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Larvicidal efficacy and phytochemical properties of *Hemidesmus indicus* root against dengue vector *Aedes aegypti*

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ABSTRACT

The Present study was carried out to study the larvicidal efficacy of Aedes aegypti on ethanol, ethyl acetate, petroleum ether and aqueous extracts of *Hemidesmus indicus* root. The root extract of *Hemidesmus indicus* with different solvents were tested for their phytochemical constituents. The analysis revealed the presence of Steroids, terpenoids, flavonoids, and carbohydrates in most prominent amount. Larval mortality was observed and recorded after 24, 48, 72 hours exposure periods. All the tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was in aqueous extract of *Hemidesmus indicus* root observed at 72 hours. The LC_{50} and LC_{90} values of *Hemidesmus indicus* root against the 1^{st} and 2^{nd} , 3^{rd} and 4^{th} instar larvae of *Aedes aegypti* LC_{50}136.74,LC_{90} 259.16 ppm and LC_{50} 101.09, LC_{90} 198.92 ppm respectively. These results revealed that larvicidal properties of *Hemidesmus indicus* root and encourages further effort to investigate the bioactive compounds in those extracts that might possess good larvicidal properties when it will be isolated in pure form.

Keywords: *Hemidesmus indicus*®, *Aedes Aegypti*, Phytochemical, Larvicidal Activity.

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INTRODUCTION

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human[1]. They do not only transmit parasites and pathogens but they also source of allergic reaction that includes local skin and systemic sensitivity[2]. The most common diseases associated with mosquitoes are dengue fever, chikungunya, yellow fever and the worst, dengue hemorrhagic fever where *Aedes aegypti* is one of the mosquito species responsible for the transmission of these vector borne diseases[3]. World Health Organization (WHO) stated that about 2/5 of the global human population are currently threaten of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes that cause the disease. Dengue is one of the most significant viral diseases transmitted by *Aedes aegypti* because it afflicts humans worldwide whose symptoms ranging from mild fever to a severe and potentially life threatening hemorrhagic disease. *Aedes aegypti* is of supreme concern because of its wide distribution and close association with humans[4]. *Aedes aegypti* is present in heavy polluted areas like Asia, America and some Pacific Islands and infested about 2/3 of the world's population[5]. Due to the pathogenic diseases and serious harms caused by mosquitoes, controlling them has been the primary subject of several new researches over the past few years[6]. The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide[7]. However, the use of synthetic larvicides imposes threats not only to human health but also to the ecosystem because when they are applied into the environment; they may stay on for a very long time or even remain there without end. Synthetic larvicides also disrupt natural biological control systems that sometimes results into a widespread development of resistance[7, 8]. This phenomenon has triggered and urged the development of alternative techniques using natural products. Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment [7,9,10]. Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and environment-friendly[11]. Chemical control is an effective strategy used extensively in mosquito control program. Many kinds of toxic chemical compounds to mosquitoes include organochlorine, organophosphorus, carbamates, and pyrethroids, respectively. However, indiscriminate usage of chemicals in the breeding site and also used in the form of adulticides, fumigants, repellents and residual spray the rate of mosquito

breeding are increased [12]. The continuous usage of chemicals disrupts natural enemies and also let to outbreaks of some insect species [13]. Plants may be an alternative source of mosquito control agents because they constitute a rich source of bioactive chemicals [14]. They are not only effective, but also greatly reduce the risk of potentially adverse ecological effects, do not have any ill effects on non-target populations are degradable, safe and easily available at low cost [15]. Medicinal plants are commonly found in tropical and sub-tropical countries. A lot of research work on medicinal plants has been carried out on agriculture pest with promising results. Some crude extract from medicinal plants are currently being used as they are potentially economical, safe and practical for control measures. Therefore, this study was designed to focus on *Hemidesmus indicus* roots and their potential specific effects on *Aedes aegypti*. *Hemidesmus indicus* commonly known as Indian sarsaparilla, belongs to the family *Asclepiadaceae*, is a common medicinal plant widely used in Indian system of medicine and also an official drug in Indian pharmacopoeia and British pharmacopoeia [16]. The entire plant is used in traditional medicine; however the root is mentioned to be the most powerful part. In the present study, we investigated larvicidal activity of petroleum ether, Ethyl acetate, Ethanol and aqueous extract of *Hemidesmus indicus* root against four instars of Aedes aegypti larvae.

