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Antimicrobial Activities of Three Ethno-Medicinal Plants of Assam against Human Test Pathogens

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Abstract: Discovery of new antimicrobial compounds is urgently required in the light of rise in the occurrence of new and re-emerging infectious diseases. This has led to renewed interest in herbal products as sources of novel drugs because 80% of the rural populace relies upon plants as primary healthcare remedies. Under this research, we aimed to examine the phytochemical screening and antimicrobial potential of selected medicinal plants used indigenously by the people of North-East India against some clinically important microorganisms. Different parts of three plants, namely *Annona reticulata*, *Houttuynia cordata,* and *Tinospora cordifolia* were collected and subjected to maceration using water, ethanol, hexane, and ethylacetate. Qualitative phytochemical analysis was performed for the different plant extracts by chemical reaction methods. Using agar well diffusion method we evaluated antibacterial and antifungal activities of the plant extracts against three clinically important microbes namely *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Candida albicans* (*C. albicans*). Alkaloids, flavonoids, saponins, tannins, terpenoids, phenols, steroids, and glycosides were found in the plant extracts based on phytochemical analysis. Overall, the extracts demonstrated substantial antibacterial and antifungal activity against the selected microbial strains. However, the ethanolic extract displayed the presence of highest amount of phytochemical compounds and the best activity against both bacteria and fungi. From our results, it is evident that the studied plants are a rich source of medicinally active compounds that exhibit remarkable antimicrobial activity against human test pathogens.

Keywords: Medicinal plants, Phytochemicals, Microbial resistance, Antimicrobial therapy, Traditional knowledge, Antifungal

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Introduction

The scenario of modern medicine and therapeutic standards to treat infections has been reformed by the discovery, and commercialization of antimicrobial compounds. But bacteria and other

pathogens have always evolved to compromise the accessibility and affordability of many currently prescribed antibiotics worldwide. Because of indiscriminate use of commercial antimicrobial drugs in infectious diseases' treatment, human pathogenic microbes have acquired a variety of drug resistance (Ginovyan *et al.*, 2017). Food poisoning, endocarditis, osteomyelitis, toxic shock syndrome, and postoperative wound infections are all mainly caused by *S. aureus*, a Gram-positive bacteria (Ortega, 2013). A Gram-negative bacteria, *E.coli* exist in intestine of human and responsible for lower Urinary Tract Infection (UTI), septicaemia, or coleocystis (Vila *et al.*, 2016). As an opportunistic fungus, *C. Albicans* inhabits the buccopharyngeal cavity, gastrointestinal tract, and vulvovaginal tract. It can cause serious systemic infections, which are often associated with immunosuppressive agents and indwelling catheters (Enfert *et al.*, 2021). According to WHO, antibiotic resistance is one of most significant public health threats of the 21st century (WHO, 2014). The emerging global health threats that these organisms pose call for the development of novel and sustainable avenues to control infection.

Plants have been essential in the maintenance of human health since ancient times because they are rich source of medicinal compounds (Kebede *et al.*, 2021). Plants contain different phytochemicals which may have anti-inflammatory, antioxidant, antimicrobial, and anti-cancerous properties, making the use of plants more beneficial. The therapeutic value of plants is attributed to phytochemicals that are some chemical substances like flavonoids, alkaloids, vitamins, glycosides, coumarins, and tannins. Phytochemicals are plant-based bioactive compounds that have antibacterial properties and a specific physiological effect on humans (Chandra *et al.*, 2017). Antimicrobial substances derived from plants have a great therapeutic potential to treat a wide range of infectious diseases and, in contrast to synthetic drugs, are not linked to any negative side effects. According to WHO, it is estimated that up to 80 per cent of the world population still relies on traditional remedies with more than 35,000 plant species being used as medicines in various human cultures (WHO, 2014).

