*European Journal of Medicinal Plants*
*18(4): 1-7, 2017; Article no.EJMP.32059*
*ISSN: 2231-0894, NLM ID: 101583475*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Antibacterial Activity of Ethnic Vegetables Consumed by the Indigenous People Living in Chittagong Hill Tracts

**Parveen Begum[1], Md Abdullah Al Noman[2], Mohiminul Adib[3], Monira Ahsan[3] and Sheikh Nazrul Islam[1*]**

*[1]Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh.*
*[2]Department of Pharmacy, University of Asia Pacific, Dhaka-1215, Bangladesh.*
*[3]Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors SNI and M. Ahsan designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author PB conducted the experiments. Authors MAAN and M. Adib managed the literature searches and proofreading of the manuscript. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/EJMP/2017/32059
Editor(s):
(1) Daniela Rigano, Department of Chemistry of Natural Compounds, University Federico II of Naples, Italy.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
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(5) Łukasz Woźniak, Institute of Agricultural and Food Biotechnology, Warsaw, Poland.
Complete Peer review History: http://www.sciencedomain.org/review-history/18838

***Original Research Article***

***Received 5th February 2017***
***Accepted 10th April 2017***
***Published 28th April 2017***

## ABSTRACT

**Aim:** Emergence of multidrug resistant bacteria has made the treatment of infectious diseases much difficult. Plant or herbal antibiotics have been reported to be effective against multiple drug resistant bacteria. In line of exploring newer antibacterial agent(s), this study describes the screening of ten selected ethnic vegetables for their antibacterial activities against fourteen bacterial strains.
**Methodology:** Fresh plant sample was extracted using aqueous and ethanol solvent by cold-extraction. Disc diffusion method was employed to screen the antibacterial activity of fresh and

**Corresponding author: E-mail: sheikhnazrul@du.ac.bd, sheikhnazrul09@gmail.com;*
*E-mail: md.alnoman@gmail.com*

ethanol extracts of ethnic vegetables consumed by the ethnic people of Chittagong Hill Tracts.
**Results:** Among the tested plant extracts, fresh extracts of Kamino (*Caesalpinia digyna* Rottler*)* leaves, Chikipung (*Rumex vesicarius* L) and Ammpata (*Mangifera indica* L*)*; and ethanol extracts of Kamino leaves showed potent antibacterial activity against most of the pathogens. Gram positive bacteria were found to be more sensitive than the gram negative strains. Fresh extract of Kamino leaves showed a strong antibacterial activity against of the bacteria tested (20 mm to 32 mm zone of inhibition). Highest antibacterial activity was observed against *Sercina lutea* (32 mm zone of inhibition). Against *Escherichia coli* and *Shigella dysenteriae* type-1, Kamino leaves indicated 24 and 21 mm zone of inhibition respectively. Ethanol extract of Kamino leaves showed mild activity (10 mm to 15 mm zone of inhibition) against the tested strains.
**Conclusion:** Samples of ethnic vegetables investigated in this study are found to possess antibacterial activity. Fresh extract of some samples, in comparison with their ethanol extract has shown higher antibacterial activity. These findings indicate the possibility of further research on these plant to find effective antimicrobial compounds from these plants.

*Keywords: Ethnic vegetables; plant products; antibacterial activity; foods.*

## 1. INTRODUCTION

The wild plants are rich in health promoting phytochemicals [1], which provide the lead molecules for modern drugs. Epidemiological studies report that people consuming a diet rich in fruits and vegetables have a lower incidence of many chronic diseases, including diabetes, cardiovascular and neurological disorders, cancers and infections [2,3]. Plant medicines have also been reported to be widely used in the management of HIV/AIDS-related opportunistic infections [4,5].

In different communities and cultures, the use of plants as traditional medicine has been extensively documented. Plant medicines continue to play an essential role in health care system. The plant-based systems play an essential role in the healthcare system and a vast majority of global people rely on the plant medicine. The World Health Organization estimates that 80% of the world population rely on traditional medicines for their health care; for the remaining 20% living in developed countries use of plant medicines also plays important role in the prevention and therapy of diseases [5]. Infectious diseases have been accounted for approximately 50% of all global deaths in tropical countries and as much as of 20% deaths in the Americas [6].

Infection is invasion and multiplication of microorganisms in body tissues, especially that causing local cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response. Contributing factors to the infectious diseases include the HIV pandemic and resultant secondary infections as well as in antibiotic resistance in acquired infections. Antimicrobial agents are commonly used to treat infection and often misused. The indiscriminate use of antimicrobial agents leads the emergence of antibiotic-resistant pathogens that make the sporadic incidence of an epidemic and make the treatment of infections difficult or a failure. This directs an ever-increasing need for new drugs. Many plant products have been reported to be effective against multiple drug-resistant bacteria [7,8]. Plant products as herbal antibiotics have also been reported to be effective agent for the treatment of multidrug-resistant bacterial infections [9]. In this study, a selected number of ethnic vegetables using in traditional treatment was tested for their antibacterial activity against a spectrum of pathogens.

