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Research Article

ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AGAINST MULTIDRUG RESISTANT BACTERIA

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ABSTRACT

The present study was conducted with a view to evaluate the therapeutic potentials of fifteen plant extracts commonly used against tested multidrug resistant bacteria by agar well diffusion method. Out of fifteen plant extracts five crude plant extracts of *Ribes grossularia* (fruit), *Piper nigrum* (fruit), *Syzygium aromaticum* (fruit), *Saroca asoca* (leaf), and *Azadirachta indica* (leaf) were found to exhibit potential antimicrobial properties against the tested multidrug resistant bacterial isolates whereas ten plant extracts of *Ocimum tenuiflorum* (leaf), *Coriandrum sativum* (leaf), *Trigonella foenumgraecum* (leaf), *Citrus limon* (fruit), *Phyllanthus debilis* (leaf), *Datura stramonium* (leaf), *Urtica dioica* (leaf), *Allium sativum* (bulb), *Cuminum cyminum* (seed) and *Zingiber officinale* (rhizome) could not inhibit the growth of tested multi drug resistant bacteria. The results demonstrate the antimicrobial potential of these plants and suggested the possibility of employing them in drugs for the treatment of infectious diseases caused by the test organisms.

Keywords: Antimicrobial activity, Plant extract, Multi-drug resistant bacteria, Well diffusion method

1. INTRODUCTION

Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability. From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products [1]. Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs [2]. In the last few decades, many bacterial organisms have continued to show increasing resistance against current antimicrobial agents [3]. Different antibiotics exercise their inhibitory activity on different pathogenic organisms [4]. Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs [5]. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents.

In light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [6]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [7]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [8]. Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides [9]. Reports are available on the use of several plant by-products, which possess antimicrobial properties, on several pathogenic bacteria and fungi [10-12]. Here, we

evaluate the potential of several plant extracts for anti-bacterial activity against important human pathogenic and phytopathogenic bacteria.

2. MATERIAL AND METHODS

2.1. Collection of Plants and Microbial Samples:

Plants were collected from different locations of Delhi & NCR (India). The plants were identified taxonomically and authenticated by Dr. Ravinder Kumar, Assistant Professor, Department of Botany, Hindu College (University of Delhi). The medicinal plants used to evaluate antimicrobial activity in this study are given below in Table 1.

The microbial samples used in this experiment were isolated from hospital wastes collected from different hospitals of Delhi & NCR.

Table 1: List of medicinal plants used to evaluate antimicrobial activity

S.No.	Local Name	English Name	Botanical Name	Plant Part Used
1.	Neem	Neem	*Azadirachta indica*	Leaf
2.	Tulsi	Basil	*Ocimum tenuiflorum*	Leaf
3.	Kali Mirch	Black Peper	*Piper nigrum*	Fruit
4.	Bhuiamla	Phyllanthus debilis	*Phyllanthus debilis*	Root & Leaf
5.	Laung	Cloves	*Syzygium aromaticom*	Fruit
6.	Dhania	Coriander	*Coriandrum sativum*	Leaf
7.	Zeera	Cumin	*Cuminum cyminum*	Seed
8.	Methi	Fenugreek	*Trigonella foenum – graecum*	Leaf
9.	Lahsan	Garlic	*Allium sativum*	Bulb
10.	Adrak	Ginger	*Zingiber officinale*	Rhizome
11.	Amla	Gooseberry	*Ribes grossularia*	Fruit
12.	Nimbu	Citrus	*Citrus limon*	Leaf & Fruit
13.	Datura	Jimsonweed	*Datura stramonium*	Leaf & Seed
14.	Sita Ashoka	Ashoka Tree	*Saraca asoca*	Leaf, Bark & Flower
15.	Bichu Buti	Stinging Nettle	*Urtica dioica*	Leaf, Stem & Root

2.2. Plant Material

Fresh leaves of *Ocimum tenuiflorum, Coriandrum sativum, Trigonella foenum-graecum, Citrus limon, Phyllanthus debilis, Azadirachta indica, Ribes grossularia, Datura stramonium, Saroca asoca* and *Urtica dioica* were collected. The fruits of *Piper nigrum* and *Syzygium aromaticum*, seeds of *Cuminum cyminum*, bulb of *Allium sativum*, and rhizome of *Zingiber officinale* were also collected. These plant parts were washed thoroughly 2-3 times with running tap water and then with sterile water. Plant parts were then shade-dried, powdered and used for extraction.

2.3. Preparation of Aqueous Plant Extracts

25g of shade dried, powder of plant materials were macerated separately with 50ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000rpm for 15min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120°C for 30min. The extract was preserved aseptically in a brown bottle at 4°C until further use.

2.4. Preparation of Solvent Extractions

25g of shade dried, powder of plant materials were filled separately in the thimble and extracted successively with 150ml each of methanol, acetone, ethyl acetate, chloroform and distilled water using a Soxhlet extractor for 48h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4°C in airtight bottles until further use. 1g of each solvent residue was dissolved in 10ml of respective solvents and were used as the test extracts for antimicrobial activity assay.

