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ANTIBACTERIAL ACTIVITY OF MIXTURE OF LEAF EXTRACTS OF NEEM (*AZADIRACHTA INDICA* LINN.) AND TANTANI (*LANTANA CAMARA*)

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Staphylococcus aureus,
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ABSTRACT: Screening of medicinal plants for their antimicrobial activity leads to development of less expensive formulations with better safety and efficacy. *A. indica* and *L. camara* shows antibacterial activity. *A. indica* is multipurpose tree with wide range of uses. Also the *L. camara* has steroidal content showing cardiotoxic, antispasmodic activity along with antibacterial activity. In the present study we have compared the individual alcoholic leaf extracts of both plants with their combination. Antibacterial activity in mixture of leaf extracts of Neem (*Azadirachta indica*) and tantani (*Lantana camara*) was tested against human pathogenic bacteria *E. coli*, *Staphylococcus aureus*, *P. aureginosa* and *Bacillus subtilis*. Ethanolic extracts of Neem and Tantani leaves were used. Varying concentrations of individual extracts and their combination of 10 mg/ml, 50 mg/ml, and 100 mg/ml were prepared. Antimicrobial assay using cup plate method was used. When compared with individual leaf extract of Neem and tantani, combination shows maximum inhibition on *E. coli*, *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Bacillus subtilis*. These good experimental results can further be explored. The mixture of these plant extracts as bactericidal can be used in formulation development in natural products.

INTRODUCTION: Neem (*Azadirachta indica*) is a tree in the mahogany family Maliaceae. Neem is used in traditional medicine as a source of therapeutic agents in the Indian culture and grows well in tropical countries. Its twig provides a chewing stick and are widely used in Indian subcontinent¹. Earlier studies on Neem have showed that it contains active substances with multiple medicinal properties. *Azadirachta indica* in folklore medicine for the treatment of diabetes. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premises¹. The antimicrobial efficacy of extracts of Neem leaf, bark and seed shows high,

moderate and low antimicrobial activities². The nanoemulsion of Neem at lower concentrations can be used as an effective antibacterial agent for treatment of pathogenic bacterial infections without any toxicity to human system³. The ethanolic extracts obtain whether from dry and fresh leaves are more efficient⁴. *Lantana camara* commonly known as wild sage, is a flowering shrub native of tropical America and is cultivated throughout the World as an ornamental. The leaves are used in treatment of itches, cuts, ulcers, swellings, bilious fever, eczema and rheumatism. Many pharmacological investigations indicated that extracts of leaves of *Lantana camara* exhibit antibacterial properties⁵. Pharmacological investigations have indicated that extracts of the shoots of *L. camara* exhibit antibacterial properties. Lancamarone, a steroid from the leaves, possesses cardiotoxic properties, while lantamine, an alkaloid from the stem and root bark, shows strong antipyretic and antispasmodic properties comparable with those of quinine⁶.

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The genus *Lantana* belongs to family Verbanaceae is used in folk medicine by local people. The leaves of this plant are used as medicine for treating skin infections caused by pus-producing organisms like staphylococci and group A streptococci⁷. The hexane extracts of *Lantana camara* leaves shows highest zone against streptococcus pneumoniae⁸. The studies on *Lantana camara* extracts demonstrate that leaves can be employed to combat antimicrobial, fungicide, insecticide, nematicide problem⁹. The purpose of present study was to investigate the antimicrobial activity of combination of *Azadirachta indica* and *L. camara* leaves extracts against human pathogenic bacteria, including *E. coli*, *Pseudomonas aureginosa*, *Staphylococcus aureus* and *Bacillus subtilis*.

MATERIAL AND METHODS:

Plant Material: The plant Neem (*Azadirachta indica*) and Tantani (*Lantana camara*) were selected for study. The plant Neem was selected from medicinal garden of JSPM's Rajarshi Shahu College of Pharmacy and Research, Tathwade, Pune - 411033 and plant *L. camara* was selected from local garden, Pune - 411019. Both the plants were authenticated by Agarkar Research Institute, Pune. [Authentication voucher specimen no. of *A. indica* (17- 186) and *L. camara* (17-187)].

