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Susceptibility of microorganism to selected medicinal plants in Bangladesh

S. M. Masud Rana[1], Md. Mustahsan Billah[1*], Mohammad Salim Hossain[1*], A. K. M. Saifuddin[2], S. K. M. Azizul Islam[2], Sujan Banik[1], Zannatul Naim[3], Golam Sarwar Raju[1]

[1]*Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh*

[2]*Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh*

[3]*Department of Pharmacy, Atish Dipankar University of Science and Technology, Banani, Dhaka, Bangladesh*

PEER REVIEW

Peer reviewer

Dr. A. K. M. Mohiuddin, Dean of Life Science Faculty, Associate Professor, Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh.
Tel: +88 0921 55399
E-mail: akmmohi@yahoo.com

Comments

This is an appreciated research in which authors have tried their best to demonstrate antimicrobial activities of selected medicinal plants in Bangladesh. Therefore, the findings of this study will encourage people to use medicinal plants, leaving synthetic medication as well as the researchers to develop better drugs against microbial infections in near future.
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ABSTRACT

Objective: To analyze *in-vitro* antimicrobial activities of some ethno-pharmacologically significant medicinal plants (methanol extract) against the pathogenic microorganisms (*Escherichia coli*, *Salmonella* spp., *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*).

Methods: The disc diffusion method was applied for antibacterial test and the poisoned food technique was applied for antifungal test.

Results: The methanol extract of *Terminalia chebula* (bark), *Phyllanthus acidus* (fruits), *Sarcochlamys pulcherrima* (leaves) and *Abelmoschus esculentus* (fruits) had significant *in vitro* antibacterial activity angainst the entire test samples in comparison to standard drug ciprofloxacin. Most of the plant extracts showed low activity against Gram negative bacteria while potential activity against Gram positive bacteria. The antifungal activities of methanol extracts of these plants and standard drug griseofulvin were determined against two pathogenic fungi, and *Polygonum lapathifolium* (leaves) and *Cinnamomum tamala* (leaves) showed maximum activity, while *Erioglossum rubiginosum* (leaves) showed no antifungal activity.

Conclusions: Further chemical and pharmacological investigations are required to identify and isolate chemical constituents responsible for these potential bioactivities and thus to determine their full spectrum of efficacy.

KEYWORDS

Susceptibility, Zone of inhibition, Percentage of inhibition, Antibacterial activity, Antifungal activity

1. Introduction

Microorganisms (*i.e.* bacteria, fungi and viruses) cause serious human and animal infections in tropical and subtropical countries and are the leading cause of death throughout the world. Antibiotic resistance has become a worldwide apprehension[1]. The clinical effectiveness of many surviving synthetic antibiotics is being vulnerable by the advent of multidrug-resistant pathogens[1]. In recent years, multiple drug resistance in human pathogenic microorganisms has been established due to unselective use of synthetic antimicrobial drugs[2]. Resistance is a capability of a microorganism by which microorganism

*Corresponding author: Mohammad Salim Hossain, PhD, Associate Professor and Chairman, Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.
Tel: +8801711-200410
E-mail: pharmasalim@yahoo.com
Md. Mustahsan Billah, Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.
Tel: +88-01832-164651
E-mail: mustahsan04pharmacy@yahoo.com
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can still alive under antibiotic and antifungal therapy. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Since most of the antibiotic and antifungal drugs are chemically synthesized or semi synthesized, resistance is not only the major problem, and adverse effects also play an important role in causing other serious diseases in human and animal (*e.g.* allergy, immune suppression, and hypersensitivity)[3].

Data from WHO illustrate that 70%-80% of the world's population use herbal medicine as alternative medicine[4]. Interest in plant-derived drugs has been increasing, mainly due to the current extensive belief that "green medicine" is safer and more dependable than costly synthetic drugs[5]. Natural and herbal products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Over the past two decades, there has been an increasing interest in the investigation of natural materials as sources of new antibacterial agents[6]. Many reports have revealed the effectiveness of natural plants against microorganisms; as a result, plants are one of the foundations for modern medicine to attain new principles[7]. Even though numbers of plant-derived antibiotics are being identified, the scientific evaluations of plant derived antibiotics still remain an area of intensive investigation[8]. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens[9]. The continuing development of resistance to existing antibacterial agents and the shortage of good antifungal agents provoke this effort toward innovation[10]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections[11].

