



Comparative Study Of Antimicrobial Activity Of Different Plants Against Multi Drug Resistant Pathogen *Staphylococcus aureus* ATCC242 Isolated From Burnt Patients And The Effect Of Different Binary Combination Of Antimicrobial Plant Extracts

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Abstract

Recently, multiple-drug resistant strains have become a serious concern. New Prototype antimicrobial agents were screened from six different plants extracts. It showed a significant clinical value in the treatment of multi-drug resistant pathogen *Staphylococcus aureus* ATCC22 isolated from burn unit of All India Institute of Medical Sciences (A.I.I.M.S). The organic extracts of different plants along with their parts included *Lantana camera* (Leaves, Flower and Berries); *Argimone mexicana* (Leaves and seeds); *Catharanthus roseus* (Leaves); *Calatropis gigantea* (Leaves and Latex); *Abutylon theophosti* (Leaves and Fruits) and *Eucalyptus* (Bark and Oil). The Zone of Inhibition was evaluated by Agar well-diffusion method. The highest antimicrobial potentials were observed for the *Eucalyptus* oil and ethanol extract of its bark and *Lantana* leaves. The combined effect of some extracts increases its resistance to test microorganism with minimal dosage of crude extract compared to individual plant extracts. The active compound responsible for the pharmacological responses in binary plant extract is used for further research work.

Keywords: Antimicrobial activity, Multi-drug resistant pathogen, Agar Well-Diffusion method, Binary plant extracts combination.

Introduction

Various microorganisms have survived for thousands of years by their being able to adapt to antimicrobial agents. They do so via spontaneous mutation or by DNA transfer. It is this very process that enables some bacteria to oppose the assault of certain antibiotics, rendering the antibiotics no longer effective. These microorganisms employ several mechanisms in attaining multidrug resistance:

- No longer relying on a glycoprotein cell wall.
- Enzymatic deactivation of antibiotics.
- Decreased cell wall permeability to antibiotics.

- Altered target sites of antibiotic.
- Efflux mechanisms to remove antibiotics.
- Increased mutation rate as a stress response.

Methicilline Resistant *Stap. aureus* (MRSA) continues to be a major cause of serious infection to man, both in hospitals and in the community. Until the early 1980's MRSA reports consisted of isolated cases, later in 1982 epidemic MRSA strains (EMRSA) were described as multi-resistant strains with special capacity to colonize patients and staff and cause widespread outbreaks of infections. (Pavillard., et al. 1982).

Staphylococcus aureus is the most frequently isolated wound pathogen, and it is becoming increasingly resistant to antibiotics. (Shanson D.C. 1981). It causes significant

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morbidity and mortality to the burn patients who have been shown to become colonized and infected more readily than other patient groups. Extensive burn injuries are particularly susceptible to infection as a result of the disruption of the normal skin barrier and accompanying depression of immune responses. (Samy A Shehab El-Din et al. 2003). Extended hospitalization and antibiotic therapy leading to nosocomial infection and have been identified as additional risk factors for MRSA carriage and infection. (Lee and Bishop, 1997).

Traditional healers have long used plants to prevent or cure infectious disease. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit the growth of pathogenic micro-organisms.

Materials and Methods

Plant Extract:

The plant materials used in this study consisted of *Lantana camera* (Leaves, Flower and Berries); *Argimone mexicana* (Leaves and seeds); *Catharanthus roseus* (Leaves); *Calatropis gigantea* (Leaves and Latex); *Abutylon theophrasti* (Leaves and Fruits) and Eucalyptus (Bark and commercially available Oil) were collected from Noida, U.P, India during mid-Jan to mid-July. The plant varieties were identified by Dr Devendar Mallik, HOD-Botany, CCRD, Muzaffarnagar, U.P. The plant parts were initially rinsed with distilled water and dried on paper towel in laboratory at $(37 \pm 1)^\circ\text{C}$ for 24hr.

Ethanol extract - After drying, the plant materials were macerated in pestle and mortar, 10gm of powdered plant material was thoroughly mixed with 100ml organic solvent. The mixtures were kept for 48 hr in tightly sealed vessels at

room temperature, protected from sunlight and mixed several times with a sterile glass rod. This mixture was filtered through Whatman no.1 filter paper and the residue, adjusted to the required concentration with the extraction fluid for further extraction and it was repeated thrice and a clear colourless supernatant extraction liquid was finally obtained. The extracted liquid was subjected to rotary evaporation in order to remove the ethanol. The semisolid extract produced was kept in a freezer at -80°C overnight and then subjected to freeze drying for 24h at -60°C in 200 ml vacuum. Then the extract was dissolved in Tris-Saline Buffer and was stored in an airtight container at 4°C in refrigerator for further use.

