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Biocidal activity of *Scoparia dulcis* and *Clerodendrum phlomidis* on human pathogens, Mosquito larvae and storage pest

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Abstract
Medicinal plants consist of chemical compounds which are used as natural medicines to treat common bacterial infections. In this study proposes *Clerodendrum phlomidis* and *Scoparia dulcis* are important medicinal plants as its extracts showed good anti-bacetral activity against all organisms like *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus vulgaris*. The Iso-propyl alcohol extracts of both *Clerodendrum pholmids* and *Scoparia dulcis* showed best result of larvicidal and pesticidal activity against the mosquito larvae *Aedes aegypti*, and the *pests Sitophilus oryzae* (Rice weevil). In the present investigation the n-butyl alcohol extacts of *Clerodendrum phlomidis* showed very good activity against all tested pathogens. This study *Clerodendrum phlomidis* in the traditional system of to treat various infectious diseases, caused by the microbes as well as in mosquitos and pest control.

Keywords: *Biocidal activity, Aedes aegypti, Sitophilus oryzae, Clerodendrum phlomidis, Scoparia dulcis*

Introduction
Plants that produce significant yield of relatively high value products such as pharmaceuticals, biologically, active materials and essential oils. Plant products also play an important role in the health care for the remaining 20% in developing countries and for those in industrialized countries as well [1]. The number of medicinal plants have been evaluated for their therapeutic effective against various diseases. Many plants especially those used by traditional healers produce pharmaceutically active compounds that have antimicrobials, antihelminthic, antifungal, antiviral, anti-inflammatory and antioxidant activity. Anti-diabetic activity [2-5] Medicinal plants have been regularly used in various system of Indian medicine because of minimal side effect and cost effectiveness which provide scientific support to therapeutic use of the plants in tribal medicine mosquitoes are carrying of diseases such as malaria, dengue fever, yellow fever, filariasis etc. They are responsible for the death and illness of millions of people through the transmission of diseases. Bio pesticides often are effective in very small quantities and often decompose quickly than by resulting in lower exposures and largely avoiding the pollution problem caused by conventional pesticides. *Scoparia dulcis* commonly known as Sweet broom weed is a perennial herb widely distributed in tropical and subtropical regions[6] Phytochemical screening has revealed that the plant contains diterpenoids, flavonoids, tannins, alkaloids, triterpenes, hexacosonol, β *B*sitosterol, ketonedulcitone, amellin and antidiabetic compound [7-9]. The main chemicals include scopadulcic acids A and B. Scopadiol, Scopadulin, Scoparic acids A-c and betulinic acid [10] Scoparia dulcis contains, coumarins, phenols, Saponins, tannins, amino acids, alkaloids, carbohydrates, glycosides [11]. Some of these compounds are likely to be active against certain bacteria, this may account for the traditional use as medicinal plants.
These herbs have been used worldwide in folk medicine since ancient times [12, 13]. They have been used as tonics, antimalarials, antihelmintics, antidiabetics and in treating wounds, bronchitis, ulcers and tuberculosis in traditional Anatolian medicine [14-17]. There are also several reports concerning the antimaterial, antioxidant, Cytotoxic, antiphyretic, analgesic, antidiabetic, antimicrobial and antifungal activities of different Scoparia species. *Clerodendrum phlomidis* is commonly known as wind killer [18] *Chlerodendrum phlomidis* is one of the highlytraded medicinal plants from tropical forests, as the leaves and roots are used in the Folklore, Ayurveda, Siddha and Unani medicines [19].

Clerodendrum has been found to have a number of biological activities mainly including anti-inflammatory, hepatoprotective, antihypertension, antioxidant, cytoxicity, antitumor and antifeedant activities and effects on central nervous system [21]. The objective of the study which deals with the effect of different solvent extracts of *Clerodendrum phlomidis* and *Scoparia dulcis* on human pathogens mosquito larvae and storage pest.

Materials and Method

Collection of plant materials

For the present study, the investiagater collected fresh and healthy plant parts from Kanyakumari District, TamilNadu. The taxonomic identification was done by referring Herbarium voucher specimen and flora of presidency of Madras (Gamble, 1928).

Preparation of Extracts

The collected plant parts were separated from undesirable materials or plant parts. They were shade dried for 40 days. The plant parts were grind into coarsely powdered with help of a suitable grinder. The powder was stored in airtight containers and kept for further studies.

The grind powder was soaked in 10 ml of different solvents such as ethanol, n-butylalcohol, iso-propyl alcohol benzene and acetone. All the solvent extracts were kept in room temperature for 20 days with periodic shaking to extracts the compound from the powdered material. After that they were centrifuged for ten minutes and filtered through whatman No. 1 filter paper, the respective solvents were evaporated with the help of heating mantle. Then it was stored in air-tight conical flask for further study.

