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Traditional healing and antimicrobial role of the herbal drug against UTIs by ethnic people of Darjeeling tea gardens, India

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The herbal age-old traditional method is practiced even today to treat Urinary Tract Infection (UTI) by the ethnic inhabitants of tea gardens of Darjeeling Himalaya, India. The aim of this study was to explore and document the traditional ways of healing UTIs. The information was collected from selected tea gardens and data was quantitatively analyzed with the help of ethnobotanical indices *viz.*, use value, plant part value, family use value, fidelity level (%), and informant consensus factor. Antibiogram of four UTI-causing bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*) was also established by Kirbye Bauer's disc diffusion method. Antibacterial activity of twelve mostly used plants like *Cheliocostus speciosus*, *Equisetum diffusum*, *Saccharum officinarum*, *Elettaria cardamomum*, *Coriandrum sativum*, *Plantago asiatica* ssp. *erosa*, *Centella asiatica*, *Achyranthes bidentata*, *Carex cruciata*, *Drymaria cordata* ssp. *diandra*, *Nephrolepis cordifolia*, *Malvaviscus arboreus* were assessed against the aforementioned bacterial strains. *Drymaria cordata* and *Centella asiatica* on comparative MIC and MBC study showed the lowest MIC and MBC value of 0.29 mg/mL each against *Staphylococcus*, representing their effectivity. *Nephrolepis cordifolia* with 100% FL showed the lowest MIC and MBC value 0.67 mg/mL each against *E. coli K12*. The uses of these plants known from the ethnomedicinal knowledge of the healers could be promoted as complementary medicine to treat UTI.

Keywords: Antibiogram, Darjeeling Himalaya, Ethnic knowledge, Ethnobotanical indices, Plant extract, Urinary tract infection

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Urinary tract infections (UTIs) have become one of the stereotypical issues among the present-day human population as it spreads as community-acquired or nosocomial infection[1]. It has been estimated that about 150 million people encounter UTI every year globally. It is also said that nearly 40% of women population worldwide develop UTI at the minimum during their life span as the women have a shorter urethra, liable to more bacterial infection than men. Urinary tract infection generally adverts to the pathological condition where the bacterial count in the urine is more than 10^5/mL[2,3]. Bacteria colonize different niches within our body from the gastrointestinal tract to the bloodstream. Symptomatic UTI is also called as cystitis or lower UTI, when bacteria colonize the bladder through the urethra. Both the male and female can suffer from bacterial cystitis. Untreated cystitis may progress to the kidneys through ureters causing pyelonephritis. Further, pyelonephritis may often lead to bacteremia, the occurrence of bacteria in the bloodstream[4]. Clinical classification of UTI includes complicated and uncomplicated UTIs. Urogenital infection when gets associated with diseases such as diabetes mellitus, sickle cell diseases, and also analgesic abuse, calculi, strictures, presence of catheters, and if remain untreated may lead to kidney damage and urosepsis and are generally considered as complicated UTI[5]. Recurrent and untreated UTI in pregnant women may also result in preterm delivery[6].

Most studies on UTI show that the *Escherichia coli* are responsible for 80-85% of infection. However, other bacteria like *Enterobacter aeruginosa*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *K. oxytoca*, *Staphylococcus aureus* and fungi like *Candida albicans* also impart the remaining infection. The etiology of UTI in adult females may include pregnancy, sexual intercourse, use of a diaphragm, spermicidal, and not maintaining personal hygiene like single-use of tampons, sanitary napkins for more than 4 h a day[7].

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Villages of tea garden of Darjeeling hills inhabit many indigenous tribes and ethnic communities rich in traditional knowledge. *Lepcha, Sherpa, Kirat, Khas, Newar, Bahun, Sunar, Yolmo, Bhutia, Tamang, Gurung, Mangar, Kami, Damai* are the aboriginal communities residing there and are very much dependent on locally available wild medicinal plants for their primary health care. Dependency on herbal drugs may be owing to inadequate medical facilities, ill mode of transport in the remote areas of the tea gardens, poor economic status, or their belief system over age-old practices of ethnomedicinal knowledge to cure health disorders[8]. This work has been executed in two ways, firstly, herbal formulae along with plant list were collected through the comprehensive survey in selected tea gardens of Darjeeling hills and their quantitative study. Secondly, *in-vitro* antibacterial activities of these plant extracts against three gram-negative bacteria such as *E. coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* and a gram-positive bacteria, *Staphylococcus aureus* were examined.

The efficacy of the ethno-medicinal formulation was recorded physically. Inclusion of quantitative analysis being the first attempt from the study area has increased the level of significance of the work. Nevertheless, the present work has revealed the hidden knowledge of the ethnic communities of the tea gardens of the Darjeeling hills to cure UTI. The present study also bridges the gap between folklore and scientific investigation. Antibacterial assay of the selected plant extracts against UTI-causing bacteria would be a novel approach to authenticate the ethno-medicinal knowledge of the tea garden workers of the Darjeeling hills.

