International Scholars Journals

International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 14 (2), pp. 001-007, February, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Phytochemical Analysis and Antibacterial Efficacy of *Vitex negundo* Extracts from Leaf and Bark in Similipal Biosphere Reserve, India

S. K. Panda[1], H. N. Thatoi[2] and S. K. Dutta[1*]

[1]Post Graduate Department of Zoology, North Orissa University, Baripada -757003, India.

[2]Post Graduate Department of Biotechnology, North Orissa University, Baripada -757003, India.

Accepted 6 January, 2025

The antimicrobial activity and phytochemicals of the leaves and bark of *Vitex negundo* L. was evaluated against three Gram-positive bacteria viz. *Staphylococcus epidermidis*, *Bacillus subtilis*, *Staphylococcus aureus* and five Gram-negative bacteria viz. *Escherchia coli*, *Salmonella typhimurium, Pseudomonas aeruginosa*, *Vibrio cholerae* and *Vibrio alginolyteus*. Both polar and nonpolar extracts viz. petroleum ether, chloroform, ethanol, methanol and aqueous extracts were prepared and studied for antibacterial activity using disc diffusion, agar cup and broth dilution methods. Results showed promising antibacterial activity of all the extracts of both leaf and bark against *E. coli*, followed by *S. aureus*. Ethanol and methanol extracts of the leaf showed inhibition activity against both Gram- positive and Gram-negative bacteria where as petroleum ether and chloroform extracts of bark had better antibacterial activity against Gram-positive bacteria. MIC showed that 5% of the extracts were active in a concentration of 0.312 mg/ml; 27.5% in a concentration of 0.625 mg/ml and 88.75% in a concentration of 1.25 mg/ml; were active against different human pathogenic bacteria. At concentration about 2.5 mg/ml, 100% of inhibition was recorded against both Gram- positive and Gram-negative bacteria. The result obtained with ethanol and methanol extracts of leaves; petroleum ether and chloroform extract of bark exhibited significant antibacterial activity, a property that supports traditional use of the plant in the treatment of some diseases as broad spectrum antibacterial agents.

Key words: Antibacterial activity, similipal biosphere reserve, MIC, *Vitex negundo,* phytochemical, medicinal plant activity.

INTRODUCTION

Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine con-tain 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 (Duraipandiyan et al., 2006). The demand for more and more drugs from plant sources is continuously increasing. It is therefore essen-tial for systematic evaluation of plants used in traditional medicine for various ailments.

The increased interest in plant derived drugs is mainly because of the wide spread belief that 'herbal medicine' is safer than costly synthetic drugs which possesses side effects. Hence, there is need to screen medicinal plants for promising biological activity. Further, there is a conti-nuous development of resistant strains which pose the need for search and development of new drug to cure diseases (Silver, 1993).

Vitex negundo L. (Verbenaceae) commonly known as nirgundi chiefly occurring throughout India (Watt, 1972; Gupta et al., 2005) is widely distributed in Similipal Bio-sphere Reserve, Orissa. Though, almost all parts of *V. negundo* are used, the leaves and the barks are the most important in the field of medicine (Chandramu et al., 2003). The decoction of leaves is considered as tonic, vermifuge and is given along with long pepper in cater-rhal fever (Chandramu et al., 2003). Water extract of ma-

*Corresponding author. E-mail: sk_dutta@yahoo.com. Fax: 067 92-253908.

ture fresh leaves exhibited anti-inflammatory, analgesic and antihistamine properties (Dharmasiri et al., 2003).

Leaves of this plant have been shown mosquito repellent effects (Hebbalkar et al., 1992) as well as antiulcerogenic (Sahni et al., 2001), antiparasitic (Parveen, 1991), antimicrobial (Rusia and Srivastava, 1998) and heaptoprotective (De et al., 1993) potentials. The methanolic root extract possessed potent snake venom (*Viper russellii* and *Naja kaouthia*) neutralizing capacity (Alam and Gomes, 2003). The acetone extract of *V. negundo* was found to possess insecticidal, ovicidal, growth inhibition and morphogenetic effects against various life stages of a noxious lepidoteron insect-pest (Prajapati et al., 2003).

