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# Antimicrobial activity and GC-MS analysis of some ethno medicinal plants used by Santali tribe of Assam

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## ABSTRACT

The increasing occurrence of antimicrobial resistance has become a major global threat, calling for the exploration of alternative antimicrobial agents derived from natural resources, including ethnomedicinal surveys of plants. This study evaluated the comparative antimicrobial activity of crude ethanolic extracts of the following ethnomedicinal plants used by Santali tribe of Kokrajhar district of Assam, India- *Clerodendrum infortunatum*, *Piper nigrum*, *Psidium guajava*, *Camellia sinensis*, *Andrographis paniculata* and *Murraya koenigi* against three bacterial pathogens, *Staphylococcus aureus, Escherichia coli* (non pathogenic), *Salmonella typhi* and two fungal pathogens, *Candida albicans* and *Aspergillus fumigatus*. Antimicrobial activity was evaluated using the agar cup/disk diffusion method, and inhibition zones were statistically analyzed by using mean data and standard deviation. *C. sinensis* showed highest antimicrobial activity (22.33±0.93 mm against *E. coli*) followed by *P. guajava* (20±1.63 mm against *E. coli*) and *A. paniculata* (19±0.81mm against *S. aureus*). *P. nigrum* and *M. koenigi* showed moderate inhibition while *C. infortunatum* exhibited comparatively lower activity. GC-MS analysis revealed several bio active compounds, including piperine, hexamethyl cyclotrisiloxane, methyl thiophene-2-carboxylate and tetradecamethyl cycloheptasiloxane. The results support ongoing efforts to identify alternative natural, plant-derived agents to tackle antimicrobial resistance.

**Keywords:** Antimicrobial, bioactive, extract, herbal, resistance

## INTRODUCTION

The human population has reached approximately 8.3 billion globally and each year millions of deaths are reported due to the re-emergence of previously controlled pathogens and the growing threat of antimicrobial-resistant microorganisms that cause severe infectious diseases (Zouine *et al*., 2024). The Antimicrobial Resistance Project of Global Research reported that, due to antimicrobial resistance, nearly one million people died each year between 1990 and 2021. This study predicted nearly 1.91 million deaths due to antimicrobial resistance in 2050 (Murray *et al*., 2022), while further studies found that 3 million children died from antimicrobial resistance in 2022 and 4.95 million people died in 2023 (Alomari *et al*., 2024). Scientists now predict that antimicrobial resistance could cause 39 million deaths globally in 2050 (Naghavi *et al*., 2024). In India nearly 224,000-310,000 deaths were attributed to antimicrobial resistance and 855,000-1,120,000 deaths were attributed with diseases associated with antimicrobial resistance in 2021. Report shows that in India largest number of deaths of antimicrobial resistance was among people aged above 70 in India and the second largest effected groups were children (Anonymous, 2023).

Various studies on antimicrobial resistance show the emergence of third-generation cephalosporin-resistant *E. coli*, which also exhibits resistance to antibiotics like co-trimoxazole, ampicillin, and fluoroquinolones; resistance in *S. typhi* to fluoroquinolones, ampicillin, and cephalosporins; methicillin-resistant *Staphylococcus aureus*; carbapenem-resistant *P. aeruginosa*; and antibiotic-resistant *Klebsiella pneumonia* etc. The rapid emergence of multi-drugresistant *Candida sp. and* amphotericin B resistant *Aspergillus spp. adding fungi in the list of drug resistant microbes*. Drug resistance is also emerging in the disease categories like HIV, tuberculosis, Malaria and neglected tropical diseases like leprosy, African trypanosomiasis, leishmaniasis *etc* (Anonymous, 2024; Marchello *et al*., 2020; Zouine *et al*, 2024). Research

showed that if we do not control the current scenario of antimicrobial resistance, it could lead to the loss of 5.5 million lives per year soon in India (Anonymous, 2023). Therefore, scientists are in constant search for alternatives to combat these cases of drug resistance which can help them to manufacture some drug with long shelf life, less side effects and more affectivity.

