ANTIBACTERIAL ACTIVITY OF VITEX NEGUNDOL. LEAVES' EXTRACTS AGAINST SOME SOIL BACTERIA

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ABSTRACT: *Vitex negundo*L. is popularly known as Nishinda in Bangladesh regarded as valuable medicinal plant from the ethnobotanical perspectives, which has been using in local areas of Bangladesh from ancient time for curing different types of diseases. The Antibacterial activity of the plant leaves have been evaluated in this study for determination of plant efficacy against some soil bacteria namely *Terribacillus* spp. 3LF, *Bacillus simplex* strain TAD155, *Bacillus cereus* strains 13635K, *Bacillus megaterium* strains pQ2, *Paenibacillus* sp. L32 and *Paenibacillus* spp. BF38. For evaluation of antibacterial activity of *Vitex negundo* ethyl acetate, chloroform, hexane and ethanol extracts were prepared from powdered leaves. Antibacterial potential of extract was checked against soil bacteria using disc diffusion method. Among different types of extracts, ethanol extract showed prominent antibacterial activity against nearly all specially the *Bacillus cereus* strain 13635K considered as the most pathogenic bacteria among the *Bacillus* genus with inhibition zones of 29mm.

Keywords: *Bacillus* species, zone of inhibition, minimum inhibitory concentration, antibacterial activity.

I. INTRODUCTION

Vitex negundo (Verbenaceae), commonly known as Nishinda in Bangladesh is an major medicinal plant which used as vital source for natural drugs, (Ayurvedic and Unani systems of medicine). Asian system of medicines (Malaysian, Chinese, Indian etc.) used its different parts (bark, fruit, leaves, roots and flowers) as medicine to treat various diseases [1], [2], [3]. Verity of medicinal properties are related with *V. negundo* such as anti inflammatory, antibacterial and pain killing [4], inhibition of enzymes [5], scavenging of nitric oxide [6], neutralization snake venom [7], anti feeding activity [8] and antiradical and anti-lipoperoxidative activity [9]. Similarly selected plant also have CNS activity [10], larvicidal, hepatoprotective and antifungal activity [11], [12], anti androgenic and mosquito repellent activity and so on. [13], [14].

Synthetic drugs are potentially toxic and have verity of side effects on human body, this develope an interest in medicinal plants natural products [15]**.** Microbiologist from all over the world searches the efficacy of natural plant products as a substitute of synthetic antimicrobial agent [16]. Due to production of resistant pathogenic microbial strains and side effects of the synthetic drugs, natural products from plants gain much attention in recent years [17]. Keeping in view medicinal importance of plants and emerging threat of

resistant microbial strains, the current study is design to investigate the antibacterial activity of leaves extract of *Vitex negundo* against soil bacteria.

II. MATERIAL AND METHODS

Collection of Plant Leaves: Leaves of *V. negundo* were collected by M.M.A. Mamun and M.M.A. Masud from Kumarkhali, Kushtia, Bangladesh and later on, these leaves were dried in shade and after complete drying these dry leaves were converted into fine powder (100g) by using grinder.

Preparation of *V. negundo* Leaf Extracts: Different organic solvents (N-hexane, chloroform, ethyl acetate and ethanol) were used for extract formation. 50g of fine powder and 100ml of solvent was used for extraction. These solutions were kept for 48 hours and after that filtered by using Whatman filter No. 1 paper.

Serial Dilution, Disk Diffusion Method and Antimicrobial Bioassay: 01ul serial dilutions were made from extracts as explains in previous literature [18]. Disc diffusion method was used to check the antibacterial activity of *V. negundo* [19]. Antibacterial activity of the test samples was tested by disc diffusion method according to previous study briefly, the soil bacterial isolates namely *Terribacillus*sp. 3LF, *Bacillus simplex* strain TAD155, *Bacillus cereus* strains 13635K, *Bacillus megaterium* strains pQ2, *Paenibacillus*sp. L32 and *P. aenibacillus*sp BF38 (previously stored in pure culture forms in the Microbiology Laboratory, Department of Biotechnology and Genetic Engineering, Islamic University, Bangladesh). These bacterial strains were cultured fresh in nutrient broth agar media at 30^0C for 24 hours. 10^8-10^9 /ml fresh bacterial inoculum was taken for activity. Sterilized media was poured in autoclaved petri plates and kept for cooling at room temperature. 100ul of fresh bacterial culture was spread on media with the help of spreader. Disc containing 10ul of plant extract were kept upon media and incubated at used 37^0C for 24 hours. Zone of inhibition diameter was used to determine the antibacterial activity. Minimum inhibitory concentration (MIC) was determined using standard protocols present in literature [20].