Materials and Methods

Source of information

The information was gathered (2016-2019) by conducting extensive surveys across the villages associated with the major tea estates of Darjeeling Himalayas like Dhajea (26º59′40″N & 88º12′79″E), Nagri (26º55′29″N & 88º12′29″), Turzum (26º94′84″N & 88º17′62″E), Longview (26°81′56″ N & 88°26′07″E), Chamong (26º47′.376″N & 88º19′.012″), Ambootia (26º87′43″ N & 88º 24′13″ E), Pankhabari (26º50′0″N & 88º16′00″), Marybong (27°03′52″ N & 88°19′22″ E), Ambiok (26°58′12.00″ N & 88°42′0.00″ E) and Mim (27º02′34″ N & 88º16′57″).

Survey for herbal drugs

There are nearly 87 tea gardens spread over Darjeeling and Kalimpong hills and tea gardens are comprised of many villages[9]. However, among the ten randomly selected tea gardens (nine from Darjeeling and one from Kalimpong) ethno-medicinal formulation could be gathered only from seven gardens (Table 1 & Fig. 1).

Prior informed consent (PIC) had been considered and the information was collected following a pre-designed questionnaire (Table 2). The structured questionnaire consisted of questions like name, occupation, age, sex, education, source of knowledge, in practice since, vernacular name of the plants, parts used, mode of use, dosage, prohibited food during medication, drug efficacy, healing period and reason behind not cure.

Collection of samples

Plant species were spotted at different hilly forested villages and adjoining areas of tea gardens, photographed. The fertile sample were collected and processed through entire conventional herbarium techniques[10]. For authentic identification, local floras were consulted and finally matched with pre-identified herbarium specimens of the University of North Bengal (NBU) herbarium[11-13]. To validate the accepted scientific name of the genus, species, families, and author citation, popular websites were consulted[14,15] and voucher specimens were deposited to NBU Herbarium (Table 1). To determine the efficacy, interviews were conducted among the patients of different tea gardens with their consent.

Quantitative data analysis

The following five quantitative ethnobotanical indices have been considered for the present study.

Use value (*UV*)

The relative importance of the plant species is determined by this factor[16]

$$Use\ value\ (UV) = \Sigma U/N$$

Where, U= number of use reports mentioned by each informants for a given species.

N= Total number of informants.

$$Plant\ part\ value\ (PPV) = \frac{\mathrm{RU}_{\mathrm{plant\ part}}}{\mathrm{RU}}$$

Where, RU= number of uses reported for all parts of the plant

$\mathrm{RU}_{\mathrm{Plant\ part}}$= Sum of the uses reported per part of the plant. The plant part having highest part value (PPV) is the most used by the informants[17].

Table 1 — Ethno-medicinal formulations practiced against UTI

Disease Type	Species [vernacular name] Family and Accession no.	Parts used	Mode of use, dosage, and time of healing
Burning with urination	*Plantago asiatica* ssp. *erosa* (Wall.) Z. Yu Li [*Sitalu/Masalejhar*]; Plantaginaceae Accession No. 10702	Leaves and roots	All the plant materials are cut into small pieces and grounded in a traditional stone-made grinder and squeezed. 250 g palm candy is pounded and dissolved in 250 mL boiled water and mixed with that plant extract. In acute cases, the *Cheilocostus* is used.
	Equisetum diffusum D. Don [*Kurkurejhar/Kharayojhar*]; Equisetaceae Accession No.10705	Rhizome	
	Cheilocostus speciosus (J. Koenig) C.D. Specht [*Betlauri*]; Costaceae Accession No. 10842	Stem	
	Coriandrum sativum L. [*Dhania*]; Apiaceae Accession No.10848	Leaves	
Burning with urination	*Equisetum diffusum* D. Don [*Kurkurejhar/Kharayojhar*] Equisetaceae Accession No.10705	Tuber	Small pieces of all the plant materials & palm candy are made, and put in 1.5-litre boiled water, and covered with a lid and kept overnight. It is then filtered and the filtrate (30 mL, early in the morning, after lunch, and after dinner) is taken and should be completed within 3 days.
	Saccharum officinarum L. [*Kaloukhoo/Gewari*]; Poaceae Accession No. 10851	Stem	
	Cheilocostus speciosus (J. Koenig) C.D. Specht [*Bet lauri*] Costaceae Accession No. 10842	Stem	
	Carica papaya L. [*Mewa*]; Caricaceae Accession No. 10855	Fruit	
Burning with urination	*Saccharum officinarum* L. [*Kaloukhoo/Gewari*] Poaceae Accession No. 10851	Stem	All the ingredients are crushed in wooden mortar and pestle. 1-litre water is boiled and to lukewarm water, all the ingredients including palm candy are soaked for 30 min and sieved and the filtrate is taken as medicines. 30 mL is taken three times, 5-10 min before each meal. Chillies, ginger, lentils should be avoided.
	Cheilocostus speciosus (J. Koenig) C.D. Specht [*Bet lauri*] Costaceae Accession No. 10842	Stem	
	Elettaria cardamomum (L.) Maton [*Chhota Alaichi*] Zingiberaceae Accession No.10844	Fruit	
	Achyranthes bidentata Bl. [*Nakarakpa*] Amaranthaceae Accession No.10755	Roots	
	Carex cruciata Wahlenb [*Harakatta*]; Cyperaceae Accession No.10757	Roots	
Burning with urination	*Equisetum diffusum* D. Don [*Kurkurejhar*]; Equisetaceae Accession No.10705	Tuber	All the ingredients are washed, cut into small pieces, and crushed and squeezed. Palm candyis dissolved in half-litre water and mixed well with the plant extracts. It can be stored in a glass/ plastic container and can be used for 2-3 days. 30 mL is taken in the morning and the evening daily.
	Saccharum officinarum L. [*Kaloukhoo*]; Poaceae Accession No. 10851	Stem	
	Cheilocostus speciosus (J. Koenig) C. D. Specht [*Bet lauri*]; Costaceae Accession No. 10842	Stem	
Burning with urination	*Equisetum diffusum* D. Don [*Kurkurejhar/Kharayojhar*]; Equisetaceae Accession No.10705	Tuber	All the plant materials are washed, crushed and soaked in 1 litre boiled water, and sieved and juice is taken as medicine, 30 mL early in the morning and the evening
	Cheilocostusspeciosus (J. Koenig) C. D. Specht [*Bet lauri*]; Costaceae Accession No. 10842	Stem	