Preparation of Natural disc

Sterile discs were obtained and stored at 4^0C. Discs were handled using a pair of presterilized forceps. Finally the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium later on these plates were kept at room temperature for 60 minutes. The extract was loaded on the disc carefully using capillary tube, without spreading out. Thus the disc completely saturated with the extract was used for testing antibacterial activity.

Synthetic disc

The synthetic discs used were Ampicillin, Streptomycin, Erythromycin, Chloramphenicol.

The isolated microorganisms were found to be *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. All the human pathogens were obtained from clinical laboratories Kanyakumari District TamilNadu.

Antibacetrial activity

The agar plates were dispensed into sterile plates and then sterile discs were impregnated with extracts. The plates were incubated at 37^0C for 24 hours. After incubation all zones were measured in millimeters. The result were expressed as average value. Disc without zone around test paper discs indicate the absence of bacterial growth and that was recorded as positive test and absence of zone as negative test.

Larvicidal assay by Serial Dilution method

The larvidicial activity was tested on the larvae of the mosquito Aedes aegypti in the bioassay laboratory of the IQB/UFAL, based on methodology described by the WHO. After 100 mosquito larvae (Aedes aegypti) in the IVth instar stage were collected from freshwater. The susceptibility of the mosquito larvae to the selected concentration of the extracts was studied by this method. Then 10 larvae of Aedes aegypti in the 4th instar stage were prepared for immense into the crude extract. Ten larvae were placed in each bowl containg different concentration of the extract (0.1% to 0.5) another set of 10 larvae were introduced into separate bowls as considered as control. Then the bowl was left undisturbed the activity of the tested extracts was estabilished based on the average percentage of mortality of the larvae after their periodic time.

Pesticidal Assay by Cotton Roll method

A set of 10 healthy *Sitophilus oryzae* were introduced into a series of dried and sterilized petri plates containing evaporated cotton rolls which were already dipped in different extracts in different concentration. Another set of 10 healthy insects were introduced separately in another sterilized petri plate without cotton roll as control. Then the plates were covered and kept undisturbed. The plates were observed through out the experiment and time of death of each pests was recorded carefully.

Studied activity

Antibacetrial activity by agar disc diffusion method [22, 23].

Result and Discussion

The antibacterial activity *Clerodendrum pholmidis* and *Scoparia dulcis* were assessed using agar disc diffusion method by measuring the diameter of inhibition zones (Table (1,2)

In the present investigation, different, plant extracts possesses antibacterial activity against tested gram positive (*Staphylococcus aureues* and gram negative strains (*Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* and *Klebsiella pneumonia*). The extracts obtained with different solvents like Ethanol, acetone, Benzene, n- butyl alcohol and iso-propyl alcohol showed varying activity (Table-1)

In our study proposes *Clerodendrum phlomidis* and *Scoparia dulcis* as important medicinal plants as its extracts showed good anti-bactrial activity against all organisms like *Escherichiacoli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphyloccus aureus* and *Proteus vulgaris*. In the isolated compounds shows good antibacterial activity than other compounds. n-butyl alcohol extracts of *Clerodendrum phlomidis* exhibited the high anti-bacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Proteus vulgaris* with zone formation of 25mm, 26mm, 29mm 17mm and 22mm respectively. The n-butylalcohol extract of Chlorodendrum phlomidis exhibited lower zone of inhibition compared to other solvent extracts the zone formation was 17mm against *Staphylococcus aureus* (Table 1, 2).

Larvicidal and Pesticidal activity

The most effective larvicidal activity and pesticidal activity *Scoparia dulcis* and *Clerodendrum phlomidis*. The iso-propyl alcohol extracts of *Scoparia dulcis* showed the best result of larvicidal activity. The mortality started from 0.1% concentration. In 0.1% of concentration 60% larvae were died with in96 minutes. In 0.2% concentration 8 larvae were died within 90 minutes. In0.3% concentration 90% of larvae were died within 65 minutes. In both 0.4% and 0.5 concentration

100% mortality occurred within various time duration. i.e 58 and 47 minutes respectively (Table 3)

The *I*so-propyl alcohol extracts of *Clerodendrum phlomidis* showed very good result of larvicidal activity, against the mosquito larvae *Aedes aegypti*. In 0.1% concentration 6larvae were died within 109 minutes. In 0.2% concentration and 0.3% concentration 70% of the larvae were died within various time duration ie 84 and 50 minutes. In both 0.4% and 0.5% concentration all the 10 larvae were died within 40 and 36 minutes respectively (Table – 4)

In Iso-propyl alcohol extracts of *Clerodendrum phlomidis* and *Scopoaria dulcis* showed better results of pesticidal activity against the pests *Sitophilus oryzae* (Rice weevil). In 0.1% and 0.2% concentration *Scoparia dulcis* extract 70% pests were died within 85 and 77 minutes. In 0.3% concentration 90% pests were died within 79 minutes In 0.4% and 0.5% concentration 100% pests were died within 69 and 53 minutes durations.

