INTRODUCTION

Beginning from the history, there has been a continual battle between humans and the multitude of microorganisms which are the basis of infectious disease. The crucial fact about the treatment of bacterial infections is the ability of bacteria to develop resistance to antimicrobial agents which is variable in different geographic areas and it has been correlated with the consumption of antibiotics in the general population [1]. Antibiotics are designed to inhibit/kill the infecting organism and to have no/minimal effect on the recipient. They are one of our most important weapons in fighting bacterial infections and have greatly benefitted the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain bacteria as they produced resistance due to target site or metabolic process which is affected by the particular drug or due to mutation/gene transfer and thereby increases chances of chronic infection and risk of morbidity. The drug resistance also increased expenditure on patient management and implementation of infection control measures [2]. Antimicrobial agents principally act by the following mechanisms: interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway and disruption of bacterial membrane structure [3]. Microbial infection may also increase generation of highly reactive oxygen species (ROS) which causes significant damage to cell structures due to environmental stress [4]. And the similarity of the eukaryotic fungal cell with human cell is the basis of toxicity with the antifungal drugs [5]. Prolong use of standard antifungal drugs such as Amphotericin-B and Fluconazole may be led to several marked adverse effects [6]. Therefore, it is essential to investigate newer drugs with minimal resistance and increased spectrum of activity with desirable properties. Drugs derived from natural sources play a significant role in the prevention and treatment of many human diseases. The plant-based technique of medicine being natural does not cause any serious side effects or problems and so are popularized worldwide [7]. North Eastern part of India comes under one of the world biodiversity hotspots which are very rich in traditional herbal medicine [8, 9].

As plants are a rich source of secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say ‘Return to Nature’. Now a days, there is a revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern synthetic medicine and now the herbal drug industries is the fastest growing sector in the international market. But unfortunately, India has not done well in this international trade of herbal industry due to lack of scientific input in herbal drugs.

Medicinal plants represent a rich source of antimicrobial agents [10]. Plant-derived antimicrobials have a long history of providing the much-needed novel therapeutics [11]. Plants of the Annona genus are the notable source of potential therapeutic agents. The plant Annona reticulata Linn. belonging to family Annonaceae, commonly known as Custard apple, Bullock's heart, and Ramphal is found in India and cultivated in Thailand and originates from the West Indies and South America. Leaves are membranous, oblange or narrow-lanceolate, 10-20 cm long 2-5 cm wide with conspicuous veins. Flowers are fragrant, slender, with 3 outer fleshy, narrow petals 2-4 cm long; light-green externally and pale-yellow with a dark-red or purple spot on the inside at the base and fruits are symmetrically heart-shaped, irregular, or nearly round, or oblate, with a depression at the base. The covering or skin of this fruit is...
quite thin and when ripe, it generally changes to yellowish or slightly brownish. Beneath the covering or the skin lies a dense, creamy white layer of flesh that is granular and akin to that of custard. This succulent encases small black solid seeds.

Various extracts of different parts of this plant have shown anti-hyperglycemic [12], cytotoxic and recombinant caspase inhibitory activity [13], anti-nociceptive [14], analgesic and CNS depressant [15], analgesic and anti-inflammatory [16], tumor inhibitor [17], and anti-proliferative [18] effects. Traditionally it is used for various purposes like:
- Crushed leaves/paste is used in internal and external wounds and boils, leaf decoction is used in gastritis.
- Leaf juice is used as vermifuge.
- Unripe dried fruits used in diarrhoea and dysentery treatment.
- Root bark is used in tooth ache.
- Seeds, leaves, young fruits have got insecticidal activity [19].

It is one of the most popular nutritional and medicinal plant which is rich in a variety of secondary metabolites including polyphenols [20]. Pinene, Myrcene, Limonene, Terpinen-4-ol, Germacene D are the bioactive metabolites commonly present in fruits. Other species of plant reported to have antibacterial potential, but A. reticulata is still not confirmed for antimicrobial potential. Therefore, the objective of the present study was to investigate the antimicrobial activity of A. reticulata fruits using different pathogenic strains of micro-organism.

MATERIALS AND METHODS
Collection of plant material
The fruits of the plant Annona reticulata were collected from Dibrugarh district of Assam in January 2017 and the fruits were thoroughly washed with water, dried in shade for 2 w and finally dried in a Tray drier at a considerably low temperature not exceeding 30 °C for 24 h.

