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Evaluation of Mosquito Larvicidal Efficacy of Different Parts of *Dalbergia sissoo* Plant

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ABSTRACT

The present study was carried out to evaluate the larval efficacy of *Dalbergia sissoo* and to identify its most potent part as a larvicide. Different solvent extracts obtained from flowers, seeds and branches of *D. sissoo* along with market procured shisham oil were tested for their larvicidal potential against *An. stephensi* larvae. There was an overall lethal effect of different extracts of *D. sissoo* against vector mosquito larvae. On the basis of the LC_{50} values, the most potent extract of the larvicidal nature was screened and analyzed by GC-MS technique (Gas Chromatography-Mass Spectrography). Hexane extract of flowers was found to be most effective with LC50 value of 55.328 ppm and LC90 value of 180.319 ppm. LC50 and LC90 values of methanol extract of seeds, acetone extract of branches and oil of shisham were 121.517 and 300.695 ppm; 114.829 and 341.972 ppm; 128.739 and 232.543 ppm respectively. The gas chromatography of the flowers extract of *D. sissoo* revealed 62 chemical constituents19 of which contributed more than 1% of the total area of the chromatogram. These constituents may be further tested for their larval efficacies and their potential could be exploited for the development of safer and effective plant based mosquito larvicide.

Keywords: *Dalbergia sissoo*, larvicide, plant extracts, vector control and Gas chromatography-Mass Spectrography.

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INTRODUCTION

Malaria, filaria, dengue and japanese encephalitis are the most important mosquito borne diseases in India which cause significant morbidity and mortality. Mosquito control is mainly insecticide dependent which has become less effective due to the development of insecticide resistance [1-3]. Further, deleterious effect caused by the regular insecticidal use at large scale creates need for some eco-friendly and non-hazardous methods of mosquito control. As an alternative, scientists have recognized the scope of plant extracts as larvicides, and thus, considerable work has been done on the mosquito larvicidal efficacy of different plant [4-6].

Several plant-based products are used for their insecticidal and repellent activities in mosquito control [7-8]. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellents and ovipositor attractant as reported by many researchers [8-10]. Compounds of plant origin such as rotenone, nicotine, anabasine, methyl anabasine and lupinine have been found effective in killing *Culex territans* (Diptera: Culicidae) [11]. Most effective compound of plant origin for the control of adult mosquitoes is pyrethrum extract (mixture of esters of pyrethrins and cinnerins) obtained from the flowers of *Chrysanthemum cinneraefolium* (Family: *Asteraceae*). This extract was first used successfully in vector control operations in South Africa [12-13] and later in India [14-15]. In India, pyrethrum extract is used for liquidation of epidemic foci under anti-malaria program [16].

The genus, Dalbergia consists of about 300 species and out of these 25 species occurs in India. *Dalbergia sissoo* is commonly known as 'Shisham' or Indian rosewood. Shisham is a native plant of India and is the most important cultivated timber tree, planted on roadsides, and as a shade tree for tea plantations. *D. sissoo* has been utilized as medicines, health products, pharmaceuticals and cosmetics [17]. In ayurveda, the leaf juice of *D. sissoo* is used for eye ailments, the wood and bark acts as abortifacient, anthelmintic, antipyretic, apertif, aphrodiasiac, expectorant and refrigerant [18]. Earlier, larvicidal and repellent properties of market procured oil of *D. sissoo* and pine has been studied by Ansari *et al* [19-20] and found the oil very effective as a mosquito repellent, inhibitor and larvicide against *Anopleles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.* Considering the potential of *D. sissoo* as a larvicide against vector mosquito species, it was thought prudent to identify and isolate most potent part of *D. sissoo* which may be useful for the vector control.

MATERIALS AND METHODS

Collection of plant parts and its extraction

Different parts of Shisham e.g. flower, seed and branches were collected from Delhi and the taxonomic identification was made by Indian Agriculture Research Institute, PUSA, Delhi. The collected plant material was washed thoroughly in de-chlorinated running tap water and dried completely in the shade. Thereafter, it was grinded to powder form in an electric grinder and was weighed. The powdered dried material was subjected to Soxhlation (Soxhlet's apparatus) in different solvent in the ratio of 1:10(w/v) like hexane, acetone and methanol subsequently for 72 hours for complete extraction. The respective solvents were evaporated after the extraction by vacuum rotary evaporator to obtain concentrated residues.

