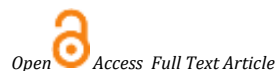


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Research Article

Evaluation of hydroalcoholic extract of *Vitex negundo* leaves for its anthelmintic activity on *Tubifex tubifex*

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Abstract

Background: The purpose of this study was to screen the anthelmintic property of a hydroalcoholic extract of *Vitex negundo* leaves. It is widely used for the treatment of anti-inflammatory, enzyme inhibitory, oxidative stress, cytotoxic impact in the ayurvedic medicine system as well as it is also used as a folk medicine in joint pain. The other parts of this plant are scientifically proven for its anthelmintic activity. The most important activities like glucose uptake, enzyme inhibitory activity, auxin transportation etc. have already been proven but still there is no scientific data available on anthelmintic activity of *Vitex negundo* leaves.

Methods: The hydroalcoholic extract of *Vitex negundo* leaves was obtained by maceration process and various phytochemical tests were performed. Limit test, main test and comparative test were performed to investigate the anthelmintic activity.

Results: The paralysis and death time of the *Tubifex tubifex* worms was noted and the minimum concentration of dose responsible for death of all the test worms within 4 hours was calculated. Albendazole suspension was used as standard drug. The data shows statistically significant by using one-way ANOVA, Dunnett's post-parametric test between test group and standard group data ($P < 0.01$).

Conclusion: Therefore, the hydroalcoholic extract of *Vitex negundo* leaves has the potential to be used as a natural anthelmintic agent.

Keywords: *Vitex negundo*, hydroalcoholic extract, anthelmintic activity, *Tubifex tubifex*, paralysis time, death time, Albendazole.

INTRODUCTION

Due to the prevalence of prescription drugs in modern countries, the interest in traditional or folk medicines has substantially expanded in recent years. Drugs are derived from tropical plants all around the world ^{1,2}. In many regions of the world, conventional plants have been employed, when they offered a safe, affordable, and trustworthy natural substitute for pharmaceutical medications ^{3, 4}. The natural world has given us several medicinal plants that become the source of treatments for all human illnesses ⁵. India is rich in flora which is widely dispersed across the entire nation. The foundation of treatment and cure has been herbal medications for a variety of illnesses and physiological states in the use of ancient practices like Ayurveda, Unani and Siddha.

Worm infections are more common in developing countries ⁶. It is seen in the people with poor hygiene. Helminth contaminations are among the commonest diseases in man, influencing an enormous extent of the total population. The helminth hurt the host by denying him of food, causing blood misfortune, injury to organs, digestive or lymphatic hindrance and by discharging poisons ⁷. Helminthiasis is seldom deadly, however is a significant reason for grimmess ⁸. Anthelmintics are the deworming agents. A vermifuge promotes expansions of worms.

There are so many drugs available in the market. At past many of the plant part is used in anthelmintic study like *Piper betel*, *Hibiscus rosasinesis*, *Coriandrum sativum* etc. These plants contain chemical constituents like alkaloid, saponin, carbohydrate etc. which are responsible for their anthelmintic property. The *Vitex negundo* also contain the same chemical constituents. Other parts of the plant are used but the leaves are not used in anthelmintic study. Hence *Vitex negundo* leaves were selected for this study.

MATERIALS AND METHODS

Worms used:

Aquarium worms (*Tubifex tubifex*) were purchased from local market and kept in an earthen pot. The length of the worms were 1-1.5 cm. These worms were acclimatized to the laboratory environment until the experiment.

Drugs and chemicals used:

Ethanol (Dr. S.C. Deb's Dilution), Albendazole oral suspension IP (Zentel 400 mg by GSK) and Distilled water. All the chemicals and drugs used were of analytical grade or laboratory grade supplied by standard manufacturers.

Preparation of plant extract:

The leaves of the plant *Vitex negundo* were collected from Bongaon, West Bengal, India. The leaves were washed with water and dried under shade at room temperature. Then the dried leaves were made coarse by hand and subject to extraction. The dried leaves were macerated using 70% alcohol and 30% water for 48 hours. Then the extract was collected, dried and used for the further investigations.