It is evident that herbal antibacterial and antifungal drug has great potential to complement synthetic medicines; it is therefore necessary to increase the production of drug from both plants and chemical synthesis. Bangladesh is home to over 5 000 medicinal plant species and their use for medicinal purposes is remarkable[12]. Bangladesh is one of the richest countries in the world regarding genetic resources of medicinal plants. The country exhibits diversity in terms of topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions enable introduction and domestication of plant varieties[1]. In recent years, medicinal plants previously with unknown pharmacological activities have been broadly studied as a rich source of medicinal agents in Bangladesh[13]. It is expected that phytomedicines with adequate antibacterial efficacy could be used for the treatment of microbial infections[14]. However, there is still lack of data on the susceptibility of microbes to commonly used medicinal plant extracts in Bangladesh.

Against the above background, the present study was undertaken to evaluate the susceptibility of selected bacterial and fungal pathogens to medicinal plant extracts commonly used as herbal medicines. It is hoped that the findings of the study will promote the use of herbal products. The ultimate goal of the present study is to establish appropriate and efficient herbal remedies that could effectively inhibit growth of pathogens.

2. Materials and methods

2.1. Collection and identification of plant material

Fresh plant/ plant parts were collected based on ethno-pharmacological importance from Noakhali, Dhaka and Cox's Bazar district, Bangladesh. The plants and the parts screened, along with their taxonomic identity, local name, family and therapeutic uses, are given in Table 1.

Table 1

Ethnobotanical information of some traditionally used Bangladeshi medicinal plant species selected for antimicrobial activity.

Plant name	Accession number	Local name	Family	Therapeutic use	Reference
P. lapathifolium	DACB 37924	Bishkatali	Polygonaceae	Anthelmintic, antiemetic, cytotoxic	[15]
S. violaceum	DACB 37751	Tit Begun	Solanaceae	Anthelmintic, antioxidant, antimicrobial, anti-inflammatory, cytotoxic	[16]
M. charantia	DACB 37656	Korola	Cucurbitaceae	Hypoglycemic, antioxidant, anti-fungal, anti-malarial, thrombolytic	[17]
A. bilimbi	DACB 34207	Bilimbi	Oxalidaceae	Antibacterial, antioxidant, cytotoxic	[18,19]
C. tamala	DACB 39290	Tejpata	Lauraceae	Antimicrobial, antidiarrhoeal, cytotoxic	[20]
E. officinalis	DACB 37912	Amalaki	Euphorbiaceae	Anti-inflammatory, antioxidant, anticancer	[21]
J. gossipifolia	DACB 35937	Jatropha	Euphorbiaceae	Anti-infertility, anti-inflammatory, antidiarrhoeal, analgesic	[22,23]
L. monopetala	DACB 39559	Mendapata	Lauraceae	Antimicrobial, anti-inflammatory, thrombolytic	[24]
E. fluctuans	DACB 37925	Helencha	Asteraceae	Antioxidant, thrombolytic, anthelmintic, analgesic, CNS depressant, antidiarrheal, antimicrobial, cytotoxic	[25-30]
D. quercifolia	DACB 35489	Pankhiraj, Pankha	Drynariaceae	Anthelmintic, antibacterial, antioxidant, cytotoxic, thrombolytic	[31]
T. chebula	DACB 37909	Maritaki, Haritaki	Combretaceae	Antiemetic, antidiarroheal, antioxidant, anti-diabetic, antimicrobial, cytotoxic	[32]
A. conyzoides	DACB 39526	Dochunti, Fulkuri	Asteraceae	Antioxidant, anti-inflammatory, analgesic, antipyretic, antiseptic	[33]
M. cordifolia	DACB 34527	Guaco	Asteraceae	Antioxidant, anti-inflammatory, analgesic, antipyretic, preventing sexually transmitted diseases	[34]
C. bonplandianum	DACB 37658	Bon Tulshi	Euphorbiaceae	Antioxidant, cytotoxic	[35]
P. acidus	DACB 34509	Arboroi, Harbori	Phyllanthaceae	Anti-inflammatory, anti-nociceptive, antioxidant, antidiarrheal, antimicrobial, cytotoxic	[36-38]
A. polystachya	DACB 37658	Pitraj	Meliaceae	Cytotoxic, anthelmintic, antimicrobial, thrombolytic, antioxidant	[39]
A. esculentus	DACB 2435	Okra, Bendi	Malvaceae	Antibacterial, antioxidant, antidiabetic, CNS depressant, analgesic	[40-42]
S. pulcherrima	DACB 35871	Jangaillya shak	Urticaceae	Thrombolytic	[43]
C. viscosum	DACB 35979	Vant, Ghetu	Lamiaceae	Antiseptic, antimicrobial, anti-inflammatory, antipyretic	[44]
E. rubiginosum	DACB 38566	Kalayo	Sapindaceae	Membrane stabilizers, antimicrobial, antioxidant, thrombolytic, cytotoxic, CNS depressant	[45,46]