Some studies have also been done on antimicrobial activity of *V. negundo* along with some other Indian medicinal plants (Ahmed et al., 1998; Rusia and Srivastava, 1998; Kumar et al., 2006; Valasraj et al., 1997). These works give little information on antimicrobial property of this plant. Hence, in the present experiment an attempt has been made to evaluate the antibacterial activity of different extract (petroleum ether, chloroform, ethanol, methanol and aqueous) against eight prominent Gram-positive and Gram-negative human pathogenic bacteria and the biological activities of the extracts in terms of MIC and MBC were also determined. Besides, phytochemical screening of the extracts were also carried out with view assess the presence of different phytochemicals in different extracts.

MATERIALS AND METHODS

Study area

The Similipal Biosphere Reserve is located in the district of Mayurbhanj in the Northern region of Orissa between 21°08" - 21°27" N latitude and 86°04" - 86°15" E longitude a unique habitat of mixed tropical forest and harbors varied flora and fauna. The ecosystem is enriched with variety of medicinal plants. The climate of Similipal Biosphere Reserve is tropical. The ambient temperature varies between a minimum of 2°C during winter to maximum 48°C during summer. The relative humidity varies from 70% - 100% with 30% in midnight in summer.

Plant material

Bark and leaves of the plant have separately been collected from Similipal Biosphere Reserve. The shed dried bark and healthy leaves were powdered separately using mechanical grinder and then were passed through sieve so that uniform powder size is maintained.

Chemicals and media

All chemicals used for extraction were of analytical grade obtained from Hi-media Lab Ltd, Mumbai, India. Muller Hinton Broth and Muller Hinton Agar procured from HI-Media and Mumbai were used in the study and prepared as per manufacturer's instructions.

Bacterial strain and inoculum preparation

The antibacterial activity was tested against *Staphylococcus epidermidis* MTCC 3615, *Bacillus subtilis* MTCC 7164, *Staphylococcus aureus* MTCC 1144, *Escherchia coli* MTCC 1098, *Salmonella typhimurium,* MTCC 3216, *Pseudomonas aeruginosa* MTCC 1034, *Vibrio cholerae* MTCC 3904 and *V. alginolyteus* MTCC 4439. These bacterial strains were obtained from MTCC, Chandigarh, India (Customer no. 4853). Test organisms were maintained on nutrient agar slants.

Preparation of extract

Sequential extraction was carried out with the same powder using solvents of increasing polarity. About 250 g of dry bark and leaf powder were sequentially extracted using petroleum ether, chloroform, ethanol, methanol and aqueous solution in Soxhlet apparatus. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation.

Phytochemical analysis

Preliminary phytochemical study was screened for presence of alkaloid, flavonoid, carbohydrates, glycosides, proteins and amino acids, steroids, vitamin C, fat and fixed oil (Trease and Evans, 1989).

Determination of antibacterial activity

Disc diffusion method

Preliminary screening of the extracts was carried out by disc diffusion method (Bauer et al., 1966). Briefly, freshly grown liquid culture of the test pathogens were seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs of eight mm diameter were soaked with 40 l of 50 mg/ml the extracts and air dried to evaporate the solvent and the discs were applied over the seeded MHA plates at equidistance.

The plates were incubated at 37°C for 18 - 24 h. After the incubation period, the plates were observed for a clearance zone around the discs which indicates a positive antibacterial activity of the respective extracts. The clearance zones formed around each disc were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Agar cup method

The agar cup method was followed to doubly ensure the antibacterial activity of the extracts (Barry, 1980). Over night Muller Hinton Broth culture of the test organisms were firmly seeded over the MHA plates. Wells of 6 mm diameter was punched over the agar plates using a sterile borer. The bottoms of the wells were sealed by pouring 50 - 100 l of molten MHA into the scooped out wells. 50 l of 50 mg/ml extracts were poured into the wells. The water was allowed to evaporate and the plates were incubated at 37°C for 18 - 24 h.

