



Research Article

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ANTIMICROBIAL EVALUATION OF DIFFERENT EXTRACTS OF NIRGUNDI LEAF AGAINST *PSEUDOMONAS AERUGINOSA*, *STAPHYLOCOCCUS AUREUS*, *ESCHERICHIA COLI* AND *KLEBSIELLA AEROGENES*

Satyapal Sharma^{1*}, Gaurav Sharma², Sudipta Kumar Rath³

¹P.G. Scholar, P.G. Department. of Dravyaguna Vigyan, National Institute of Ayurveda, Jaipur, Rajasthan, India

²Pharmacologist, P.G. Department of Dravyaguna Vigyan, National Institute of Ayurveda, Jaipur, Rajasthan, India

³Assistant professor, P.G. Department of Dravyaguna Vigyan, National Institute of Ayurveda, Jaipur, Rajasthan, India

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*Corresponding author

E-mail: drsatyapal04@gmail.com

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ABSTRACT

Aqueous & alcoholic extracts of *Vitex negundo* Linn (Nirgundi leaf) and mixture of both extract was subjected to in vitro antibacterial assay against human pathogens *Pseudomonas aeruginosa* MTCC No. 1034, *Staphylococcus aureus* MTCC No 6908. *Escherichia coli* MTCC No 10239 and *Klebsiella aerogenes* MTCC No 39 by agar well diffusion method. Results showed that leaf extracts (Aq. & Alc.) exhibited antimicrobial effect against bacteria at all the concentrations tested (10%, 20% and 30% w/v). Our results suggest that aqueous extract of *Vitex negundo* leaf showed significant antimicrobial activity at 30% w/v concentration and mixture of both extract showed significant antimicrobial effect at 30% w/v concentration.

Keywords: Antimicrobial activity, agar well diffusion method, *Vitex negundo* leaf and micro-organism.

INTRODUCTION

Infectious diseases have long been a major health concern to entire human population, more so in under developed & developing countries of the world like India. Infections occurring during & after surgery are also a major problem for already suffering patients, which can even cost life of the patient or make him/her physically disturbed.

Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases.

According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments¹. Many infectious diseases are known to be treated with herbal remedies. Hence, there is need to screen medicinal plants for promising biological activity.

Vitex negundo Linn. belong to family Verbenaceae commonly known as Nirgundi. It is an aromatic large shrub or small tree about 3m in height with quadrangular branches and almost found throughout India, ascending to 1500m in the outer Himalaya, fairly common in waste lands, on road side, the banks or streams or in moist places near deciduous forests².

The leaves are aromatic, tonic, vermifuge and useful in rheumatism, arthritis, catarrhal fever, cephalagia, sprains, orchitis, syphilis, inflammations and ulcers³.

Charak has described of Nirgundi as Krimighna Mahakshaya⁴. Most of the bacterial pathogens like *Salmonella paratyphi*, *Klebsiella pneumonia*, *Vibrio cholera*, *Streptococcus mutans* and

E.Coli were found to be susceptible in leaf extracts of the *Vitex negundo*⁵.

Antimicrobial properties of different parts of *Vitex negundo* were evaluated on bacterial strains⁶.

Antimicrobial capabilities were also evaluated. Rideout *et al.* (1999) reported antibacterial & antifungal activity of Vitexilactone & Casticin from the chloroform extract of *Vitex negundo* leaves against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* using agar plate method.

Antibiotics have many toxic effects not only against microbes but also to the human being themselves. To over-come this research is on for finding a drug which is effective against the diseases causing pathogens & at the same time does not produce toxic effect.

Aims & objectives

Reconfirmation of the anti- microbial activity of Nirgundi extracts individually against the four target micro-organisms.

Study of anti-microbial activity against the four target micro-organisms of the combination of the extracts.

MATERIALS AND METHODS

Collection of Samples

The authentication of plant material collected for study was done at Herbarium section, Botany department of Rajasthan University, Jaipur with authentication number RUBL11527.