MATERIALS AND METHODS

Collection of Plant Materials

Roots of *Hemidesmus indicus* were collected from Ayurvedic stores, Vellore, Tamilnadu. Their identity was confirmed at Voorhees College, Vellore, Tamilnadu. Matured roots were collected and washed, shade dried and then milled into course powder by a mechanical grinder. Collected samples were subjected to phytochemical Analysis.

Preparation of Solvent Root Extraction

The *Hemidesmus indicus* roots were properly washed with distilled water then after it was shade dried for two weeks. The roots were finely powdered and stored at room temperature for extraction. The dried root powder of 50 grams each was soaked separately in 200 ml of Ethanol, Ethyl acetate, Petroleum Ether and Aqueous by increasing order of their polarity[17].Each solvent in separate flasks with powdered root sample were kept for 3 days. The extracts were filtered throughwhat man No1 filter paper to remove the impurities, separate the roots and dried in room temperature.The organic solvents were concentrated in vacuum using a rotary evaporator, while aqueous extract was dried using water bath.

Collection of eggs and maintenance of *Aedes Aegypti* larvae

Aedes aegypti immature stages collected from zonal entomological team in Vellore, Tamilnadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified and species confirmed before rearing. Cyclic generations of *Aedes aegypti* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of 27±2 ºC and a relative humidity of 70- 80 percent were maintained in the insectary. The adult mosquitoes were fed on ten percent glucose solution. The adult mosquitoes were blood fed with the laboratory rearing albino mice. Ovitraps were placed inside the cages for eggs lying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with dog biscuits and yeast in 3:1 ratio. Twenty five 1st and 2nd, 3rd and 4th instar larvae were exposed to separate experimental trays. Control was maintained separately.

Qualitative phytochemical Analysis

Phytochemical test were carried out using Aqueous, Ethanol, ethyl acetate and petroleum ether extracts on *Hemidesmus indicus* root. The powdered specimens were screened under standard procedures to identify the constituents[18, 19].

Test for alkaloids

Mayer's Test: Few ml of test solution, a drop of Mayer's reagent (Potassium Mercuric iodide) was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

Test for Carbohydrates

Molisch's Test: Test solution with few drops of molisch's reagent and 2ml of conc. sulphuric acid is added slowly from the sides of the test tube shows a purple ring at the junction of two liquids.

Test for Saponins

Foam Test: The extract was diluted with distilled water and made up to 20ml. the suspension was shaken in a graduated cylinder for 15 min. 2cm layer of foam indicates the presence of saponins.

Test for Steroids

Salkowaski Test: 2ml of conc. sulphuric acid is added to the test solution, shaken and allowed to stand, lower layer turns red indicating the presence of steroids.

Test for Phenolic Compounds

Ferric Chloride Test: Test Solution when treated with Ferric chloride. The formation of Red, Blue, green or purple indicates the presence of phenolic Compounds.

Test for Tannins

Lead Acetate Test: Test Solution when treated with solution of lead acetate was added, Formation of a yellow or red precipitate indicated the presence of tannins.

Test for Flavonoids

Alkaline Reagent Test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute hydrochloride.

Test for Terpenoids

Liebermann Burchardt Test: The test solution treated with acetic anhydride, mixed well and conc. sulphuric acid is added from the sides of the test tube. Deep red colour forms.

Test for Cardiac glycosides

Keller Killiani Test: The test solution with few drops glacial acetic acid and 2ml of ferric chloride solution and conc. Sulphuric acid is added from the sides of the test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green.

Test for Proteins

Xanthoprotein Test: Test solution treated with 2ml of conc. nitric acid and on boiling gives yellow precipitate.