Screening for the Antimicrobial potential of the Plant Extracts:

Test microorganism:

S. aureus (ATCC242) was used as the test organism and was obtained from Microbiology Laboratory of All India Institute of Medical Sciences, (A.I.I.M.S) college and Hospital, Ansari Nagar, New Delhi-29. The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C .

Antimicrobial activity

Agar-well Diffusion Method - The antimicrobial activity of the crude extract was determined by agar well diffusion method (Perez et al. 1990). The test microbes were removed aseptically with an inoculating loop and transferred to a test tube containing 5ml of sterile distilled water. Sufficient inoculum's were added until the turbidity equalled 0.5 McFarland (10^8 CFU/ml). One millilitre of the test tube suspension was added to the 15-20ml of nutrient agar plates. Wells (6 mm diameter) were punched in the agar and filled with 25, 50, 75, 100 and 150 μl of 2000 $\mu\text{g/ml}$ plant extracts. The plates were incubated at 37°C overnight and examined for zones of growth inhibition.

Results

Table 1 Antimicrobial activity of different extracts against Multi Drug resistant pathogen *Staphylococcus aureus* ATCC 242:

SL NO.	PLANTS	PARTS	MEASUREMENT OF ZONE OF INHIBITION (MM)				
			25 μl	50 μl	75 μl	100 μl	150 μl
1	Lantana	leaves	+	+	++	++	++++
		Flower	+	+	+	+	+
		Berries	+	+	+	+	+
2	Abutylon	Fruits	-	-	-	-	+
		Leaves	-	-	+	+	+

3	Calotropis	Leaves	+	+	+	+	+
		Latex	-	-	-	-	-
4	Argimone	Leaves	-	+	+	+	+
		Seeds	-	+	++	++	++
5	Eucalyptus	Oil	++	+++	+++	+++	++++
		Bark	-	-	-	-	++++
6	Catharanthus	Leaves	+	+	+	+	+

+ = Between 5-15 mm; ++ = Between 16-25 mm; +++ = Between 26-35 mm;
 ++++ = Above 35 and - = Nil.

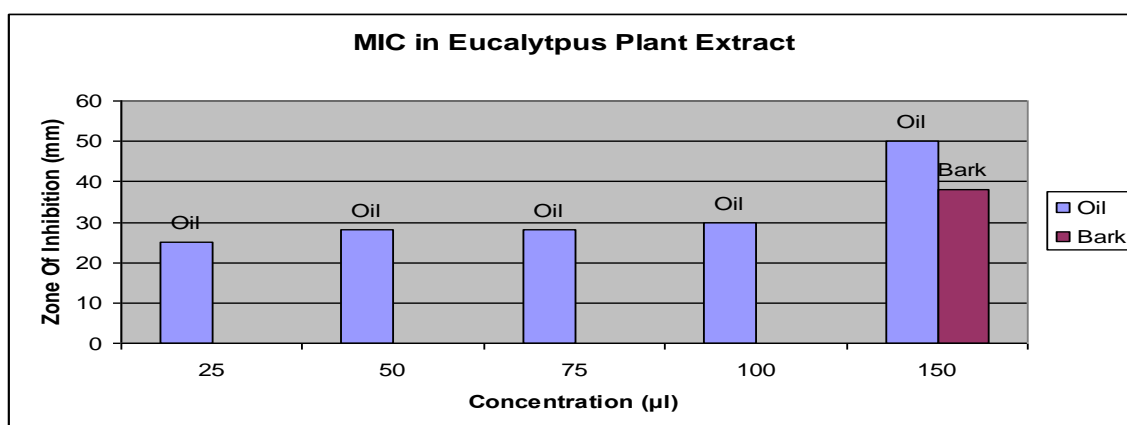


Figure 1 Graphical representation of Eucalyptus plant extract against MDR pathogen *Staph. aureus* ATCC 242