After the incubation period formation of zones around the wells, confirms the antibacterial activity of the respective extracts. The same procedure was followed for each strain and extract. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum Inhibitory Concentration was determined by agar dilution

method (Vander-Berghe and Vlietinck, 1991). In this study, the test compounds were taken at different concentration ranging form 0.156 to 5.0 mg/ml. It involves a series of six tubes for each test compound against each strain. To the first assay tube 1.9 ml of seeded broth was transferred and than 0.1 ml of test extracts of 50 mg/ml as added to it and mixed thoroughly. To the remaining five assay tube 1 ml of seeded broth was transferred.

From the first tube on 1 ml of the content was pipette out into second test tube and this was mixed thoroughly. This two fold serial dilution was repeated up to sixth tube. 10 l of the broth from each culture tube exhibiting MIC and control tubes were taken aseptically and were plated on one day old MH agar plate as a point inoculum and allowed to dry for 10 min under the laminar air hood. These were then sealed and incubated at 37°C for 24 h and observed for growth of the bacteria.

RESULTS

Phytochemical screening of five extracts (petroleum ether, chloroform, ethanol, methanol and aqueous) of both leaf and bark has been summarized in Table 1. Eva-luation of photochemical such as alkaloid, flavonoid, car-bohydrates, glycosides, proteins and amino acids, ste-roids, vitamin C, fat and fixed oil revealed presence of most of constitutes studied in polar extracts such as etha-nol, methanol and aqueous extracts compared to non polar extracts (petroleum ether and chloroform).

However, flavonoid was found to be universally occuring in all the extracts irrespective of plant part used (Table 1). Percentage yield of different extracts varies with leaf and bark. In case of leaf the order of yield of extracts were ethanol > chloroform > aqueous > methanol > petroleum ether while for bark the yields were ethanol > aqueous > methanol > chloroform > petroleum ether.

Preliminary screening of antimicrobial activity was evaluated by using disc diffusion and agar cup methods against eight human pathogenic bacteria are given in Tables 2 and 3 respectively. In disc diffusion method, zone of inhibitions were maximum for *E. coli* followed by *S. epidermidis* followed by *P. aeruginosa*. On the con-trary, *S. aureus* and *S. typhi* showed no zone of inhibition for these extracts. From disc diffusion method results obtained that, methanol extract of bark and ethanol extracts of both leaves and bark showed moderate zone of inhibition against Gram-positive bacteria.

Similarly ethanol extract showed complete inhibition against Gram-negative strain where as other extracts showed moderate inhibition against at least two Gram-negative bacteria (Table 2). Agar cup method also revealed almost similar form which however, varies with respective to the zone of inhibition against different bacteria. Similar to disc diffusion method maximum inhibition was observed against *E. coli* followed by *S. epidermidis* followed by *V. alginolytius.*

The results of MIC showed that in a concentration of 0.312 mg/ml, 5% of the extracts were active against Gram-negative bacteria, while no extracts were active against Gram-positive bacteria (Table 4). The difference was more pronounced at higher concentration. At concentration 0.625 mg/ml, the activity was 7.5 and 20% against Gram-negative bacteria and Gram-positive bacteria respectively and with 1.25 mg/ml; the activity was 32.5 and 56.25% respectively against Gram-negative bacteria and Gram -positive bacteria. At concentration about 2.50 mg/ml, 37.5% of inhibition was recorded against both Gram-positive and 62.5% against Gram-negative bacteria (Table 4) . The results of MBC showed that in a concentration of 5.0 mg/ml about 46.25% of organisms were killed and rest 53.75% were inhibited at a concentration of 5.0 mg/ml.

DISCUSSION

The negative results obtained against Gram- negative bacteria were not unexpected since this class of bacteria is usually more resistant then Gram-positive bacteria (Tomas-Barberan, 1988). Similar results were also obtained from agar cup method (Table 5). *E. coli* was completely inhibited by all the extracts of both leaf and bark. *S. aureus* was the second most inhibited bacteria with most of the extracts. Ethanol and methanol extracts of the leaves were most active inhibiting agent against both Gram-positive and Gram -negative bacteria. On other hand petroleum ether and chloroform extracts had better antibacterial activity against all Gram-positive bac-teria.