Leaves were collected from the *Vitex negundo* small tree in the college campus of National institute of Ayurveda, Jaipur. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly and a

particular amount of leaves dried under shadow and some fresh leaves kept.

Preparation of extracts⁷

Preparation of Aqueous extracts

Macerate 10 g of the air-dried drug, coarsely powdered, with 200 ml of distilled water the specified strength in a closed flask for twenty-four hours, kept on a rotatory shaker at 190-210 rpm shaking frequently for six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 100 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dry at 100 °C, to constant weight and weight.

Preparation of Ethanol Extracts

Macerate 10 g of the air-dried drug, coarsely powdered, with 200 ml of solvent the specified strength in a closed flask for twenty-four hours, kept on a rotary shaker at 190-210 rpm shaking frequently for six hours and allowing to stand for eighteen hours filter rapidly, taking precautions against loss of solvent, evaporate 100 ml of the filtrate to dry in a tarred flat bottomed shallow dish and dry at 105 °C, to constant weight and weigh.

Preparation of media and media Plates

Mueller Hinton Agar is used for determination of susceptibility of microorganisms to antimicrobial agents. Agar well-diffusion method was followed to determine the antimicrobial activity. About 15-20 ml of nutrient agar medium was poured in the sterilized petri dish and allowed to solidify. One drop of bacterial strains was spread over the medium by a sterile cotton swab. Wells of 5mm in diameter and about 2 mm was punctured in the culture medium using sterile cork borer. About 30 µl of plant extracts was added help of micropipette to the wells. Plates were incubated at 35°C for 48 hours. Antibacterial activities were evaluated by measuring the diameter of zone of inhibition in mm.

Antimicrobial Activity

Purpose

To lay down the procedure to perform is Antimicrobial activity to be performed in our samples with reference of using standard culture.

Scope

It is applicable to the laboratory of Dravyaguna vigyana department of NIA for Antimicrobial effect.

Procedure

Precaution has taken during antimicrobial activity. Glassware to be used shall be sterilized. Media to be used shall be pre-incubated. Microbial area should be sterilized before testing.

Test organisms used

Use cultures of the following microorganisms- *Pseudomonas aeruginosa* MTCC No. 1034, *Staphylococcus aureus* MTCC No 6908, *Escherichia coli* MTCC No 10239 and *Klebsiella aerogenes* MTCC No 39.

Standard Note

Distilled water, ethanol is used as negative control did not/slightly show activity against test organism.

Povidone Iodine solution served as a positive control.

Aqueous & Alcoholic extract of Nirgundi leaves 10%, 20% and 30% w/v in concentration.

Mixture of both extracts (Aq. & Alc.) was used at 30% w/v concentration.

Antimicrobial study results- Zone of inhibition (ZOI) in mm.