CNS: Central nervous system. *P. lapathifolium*: *Polygonum lapathifolium*; *S. violaceum*: *Solanum violaceum*; *M. charantia*: *Momormdica charantia*; *A. bilimbi*: *Averrhoa bilimbi*; *C. tamala*: *Cinnamomum tamala*; *E. officinalis*: *Emblica officinalis*; *J. gossipifolia*: *Jatropha gossipifolia*; *L. monopetala*: *Litsea monopetala*; *E. fluctuans*: *Enhydra fluctuans*; *D. quercifolia*: *Drynaria quercifolia*; *T. chebula*: *Terminalia chebula*; *A. conyzoides*: *Ageratum conyzoides*; *M. cordifolia*: *Mikania cordifolia*; *C. bonplandianum*: *Croton bonplandianum*; *P. acidus*: *Phyllanthus acidus*; *A. polystachya*: *Aphanamixis polystachya*; *A. esculentus*: *Abelmoschus esculentus*; *S. pulcherrima*: *Sarcochlamys pulcherrima*; *C. viscosum*: *Clerodendrum viscosum*; *E. rubiginosum*: *Erioglossum rubiginosum*.

The selected plants were identified and authenticated by Taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

2.2. Preparation of extracts

After collection, different parts of these plants were thoroughly washed with water. After drying at 37 °C for 24 h, all the plant materials were coarsely fine-grained individually and extracted by dissolving with methanol for fifteen days with occasional shaking and stirring. The sediments were filtered and also the filtrates were dried at 40 °C during a water bathtub. The solvent was utterly removed by filtering with Whatman paper (Bibby RE200, Sterilin Ltd., UK) and obtained dried crude methanol extracts were used for further experiment.

2.3. Test microorganisms

Two strains of Gram-positive bacteria [*Bacillus cereus* ATCC 10876 (*B. cereus*), *Staphylococcus aureus* ATCC 29740 (*S. aureus*)], two strains of Gram-negative bacteria [*Escherichia coli* ATCC 25922 (*E. coli*), *Salmonella* spp. ATCC 14028] and two strains of fungi [*Aspergillus niger* ATCC 16404 (*A. niger*), *Candida albicans* ATCC 10231 (*C. albicans*)] were used to evaluate the antimicrobial activity. They were collected from the Department of Microbiology, Poultry Research and Training Center, Chittagong Veterinary and Animal Sciences University, Bangladesh. All the microorganisms were maintained at 4 °C on nutrient agar slants.

2.4. Antibacterial culture media

Nutrient agar media and Muller-Hinton agar media were used. Ciprofloxacin antibiotic solutions were prepared prior to incorporation into the liquid medium.

2.5. Antifungal culture media

Potato dextrose agar media and savored agar media were used. Griseofulvine antifungal solutions were prepared with incorporation into water.

2.6. Antibacterial activity

Antibacterial activity was determined by the disc diffusion method against Gram positive (*B. cereus*, *S. aureus*) and Gram negative bacteria (*E. coli*, *Salmonella* spp.)[47]. Ciprofloxacin was used as standard. The bacteria cultures were grown in agar medium at 37 °C. After 6 h of growth, at a concentration of 10^6 cells/mL, each microorganism was inoculated on the surface of Muller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated with extract (100 μg) were placed on surface of each inoculated plate. The plates were incubated at 37 °C for 24 h. Then it was possible to observe inhibition zone.