Larval Bioassay

Larvae of *An. stephensi* reared in National Institute of Malaria Research, Delhi insectory for regular availability was used for bio-assay tests. Stocks of the desired concentrations were prepared either in ethanol or acetone for each extract from respective concentrated residues. The stock solution was further diluted to prepare a range of various test concentrations i.e. 50 – 250 ppm. One ml. of these test concentrations was added to 249 ml of water in 500 ml capacity beakers to obtain the concentration to which the larvae were exposed. Twenty five 3rd instar larvae of *An. stephensi* were exposed to the above-mentioned concentrations of each extract along with control according to standard procedure [21]. Market procured Oil of Shisham was also tested along with the above extracts as per the same protocol. Experiments were carried out in triplicate along with the control in each series at 27±2 ^{0}C and 85±5% relative humidity. Larval mortality was recorded after 24 hours and lethal concentration (LC50 and LC90) were calculated according to Probit Analysis [22]. In case of >20% mortality in control, corrected % mortality was calculated using Abbott's formula [23]. On the

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basis of the LC_{50} values, the most potent larvicidal extract was screened further and analyzed by GC-MS technique (Gas chromatography-Mass Spectrograph) for the identification of the chemical composition of the extract. The GC-MS analysis was done at the Advanced Instrumentation Research Facility of Jawaharlal Nehru University, Delhi.

RESULTS

Larvicidal activity of acetone extract of branches, hexane extract of flowers, methanol extract of seeds and *D. sissoo* oil is given in table 1. At 50 ppm dose, maximum mortality (51.6%) was observed with hexane extract of flowers followed by 20, 11.1 and 6% mortality with acetone extract of branches, methanol extract of seeds and oil respectively. In case of flower extract the percent mortality was recorded as 51.6%, 66.6 %, 80%, 95% and 98% respectively while in control the percent mortality was 6.6%. In case of seed extract the percent mortality recorded was 11.1%, 44.4 %, 48.9%, 77.8% and 88.9% respectively while in control the percent mortality was 6.7%. Hexane extract of flowers was found to be most effective with LC50 value of 55.328 ppm and LC90 value of 180.319 ppm. LC50 and LC90 values of methanol extract of seeds, acetone extract of branches and oil of shisham were 121.517 and 300.695 ppm; 114.829 and 341.972 ppm; 128.739 and 232.543 ppm respectively (table 2). The gas chromatography of the flower extract of *D. sissoo* revealed 61 chemical constituents 19 of which contributed more than 1% of the total area of the chromatogram (Figure 1). The 19 chemicals were 1H-benzocycloheptene, 2,4A,5,6,7,8,9,9A-octahydro-3,5,5-trimethyl-9-methylene-; Himachalene <gamma->; 1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-,[3R-(3α,3aβ,7β,8aα)]-; 1H-benzocycloheptene,2,4A,5,6,7,8-hexahydro; Bisabolene <(E)-, alpha->; (.+-.)-DIHYDRO-AR-TURMERONE; Acorenol <alpha->; Allohimachalol; Tumerone <ar->; Atlantone<(Z)-gamma->; Deodarone; Atlantone<(E)-gamma->; Atlantone <(Z)-alpha->; Carbonic acid, 4-isopropylphenyl propargyl ester; Trans Alpha Atlantone; 8-α-Acetoxyelemol; (E)-10,11-dihydroatlantone; Deodarone; 9,10-Dimethyl-1,2,3,4,5,6,7,8-octahydroanthracene.

Table 1: Larvicidal activity of different parts of *D. sissoo* against *An. stephensi*

Dose (ppm)	% mortality with Acetone extract of branches	% mortality with Hexane extract of flowers	% mortality with Methanol extract of seeds	% mortality with *D. sissoo* oil
50	20	51.6	11.1	6
100	34	66.6	44.4	24
150	66	80	48.9	52
200	76	95	77.8	86
250	98	98.3	88.9	98
control	0	6.6	6.7	4