Authentication of plant material
The plant was authenticated by Prof. Dr. P. P. Baruah, Department of Botany, Gauhati University. A voucher specimen (Acc-18384, Dated: 23/11/2017) was kept in Department of Botany, Gauhati University for future reference.

Chemicals and reagents
Petroleum Ether (PE), Ethanol (ET), dimethyl sulfoxide (DMSO), hydrochloric acid, Dragendorff reagent, Mayer’s reagent, Wagner’s reagent, Benedict’s reagent, sulphuric acid, lead acetate, Molisch’s reagent, Fehling solution A and B, sodium citrate, copper sulphate, ferric chloride, sodium hydroxide, glacial acetic acid, benzene, chloroform, ammonia, nitric acid, potassium nitrite, gelatine, Beef extract, Peptone and agar. All the chemicals and solvents used were of standard analytical grades.

Preparation of the plant extracts
The fruits of the plant materials were collected washed, shade dried and then kept at tray drier at 37 °C for 48 h. The dried plant materials were grounded to a fine powder using an electric grinder. The dried and powdered plant material was defatted by using petroleum ether and then was subjected to Soxhlet extractor using the solvent ethanol. The ethanolic extract was concentrated by using water bath and stored in the refrigerator for further analysis.

The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material using the formula:
\[ \text{Yield} = \frac{\text{Dry weight of the extract}}{\text{Dry weight of leaf sample}} \times 100. \]

Qualitative phytochemical screening
The plant extracts were analysed for the presence of alkaloids, saponin, flavonoids, phenol, carbohydrates, proteins, cardiac glycosides, steroids, anthraquinone and terpenoids using the standard methods.

Detection of alkaloids
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer’s Test-Filters were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner’s Test-Filters were treated with Wagner’s reagent (Iodine in Potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendorff’s Test-Filters were treated with Dragendorff’s reagent (solution of Potassium Bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

Detection of saponins
Foam Test-0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of flavonoids
Lead acetate Test-Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of carbohydrates
Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch’s Test-Extracts were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict’s Test-Extracts were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling’s Test-Extracts were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling’s A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides
Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified borntrager’s test
Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of phytosterols
Salkowski’s test-Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpene.

Detection of proteins and amino acids
Xanthoproteic test-The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of tannins
Gelatin test-To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins [21].

Antimicrobial activity
Microbial strains
Three bacterial strains [Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922) and one fungi [Candida albicans]
Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati. [ATCC 10231] were used. Stains were obtained from Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati.

Preparation of media

- Beef Extract, peptone were accurately weighted out and in 1000 ml of distilled water beef extract, peptone are added.
- pH was checked by using pH meter (pH 7.4), and finally Agar was added to the flask.
- Boil the media to dissolve and allow it to cool for few minutes.
- The media is placed in an autoclave for sterilization, while the agar is still warm, but not hot for 20 min.
- Then the media is being taken from the autoclave and pour it into the Petridish with labeled as test, control and standard.
- The antimicrobial activities of the test agents were determined by measuring the diameter of the zone of inhibition.

Antimicrobial activity

Disc diffusion method

The antimicrobial action of the plant extract was measured by agar well diffusion technique as described in NCCLS, 1993. 1 ml of bacteria suspension was uniformly spread on the sterile nutrient agar media. Amikacin (30 µl) and DMSO were used as positive and negative controls respectively. Fruits extracts were prepared in DMSO (stock: 1 mg/ml DMSO). The plant extracts of 25, 50, and 100 µg/ml concentrations were added. Sterile filter paper disc (Whatman no.1, diameter 6 mm) was soaked with the extract solution and the solvent allowed drying. The disc was placed on the nutrient agar media Petri dish inoculated with bacteria suspension to allow the extracts to diffuse into the media. The Petri dish was then placed in an incubator for 24 h at 37 °C. At the end of the incubation period, the inhibition diameter was measured using calipers and expressed in millimeters. Positive antibacterial was established by the presence of measurable zones of inhibition.

Antifungal activity

The potato dextrose agar plates were prepared and inoculated with a fungal culture. Wells of approximately 10 mm was bored using a well cutter. Plant extracts were prepared in DMSO (stock: 1 mg/ml DMSO). The plant extracts of 25, 50, and 100 µl concentrations were added. The plates were then incubated at 37 °C for 48 h. The antifungals present in the plant extract are allowed to diffuse out into the medium and interact with the test organisms in the freshly seeded plate. The antifungal activity was reported after 48 h of incubation and compared with that of standard antifungal (Fluconazole) (10 µg/ml) which was used as positive control and DMSO as the negative control [22].