(*contd.*)

Table 1 — Ethno-medicinal formulations practiced against UTI (*contd.*)

Disease Type	Species[vernacular name] Family and Accession no.	Parts used	Mode of use, dosage, and time of healing
Burning with urination	*Coriandrum sativum* L. [*Dhania*]; Apiaceae Accession No. 10848	Seeds	Coriander (250 g) and palm candy are soaked in 200 mL water over night. *Kabab Chini*, and all the plant parts are crushed separately and put in already prepared palm candy water and kept for 15 min and sieved. Thus, the prepared juice is taken 2-4 days, two times a day before meal or throughout the day.
	Elettaria cardamomum (L.) Maton [*Chhotaalaichi*]; Zingiberaceae Accession No. 10844	fruits / seed	
	Centella asiatica (L.) Urb. [*Ghodatapre/Aathanejhar*]; Apiaceae Accession No. 10847	Leaves	
	Drymaria cordata ssp. *diandra* (Bl.) J. A. Duke [*Abhiijaalo*]; Caryophyllaceae Accession No. 10850	leaves & stem	
Burning with urination	*Cheilocostus speciosus* (J. Koenig) C .D. Specht [*Bet lauri*]; Costaceae Accession No. 10842	Stem	All the ingredients are cut into small pieces and sundried for 1-2 days and pounded to powder and small balls are made. 5 balls are given for five days. 1 ball is dissolved in 1 litre cold water and taken throughout the day. Lentils, dairy products, eggs, chicken, and hot food products are strictly avoided.
	Abutilon pictum (Gillies ex Hook.) Walp. [*Ghantiful*]; Malvaceae Accession No. 10841	Flower	
Burning with urination & Leukorrhea	*Cheilocostus speciosus* (J. Koenig) C.D. Specht [*Bet lauri*] Costaceae Accession No. 10842	Stem	Both the ingredients are mixed, crushed, and soaked in 1 litre hot water for 15-20 min, sieved and the extract is taken as medicine. 30 mL is taken empty stomach and in the evening. Prohibited foods: lentils, hot chillies, chicken, mutton, beef, buff, egg.
	Nephrolepis cordifolia (L.) C. Presl [*PaaniAmala /BhuiAmala*]; Nephrolepidaceae Accession No.10337	Tuber	
UTI	*Drynaria quercifolia* (L.) J. Sm. [*Tarwareunieu*]; Polypodiaceae Accession No.10849	Rhizome	All the plant materials are washed, crushed in a mortar and pestle (iron) and 50 g palm candy is added to the mixture, soaked in 2-3 litre boiled water for 15-20 min, sieved and the filtrate is taken as medicines. Hot, spicy, sour food items and lentil should be avoided.
	Equisetum diffusum D Don [*Salli-bisalli*]; Equisetaceae. Accession No.10705	tuber	
	Scoparia dulcis L. [*Mishrijhar*]; Plantaginaceae Accession No.10770	Roots and leaves	
	Elettaria cardamomum (L.) Maton [*Chhotaalaichi*]; Zingiberaceae Accession No. 10844	Fruits / seed	
UTI	*Malvaviscus arboreus* Cav. [*Bhalephul*]; Malvaceae Accession No.10840	Flowers	The two flowers are chewed and swallowed every morning during infection.

***Family use value* (*FUV*)**

It helps to identify the significance of the plant families. It is calculated as[18]

$$\text{Family Use Value (FUV)} = \frac{\text{UVs}}{\text{Ns}}$$

Where, UVs = UV is the number of respondents mentioning the family.

Ns is the total number of species within the family.