In *Clerodendrum phlomidis* extracts in 0.1%, and 0.2% concentration 70% mortality occurred within various time duration 82 and 66 minutes from 0.3% to 0.5% concentration 100% mortality occurred within various time duration within 65, 64 and 85 minutes (Table 5,6)

The medicinal plant *Clerodendrum phlomidis* and *Scoparia dulcis* have previously been studied for a number of biological activities. This study suggested that the chemical constituent investigated can be utilized in biological pesticide formulations.

Table 1: Comparative Antibacterial Activity of Different solvent extracts of *Clerodendrum phlomidis* and *Scoparia dulcis*

Human pathogens	Solvents	Plant extracts	
		Clerodendrum phlomidis	***Scoparia dulcis***
Escherichia coli	Ethanol	31mm	34mm
	Acetone	21mm	28mm
	Benzene	31mm	20mm
	N-butyl alcohol	25mm	-
	Iso-propylalcohol	26mm	22mm
Pseudomonas aeruginosa	Ethanol	17mm	-
	Acetone	17mm	16mm
	Benzene	21mm	21mm
	N-butyl alcohol	26mm	11mm
	Iso-propylalcohol	15mm	22mm
Klebsiella pneumonia	Ethanol	-	-
	Acetone	27mm	22mm
	Benzene	34mm	26mm
	N-butyl alcohol	29mm	-
	Iso-propylalcohol	26mm	-
Staphylococcus aureus	Ethanol	-	-
	Acetone	-	-
	Benzene	-	15mm
	N-butyl alcohol	17mm	-
	Iso-propylalcohol	-	11mm
Proteus vulgaris	Ethanol	24mm	17mm
	Acetone	13mm	14mm
	Benzene	18mm	23mm
	N-butyl alcohol	22mm	10mm
	Iso-propylalcohol	22mm	18mm

Table 2: Drug sensitivity of Human Pathogens against Antibiotics

Human pathogens	Antibiotics zone formation (mm)			
	CH	**AM**	**ER**	**SM**
Escherichia coli	37mm	15mm	14mm	23mm
Pseudomonas aeruginosa	21mm	R	23mm	24mm
Klebsiella pneumonia	30mm	218mm	20mm	36mm
Staphylococcus aureus	19mm	R	11mm	R
Proteus vulgaris	15mm	R	12mm	24mm

CH – Chloramphenicol
AM – Ampicillin
ER – Erythromycin
SM – Streptomycin
R – Non-Resistance

Table 3: Biolarvicidal activity of Iso-propyl alcohol extracts of *Scoparia dulcis* in 0.1% concentration to 0.5% Concentration

Concentration	0.1%				0.2%				0.3%				0.4%				0.5%			
S. No	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)
1	2.14	3.24	70	1	2.20	3.25	65	1	2.22	3.02	40	1	2.22	2.58	36	1	2.25	2.50	25	2
2	2.14	3.28	74	2	2.20	3.31	71	2	2.22	3.05	43	2	2.22	3.00	38	2	2.25	2.53	28	3
3	2.14	3.30	76	3	2.20	3.33	73	3	2.22	3.10	48	3	2.22	3.03	41	3	2.25	2.58	33	4
4	2.14	3.40	86	4	2.20	3.37	77	4	2.22	3.13	51	4	2.22	3.05	43	4	2.25	3.00	25	5
5	2.14	3.44	90	5	2.20	3.40	80	5	2.22	3.16	54	5	2.22	3.08	46	5	2.25	3.03	28	6
6	2.14	3.48	94	6	2.20	3.44	84	6	2.22	3.20	58	6	2.22	3.10	48	6	2.25	3.05	40	7
7	2.14				2.20	3.46	86	7	2.22	3.22	60	7	2.22	3.12	50	7	2.25	3.07	42	8
8	2.14				2.20	3.50	90	8	2.22	3.25	63	8	2.22	3.15	53	8	2.25	3.09	44	9
9	2.14				2.20				2.22	3.27	65	9	2.22	3.20	58	10	2.25	3.12	47	10
10	2.14				2.20				2.22				2.22				2.25			

Table 4: Bio Pesticidal activity of Iso-propyl alcohol extrcats of Clerodendurm phlomidis in 0.1% to 0.5% Concentration