However, *S. epidermidis* and *B. subtilis* were inhibited completely by petroleum ether and chloroform extracts of bark as well as ethanol and methanol extracts of leaves. Infection caused by *P. aeruginosa* are among the most difficult to treat with conventional antibiotics (Levison and Jawetz, 1992). The growth of *P. aeruginosa* was partially inhibited by petroleum ether and ethanol extracts of leaves and bark and moderately inhibited by chloroform, methanol and aqueous extracts. So the plant *V. negundo* can be used as a source which could yield drugs that could improve the treatment of infection caused by this organism. *B. subtilis* is the common bacteria found in most natural environments including soil, water plant and animal tissues.

This bacterium has been known to act as a primary invader or secondary infection agents in a number of diseases and has been implicated in some cases of food poisoning (Turnbull and Kramer, 1991). The growth of *B. subtilis* was moderately inhibited by petroleum ether extract and methanol extracts of leaves and partially inhibited by chloroform and ethanol extracts. All the tested bacteria were susceptible to ampicillin, which was used as a control antibiotics and that exerted higher inhibitory effect on bacterial growth, having 11 - 26 mm in diameter in disc diffusion and agar cup methods where as MIC value ranging from 0.005 to 0.023 mg/ml. The low bio-activity of the crude extracts would result either from dilutions of its active constituents as form the antagonism in the bioactivity among extracts constituents (Ibrahim et

Table 1. Screening of phytochemicals of *V. negundo* bark and leaf.

Solvent	Plant Part	Color	% of yield	Alkaloids	Carbohydrates	Tannin and Phenol	Glycosides	Flavonoid	Saponin	Steroid	Gums and Mucilage
Petroleum ether	Bark	Blackish	1.07	-	-	-	-	++	-	ND	-
	Leaf	Greenish	1.24	-	-	-	-	++	-	++	+
Chloroform	Bark	Blackish	1.52	-	-	-	-	++	+	ND	-
	Leaf	Deep greenish	9.86	-	-	-	-	++	+	++	-
Ethanol	Bark	Blackish	5.86	+	-	++	+	++	+	ND	-
	Leaf	Greenish black	14.88	++	-	+++	+	++	+	+	+
Methanol	Bark	Greenish black	4.34	++	+	+++	+	++	+	ND	-
	Leaf	Greenish	7.84	+++	++	++	+	++	+	-	+
Aqueous	Bark	Greenish black	4.82	++	+++	+++	-	++	-	ND	-
	Leaf	Greenish black	8.64	+++	++	++	-	++	-	-	+

(+++) Present in high amount; (++) Present in medium amount; (+) Present in trace amount; (-) Absent; ND - Not determined.

Table 2. Antibacterial activity of *V. negundo* bark and leaf by disc diffusion method. *Typhimurium*

Strain used	Petroleum ether		Chloroform		Ethanol		Methanol		Aqueous		Ampicillin	
	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf
Bs	10.3±1.15	8.6±0.57	9.6±2.08	10.3±0.57	11.6±0.57	11.6±0.57	13.0±1.00	9.3±0.57	-	11.6±0.57	16.6±3.0	19.3±2.3
Sa	11.6±0.57	-	10.3±0.57	-	-	-	11.3±1.15	8.0±0.00	10.0±0.00	-	12.6±1.10	11.6±0.57
Se	11.6±0.57	11.3±0.57	13.6±0.57	11.6±0.57	11.6±0.57	13.6±0.57	13.6±0.57	11.3±1.15	11.6±0.57	11.3±0.57	16±2.60	17.6±1.5
Ec	12.6±0.57	12.3±0.57	13.6±0.57	13.0±1.00	13.6±0.57	16.3±1.52	12.3±0.57	12.3±0.57	13.6±0.57	13.6±0.57	14.3±1.50	15.3±1.10
Pa	11.0±0.00	11.0±0.00	10.6±0.57	12.3±0.57	13.0±0.00	10.6±0.57	10.6±1.15	13.3±0.57	12.6±0.57	12.3±0.57	19.6±1.50	21.0±1.00
St	-	10.0±1.73	-	-	11.3±0.57	11.6±0.57	11.6±0.57	9.0±1.00	11.3±0.57	10.3±0.57	16.0±0.00	15.3±1.10
Vc	11.0±0.00	11.0±0.00	9.6±0.57	11.6±0.57	11.6±0.57	9.6±0.57	13.6±0.57	11.6±0.57	13.6±0.57	10.3±0.57	22.6±1.10	22.3±1.50
Va	12.6±0.57	13.0±0.57	12.3±1.52	11.6±0.57	11.6±0.57	11.6±0.57	9.6±0.57	11.3±1.15	12.6±0.57	12.6±0.57	16.6±2.30	13.6±0.57