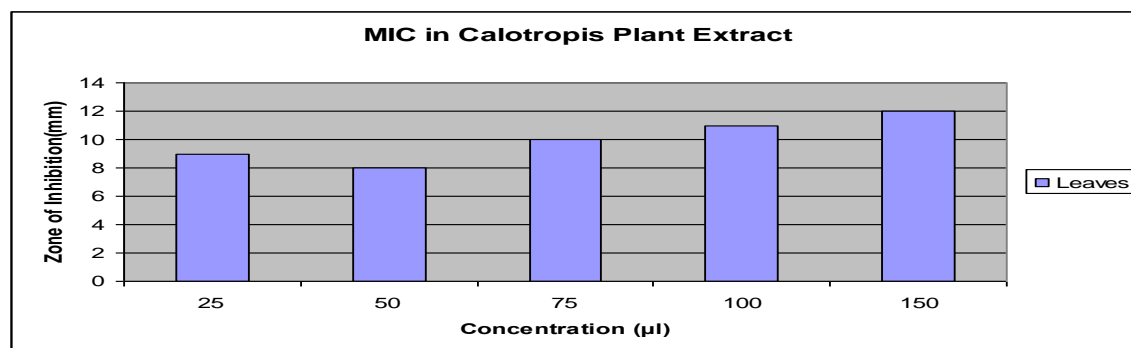


Figure 2 Graphical representation of Calotropis plant extract against MDR pathogen *Staph. aureus* ATCC 242

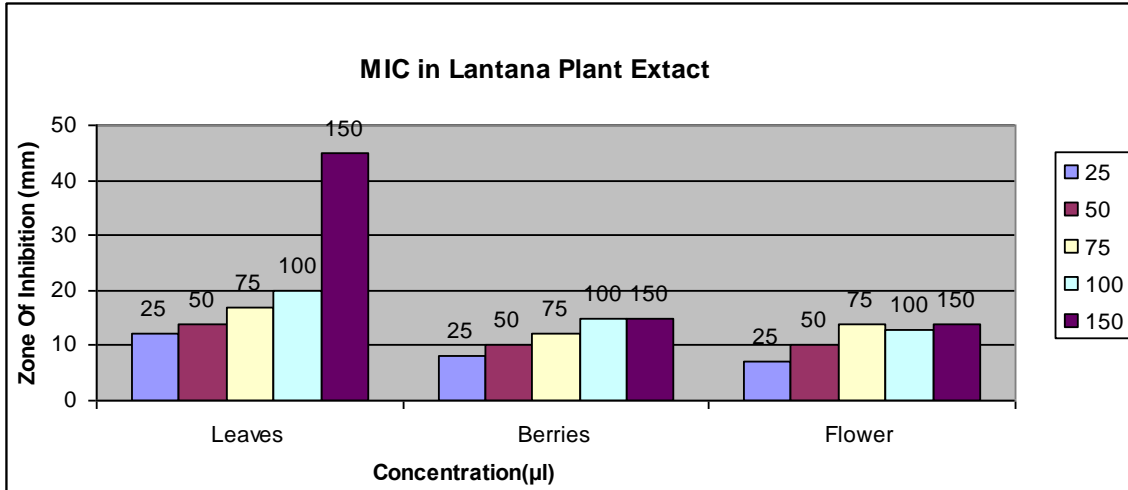


Figure 3 Graphical representation of Lantana plant extract against MDR pathogen *Staph. aureus* ATCC 242

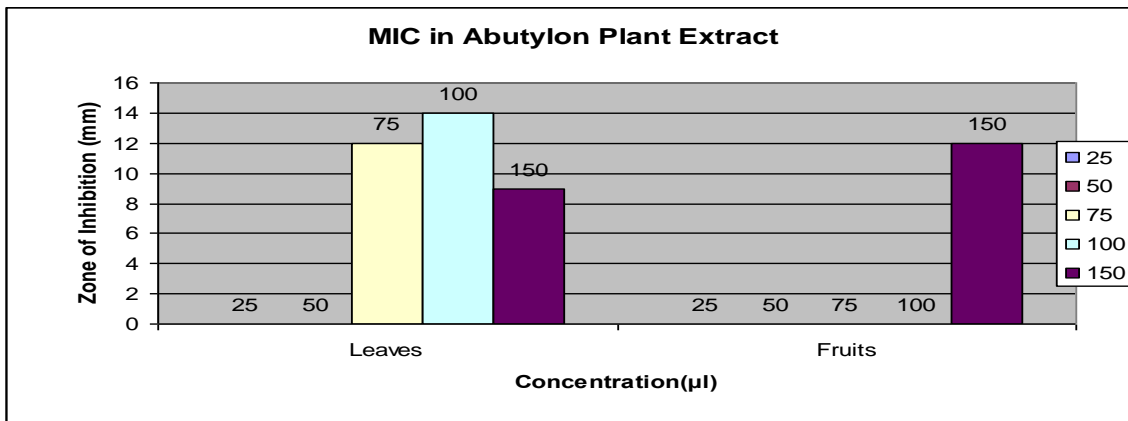


Figure 4 Graphical representation of Abutylon plant extract against MDR pathogen *Staph. aureus* ATCC 242