India, one of the world's richest countries in terms of genetic resources of medicinal plants, is an emporium of a wide variety of medicinal plants. Because of its biogeographic position, North-East India in particular is endowed with an enormous biodiversity of medicinal plants. As a result, the area is rich in all the 3 levels of biodiversity: species diversity, habitat diversity, and genetic diversity (Chandra *et al.*, 2017; Rather *et al.*, 2023). More importantly, the region is populated with many tribal people belonging to different ethnic groups that have been using traditional plants like *Annona reticulata*, *Houttuynia cordata,* and *Tinospora cordifolia* as primary healthcare remedies for ages. These plants have a variety of bioactive principles and have been proven medicinally essential plants. Few studies from North East India have reported a detailed study on these plants (Sajewm and Gosai. 2006; Lamo *et al.*, 2023). The present study aimed to examine the phytochemical screening and antimicrobial potential of selected medicinal plants namely *Annona reticulata*, *Houttuynia cordata,* and *Tinospora cordifolia.*

Identification of the studied plants by their scientific and common names, place of collection, parts used, traditional use, and previous reports on antimicrobial activity is mentioned in Table 1.

Materials and Methods

Plant collection and identification

Healthy plant samples *A. reticulata*, *H. cordata* and *T. cordifolia* were collected from three different sites of Assam, namely, Ganeshpara (26°9'32"N and 91°43'58"E), Udalguri (26°45'13.21"N and 92°6'7.74"E) and Changsari (26°15'42.43"N and 91°42'1.33"E), respectively during the period February-April 2022. A taxonomist used the morphology of the leaves and flowers to identify the collected plant species, which were then stored in the Gauhati University Herbarium. The samples were brought and processed to isolate

Table 1: Ethnobotanical information of the studied plant species

Plant species (Family); Place of collection	Common name	Parts used	Traditional uses	Previous reports on antimicrobial activities
Annona reticulata (Annonaceae); Ganeshpara, Assam	Ramphal, sitaphal, custard apple	Leaves	Antihyperglycemic, cytotoxic and recombinant caspase inhibitory activity, analgesic, CNS depressant anti-inflammatory and tumour inhibitor.	Methanolic and Ethanolic extract: *S. aureus, E. coli, C. albicans*
Houttuynia cordata (Saururaceae); Udalguri, Assam	Mosundari	Whole plant	Diarrhoea, dysentery, cholera, immunity enhancer	Ethanolic extract: *S. aureus, E. coli, C. albicans*
Tinospora cordifolia (Menispermeceae); Changsari, Assam	Giloy, amarlata, heart-leaved moonseed.	Stem	Immunomodulatory activities, treats diabetes mellitus, rheumatoid arthritis, urinary tract infection, treats inflammation and candidiasis.	Aqueous and Ethanolic extract: *S. aureus, E. coli, C. albicans*

plant parts at the lab immediately.

Preparation of plant extracts

After collection, plant parts were cleaned with the help of double distilled water, then air dried at room temperature as well as coarsely powdered. From each sample, 10 g was sequentially extracted distinctly with 100 ml (1:10 w/v) of different organic solvents starting with polar to non-polar (Trease and Evans, 1989; Talukdar and Tayung, 2019). Extraction was done at room temperature in an orbital shaker with agitation at 140 rpm for 72 h. Three cycles of maceration were performed for each solvent. As the water extract was lyophilized, the filtrate from each organic solvent was combined and evaporated in a rotary evaporator. To estimate phytochemicals and antimicrobial activity, the extracts that were collected have been filtered with the help of Whatman No. 1 filter paper. 10% (DMSO) dimethylsulphoxide was added to the extracts at a concentration of 5mg/ml & stored at -20°C for further analysis.

Preliminary phytochemical screening

Preliminary qualitative phytochemical screening was performed using the methods mentioned below [(Trease and Evans, 1989; Talukdar and Tayung, 2019):

Test for flavonoids: In a test tube 1 ml of plant extract was taken, 20% sodium hydroxide (NaOH) was added as few drops and observed for intense yellow coloration. Then few drops of dilute hydrochloric acid (HCl) were added and observed for disappearance of yellow color.

