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Evaluation of mosquito Larvicidal potential against *Culex quinquefasciatus* and phytochemical profiling in *Sesbania sesban* Merr.

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Abstract
The aim of the present study was to investigate the *Sesbania sesban* plant for preliminary phytochemical screening, GC-MS analysis and Larvicidal activity against *Culex quinquefasciatus*. The presence of phytochemical compounds was screened by qualitative method. The results showed the presence of phytochemical compounds like alkaloids, steroids, glycosides, saponins, and tannins are present in petroleum ether and ethanol extracts. The flavonoids and phenols were present only in petroleum ether extract and absent in ethanolic extract. In GC-MS analysis there are three bio active phytochemical compounds were identified in ethanolic extract of *S. sesban*. The extract were subjected for screening of Larvicidal activity against *Culex quinquefasciatus* at the concentration of 2%, 4%, 8%, 10%, 15%, 20%, were analyzed the highest mortality was observed after 72hours in petroleum ether extract. But Low level of mortality was observed in ethanolic extract after 48hours.

Keywords: *Sesbania sesban*, GC-MS, larvicidal activity, *Culex quinquefasciatus*, ethanolic extract

1. Introduction
Medicinal plants are plants containing built in active ingredients familiarized to cure disease and relieve from pain [1]. The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed [2]. Modern-day pharmacopoeia however contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated from plants. Involvement ion medicinal plants as a re-budding health assistance has been fuelled with the rising charges of prescription drugs in the safeguarding of personalized health and wellbeing and the bio prospecting of new plant derived drugs [3]. The ongoing development recognition medicinal plants is due to various reasons; include increasing faith in herbal medicine [4]. On the top of that, an increasing dependence on the use of these medicinal plants in the industrialized organizations has been traced towards the extraction and development of drugs and chemotherapeutics from these plants as well as from conventionally used herbal remedies [5]. The therapeutic properties of plants could be based on their anti-oxidant, anti-microbial, antipyretic effects of the phytochemicals constituents in them [6]. According to World health organization, medicinal plants would be the greatest source to obtain an array of drugs. Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness [7].
Plants contain many chemicals which are important in their defense against insects. Insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent against vectors of public health importance. The use of plant extracts for vector control has several appealing features as they are easily degradable, less hazardous and rich stock house of chemicals of diverse biological activity. These are considered environmentally safe and economical as well as practical in application. Therefore biologically active plant materials have attracted considerable interest in mosquito control programs in the recent time [8]. Weeds become dominant due to their wide adaptability to adverse environmental conditions, resistant to microbes (not a host to plant pathogen) and insect predators. Using weeds as botanical Larvicidal against mosquitoes have several advantages, as these are easily available, required little technical input and time for cultivation and procurement. The main objective of the present study was to analyses the bioactive compounds of *Sesbania sesban* and its potentiality against *Culex quinquefasciatus.*

2. Materials and Methods
2.1 Collection of plant materials
The selected medicinal plant like *S. sesban* was collected in Othimali, Annur, Coimbatore district, Tamil Nadu, India.

2.2 Preparation of plant extracts
50g of powdered *S. sesban* whole plant was successively extracted using 500ml of petroleum ether and ethanol using the Soxhlet extractor for 8–10h. The extract was filtered through Whatman No. 1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent.

2.3 Preliminary phytochemical analysis
The ethanol and petroleum ether extracts were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the powdered *S. sesban* which was followed by [9].

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis
GC-MS analysis of the ethanol extract of *S. sesban* was performed using Shimadzu Japan GC QP2010 plus with a fused GC column coated with poly methyl silicone (0.25 nm × 50 m) and the conditions were as follows: Temperature programming from 80 to 200°C held at 80°C for 1 min, rate

5°C/min, and at 200°C for 20 min. Field ionization detector temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a flow rate of 1 ml/min, and split ratio of 1:75 GC-MS were conducted using GCMS-QP 2010 plus Shimadzu Japan with an injector temperature of 220° and carrier gas presence of 116.9 kpa. The color length is 30 m with a diameter of 0.25 mm and flow rate of 50 ml/min. Elutes were automatically passed into a MS with a dictator voltage set at 1.5kv and sampling rate 0.2 s. The MS was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

3. Results

3.1 Preliminary phytochemical analysis of *Sesbania sesban*

The preliminary phytochemical analyses in petroleum ether and ethanol extract of *S. sesban* were presented in the Table No.1. Qualitative phytochemical analysis of *S. sesban* confirms the presence of various secondary metabolites. According to qualitative analysis in ethanalic extract showed positive results with all the compounds like alkaloids, flavonoids, steroids, glycosides, saponins, phenols and tannins. Glycosides, saponins, steroids, and alkaloids were present in both Petroleum Ether and Ethanol extract. Flavonoids, phenols, present only in ethanolic extract but absent in Petroleum Ether extract. Petroleum Ether extract showed the presences of alkaloids, steroids, saponins, and tannins it was concluded that the *S. sesban* extracts contain important secondary metabolites.

