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# Synergistic Antibacterial Activity of *Lantana camara* L., *Parthenium hysterophorus* L., *Cannabis sativa* L. and *Justicia adhatoda* L. Leaves Extract against Procured Multi-drug Resistant Bacteria *In Vitro*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author JC designed the study performed the statistical analysis and wrote the first draft of the manuscript. Author PS managed the analyses of the study and contributed in performing the study. Author RG wrote the protocol and contributed in the analyses of the study. Author NS managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The scientific study of this research has been focused on synergistic antibacterial activity of two weed plants, *Lantana camara* L., *Parthenium hysterophorus* L. alongwith two medicinal plants, *Cannabis sativa* L., *Justicia adhatoda* L. against multi- drug resistant (MDR) bacteria. Dried leaf powders of the plants were extracted using air-dried method followed by the ethanol- solvent extraction method for the crude extract of the leaves. The crude extracts were tested for antibacterial activity against three MDR bacteria, that is, one Gram positive bacteria- *Staphylococcus aureus* and two Gram negative bacteria- *Escherichia coli* and *Proteus mirabilis*. Out of 18 antibiotics tested against procured bacteria, *Staphylococcus aureus* was resistant to 10 out of 10 tested antibiotics, *Escherichia coli* was resistant to 4 out of 12 tested antibiotics and *Proteus mirabilis* was resistant to 9 out of 10 tested antibiotics. The tested weed plants and the medicinal plants when combined together showed more zone of inhibition against multidrug

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resistant bacteria ( Two combinations of phytochemicals *Lantana camara*, *Cannabis sativa* and *Lantana camara*, *Cannabis sativa*, *Justicia adhatoda*, *Parthenium* hysterophorus showed maximum zones of inhibition, that is, 30 mm) as compared to when these plants were tested solitarily, showing pronounced antibacterial activity. These findings showed that the antibacterial activity enhanced when they were combined together and this potential could be used against various infectious diseases with more research and modification in this area. Weed plants also holds as much importance as the medicinal plants although not to that extent, but they clearly inhibit the growth of bacteria and this property of weeds along with the medicinal plants holds a promising future in treating many diseases caused by multi-drug resistant bacteria on the pharmaceutical level.

*Keywords: Antibacterial activity; combined effect; multi-drug resistant bacteria; Lantana camara L.; Parthenium hysterophorus L.; Cannabis sativa L.; Justicia adhatoda L.; zone of inhibition.*

## ABBREVIATIONS

*MDR : Multi-Drug Resistance*
*ZOI : Zone of Inhibition*
*AST : Anti-Susceptibility Test*
*L. camara : Lantana camara*
*P. hysterophorus : Parthenium hysyerophorus*
*C. sativa : Cannabis sativa*
*J. adhatoda : Justicia adhatoda*
*E. coli : Escherichia coli*
*Staph. Aureus : Staphylococcus aureus*
*P.mirabilis : Proteus mirabilis*

## 1. INTRODUCTION

Multiple drug resistance, multidrug resistance or multi resistance is an antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories [1]. The MDR types most threatening to public health are those that resist multiple antibiotics, other types include MDR viruses, parasites (resistant to multiple antifungal, antiviral and antiparasitic drugs of a wide chemical variety) [2]. Various microorganisms have survived for thousands of years by their ability to adapt to antimicrobial agents and they do so via spontaneous mutation or by DNA transfer and this process enable some bacteria to oppose the action of certain antibiotics, rendering the antibiotics ineffective [3].

