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Research Article

COMPARATIVE ANTIBACTERIAL ACTIVITIES OF THE COMBINED CRUDE LEAF EXTRACT OF *BIXA ORELLANA, AZADIRACHTA INDICA* AND *OCIMUM SCANTUM*

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ABSTRACT

In the present paper, we analyze the past, present and future of medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents. Ethanol extracts of the leaves of *Bixa orellana, Azadirachta indica* & *Ocimum scantum* were investigated for their invitro antimicrobial properties. Fresh leaves were collected randomly from Bhimavaram region, India. Plants were compared with voucher specimens deposited by Dr. B. Sarveswara Lingam at Department of Botany, K.G.R.L College (Autonomous), Bhimavaram, Andhra Pradesh, India. The aim of study was to scientifically test whether plant used in traditional medicine for the treatment of infection showed antibacterial activity. Extracts of sample of 3 species traditionally used as antibacterial were screened for activity against *Esherichia coli, Pseudomonas aeruginosa* & *Staphylococcus aurues.* The study confirms that simple laboratory methods are very well suited to assess the efficacy of traditionally used medicinal plants to inhibit bacterial growth. A comparison to the traditional uses also indicate that local knowledge can give important leads for the development of new treatments. Further tests, especially with regards to toxicity, are needed to verify the safety of the traditional preparations.

KEYWORDS: Antibacterial activity, Medicinal plants, Bhimavaram, Traditional medicine.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine [1]

The search for eternal health and longevity and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, minerals etc. and the development of a variety of therapeutic agents [2.]

A total of 122 biologically active compounds have been identified, derived only from 94 species of plants. A conservative estimate of the number of flowering plants occurring on the planet is 2,50,000. Of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically. Consistent findings should be carried out to discover a probable abundance of medicinal extracts in these plants [3.]

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency [4,5, 6.]

In the present study, ethanol extracts of 3 medicinal plants were screened for antimicrobial activity against 3 different standard strains of microorganisms. Further, all the three plant extracts were mixed and screened for antimicrobial activity against 3 standard strains of microorganisms.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves were collected randomly from Bhimavaram region, India (K.G.R.L College campus, Dirsumarru Road). The details of the plant/plant parts screened, their families and their locality are given in Table-1 [7,8,9.] Plants were compared with voucher specimens deposited by Dr. B. SarveswaraLingam at Department of Botany, K.G.R.L College (Autonomous), Bhimavaram, Andhra Pradesh, India. Fresh plant materials were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Ethanol Extraction

Thoroughly washed fresh leaves of 3 plants *Bixa orellana, Azadirachta indica* & *Ocimum scantum* of plant materials dried in shade for two weeks & then powdered with the help of waring blender, 100gm of shade dried powder was mixed with 250 ml ethanol & taken in soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure & preserved at 5 °C in airtight bottle until further use.

Source of Micro Organism

The organisms used *were Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aurues.*organisms were obtained from K.G.R.L Degree microbiology lab & K.G.R.L pharmacy microbiology lab.

Medium preparation

Accurately weigh 55gr of macconkey agar and make up to the volume (1000ml)using distilled water(used for *Esherichia coli*). Nutrient agar medium is prepared(used for *Pseudomonas aeruginosa*, *Staphylococcus aurues)* and the components are Agar (15gr), Beef extract (3gr), Sodium chloride (5gr), Peptone (5gr), make up to 1lt using distilled water. Above prepared mediums are autoclaved at 121°C temperature, 15lb pressure for 15 minutes.

Stock preparation

Stock solutions of various organic crude extracts were prepared by mixing well the appropriate amounts of dried extracts and suitable solvent(distilled water)to give rise the

final concentrations. various concentrations of dried crude extracts are made 1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml.

Determination of antimicrobial activity [10]

Petri dishes (size 100 mm diameter) containing 18 ml of cool and molten Agar (at 40°C) were seeded with 100 µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 1.0 x 108 CFU/ml). Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Wells of 6 mm diameter were cut into solidified agar media with the help of sterilized core borer.

Aliquot 100 µl of each extract was poured in the respective well and the plates were incubated at 37°C overnight. Solvents (distilled water), in which extracts concentration were prepared, were used as negative control while Streptomycin antibiotic of one unit strength was used as positive control. The experiment was performed in triplicate under strict aseptic conditions. The antibacterial activity for each of the extract evaluated was expressed in terms of the average of the diameter of zone of inhibition (in mm) produced by the respective extract at the end of incubation period. Standard deviations were also calculated and represented in the respective table against each extract. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm.All the assays were performed in triplicate and expressed as average values

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition. The MIC had done by 96 well U bottom plates. The MIC plates were filled with Macconkey Agar, agar and various concentrations of plant extracts, antibiotics-Streptomycin or solvent control. Finally the MHB medium with overnight test organism (10-8cfu/ml-1) was equally distributed. All the samples were prepared in triplicates and incubated at 37°C for 24 hrs. The least concentration (highest dilution) of the extract that inhibits colony formationon a solid agar medium after incubation at 37°C for 24 hr was considered as MIC.