Scientists are now interested in plant extracts as an alternative to synthetic drugs because plants naturally produce a diverse array of secondary metabolites, such as alkaloids, flavonoids, terpenoids, and essential oils, that kill different plant pathogens. These plants extracts exhibit multiple modes of action against various plant pathogens unlike the synthetic drugs targeting a specific target pathogen (Aslam *et al*., 2025; Dheeraj *et al*., 2024). In the search of plant alternatives to reverse the ill effects of drug resistant synthetic medicines scientists are emphasizing on the extensively screening medicinal plants from traditional ethnomedicinal systems across the globe as native people are using those plants from time immemorial to kill or control various disease causing microbes which gives a clear idea of that plants antimicrobial capacity (Zouine *et al*., 2024). Countries rich in biodiversity hotspots are actively participating in this search by exploring and documenting the ethnomedicinal knowledge of their native peoples. The ethnic people were using plants to target specific diseases. They use single plant or sometime mixture of different plant and animal parts to cure specific diseases or disorders. Extensive global ethnomedicinal documentation has documented many plants with antimicrobial activity, and researchers have confirmed this using antimicrobial assays and phytochemical analysis (El Amri *et al*., 2025; Rattana chitthawat *et al*., 2022; Kabede *et al*., 2021). Therefore, the present investigation was to validate the claims of ethnomedicinal knowledge of the Santali tribe of Kokrajhar District of Assam, India through antimicrobial assay and phytochemical analysis.The aim of this study is to examine the antimicrobial activity of some medicinal plants used by Santali tribe of Kokrajhar District of Assam for the treatment of diseases caused by most prevalent microorganisms.

## MATERIAL AND METHODS:

**Collection of plant samples**

Based on an ethnomedicinal survey, 12 medicinal plants were selected and collected from different parts of the study area. Then the plant samples were cleaned properly, shade dried, made into powder using a grinder and packed in airtight containers.

**Extraction of crude metabolites**

Crude metabolites were extracted by maceration using 1:5 ratio of plant powder to 96% ethanol (Zazharskyi *et al*., 2019). The plant powder was then soaked in ethanol for 7 days, and shaken regularly (Rudraswamy *et al*., 2021). After 7 days, the mixture was filtered through Whatman No. 1 filter paper, and the ethanol was dried. Then we dissolved the extract in 10% DMSO solution and stored in 4°C for further use (Manilal *et al*., 2023).

**Test pathogens and maintenance**

We selected human pathogens based on their prevalence and collected plants to target diseases caused by them. The human test pathogens which used in this study consisted of three pathogenic bacteria, *Escherichia coli* (MTCC–443), *Salmonella typhi* (MTCC- 323), *Staphylococcus aureus* (MTCC-737) and two pathogenic fungi, *Candida albicans* (MTCC – 227) and *Aspergillus fumigatus* (MTCC-870) purchased from IMTECH, Chandigarh. The test bacterial and fungal pathogens were grown and maintained in Mueller Hinton Slant and Potato Dextrose Agar medium respectively. For determination of antimicrobial activity, we streaked freshly cultured microorganisms on the growth medium and incubated for 24 hours and 48 hours at 37°C for fungi and bacteria respectively. The OD of the bacterial inoculums was maintained at 0.8 at 600nm for susceptibility testing. For fungal culture final inoculums size was maintained between $(0.5\text{-}1)\times10^{8}$CFU/ml at 0.5 McFarland standards. The lowest concentration of the extract which inhibits the pathogens was considered as minimum inhibitory concentration (MIC).