***Fidelity level* (*FL*) (*%*)**

Fidelity level is calculated as percentage of the informants citing the particular plant species for treating the given disease and the total number of informants who mentioned the same plant for treating any other disease. It is determined by the following formula[19]

$$Fidelity\ Level\ (\%) = \frac{I_p}{Iu} \times 100$$

Where, I_p is the total number of informants who claimed the particular plant for treating the same

Fig. 1 — A. *Carex cruciata* B. *Saccharum officinarum* C. *Cheilocostus speciosus* D-J. Preparation of medicines by Mr. Khem (Formulation-3) E. Healer with root of *Achyranthes bidentata* F. Cutting of plant materials G. Crushing all the ingredients in wooden mortar and pestle H. Crushed materials I. Sieving of sample J. Healer praying the Mother Nature

Table 2 — Healer's information

Formulation	Informer's name and address	Age	Sex	Education	Occupation	Source of knowledge
1	Sitaram Sharma; Nagri T.E. (Bahungaon)	55	M	Class VIII	TG worker	Inherited & by trial and error methods
2	Arjunsingh Tamang Marybong T.E.	63	M	Class V	Retd. TG worker	
3	Khem Thapa Dhajea T.E.	80	M	Class V	Retd. TG Chowkidar	
4	Chandra Bir Chettri, Dhajea Ranibon	68	M	Unread	Retired TG worker	
5	Nima Sherpa Mim T.E.	71	M	Unread	Retired TG worker	
6	Roma Tamang Nagri T.E.	61	F	Class X	Homemaker	
7	Kalyan Lama Pankhabari T.E.	48	M	Class IX	Taxi Driver	
8	Bhim Bahadur Tamang & Niru Tamang	Both 64 yrs	M & F	Unread	Tamang priest (Lama) & TG (Retd.) worker	
9	Bom Bahadur Rai, Upper Ambiok Busty, Dhip Dara	59	M	B. Com	Farmer	
10	Rahar Bahadur Darjee, Paanch Ghare, Ambootia T.E.	64	M	Unread	Carpenter (Retd.)	

major ailment and I_u is the total no of informants who uses the same plant for treating any other ailment.

***Informant consensus factor* (*ICF*)**

Informant consensus factor was used to see the level of homogeneity among the informants for using the plant species for treating the particular ailment category. Its value ranges from 0 to 1. ICF near to 1, means a large number of informants agree with the use of particular plant against the given ailment category. A low value indicates that there was no

exchange of information among the healers. ICF is calculated as[19]

$$Informant\ Consensus\ Factor\ (ICF) = \frac{\mathrm{Nur} - \mathrm{Nt}}{\mathrm{Nur} - 1}$$

Where, Nur is the number of use reports for a particular disease category and Nt is the number of taxa used by the informants interviewed for that category.

Preparation of plant extract

Plant parts were dried separately at ambient temperature and pounded to powder using a mixer grinder. They were then filtered through a 40 µm mesh sieve and thus fine powder was obtained. The powdered materials are then stored in air-tight containers at 4°C to prevent the growth of fungi and other microorganisms. Original stock solutions of plant extracts were prepared with methanol, using 44mg plant extract/mL of 10% DMSO solution, and distilled water. Each stock solution was diluted to obtain final concentrations of 0.29, 0.67, 1.51, 3.41, 4.27, 9.63, and 21.67 mg/mL with the DMSO solution.

Growth condition of selected bacterial strains

UTI-causing strains of four pathogenic bacteria (*Staphylococcus aureus* MW073410, *E. coli* K12 MTCC1302, *Pseudomonas aeruginosa* MW073400 and *Proteus vulgaris* MN809382) were considered for the antibacterial activity test. All the strains were cultured in Nutrient Agar slants at 4°C. *E. coli* K12 MTCC1302 was purchased from MTCC while the other bacteria were isolated from stagnant pond water of the University. The strains were sub-cultured in fresh Nutrient Agar (NA) slants at 37°C and 24 h culture was used for further experiments.

Antibiotic sensitivity test

All bacterial strains were subjected to antibiotic sensitivity tests by Kirbye Bauer's disc diffusion method[20]. Inoculums of respective strains were prepared by inoculating Luria Broth with respective bacterial cultures and allowed to grow up to 2-4 h. Mueller Hinton Agar media was sterilized and then poured on sterile Petri plates (200 mm diameter) and allowed to solidify at room temperature. After solidification, prepared inoculums of respective strains were swabbed on plates and allowed to dry for 15 min.

After drying, different antibiotic discs *viz.*, Cefotaxime CTX (30 µg), Cefuroxime CXM (30 µg), Chloramphenicol C (30 µg), Gentamycin GEN (10 µg), Nalidixic Acid NA (10 µg), Norfloxacin NX (10 µg), Penicillin GP (10 µg), Imipenim IM (10 µg), Tetracycline TE (30 µg), Trimethoprim TR (5 µg), Amoxicillin AX (10 µg) and Streptomycin S (25 µg) were placed carefully over the inoculated media. The plates were incubated at 30°C for 24-48 h and observed for the formation of clear zones around the antibiotic discs and the zone diameter (in mm) was recorded (Fig. 2). Resistance and sensitivity of the isolates towards the antibiotics were assessed according to the antibiotic disc manufacturer's protocol[21].