RESULTS AND DISCUSSION

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, pathogenic microorganisms are constantly developing resistance to these drugs [11] because of indiscriminate use of antibiotics [12,13].

Results obtained in the present study relieved that the antibacterial activities of *Bixa orellana, Azadirachta indica* & *Ocimum scantum* showed that Ethanolic extract shown good inhibitory activity on the test organisms taking reference Streptomycin as standard drug. The MIC was observed in 100 µg/ml in ethanol extracts & acetone extracts.

Combination of ethanolic *Bixa orellana, Azadirachta indica* & *Ocimum scantum* extracts shown better antibacterial activity compared to acetone extracts of *Bixa orellana, Azadirachta indica* & *Ocimum scantum,* whereas ethanolic combination extract shown better inhibitory zone compared to individual extract taking referance Streptomycin as standard & normal saline as control.

Table-1

Name of plant	Plant parts	Family	locality
Ocimum Scantum	Leaf	Lamiaceae	Dirusumarru (BVRM)
Bixa Orellana	Leaf	Bixaceae	K.G.R.L Campus
Azadirachta indica	Leaf	Meliaceae	K.G.R.L Campus

Table: 2 Minimum inhibitory concentration (MIC) of *Bixa orellana, Azadirachta indica, Ocimum scantum* & Streptomycin

Compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Bixa orellana,	100	100	100
Azadirachta indica	75	100	100
Ocimum scantum	100	100	100
Streptomycin	10	10	10

The value of all the compounds is in µg/ml

Table: 3 Combination antibacterial activity (Ethanol Extract)

Bacteria	Bixa+Neem+Tulsi Leaves Ethanol extract				Streptomycin (Std) & 10µg/ml	Saline (Control)
	100 µg/ml	250 µg/ml	500 µg/ml	1000µg/ml		
Escherichia coli	7.0 ±0.57	10.6±0.8	14.6±0.6	17.6±0.8	22.3±0.88	0.33±0.33
Pseudomonas aeruginosa	6.6±3.33	11.0±1.52	13.6±1.45	17.6±0.8	21.0±1.5	0.33±0.33
Staphylococcus aureus	5.00±0.57	11.6±1.45	13.6±1.45	16.0±1.33	20.3±1.2	0.33±0.33

