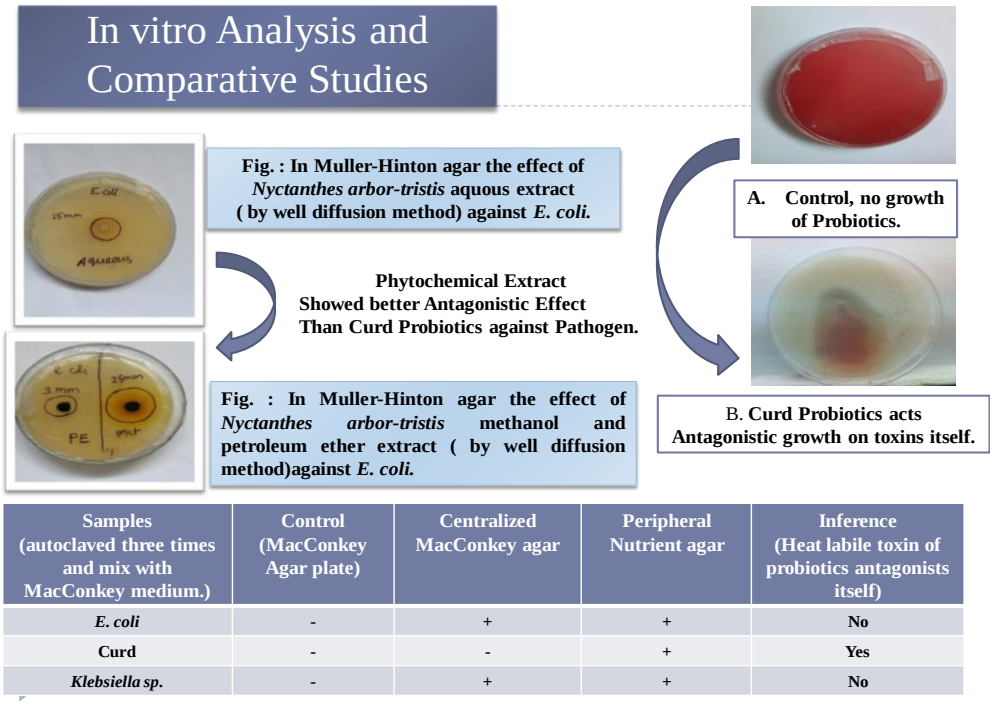


Figure 5.1.3: Graphical review of antagonistic action of bio active compound against *E. coli.*



Samples (autoclaved three times and mix with MacConkey medium.)	Control (MacConkey Agar plate)	Centralized MacConkey agar	Peripheral Nutrient agar	Inference (Heat labile toxin of probiotics antagonists itself)
E. coli	-	+	+	No
Curd	-	-	+	Yes
Klebsiella sp.	-	+	+	No

Figure 5.1.3: Review of invitro analysis and comparative studies on antagonistic action of bio active compound against *E. coli* and curd probiotic against *Klebsiella sp.*

The plant sample was collected from the Noonmati, Gauhati. The plant extracts were assayed for phytochemical compounds and antibacterial activity. Antibacterial activity was screened against *Escherichia coli*. Plant leaves were used foe extraction of antibacterial metabolites using methanol and petroleum ether. All the extracts showed effect against the isolated bacteria of all the extracts. Methanol extracts was most effective for its antibacterial activity against E. coli. Methanol extract showed a zone of 25mm against *E. coli.* The findings support the use of this plant against *Escherichia coli* infections. The result revealed that the selected phytoextract could be used for the biocontrol of the tested E. coli. The plant can be used to discover bioactive products; those may serve in new drug formulations and lead to the development of new pharmaceuticals against the pathogens. The extract which showed antimicrobial activity can be identified and characterized for further studied.

DISCUSSION

The present study is aimed at the evaluation of the antibacterial activity of *Nyctanthes arbor-tristis* leaf extracts against the bacteria *Escherichia coli* collected from stock collection of the laboratory. The test strain is identified by morphological and Biochemical tests.

In this present study, various phytochemical tests were performed against the *Nyctanthes arbor-tristis* leaf extracts. Phytochemical analysis of *N. arbour-tristis* leaf showed the presence of alkaloids, flavonoids, saponnin, glycosides, protein and carbohydrates. Extracts were prepared from dried leaves of *N. arbour-tristis* plant using methanol and petroleum ether. The antimicrobial activity of the extracts was determined by agar-well diffusion method. Methanol and petroleum ether were taken for the antibacterial studies against *Escherichia coli.*
In the present study, methanol extracts turn out to be more effective for its antibacterial activity against *Escherichia coli.* Widest zone of inhibition with a diameter of 25mm. the petroleum ether extract was less effective for antibacterial activity as compared to Methanol.
Expected research is newly implemented of probiotic, studying preferably antagonistic action on against urinary tract and gut infection. Here researcher stands for comparing studies and result of curd probiotic along with antibiotics susceptibility, which may result death of normal micro flora in gut and its sensitized toxin acts itself against normal gut flora resulting antagonistic action but not against pathogenic gram negative entity *Klebsiella sp.* and *E. coli.*

Our conclusive study provides that probiotics toxin antagonistic against itself, but k toxin of *Klebsiella sp*. non antagonist by itself to it. So it is impossible to develop killed vaccine from this pathogen. Gram-positive spore forming probiotic has no antagonistic effect on Gram negative pathogen. But phytomedicinal bioactive compound degrades slowly to produce antagonistic effect better than food producing probiotic such as example curd. Here researcher conclusion is for to analyze and categorize antagonistic activities of gut acting curd probiotic against pathogenic *Klebsiella sp*., and *E. coli* responsible for urinary tract infection to take a part of study in molecular based and phytomedicinal based of study.

CONCLUSION

The plant extracts were assayed for phytochemical screening and antibacterial activity against *Escherichia coli*. All the extracts showed effect against the isolated bacteria of all the extracts. Methanol extracts was most effective for its antibacterial activity against *E. coli* compared to aqueous and petroleum ether extracts where as *Klebsiella sp.* non antagonist and non-resist against food probiotics and its toxins. Here researcher on his conclusive view, stands for excessive doses of probiotics along with antibiotics causes death of probio and produced toxin itself against normal gut flora resulting antagonistic action. Respective doctors and Practitioner carry out their treatment against urinary tract pathogen along with probiotics supplements. Here researcher conclusion is for comparative analyzed between phytochemicals and food probiotics, to asses for medicinal residues by in vitro studies.

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