3.2 GC-MS Analysis of *S. sesban*

The ethanolic extract of *S. sesban* were analyzed by GC-MS and which revealed the presence of three compounds (GC-MS chromatogram Fig.1). The spectra of compounds were compared with mass spectra of NIST and Willy library. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), peak area (%), structure and medicinal uses were tabulated (Table No.2).

3.3 Larvicidal activity of *S. sesban*

The petroleum ether and ethanolic extracts of *S. sesban* was taken in eight concentrations (0%, 2%, 4%, 6%, 8%, 10%, 15%, 20%) including control. In different concentration of the plant extract 20 mosquito second stage live larva were allowed to survive. The mortality rate was observed for every 24hrs intervals to 96hrs (4days) without disturbing the set up. This observation was made in both ethanolic extract treatment and petroleum ether extract treatment (Table No. 3 to 8).

The efficacy of the different concentration of *S. sesban* was studied and results revealed that compare to petroleum ether extract ethanol extract was quite promising and lower concentration of the ethanol extract (4%) take 24hrs. Were as the same results were observed in petroleum ether extract (8%) in 48hrs. 100% mortality was observed in ethanol extract, 8% with in 48hrs. Were as in petroleum ether extract of 10% concentration in 48hrs showed 100% mortality. If the plant extract concentration increases (10%, 15%, 20%) it directly affects the mortality rate with in 24hrs. It showed 100% death.

4. Discussions

The phytochemical analysis carried out in the dry plant petroleum ether extract and ethanolic extract showed the presence of some bioactive compounds in *S. sesban*. In the two forms of extract, eight bioactive constituents were tested for, out of which only three were present in the two extractions (Table 1). Analysis of tannins in the two extracts was positive but higher color intensity was observed in the ethanolic extract than the petroleum ether extract. Presence of tannins suggests the ability of this plant to play a major role as antidiarrhoeic and antihaemorrhagic agent [10]. Saponins though positive for both extracts, persistent frosting was intense in the ethanolic extract than the dry leaf water extract. This compound has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties [11,12]. This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension. It was also found that alkaloids were present in petroleum ether and ethanolic extracts.

The findings of the present investigation revealed that *S. sesban* has potent Larvicidal activity against *C. quinquefasciatus.* Many plant extracts and essential oils manifest repellency activity against different mosquito species. The present results are in accordance with such results obtained by [13] using neem oils against mosquito bites of *Anopheles spp., culex spp*. And *Ae spp*., [14] using ethanol extract of fruits from *Foeniculum vulgarea* against hungry *A. Aegypti*, [15] using methanol extracts from 23 aromatic medicinal plant species against female blood-starved *A. Aegypti*, [16] using repellent activity of selected essential oils from ten plant species against *A. Aegypti*, [17] using neem tree (*Azadirachta indica*) oil against the Asian tiger mosquito (*A. Albopictus*), [18] using acetone leaves extract of *T. terrestris* against *A. Aegypti* and [19] using 16 ethanolic and petroleum ether extracts of 4 indigenous plants as a repellent in the filed against wild mosquitoes.

The insecticidal efficacy of EO containing piperitenone oxide has been explored by some other authors. For example, [20] mentioned that EO obtained from *Mentha microphylla* with a majority share of piperitenone oxide was significantly more effective against *Sitophilus oryzae* and *Tribolium castaneum* than other tested EO [21].