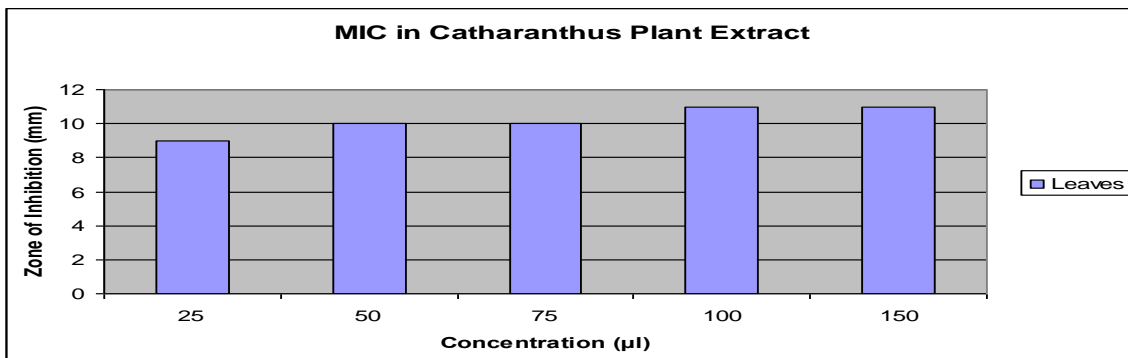


Figure 5 Graphical representation of Catharanthus plant extract against MDR pathogen *Staph. aureus* ATCC 242

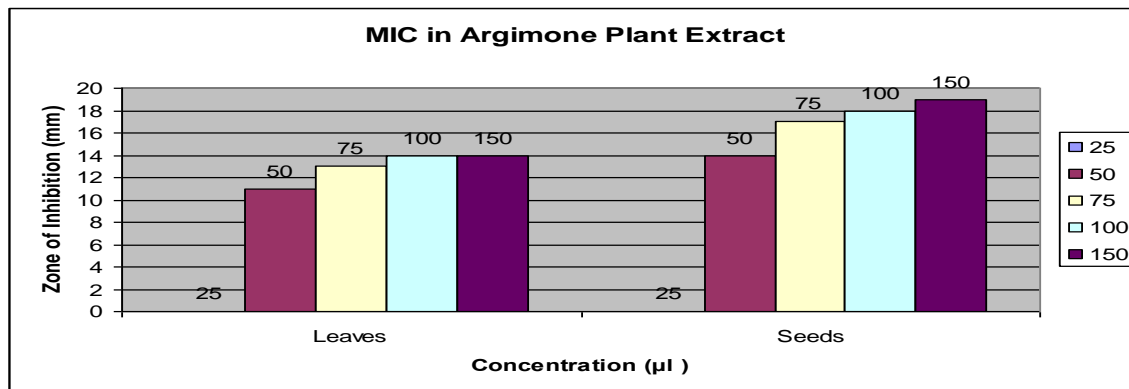


Figure 6 Graphical representation of Argimone plant extract against MDR pathogen *Staph. aureus* ATCC 242

Discussion

Since multiple drug resistance of *Staphylococcus aureus* ATCC 242 is a major medical problem, screening of natural product in a search for new antibacterial agents that would be active against pathogen is the need of hour. The present study reveals the antimicrobial potential of different parts of *Lantana camera*, *Catharanthus roseus*, *Eucalyptus*, *Calotropis procera*, *Argimone maxicana*, *Abutylon theophrasti* against *Staphylococcus aureus*. Almost all parts of plant showed antimicrobial potential (Figure 1-6), except *Calotropis latex*. (Table 1).

The highest antimicrobial potential was shown in *Eucalyptus* oil and ethanol extraction of *Eucalyptus* bark and *Lantana* leaves. (Figure 1 & 3).

Hence, MDR *S. aureus* ATCC 242 infection could be treated by the *Eucalyptus* Oil, Bark and *Lantana* Leaves and may be used as an antibacterial agent in known dosages, especially in rural communities where conventional drugs are unaffordable or unavailable and the health facilities are inaccessible. The results presented here indicate that the natural products analyzed seem to be a good choice for the development of new strategies to treat MDR *S. aureus* ATCC 242 infections.

Besides this, the effect of different binary combination of antimicrobial plant extracts is still in the mode of trial. These combined effects will surely going to lower the individual drug dosages quantity used earlier and will be more efficiently against MDR pathogen.

ACKNOWLEDGEMENTS

We take this opportunity to express our gratitude to Dr. Rai Ajit Kumar Srivastav, Director of Clonogen Biotechnology Pvt Ltd, Noida for providing us an opportunity to work in this prestigious project.

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