Test for alkaloids: Addition of 0.5 ml of Mayer's reagent to 1 ml extract followed by dilute hydrochloric acid and the formation of a creamish precipitate was observed.

Test for glycosides: 2 ml of chloroform and 2 ml of glacial acetic acid were added to 1 ml of extract. Then few drops of concentrated hydrochloric acid were added and appearance of a brown ring was observed.

Test for saponin: After shaking 2 ml of the test solution with 2 ml of distilled water, the mixture was observed for frothing.

Test for steroids: 1 ml of extract, 2 ml concentrated sulfuric acid (H_2SO_4) and 2 ml chloroform was mixed. After this formation of a ring was observed.

Test for terpenoids: 2 ml chloroform was mixed with 5 ml extract. Subsequently, concentrated H_2SO_4 was carefully poured to create a layer and appearance of reddish brown colouration at the interface was observed.

Cultivation of test organisms

As test pathogens, we involved a pathogenic fungus *C. albicans* (MTCC 227), a Grampositive bacterium *S. aureus* (MTCC 737), and a Gram negative bacteria *E. coli* (MTCC 443). These strains were acquired from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial test pathogens were grown in freshly prepared nutrient broth (NB), while the fungal test pathogen was grown on Sabouraud's Dextrose Broth (SDB).

Initial evaluation of plantsantibacterial and antifungal activity

Agar cup diffusion method was employed for initial determination of antimicrobial activity of the plant extracts against above-mentioned human test pathogens. Different agar plates for bacterial and fungal pathogens, [for bacteria: nutrient agar (NA) plates and for fungus: (SDA) Sabouraud's dextrose agarplate] were inoculated with 0.2 ml of overnight grown culture containing 1.0×10^6 cells. A sterile cotton swab was used to evenly spread the test organisms on respective plates. With the help of a sterile cork borer (7 mm in diameter), preparation of agar cups was done, followed by filling with 100 µl of the plant extracts. The agar cups were incubated at 37±1°C for 24 h for bacterial pathogens whereas at 28±1°C for 48 h for fungal pathogens. The antimicrobial activity of the extracts was determined by observing a clear zone of inhibition around the agar cups against the target organism. Fluconazole (25 µg) and Streptomycin (10 µg) were used as positive control for bacteria and fungus respectively whereas DMSO was used as the negative control (Soyingbe *et al*., 2018; Talukdar *et al*., 2021).

Results and Discussion

Qualitative phytochemical composition

In this study, we evaluated the presence of phytochemical composition along with antimicrobial activities of three important medicinal plants used indigenously by the people of Assam, namely *A. reticulata*, *H. cordata,* and *T. cordifolia*. The naturally occurring complex phytochemicals in plants have been found to have a wide array of benefits and hence are important derivatives of new drug entities. Even though water is the preferred solvent by most traditional healers as it is readily available, nevertheless not all non-polar bioactive compounds can be extracted using this method (Das *et al*., 2021). As a result, we decided to extract compounds of different polarities from the selected plant parts using other solvents, specifically ethanol, ethyl acetate, and hexane. Table 1 lists the ethnobotanical information of the selected plants. Phytochemical screening was performed on freshly produced extracts to detect various metabolites. Our results indicated that all extracts of plant species under study contained phytochemical substances such as alkaloids, flavonoids, tannins, steroids, phenols, terpenoids, and glycosides (Tables 2, 3, 4). Interestingly, our study showed that only the stem extract of *T. cordifolia* has another component called saponin, an important plant metabolite. These differences may arise due to variations in genetic makeup,

Table 2: Phytochemical analysis of leaf extract of *Annona reticulata*

S. No.	Phytochemical Constituent	Ethanol Extract	Ethyl acetate Extract	Hexane extract
1	Flavonoid	+	+	+
2	Alkaloid	+	+	+
3	Saponin	-	-	-
4	Steroid	+	+	+
5	Tannin	+	+	+
6	Phenol	+	+	+
7	Terpenoid	+	-	+
8	Glycoside	+	-	+