Table 1: Preliminary Phytochemical analysis in petroleum ether and ethanolic extracts of *S. Sesban*

S. No.	Name of the Test	Petroleum Ether extract	Ethanolic extract
1.	Test of the Alkaloids	✓	✓
2.	Test of the Flavanoids	-	✓
3.	Test of the Steroids	✓	✓
4.	Test of the Glycosides	✓	✓
5.	Test of the Saponin	✓	✓
6.	Test for Tanins	✓	✓
7.	Test for Phenols	-	✓

✓ - Present,
- - Absent

Table 2: GC-MS analysis of *S. Sesban*

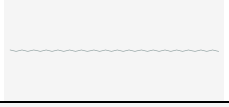
S. No	Retention time	Name of the chemical Compound	Molecular Formula	Molecular weight	Peak Area	Structure of the Compound	Properties of the chemical Compound
1.	24.337	Squalene	$C_{30}H_{50}$	410	60.554		Industrial intermediate in the vitamin E synthesis). Squalene is commercially extracted from fish oil, and in particular shark liver. (PubChem)
2.	24.787	Hexatriacontane	$C_{36}H_{74}$	490	21.563		No biological activity
3.	25.978	Lauroyl Peroxide	$C_{24}H_{46}O_4$	172	17.883		Plastic and rubber products not covered elsewhere. Ingestion causes irritation of mouth and stomach. (PubChem)

Table 3: Mortality rate of *Culex quinquefasciatus* larvae in water (Control)

S. No	Hours	Treatment	No. of Mosquito larvae	Rate of death	Rate of survival	No. of Mosquito larvae in Active	No. of Mosquito larvae in Inactive
1.	24 hours	100ml water	20	0	0	20	0
2.	48 hours	100ml water	20	0	0	20	0
3.	72 hours	100ml water	20	0	0	20	0
4.	96 hours	100ml water	20	0	0	20	0

Table 4: Mortality rate of *Culex quinquefasciatus* larvae in ethanolic extract of *S. Sesban* (24hrs)

S. No	Treatment	No. of Mosquito larvae	Rate of death	Rate of survival	No. of Mosquito larvae in Active	No. of Mosquito larvae in Inactive
1	2%	20	5	15	10	5
2	4%	20	12	8	6	2
3	8%	20	17	3	2	1
4	10%	20	20	0	0	0
5	15%	20	20	0	0	0
6	20%	20	20	0	0	0

Table 5: Mortality rate of *Culex quinquefasciatus* larvae in ethanolic extract of *S. Sesban* (48hrs)

S. No	Treatment	No. of Mosquito larvae	Rate of death	Rate of survival	No. of Mosquito larvae in Active	No. of Mosquito larvae in Inactive
1	2%	20	1	19	17	2
2	4%	20	3	17	15	2
3	8%	20	8	12	10	2
4	10%	20	13	7	4	3
5	15%	20	20	0	0	0
6	20%	20	20	0	0	0

Table 6: Mortality rate of *Culex quinquefasciatus* larvae in Petroleum ether extract of *S. Sesban* (24hrs)

S.No	Treatment	No. of Mosquito larvae	Rate of death	Rate of survival	No. of Mosquito larvae in Active	No. of Mosquito larvae in Inactive
1	2	100	20	0	0	0
2	4	100	20	0	0	0
3	8	100	20	0	0	0
4	10	100	20	0	0	0
5	15	100	20	0	0	0
6	20	100	20	0	0	0

Table 7: Mortality rate of *Culex quinquefasciatus* larvae in Petroleum ether extract of *S. Sesban* (48hrs)

S.No	Treatment	No. of Mosquito larvae	Rate of death	Rate of survival	No. of Mosquito larvae in Active	No. of Mosquito larvae in Inactive
1	2%	20	15	5	3	2
2	4%	20	17	3	2	1
3	8%	20	20	0	0	0
4	10%	20	20	0	o	0
5	15%	20	20	0	0	0
6	20%	20	20	0	0	0

Table 8: Mortality rate of *Culex quinquefasciatus* larvae in Petroleum ether extract of *S. Sesban* (72hrs)

S.No	Treatment	No. of Mosquito larvae	Rate of death	Rate of survival	No. of Mosquito larvae in Active	No. of Mosquito larvae in Inactive
1	2%	20	18	2	0	2
2	4%	20	19	1	0	0
3	8%	20	20	0	0	0
4	10%	20	20	0	0	0
5	15%	20	20	0	0	0
6	20%	20	20	0	0	0

5. Conclusion

The extracts of *Sesbania sesban* has potent larvicidal activity against *culex quinquefasciatus* there results that larvicidal activity of *Sesbania sesban* may contribute to their claimed medicinal property. Further studies on the screening isolation and purification of bio active compounds followed by in depth and filed bio assay are needed as the present studied shows that there is scope to use *Sesbania sesban* to control the vector mosquito.

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7. References

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