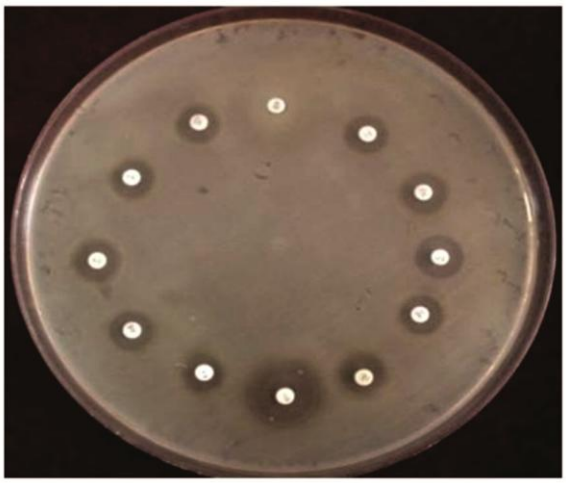

Fig. 2 — Antibiotic susceptibility of the test pathogens *E. coli* K12 against twelve antibiotics (Nalidixic acid, Imipenim, Gentamycin, Cefotaxime, Tetracyclin, Kanamycin, Trimethoprim, Amoxicillin, Streptomycin, Chloramphenicol, Cefuroxime, and Norfloxacin)

Antibacterial test of plant extracts

Each bacterial strain was found resistant against most of the tested antibiotics and these bacterial strains were further tested against methanolic extracts of different parts of 12 plant species by the agar-well diffusion method[20]. The antibacterial activity profile was assessed like before and the final results are presented.

Determination of MIC and MBC of plant extracts

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the active plant extracts were determined[22]. A separate experiment was conducted for each plant extract. An aliquot of 80 µL of each dilution of a plant extract was released into a well on a 96-well microtiter plate, along with an aliquot of 100 µL MH broth, an aliquot of 20 µL bacterial inocula (10^9 colony forming

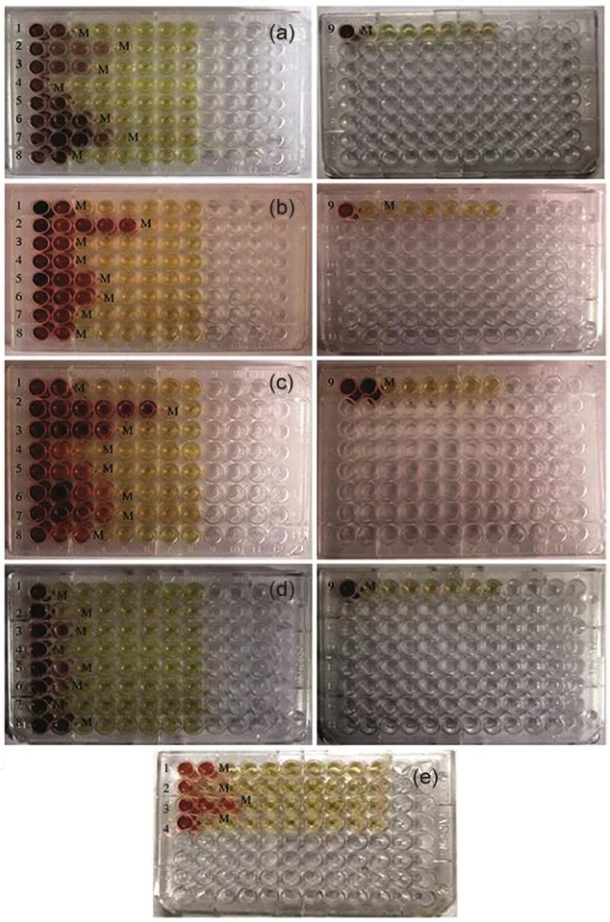

Fig. 3 — Determination of minimum inhibitory concentrations in a 96-well microtiter plate of nine selected plant extracts against 4 test pathogens (A: *P. vulgaris*, B: *P. aeruginosa*, C: *E. coli* K12, and D: *S. aureus*) and E represents the MIC value of Chloramphenicol (standard) against the pathogens. M= MIC at numbers that signifies the lowest concentration of leaf extract

units/mL), and a 5 µL aliquot of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC). After pouring all the aforementioned ingredients into a well, the microplate was incubated at 37°C for 18 h. The development of red coloration due to TTC indicated bacterial growth, whereas the absence of the color indicated inhibition of bacterial growth. The first well of the microplate was the control without any plant extract (Fig. 3). The MIC value was noted at the well where no colour appeared. Further, bacteria from each well of the microplate were sub-cultured on a nutrient agar plate; the level of dilution, where no bacterial growth on the nutrient agar plate was observed, was noted as the MBC value[23].

Statistical analysis

All experiments were done in triplicates. For antibiotic sensitivity test, data from three independent experiments were averaged. Standard error was calculated using the statistical software SPSS version 18.0. Data are represented as triplicate of mean ± SD.

Results

Urinary tract infections (UTIs) or inflammation-related all possible information were carefully documented from various patients of different ages and sex groups to understand the efficacy of the traditional herbal drugs.