**Determination of antimicrobial activity**

We evaluated the plant extracts for antimicrobial activity using the agar cup diffusion method (Heatley, 1944). We cultured the bacterial pathogens in freshly prepared Mueller Hinton broth and fungal pathogen in freshly prepared Potato Dextrose Broth. Media were prepared as per the manufacturer's instructions. Broth cultures of bacterial and fungal pathogens were inoculated onto the respective MHA and PDA plates using a spreader. Agar cups were made with a sterile cork borer of 7mm diameter. 100 µl of crude plant extracts were loaded on each cup. The fungal and bacterial pathogens were incubated at 37°C overnight and 35°C for 24 hours respectively. The zone of inhibition was observed carefully and the inhibition zones were measured in millimeters.

***Minimum Inhibitory Concentration (MIC) Assay***

The MIC assay was done for the plant extracts which showed high antimicrobial activity against the selected test pathogens by the Kirby–Bauer's disc diffusion method (Baur *et al*., 1966). The prepared plates containing bacterial

and fungal test organism were incubated at 35°C for 24 hours. Extracts were prepared in ethanol at concentrations of 0.1g/ml, 0.05g/ml, 0.025g/ml, 0.012g/ml, and 0.006g/ml and loaded onto the disks.The lowest concentration of the plant extract which produced a clear zone of inhibition was considered as the MIC (Sukeri *et al*., 2021). A control experiment was also run in parallel, using various controls to assess the growth of the selected test pathogens (Shihabudeen *et al*., 2010). Ampicillin and Flucanazole are used as bacterial and fungal positive control respectively and 10% (*v/v)*DMSO were applied as negative control. The research work was conducted between the years 2024-2025.

**Plant extract analysis by GC-MS**

Gas Chromatography–Mass Spectrometry (GC–MS) analysis was conducted to determine the bioactive compounds present in the plant extracts of the selected plants. For this analysis, the instrument used was a Gas Chromatography–Mass Spectrometry (GC–MS) system, manufactured by Agilent technologies where the GC Model was Agilent 7890B and Mass Spectrometer model was Agilent 5977B Mass Selective Detector (MSD). GC Column name was HP-5MS capillary column bearing length of 30 m; internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier Gas was Helium. The initial temperature was50 °C, holding time: 2 min, temperature ramp: 10 °C min ¹ 20, final temperature: 280 °C and final holding time was 5 min 22 for GC oven temperature program. The injection conditions are- injection volume: 1 µL 24, injector temperature: 250 °C 25 and injection mode was Splitless; Ionization mode was Electron Ionization with electron energy f 70 eV. Mass Ranges are- acquisition mode: full-scan mode, mass scan range: m/z 40–550, ion source temperature: 230 °C and quadrupole temperature was 150 °C. Compound Identification Methods used were- library/database: NIST Mass Spectral Library and the detected compounds were identified by comparing their obtained mass spectra with reference spectra available in the NIST Mass Spectral Library. Identification was based primarily on mass spectral matching characteristic fragment ions.

## RESULTS AND DISCUSSION

After collecting information on medicinal properties, 12 plant species were selected for antimicrobial activity testing. The list of plant species is given in Table 1-

**Table 1: List of ethnomedicinal plants with the medicinal uses selected for the study.**

| Scientific name | English name | Common/ Vernacular name | Economic part | Uses |
|---|---|---|---|---|
| *Andrographis paniculata* | Green chiretta | Chirota | Leaf and stem | Stomach issue, skin disease |
| *Clerodendrum infortunatum* | Hill glory bower | Varni | Tender leaf | Skin disease |
| *Piper nigrum* | Black pepper | Golmorich | Seed | Cough, sore throat, Asthma |
| *Morinda angustifolia* | Narrow-leaf Morinda | Kerry | Bark | Jaundice, kidney stone, gallbladder stone, Tuberculosis, skin disease, problem of irregular and heavy period |
| *Streblus asper* | Sandpaper tree | Shewra | Leaf | Typhoid, jaundice, kidney stone, gallbladder stone, Tuberculosis, skin disease, problem of irregular and heavy period |