Ethno pharmacology is a related study of ethnic groups and their use of plant compounds and it is linked to medicinal plant use and ethnobotany, a source of lead compounds for drug discovery [4]. Emphasis has long been on traditional medicines, although the approach also has proven useful to the study of modern pharmaceuticals [5]. *Lantana camara* L., a species of flowering plant within the verbena family (Vebenaceae), is a native to the American tropics [6,7]. It has spread from its native Central and South America to around 50 countries, where it has become an invasive species [8,9]. *L. camara* can outcompete native species, leading to a reduction in biodiversity [10]. Studies conducted in India have found that *Lantana* leaves can display antimicrobial, fungicidal and insecticidal properties [11,12]. *L. camara* L. which is widely spread across the whole globe has the potential to be used as an antibiotic drug against many bacterial pathogens and especially against MDR bacteria which poses a great threat in today's health of the people [13]. *Parthenium hysterophorus* is a species of flowering plant in the aster family, Asteraceae and it is native to the American tropics [14]. The pollen grains of *Parthenium hysterophorus* invades disturbes land, including roadsides, it infests pastures and farmland, causing often disastrous loss of yield, as reflected in common names such as famine weed [15]. As an invader, it first appeared as a contaminant in imported wheat [16]. It is being investigated as a means of removing heavy metal dyes from the environment, control of aquatic weeds, commercial enzyme production, an additive in manure for biogas production, as a biopesticide and as a green manure and compost [17]. *Cannabis sativa* L. is an annual herbaceous flowering plant indigenous to Eastern Asia but now of cosmopolitan distribution due to widespread cultivation [18]. Although the main psychoactive constituent of *Cannabis* is tetrahydrocannabinol (THC), the plant is known to contain more than 500 compounds, among them at least 113 cannabinoids; however, most of these "minor" cannabinoids are only produced in trace amounts [19]. The flowers and fruits (and to a lesser extent the leaves, stems and seeds) contain psychoactive chemical compounds known as cannabinoids that are consumed for recreational, medicinal and spiritual purposes, in

traditional medicine of India in particular *C. sativa* has been used as hallucinogenic, hypnotic, sedative, analgesic and anti-inflammatory agent [20]. *Justicia adhatoda* L., commonly known in English as Malabar nut, adulsa, adhatoda, is a medicinal plant native to Asia, widely used in Siddha Medicine, Ayurvedic, Homeopathy and Unani systems of medicine [21]. The leaves of *J. adhatoda* contain phytochemicals such as alkaloids, tannins, saponins, phenolics and flavonoids [22].

In this study, antibacterial activities of the ethanolic leaf extract of the plants were tested against MDR bacteria. The leaves extract were tested solitarily and in combination to observe and compare the ZOI against MDR bacteria.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material and Preparation of the Leaves Extracts

The plants *L. camara, P. hysterophorus, C. sativa,* and *J. adhatoda*, were collected from the city Dehradun of the state Uttarakhand from the country India. The leaves were shade-dried powdered and the powdered leaves were stored in polyethene bags at room temperature for further use. Leaf extracts were obtained by the laboratory water bath method using ethanol as a solvent. The sticky masses obtained were 2.0 g (*L. camara)*, 2.0 g (*P. hysterophorus*), 1.23 g (*C. sativa)* and 1.95 g (*J. adhatoda)* of extract for ethanol, respectively. The concentrated sticky mass for each plant was diluted with a volume of 3% (v/v) mother solvent (ethanol) to produce a 25 mg/ml stock solution which was stored at -4 °C for further test.

### 2.2 Anti-susceptibility Test of Pathogenic Bacteria

The three strains of bacteria, that is, *E. coli*, *Staph. aureus* and *P. mirabilis* were precured from DNA Labs- A Center for Applied Sciences, Dehradun, Uttarakhand, India. These strains were subjected to AST by the Kirby-Bauer's disc diffusion method, on 2mm thick Mueller-Hinton agar (MHA) medium (Microgen, New Delhi) [23]. A fraction from a growing culture was spread on agar for the developoment of bacterial lawn at 37 °C in a BOD incubator. On the lawn of agar plate, 12 antibiotics discs for *E. coli* and 10 antibiotics discs for *Staph. aureus* and *P. mirabilis* were placed at equal distances from one another. These plates were incubated at 37 °C. The ZOI were examined around each disc, according to the antibiotic susceptibility test chart of Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2011).

### 2.3 Antibacterial Activity Test by Agar Well Diffusion Method

The antibacterial activities of the ethanolic solvent extracts of four plants were tested using the agar well diffusion method [24,25]. The strain of the bacterial species which showed maximum number of resistance against antibiotics was taken for observing antibacterial activities of plant extracts. The agar plate surface was inoculated by spreading a volume of microbial inoculum over the 6mm thick agar surface. Wells 6mm deep were punched with a diameter of 8mm with a sterile tip and a volume of 30 µl for solitary plant extract and 20 µl for the combinational plant extract in a ratio of 1:1 was introduced into the well. Plates were incubated at 37 °C for 24-48 h. The inhibition zones were measured for the evaluation of antibacterial activities of the leaf extracts. No reference controls were taken as the method was applied to observe and compare the ZOI of different combinations.