RESULTS

The present investigation shows the phytochemical analysis, antimicrobial activity of the ethanolic extract of the plant *Annona reticulata*. The yield % of the extraction of ethanol was 3.26%. It was a light green solid powder (ET) in appearance.

Phytochemical analysis

Different photochemical investigations with the ethanolic plant extract were demonstrated the presence of alkaloids, saponin, flavonoids, carbohydrate, glycosides, steroids, amino acids, tannins (table 1).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical test</th>
<th>Reagent used</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>Creamy white precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
<td>Reddish brown precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendoroff’s test</td>
<td>Orange brown precipitate</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>Foam Test</td>
<td>Froth formation</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavanoid</td>
<td>Lead Acetate test</td>
<td>Yellow precipitate</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrate</td>
<td>Molisch’s test</td>
<td>Formation of violet ring</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benedict’s test</td>
<td>Formation of orange</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>Red precipitate</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>Modified Borntrager’s test</td>
<td>Formation of pink colour</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>Salkowski’s test</td>
<td>Formation of green colour</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Amino acids</td>
<td>Xanthoproteic test</td>
<td>Formation of yellow colour</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>Gelatin test</td>
<td>Formation of green colour</td>
<td>+</td>
</tr>
</tbody>
</table>

+ sign indicates presence and – sign indicates absence.

Antimicrobial activity

Plant extract is generally rich in antimicrobial compounds. The *in vitro* antimicrobial activity of the ethanolic extract and petroleum ether extracts of *Annona reticulata* fruit under different concentration with the DMSO and standard was determined against bacterial and fungal strains and recorded as zone of inhibition. The results are appeared in table 2.

Table 1: Phytochemical screening of ethanolic (ET) extract of fruits parts of *Annona reticulata*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical test</th>
<th>Reagent used</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>Creamy white precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
<td>Reddish brown precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendoroff’s test</td>
<td>Orange brown precipitate</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>Foam Test</td>
<td>Froth formation</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavanoid</td>
<td>Lead Acetate test</td>
<td>Yellow precipitate</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrate</td>
<td>Molisch’s test</td>
<td>Formation of violet ring</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benedict’s test</td>
<td>Formation of orange</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>Red precipitate</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>Modified Borntrager’s test</td>
<td>Formation of pink colour</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>Salkowski’s test</td>
<td>Formation of green colour</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Amino acids</td>
<td>Xanthoproteic test</td>
<td>Formation of yellow colour</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>Gelatin test</td>
<td>Formation of green colour</td>
<td>+</td>
</tr>
</tbody>
</table>

+ sign indicates presence and – sign indicates absence.

Table 2: Antimicrobial activity of *Annona reticulata* extract using disc diffusion method

<table>
<thead>
<tr>
<th>Name of the compounds with concentration</th>
<th>Anti-bacterial activity diameter of zone of inhibition (mm)</th>
<th>Anti-fungal activity diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Amikacin (30 µl)</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Fluconazole (10 µl)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eth. extract (25 µl)</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Pet. ether extract (25 µl)</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Eth. extract (50 µl)</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Pet. ether extract (50 µl)</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Eth. extract (100 µl)</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Pet. ether extract (100 µl)</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

The zone of inhibition of ethanoic extract was against the strains of bacteria were in the range of 6-13 mm and that of petroleum ether extract was 5-12. The ethanolic and petroleum ether extract exhibited potent inhibitory activity against E. coli.

The zone of inhibition of ethanolic and petroleum ether extracts against fungal strain was found within the range 10-13.

DISCUSSION

In recent years, multiple drug resistance against pathogenic microorganisms developing due to indiscriminate use of commercial antimicrobial drugs. This situation has necessitated a search of effective antimicrobial compounds. Medicinal plants can produce a varities of secondary metabolites with antimicrobial activity that are active against pathogens [23]. Every parts of plant individually or in combination show antimicrobial properties. A significant part of the chemical diversity produced by plants if thought to protect plants against pathogenic microbes.