The experiment was aimed to find the antimicrobial potential in these plants which might substantiate their traditional use or explore novel potential or encourage further research for the finding of effective antimicrobial compounds.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

On the basis of using as traditional healing agents for many ailments, ten ethnic vegetables were selected for screening against thirteen pathogens (Tables 1, 2). Plant samples were identified and collected from the weekly local markets at Bandarban, Rangamati and Khagrachari districts of Chittagong Hill Tracts. Vegetables samples were collected from each of the markets for every plant. These samples were then certified by a Taxonomist of Dhaka University.

**Table 1. Plant tested for antibacterial activity**

| Local name | Scientific name | Edible portion | Traditional use [10] |
|---|---|---|---|
| Khoropata | *Cissus repens* Lam. | Leaves | Perineal healing, rectraction of the uterus |
| Kamino | *Caesalpinia digyna* Rottler | Leaves | Hypoglycemic, snakebite, rheumatism |
| Moroi | *Foeniculum vulgare* mill. | Leaves | Memory enhancing, antimicrobial, anti-inflammatory, cytotoxic, hypotensive etc |
| Yangfo | *Ficus benghalensis* L. | Leaves | Diabetic, constipation, antibacterial, costharoag |
| Ozonshak | *Spilanthes calva* DC. | Leaves | Inflammation, toothache, skin diseases, purgative, diuretic, lithotripter and dysentery |
| Mimini | *Centella assiatica* L. | Leaves | Management of central nervous system, skin and GITs disorder |
| Chikipung | *Rumex vesicarius* L. | Leaves | Cooling agent, curing stomach heat, toothache and to check nausea diabetes fever |
| Takbagun | *Solanum virginianum* L | Fruit | Epilepsy, pain relieving, headache, migraine, hair fall, bronchial asthma, skin problem, cough and other diseases |
| Aampata | *Mangifera indica* L | Leaves | Mouth infections, Gastrointestinal disorder, diabetes, diarrhea, scurvy, typhoid fever, sore throat, dysentery etc |

**Table 2. Pathogenic bacteria used in sensitivity test**

| Gram positive bacteria | Strain | Gram negative bacteria | Strain |
|---|---|---|---|
| *Bacillus subtilis* | BTCC 17 | *Shigella flexneri* | BTCC 499 |
| *Bacillus megateri* | BTCC 18 | *Shigella dysenteriae type-1* | BTCC 500 |
| *Bacillus cereus* | BTCC 19 | *Shigella sonnei* | BTCC 497 |
| *Staphylococcus aureus* | BTCC 43 | *Shigella boydii* | BTCC 498 |
| *Sarcinalutea* | BTCC 484 | *Escherichia.coli* | BTCC 482 |
| *Bacillus polymyxa* | BTCC 16 | *Salmonella B* | BTCC 495 |
| | | *Klebsiella species* | BTCC 13 |
| | | *Serratia species* | BTCC 198 |

## 2.2 Preparation of Crude Plant Extracts

### 2.2.1 Fresh extract

Three samples of each plant were pooled together to make a composite sample, from which fresh tender one was sorted out, washed and rinsed with distilled water. Excess water was wiped out with tissue paper and air-dried. A 500 g of the air-dried sample was cut into small pieces and blended in a mortar with a pestle. Fresh extract was collected by filtering through a muslin cloth and then by a filter paper under vacuum. The extract, thus obtained, was aliquoted into screw-capped glass vials and stored in a refrigerator.

### 2.2.2 Ethanol extracts

Fresh plant samples were collected, sorted, washed and rinsed as before. A 500 g of the air-dried sample was cut into small pieces and blended with twice the amount of ethanol in a mortar by a pestle. Ethanol extract was collected by filtering through a muslin cloth and then by a filter paper under vacuum. The extract, thus obtained, was aliquoted into screw-capped glass vials and stored in a refrigerator.

## 2.3 *In Vitro* Antibacterial Screening

### 2.3.1 Test materials and bacterial cultures

Sterile Matricel filter paper discs (5 mm dia) were impregnated with 20 µl (~100 µg/disc) of respective fresh and ethanol extracts. Ciprofloxacin (30 µg/disc) was used as standard antibiotic in the experiment. Bacterial cultures were collected from Bacterial Type Culture Collection (BTCC) from the Microbiological Laboratory at the Institute of Nutrition and Food

Science, University of Dhaka. Nutrient agar medium (Difco Laboratories, Detroit, MI, USA) was used to sub-culture the bacteria and to test the sensitivity of the bacteria to the plant extracts.

