



Antibacterial activity of four ethnobotanical drugs of Rayalaseema, Andhra Pradesh, India.

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Abstract: The study aimed to evaluate the antibacterial activity by crude aqueous extract of ethnobotanical drugs prepared and prescribed by ethnic healers of Rayalaseema region. Ethnic drugs prepared from whole plant parts of *Litsea deccanensis* (LD), *Atalantia racemosa* (AR), *Hiptage benghalensis* (HB) and *Lagerstroemia parviflora* (LP) at dosages of 25,50,75 mg/mL were tested against pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*) using agar well diffusion technique. Results reported significant antibacterial effect at 50 & 75 mg/mL for all the drugs tested and recommend these crude drugs as antibacterial agents.

Key words: Ethnobotanical drugs; Rayalaseema; Antibacterial activity.

Introduction

The existing antimicrobial agents depriving its potency and efficacy against new microbial evolution and resistance resulting need to optimize microbial infestations and its control by developing novel antimicrobial agents (Balouiri *et al.*, 2016; Mostafa *et al.*, 2018; Boakye *et al.*, 2019). The mining of novel antimicrobial agents from traditional medicinal plants is critically needed due to the surge in antimicrobial resistance in global health concerns and there is a vital need to replenish our arsenal of antimicrobial agents. Plant derived formulations proved to be historical pharmacopoeias with potential therapeutic properties with ample diversity of metabolites which act as antimicrobial agents (Valgas *et al.*, 2007; Sinha *et al.*, 2013; Dubey *et al.*, 2018; Elhidar *et al.*, 2019). In the vision of ethnic healing commodities, there are plenty of ethnic drugs prepared from the locally available plants setting up by varied formulation, dosage and citing precautions.

Research concerning the biological efficiency of whole plant crude drugs proposed by ethnic healers of rayalaseema were scarce which made to take up this investigation. Studies on the ethnobotanical crude drugs which were prepared from whole plant parts are not properly documented.

In the present paper, we have attempted to scientifically validate the ethnic antibiotic plant crude drugs prescribed and recommended by ethnic healers of Rayalaseema region of Andhra Pradesh.

Materials and Methods

Ethnobotanical exploration in Rayalaseema revealed few commonly used crude ethnobotanical drugs prescribed for different microbial infestations and for wounds. Data on ethnic plant drugs and its dosage, composition, applied conditions in intake were collected from different ethnobotanical healers and village

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vaidyas by interviews. we have selected four highly recommended crude drugs as prescribed by ethnic healers which were prepared by maceration and drying of whole plant parts (Root: stem: Leaf in 1:1:1 ratio) of *Litsea deccanensis* (LD), *Atalantia racemosa* (AR), *Hiptage benghalensis* (HB) and *Lagerstroemia parviflora* (LP). The plant taxa which were used in the drugs preparation was revealed by them and identified on critical examination thorough scrutiny of relevant taxonomic literature, flora's (Pullaiah et al., 2007; Chetty et al., 2015). Voucher specimens of the identified taxa were deposited in the Herbarium, Department of Botany, Sri Venkateswara University, Tirupati.

Determination of Antibacterial Assay

In-vitro antibacterial activity of the crude aqueous extract was investigated against four bacterial strains (Gram positive - *Bacillus subtilis*, *Staphylococcus aureus*; gram negative bacteria- *Escherichia coli* and *Klebsiella pneumonia*) by the agar well diffusion method followed in various research studies (Perez et al., 1990; Balouiri et al., 2016; Valgas et al., 2007; Oses et al., 2016) with slight modifications. Mueller Hinton agar (MHA) (Hi Media, India) was used as the bacteriological medium. The MHA was melted and cooled to 48°C - 50°C and a standardized inoculum (1.5×10^8 CFU/mL, 0.5 McFarland standard) was then added aseptically to the molten agar and transferred into sterile Petri dishes to give a solidified plate. Four wells (6mm) were prepared in the seeded agar plate. The testing material (25 - 75 mg/mL) was introduced in the well accordingly and the seeded plates were incubated overnight at 37°C. Bacterial zones of inhibitions were measured in mm (Kirby et al., 1957). Commonly used antibiotics Ciprofloxacin (CF) (0.4 µg/mL) and Gentamycin (GM) (0.4 µg/mL) were considered for control and comparison was done with selected susceptibility testing methods were performed as recommended by CLSI (M07-A). The bacterial growth was assessed by a stereo

microscope after the incubation period. All tests were done in triplicates and the mean value is scored.