Concentration	0.1%				0.2%				0.3%				0.4%				0.5%			
S. No	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)
1	11.34	12.26	52	1	11.35	12.06	31	1	11.36	11.56	20	1	11.40	11.55	15	1	11.40	11.51	11	1
2	11.34	12.57	83	2	11.35	12.26	51	2	11.36	11.58	22	2	11.40	11.59	19	2	11.40	11.55	15	2
3	11.34	1.09	95	3	11.35	12.37	62	3	11.36	12.00	24	3	11.40	12.00	20	3	11.40	11.58	18	3
4	11.34	0.19	105	4	11.35	12.45	70	4	11.36	12.05	29	4	11.40	12.02	22	4	11.40	12.02	22	4
5	11.34	1.20	106	5	11.35	12.48	73	5	11.36	12.08	32	5	11.40	12.08	28	6	11.40	12.06	26	5
6	11.34	1.23	109	6	11.35	12.55	80	6	11.36	12.12	36	6	11.40	12.12	32	7	11.40	12.10	30	6
7	11.34				11.35	12.59	84	7	11.36	12.26	50	7	11.40	12.16	36	8	11.40	12.12	32	7
8	11.34				11.35				11.36				11.40	12.20	40	10	11.40	12.16	36	9
9	11.34				11.35				11.36				11.40				11.40			10
10	11.34				11.35				11.36				11.40				11.40			

Table 5: Bio pesticidal activity of Iso-propyl alcohol extracts of *Scoparia dulcis* in 0.1% to 0.5% Concentration

Concentration	0.1%				0.2%				0.3%				0.4%				0.5%			
S. No	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)
1	2.00	2.56	56	1	2.00	2.52	52	1	2.01	2.49	48	1	2.01	2.41	40	1	2.02	2.32	30	1
2	2.00	3.00	60	2	2.00	2.58	58	2	2.01	2.54	53	2	2.01	2.44	43	2	2.02	2.36	34	2
3	2.00	3.03	63	3	2.00	3.03	63	3	2.01	2.58	57	3	2.01	2.46	45	3	2.02	2.40	38	3
4	2.00	3.13	73	4	2.00	3.08	68	4	2.01	3.04	63	4	2.01	2.50	49	4	2.02	2.43	41	4
5	2.00	3.20	80	5	2.00	3.10	70	5	2.01	3.10	69	5	2.01	2.53	52	5	2.02	2.45	43	5
6	2.00	2.22	82	6	2.00	3.13	73	6	2.01	3.13	72	6	2.01	2.57	56	6	2.02	2.47	45	8
7	2.00	3.25	85	7	2.00	3.17	77	7	2.01	3.15	74	7	2.01	2.59	58	7	2.02	2.50	48	9
8	2.00				2.00				2.01	3.19	78	8	2.01	3.03	62	8	2.02	2.55	53	10
9	2.00				2.00				2.01	3.20	79	9	2.01	3.10	69	10	2.02			
10	2.00				2.00				2.01				2.01				2.02			

Table 6: Bio pesticidal activity of Iso-propyl alcohol extracts of *Clerodendrum phlomidis* in 0.1% to 0.5% Concentration

Concentration	0.1%				0.2%				0.3%				0.4%				0.5%			
S. No	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)	Initial time	Final time	Time duration (minutes)	Death Rate (No)
1	1.00	1.50	50	1	1.00	1.42	42	1	1.00	1.40	40	1	1.01	1.38	37	1	1.01	1.35	34	1
2	1.00	1.58	58	2	1.00	1.48	48	2	1.00	1.43	43	2	1.01	1.40	39	2	1.01	1.38	37	2
3	1.00	2.02	62	3	1.00	1.52	52	3	1.00	1.46	46	3	1.01	1.44	43	3	1.01	1.40	39	4
4	1.00	2.10	70	4	1.00	1.56	56	4	1.00	1.50	50	4	1.01	1.49	48	4	1.01	1.44	43	5
5	1.00	2.14	74	5	1.00	1.59	59	5	1.00	1.54	54	6	1.01	1.55	54	5	1.01	1.48	47	7
6	1.00	2.10	80	6	1.00	2.00	60	6	1.00	2.00	60	8	1.01	1.59	58	7	1.01	1.53	52	8
7	1.00	2.22	82	7	1.00	2.06	66	7	1.00	2.05	65	10	1.01	2.04	63	8	1.01	1.55	54	9
8	1.00				1.00				1.00				1.01	2.05	64	10	1.01	1.59	58	10
9	1.00				1.00				1.00				1.01				1.01			
10	1.00				1.00				1.00				1.01				1.01			

Conclusion

The anti-bacterial activity of medicinal plants such as *Scoparia dulcis* and *Clerodendrum phlomidis* were studied against the human pathogens such as *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In the present investigation the n- butyl alcohol extract is greater activity against for tested pathogens expected *Klebsiella pneumonia*. The most powerful larvicidal and pesticidal activity of *Scoparia dulcis* and *Clerodendrum phlomidis* were shown by iso-propyl alcohol extracts. The other extracts like Acetone, Benzene, n-butyl alcohol and Ethanol were showed moderate level of larvicidal and pesticidal activity. In conclusion findings of the present study will helpful in future ethanopharmacological studies in India.

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