Test for Amino Acids

Ninhydrin reagent Test: Test Solution treated with Ninhydrin reagent gives blue colour.

Test for Reducing Sugars

Benedict's test: Test solution treated with Benedict's reagent and boiling on a water-bath shows reddish brown precipitate.

Test for Fat & Oil

Test Solution when treated with Copper sulphate and sodium hydroxide was added. Formation of clear blue solution indicated the presence of fat & Oil.

Larvicidal Activity

The larvicidal bioassay was performed on the first to four instars of *Aedes aegypti* larvae in accordance with the procedure described by WHO[20] with slight modifications. Twenty five 1st to 4thinstar larvae of *Aedes aegypti* were released separately in 500ml capacity of beaker containing 249 ml of water and to treat in various concentrations of plant extracts,and then were distributed in each of the replicates. The control was prepared by the addition of acetone to water. Larval mortality was recorded after 24, 48, 72 hours and control mortality was corrected by using Abbott's formula.

$$\text{Corrected Mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

Percentage Mortality = Number of dead larvae / Number of larvae introduced X 100

Statistical analysis

The larvicidal bio-assays and per cent control mortality were calculated using Abbott's transformation [21]. LC_{50} and LC_{90} (lethal concentration causing 50 and 90 per cent mortality) were calculated using Probit analysis [22]. Data from larval mortality was subjected to an analysis of variance. Statistical software SPSS 20.0 was used for data analysis.

RESULTS AND DISCUSSION

Qualitative phytochemical Analysis

The *Hemidesmus Indicus* ® extract in different solvents were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of steroids, terpenoids, flavonoids and carbohydrates in most prominent amount, while alkaloids and Cardiac Glycosides are not prominent. The qualitative phytochemical Analysis of the root extract of *Hemidesmus indicus* is documented in Table 1.0.

Table 1: Qualitative phytochemical analysis of H. Indicus ® extract

Chemical Test	Petroleum Ether Extract	Ethyl Acetate Extract	Ethanol Extract	Aqueous Extract
Alkaloids	-	-	-	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	-
Phenolic Compounds	-	+	+	+
Tannins	-	+	+	+
Fat & Oil	+	-	-	-
Cardiac Glycosides	-	-	-	-
Proteins	-	+	+	+
Carbohydrates	+	+	+	+
Amino Acids	-	-	-	+
Reducing Sugars	-	+	+	+

* Present – +; Negative – -.

Larvicidal Activity

The larvicidal activity of aqueous, ethanol, petroleum ether and ethyl acetate extracts of *hemidesmus indicus* root against *Aedes aegypti* larvae reveals that the aqueous extract indicates higher mortality rates compared to the other solvent extracts.Table 2 indicates, the effect of