Quantitative data analysis

The highest UV was shown by *Cheilocostus speciosus* (0.7), followed by *Equisetum diffusum* (0.5), *Saccharum officinarum* (0.3) (Table 3). Among the various plant parts, the leaf, root and flower showed the highest PPV, 0.5 each. This was followed by rhizome, tuber, stem and fruit (0.25 each). The least PPV was shown by the seed (0.12) (Supplementary Fig. S1). The highest FUV (7) was shown by Coastaceae followed by Equisetaceae (5), Poaceae and Zingiberaceae (3 each) (Supplementary Fig. S2). The fidelity level (%) varied from 16.66 to 350. The percentage value more than 100 occurred as many respondents claimed the use of same plant for treating the given disease category and fewer claimed to treat the any other disease categories. The low FL level indicates that the individual informant is using the particular plant for treating UTI in the study area and the same plant is being used against many other disorders by the other informants. The Informant consensus factor was recorded as 0.5.

Drug efficacy test

Interview with the healers and accessible patients helped to determine the efficacy of the formulations against UTI theoretically. The vulnerability of infection was found more in females than in males (Table 4). Further, the risks of infection in matured females are higher than the young ones. Of the enlisted formulas, formulation 9 has high efficacy as it showed 100% cure. However, a decrease in efficacy may be due to an increase in the number of patients thereby encountering more complications, or maybe due to not following the proper prescription.

Antibiotic sensitivity test

Antibiotic sensitivity test showed that the studied microorganisms responsible for causing UTI were found resistant to most antibiotic discs (Table 5).

Table 3 — Quantitative indices of the recorded plants used to treat UTI. [*Abbreviation used*: UV: Use value, PPV: Plant Part value, FL: Fidelity level, ICF: Informant consensus factor]

Taxa	No. of Citations (Nur)	Total no of informants interviewed	Parts used	Quantitative Indices				
		10		UV	PPV	FUV	FL (%)	ICF
Plantago asiatica ssp. *erosa*	1		Leaves & Roots	0.1	0.5 (L) & 0.5 (R)	1	200	0.5
Equisetum diffusum	5		Rhizome & Tuber	0.5	0.25 (Rh) &) 0.25 (T)	5	250	
Cheilocostus speciosus	7		Stem	0.7	0.25	7	350	
Coriandrum sativum	2		Leaves	0.2	0.5	1.5	200	
Saccharum officinarum	3		Stem	0.3	0.25	3	150	
Carica papaya	1		Fruit	0.1	0.25	1	100	
Elettaria cardamomum	3		Fruits & Seeds	0.3	0.25 (F) & 0.12 (S)	3	300	
Achyranthes bidentata	1		Roots	0.1	0.5	1	100	
Carex cruciata	1		Roots	0.1	0.5	1	100	
Centella asiatica	1		Leaves	0.1	0.5	1.5	20	
Abutilon pictum	1		Flower	0.1	0.5	1	100	
Nephrolepis cordifolia	1		Tuber	0.1	0.25	1	100	
Drynaria quercifolia	1		Rhizome	0.1	0.25	1	100	
Scoparia dulcis	1		Leaves & Roots	0.1	0.5 (L) & 0.5 (R)	1	16.66	
Malvaviscus arboreus	1		Flowers	0.1	0.25	1	100	
Drymaria cordata ssp. *diandra*	1		Leaves & Stem	0.1	0.5 (L) & 0.25 (St)	1	16.66	
NtΣ16	Σ31							

Table 4 — Physical investigation among the patients for drug Efficacy rate test [*Abbreviation used*: NFD= Not following drug; SC= Severity of cases; PP= Patients physiology; ID= Improper diet; CMA= Cases may be acute; NC= Not cured; PC=Partially cured; FC=Fully cured]

Formulations	Healers name (Sub-community)	Patients treated												
		Age groups in years						Sex			Efficacy			Reason behind NC
		5 –14	15 -25	26 – 36	37 - 47	48 – 58	>59	M	F	T	FC	PC	NC	
1	Sita Ram Sharma (*Bahun*)	2	1	3	4			3	7	10	7	-	3	NFD
2	Anjursingh Tamang (*Tamang*)	20	30	40	50	35	20	80	115	195	120	45	30	NFD
3	Khem Thapa (*Mangar*)	12	20	11	12	15	11	30	51	81	50	20	11	NFD
4	Chandra Bir Chettri (*Khas*)	-	1	2	1	3	2	3	6	9	3	5	1	-
5	Nima Sherpa (*Sherpa*)	-	2	4	7	5	2	5	15	20	15	3	2	SC, NFD
6	Roma Tamang (*Tamang*)	2	4	5	3	-	-	6	8	14	10	3	1	NFD
7	Kalyan Lama (*Tamang)*	5	10	9	7	5	4	10	30	40	25	10	5	NFD , PP, ID, SC
8	Bhim Bahadur Tamang & Niru Tamang (*Tamang*)	3	5	7	6	5	3	11	18	29	20	5	4	NFD, ID
9	Bom Bahadur Rai (*Kirat*)	3	4	3	2	1	2	6	9	15	10	5	_	NFD
10	Rahar Bahadur Darjee (*Damai*)	2	3	-	-	2	-	-	-	7	5	1	1	CMA

E. coli K12 was resistant against antibiotics such as Nalidixic acid (NA), Imipenim (IM), Gentamycin (GEN), Cefotaxime (CTX), Tetracyclin (TE), Kanamycin (K), Amoxicillin (AX), Streptomycin (S), Cefuroxime (CXM), Norfloxacin (NX). But it was susceptible to Trimethoprim (TR) and Chloramphenicol (C). *Pseudomonas aeruginosa* was susceptible to Tetracyclin, Chloramphenicol, and