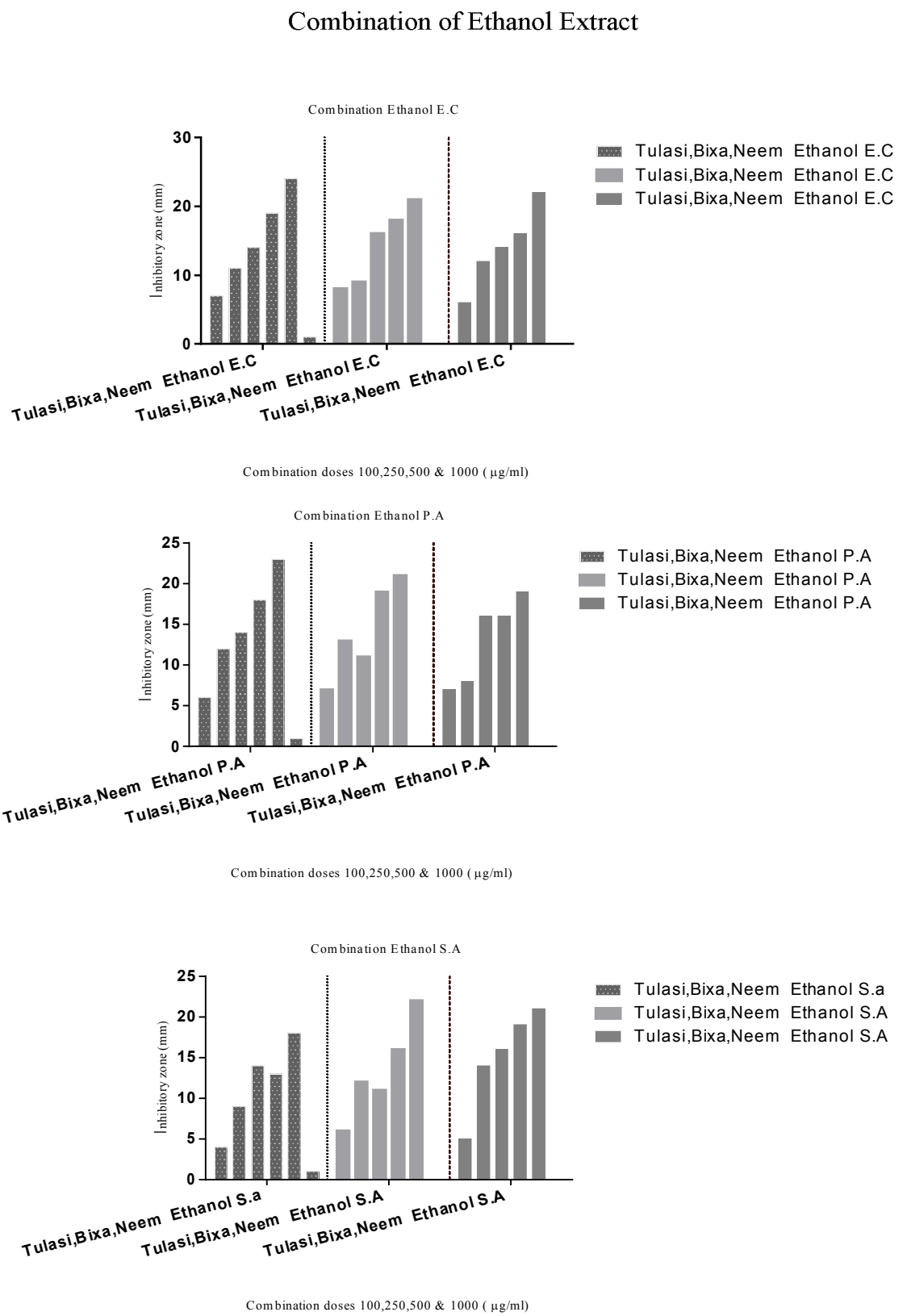
The values represents mean of sample±SD for n=3. Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample.

Table: 4 Combination antibacterial activity (Acetone Extract)

Bacteria	Bixa+Neem+Tulsi Leaves Acetone extract				Streptomycin(Std) & 10µg/ml	Saline(Control)
	100 µg/ml	250 µg/ml	500 µg/ml	1000µg/ml		
Escherichia coli	3.0 ±0.57	6.3±0.8	11.0±1.5	14.0±1.5	21.0±0.5	0.33±0.33
Pseudomonas aeruginosa	2.3±0.33	4.3±0.8	10.3±1.8	12.6±2.0	22.0±1.1	0.33±0.33
Staphylococcus aureus	3.3±0.3	6.0±0.5	10.6±0.8	14.3±0.8	20.3±1.2	0.33±0.33

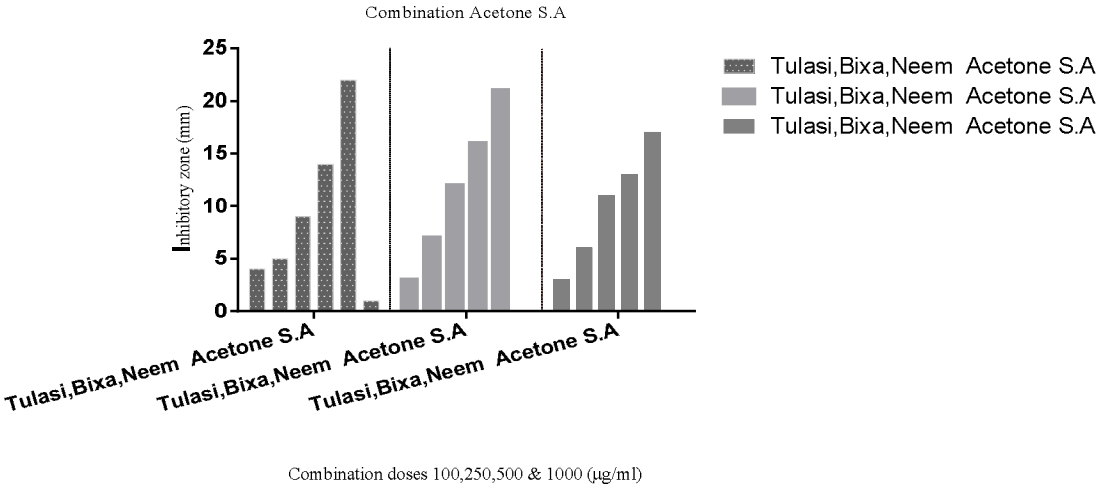
The values represents mean of sample±SD for n=3. Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample.