### 2.4 Statistical Analysis

Each experiment was performed at least three times and the data was analysed on the basis of ±Standard Deviation with P values > 0.05 considered to be significant.

## 3. RESULTS

Antibiotic susceptibility test one Gram Positive and two Gram Negative bacteria were carried out. The result of these tests has been given in Table 1 as an antibiogram. *E. coli* was resistant to 4 out of 12 tested antibiotics, *Staph. aureus* was resistant against 10 out of 10 tested antibiotics and *P. mirabilis* was resistant against 9 out of 10 tested antibiotics.

The zones of inhibitions were measured of ethanolic leaves extracts for the evaluation of antibacterial activity of the four plants against MDR bacteria. The maximum zone of inhibition was recorded by the combination of the plants *L. camara* and *C. sativa*, 30 mm±3.55 mm against the MDR bacteria *P. mirabilis* and *L. camara*, *P. hysterophorus*, *C. sativa*, 33 mm±4.54 mm against *Staph. aureus*. The ZOI of all the plants has been recorded in Table 2. And the

comparison of the ZOI of different plant extracts (leaves) has been presented in Fig. 1 against tested MDR bacteria.

The least inhibition has been shown by the plant combination *P. hysterophorus, J. adhatoda*, 10mm, against *P. mirabilis*.

## 4. DISCUSSION

The combined ethanolic leaves extracts showed more ZOI as compared to solitary leaves extracts. *C. sativa*, a medicinal plant has shown the maximum antibacterial activity, solitarily, against the MDR bacteria *E. coli,* that is, 29 mm. In combination, the maximum ZOI has been showed by the plants, *L. camara, P. hysterophorus, C. sativa,* that is, 33 mm, against the MDR bacteria *Staph. aureus*. The minimum ZOI has been shown by the combination of plants, *P. hysterophorus, J. adhatoda.* Therefore, this work has prevailing importance over other similar research into MDR bacteria and the antimicrobial activity which is widely available in other papers [26,27]. Long term hospitalization leads to increased susceptibility of patients to both Gram Positive and Gram Negative bacteria [28]. *L. camara* exhibited considerable antibacterial activity against MDR strains of bacteria [26]. Ethanolic leaf extract of the invasive plant *P. hysterophorus* showed the considerable amount of antibacterial activity against MDR strains as has been studied [29]. *C. sativa* leaf extract has showed the pronounced inhibition against MDR strain of *Staph. aureus* and *E. coli* in this study [30] but in our work, ethanolic leaf extract has also shown the considerable amount of antibacterial activity. The leaves of *J. adhatoda* showed minimum inhibitory concentration against MDR strain of *E. coli* and highest minimum inhibitory concentration against MDR strain of *Staph. aureus* [31] but in our study it has shown moderate antibacterial activity when tested alone and pronounced activity when tested in a combination with other plants. More than 95% of the medicinal plants are collected from the wild; a number of them have become endangered in their natural habitats [32]. The analysis of antibacterial activity of weed plants carried out in this work could be helpful in the biodiversity conservation of medicinal plants by increasing the use of weed plants as compared to that of medicinal plants and hence, could be considered as significant from the ecological point of view.

The readings may vary if this work would be carried out elsewhere for research purpose as conditions may differ with different factors involved. Many studies have been carried out by using different plants but they all were carried out using only single plant as we have discussed previously. This study was carried out to observe the leaves extracts but in combination with other plants to see the impact against MDR bacteria. In future medicine, the combination of effective plants could be used against MDR pathogens with more modifications.