2.7. Antifungal activity

Antifungal activity was determined against *A. niger* and *C. albicans* by the poisoned food technique with slight modification[48]. First step is the plate preparation; potato dextrose agar was prepared and placed in Petri dish. The extract was mixed with media and dried up for 30 min. Second is the test suspension preparation; a few colonies of fungus were selected and placed in savored agar media, then incubated at room temperature for 3 d and matched with standard (griseofulvin). The last is streaking the plates; all plates with test organism were streaked and dried up for 30 min, then incubated at room temperature for 3 d. Zone of growth was recorded and finally percentage of inhibition was calculated by following formula:

$$I=\frac{C-T}{C}\times 100$$

Where, C stands for diameter of control (8 cm); T stands for diameter of zone of growth; I stands for percentage of inhibition.

3. Result

3.1. Antibacterial activity

The antibacterial activities of methanol extracts of selected plants (100 μg/disc) and standard ciprofloxacin (30 μg/disc) were determined against two Gram negative and two Gram positive bacteria, and Gram negative bacteria showed potential susceptibility to *T. chebula*, *A. conyzoides*, *M. cordifolia*, *C. bonplandianum*, *P. acidus*, *A. polystachya*, *A. esculentus*, *S. pulcherrima*, *E.*

rubiginosum (bark). Among them, *T. chebula* exhibited highest [(14.12±0.20) mm] zone of inhibition against *E. coli* followed by zone of inhibition [(15.02±0.15) mm] against *Salmonella* spp. Almost all plant extracts except *E. rubiginosum* (leaves) showed potential activity against Gram positive bacteria. *P. acidus* revealed highest zone of inhibition [(29.14±0.20) mm and (31.20±0.15) mm] against *B. cereus* and *S. aureus* respectively in comparison with ciprofloxacin [(45.05±0.11) mm and (46.12±0.03) mm]. In all cases, *T. chebula*, *P. acidus* and *S. pulcherrima* demonstrated more significant and *E. rubiginosum* (leaves) showed minimum antibacterial activity (Table 2).

Table 2

Antibacterial activity of methanol extracts of selected medicinal plants.

Plant name	Part	Zone of inhibition (mm)			
		Gram negative bacteria		Gram positive bacteria	
		E. coli	*Salmonella* spp.	*B. cereus*	*S. aureus*
P. lapathifolium	Root	0.00±0.00	0.00±0.00	8.06±0.10	10.11±0.03
	Leaves	0.00±0.00	0.00±0.00	9.01±0.08	8.09±0.04
	Flower	0.00±0.00	0.00±0.00	13.14±0.01	12.11±0.12
S. violaceum	Whole plant	3.02±0.01	1.01±0.03	11.01±0.02	15.11±0.05
M. charantia	Fruits	0.00±0.00	0.00±0.00	9.03±0.07	13.01±0.03
A. bilimbi	Fruits	0.00±0.00	0.00±0.00	4.01±0.03	6.05±0.40
C. tamala	Leaves	0.00±0.00	0.00±0.00	10.02±0.10	11.10±0.13
E. officinalis	Whole plant	0.00±0.00	0.00±0.00	15.11±0.20	17.03±0.17
J. gossipifolia	Bark	0.00±0.00	0.00±0.00	20.13±0.12*	16.16±0.30
	Leaves	0.00±0.00	0.00±0.00	19.13±0.17	17.16±0.21
L. monopetala	Whole plant	0.00±0.00	0.00±0.00	10.10±0.11	12.01±0.03
E. fluctuans	Whole plants	0.00±0.00	0.00±0.00	9.02±0.03	7.12±0.10
D. quercifolia	Leaves	0.00±0.00	2.01±0.04	22.02±0.15**	14.12±0.11
T. chebula	Bark	14.12±0.20**	15.02±0.15**	21.02±0.14**	24.08±0.40**
A. conyzoides	Leaves	4.01±0.10	7.12±0.13	15.01±0.12	17.12±0.23
M. cordifolia	Leaves	9.03±0.14	7.01±0.11	17.01±0.16	19.02±0.03
C. bonplandianum	Whole plants	5.03±0.02	5.34±0.01	15.23±0.12	18.02±0.03
P. acidus	Fruits	12.15±0.40**	15.03±0.19**	29.14±0.20**	31.20±0.15**
A. polystachya	Bark	3.01±0.01	5.00±0.05	15.40±0.13	17.11±0.15
A. esculentus	Fruits	10.03±0.03	7.01±0.07	22.05±0.10**	30.01±0.07**
S. pulcherrima	Leaves	13.01±0.12**	11.04±0.07*	25.03±0.15**	29.05±0.17**
C. viscosum	Leaves	0.00±0.00	12.02±0.11	20.01±0.13*	0.00±0.00
E. rubiginosum	Leaves	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Bark	5.03±0.03	7.06±0.05	0.00±0.00	17.02±0.03
Ciprofloxacin (standard)		39.02±0.12	37.04±0.05	45.05±0.11	46.12±0.03

