Extraction of Phytochemicals from the Stem and Leaves of the Ayurvedic Medicinal Plant Vitex negundo as Possible Therapeutic against Vibrio cholera

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Abstract: The emergence of multidrug resistance (MDR) among enteric bacteria presents a serious challenge and Infectious diarrehoeal diseases are responsible for greater patient morbidity and mortality, principally in developing countries [1]. In this stance, it is imperative to develop alternative therapeutic agents and phytochemicals offer a newer class of anti infective agents[2]. In the present paper, we report the efficacy of the phytochemicals present in the cold methanlic extract of the Ayurvedic medicinal plant, *Vitex negundo* against clinical isolate of *Vibrio cholera.* The anticholera activity shown by the extract was compared to that of antibiotics of known potencies. The diameter of zone of inhibition was 20 mm for the extract where as only three antibiotics *viz,* Amikacin, chloramphenicol and carbenicillin were proved to be effective and the respective zone of inhibitions were 19, 17 and 17mm. This study showed that *V.cholera* exhibited resistance to most of the antibiotics evaluated in this study underlining the presence of MDR traits in them. The anti oxidant activity of the extract was evaluated by the DPPH method and 76.12 % inhibition was obtained at concentration of 100mg/mL with an IC_{50} value of 50.92 mg/mL which may enhance the anti bacterial activity. The volatile components present in the extract were analysed by GCMS.

Key words - Multiple drug resistance, plant extract , phytochemicals, antibacterial.

I. INTRODUCTION

Multiple surveillance studies have demonstrated that resistance among prevalent pathogens is increasing at an alarming rate, leading to greater patient morbidity and mortality from nosocomial infections. Repeated and excessive use of antibiotics can disturb the person's own immunity leading to harmful side effects to some crucial organs and other internal ecology, even harming the beneficial bacteria inside our body. Adverse antibiotic-induced reactions are a concern because they cause host injury and interrupt and complicate therapy as well. This often necessitate alternative, more expensive agents that have the ability to foster the emergence and spread of drug-resistant organisms [1,2]. The growing awareness about increased drug resistance among bacteria and the after effects of modern therapy and the standardization of the activity of herbal based formulations as therapeutic agents against virulent bacterial species, stimulate a lot of potential confronting current research.

Only a few plant species have been thoroughly investigated for their medicinal properties. India exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. It is an emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of medicinal plants. In India, there is a common practice of the vast traditional knowledge of use of herbal medicine for cure of various diseases[4]. But the major problem that we face today is that most of these valuable plant species are endangered, through this project we aim to highlight the importance of such medicinal plants and thus to conserve them.The recent researches focused on natural products have shown a useful way to obtain a potentially rich source of drug candidates, where alkaloids and flavanoids have been found more effective[5]. Current literature of natural product provide a growing research on plant derived antimycobacterial

alkaloids and many groups are actively engaged in screening of natural product extracts as the preliminary step to finding new lead compounds.

The present study has been undertaken with the following objectives:

a. To carry out the phytochemical screening of the extracts of the plant, *Vitex negundo*.
b. To evaluate the efficacy of the extracts against potent enterobacteria.
c. To screen the antioxidant activity of the extracts.
d.

II. MATERIALS AND METHODS

2.1 The potent plant:

The plant, *Vitex negundo* was identified by the Taxonomist of Amrutha Arya Drugs, Koodal, Pathanamthitta, Kerala, India. It was collected from Pathanamthitta District, Kerala, India in December 2011. The leaves, stem and bark were separated and dried at 40^0C and employed for further extraction. Fig. 1. represents the photograph of the plant.

Fig.1. *Vitex negundo* Plant

2.2 Extraction

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.The Soxhlet extraction method was employed for the pulverized and dried plant parts using a sequential method with solvents in the increasing polarity as hexane, dichloromethane, chloroform, ethyl acetate and methanol. The residue was obtained by solvent removal or evaporation under reduced pressure.

2.3 Analytical methods

The phytochemical analyses of the hexane, dichloromethane, chloroform, ethylacetate and cold methanolic extracts of *V. negundo* for alkaloids, saponins, tannins, glycosides, anthraquinones, terpenes, and flavonoids were carried out using the methods described by Harborne 1993 [26]; Sofowara 1982 [27] and Trease and Evans 1983[28].. The powdered root was extracted with the required solvent and necessary reagent added to the right quantity of the extract. All observations were recorded.

2.4 Antioxidant activity studies

The Free radical scavenging activity of the residues were evaluated on the basis of the DPPH model of Robards, Sanchez-Moreno, Larrauri and Calixto with some modifications. The general procedure employed is as follows: 2mL methanolic solution of DPPH (0.1 mM) was mixed with 200µL of the test samples (0.1mg/mL) and was made to a volume of 3 mL with methanol. After 60 min in the darkness, the absorbance was measured at 517 nm against methanol as blank. Vitamin C is used as the positive control. Free radical scavenging activities of the residues were evaluated by comparison with a control (2 mL DPPH solution and 1 mL methanol). All measurements were taken in triplicates and an average value was taken.