### 2.3.2 Procedure for sensitivity testing

Sensitivity of bacteria to the plant extracts was determined by the disc diffusion method. Nutrient agar plates (120 mm diameter) were seeded with respective bacterial culture using sterile cotton swabs. The test materials and antibiotic discs were placed on the agar plates, which were then kept in a freezer (at 4℃) overnight for diffusion of test materials into agar medium. The plates were then incubated at 37℃ for 24 hours to allow maximum growth of the bacteria. The test materials having antibacterial activity inhibited the growth of bacteria, and a clear zone of inhibition was shown around the discs. The bacterial sensitivity to the extracts was estimated by measuring the zone of growth inhibition [11].

However, since the bacterial growth inhibition does not mean the bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects.

Moreover, the agar disk-diffusion method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium. Nevertheless, an approximate MIC can be calculated for some microorganisms and antibiotics by comparing the inhibition zones with stored algorithms [12].

## 3. RESULTS AND DISCUSSION

It was shown that most of the plant extracts showed strong or moderate antibacterial activity against either a single or more bacterial strains (Table 3). Of the tested materials, fresh extracts of Kamino (*Caesalpinia digyna Rottler)* leaves, Chikipung (*Rumex vesicarius* L) and Ammpata (*Mangifera indica* L*)*; and ethanol extracts of Kamino leaves showed significant activity against most of pathogens used. Gram positive bacteria were found to be more sensitive than the gram negative strains. Fresh extract of Kamino showed potent antibacterial activity (20 to 32 mm diameter zone of inhibition) against most of the bacterial strains. Highest activity was noted against *Sarcina lutea* (32 mm diameter of zone of inhibition). Against *Escherichia coli* and *Shigella dysenteriae type-1*, Kamino indicated 24 mm and 21 mm zone of inhibition respectively. Ethanol extract of Kamino demonostrated mild antibacterial activity (10 to 15 mm zone of inhibition) against the tested organisms. Mild antibacterial activity was also recorded for fresh extract of Khoropata. Other extracts showed a poor or no antibacterial activity. It was noted that fresh extracts were found to be more active than the ethanol extracts.

The use of natural therapeutic products is as ancient as human civilization. Plants have always been a common source of medicaments either in the form of traditional preparations or as pure active principles [13]. A large number of currently used drugs are derived from plants [13-15]. About 25% of the drugs prescribed worldwide derive from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusive of plant origin and a significant number are synthetic drugs obtained from natural precursors [15].

Plants possess a wide variety and content of phytochemicals that make the variation in their medicinal value [16]. Leafy vegetables are natural source of antioxidants and rich in phytochemicals, which possess preventive or curative properties against many chronic diseases [3]. Plant has antimicrobial potential and the phytochemicals, particularly polyphenols and triterpenoids candidate antimicrobial principles [7].

In the present study, a significant variation in the antibacterial activity was observed for different plants, even for the fresh and ethanol extracts. Kamino leaves demonstrated highest antibacterial activity against most of the bacterial strains included in the experiment, while other plants showed lower or poor activity. Antibacterial activity for different plant products against different bacterial species has been documented by several other researchers [17-20]. The variation in the spectrum of antibacterial activity of different plant extracts was possible because of different varieties and contents of phytochemicals in them and was due to different mechanisms of antimicrobial action. Ethanol extract has shown less antibacterial action than fresh extract; this is probably due to the absence of some antimicrobial compounds in ethanol extract due for their insolubility. It is reported that

phytochemicals may act by inhibiting microbial growth, inducing cellular membrane perturbations, interference with certain microbial metabolic processes, modulation of signal transduction or gene expression pathways [21-22].

