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# Anti-MRSA activity of few Indian medicinal plants

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## Abstract

The report of emergence of resistant strains of *S.aureus* like MRSA is increasing day by day. With a view to get new antimicrobial, plants belonging to different families alleged to possess antimicrobial properties were studied against the antibiotic-resistant strains of bacteria. The eighteen plants were selected for anti bacterial activity based on the literature search from different regions of India. The various plant parts were collected, dried and than extracted using three different solvents viz. methanol, hydro-methanol and aqueous. The extracts so prepared were tested against the bacterial strains which are standard ATCC cultures procured and maintained in the lab. For anti bacterial screening, we used strains of Gram positive bacteria -*S.aureus* 209P and Methicillin Resistant S.aureus (MRSA) strains. Out of the eighteen plants studied seven were found to be active with zone of inhibition against all the tested bacterial species. New sources of anti infectious agents should be studied with a view to get potent antimicrobials which can tackle resistance problem.

## INTRODUCTION

Infectious diseases caused by pathogenic bacteria have been the leading cause of morbidity and mortality in human history [1]. The discovery of antimicrobial agents was one of the major events of the twentieth century. However, over 60 years of application of antibiotics has lead to the development of antibiotic resistance of many bacterial pathogens. Consequently, bacterial infections have again become the most common and deadly causes of human diseases. Bacterial resistance is beyond doubt the consequence of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics. We are now faced with the global problem of emerging resistance in virtually all pathogens [2]. Two of the most lethal hospital infections are caused by Methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) [1]. Staphylococci continue to be a clinical and therapeutic problem and have been increasingly associated with nosocomial infections since the early 1960s. The coagulase positive Methicillin resistant *Staphylococcus aureus* has long been problematic in both community acquired and nosocomial infections. Vancomycin was the choice of antibiotic for MRSA infections until recently when resistant species to Vancomycin are increasingly reported [3].

The emergence of both "new pathogens" and resistant strains from "old pathogens" demands new antibacterial compounds to deal with this crisis. Because of the limited life span of antibiotics, it is of utmost importance to find appropriate solutions to impede, or perhaps even reduce, the development of drug resistance associated with many microbial species. Given the fact that most commonly used antibiotics are isolated from microorganisms, it is important to search for new antibacterial compounds from new bio-resources [1].

Since time immemorial, medicinal plants have been a dependable source of therapeutics for the treatment of various ailments but since the advent of the use of fermentation-based antibiotics, work on anti-microbial agents from plants sources has been greatly overshadowed. The rapid propagation in antibiotic resistance and the increasing interest in natural products, however, have placed medicinal plants back in the front lights as a reliable source for the discovery of active anti-microbial agents and possibly even novel classes of antibiotics [4]. Plants are complex chemical storehouses of undiscovered biodynamic compounds with unrealized potential for use in modern medicine. It has long been established that naturally occurring substances in plants have anti-bacterial and anti-fungal activities [4]. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency**.** Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannins [5]. Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. They are effective, yet gentle. Many plants have tropisms to specific organs or systems in the body. Phytomedicines usually have multiple effects on the body. Their actions often act beyond the symptomatic treatment of disease. An example of this is *Hydrastis canadensis*. *Hydrastis* not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the spleen to release mediating compounds [6].

The increased resistant infections can be checked by a multi-approached program of developing new therapeutic compounds. Studies using plants as a source of novel compounds for resistant organisms needs to be evaluated more and more. Hence screening of plants for antimicrobials against resistant bacterial strains was conducted here. Some of the plants studied, show promising activity and needs further investigation.

## MATERIALS AND METHODS

### *Plant collection and sample preparation*

Based on literature search and ethanobotanical data varied plant species were collected from different regions of India. The plants were identified and authenticated by comparing the botanical descriptions with the known data and voucher specimens deposited in the Piramal LifeSciences Ltd. herbarium by the botanist in charge. The plants studied along with their families are listed in table no.1. The plant part samples were air dried and pulverized in to coarse powder of mesh size 30-40 before use. Each plant sample was extracted using three different solvents viz. methanol, aqueous-methanol and water. The resulting extracts were filtered and the filtrate was collected. The filtrate was vacuum-dried under pressure to obtain solid extract of each plant sample. The stock solution of 20 mg/ml of each extract was prepared in methanol. Various dilutions were further prepared to get a series of concentration.