2.5. Anti-bacterial Activity Assay

Antibacterial activity of aqueous extract and solvent extracts; methanol, acetone, ethyl acetate, chloroform and distilled water was determined by agar well diffusion method on nutrient agar medium (Anonymous, 1996). The bacteria cultures were grown in nutrient broth medium at 37°C. After 6 h of growth, each microorganism, at a concentration of 10^6 cells/mL, was inoculated on the surface of nutrient agar

plates with a sterile swab, and Wells were punched into nutrient agar plates using sterile cork borer (5 mm). Then 50µl each of all aqueous and solvent extracts were placed in the wells made in inoculated plates. The treatment also included 50µl of solvents served as control and methanol as a standard control. The plates were incubated at 37°C for 24h. After this period, it was possible to observe inhibition zone. Each treatment consists of three replicates and repeated at least twice.

3. RESULTS

3.1. Antimicrobial Activity of Plant Extracts

The results showed that the most active organic solvent to extract the antibacterial compounds from tested plants was methanol followed by ethyl acetate. As shown in Table 1.2, the aqueous extracts of *Ocimum tenuiflorum, Coriandrum sativum, Trigonella foenum-graecum, Citrus limon, Saroca asoca, Urtica dioica, Phyllanthus debilis, Cuminum cyminum, Allium sativum,* and *Zingiber officinale* could not inhibit the growth of tested multi drug resistant bacteria. While as the aquous extracts of *Ribes grossularia* showed significant inhibition against tested organisms and *Piper nigrum* and *Syzygium aromaticum* and *Azadirachta indica* showed moderate inhibition.

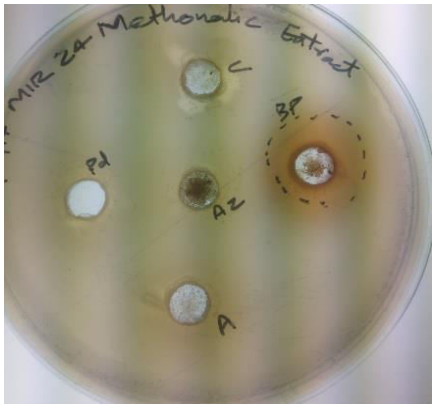

Fig. 1: Zone of inhibition of plant extracts in methanol

Table 2: Zone of inhibitory activity (in millimeter) of different plant extracts against MDR's isolated

Plants	Extracts	C2	C3	C18	C22	C24
Ribes grossularia	Aqueous	25mm	24mm	14mm	21mm	24mm
	Methanol	31mm	30mm	20mm	27mm	26mm
	Acetone	24mm	21mm	19mm	*	*
	Chloroform	19mm	19mm	20mm	23mm	24mm
	Ethyl Acetate	20mm	21mm	24mm	24mm	26mm
Piper nigrum	Aqueous	-	11mm	-	*	17mm
	Methanol	17mm	18mm	18mm	16mm	18mm
	Acetone	18mm	16mm	15mm	16mm	18mm
	Chloroform	*	-	13mm	-	12mm
	Ethyl Acetate	18mm	17mm	16mm	*	*
Azadirachta indica	Aqueous	-	15mm	-	17mm	*
	Methanol	17mm	18mm	15mm	*	14mm
	Acetone	15mm	14mm	15mm	13mm	14mm
	Chloroform	*	*	-	-	-
	Ethyl Acetate	15mm	18mm	15mm	15mm	-
Syzygium aromaticom	Aqueous	-	-	16mm	16mm	-
	Methanol	16mm	16mm	18mm	18mm	16mm
	Acetone	15mm	14mm	-	14mm	16mm
	Chloroform	-	-	-	-	-
	Ethyl Acetate	12mm	*	-	16mm	18mm
Saraca asoca	Aqueous	-	-	-	-	-
	Methanol	18mm	18mm	15mm	14mm	15mm
	Acetone	14mm	*	14mm	*	12mm
	Chloroform	*	14mm	-	15mm	-
	Ethyl Acetate	20mm	*	16mm	*	-
Coriandrum sativum	Aqueous	-	-	-	-	-
	Methanol	*	*	-	14mm	-
	Acetone	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	-	-	-	-	-