**Table 3. Antimicrobial activity of the extracts of the ethnic vegetables**

| Plant extract/ bacterial strain | Fresh extract (100 µg per disc) | | | | | | | | | Ethanol extract (100 µg per disc) | | | Ciprofloxacin (30 µg per disc) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 1 | 2 | 7 | |
| *Bacillus subtilis* | 12 | 24 | 7 | 8 | 13 | 11 | 14 | 9 | 18 | 10 | 15 | - | 39 |
| *Bacillus cereus* | 12 | 17 | - | 8 | 12 | 8 | 12 | 8 | 26 | 12 | 13 | 11 | 42 |
| *Bacillus megateri* | 11 | 23 | - | 16 | 17 | 7 | 13 | 7 | 22 | 9 | 13 | 8 | 28 |
| *Bacillus polymyxa* | 11 | 28 | - | - | 7 | 9 | 9 | - | 17 | 9 | 15 | 7 | 43 |
| *Staphylococcus aureus* | 7 | 29 | - | 13 | 7 | 8 | 9 | - | 17 | - | 11 | - | 36 |
| *Sercina lutea* | 13 | 32 | - | | | 11 | 13 | - | 18 | 8 | 12 | 9 | 33 |
| *Shigella Flexneri* | 10 | 21 | - | - | | - | 8 | - | 15 | - | 10 | - | 26 |
| *Shigella dysenteriae* type-1 | 10 | 21 | - | - | 7 | - | 11 | - | 16 | 7 | 10 | 8 | 36 |
| *Shigella sonni* | | 12 | 15 | - | - | - | - | 11 | - | 8 | 14 | - | 51 |
| *Shigella boydii* | 9 | 20 | - | - | - | - | 13 | - | 12 | 7 | 10 | 9 | 50 |
| *Escherichia coli* | 9 | 24 | - | 8 | - | | 10 | - | 15 | 8 | - | - | 60 |
| *Selmonella B* | 12 | 16 | - | - | - | - | 8 | - | 17 | 11 | 10 | - | 14 |
| *Klabsiella S* | 10 | 13 | - | - | - | 7 | 14 | - | 18 | 10 | 14 | 8 | 37 |

*'- ' indicated no zone of inhibition (extracts were not active)*

*Plant id represented as: 1- Khoropata (Cissus repens Lam.), 2- Kamino (Caesalpinia digyna Rottler), 3-Moroishak(Foeniculum vulgare Mill), 4- Yangfoo (Feics benghalensis L.), 5- Ozonshak (Spilanthes calva DC.), 6-Mimini (Centella assiatica L.), 7- Chikipung (Rumex vesicarius L.), 8- Takbagun (Solanum virginianum L.), 9- Ammpata (Mangifera indica L.)*

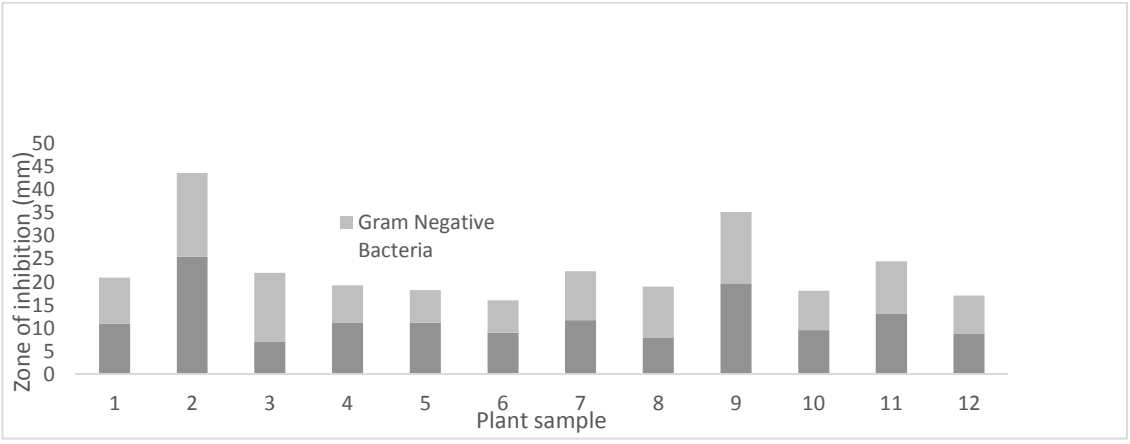


**Fig. 1. Antimicrobial activity of ethnic vegetables**

*'- ' indicated no zone of inhibition (extracts were not active)*

*Plant id represented as: 1- Khoropata (Cissus repens Lam.), 2- Kamino (Caesalpinia digyna Rottler), 3-Moroishak(Foeniculum vulgare Mill), 4- Yangfoo (Feics benghalensis L.), 5- Ozonshak (Spilanthes calva DC.), 6-Mimini (Centella assiatica L.), 7- Chikipung (Rumex vesicarius L.), 8- Takbagun (Solanum virginianum L.), 9. Ammpata (Mangifera indica L.) 10- Ethanol extract of 1, 11, Ethanol extract of 2, 12, ethanol extract of 7.*

## 4. CONCLUSION

These vegetables consumed by ethnic population of Bangladesh are mostly found to possess potential antimicrobial activity. Their traditional application in different diseases as well as infectious ones like the application of Aampata in mouth infection can be attributed to their antimicrobial action. Also further research aiming toward isolation of antimicrobial compounds from these plants is another prospect to look forward to.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The study was approved by the ethical committee of the Institute of Nutrition and Food Science.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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