

Original Research Article

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

ABSTRACT

Aim: Emergence of multidrug resistant bacteria makes the treatment of infections failure or 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: Disc diffusion method was employed to screen the antibacterial activity of fresh and 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) shak, Chikipung (*Rumex vesicarius* L) and Kochi ammpata (*Mangifera indica* L); and ethanol extracts of Kamino shak showed potent antibacterial activity against most of pathogens. Gram positive bacteria were found to be more sensitive than the gram negative strains. Fresh extract of Kamino 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 shak indicated 24 and 21 mm zone of inhibition respectively. Ethanol extract of Kamino showed mild activity (10 mm to 15 mm zone of inhibition) against the tested strains.

Conclusion: Ethnic vegetables have promising antibacterial activity. Fresh plant extracts are found to be more effective than the ethanol extracts, thus it is suggested to use fresh plant part.

Keywords: Ethnic vegetables; Plant products; Antibacterial activity, Foods

1. INTRODUCTION

Ethnic people are healthy and have less morbidity and higher life expectancy. They consume almost all of the plant and animal foods, especially those consumed by birds and monkey; thus they naturally screen edible and poisonous foods. The wild plants are rich in health promoting phytochemicals [1], which provide the lead molecules for modern drugs. Epidemiological studies reports 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 healthcare system and vast majority of global people rely on the plant medicine. The World Health Organisation estimates that 80% of the world population rely on traditional medicines for their health care; the remaining 20% people living in developed countries also depend on plant medicines [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 sporadic incidence of epidemic and make the treatment of infections difficult or 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.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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 (Table 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	<i>Cissus repens</i> Lam.	Leaves	Perineal healing, retraction of the uterus
Kamino	<i>Caesalpinia digyna</i> Rottler	Leaves	Hypoglycemic, snakebite, rheumatism
Moroi	<i>Foeniculum vulgare</i>	Leaves	Memory enhancing, antimicrobial, anti-inflammatory, cytotoxic, hypotensive etc
Yangfo	<i>Ficus benghalensis</i>	Leaves	Diabetic, constipation, antibacterial, costharaog
Ozonshak	<i>Spilanthes calva</i>	Leaves	inflammation, toothache, skin diseases, purgative, diuretic, lithotripter and dysentery
Mimini	<i>Centella asiatica</i>	Leaves	Management of central nervous system, skin and GITs disorder
Chikipung	<i>Rumex vesicarius</i> L	Leaves	Cooling agent, curing stomach heat, toothache and to check nausea diabetes fever
Takbagun	<i>Solanum virginianum</i> L	Fruit	Epilepsy, pain relieving, headache, migraine, hair fall, bronchial asthma, skin problem, cough and other diseases
Aampata	<i>Mangifera indica</i> L	Leaves	Mouth infections, GITs disorder, diabetes, diarrhea, scurvy, typhoid

			fever, sore throat, dysentery etc
Balapata	<i>Pouzolzia hirta</i>	Leaves	Ulcer, syphilis, gonorrhoea, sores, stomachache

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53 2.2.Preparation of crude plant extracts

54 2.2.1. Fresh extract

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

61 2.2.2. Ethanol extracts

62 The plant materials were processed as of the fresh extract. After blending the sample, ethanol was
63 added to it in a ratio of 1:2 to soak for 48 hours. The extract was collected by filtering through a muslin
64 cloth and filter paper under vacuum and then refrigerated.

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66 Table 2: Pathogenic bacteria used in sensitivity test

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

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68 2.3 In vitro antibacterial screening

69 2.3.1 Test materials and bacterial cultures

70 Sterile Matricel filter paper discs (5 mm dia) were impregnated with 20 µl (~250 µg/disc) of respective
71 fresh and ethanol extracts. Ciprofloxacin (30 µg/disc) was used as standard antibiotic in the
72 experiment. Bacterial cultures were collected from Bacterial Type Culture Collection (BTCC) from the
73 Microbiological Laboratory at the Institute of Nutrition and Food Science, University of Dhaka.
74 Nutrient agar medium (Difco Laboratories, Detroit, MI, USA) was used to sub-culture the bacteria and
75 to test the sensitivity of the bacteria to the plant extracts.