Table 5 — Antibiotic susceptibility test [*Abbreviations used*: NA= Nalidixic acid, IM = Imipenim, GEN = Gentamycin, CTX= Cefotaxime, TE =Tetracyclin, K= Kanamycin, TR= Trimethoprim, AX = Amoxicillin, S= Streptomycin, C= Chloramphenicol, CXM = Cefuroxime, NX= Norfloxacin, R= Resistant, S = susceptible]

Antibiotics	*E. coli*	*P. aeruginosa*	*P. vulgaris*	*S. aureus*
NA	R	R	R	R
IM	R	R	S	S
GEN	R	R	R	R
CTX	R	R	R	S
TE	R	S	R	R
K	R	R	R	R
TR	S	R	R	R
AX	R	R	R	R
S	R	R	R	R
C	S	S	S	S
CXM	R	R	R	S
NX	R	S	R	R

Norfloxacin whereas resistant against the rest of the antibiotics. *Proteus vulgaris* on the other hand was susceptible to Imipenim and Chloramphenicol while resistant against NA, GEN, CTX, TE, K, AX, S, CX M, and NX. Similarly, *Staphylococcus aureus* was resistant against NA, GEN, TE, K, TR, AX, S, and NX but susceptible to IM, CTX, C and CXM.

Antibacterial test of plant extracts

Among the twelve tested plants, the three plants *viz*., stem of *Cheilocostus speciosus*, the tuber of *Equisetum diffusum*, and seeds of *Coriandrum sativum* did not show a clear zone of inhibition (Fig. 4) as compared to the other nine species. Therefore, those three were excluded from the subsequent MIC and MBC tests.

MIC and MBC of plant extract

The results of MIC and MBC of the nine effective plant extracts are given in Table 6. Methanol extract of the tuber of *N. cordifolia* had the lowest MICs 0.29 mg/mL against *S. aureus* and 0.67 mg/mL each against *E. coli*, *P. aeruginosa* and *P. vulgaris*. It also showed MBC 0.67 mg/mL against *E. coli* and *S. aureus, A. bidentata* exhibited the lowest MIC 0.67 mg/mL against *S. aureus*. Methanolic extract of *E. cardamomum* seeds showed the lowest MIC and MBC 0.67 mg/mL against *P. aeruginosa*. *D. cordata* showed the lowest MIC and MBC 0.29 mg/mL each against *S. aureus*. Similarly, *C. asiatica* showed the lowest MIC and MBC 0.29 mg/mL against *S. aureus*. *C. cruciata* showed the highest MBC value 3.41 mg/mL against *E. coli*, *P. aeruginosa*, and *S. aureus* and the same MIC value against *E. coli*. It showed MIC and MBC values 1.51 mg/mL against

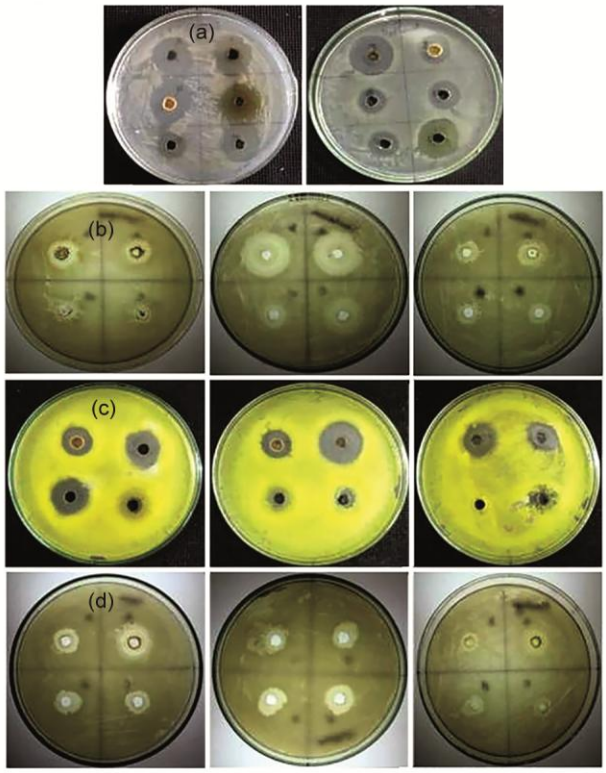

Fig. 4 — Antibacterial activity of the 12 plant extracts against 4 test pathogens; a) *E. coli* K12, b) *Staphylococcus aureus* c) *Pseudomonas aeruginosa* and d) *Proteus vulgaris*

P. vulgaris whereas MIC 1.57 mg/mL against *P. aeruginosa*. *M. arboreus* showed the lowest MIC 0.29 mg/mL against *S. aureus* but comparatively highest MIC 3.41 mg/mL against *E. coli* and *P. vulgaris*, whereas 0.67 mg/mL against *P. aeruginosa*. It showed the relatively highest MBC 9.63 mg/mL against *E. coli*. *S. officinarum* showed MIC 0.67 mg/mL against *P. aeruginosa*, *P. vulgaris*,