Current research is focusing over plants as a potential source of medicine, as phytochemicals are generally safe with no or few side effects. A. reticulata is a most popular possessing nutritional and medicinal value. The antibacterial and antifungal effect of ethanolic extract of A. reticulata against different pathogenic bacterial and fungal strains had supported traditional claims of plant as a medicine. The potent inhibitory effects of ethanolic extract against the bacterial strains are attributed due to the presence of phenolic and polyphenolic compounds in the extract. It is already reported that polyphenolic compounds are biologically active and possesses antimicrobial property [24] suggesting that antibacterial and antifungal activity of ethanolic extract may be due to the presence of some phenolic compounds. There are several mechanisms that explained the antimicrobial effect of polyphenols that mainly includes ability of polyphenols to neutralize bacterial toxins, biofilm inhibition, and reduction of host ligands adhesion, enzyme inhibition and interaction with eukaryotic DNA and membrane disruption [25]. The antimicrobial activity of fruits of the plant has been assessed. Phyto-constituents exhibit in plants to be specific alkaloids, flavonoids, tannins and triterpenoids are delivering an energizing open door for expansion of modern therapies against an extensive variety of microorganism.

In the present study, antimicrobial activity was studied in ethanol, petroleum ether fruit extract of Annona reticulata. One Gram-positive, one Gram-negative bacterium and one fungal strains were chosen start with Staphylococcus aureus, Escherichia coli, Candida albicans for screening antimicrobial impact of the extract to see antimicrobial spectrum.

To study the antimicrobial properties disc diffusion method was used to observe the growth of tested microorganism. Table 2 showed that the ethanolic extract of the fruits Annona reticulata had greater zone of inhibition than the petroleum ether extract. All the extracts are active against all the tested pathogens. E. coli was strongly influenced by fruit extract with the inhibition zone of 10 mm, 12 mm, 13 mm for ethanolic extract of 25,50,100μl respectively and zone of inhibition of 9 mm, 11 mm, 12 mm for petroleum ether extract respectively. And zone of inhibition of 23 mm for standard (Amikacin). Against Staphylococcus aureus zone of inhibition of 6 mm, 8 mm, 10 mm for the ethanolic extract of 25, 50,100μl respectively and zone of inhibition of 5 mm, 7 mm, 8 mm for petroleum ether extract of 25,50,100μl respectively. And zone of inhibition of 21 mm for standard (Amikacin). Among the various concentration of the ethanolic extract concentration 25 μl exhibit minimum zone of inhibition whereas 100 μl exhibit maximum zone of inhibition against bacterial strain of Escherichia coli and Staphylococcus aureus. And Gram-negative bacteria were more susceptible than Gram-positive bacteria.

Against the fungal strain Candida albicans, zone of inhibition was found to be 11 mm, 12 mm, 13 mm for ethanolic extract of 25,50,100μl respectively and zone of inhibition of 10 mm, 11 mm, 11 mm for petroleum ether extract of 25,50,100μl respectively. And zone of inhibition of 13 mm for standard (Fluconazole). In case of fungal strain concentration 25 μl exhibit minimum zone of inhibition whereas 100 μl exhibit maximum zone of inhibition against fungal strain of C. albicans. The exhibit of antimicrobial activity against both Gram-positive and Gram-negative bacteria and on various fungal strains might be demonstrative of the presence of broad spectrum antibiotic compounds in the extract.

CONCLUSION

On the basis of data obtained by the study, it can be concluded that tested fruit extract has beneficial effect on tested pathogens and leaves of A. reticulata could be source of bioactive antimicrobial components. Ethanol leaf extract possessed strong antimicrobial activity. Different classes of phenolics such as flavonoids, steroids and saponin present in tested extract are most likely to the active substance inhibiting the growth of Staphylococcus aureus, Escherichia coli and Candida albicans. This study reveals potential use of these fruits for developing new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. Finally, the results obtained in the present study demonstrated that Annona reticulata possesses good antimicrobial activity. Be that as it may, additionally studies are required to toss light on the biological activity of Annona reticulata.
and its bioactive compounds against various diseases and also to explore precise mechanisms and component underlying these beneficial biological effects.

ACKNOWLEDGEMENT
The authors thank to Dr Gouranga Das, Principal Incharge of Girijananda Chowdhary Institute of Pharmaceutical Science Azara, Guwahati, India for providing facilities to carry out the research work, Mr. Bishwajit Dash, Assistant Professor Department of Pharmaceutical Chemistry, Girijananda Chowdhury Institute of Pharmaceutical Science Azara, for his support and guidance provided during this work and Head of the Department, Guwahati Pharmaceutical Science, Azara, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati for conveying this exploration work.

AUTHORS CONTRIBUTIONS
All the author have contributed equally

CONFLICT OF INTERESTS
There is no conflict of interest

REFERENCES