III. RESULTS

Six different bacterial strains (*Terribacillus*sp. 3LF 22T, *Bacillus cereus* strain 13635K, *Bacillus megaterium* strain pQ2, *Bacillus simplex* strain TAD155, *Paenibacillus* sp. BF38 and *Paenibacillus* sp. L32) were used to determined the antibacterial effect of *V. negundo* leaves extracts in four solvents (N-hexane, ethyl acetate, ethanol and chloroform). Minimum inhibitory concentration (MIC), zone of inhibition (ZI) and standared deviation was measured (Table 1, 2, 3, 4, 5).

Table 1 Antibacterial activities of ethanol leaf extracts of *V. negundo*.

Tested Bacterial Strains	*Zone of Inhibition (mm)*						
	Dose Level (µg/ml)					Amoxycillin (µg/disc)	Negative Control (µl/disc)
	6,700[a]	670	512	256	128	10	10
***B. simplex* strain TAD 155**	14±1	13±1	14±1	10±1	9±1	-	-
***B.* cereus**	9±1	19±1	29±1	24±1	29±1	12	-
***Terribacillus* 3LF 22T**	19±1	7±1	8±1	9±1	14±1	-	-
B. megaterium	7±1	7.5±0.5	9±1	-	-	15	-
P. sp. L32	-	-	-	-	-	-	-
P. sp. BF38	-	-	-	-	-	29	-

a = mother solution, (SD = Standard Deviation) (Zone of inhibition of mean ± SD in mm), (-) No zone of inhibition; Zone of inhibition (ZI) including 6 mm disc in diameter, *B = Bacillus, P = Paenibacillus*

Table 2 Antibacterial activities of chloroform leaf extracts of *V. negundo*.

Tested Bacterial Strains	Zone of Inhibition (mm)							
	Dose Level (µg/ml)						Amoxycillin (µg/disc)	Negative Control (µl/disc)
	5,400[a]	540	512	256	128	64	10	10
***B. simplex* strain TAD 155**	-	-	-	-	-	-	-	-
***B.* cereus**	8±1	7±1	-	-	9±1	-	12	-
***Terribacillus* 3LF 22T**	7±1	7±1	-	-	7±1	-	-	-
B. megaterium	7±1	-	-	-	-	8±1	15	-
P. sp. L32	-	-	-	-	-		-	-
P. sp. BF38	9±1	10±1	10±1	8±1	9±1	10±1	29	-

a = mother solution, (SD = Standard Deviation) (Zone of inhibition of mean ± SD in mm), (-) No zone of inhibition; Zone of inhibition (ZI) including 6 mm disc in diameter, *B = Bacillus, P = Paenibacillus*

Table 3 Antibacterial activities of ethyl acetate leaf extracts of *V. negundo*.

Tested Bacterial Strains	*Zone of Inhibition (mm)*						
	Dose Level (µg/ml)					Amoxycillin (µg/disc)	Negative Control (µl/disc)
	7,700[a]	770	512	256	128	10	10
***B.simplex* strain TAD 155**	7±1	-	-	7±1	-	-	-
***B.* cereus**							

	-	-	-	-	8±1	12	-
***Terribacillus* 3LF 22T**	-	-	-	-	-	-	-
B. megaterium	7±1	7±1	8±1	-	-	15	-
P. sp. L32	-	-	-	-	-	-	-
P. sp. BF38	-	-	-	-	7±1	29	-

a = mother solution, (SD = Standard Deviation) (Zone of inhibition of mean ± SD in mm), (-) No zone of inhibition; Zone of inhibition (ZI) including 6 mm disc in diameter, *B = Bacillus, P = Paenibacillus*

Table 4. Antibacterial activities of hexane leaf extracts of *V. negundo*.

Tested Bacterial Strains	***Zone of Inhibition (mm)***					
	Dose Level (µg/ml)				**Amoxycillin (µg/disc)**	**Negative Control (µl/disc)**
	3,900[a]	**512**	**256**	**128**	**10**	**10**
***Bacillus simplex* strain TAD 155**	-	-	-	-	-	-
***Bacillus* cereus**	20±1	7±1	8±1	-	12	-
***Terribacillus* 3LF 22T**	-	-	-	-	-	-
B. megaterium	-	-	-	-	15	-
P. sp. L32	-	-	-	-	-	-
P. sp. BF38	-	-	-	-	29	-

a = mother solution, (SD = Standard Deviation) (Zone of inhibition of mean ± SD in mm), (-) No zone of inhibition; Zone of inhibition (ZI) including 6 mm disc in diameter, *B = Bacillus, P = Paenibacillus*

Table 5 Minimum Inhibitory Concentrations (MIC) of ethanol, chloroform, ethyl acetate and hexane extracts of *V. negundo* leaves against the identified bacteria.