Values are the mean of three replicates±SD. *: $P<0.05$ (significant), **: $P<0.01$ (more significant).

3.2. Antifungal activity

The antifungal activities of methanol extracts of selected plants (200 μL/plate) and standard griseofulvin (30 μL/plate) were determined against two pathogenic fungi where, *P. lapathifolium* (leaves) and *C. tamala* (leaves) showed the maximum antifungal activity, while *E. rubiginosum* (leaves) exposed no antifungal activity (Table 3).

Table 3

Antifungal activity of methanol extracts of screened medicinal plants.

Plant name	Part	Inhibition (%)	
		A. niger	*C. albicans*
P. lapathifolium	Root	37.5%	41.0%
	Leaves	100.0%	89.0%
	Flower	37.5%	56.5%
S. violaceum	Whole plant	50.0%	45.5%
M. charantia	Fruits	37.5%	49.0%
A. bilimbi	Fruits	25.0%	39.0%
C. tamala	Leaves	100.0%	92.0%
E. officinalis	Whole plant	0.0%	13.0%
J. gossipifolia	Bark	0.0%	20.0%
	Leaves	12.5%	17.0%
L. monopetala	Whole plant	0.0%	15.0%
E. fluctuans	Whole plant	25.0%	30.5%
D. quercifolia	Leaves	19.0%	22.0%
T. chebula	Bark	32.0%	29.0%
A. conyzoides	Leaves	25.5%	27.0%
M. cordifolia	Leaves	29.0%	21.0%
C. bonplandianum	Whole plant	25.5%	25.0%
P. acidus	Fruits	35.5%	32.5%
A. polystachya	Bark	21.0%	25.5%
A. esculentus	Fruits	29.0%	35.0%
S. pulcherrima	Leaves	39.0%	44.0%
C. viscosum	Leaves	25.0%	20.5%
E. rubiginosum	Leaves	0.0%	0.0%
	Bark	25.0%	30.5%
Griseofulvin (standard)		100.0%	98.50%

4. Discussion

Making the sense for searching the new broad spectrum antimicrobial to treat the microbial disease, much attention has been focused toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a dynamic role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms[49].

So, we tried to evaluate the susceptibility of different microorganism specially *E. coli*, *Salmonella* spp., *B. cereus*, *S. aureus*, *A. niger* and *C. albicans* against 24 methanol extracts of different plants which have ethnopharmacological importance in Bangladesh.

In antibacterial susceptibility testing, the methanol extract of *T. chebula* (bark), *P. acidus* (fruits), *S. pulcherrima* (leaves) and *A. esculentus* (fruits) had significant *in vitro* antibacterial activity among the entire test sample in comparison to ciprofloxacin standard. Most of the plant extracts showed low activity against Gram-negative bacteria while potential activity against

Gram-positive bacteria. This suggested that the Gram-positive bacteria were more susceptible to the extract than the Gram-negative bacteria. In general, Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria[50]. The resistance is due to the differences in their cell wall composition. In Gram-negative bacteria, the outer membrane acts as a great barrier to many environmental substances including antibiotics[51]. Presence of thick murine layer in the cell wall inhibits the entry of the entry of the inhibitors[49]. The efficacy of the extracts to exhibit antibacterial activity against the bacteria suggested the presence of hydrophilic and hydrophobic antibacterial compounds such as luteolin, apigenin *etc*[52]. Previous study on the preliminary phytochemical screening of these extracts revealed the presence of wide range of phyto-constituents along with antibacterial compounds.