| Scientific name | English name | Common/ Vernacular name | Economic part | Uses |
|---|---|---|---|---|
| *Murrayakoenigi* | Curry tree | Narsingha | Leaf | Jaundice, kidney stone, gallbladder stone, Tuberculosis, skin disease, problem of irregular and heavy period |
| *Cheilocostus speciosus* | Wild ginger | Orrot | Bulb | kidney stone, gallbladder stone, Tuberculosis, skin disease, problem of irregular and heavy period |
| *Cassia fistula* | Golden shower tree | Bandorer lathi | Bark | Urination problem |
| *Psidium guajava* | Guava | Pyaara | Tender leaf | Gallbladder stone, period problem |
| *Camellia sinensis* | Tea tree | Chah paat | Leaf | Diarrhea |
| *Azadirachta indica* | Indian lilac | Neem | Leaf | Skin disease |
| *Citrus maxima* | Pomelo | Jambura | Bark | Asthma |

The ethanol extracts of 12 ethnomedicinal plants were tested against common pathogenic microbes based on their claims by ethnomedicinal practitioners, viz. *Staphylococcus aureus* causes skin infections, food poisoning, and pneumonia; *Escherichia coli* causes gastrointestinal diseases such as diarrhea, vomiting, and urinary tract infection; *Salmonella typhi* causes typhoid; *Candida albicans* causes candidiasis and urinary tract infection; and *Aspergillus fumigatus* causes allergic conditions in people with asthma and lung infections. Plant metabolites were extracted by maceration using 40% ethanol. The agar cup diffusion method was then used in the preliminary assay to determine the plant extracts' antimicrobial activity. The result indicated that plant extracts of all the 11 medicinal plants tested against the targeted human test pathogens showed antimicrobial activity except *Citrus maxima* which did not show any significant activity against *A. fumigatus*, it can be due to the fact that the traditional healer combines many plants while treating a disease, examination of the synergistic effect of *Citrus maxima* may lead to a promising result in future studies (Table 2).

**Table 2: Preliminary antimicrobial screening of plant extracts against human test organisms by agar cup diffusion method**

| Name of medicinal plant | Human bacterial test organism | | | Human fungal test organism | |
|---|---|---|---|---|---|
| | *S.aureus* | *E. coli* | *S. typhi* | *A.fumigatus* | *C. albicans* |
| *Streblus asper* | + | ND | + | + | + |
| *Andrographis paniculata* | ++ | ND | ND | ND | ND |
| *Azadirachta indica* | + | ND | ND | ND | ND |
| *Clerodendrum infortunatum* | + | ND | ND | ND | ++ |
| *Morinda angustifolia* | ND | ND | + | + | + |
| *Murraya koenigii* | ND | ND | ++ | + | ND |
| *Camellia sinensis* | ND | ++ | ND | ND | ND |
| *Psidium guajava* | ND | ++ | ND | ND | ND |
| *Cheilocostus speciosus* | ND | ND | + | NA | + |
| *Cassia fistula* | ND | ND | ND | ND | + |
| *Citrus paradise* | ND | ND | ND | NA | ND |
| *Piper nigrum* | ND | ND | ND | ++ | ND |

+ sign indicates sensitivity, ++ indicates more sensitivity than the other plant extract, ND indicates not determined, NA indicates not applicable.