Preliminary phytochemical studies:

The hydroalcoholic extract of *Vitex negundo* leaves were subjected to preliminary phytochemical screening for the detection of various plant constituents like alkaloids, glycosides, flavonoids, saponins, carbohydrates, amino acids, fats, and steroids ⁹.

Anthelmintic activity:

The anthelmintic activity of the *Vitex negundo* leaves hydroalcoholic extract was evaluated by limit test, main test and comparative test according to OECD guidelines ¹⁰. The worms were selected randomly for the experiment. The minimum dose needed to paralyze and killed all the worms within 4 hours was calculated as well as the maximum paralysis and death time were noted. When the worms stopped their usual movements, pre-complete paralysis was thought to be started. Full paralysis time was confirmed by applying external stimuli frequently to make an aquatic worm move. The death time was noted when the worms stopped all their motion, body color disappeared and showed no sign of movements when immersed in hot water (50°C). The activity of the leaves extract was compared to control and standard drug Albendazole.

In the limit test a dose of 5000 mg/kg was taken in six Petri dishes containing 20 ml of distilled water in each. Each Petri dish contained one worm. The study was carried out for 4 hours. All the worms were died in this study. Then the main test was performed.

For the main test 4 Petri dishes containing 20 ml distilled water were taken and 175 mg/kg, 550 mg/kg, 2000 mg/kg, and 5000 mg/kg doses were added in those Petri dishes respectively. In each Petri dish six worms were placed and the LD₁₀₀ dose was determined. This study was carried out for 4 hours and the paralysis and death time were noted. In this study the minimum dose of LD₁₀₀ was determined by various division of doses and 550 mg/kg dose was found to be the minimum dose where all the worms died.

After the main test the comparative test was performed. Here the minimum LD₁₀₀ dose of *Vitex negundo* leaves hydroalcoholic extract was compared with standard drug. In 2 Petri dishes containing 20 ml of distilled water were taken and 550 mg/kg dose of *Vitex negundo* leaves hydroalcoholic extract and Albendazole suspension (same dose as plant extract, i.e. 550 mg/kg) were taken into the Petri dish as test and standard drug respectively. Then six worms were placed in each Petri

dish and their paralysis and death time were recorded for 4 hours.

Statistical analysis:

Experimental results are presented as the mean $\pm$ SEM (Standard error of mean) for each treatment group. Significance of activity was assessed using one-way ANOVA (Analysis of variance), Dunnett's post-parametric test between test group and standard group data. $P < 0.01$ was considered statistically significant ¹¹.

RESULTS

Extract preparation:

The *Vitex negundo* leaves were subjected to maceration extraction by using 70% ethanol for consecutive 48 hours and 9.01% yield was obtained.

Preliminary phytochemical investigation:

The extract obtained from the *Vitex negundo* leaves was subjected to preliminary phytochemical investigation and the following phytoconstituents were found.

Table 1: Result of preliminary phytochemical investigation

Sl. No.	Phytoconstituents	Result
1	Alkaloids	+
2	Carbohydrates	+
3	Glycoside	+
4	Proteins and amino acids	-
5	Flavonoids	+
6	Saponins	-
7	Steroids	+
8	Lipid or fat	-

Effect of the extract:

In this study, the hydroalcoholic extract of *Vitex negundo* leaves produced paralysis as well as death of the *Tubifex tubifex* worms. All the worms were died in the preliminary limit test where the dose of *Vitex negundo* leaves extract (VNLE) was 5000 mg/kg. Then the main test was performed with 175 mg/kg, 550 mg/kg, 2000 mg/kg, and 5000 mg/kg doses of *Vitex negundo* leaves extract (VNLE). Various dose division were processed to find out the minimum dose where 100% worms died within 4 hours. The hydroalcoholic extract of *Vitex negundo* leaves showed significant anthelmintic activity at minimum dose of 550 mg/kg and exhibited 100% paralysis and death of *Tubifex tubifex* worms within 103 ± 6.84 minutes and 128 ± 6.95 minutes respectively (Table 2).