aqueous extract of *Hemidesmus indicus* ®on 1st and 2nd instar larvae of *Aedes aegypti*. It is clear that the highest larvicidal potency with LC_{50} and LC_{90} was depicted after 24 hours was 236.67 ppm and 417.48 ppm. While after 48 hours the LC_{50} and LC_{90} indicated 182.65 ppm and 338.46 ppm. Whereas, after 72 hours LC_{50} and LC_{90} was observed as 136.74 ppm and 259.16 ppm.Table 3 indicates, the effect of aqueous extract of *Hemidesmus indicus* ® on 3rd and 4th instar larvae of *Aedes aegypti*. It is clear that the highest larvicidal potency with LC_{50} and LC_{90} was depicted after 24 hours was 203.65 ppm and 372.43 ppm. While after 48 hours the LC_{50} and LC_{90} indicated 146.86 ppm and 269.39 ppm.Whereas, after 72 hours LC_{50} and LC_{90} was observed as 101.09 ppm and 198.92ppm respectively. The regression equation values were also calculated. The results of larvicidal activity clearly indicates that the percentage of mortatily being directly proportional to the concentration of the extracts. This proves that concentration plays major role in larvicidal activity. Larvicidal effect and Mortality of *Aedes aegypti* larvae treated in *Hemidesmus indicus*® extract in Ethyl acetate, Ethanol, Petroleum ether and aqueous showed in Table 2&3.Moderate mortality rate was observed in petroleum ether and ethyl acetate after 24,48,72 hours in first to four instar larvae of *Aedes aegypti*. The aqueous and ethanol extracts depicted highest mortality when compare to other solvents. *Hemidesmus indicus*® showed larvicidal activity, with higher effect seen in aqueous,ethanol extracts followed by ethyl acetate and petroleum ether. The phytochemical analysis of the promising aqueousand ethanol extract of *Hemidesmus indicus* root were positive for carbohydrates, steroids, terpenoids, tannins, flavonoids, proteins, phenolic compounds. The comparative study of larvicidal activity of root extract of *Hemidesmus indicus* leaves extracts of *G. sylvestre* and *E. prostrata* was established against *Culex quinquefasciatus* mosquito [23]. Here, this result showed that the plant *Hemidesmus indicus* having high larvicidal activity, soit is suggested that the aqueous extract of this medicinal plant can be used as eco-friendly and sustainable insecticide to control mosquito. Reports are available in support of antibacterial activity of several phytochemical present in plant extracts [24, 25]. Mosquito borne diseases are one of the public health issues in developing countries. It can be controlled by preventing mosquito bite using repellent, causing larval mortality and killing mosquitoes. Higher plants are a rich source of novel substances that can be used to develop environmental safe methods for insect control. The deleterious effects of plant extracts or pure compounds from plants on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility. It has been arrived that there is a strong connection between medicinal and pesticidal plants[26, 27]. It is observed that the carbohydrate, steroids, terpenoids and flavonoids are having mosquito larvicidal activity.

From the results, it may be concluded that the presence of phytoconstituents in the solvent extracts of *Hemidesmus indicus* ® might be the reason for larvicidal activity. The plants could be used for beneficial effect to control vector borne disease such as filarial, Dengue etc. The transmission of mosquito-borne diseases can be interrupted by the potential insecticides of herbal origin at the individual as well as at the community level [28]. Recently the natural insecticides of plant origin have been given importance due to their eco-friendly nature and biodegradability as a substitute of synthetic insecticides for the control of vectors of public health importance. Different types of phytochemical of plant either from the whole part or from the specific parts come out with solvent during chemical extraction depending on the polarity of the solvent [29,30]. These phytochemical generally act as a toxicant for adult, pupa as well as larval form of mosquitoes, while some interfere with the growth (growth inhibitory) and reproduction (ovicidal deterrent) bio-control.It was clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for control of mosquitoes [31, 32]. The plant extract are eco-friendly and are not toxic to vertebrates. In this study, the result showed that the aqueous extract of *Hemidesmus indicus* ® indicates the high percentage of blocking the development by induction of great mortality of larvae. Most of the epithelial cells degenerated and vacuolated after the treatment of 48h Root extract of *Hemidesmus indicus*. Histological changes were seen in the anterior and posterior regions of the mid-gut included separation in the epithelial cells from the basement membrane with damage of the peritrophic membrane. It was investigated that the mixing of the gut contents with hemolymph caused the larval mortality as reported.

Table.2. Lethal Concentration values of H. Indicus root extract against Aedes Aegypti 1st& 2nd Instars larvae

Exposed Hours	Solvents used for extraction	LC_{50} (ppm) (LCL – UCL)	LC_{90} (ppm) (LCL – UCL)	Regression Equation
24 Hrs	Ethanol	240.50 (206.72 - 291.65)	420.82 (351.69 – 560.61)	y = 0.052x – 0.162
	Petroleum Ether	323.30 (263.23 – 474.72)	572.17 (438.31 – 957.50)	y = 0.033x + 0.429
	Ethyl Acetate	322.24 (261.06 – 476.67)	577.12 (440.04 – 977.61)	y = 0.033x + 0.610
	Aqueous	236.67 (203.18 – 286.58)	417.48 (349.08 – 555.08)	y = 0.053x – 0.048
48 Hrs	Ethanol	184.67 (157.05 – 215.87)	338.58 (291.79 – 420.28)	y = 0.066x + 0.524
	Petroleum Ether	274.68 (226.71 – 372.04)	515.36 (404.65 – 796.79)	y = 0.040x + 1.095
	Ethyl Acetate	272.03 (223.92 – 369.74)	516.82 (404.69 – 804.28)	y = 0.040x + 1.276