Tested Bacterial Strains	**Minimum Inhibitory Concentration (µg/ml)**			
	Ethanol Extract	**Chloroform Extract**	**Ethyl acetate Extract**	**Hexane Extract**
***Bacillus simplex* strain TAD 155**	128	-	256	-
***Bacillus* cereus**	128	128	-	-
***Terribacillus* 3LF 22T**	512	64	512	-
B. megaterium	128	128	128	256
P. sp. L32	-	-	-	-
P. sp. BF38	-	64	-	-

(-) No zone of inhibition; Zone of inhibition (ZI) including 6 mm disc in diameter, *B = Bacillus, P = Paenibacillus*

IV. DISCUSSION

Production of antibiotic resistant pathogens is the major concernof the present time both in hospitals and community sittings (Alanis, 2005). Again and again expousre of bacteria to same antibiotic makes the germ more potent that they are not killed by this bacteria. Bacteria adopt different mechanisms to resist antibiotics, that inturn decrease the eficacy of drugs. This problem had led many of scientest to use new natural resources espacially plants to produce novel drugs that combat such antibiotic resistant starins.

Vitex negundo has been using as medicinal plant in Indian subcontinent since ancient period of time and many pharmacological evidences have been already attributed to it. In this experiment all antibacterial activities related to *V. negundo* leaf extracts (ethanol, chloroform, ethyl acetate and hexane) in comparison to the antibiotics amoxicillin and their minimum inhibitory concentrations (MIC) have been shown (Table 1, 2, 3, 4 & 5). The ethanol extract forms a zone of inhibition nearly 29mm (MIC~128 µg/ml) while the amoxicillin only 12mm meaning that the ethanol extract contains bioactive compounds that can hinder the growth of *B. cereus*. The chloroform and ethyl acetate extracts does not show considerable result against *B. cereus* where hexane extract show 20mm zone of clearance against it. It is interesting to report that in case of *B. simplex* and *Terribacillus* sp. 3LF,the antibiotic used in this study exhibits no zone of inhibition reflecting they are antibiotic resistant whereas the ethanol extract shows bactericidal activity by forming zones of clearance ~ 14mm and 19mm. This suggests that the ethanol extract possesses such phytochemical constituents that are more active as well as potent than amoxicillin or contains such compounds that are absence in amoxicillin. There were vast studies about the medicinal values of *Vitex negundo* but a few or no study has been performed about antibacterial activity of *Vitex negundo* specifically on *B. cereus* indicating novel work on this bacteria. Therefore, the ethanol extract and ethanol dilute of essential oil of *Vitex negundo* leaves can be strong candidate for therapeutic as well as drug development purpose because β-lactamase abroad spectrum antibiotic resistance chemical produced by *Bacillus cereus* meke this to panicillin resistant and this bacteria cause common oppertunastic infections and food poisoing [21]. Domastic animals are also infected by *Bacillus cereus*, this bacteria is a well known agent that cause abortion and masititis in cattles and same conditions inother livestocks [22]. The *Bacillus cereus* has been also reported to be associated with different of human and animal diseases and it also causes significant morbidity and mortality worldwide. Some reviews about the resistances of Bacillus cereus show that Bacillus cereus isolates are resistant to penicillin, ampicillin, trimethoprim and cephalosporins [21]. Unpricribed and over use of antibiotic is major cause that produces multi-drug resistant bacterial starins in and other microorganism in Bangladesh.

Main basis for tharpy of microbial (bacterial and fungal) infection is antibiotics. Medical community believed since the discovery of antibiotics that they are completly erdicates the infectious microorganisms. However continous use of antibiotics is a main cause of antibiotics resistant bacterial strains [23]. Syntahtic drugs are expensive, toxic and have alot of side effects on host body is a major cause that reborn the interest in natural products of plants [15]. This turn the attention of scientest in formulation of new antibacterial drugs using plant products as substitute of synthetic drugs [24]. Herbla medicins got much attention due to failure of synthetic medicines [25]. The experiment shows that *V. negundo* contains (unknown) bioactive compound(s) that can be useful for pharmaceutical purposes for which further research is needed.

V. CONCLUSION

Findings of current work support to certain degree that palnts can be used to treat both animals and human diseases reinforce that ethnobotanical approach to isolate bioactive compounds from plants that can develop the novel drugs for variuos multi-drug resistant microbial strains.

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