Table 2: Effects of various doses of *Vitex negundo* leaves extract (VNLE) on *Tubifex tubifex* worms

Treatment	Paralysis time (Minute)	Death time (Minute)
VNLE 175 mg/kg	-	-
VNLE 550 mg/kg	$103 \pm 6.84^{**}$	$128 \pm 6.95^{**}$
VNLE 2000 mg/kg	$50 \pm 6.65^{**}$	$110 \pm 5.01^{**}$
VNLE 5000 mg/kg	$38 \pm 4.32^{**}$	$60 \pm 3.48^{**}$

Results are expressed as mean $\pm$ SEM, n=6, $^{**}P < 0.01$ was considered statistically significant.

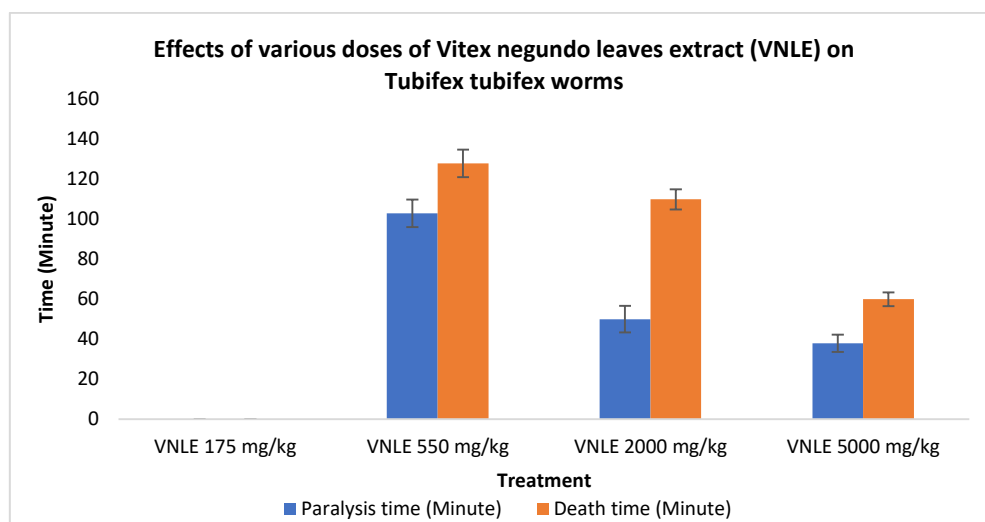


Figure 1: Effects of various doses of *Vitex negundo* leaves extract (VNLE) on *Tubifex tubifex* worms

In the comparative test both of standard drug albendazole and *Vitex negundo* leaves extract showed significant anthelmintic activity with 100% paralysis and death of *Tubifex tubifex* worms at 550 mg/kg dose respectively. The paralysis time shown by same dose of albendazole and *Vitex negundo* leaves extract were 65 ± 6.81 minutes and 103 ± 6.84 minutes

respectively (Table 3) and the death time were 100 ± 6.65 minutes and 128 ± 6.95 minutes respectively. As shown in the Table 3, the extract has exhibited prominent anthelmintic property as compare to standard drug albendazole. All the data were found to be statistically significant at $P < 0.01$.

Table 3: Effect of albendazole and *Vitex negundo* leaves extract (VNLE) on *Tubifex tubifex* worms

Treatment	Paralysis time (Minute)	Death time (Minute)
Albendazole	$65 \pm 6.81^{**}$	$100 \pm 6.65^{**}$
VNLE	$103 \pm 6.84^{**}$	$128 \pm 6.95^{**}$

Results are expressed as mean $\pm$ SEM, n=6, $^{**}P < 0.01$ was considered statistically significant.