### *Bioactivity by agar well diffusion method*

The plant extract samples obtained by above method were checked for their activity by agar-well-diffusion method as follows:

### *Microorganisms*

The plant samples were tested against bacterial species, including *Staphylococcus aureus* 209P, Methicillin resistant *Staphylococcus aureus* (MRSA) 3710 and Vancomycin resistant Enterococci (VRE) were obtained as clinical isolates from the hospitals of Mumbai, India and maintained in the laboratory. Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 33591 procured from American Type Culture Collection.

### *Agar-well diffusion method*

Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA) and colonies from subcultures were suspended in saline and vortexed to give an optical density of 0.5 at 600nm ($A_{600nm}$ 0.5 $10^8$ cfu/ml). Predefined volume of inoculum was mixed with molten agar at $40^0$C and poured into sterile petri plates. Plates were allowed to set and solidify. Then wells (six mm in diameter) were cut from the agar using a sterile well-borer and 0.05ml of extract solution was delivered into them. After

**TABLE 1:** List of plants studied along with the family name and parts used.

| Sr.No. | Plant name | Family | Parts used |
|---|---|---|---|
| 1 | *Baliospermum montanum* (Willd.) Müll.Arg. | Euphorbiaceae | Root |
| 2 | *Cassia auriculata* L. | Leguminosae | Stem |
| 3 | *Erythrina suberosa* Roxb. | Leguminosae | Stem |
| 4 | *Haldina cordifolia* Roxb. | Rubiaceae | Stem |
| 5 | *Holoptelea integrifolia* Planch. | Ulmaceae | Leaves |
| 6 | *Holoptelea integrifolia* Planch. | Ulmaceae | Stem |
| 7 | *Ixora brachiata* Roxb. | Rubiaceae | Stem |
| 8 | *Mahonia leschenaultia* (Wall. ex Wight & Arn.) | Berberidaceae | Stem |
| 9 | *Millingtonia hortensis* L.f | Bignoniaceae | Stem |
| 10 | *Murraya paniculata* (L.)Jack | Rutaceae | Stem |
| 11 | *Operaculina turpethum* L. | Convolvulaceae | Aerial part |
| 12 | *Pedalium murex* L. | Pedaliaceae | Fruits |
| 13 | *Premna herbacea* Roxb. | Verbenaceae | Root nodules |
| 14 | *Rungia repens* (L.) | Acanthaceae | Whole plant |
| 15 | *Schrebera swietenioides* Roxb. | Oleaceae | Stem |
| 16 | *Uraria picta* (Jacq.) DC | Fabaceae | Roots and leaves |
| 17 | *Viscum articulatum* Burm.f | Loranthaceae | Aerial parts |
| 18 | *Wattakaka volubilis* (L.f.) Stapf | Asclepiadaceae | Stem |

incubation for 24 hours at 37°C, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters. Positive control of Ampicillin (500 and 50 mcg/ml) and solvent control were included in the study.

## RESULTS AND DISCUSSION

The plants collected from various regions were studied for their anti bacterial property. Total eighteen plants were selected based on the literature search (table 1).

The solvents methanol and the mixture of methanol and water (aqueous-methnaol) are known to extract pharmacologically important compounds like phenolics and anti oxidants from plants [7]. Water was also used as a solvent since the plants were selected based on their ethanobotanical usage where mainly water is used for extraction. Hence these three solvents were used for

**TABLE.2:** Activity profile of the seven active plants

| Plant name | Extract | Concentration (mg/ml) | *S.aureus* 209P | MRSA ATCC 33591 | MRSA 3710 | VRE |
|---|---|---|---|---|---|---|
| *Ixora brachiata* | Methanol | 5 | 11 | 11 | 10 | 11 |
| | | 10 | 13 | 14 | 14 | 11 |
| | | 20 | 15 | 15 | 15 | 13 |
| | Hydro-methanol | 5 | 11 | 10 | 9 | 10 |
| | | 10 | 13 | 13 | 12 | 12 |
| | | 20 | 15 | 15 | 13 | 14 |
| | Aqueous | 5 | 11 | 11 | 11 | 9 |
| | | 10 | 12 | 12 | 11 | 12 |
| | | 20 | 15 | 15 | 14 | 13 |
| *Mahonia leschenaultii* | Methanol | 5 | 18 | 18 | 18 | 13 |
| | | 10 | 20 | 18 | 18 | 14 |
| | | 20 | 25 | 24 | 22 | 18 |
| | Hydro-methanol | 5 | 15 | 14 | 13 | 12 |
| | | 10 | 18 | 17 | 16 | 14 |
| | | 20 | 20 | 20 | 20 | 16 |
| *Wattakaka volubilis* | Methanol | 5 | 11 | 10 | 10 | 9 |
| | | 10 | 14 | 14 | 14 | 11 |
| | | 20 | 14 | 14 | 14 | 12 |
| *Erythrina suberosa* | Methanol | 5 | 10 | 8 | - | - |
| | | 10 | 12 | 11 | 10 | 10 |
| | | 20 | 16 | 15 | 13 | 14 |
| *Holoptelea integrifolia* | Methanol | 5 | 11 | 10 | 8 | - |
| | | 10 | 13 | 11 | 10 | 9 |
| | | 20 | 18 | 16 | 14 | 15 |
| *Pedalium murex* | Methanol | 5 | 13 | 13 | 12 | 12 |
| | | 10 | 14 | 15 | 13 | 12 |
| | | 20 | 16 | 18 | 16 | 14 |
| *Millingtonia hortensis* | Hydro-methanol | 5 | - | - | - | - |
| | | 10 | 12 | 11 | 10 | 11 |
| | | 20 | 16 | 14 | 12 | 13 |