(Zone of inhibition of mean ± SD in mm), (-) No zone of inhibition; Zone of inhibition including 8 mm disc, Bs- *B. subtilis*; Sa- *S. aureus*; Se- *S. epidermidis*; Ec- *E. coli*; St- *S. typhimurium* ; Pa- *P. aeruginosa*; Vc- *V. cholerae* ; Va- *V. alginolyteus* .

al., 1997). In general the plant antibiotic substances appear to be more inhibiting to Gram-positive organisms than to the Gram-negative type (Kumar et al., 2006) . It may be remembered that penicillin and some of the other prominent antibiotic agents of fungal origin are also rather selective in their inhibitory action, most of them being inhibited to Gram-positive bacteria. Unlike gram -positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major fungal origin are also rather selective in their inhibitory action, most of them being inhibited to Gram -positive bacteria. Unlike gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Burn, 1988). Access of the most compounds to the peptidoglycan layer of the cell wall is hin-

Table 3. Antibacterial activity of *V. negundo* bark and leaf by agar cup method.

Strain	Petroleum ether		Chloroform		Ethanol		Methanol		Aqueous		Ampicillin	
used	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf
Bs	11.3±0.57	13.6±0.57	11.6±0.57	9.6±0.57	11.3±2.08	11.3±0.57	10.6±0.57	12.3±0.57	9.3±1.15	10.6±0.57	19.3±1.15	18.6±3.00
Sa	11.6±0.57	-	13.6±0.57	9.3±0.57	11.3±0.57	-	10.6±0.57	9.3±0.57	9.6±0.57	9.6±1.52	15.6±0.57	15.6±0.57
Se	13.6±0.57	11.3±0.57	10.3±0.57	11.0±0.00	11.6±0.57	13.0±1.00	13.0±0.00	12.3±0.57	11.3±3.21	12.0±0.00	20.0±0.00	18.6±1.15
Ec	13.6±0.57	12.3±0.57	9.6±0.57	10.6±0.57	13.6±0.57	13.3±0.57	12.6±0.57	13.0±0.00	10±1.73	10.6±2.3	16.6±2.30	17.33±1.15
Pa	12.3±1.52	11.3±0.57	13.6±0.57	12.0±1.73	13.6±0.57	120.3±0.57	12.6±0.57	9.3±0.57	12.6±0.57	10.3±0.57	20.0±0.00	20.6±1.15
St	-	9.6±0.57	-	-	11.3±0.57	10.3±0.57	10.3±0.57	9.6±0.57	9.0±0.00	9.0±0.00	17.3±1.13	16.6±1.15
Vc	15.6±0.57	14.3±1.00	11.6±0.57	11.6±0.57	12.6±0.57	12.6±0.57	10.6±0.57	10.6±0.57	10.6±0.57	10.6±0.57	23.3±1.15	24.6±1.15
Va	13.0±0.57	13.6±0.57	12.6±0.57	12.6±0.57	11.6±0.57	11.6±0.57	9.6±0.57	-	10.6±0.57	-	15.3±2.30	15.3±2.30

(Zone of inhibition of mean ± SD in mm), (-) No zone of inhibition; Zone of inhibition including 6 mm disc, Bs- *B. subtilis*; Sa- *S. aureus*; Se –*S. epidermidis*; Ec- *E. coli*; St- *S. typhimurium*; Pa- *P. aeruginosa*; Vc- *V. cholerae* ; Va- *V. alginolyteus.*

Table 4. Minimum inhibitory concentration of *V. negundo* bark and leaf.