Table 6 — MIC and MBC values of selected nine ethno-medicinal plants and standard against pathogenic bacteria

Plants/Standard	*E. coli*		*P. aeruginosa*		*P. vulgaris*		*S. aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Nephrolepis cordifolia	0.67	0.67	0.67	1.51	0.67	1.51	0.29	0.67
Acyranthes bidentata	9.63	21.67	4.27	9.63	3.41	9.63	0.67	1.51
Elettaria cardamomum	3.41	4.27	0.67	0.67	1.51	4.27	0.67	1.51
Drymaria cordata ssp. *diandra*	0.67	1.51	0.67	3.41	0.29	1.51	0.29	0.29
Plantago asiatica ssp. *erosa*	1.51	3.41	1.51	4.27	0.67	1.51	0.67	1.51
Carex cruciata	3.41	3.41	1.57	3.41	1.51	1.51	0.67	3.41
Malvaviscus arboreus	3.41	9.63	0.67	1.51	3.41	4.27	0.29	1.51
Saccharum officinarum	1.51	4.27	0.67	0.67	0.67	1.51	0.67	1.51
Centella asiatica	0.67	3.41	0.29	3.41	0.29	1.51	0.29	0.29
Chloramphenicol (Standard)	0.67	1.51	0.15	0.67	0.29	1.51	0.15	0.29

and *S. aureus* and also MBC 0.67 mg/mL against *P. aeruginosa.* Lower MICs and MBC signify the effectivity of the plant extract that the minimum concentration of the plant extract is enough to control the UTI causing bacteria whereas the high value signifies the high dosage is required to inhibit their growth.

Discussion

In quantitative ethnobotany, high use value of the species generally means the mostly cited one for the particular disease cluster. However, the present quantitative study did not correlate the experimental outcomes. Plant with moderate use value (*Saccharum* 0.3) or with comparatively less use value (*Nephrolepis, Centella* and *Drymaria* 0.1 each) showed the antagonistic effect against the tested bacterial strains compare to the species having high UV scores (*Cheilocostus* 0.7, *Equisetum* 0.5 and *Coriandrum* 0.3). However, the plants with fidelity level ranging from 16.66% to 150% have showed the remarkable antimicrobial activity. The informant consensus factor 0.5 indicates that 50% of the informants were agreeing to use those plants for the treatment of UTI.

Among the tested plants the most effective plants with the lowest MIC/MBC values were *Nephrolepis cordifolia*, *Drymaria cordata* ssp. *diandra, Centella asiatica* and *Saccharum officinarum*. These plant extracts may have an anti-adhesive effect and may inhibit the chaperon–usher system of UTI-causing pathogens[24]. Further, Oral administration of the formulation of these plants may stimulate the production of more THP (syn. Uromodulin) in the renal tubules thereby inhibiting the binding of type 1 fimbriated UPEC to uroplakins of uroepithelial cells[25]. These plants may also contain natural quorum-sensing substances that could inhibit the virulence of the pathogens[26].

Cheilocostus speciosus was used by the majority of the healers and the quantitative study also showed high UV score (7) and FL 350% but its methanolic extract did not show antimicrobial activity against the studied bacteria. Similarly, the extract of seeds of *Coriandrum sativum* (UV score 0.2; FL 200%) and *Equisetum diffusum* tuber (UV score 0.5; FL 250%) did not show any antimicrobial activity. Though *Cheilocostus speciosus* and *Equisetum diffusum* are highly recommended by the healers but failing to show inhibition is quite unlikely.

Many plants used by the healers of the tea gardens to treat UTIs have been found to cure other types of ailments too[27]. The root juice of *Achyranthes bidentata* is used as a pain reliever against toothache, to cure indigestion and asthma in Nepal[28]. In Ayurveda, *Centella asiatica* is used as brain tonic, anti-gastric ulcer, anti-cancerous, anti-inflammatory and wound-healing medicines[28,29]. In Tamil Nadu, tuber extract is used to cure stomach disorder and urinary problems. The raw *Nephrolepis cordifolia* tuber is eaten by the children of tea garden workers and juice is found to have a diuretic property[30].

Conclusion

Among the nine tested ethno-medicinal plants, *Nephrolepis cordifolia*, *Drymaria cordata* ssp. *diandra, Saccharum officinarum*, and *Centella asiatica* were found most effective with the lowest MICs and MBCs. However, the underlying mechanisms of these plant extracts, whether it inhibits the adhesive and virulence factors of the pathogens, or contain natural quorum sensing substances, or stimulates the renal tubules to secrete more THP are not known for which further exploration is needed.

Present work is preliminary and in-depth pharmacological research is required for discovery of novel antimicrobial compounds from herbs to control UTI.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23(02)(2024)170-181_SupplData.pdf

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Conflict of Interest

The authors do not have any conflict of interest.

Author Contributions

MC conceptualized the study. DC conducted the ethno-botanical survey and analysed the field data. DC and SM designed & conducted laboratory experiments for the anti-bacterial test and analysed the data. All the authors participated in the writing of the manuscript. MC and DS have read and approved the final manuscript.

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