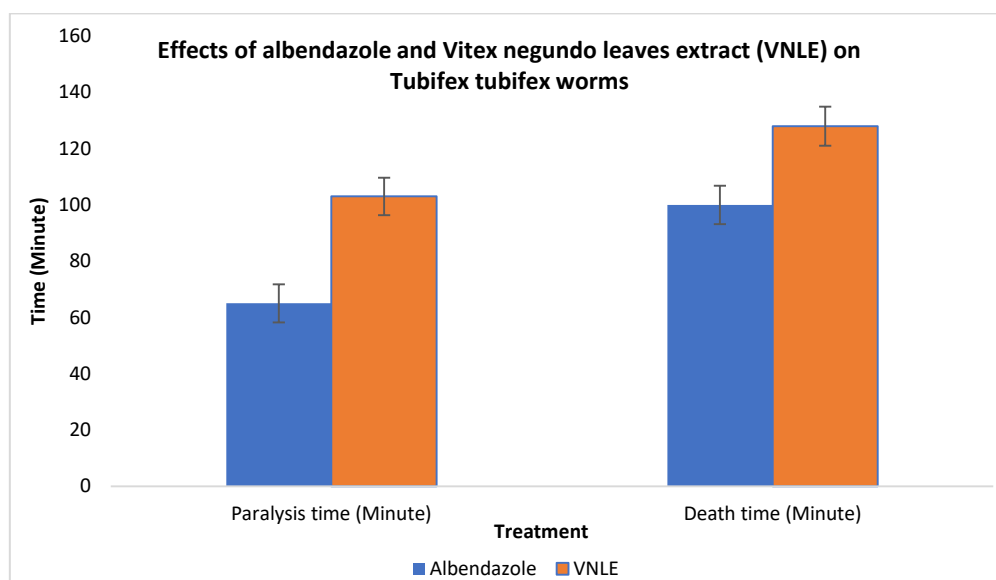


Figure 2: Effects of albendazole and *Vitex negundo* leaves extract on *Tubifex tubifex* worms

DISCUSSION

In this study, the anthelmintic activity was performed on aquarium worms, *Tubifex tubifex* due to its similar anatomical and physiological characteristics with the intestinal roundworm parasite¹². The easy availability of this worm was also an important factor for the selection.

Albendazole suspension was used as a standard anthelmintic drug. Anthelmintics like albendazole generally bind with β -tubulin of the parasite and inhibit the synthesis of micro tubules and also inhibits the glucose uptake in the parasite body¹³. All these actions create paralysis and death of the worm.

The results of the study indicate that hydroalcoholic extract of *Vitex negundo* leaves possess significant anthelmintic activity as compare to standard drug albendazole. The mechanism of action of *Vitex negundo* leaves extract is not yet fully understood, but the anthelmintic activity of the plants may be due its various chemical constituents. Phytochemical screening of the leaves extract has shown the presence of various chemical constituents like alkaloids, carbohydrates, glycosides, flavonoids and steroids. Some of these chemical constituents stand responsible for anthelmintic effect. Alkaloids show anthelmintic activity by targeting acetylcholine receptor and surpassing glucose uptake. Thus, the helminth die due to starvation¹⁴. Flavonoids inhibit glucose uptake and auxin transport and show its anthelmintic activity by blocking the phosphorylation reaction that inhibits the energy production within the parasite leading to death^{15, 16}. The carbohydrate and glycosides have potent activity against different helminths. Glycosides cause disturbance of sodium and potassium ion transportation into helminths¹⁷.

In a nutshell we can come to the point that *Vitex negundo* is a miracle herb having multiple pharmacological effect. Some of them are established but the anthelmintic property of the leaves of this plant was not revealed previously but from all the above findings it is clear that hydroalcoholic extract of *Vitex negundo* leaves has profound anthelmintic effect against tested worm species. It justifies its use in the treatment of helminth infection as a traditional remedy. Further studies are required to determine the mode of action and isolation of phytoconstituents responsible for its anthelmintic property.

CONCLUSION

In this study hydroalcoholic extract of leaves of *Vitex negundo* has been evaluated for anthelmintic activity. Albendazole suspension was used as a standard drug. *Vitex negundo* has shown significant anthelmintic activity by parolization and death of the *Tubifex tubifex* worms. The death time of *Tubifex tubifex* by hydroalcoholic extract of *Vitex negundo* leaves has shown comparable effect of albendazole oral suspension. This activity may be due to the presence of active chemicals like alkaloid, carbohydrate, glycoside, steroids in it. The potential of this drug may bring a beam of hope in the anthelmintic activity.

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CONFLICT OF INTEREST

The authors have no conflict of interest regarding this investigation.

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