Results and Discussion

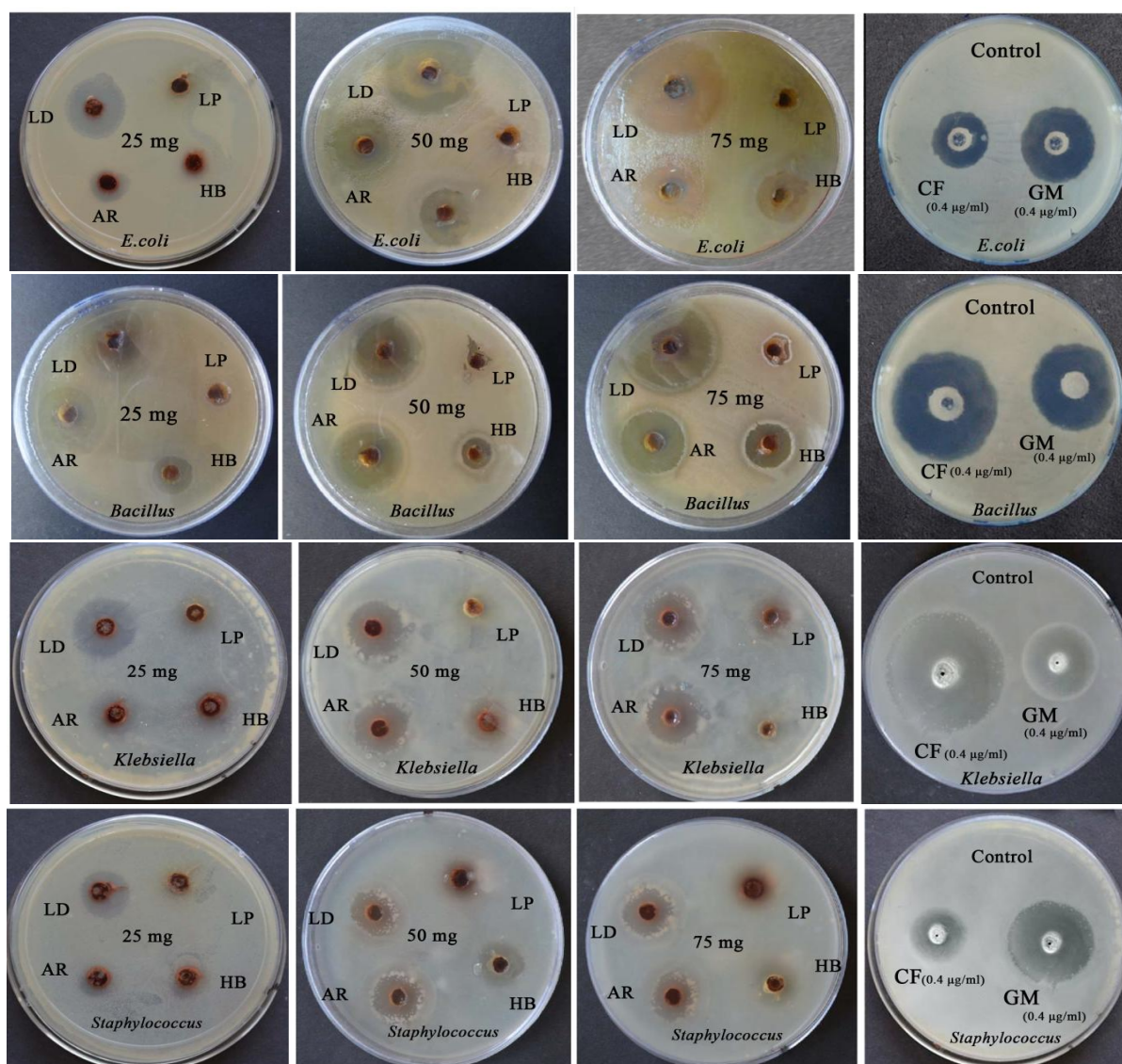
In our observation, whole plant drugs are prominent in ethnic hamlet of rayalaseema tribal hamlets. Inorder to validate scientifically, and to evaluate the antibacterial activity by aqueous crude drug extracts prepared with whole plants of *L.deccanensis* (LD), *A. racemosa* (AR), *H. benghalensis* (HB) and *L. parviflora* (LP) were tested against gram positive (*B.subtilis*, *S.aureus*) and gram negative bacteria (*E.coli* and *K. pneumonia*) at 25 mg/mL, 50 mg/mL, 75 mg/mL. Results showed the inhibitory effect of aqueous extracts of the selected ethnobotanical drugs increased with increasing the concentration from 25 to 75 mg/mL. (Table 1; Fig. 1).