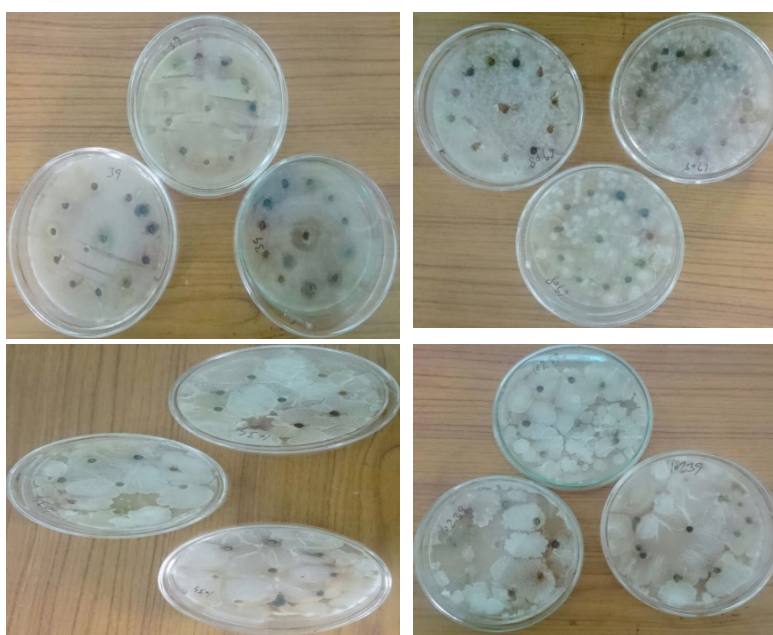


Figure 1: Some pictures showing zone of inhibition

Table 1: ZOI of various extract/conc. of Nirgundi leaf against target bacteria with negative control (Aq./Alc.) and positive control (Povidone iodine)

S. No	Test Sample		<i>Pseudomonas Aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia Coli</i>	<i>Klebsiella Aerogenes</i>
1	10% conc.	Aq.	9	0	0	0
2		Alc.	8	7	9	0
3	20% conc.	Aq.	9	8	10	0
4		Alc.	8	7	7	7
5	30% conc.	Aq.	10	9	10	7
6		Alc.	8	9	8	8
7	Aq. -ve control		0	0	0	0
8	Alc. -ve control		6	0	6	7
9	Povidone iodine +ve control		12	14	13	11

Table 2: ZOI of mixture (Aq. & Alc. extract) of Nirgundi leaf at 30% w/v conc. against target bacteria with negative control (Aq.) and positive control (Povidone iodine)

S. No	Test sample	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia Coli</i>	<i>Klebsiella aerogenes</i>
1	Mixed 30% conc.	9	10	7	7
2	Aqueous -ve control	0	0	0	0
3	Povidone iodine +ve control	13	15	14	11

Determination of the activity index⁸

The activity index of the test samples extract was calculated as-

$$\text{Activity index (AI)} = \text{Zone of inhibition of the extract} / \text{Zone of inhibition obtained for standard Povidone iodine}$$

Table 3: Activity index of the Aqueous & Alcoholic extracts at various concentrations of Nirgundi leaf

Concentration	Extract	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>E. coli</i>	<i>K.aerogenes</i>
10%	Aq.	0.75	0.00	0.00	0.00
	Alc.	0.67	0.50	0.69	0.00
20%	Aq.	0.75	0.57	0.77	0.00
	Alc.	0.67	0.50	0.54	0.55
30%	Aq.	0.83	0.64	0.77	0.64
	Alc.	0.67	0.64	0.62	0.55

Table 4: Activity index of mixed aq. & alc. extract of Nirgundi leaf

Concentration	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>E. coli</i>	<i>K.aerogenes</i>
30%	0.69	0.67	0.50	0.64

RESULTS AND DISCUSSION

To prevent post-surgical wounds from infection, a broad spectrum antiseptic formula is required. But in Ayurveda, no such formulation with proven efficacy is currently available are widely used drugs for their Krimighna activity in different ailments and have shown anti-microbial activity against the four organisms commonly responsible for infection in ano-rectal surgical wounds which are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella aerogens*. Therefore, Nirgundi leaf was selected in order to study the anti-microbial effect on target microorganisms.

Aqueous extract of Nirgundi leaf showed ZOI of 9mm, 8mm and 10mm at 20% w/v conc. against *P. aeruginosa*, *S.aureus*, *E.coli* respectively.

Aqueous extract of Nirgundi leaf showed ZOI of 10mm, 9mm and 10mm at 30% w/v conc. against *P. aeruginosa*, *S.aureus*, *E.coli* respectively.

Alcoholic extract of Nirgundi leaf showed activity at 30% w/v concentration as 8mm, 9mm and 8mm of ZOI against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*.

Mixture of both extract at 30% w/v concentration showed ZOI of 9mm, 10mm, 7mm and 7mm against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli* and *K. aerogenes* respectively.

CONCLUSION

The results of this study suggest that the leaf of *V. negundo* can be used as an antibacterial agent in aqueous extract at 30% w/v concentration against infections caused by *P. aeruginosa*, *S. aureus* and *E. coli*.

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