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Research Paper

Isolation and Characterization or Bioactive Compounds from Medicinal Plants

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ABSTRACT

The isolation and characterization of bioactive compounds from medicinal plants are critical processes in the discovery of novel therapeutic agents. Medicinal plants are rich sources of phytochemicals such as alkaloids, flavonoids, terpenoids, glycosides, and phenolics, many of which exhibit significant pharmacological activities. This study focuses on the systematic extraction, isolation, purification, and structural elucidation of these bioactive constituents using various chromatographic and spectroscopic techniques. The research highlights the potential of natural compounds in the development of new drugs, particularly in combating antimicrobial resistance, inflammation, and chronic diseases. Advanced techniques such as HPLC, GC-MS, NMR, and FTIR play a vital role in identifying the structure and activity relationships of these compounds. This approach not only preserves traditional knowledge but also integrates it with modern scientific methodologies to harness the medicinal potential of plants effectively.

INTRODUCTION

Mosquitoes are insects in the order Diptera, family Culicidae. There are approximately 3,500 described species of mosquitoes in the world, about 50 of which occur in California. Not all these are of public health importance, either because they occur only in remote areas far from large populations of people, or because the female mosquitoes rarely, if ever, bite people. On the other hand, a number of species are severe pests and vectors, and life as we know it where they occur would be much degraded were it not for the concentrated efforts of mosquito abatement agencies in the state. In this manual, pests refer to arthropods that are of direct public health importance. Mosquito pests that transmit organisms that result in infectious diseases in humans and other vertebrate animals are known as vectors (Govindarajan et al., 2008). Vector-borne

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diseases are illnesses caused by pathogens and parasites in human populations. Every year there are more than 1 billion cases and over 1 million deaths from vector-borne diseases such as malaria, dengue, schistosomiasis, human African trypanosomiasis, leishmaniasis, Chagas disease, yellow fever, Japaneseencephalitis and onchocerciasis, globally. Vector-borne diseases account for over 17% of all infectious diseases. Distribution of these diseases is determined by a complex dynamic of environmental and social factors. Globalization of travel and trade, unplanned urbanization and environmental challenges such as climate change are having a significant impact on disease transmission in recent years. Some diseases, such as dengue, chikungunya and West Nile virus, are emerging in countries where they were previously unknown. Changes in agricultural practices due to variation in temperature and rainfall can affect the transmission of vector-borne diseases. Climate information can be used to monitor and predict distribution and longer-term trends in malaria and other climate-sensitive diseases. The brief description below does not cover all vector- borne diseases. For moroe detailed information, please refer to fact sheets for specific diseases. Mosquitoes can be found all over the world from the Tropics to the Arctic. Some mosquitoes can be found 200 miles from their birthplace. Of all the harmful creatures on earth, this little “vampire” probably poses the greatest threat to mankind. There are more than 3,500 species in the culicid, or mosquito family, worldwide and mosquito-borne diseases infect about 700 million people each year and kill 3 million according to the Centers for Disease Control. On average, one person dies every 10 seconds as a result of a little mosquito “bite”. Mosquito borne diseases continue to be a major problem in almost all tropical and subtropical countries. They are responsible for the transmission of the pathogens causing some of the most life – threatening and debilitating diseases of man, like malaria (Anopheles), yellow fever, dengue fever, chikungunya (Aedes), filariasis, encephalitis (Culex), etc. Mosquitoes can be found all over the world from the Tropics to the Arctic. Some mosquitoes can be found 200 miles from their birthplace. Of all the harmful creatures on earth, this little “vampire” probab poses the greatest threat to mankind. There are more than 3,500 species in the culicid, or mosquito family, worldwide and mosquito-Kborne diseases infect about 700 million people each year and kill 3 million according to the Centers for Disease Control. On average, one person dies every 10 seconds as a result of a little mosquito “bite”. Mosquito borne diseases continue to be a major problem in almost all tropical and subtropical countries. They are responsible for the transmission of the pathogens causing some of the most life – threatening and debilitating diseases of man, like malaria (Anopheles), yellow fever, dengue fever, chikungunya (Aedes), filariasis, encephalitis (Culex), etc.**. Vector mosquitoes**

1m Culex quinquefasciatus

Culex quinquefasciatus, the Southern House Mosquito, is the vector responsible for transmission of filariasis and a number of viruses including West Nile virus, St. Louis encephalitis and Japanese encephalitis.

Aedes aegypti

The yellow fever mosquito, Aedes aegypti is a mosquito that can spread the dengue fever, Chikungunya and yellow fever viruses, and other diseases. The mosquito is a small, dark mosquito of approximately 4 to 7 millimeters with typical white markings on the legs and a marking of the form of a lyre on the thorax. Females are larger than males, and can be distinguished by small

palps tipped with silver or white scales.1.19 In most cases, malaria i s t r a n s m i t t e d t h r o u g h t h e b i t e s o f female Mosquitos; around 30 are malaria vectors of major importance. All of the important vector sp1.2.4.

Dengue

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, Incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present Decade, from urban to rural settings. An estimated 50 million dengue infections occur annually and Approximately 2.5 billion people live in dengue endemic countries

Chikungunya

Chikungunya, also called chikungunya virus disease or chikungunya fever, is a viral illness that is spread by the Bite of infected mosquitoes. The disease resembles dengue fever, and is characterized by severe, sometimes Persistent, joint pain (arthiritis), as well as fever and rash. It is rarely life-threatening. Chikungunya occurs in Africa, India and Southeast Asia. It is primarily found in urban /peri-urban areas. There is no specific treatment

For **chikungunya**.