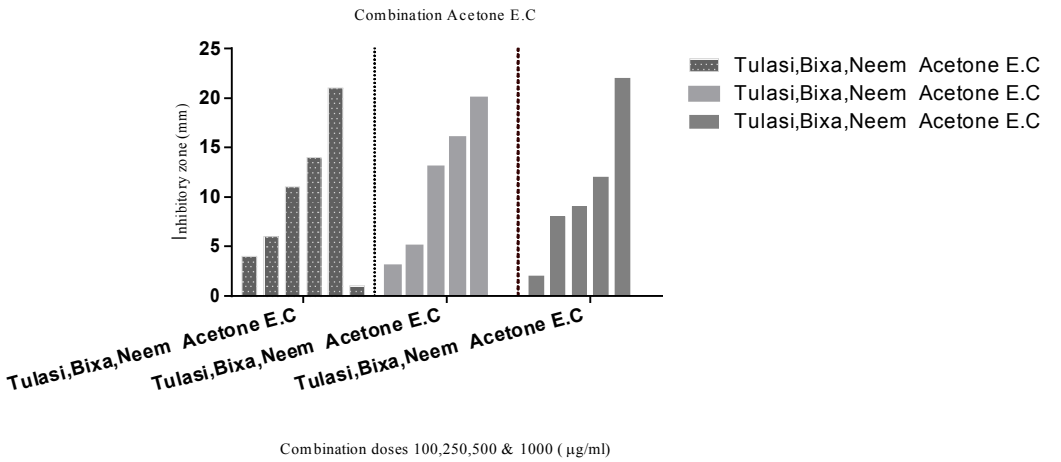
Screening of combination extracts for antimicrobial property

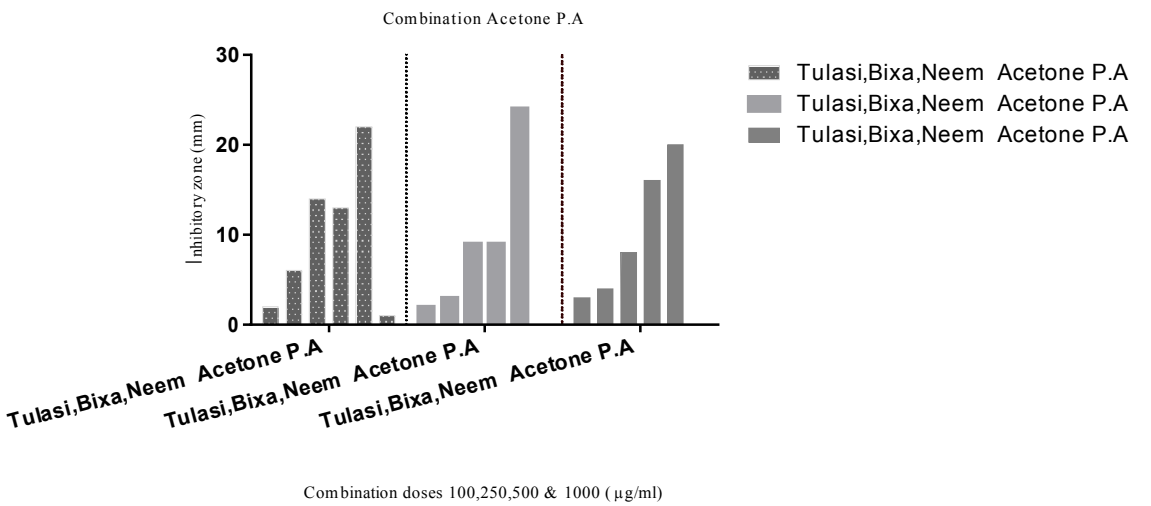


Figures: 1-3 (Ethanolic Extracts of Bixa+Neem+Tulsi) & Streptomycin 10µg/ml

Combination of acetone Extract







Figures: 4-6(Acetone Extracts of Bixa+Neem+Tulsi) & Streptomycin 10µg/ml

CONCLUSION

On the basis of the results obtained, it can be concluded that ethanol & acetone can be used for extracting antimicrobial compounds from leaves of three plants. The present study shows that ethanolic plant extracts possessed good antimicrobial activity compared to acetonic extracts against bacteria such as *Escherichia coli, Pseudomonas aeruginosa* & *Staphylococcus aureus*. Therefore, it suggests that the plant can be a source of oral drugs to be used in the treatment of opportunistic infections and may be a source for future drug formation.

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