After preliminary screening, we had selected 6 plants based on their higher activity against the target test organism. The plant extracts were then subjected to final screening to determine zone of inhibition against the human test organisms by following the agar cup diffusion method and GC-MS technique was applied to analyze their bioactive compounds. The final antimicrobial screening of the selected plants revealed that *C. sinensis* showed zone of activity of 22.33±0.93 mm against *E. coli* which was also confirmed by Hope *et al*., 2022 where ethanolic extract of *C. sinensis* showed 20 mm zone of inhibition against *E. coli*. Followed by *A. paniculata* showing 19±0.81mm zone of inhibition against *S. aureus* similar work by Rakhmawati and Pertiwi (2025) showed that ethanol extracts of *A. paniculata* showed zone of inhibition varying from 2.17±0.77 to 10.59±0.26 against *S. aureus*; *Piper nigrum* showed18.33±0.45 mm zone of inhibition against *A. fumigatus*, Rani *et al*., 2013 examined the piperine extract against *Aspergillus niger* and *Aspergillus flavus* which showed 30 mm and 38 mm zone of inhibition but no report on *A. fumigatus* was found. *P. guajava* showed 20±1.63 mm zone of inhibition against *E. coli*; the study by Buah *et al*., 2023 showed that ethanolic extract of *P. guajava* showed 18 mm zone of inhibition against *E.* coli (Fig.1). *M. koenigi* showed 13.66±1.69 mm zone of inhibition against *S. typhi*, similarly Bansode and Chavan (2014) showed that ethanol extract of *M. koenigi* showed a zone of inhibition of 6 mm against *S. typhi*. *C. infortunatum* showed 12.33±0.42 mm zone of inhibition against *C. albicans* while work by Waliullah *et al*., 2014 showed that highest zone of inhibition of ethanol extract of *C. infortunatum* was 11 mm against *C. albicans*. Our study revealed significant zones of activity against all test pathogens, which may be due to environmental conditions in the plant habitat and solvent composition (Table 3).

**Table 3: Antimicrobial activity of plant extracts against selected human test organisms by agar cup diffusion method.**

| Name of medicinal plant | Human test organism | Zone of inhibition(mm) |
|---|---|---|
| *Clerodendrum infortunatum* | *C. albicans* | 12.33±0.42 |
| *Piper nigrum* | *A. fumigates* | 18.33± 0.45 |
| *Psidium guajava* | *E. coli* | 20±1.63 |
| *Camellia sinensis* | *E. coli* | 22.33±0.93 |
| *Andrographis paniculata* | *S. aureus* | 19±0.81 |
| *Murraya koenigi* | *S. typhi* | 13.66±1.69 |

NB. The data are expressed as Mean ± SD

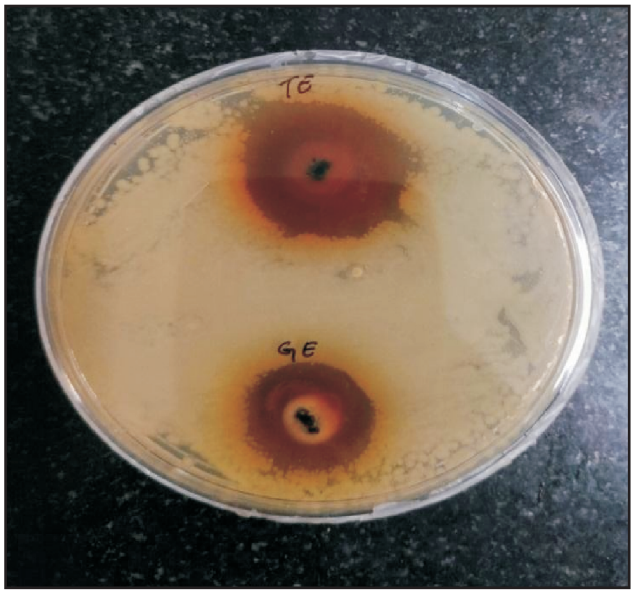
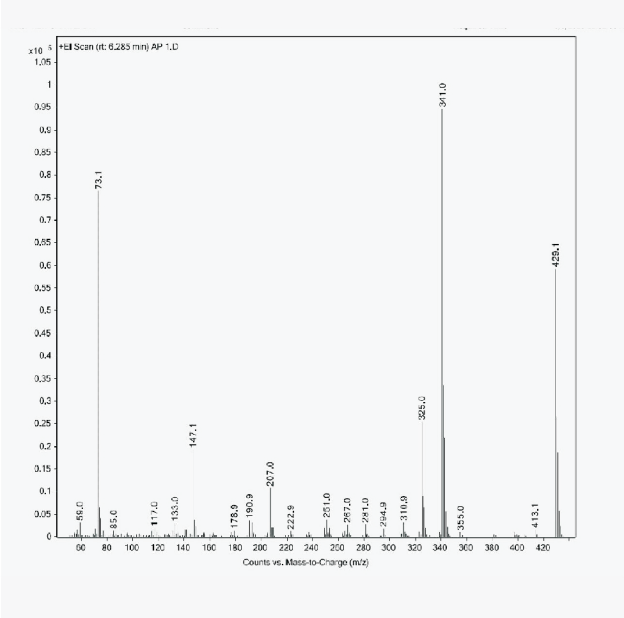