+ indicates present; - indicates absent

Table 3: Phytochemical analysis of whole plant of *Houttuynia cordata*

S. No.	Phytochemical Constituent	Ethanol Extract	Ethyl acetate Extract	Hexane extract
1	Flavonoid	+	+	+
2	Alkaloid	+	+	+
3	Saponin	-	-	-
4	Steroid	+	+	+
5	Tannin	+	+	+
6	Phenol	+	+	+
7	Terpenoid	+	+	+
8	Glycoside	+	+	+

+ indicates present; - indicates absent

weather conditions, and geographical locations as well as due to different extraction processes. The phytoconstituents found in the plant extracts are antibiotic principles of plants, which exert their therapeutic action by destroying the microbial cell membrane, membrane proteins, and enzymes (Sundar *et al*., 2021).

Many previous reports have explained the therapeutic potential of these studied plant extracts. For example, *A. reticulata*, commonly known as custard apple is famous for the presence of acetogenin, a potent antimicrobial and cytotoxic

Table 4: Phytochemical analysis of stem extract of *Tinosporacordifolia*

S. No.	Phytochemical Constituent	Ethanol Extract	Ethyl acetate Extract	Hexane extract
1	Flavonoid	+	+	+
2	Alkaloid	+	+	+
3	Saponin	+	+	+
4	Steroid	+	+	+
5	Tannin	+	+	+
6	Phenol	+	+	-
7	Terpenoid	+	+	+
8	Glycoside	+	+	+

+ indicates present; - indicates absent

compound. Many studies have shown that this plant presents remarkable antitumor and antimicrobial activities along with other phytomedicinal potentials (Sofowara, 1993; Sit *et al*., 2017). *H. cordata* can be used against cancer, inflammation, severe acute respiratory syndrome (SARS), obesity, diabetes, pneumonia, stomach ulcer, muscular sprain, etc. Furthermore, it has been reported to be active against various viruses, that include the mouse hepatitis virus, SARS coronavirus, dengue virus type 2, influenza virus, human immunodeficiency virus type 1 and herpes simplex virus type 1 (Bonvicini *et al*., 2014; Sundar *et al*., 2021). In ayurvedic medicine, *T. cordifolia* is considered as 'Rasayana', a Sanskrit term which means it can provide a general well-being condition and defense against diseases (Dongre and Pande, 2020). Many studies have demonstrated the immunomodulatory, immuno-stimulatory, cardioprotective, antidiabetic, and antimicrobial properties of this plant (Policegoudra *et al*., 2012; Kattupalli *et al*.,2019). Our present finding of presence of many important phytoconstituents in the studied plant extracts corroborates the above stated previous reports.

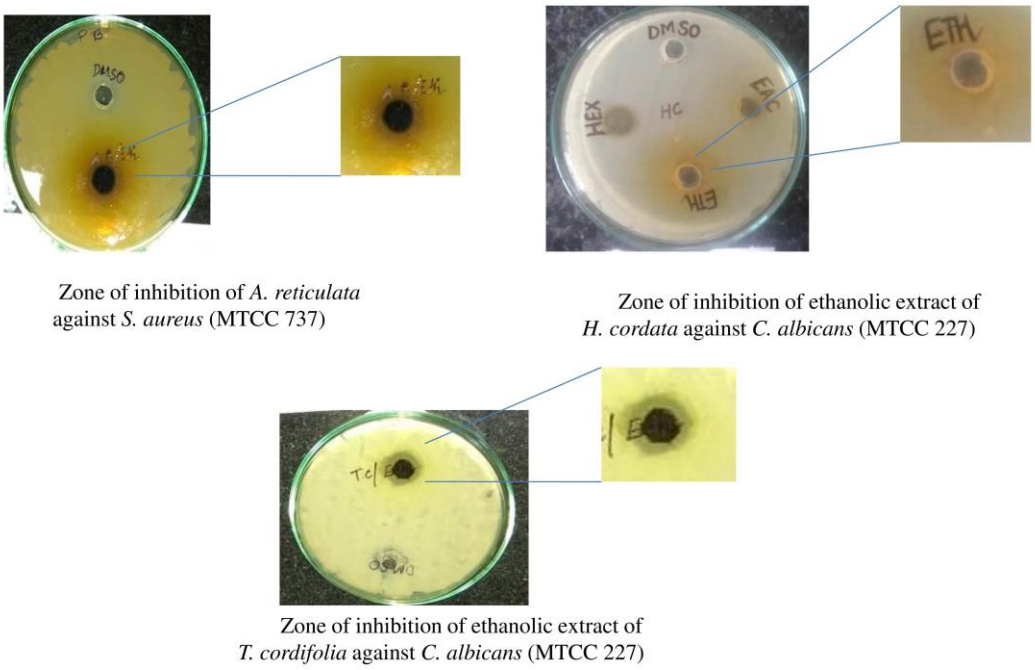
In vitro antimicrobial activity of the plant extracts