Preparation of Extracts: The leaves of both the plants were air dried at room temperature and grinded them into powder form with help of mechanical grinder. Both powdered plant leaves were extracted with the ethanol in rotary shaker at 100 rpm for 3 days¹⁰. The obtained liquid extracts were subjected to rotary evaporator at reduced pressure (in vacuum at 40 °C) and evaporated to dryness^{11, 12}.

RESULTS:

TABLE 1: DIAMETER OF ZONE OF INHIBITION (mm) OF A. INDICA, L. CAMARA, MIXTURE AT CONCENTRATION OF 10 mg/ml, 50 mg/ml, 100 mg/ml [1. ESCHERICHIA COLI, 2. BACILLUS SUBTILIS, 3. STAPHYLOCOCCUSA AUREUS, 4. PSEUDOMONAS AUREGINOSA]

Extracts	Conc. (mg/ml)	Diameter of zones of inhibition /mm (±0.5 mm)			
		1	2	3	4
<i>A. indica</i>	10	14	12	09	13
	50	18	15	12	17
	100	21	18	17	20
<i>L. camara</i>	10	16	14	12	15
	50	19	16	17	18
	100	20	21	22	23
Mixture (<i>A. indica</i> and <i>L. camara</i>)	10	17	15	18	16
	50	20	18	19	19
	100	22	23	23	24

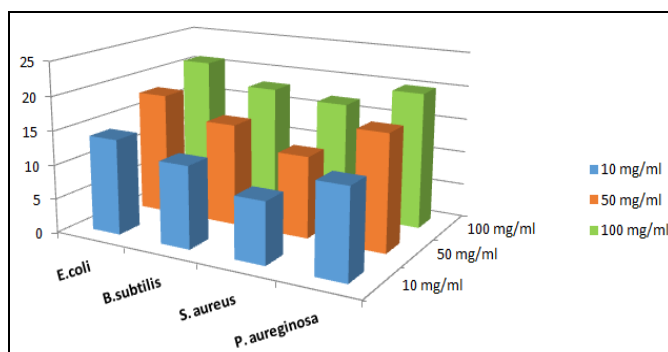
The dried crude extract of *L. camara* was sterilized overnight under UV radiation². Both dried extracts were stored at refrigeration temperature¹.

Preparation of Concentrations: In the antibacterial study, both the extracts were diluted in dimethyl sulfoxide (DMSO)^{13, 14}. The concentrations were taken as 10 mg/ml, 50 mg/ml, and 100 mg/ml of individual extracts and mixture of both (*A. indica* and *L. camara*).

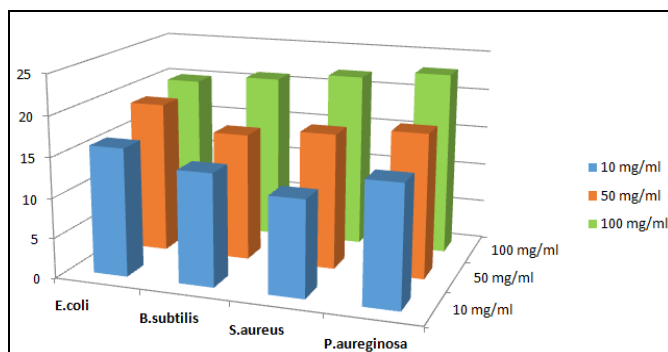
Test Bacterial Strain: The following bacterial strains were used for antibacterial study, *Escherichia coli*, *Pseudomonas aureginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. All the bacterial strain were obtained from National Chemical Laboratory, Pashan.

Antibacterial Screening:

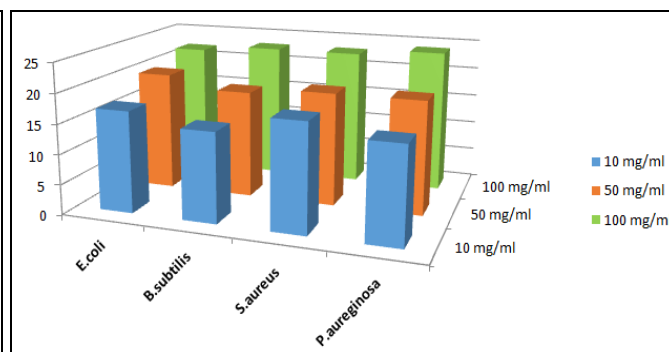
Cup Plate Method: The antibacterial effects were tested by cup plate method¹³. Firstly, the bacteria to be tested were inoculated into Nutrient broth and incubated at 37 °C for 24 h. Petri dishes containing nutrient agar media were impregnated with these bacterial suspensions. Cups were made into the agar media with the help of borer (7 mm diameter)¹⁴. Wells / bore were impregnated with different concentrations of each individual extract and mixture of both extracts. Blank well/cup was impregnated with DMSO as negative control and well/cup of chloramphenicol (10, 50, 100 mg/ml per cup) as positive control. The entire test plates were incubated at 37 °C for 24 h and diameter of zones of inhibition were measured. The inhibitory effects of both individual extracts of *Azadirachta indica* and *Lantana camara* and their mixtures on four bacterial strains in nutrient agar media are shown in **Table 1**.



GRAPH 1: SHOWING THE BACTERIAL STRAINS TESTED WITH 10, 50, 100 mg/ml CONC. OF AZADIRACHTA INDICA ON HORIZONTAL AXIS AND ZONE DIAMETER (mm) ON VERTICAL AXIS



GRAPH 2: SHOWING THE BACTERIAL STRAINS TESTED WITH 10, 50, 100 mg/ml CONC. OF LANTANA CAMARA ON HORIZONTAL AXIS AND ZONE DIAMETER (mm) ON VERTICAL AXIS



GRAPH 3: SHOWING THE BACTERIAL STRAINS TESTED WITH 10, 50, 100 mg/ml CONC. OF MIXTURE OF A. INDICA & L. CAMARA ON HORIZONTAL AXIS AND ZONE DIAMETER (mm) ON VERTICAL AXIS