Strain	Petroleum ether in mg/ml		Chloroform in mg/ml		Ethanol in mg/ml		Methanol in mg/ml		Aqueous in mg/ml		Ampicillin in mg/ml
used	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Leaf and Bark
Bs	2.50	2.50	1.25	1.25	0.625	0.625	1.25	1.25	1.25	2.50	0.005
Sa	1.25	1.25	1.25	0.625	1.25	0.625	1.25	1.25	1.25	1.25	0.005
Se	1.25	2.50	1.25	0.625	1.25	0.625	1.25	1.25	1.25	1.25	0.025
Ec	0.625	0.312	0.625	0.312	0.625	0.312	0.625	0.625	0.625	0.312	0.005
Pa	2.50	2.50	1.25	1.25	0.625	0.625	2.50	2.50	1.25	1.25	0.005
St	1.25	2.50	1.25	1.25	1.25	1.25	0.625	1.25	0.625	1.25	0.012
Vc	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	0.005
Va	1.25	1.25	1.25	1.25	1.25	1.25	0.625	1.25	0.625	1.25	0.005

Bs- *B. subtilis*; Sa- *S. aureus*; Se- *S. epidermidis*; Ec- *E. coli*; St- *S. typhimurium*; Pa- *P. aeruginosa*; Vc- *V. cholerae* ; Va- *V. alginolyteus*.

dered by the outer lipopolysaccharide layer. This explains the resistance of Gram-negative bacteria to the lytic action of most extracts exhibiting the activity. Antibacterial extracts from the tested plants can be useful in warding of infectious dis-eases and there is therefore a compelling reason to suppose that, as anti-infective agents from test-ed plants are active against human pathogens, it can be assumed that these plants could be useful in warding of infectious diseases (Kirtikar and Basu, 1965; Nadkarni, 1997).

The phytochemical constitute such as alkaloid, flavonoid, tannin and phenolic compound have been reported to be important compounds in many Other medicinal plants (Barnabas and Nagarjnl, 1988; Burapedjo and Bunchoo, 1995). The results of the present investigation ethanol extracts of both leaves and bark possesses these compounds which might shows antibacterial activity. Although previously some reports concerning the antibacterial activity of *V. negundo* are present but our finding supports the efficacy.

Kumar et al. (2006) studied the antibacterial activity of dichloromethane: methanol (1:1 v/v) ex-

Table 5. Minimum bactericidal concentration of *V. negundo* bark and leaf.

Strain used	Petroleum ether in mg/ml		Chloroform in mg/ml		Ethanol in mg/ml		Methanol in mg/ml		Aqueous in mg/ml		Ampicillin in mg/ml
	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Leaf and Bark
Bs	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	1.25	> 5.0	> 5.0	> 5.0	> 5.0	0.005
Sa	> 5.0	2.50	2.50	2.50	> 5.0	1.25	> 5.0	> 5.0	> 5.0	> 5.0	0.005
Se	1.25	> 5.0	> 5.0	2.50	2.50	1.25	2.50	> 5.0	> 5.0	> 5.0	0.025
Ec	0.625	0.625	5.0	0.625	2.50	0.625	1.25	2.50	0.625	0.625	0.005
Pa	5.0	5.0	5.0	1.25	2.50	0.625	> 5.0	> 5.0	> 5.0	> 5.0	0.005
St	2.50	> 5.0	> 5.0	1.25	2.50	1.25	> 5.0	> 5.0	1.25	> 5.0	0.012
Vc	> 5.0	> 5.0	> 5.0	> 5.0	2.50	1.25	> 5.0	2.50	> 5.0	> 5.0	0.005
Va	> 5.0	> 5.0	> 5.0	> 5.0	2.50	1.25	> 5.0	> 5.0	2.50	> 5.0	0.005