extraction procedure.

The results of the screening study demonstrated potential anti bacterial activity of seven of the plants. The methanolic extract was the most potent extract followed by hydro-methanolic amongst the three extracts. The water extracts of all the plants tested except *I.brachiata* does not show any activity against the tested organisms. The seven active plants out of total eighteen plants screened were active against all the bacterial strains tested including the Methicillin resistant species. These plants showed activity even at the lowest concentration tested which is 5 mg/ml (table 2).

Most of the species that were screened in this study have previously been surveyed for antimicrobial properties. *Ixora brachiata* was the most active plant with all of its three extracts of stem parts showing activity against all the bacteria tested. The data correlates with the previous work done on this plant by various authors where the plant has shown good anti bacterial activity against *Staphylococcus aureus* and dermatophyte species [8, 9]. In our study the plant was also active against the MRSA and VRE strains at the same concentration and showed the same inhibition-zone value as of for normal *Staphylococcus aureus* 209P strain. This indicates the plants potential as a candidate for anti bacterial agent as it is active against the antibiotic-resistant strains of bacteria.

The two extracts of *Mahonia leschnaultii* were active against the bacteria species with the highest zone of inhibition diameter amongst the tested plants. The zone diameters of greater than 22 mm were recorded for the methanolic extract of this plant. The antibacterial activity of this plant is well reflected in the previous work done by Duraiswamy et al [10]. The plant should be further tested for its specific activity with the MIC values to get an actual idea of the anti bacterial activity.

The methanolic extracts of the four plants, *Wattakaka volubilis, Erythrina suberosa, Holoptelea integrifolius* and *Pedalium murex* were active against the bacterial strains with moderate zone of inhibition. The other two extracts of these four plants did not show any anti bacterial activity. *Wattakaka volubilis* has been studied extensively for its anti diabetic and anti inflammatory activity [11], one work done by R.Jeyachandran on leaf extract of this plant shows anti bacterial activity against some bacterial pathogens but no activity was recorded for *Staphylococcus* species [12]. *Holoptelea integrifolius* has shown broad spectrum anti bacterial activity in the study carried out by Reddy et al which matches with our results of methanolic extract of the stem part of the same plant [13]. However the leaf part studied of the same plant did not show any activity. The same plant has shown anti bacterial activity in the aqueous extract in the work of Sharma M.C. et al whereas no activity was found in the water extract of our plant studied [14]. The results of methanolic extract of fruits of *Pedalium murex* which showed good anti bacterial activity matches well with the previous work done on this plants leaf and root extract by some other workers [15, 16]. The aqueous methanol extract of stem bark of *Millingtonia hortensis* showed moderate anti bacterial activity in our study whereas some other authors like Nagaraja et al have got broad spectrum activity for all the extracts including polar and non-polar solvent-extracts for this same plant [17].

Although these active plants have been previously studied for their anti bacterial activity, the most important observation of ours was that these plants were also active against the resistant strains of bacteria at the same concentration and intensity in this screening study.

The plants potential as anti bacterial agents with activity against Methicillin and Vancomycin resistant strains should be further tapped to get a new antibiotic which can handle the infections caused by resistant species that are alarmingly increasing.

Some of these active plants, like *Ixora brachiata*, *Mahonia leschnaultii* and *Wattakaka volubilis* have been reported to show anti inflammatory activity. Such plants with potent anti bacterial activity supported by anti inflammatory activity can be considered for development of a good dermal anti infective agent like for acne conditions. The results obtained further justify the ethanomedicinal use of these plants as anti infectious agents. This preliminary investigation of the activity of these crude extracts indicates the importance of screening plants as an alternative for finding new antimicrobials.

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