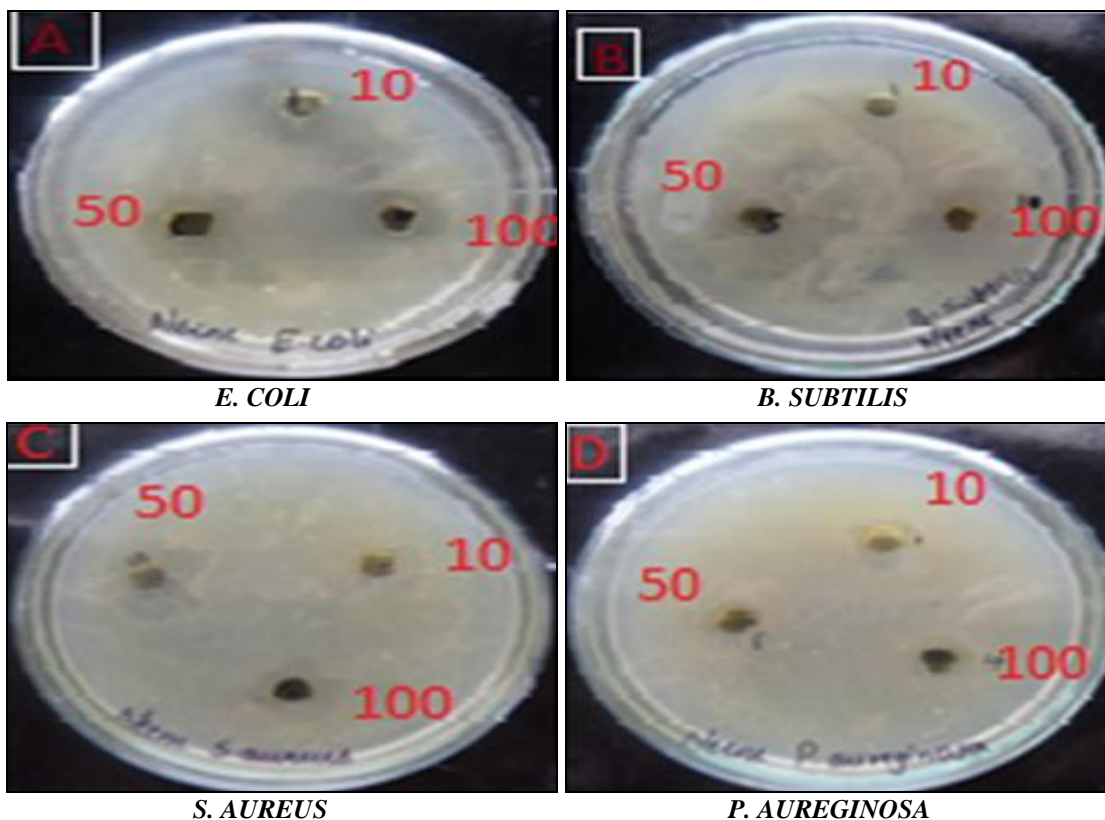


FIG. 1: AZADIRACHTA INDICA

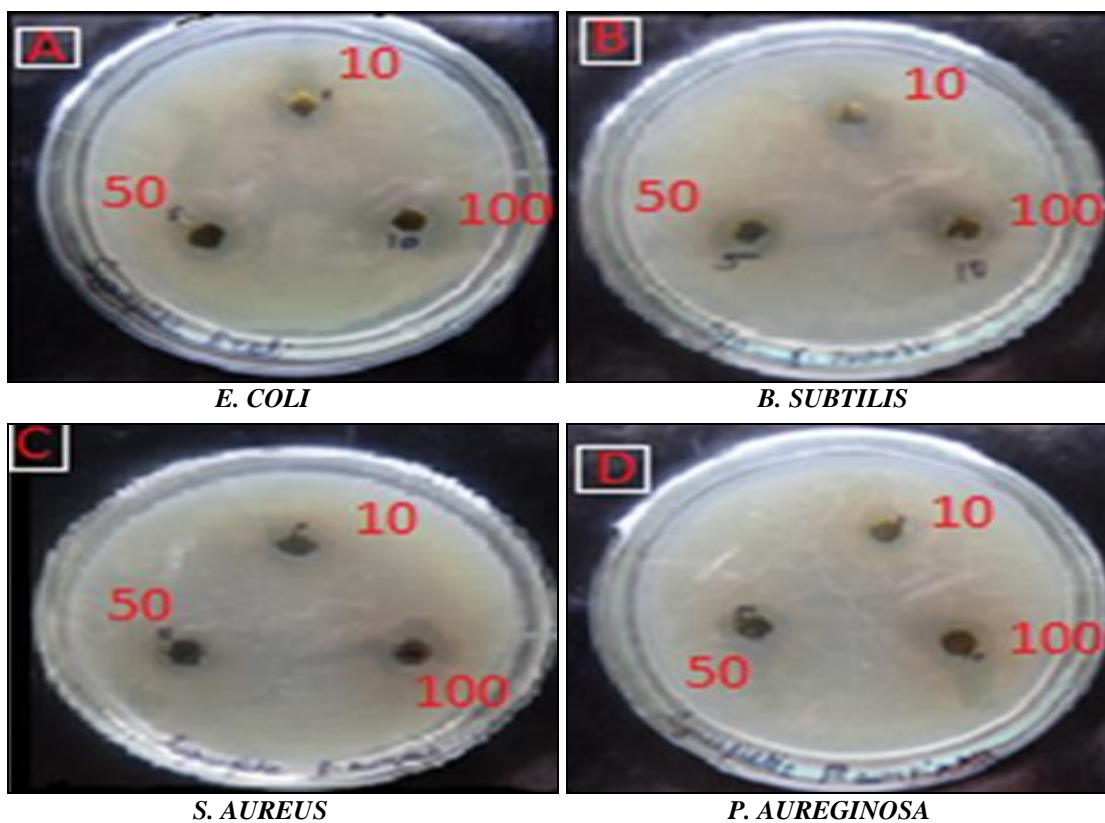


FIG. 2: LANTANA CAMARA

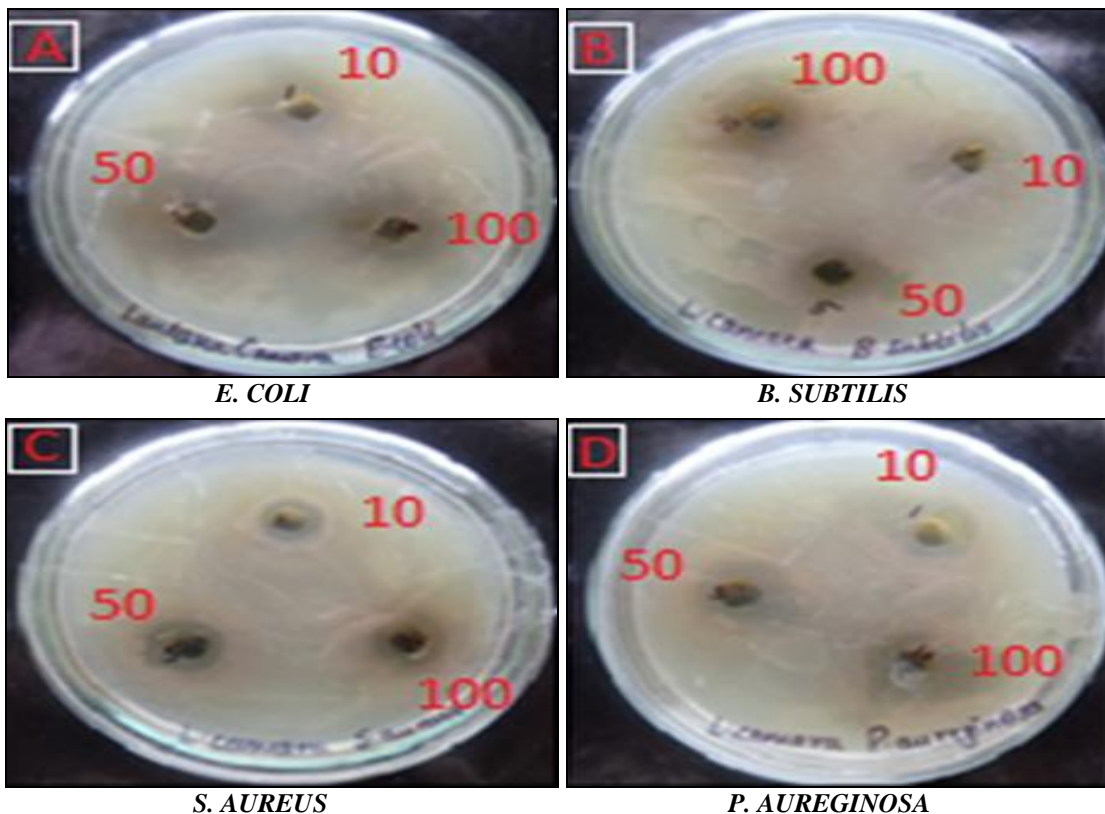


FIG. 3: MIXTURE

DISCUSSION AND CONCLUSION: Many of the existing synthetic drugs cause various side effects. Hence, natural drug plant based compounds could be useful in meeting this demand for newer

drugs with minimal side effects¹⁵. *A. indica* leaves possess good antibacterial activity confirms the great potential of bioactive compounds and is useful for rationalizing the use of this plant in

primary health care¹⁶. Neem and its ingredient have been traditionally used worldwide especially in India the role of its active ingredients as chemopreventive effects has been noticed in various tumor *via* modulations of numerous cell signaling pathway¹⁷. Also the antibacterial activity of *L. camara* extracts varied with solvents used for extraction. Crude preparation of leaves of plant containing both active and non-active components too have high efficacy than semi crude drugs or pure plant substances¹⁸. Many authors have mentioned the secondary metabolites of extract effective against micro organism were evaluated and recognized as saponins, tannins, cardiac glycosides, alkaloid, flavonoides, steroids, terpenoids¹⁹. However, most of pharmacological studies were preliminary and require intensive preclinical and clinical studies to evaluate the efficacy and toxicity of these plant products²⁰. The results of this experimental study showed that the effectiveness of extracts was dependent of concentration used, thus increase in extract concentration leads to increase the inhibition zone. The purpose of this study was to evaluate the effect of combination of leaf extracts on bacterial strain and was found that, the combination of leaf extract of *L. camara* and *A. indica* shows higher zone of inhibition than individual leaf extracts. This shows synergistic antibacterial effect on bacterial strain *Escherichia coli*, *P. aureginosa*, *Staphylococcus aureus* and *Bacillus subtilis*.

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CONFLICT OF INTEREST: Nil

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