Zone of inhibition was measured to determine antimicrobial activity of *A. reticulata*, *H. cordata,* and *T. cordifolia* plant crude extract against three clinically important pathogenic human test organisms. The results are shown as mean ± standard error mean, and each experiment was conducted in triplicate. Agar well diffusion method was performed for the initial evaluation of plants' anti-bacterial as well as anti-fungal activity against one Gram-positive bacteria, one Gram-negative bacteria, and one fungal species (Table 5). Solvent extracts of each plant showed substantial activity against the three microbial strains, out of which ethanol extracts of all three plants displayed the strongest antibacterial and antifungal activity yielding 10.6 mm to 14.6 mm diameter of IZ. The highest activity of ethanolic extract of *A. reticulata* was observed for *S. aureus* (11.6±0.5 mm). Whereas, ethanolic extracts of *H. cordata* and *T. cordifolia* exhibited maximum activity for *C. albicans* (14.3±2.0 mm and 14.6±2.5 mm, respectively). Our results dictate that greater activity resides in ethanolic extracts as compared to ethylacetate and hexane. *A. reticulata*, ethanolic

Table 5: Antimicrobial activity of the plant extracts against human pathogens

Medicinal plants	Solvent extract	*S. aureus*	*E. coli*	*C. albicans*
A. reticulata	Ethanol	11.6±0.5	10.6±0.5	11.3±1.1
	Ethyl acetate	9.3±0.5	8.3±0.5	9.3±0.5
	Hexane	11.3±1.1	9.6±0.5	11.6±0.5
H. cordata	Ethanol	13.3±1.5	13.0±3.0	14.3±2.0
	Ethyl acetate	7.3±0.5	9.3±2.5	9.0±1.0
	Hexane	8.6±2.0	10.3±1.1	11.6±2.5
T. cordifolia	Ethanol	12.3±2.3	11.6±1.1	14.6±2.5
	Ethyl acetate	12.0±0.1	8.3±1.5	7.6±1.1
	Hexane	10.0±1.0	1.3±2.3	8.6±2.3

Data indicate mean diameter of inhibition zone (IZ) in mm, ± standard deviation



Zone of inhibition of *A. reticulata* against *S. aureus* (MTCC 737)

Zone of inhibition of ethanolic extract of *H. cordata* against *C. albicans* (MTCC 227)

Zone of inhibition of ethanolic extract of *T. cordifolia* against *C. albicans* (MTCC 227)

Fig. 1: Antimicrobial activity of ethanolic extract of the studied plant parts against different microbial strains, shown as zone of inhibition of growth (in mm). Positive control-Fluconazole (25 µg) and Streptomycin (10 µg) for bacteria and fungus, respectively. Negative control- DMSO.