Bs- *B. subtilis*; Sa- *S. aureus*; Se- *S. epidermidis*; Ec- *E. coli*; St- *S. typhimurium*;
Pa- *P. aeruginosa*; Vc- *V. cholerae* ; Va- *V. alginolyteus.*

tracts of *V. negundo* against different bacterial strains. Their finding conclude that non of the micro-organisms including the bacterial strains like *B. subtilis, S. aureus, S. epidermidis, E. coli* and *P. aeruginosa* were inhibited by dichlorome-thane: methanol extract.

Ahmad et al. (1998) studied the antibacterial activity of *V. negundo* whole plant of hexane, alcoholic and aqueous extracts against *B. subtilis, E. coli, Proteus vulgaris, S. typhimurium, P. aeruginosa* and *S. aureus* had no activity. Valasraj et al. (1997) studied antibacterial activity of ethanol extracts of *V. negundo* leaf using agar dilution method against four bacteria *B. subtilis*, *S. epidermidis, E. coli* and *P. aeruginosa.*

They conclude that antibacterial activities against Gram -positive bacteria were more pro-nounced then against Gram-negative. Their find-ing showed that at concentration 6.25 mg/ml inhi-bition was found against *B. subtilis* where as other organism's viz. *S. epidermidis, E. coli* and *P. aeruginosa* were inhibited at a concentration of 25.0 mg/ml.

So far the antibacterial activity on *V. negundo* tested by Kumar et al. (2006) and Ahmad et al. (1998) resulted in negative results. On the other hand, Valasraj et al. (1997) reported positive response with four strains only.

However, our results obtained have better inhibitory effect as compared to Valasraj et al. (1997).

Comparison of the data obtained in this study with previously published result is problematic. First, the composition of the plant extracts is known to vary according to local climatic and environmental conditions (Janssen et al., 1987; Sivropoulou et al., 1995). Secondly, the method used to assess antibacterial activity and the choice of the test organisms also varies (Janssen et al., 1987). Most frequently used methods to antibacterial activity are agar diffusion techniques and broth dilution methods. The results obtained by each of these methods may differ as many fac-tors vary between assays (Janssen et al., 1987; Hili et al., 1997). *In vivo* studies may be re-quired to confirm the values of the some of the results obtained

Conclusion

Our results allow us to conclude that the crude extracts of ethanol and methanol of leaves and petroleum ether, chloroform extracts of bark exhibited significant antibacterial activity and properties that support folkloric use in the treatment of some diseases as broad spectrum antibacterial agents. This probable explains the use of this plant by indigenous peoples against a number of infectious diseases since generation.

Also the experiment provides some scientific justification for the utilization of extracts from both bark and leaves to treat infectious diseases.

However, it is important to point out that the crude extracts such as these need to be further purified through antibacterial activity guided fractionation to isolate and identify the compounds responsible for antibacterial activity.

ACKNOWLEDGEMENTS

The present research has been funded by the Department of Science and Technology, Government of Orissa (Grant No. 2818/28.6.2006). We are also grateful to the authorities of North Orissa University for providing necessary facilities to carryout this research.

REFERENCES

Ahmad I, Mehamood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62:183–193.

Alam MI, Gomes A (2003). Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts. J. Ethnopharmacol. 86:75-80.

Barnabas CG, Nagarajan S (1988). Antimicrobial activity flavonoids of some medicinal plants. Fitoterapia 3:508-510.

Barry AL (1980). Procedure for testing antimicrobial Agents in Agar media. In: Antibiotics in Laboratory Medicine. Lorin V (eds), Williams Wilkins Co. Baltimore: USA, pp. 1-23.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

Burapedjo S, Bunchoo A (1995). Antimicrobial activity of tannins from *Terminalia citrine*. Planta Med. 61:365-366.