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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°C) overnight for diffusion of test materials into agar medium. The plates were then incubated at 37°C 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.

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*)shak, Chikipung (*Rumex vesicarius* L) and Kochi ammpata (*Mangifera indica* L); and ethanol extracts of Kamino shak 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 demonstrated 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.

Table 3: Bacterial sensitivity to the ethnic vegetables (expressed in zone of inhibition in mm diameter)

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	
<i>Bacillus subtilis</i>	12	24	7	8	13	11	14	9	18	10	15	-	39
<i>Bacillus cereus</i>	12	17	-	8	12	8	12	8	26	12	13	11	42
<i>Bacillus megateri</i>	11	23	-	16	17	7	13	7	22	9	13	8	28
<i>Bacillus polymyxa</i>	11	28	-	-	7	9	9	-	17	9	15	7	43
<i>Staphylococcus aureus</i>	7	29	-	13	7	8	9	-	17	-	11	-	36
<i>Sercinalutea</i>	13	32	-			11	13	-	18	8	12	9	33
<i>ShigellaFlexneri</i>	10	21	-	-		-	8	-	15	-	10	-	26
<i>Shigelladysenteriae type-1</i>	10	21	-	-	7	-	11	-	16	7	10	8	36
<i>Shigellasonni</i>		12	15	-	-	-	-	11	-	8	14	-	51
<i>Shigellaboydii</i>	9	20	-	-	-	-	13	-	12	7	10	9	50

<i>Escherichia coli</i>	9	24	-	8	-		10	-	15	8	-	-	60
<i>Salmonella B</i>	12	16	-	-	-	-	8	-	17	11	10	-	14
<i>Klasiella S</i>	10	13	-	-	-	7	14	-	18	10	14	8	37

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104 ‘-’ indicated no zone of inhibition (extracts were not active)

105 Plant id represented as: 1- Khoropata shack (*Cissusrepens Lam.*), 2- Kamino shack
 106 (*Caesalpinia digyna*), 3-Moroishack(*Foeniculum vulgare*), 4- Yangfoo(*Feicse benghalensis*), 5- Ozon
 107 shack (*Spilanthes calva*), 6-Mimini (*Centella asiatica*), 7- Chikipung (*Rumex vesicarius L.*), 8-
 108 Takbagun (*Solanum virginianum*), 9- Kochi ammpata (*Mangifera indica*)

109 The use of natural therapeutic products is as ancient as human civilization. Plants have always been
 110 a common source of medicaments either in the form of traditional preparations or as pure active
 111 principles [1]. A large number of currently used drugs are derived from plants [11-13]. About 25% of the
 112 drugs prescribed worldwide derive from plants, 121 such active compounds being in current use. Of
 113 the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are
 114 exclusively of plant origin and a significant number are synthetic drugs obtained from natural
 115 precursors [13].

116 Plants possess a wide variety and content of phytochemicals that make the variation in their
 117 medicinal value [14]. Leafy vegetables are natural source of antioxidants and rich in phytochemicals,
 118 which possess preventive or curative properties against many chronic diseases [3]. Plant has
 119 antimicrobial potential and the phytochemicals, particularly polyphenols and triterpenoids candidate
 120 antimicrobial principles. [7].

121 In the present study, a significant variation in the antibacterial activity was observed for
 122 different plants, even for the fresh and ethanol extracts. Kamino shack demonstrated highest
 123 antibacterial activity against most of bacterial strains included in the experiment, while other plants
 124 showed lower or poor activity. Antibacterial activity for different plant products against different
 125 bacterial species has been documented by several other researchers [15-18]. The variation in the
 126 spectrum of antibacterial activity for different plant extracts was possibly because of different varieties
 127 and contents of phytochemicals in them and was due to different mechanisms of antimicrobial action.
 128 It is reported that phytochemicals may act by inhibiting microbial growth, inducing cellular membrane
 129 perturbations, interference with certain microbial metabolic processes, modulation of signal
 130 transduction or gene expression pathways [19,20].

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4. CONCLUSION

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Ethnic vegetables, consumed as well as used in traditional treatment by the ethnic people, may lead to be promising antibacterial agent(s). As fresh plant extracts are found to be more effective, it is suggested to use fresh plant part.

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