Cuminum cyminum	Aqueous	-	-	-	-	-
	Methanol	*	-	-	*	-
	Acetone	-	-	-	12mm	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	-	-	-	-	-
Allium sativum	Aqueous	-	-	-	-	-
	Methanol	17mm	-	17mm	14mm	*
	Acetone	11mm	-	-	-	15mm
	Chloroform	-	-	-	-	-
	Ethyl Acetate	*	-	-	-	*
Zingiber officinale	Aqueous	-	-	-	-	-
	Methanol	*	-	-	-	-
	Acetone	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	*	*	*	-	-
Ocimum tenuiflorum	Aqueous	-	-	-	-	-
	Methanol	-	-	-	-	-
	Acetone	-	-	-	-	16mm
	Chloroform	-	-	-	-	-
	Ethyl Acetate	*	-	*	-	-
Citrus limon	Aqueous	-	-	-	-	-
	Methanol	-	*	-	*	-
	Acetone	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	13mm	-	-	-	-
	Aqueous	-	-	-	-	-
	Methanol	-	-	-	-	-
	Acetone	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	12mm	-	12mm	12mm	-
Phyllanthus debilis	Aqueous	-	-	-	-	-
	Methanol	*	-	14mm	13mm	*
	Acetone	-	-	-	-	-
	Chloroform	*	-	-	-	-
	Ethyl Acetate	-	-	-	-	*
Urtica dioica	Aqueous	-	-	-	-	-
	Methanol	*	-	*	-	*
	Acetone	-	-	18mm	-	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	12mm	*	12mm	*	*
Trigonella foemun graecum	Aqueous	-	-	-	-	-
	Methanol	-	-	-	-	*
	Acetone	-	*	-	12mm	-
	Chloroform	-	-	-	-	-
	Ethyl Acetate	-	-	*	-	*

*- = Not active; * = shows poor inhibition of bacterial growth.*

The acetone and chloroform extracts of *Ocimum tenuiflorum, Coriandrum sativum, Trigonella foenum-graecum, Citrus limon, Azadirachta indica, Saroca asoca, Urtica dioica, Phyllanthus debilis, Cuminum cyminum, Allium sativum,* and *Zingiber officinale* did not show any significant growth of inhibition against tested organisms.

The acetone extract of *Piper nigrum, Syzygium aromaticum* and acetone as well as chloroform extracts of *Ribes grossularia* showed significant growth of inhibition.

However acetone extracts of *Saroca asoca* and *Allium sativum* showed moderate growth of inhibition against tested isolates.

The methanolic and ethyl acetate extracts of *Coriandrum sativum, Trigonella foenum-graecum, Citrus limon, Urtica*

dioica, Phyllanthus debilis, Cuminum cyminum, and *Zingiber officinale* could not inhibit the growth of tested multi drug resistant bacteria. However the methanolic and ethyl acetate extracts of *Ribes grossularia* and *Piper nigrum* showed significant growth of inhibition against tested organisms. The methanolic and ethyl acetate extracts of *Saroca asoca, Ocimum tenuiflorum, Azadirachta indica* and *Allium sativum* showed moderate growth of inhibition against tested isolates.

The maximum growth of inhibition was recorded for *Ribes grossularia* followed by *Piper nigrum, Syzygium aromaticum, Saroca asoca* and *Azadirachta indica.*

4. DISCUSSION

Increasing the number of multi-drug resistance pathogenic microbes in human and animal as well as unwanted side effects of certain antibiotics has encouraged enormous interest to search for new antimicrobial drugs of plant origin [13]. The results showed that the most active organic solvent to extract the antibacterial compounds from tested plants was methanol followed by ethyl acetate. The aqueous, methanol, ethyl acetate, chloroform and acetone extracts of *Ocimum tenuiflorum, Coriandrum sativum, Trigonella foenum-graecum, Citrus limon, Azadirachta indica, Urtica dioica, Cinnamomum verum, Cuminum cyminum,* and *Zingiber officinale* could not inhibit the growth of tested multi drug resistant bacteria. However, all extracts of *Ribes grossularia* shows significant inhibition against tested organisms and *Piper nigrum* and *Syzygium aromaticum* shows moderate inhibition for aqueous extract. The acetone extract of *Piper nigrum, Syzygium aromaticum* and acetone as well as chloroform extracts of *Ribes grossularia* showed significant growth of inhibition. However, acetone extracts of *Saroca asoca* and *Allium sativum* showed moderate growth of inhibition against tested isolates. The methanolic and ethyl acetate extracts of *Ribes grossularia* and *Piper nigrum* shows significant growth of inhibition against tested organisms. The methanolic and ethyl acetate extracts of *Saroca asoca, Ocimum tenuiflorum, Azadirachta indica* and *Allium sativum* showed moderate growth of inhibition against tested isolates.

The maximum growth of inhibition was recorded for *Ribes grossularia* followed by *Piper nigrum, Syzygium aromaticum, Saroca asoca* and *Azadirachta indica.* The extracts of these plants especially methanolic and ethyl acetate possess a broad spectrum of activity against a panel bacteria responsible for the most common bacterial diseases. These primary extracts open the possibility of finding new clinically effective anti-bacterial compounds. A continues exploration of plant-derived antimicrobials is the need of an hour. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However the present study of in vitro antibacterial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

5. CONCLUSION

Plant extracts contains a very complex structure with the active ingredients present in the form of natural organic compounds. The process of extraction for a particular compound is dependent on the solubility of the component in the solvent (aqueous or organic solvent). The process and extraction system are constantly different with every product and compound. The crude extracts of the tested plants demonstrated good potential antibacterial activities. The potential to develop antimicrobial compounds from plants appears rewarding as it will propel to the expansion of a phytomedicine to turn against multidrug resistant microbes.

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