Burn P (1988). Amphitropic protein: A new class of membrane proteins. Trends Biochem. Sci. 13:79-83.

Chandramu C, Manohar RD, Krupadanam DGL, Dashavantha RV (2003). Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. Phytother. Res. 17:129-134

De S, Ravishankar B, Bhavsar GC (1993). Plants with hepatoprotective activity: a review. Ind. Drugs. 30:355-363.

Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD (2003). Anti -inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J. Ethnopharmacol. 87: 199-206.

Duraipandiyan V, Ayyanar M, Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Comp. Alter. Med. 6:35-41.

Gupta AK, Tandon N, Sharma M (2005). Quality standards of Indian medicinal plants. Indian Council of Medical Research, New Delhi, India.

Hebbalkar DS, Hebbalkar GD, Sharma RN, Joshi VS, Bhat BS (1992). Mosquito repellant activity of oils from Vitex negundo Linn leaves. Ind. J. Med. Res. 95: 200-203.

Hili P, Evans CS, Veness RG (1997). Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Lett. Appl. Microbiol. 24:269-275.

Ibrahim MB, Owonubi MO, Onaolapo A (1997). Antimicrobial effects of extracts of leaf, stem, bark of *Anogiessus leicarpus* on *Staphylococcus aureus* NCTC 8198, *Eschechia coli* NCTC 10408 and *Proteus vulgaris* NCTC 4636. J. Pharma. Res. Dev. 2:20-26.

Janssen AM, Scheffer JJC, Baerheim-Svendsen A (1987). Antimicrobial activity of essential oils: a 1976-86 literature review. Planta Med. 53:395-398.

Kirtikar KR, Basu BD (1968). Indian medicinal plants (vols. 1 and II). Lalit Mohan Basu, Allhabad, India.

Kumar VP, Chauhan NS, Padhi H, Rajani M (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. J. Ethnopharmacol. 67:241-245.

Levison WE, Jawetz E (1992). Medical Microbiology and immunology, Appleton and Lange, New york.

Nadkarni AK (1997). Nadkarni's Indian Materia Medica, (vol. I and II) Popular prakashan, Bombay, India.

Parveen N (1991). Antifilarial activity of Vitex negundo. Fitoterapia. 62, 163-165.

Prajapati V, Tripathi AK, Khanuja SPS, Kumar S (2003). Anti-insect screening of medicinal plants from Kukrail Forest, Lucknow, India. Pharma. Biol. 41:166-170.

Rusia K, Srivastava SK (1998). Antimicrobial activity of some Indian medicinal plants. Ind. J. Pharm. Sci. 60:57-58.

Sahni YP, Srivastava DN, Gaidhani SN (2001). Protective activity of some indigenous medicinal plants on gastric ulceration in albino rats. J. Med. Arom. Plant Sci. 22:89-90.

Silver LL (1993). Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimicrob. Agents. Chemother. 37: 377-383.

Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1995). Antimicrobial activity of mint essential oils. J. Agric. Food Chem. 43: 2384-2388.

Tomas-Barberan FA, Msonthi JD, Hostettman NK (1988). Antifungal epicuticular methylated flavonoids from three Spanish Helichrysum species. Phytochemistry 27:753-755.

Trease GE, Evans WC (1989). Text book of Pharmocognosy.13th (eds). Alden Press; Oxford; pp. 512-513.

Turnbull PCB, Kramer JM (1991). Bacillus. In: Barlows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ (eds), Manual of Clinical Microbiology, 5th (eds). Washington DC: American Society of Microbiology. pp. 345-355.

Valsaraj R, Pushpangadan P, Smitt UW, Adersen A, Nyman U (1997). Antimicrobial screening of selected medicinal plants from India. J. Ethnopharmacol. 58: 75-83.

Vander-Berghe Da, Vlietinck N (1991). Screening methods for antibacterial and antiviral agents from higher plants. In: Dey PM, Harborne JB (eds). Methods in plant biochemistry, London: Academic Press.

Watt G (1972). A Dictionary of the Economic Products of India. (Vol. IV & VI), Cosmo Publications, New Delhi, India.