**Figure 1:** Photograph showing zone of inhibition of ethanol extract of *Camellia sinensis (TE)* and *Psidium guajava (GE)* against *E. coli.*

After final screening, we determined the MIC of the selected plants against the respective pathogens. The MIC of the chosen plant species demonstrated that *M. koenigi and C. infortunatum* needed the lowest concentration of extract (0.006 g/ml) to prevent the growth of *S. typhi*and *C. albicans* respectively, followed by 0.012 g/ml concentration of *C. sinensis* against *E. coli*while *A. paniculata* needed the highest extract concentration (0.1 g/ml) to kill *S. aureus* (Table 4).

| Name of medicinal plant | MIC | Pathogen |
|---|---|---|
| *Clerodendrum infortunatum* | 6 mg/ml | *C. albicans* |
| *Piper nigrum* | 50 mg/ml | *A. fumigates* |
| *Psidium guajava* | 25 mg/ml | *E. coli* |
| *Camellia sinensis* | 12 mg/ml | *E. coli* |
| *Andrographis paniculata* | 10 mg/ml | *S. aureus* |
| *Murraya koenigi* | 6 mg/ml | *S. typhi* |

GC-MS was then used to identify the key bioactive compounds in the selected plants. The GC-MS analysis of *Clerodendrum infortunatum* revealed four compounds with antimicrobial activity, hexamethyl cyclotrisiloxane, dodecamethyl cyclohexasiloxane, octamethyl cyclotetrasiloxane and piperine (Lingfa *et al*., 2024; Periferakis *et al*., 2025). Similarly, GC-MS analysis of *Psidium guajava* showed three compounds hexamethyl cyclotrisiloxane, octamethyl cyclotetrasiloxane and dodecamethyl cyclohexasiloxane (Lingfa *et al*., 2024); *Piper nigrum* contained piperine, octamethyl cyclotetrasiloxane and dodecamethylcyclohexasiloxane (Lingfa *et al*., 2024; Periferakis *et al*., 2025); *Camellia sinensis* revealed compounds like tetradecamethyl cycloheptasiloxane, hexamethyl cyclotrisiloxane, octamethyl cyclotetrasiloxane and dodecamethyl cyclohexasiloxane (Donio *et al*., 2022; Lingfa*et al*., 2024); *Andrographis paniculata* (Fig.2) showed methyl thiophene-2-carboxylate, tetradecamethyl cycloheptasiloxane, hexamethyl cyclotrisiloxane, octamethyl cyclotetrasiloxane and dodecamethyl cyclohexasiloxane (Molina- panadero *et al*., 2024; Donio *et al*., 2022; Lingfa *et al*., 2024) and *Murraya koenigi* showed hexamethyl cyclotrisiloxane, octamethyl cyclotetrasiloxane and dodecamethyl cyclohexasiloxane (Lingfa *et al*., 2024) as potent antimicrobial agent.




## CONCLUSION

From the above observations, we conclude that the ethnomedicinal plants used by the Santali tribe of Kokrajhar district, Assam, show significant activity against various common pathogens with antimicrobial resistance. This knowledge can be applied in the future for drug docking and formulation of new natural drugs. Moreover, due to rapid urbanization and modernization, these medicinal plants are being displaced from their natural habitat and heading toward extinction, so we should emphasize conservation efforts, such as commercial cultivation, transfer to botanical gardens, or protection in their natural habitat through heritage site recognition.

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