Antifungal susceptibility testing is a dynamic field of medical mycology. Development and standardization of antifungal susceptibility tests have shown remarkable progress in the field of medical mycology[53]. Broth macro- and micro-dilution assays can be used to determine antifungal susceptibility of dermatophytes, but these methods are expensive and require specific media and equipment such as RPMI, MOPS buffer, and micro plate trays. The poisoned food technique constitutes a good model to be used for investigational purposes to investigate fungal genera and drugs as well. This assay can be adapted for routine diagnosis in the laboratory and for assessment of dermatophyte resistance against antifungal drugs.

In our present study, almost all plant extracts except *E. officinalis* (whole plant), *J. gossipifolia* (bark), *L. monopetala* (whole plant), and *E. rubiginosum* (leaves) showed antifungal activity where, *P. lapathifolium* (leaves), *C. tamala* (leaves) and *S. violaceum* (whole plant) showed significant activity in comparison with griseofulvin. Earlier study on the primary phytochemical screening of these extracts exposed the presence of wide range of phyto-constituents along with antifungal compounds.

So, our present study justifies that methanolic extracts of *T. chebula* (bark), *P. acidus* (fruits), *S. pulcherrima* (leaves), *A. esculentus* (fruits), *P. lapathifolium* (leaves), *C. tamala* (leaves) and *S. violaceum* (whole plants) possess promising activity against a wide range of microbes responsible for most common microbial diseases and consequently provide prospect of finding new clinically effective antimicrobial compounds. Therefore, further research is necessary to identify the responsible compounds within these plants and to determine their full spectrum of efficacy as well. Thus our findings form a major platform for more phytochemical and pharmacological research studies and encourage cultivation of the highly valuable plant in large-scale to increase the economic status of cultivars in the country.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Medicinal plants contain wide variety of phytochemical constituents and biological properties that are used to treat numerous diseases of human and animals without or less side effects. However, there is serious lacking of documentary evidence in a well-organized way on the susceptibility of microbes to commonly used medicinal plants extracts before this study in Bangladesh.

Research frontiers

The present research work shows antibacterial and antifungal acivity of various native medicinal plants. This study depicts that extracts of *T. chebula* (bark), *P. acidus* (fruits), *S. pulcherrima* (leaves), *A. esculentus* (fruits), *P. lapathifolium* (leaves), *C. tamala* (leaves) and *S. violaceum* (whole plant) possess promising antimicrobial activity.

Related reports

All selected plant extracts except *E. rubiginosum* (leaves and barks) showed potential activity against Gram-positive bacteria. *T. chebula* (bark), *P. acidus* (fruits) and *S. pulcherrima* (leaves) proved more significant antibacterial activity. *P. lapathifolium* (leaves) and *C. tamala* (leaves) exhibited the maximum antifungal activity. These plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective

microorganisms and thus can be a great remedy for various infectious diseases (*e.g.* allergy, immune suppression, and hypersensitivity).

Innovations and breakthroughs

The present study provides prospect of finding new clinically effective antimicrobial compounds and forms a major platform for more phytochemical and pharmacological research studies. So, further research is necessary to identify the responsible compounds within these plants with their full spectrum of efficacy.

Applications

From the literature survey, it has been found that medicinal plants are safe for human as well as animal. This scientific study initiates and provokes the use of medicinal plants as medication for wide variety of purposes as well as decreases the devastating effect of drug resistance.

Peer review

This is an appreciated research in which authors have tried their best to demonstrate antimicrobial activities of selected medicinal plants in Bangladesh. Therefore, the findings of this study will encourage people to use medicinal plants, leaving synthetic medication as well as researchers to develop better drugs against microbial infections in near future.

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