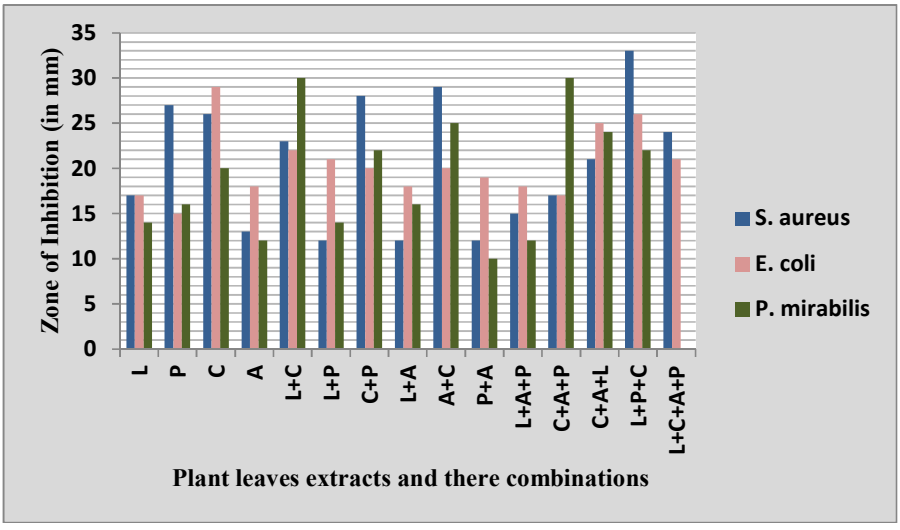


**Fig. 1. Bar graph depicting the comparison of ZOI produced by four plant leaves extracts, solitarily and in combination**

**Table 1. Antibiogram of Precured Pathogenic Bacteria**

| Bacterium | Tested Antibiotics | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MR | CFM | AMP | LE | NX | Ak | GEN | CLM | ETP | OF | AMC | CZ | TE | CIP | IMP | LZ | TR | VA |
| *Stap.aureus* | NT | NT | R | NT | NT | R | R | NT | R | R | R | R | R | R | R | NT | NT | NT |
| *E. coli* | R | R | R | S | R | S | S | S | S | S | S | S | NT | NT | NT | NT | NT | NT |
| *P. mirabilis* | R | R | R | R | NT | NT | R | R | NT | NT | NT | NT | R | NT | NT | R | R | S |

*R- Resistant; S- Sensitive; NT- Not Tested; Antibiotics(µg/disc): MR- Meropenem 10; CFM- Cefixime 5; AMP- Ampicillin 10; LE- Levofloxacin 5; NX- Norfloxacin 10; Ak- Amikacin 30; GEN- Gentamicin 10; CLM- Colistin 10; ETP- Ertapenem 10; OF- Ofloxacin 5; AMC- Amoxyclav 30; CZ- Cefazolin 30; TE- Tetracycline 30; CIP- Ciprofloxacin 5; IMP- Imipenem 10; LZ- Linezolid 30; TR- Timethoprim 5; VA- Vancomycin 30*

**Table 2. Antibacterial assay of the plant leaves extracts (ethanol solvent) by agar well diffusion method**

| MDR Bacteria | Zone of Inhibition by the plant leaves extracts(ethanol solvent) in mm | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | L | P | C | A | L+C | L+P | C+P | L+A | A+C | P+A | L+A+P | C+A+P | C+A+L | L+P+C | L+C+A+P |
| *Staph.aureus* | 17 | 27 | 26 | 13 | 23 | 12 | 28 | 12 | 29 | 12 | 15 | 17 | 21 | 33 | 24 |
| *E. coli* | 17 | 15 | 29 | 18 | 22 | 21 | 20 | 18 | 20 | 19 | 18 | 17 | 25 | 26 | 21 |
| *P.mirabilis* | 14 | 16 | 20 | 12 | 30 | 14 | 22 | 16 | 25 | 10 | 12 | 30 | 24 | 22 | 30 |

***L***- *L.camara;* ***P***- *P.hysterophorus;* ***A***- *J. adhatoda;* ***C***- *C. sativa*

## 5. CONCLUSION

The effective *in vitro* control of MDR strains, *Staph. aureus, E. coli, P. mirabilis* by plants extract *L. camara, P. hysterophorus, C. sativa* and *J. adhatoda* has been recorded through this study. It could be concluded from this study that when the plants showed pronounced antibacterial activity in combination as compared to when tested solitarily, somehow the phytochemicals enhanced the inhibiting property of each other. These findings could be applied as a complementary medicine with more research and modifications against MDR pathogens.

## CONSENT

It's not applicable.

## ETHICAL APPROVAL

It's not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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