extract showed maximum activity against *S. aureus* (IZ 11.6±0.6 mm), followed by *C. albicans* (IZ 11.3±1.1 mm) and *E. coli* (IZ 10.6±0.5 mm). However, in case of *H. cordata* and *T. cordifolia*, we observed that the most potent antimicrobial activity was against *C. albicans* (IZ 14.3±2.0 mm and 14.6±2.5 mm, respectively), followed by *S. aureus* and *E. coli*. The images of antimicrobial activity of the ethanolic extract for all three plants on cell culture plates are shown in Figure 1. The strong efficacy of ethanolic extracts against all studied microbial strains may be due to high polarity of ethanol solvent which can draw a lot of plant constituents compared to other solvents. The fact that chemical constituents responsible for antimicrobial activity are more soluble in ethanol

extracts can be one of the factors responsible for their remarkable antimicrobial properties. These serve as the defensive mechanism of plants against different pathogens, which may justify their antimicrobial properties (Kebede *et al*., 2021). According to Policegoudra *et al.* (2012), nonpolar extracts contain polysaccharides, lectins, and polypeptides that are more effective at inhibiting pathogen adsorption, while polar extracts, such as water, are less effective against microbes (Kumar *et al*., 2021).

There are previous studies that have evaluated antimicrobial properties of the plant extracts used in our study. For example, in one such study, the antimicrobial mechanism action of leaf extract of *A. reticulata* to phenolic compounds is reported to have been attributed to microbial membrane disruption, cytoplasmic leakage, and coagulation of cytoplasmic components. All of these disrupt microbial cellular metabolism and hinder anti-quorum sensing activity (Parthiban *et al*., 2018). Additional investigations would indicate the potential relevance and applications of *A. reticulata* extract in preventing foodborne bacterial diseases due to its ability to scavenge H_2O_2 within a range of 45-55% (Ruddaraju *et al*.,2019; Bhagawati and Sarmah, 2021). In addition, *H. cordata* extracts were also shown to have both antibacterial and anti-viral activities against some serious pathogens such as methicillin-resistant *Staphylococcus aureus*, corona, and dengue viruses etc. (Sekita *et al*., 2016; Lyngdoh *et al*., 2020; Řebíčková *et al*. 2020). It was found that *H. cordata* extract contains numerous essential oils (phenols terpenoids, alcohols, hydrocarbons, acids, ketones, esters, aldehydes, ethers, and mixed compounds) and flavonoids (isoquercitrin, rutin,quercitrin, hyperin, as well as afzelin). Out of these, aldehydes likedecanoyl acetaldehyde (houttuynin) and lauryl aldehyde have antibacterial effects against Gram-positive bacteria along with antifungal effects (Sharma and Prajapati, 2016). Due to its antidiabetic, immunomodulatory, and hepato-protective properties, *T. cordifolia,* a herb is extensively used in the Indian system of medicine. The herb's extract of different parts is widely used in curing variousdiseases (Sharma *et al*., 2019; Shital and Daksha, 2022). The active principles like alkaloid (berberine), bitter compounds tinosporic acid, tinosporin, tinosporal, a combination of fatty acids, as well as essential oil are considered to be accountable for their medicinal properties.

Conclusion

Since ancient times, man has relied on nature and remarkable remedies in the form of medicinal plants as a primary health care remedy. North East India, having a rich diversity of flora and fauna, is recognized as a global biodiversity hotspot. However, only a few of these have had their antimicrobial properties thoroughly evaluated; the vast majority still needs to be investigated. Thus, we studied the phytochemical composition and evaluated the antibacterial and antifungal properties of 3 important medicinal plants used indigenously by people of North East India against three clinically significant test pathogens. Our results revealed encouraging results showing presence of important phytochemicals in the extracts and appreciable antimicrobial properties of the studied plant extracts. The myriad of bioactive compounds that give these extracts their therapeutic potential and synergistic effects with commonly used antibiotics should be thoroughly researched. Therefore, we conclude by stating that drug discovery from medicinal plants is a promising strategy to overcome socioeconomic problems and long-term negative impacts as a result of harmful pathogens.

Ethical Statement

We hereby declare that this study did not include any animal species.

Author Contributions

Barman Dhanusmita, *Das Mrigakshi* and *Bhuyan Pratyasha* collected the plant species, conducted all experiments and wrote the manuscript. *Shireen Tahsina* conceptualized and designed the study, reviewed and edited the manuscript. All authors

have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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