The antimicrobial spectrum of the drugs extract was determined in terms of zone sizes around each well (Fig 1 : A, B, C, D). The diameters of zone of inhibition produced by the drugs were compared with the zone diameter showed by the control Ciprofloxacin (CF) and Gentamycin (GM). Dose response of the crude drugs against pathogenic bacteria has been illustrated in Table 1. and Figure 1. The experiment was performed three times to minimize the error and the mean values are presented in Table 1.

In general, all the selected bacterial strains were highly susceptible at 75 mg/mL against all the four drugs (LD, AR, HB, LP) possessing great inhibitory effect where as intermittently susceptible at 50mg/mL with less inhibitory effect. Three drugs LD, AR and HB showed good antibacterial effect for all the concentrations (25,50& 75mg/mL). The drug LD formulated with *L. deccanensis* is found to be highly anti-bacterial agent among all the drugs. Bacterial strains *E. coli* and *B.subtilis* found to be resistant against LP at 25 mg/mL and are susceptible at 50 and 75 mg/mL only. Due to this, antibacterial activity of the drug prepared

Table 1: Antibacterial effect of ethnobotanical drugs of Rayalaseema

Bacteria	Conc. of extract mg/mL)	<i>Litsea deccanensis</i>	<i>Atalantia racemosa</i>	<i>Hiptage benghalensis</i>	<i>Lagerstroemia parviflora</i>
<i>E. coli</i>	25	10.33±1.52	4.33±0.58	1.23±0.58	0
	50	13.64±2.08	8.82±0.58	4.32±1.15	1.22±0.58
	75	16.33±1.15	13.33±1.15	6.88±1.53	1.68±1.15
<i>Bacillus subtilis</i>	25	11.21±0.58	18.33±0.58	2.22±0.58	0
	50	15.14±1.15	8.33±0.58	3.22±0.58	1.2±1.15
	75	18.42±1.15	11.87±1.15	4.3±0.58	1.6±1.15
<i>Klebsiella pneumonia</i>	25	6.67±1.15	4.83±0.58	1.7±0.58	5.33±1.15
	50	8.33±0.58	6.2±1.15	2.9±0.58	7.33±0.58
	75	9.2±1.53	7.2±0.58	3.1±0.58	9.67±0.58
<i>Staphylococcus aureus</i>	25	4.33±0.58	1.66±0.58	1.7±0.58	1.1±0.58
	50	5.13±0.58	3.66±0.58	2.9±0.58	2.53±1.15
	75	8.33±1.058	4.78±3.21	3.12±0.58	3.33±1.15

**Figure 1.** A, B, C, D. Illustrates antimicrobial activity of four selected ethnic drugs (LD, AR, HB, LP) at 25, 50, 75 mg/ml against pathogenic bacteria *E. coli*, *B. subtilis*, *K. pneumonia* and *S. aureus* and compared with standard antibiotics Ciprofloxacin (CF) & Gentamycin (GM).

from *L. parviflora* is very less and showed moderate antibacterial activity at 50 mg/mL. It is interesting to note that extracts were more effective against bacteria equal to the antibiotics CF and GM at 50 mg/mL. (Fig 1.A,B,C,D). The overall anti-bacterial effect (when compared with zone size of inhibition) for the four selected ethnic drugs were in the order: LD > AR > HB > LP in terms of activity when compared between them.

In this experiments the tested product are crude drugs, where the rate of diffusion depended on its solubility as opined by Valgas *et al.*, (2007). Previously, we have done comparative morpho-anatomical studies of these four taxa (NagaPadmavathi *et al.*, 2018; NagaPadmavathi and Madhavachetty, 2019).

This promissory crude drug extracts which were prepared in equal ratio of vegetative parts of *Litsea deccanensis* (LD), *Atalantia racemosa* (AR), *Hiptage benghalensis* (HB) and *Lagerstroemia parviflora* (LP) laid the possibility of finding new clinically effective antibacterial compounds and ingredients. Additional research on these ethnic crude drugs prescribed by the tribal healers in rayalaseema region are necessary concerning the application of different extraction methods, isolation, purification of antibacterial compounds to testify and formulate the standard antimicrobial drugs.

Conclusion

In conclusion, scientific validation has been done on the four crude drugs prescribed by ethnic healers of rayalaseema and demonstrated as the reliable source for the ethnic treatments against infectious diseases.

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