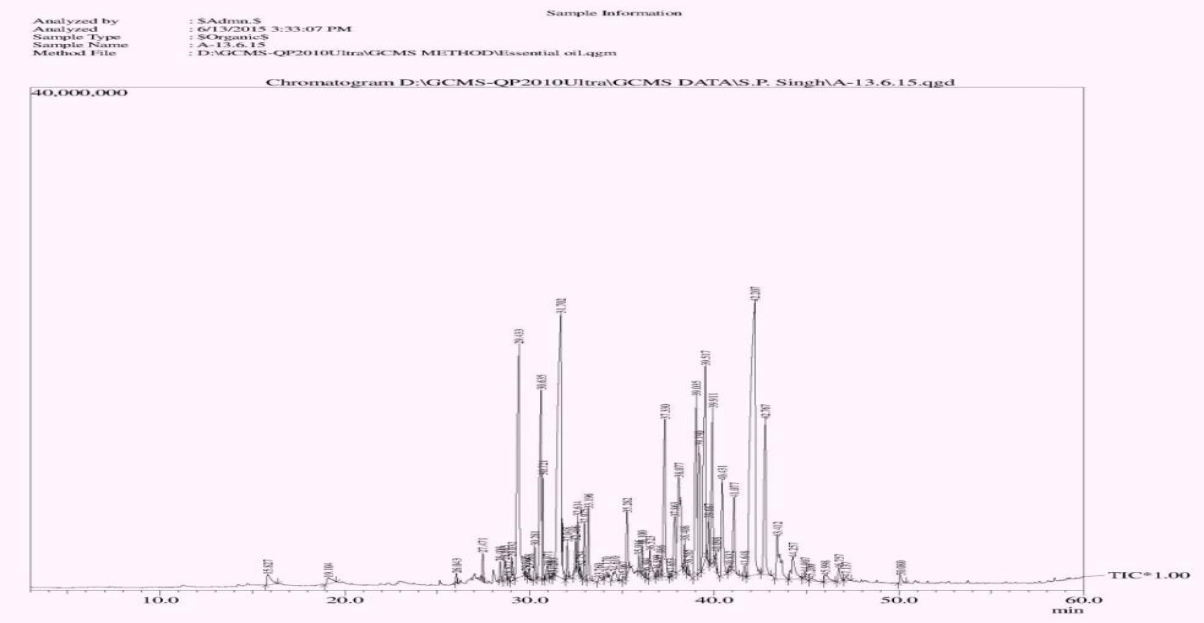

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Figure 1: GC-MS chromatogram of *D. sissoo* flower extract

Table 2: Larvicidal efficacy of different extracts of *D. sissoo* against *An. stephensi*

Extracts of D. sissoo	LC50 (lower and upper fiducial limits)	LC90 (lower and upper fiducial limits)
Acetone extract of branches	114.829 (97.232-135.718)	341.972 (253.330-593.678)
Hexane extract of flowers	55.328 (11.777- 83.913)	180.319 (121.323- 678.271)
Methanol extract of seeds	121.517 (105.259-138.325)	300.695 (245.791-411.889)
D. sissoo oil	128.739 (86.559-172.252)	232.543 (173.469-578.214)

LC_{50} – Lethal concentration for 50% mortality, LC_{90} – Lethal concentration for 90% mortality

DISCUSSION

The evaluation of extracts of different parts of *D. sissoo* revealed an overall lethal effect against *An. stephensi* larvae. The hexane extract of flowers resulted in most lethal activity as compared to other extracts. The methanol extract of seeds and acetone extracts of branches also exhibited lethal activity to a lesser extent against these premature insects. Even though the number of reports on aromatic plants used in different countries for larval mosquitocidal activities continued to increase, to the best of our knowledge, no studies have been carried out on the lethal activity of *D. sissoo* plant against the larvae of malaria vector, *An. stephensi*. However, larvicidal and repellent propensities of market procured *D. sissoo* oil against different mosquito species has been studied by Ansari e*t al.* [19]. Rate of application of pure oil was 0.4-0.5 ml/m^2 on water surface, and the results of larvicidal activity were found directly proportional to application dosages. Hundred percent mortality of C*x. quinquefasciatus* immature was observed within 24 hrs at 4 ml/m^2, followed by *Ae. aegypti* (90%) and *An. stephensi* (60%), and pupation was totally inhibited. The oil also showed strong repellent action when 1 ml oil was applied on exposed parts of human volunteers and they were protected from mosquito bites for 8-11 hrs.

Different parts of *D. sissoo* exhibited LC50 value ranging from 55-128ppm against *An. stephensi* larvae. These results are comparable with Govindrajan *et al.* experiments with *Ficus benghalensis* leaf extracts for larvicidal activity against *Cx. quinquefasciatus* (LC50 -74.32ppm), *Ae. aegypti* (LC50- 70.29ppm) and *An. stephensi* (LC50- 89.55ppm) [24]. In another study hexane extract of leaves of *Eucalyptus citriodora* exhibited LC50 value of 69.86ppm against *An. stephensi* [25]. The LC50 values of hexane extract of *Momordica charantia* against IV instar larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* were 66.05, 96.11 and 122.45 ppm, respectively [26]. Ansari *et al.* observed the larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Ae. aegypti* (LC50- 82.1 ppm), *Cx. quinquefasciatus* (LC50- 85.7 ppm), and *An. stephensi* (LC50- 112.6 ppm) [20]. Crude benzene extract of leaves of *Caesalpinia pulcherrima* was assayed for toxicity against three important vector mosquitoes, viz., *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* with the LC50 values 150.47, 135.24, and 119.27 [27]. Ethanolic extract of the fruits of *D. sissoo* exhibited molluscicide effect against eggs of the freshwater snail *Biomphalaria pfeifferi* [28]. This shows that *D. sissoo* have multidimensional potential including larvicidal propensity.

CONCLUSION

In the present study, the larvicidal efficacy of hexane extract of flowers of *D. sissoo* was found to be higher than acetone extract of branches, methanol extract of seeds and *D. sissoo* oil. The LC50 values indicated nearly 2 fold enhanced toxicity of hexane extract as compared to other extracts. In conclusion, our findings showed that the hexane extract of the flowers of *D. sissoo* was most effective part for larval control of *An. stephensi*. The feasibility of its use in field, however, needs field trials. Extracts of *D. sissoo* can also be tested for control of *Ae. aegypti* mosquito which is a containers breeder and inhabits breeding sites like that of *An. stephensi*. The gas chromatography of the flowers extract of *D. sissoo* revealed 62 chemical constituents, 19 of which contributed more than 1% of the total area of the chromatogram. The efficacy of different 19 ingredients needs to be further tested for their larval control for the development of safer and effective plant based mosquito larvicide.

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