	Aqueous	182.65 (154.75 – 213.97)	338.46 (219.92 – 421.27)	y = 0.066x + 0.714
72 Hrs	Ethanol	138.27 (113.75 – 161.82)	260.91 (228.35 – 312.28)	y = 0.078x + 1.705
	Petroleum Ether	217.83 (182.62 – 268.40)	420.06 (345.90 – 574.66)	y = 0.052x + 1.324
	Ethyl Acetate	220.78 (183.14 – 277.37)	438.08 (356.23 – 619.65)	y = 0.049x + 1.800
	Aqueous	136.74 (112.19 – 160.22)	259.16 (136.74 – 310.37)	y = 0.077x + 1.819

Table.3. Lethal Concentration values of H. Indicus root extract against Aedes Aegypti 3rd& 4th Instars larvae

Exposed Hours	Solvents used for extraction	LC_{50} (ppm) (LCL – UCL)	LC_{90} (ppm) (LCL – UCL)	Regression Equation
24 Hrs	Ethanol	221.74 (189.73 – 266.13)	400.08 (336.30 – 524.62)	y = 0.056x+ 0.257
	Petroleum Ether	261.40 (221.18 – 332.09)	466.73 (379.37 – 662.52)	y = 0.045x + 0.267
	Ethyl Acetate	257.92 (217.73 – 328.05)	466.26 (378.39 – 663.55)	y = 0.046x + 0.438
	Aqueous	203.65 (173.79 – 241.22)	372.43 (316.42 – 476.58)	y = 0.060x + 0.438
48 Hrs	Ethanol	167.78 (139.23 – 198.25)	326.53 (280.13 – 407.22)	y = 0.067x + 1.495
	Petroleum Ether	215.61 (181.86 – 262.37)	408.75 (339.70 – 548.06)	y = 0.054x + 1.057
	Ethyl Acetate	207.32 (174.81 – 250.07)	394.25 (329.85 – 520.62)	y = 0.056x + 1.095
	Aqueous	146.86 (94.67 – 198.31)	269.39 (213.43 – 418.52)	y = 0.077x + 1.086
72 Hrs	Ethanol	126.40 (102.81 – 148.64)	239.63 (209.75 – 286.20)	y = 0.079x + 2.219
	Petroleum Ether	158.94 (100.38 – 203.98)	399.51 (315.84 – 492.89)	y = 0.056x + 3.200
	Ethyl Acetate	166.60 (136.27 – 198.59)	336.26 (286.31 – 425.93)	y = 0.063x + 2.133
	Aqueous	101.09 (78.88 – 121.55)	198.92 (172.32 – 240.59)	y = 0.079x + 3.905

Control is Nil mortality significant at $p<0.05$ level; Values were based on different concentrations and for six replicates with 25 larvar in each; LC50 lethal concentration that kills 50 percent of the exposed larvae in 24 hours; LC90 lethal concentration that kills 50 percent of the exposed larvae in 24 hours; LCL – lower confidential limit; UCL - Upper confidential limit.

CONCLUSION

The present study was carried out on the root extract of *Hemidesmus indicus* revealed the presence of medicinally active constituents. In this present study, the preliminary phytochemical screening

of all extract showed the presence of bioactive compounds which may retain a wide range of actions. The secondary metabolites were found rich in *Hemidesmus indicus* root had shown the presence of carbohydrate, steroids, terpenoids and flavonoids in all the extracts tested. The Aqueous & ethanol extract of *Hemidesmus indicus* ® indicates the highest mortality rate than all other extracts. Interestingly root extract was found particularly rich in steroids. The presence of steroidal compounds would have effective larvicidal activity as cited in the study. High amount steroids metabolite may increase the high percentage mortality rates of *Aedes aegypti* larvae.

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