2.5 Antibacterial activity studies

2.5.1 Preparation of Inoculam

The colonies of identified bacterial strains were obtained by culturing the isolates of the respective isolates on separate nutrient agar plates and incubated at 37oC for 48 hrs and checked for the appearance of colonies. A

loopful of isolated colonies was inoculated into 4 mL of peptone water, incubated at 37°C for 4 h. This actively growing bacterial suspension was then adjusted with peptone water so as to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 mL of 1.75% (w/v) barium chloride dihydrate (BaCl2. 2H2O) with 99.5 mL of 1% (v/v) sulphuric acid (H2SO4). This turbidity is equivalent to approximately 1–2 × 108 colony forming units per ml (CFU/mL).

2.5.2 Evaluation of Antibacterial activity of Extracts- Well diffusion method

Agar diffusion method was employed to evaluate the antibacterial activity. Clinical isolates were tried in this study. Wells of 8mm diameter was incised on agar. The bacteria growth was swabbed on Mueller Hinton agar and 100µl of the 10% DMSO solution of the residue was added into the wells. 10%DMSO served as control. After incubation at 37oC for 24 hrs, diameter of zone of inhibition was measured and consequently antibacterial activity was assessed. The average value of diameter of zone of inhibition exhibited by sensitive isolates to antibiotics and formulations were recorded. All the experiments were conducted in triplicates and an average value was taken. A comparative study was carried out by disc diffusion against antibiotics of known potencies.

III. RESULTS AND DISCUSSION

3.1 Chemical analysis

The results of the chemical analyses are prearranged in Table 1.

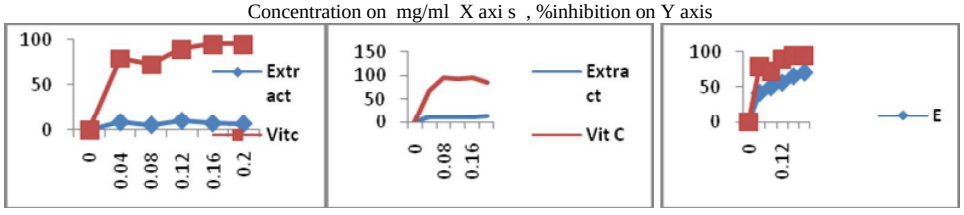
Table . 1: Phytochemical analysis of *Vitex negundo* stem and leaf extracts

Extract	**Phenols**	**Tannins**	**Alkaloids**	**Saponins**	**Flavanoids**	**Reducing sugar**	**Amino Acids**	**Steroids**
Hexane	√	×	√	√	√	√	×	√
Dichloromethane	√	√	√	×	√	√	×	√
Chloroform	×	√	×	√	×	√	×	×
Cold Methanol	√	√	√	×	√	√	√	√

3.2 Antioxidant Activity Studies

The DPPH model was employed in the evaluation of anti oxidant activities of the residue obtained from each extraction. The observed activity was compared graphically with that of vitamin C measured under identical conditions.

Concentration on mg/ml X axi s , %inhibition on Y axis



Hexane extract Dichloromethane Extract Methanol Extract

3.3 Anti bacterial activity Studies:

The efficacies of the residues were screened against clinical isolates of *Vibrio cholera, Salmonella paratyphi and Escherichia coli.* The antibiogram of the various residues are given in Table2.

Table 2: Antibiogram of Various extracts of stem and leaves of *Vitex negundo*

Sl. No.	Name of the bacteria	Extract	Diameter of zone of inhibition (mm)
1	*Vibrio cholerae*	Dichloromethane	18
2	*Salmonella paratyphi*	Dichloromethane	15
3	*Escherichia coli*	Dichloromethane	16
4	*Vibrio cholerae*	Methanol	20
5	*Salmonella paratyphi*	Methanol	16
6	*Eschericia coli*	Methanol	17

Concentration of residue was 100 μ_g per mL

Table 3: Antibiotic sensitivity of potent antibiotics

Sl.No.	Name of the antibiotic	Code and potency	Diameter of zone of inhibition (mm)		
			V.cholerae	*S. paratyphi*	*E. coli*
	Meropenem	(M-10mg)	- R	12- R	11- R
	Norfloxacin	(Nx- 10mg)	- R	17 S	13 M
	Amikacin	(Ak-10mg).	19 S	18 S	19 S
	Gentamycin	(G- 10)	- R	12 R	11 R
	Carbenicillin	(Cb- 100 µg),	17- S	19 S	13 R
	Chloramphenicol	(C-30mg),	17- S	12- R	11- R
	Ciprofloxacin	(Cf-5mg),	- R	14 R	13 R
	Netilmicin	(N- 30mg),	- R	11 R	12 R

*R- resistant, M- moderate, S- sensitive

IV. CONCLUSION

Phytochemical analysis of Vitex negundo stem and leaf extracts was done and Antioxidant Activity Studies, Anti bacterial activity Studies were also done. From this we can confirm potency of Vitex negundo stem and leaf extracts as a therapeutic drug.

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