Yellow fever Yellow fever is a serious disease caused by the yellow fever virus. It is found in certain parts of Africa and South America. Yellow fever is spread through the bite of an infected mosquito. It cannot be spread person to person by direct contact. People with yellow fever disease usually have to be hospitalized. Yellow fever can cause: fever And flu-like symptoms, jaundice (yellow skin or eyes), bleeding from multiple body sites, liver, kidney, Respiratory and other organ failure and death (20% - 50% of serious cases). 1Vector control The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, Mortality, economic loss and social disruption such as malaria, lymphatic filariasis and viral diseases is well Documented. Rapid increases in population, limited funds, and know-how together with environmental change And an increase in the resistance of vectors and pathecies bite between dusk

MATERIAL & METHODS

3.1.1. Collection and identification of plants

The plants, Portulaca oleracea, Chrozophora rottleri, Flemingia macrophylla, and Girardinia diversifolia were collected from various places of Nilgiris district, Tamilnadu, India (Plate 3.1). The plants are taxonomically identified by a taxonomist, Department of Botany and voucher specimens have been deposited in the Plant Phytochemistry Unit, Department of Zoology, Annamalai University shows the list ofplant species, vernacular name and plant parts used.

Preparation of plant extracts

The fully developed fresh leaves of the plants P. oleracea, C. rottleri, F. macrophylla, and G. diversifolia were collected and washed with tap water, shade dried at room temperature (Plate 3.3). The dried leaves were powdered with the help of electrical blender. The powdered leaf material (1.0 kg) was then subjected to extraction in various solvents viz, hexane, ethyl acetate, benzene, chloroform and methanol (5.0 L) using soxhlet extraction apparatus for 8 hours individually. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator. The residue was then made in to a 1% stock solution with ethanol.

From these stock solutions, different concentrations were prepared and these solutions were used for mosquitocidal activities. For the repellent activity the various range of stock solutions (1.0, 2.5 and 5.0 mg/cm2) were prepared by dissolving the residues in ethanol.the yield of four different plants (Leaves) with five different solvents. The results indicated that the yield (Crude residue) ranged from 80.50 to167.27. The yield of P. oleracea were 103.60, 98.52, 117.87, 115.93 and 132.42g, C. rottleri yield were 84.60, 107.38, 99.37,114.96 and 136.28g, F. macrophylla yield were 97.22, 128.86, 105.35

Laboratory colonization of mosquitoes

For the different bioassays enormous amount of different stages of mosquito colony needed. The egg raftrs/eggs of three mosquito species such as An. stephensi, Ae. aegypti and Cx. quinquefasciatus, were obtained from vector control laboratory, Department of Zoology, Annamalai University, Tamilnadu, India. The laboratory colony was maintained at 70-85% RH, 28±20 C temperature and 12:12 light and dark photoperiod cycle. The larvae were fed on powdered mixture of dog biscuits and yeast powder in 3:1 ratio. The adults were provided with 5% glucose solution and honey was given to male and females with one-week old chick for blood meal. Eggs, larvae and adult females were continuously available for the bioassays from these laboratory colonized mosquitoes.

Bioassay

3.2.1. Larvicidal activity

The larvicidal activity of four plant crude extracts and the compounds were evaluated as per the method recommended by World Health Organization (2005). All the four plant extracts were tested for larvicidal activity against An. stephensi, Ae. aegypti and Cx. quinquefasciatus. Bathes of 25 third instar larvae of An. stephensi, Ae. aegypti and Cx. quinquefasciatus were separately transferred to a small disposable test cups, each containing 200ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, starting with the lower concentration (five concentrations). The larval mortality was observed and recorded after 24 h. Each test was replicated six times and equal number of controls was set up simultaneously using tap water. To this 1 ml of ethanol was added. The percentage of mortality was calculated by using Abbott's formula (Abbott, 1925). The LC50, LC90, 95 percent confidence limit of lower confidence limit (LCL) and upper confidence limit (UCL) and chi-square values were calculated by using probit analysis (Finney, 1971).

Repellent activity

Control of mosquitoes is something of utmost importance in the present day with rising number of mosquito borne illnesses. Deforestation and industrialized farming are also two of the factors causing an alarming increase in the range of mosquitoes. Specialty products like mosquito repellent used to combat mosquitoes are required. Each of the products used for mosquito control have varying degrees of effectiveness. Carbon dioxide and lactic acid present in sweatin warm-blooded animals act as an attractive substance for mosquitoes. The perception of the odor is through chemo receptors present in the antennae of mosquitoes. Insect repellents work by masking human scent; a number of natural and chemical mosquito repellents were studied in this review that work to repel mosquitoes. Chemical mosquito repellents has a remarkable safety profile , but they are toxicity against the skin and nervous system like rashes, swelling,eye irritation, and worse

problems, though unusual including brain swelling in children, anaphylactic shock, and low blood pressure. Hence it was concluded that natural mosquito repellents were preferred over chemical mosquito repellents.

Plant discription

3.3.1. Portulaca oleracea L. (Family: Portulacaceae) M Portulaca oleracea commonly known as purslane, is an herbaceous weed. The plant grows wild and/or cultivated throughout much of the world. The World Health Organization has indicated that it is one of the most widely used medicinal plants, calling it a "global panacea". It is ranked eighth among the most commonly distributed plants in the world; it can be eaten both fresh and dried (Baytop, 2000; Dweck, 2001; Samy et al., 2004). It is palatable and has a mild flavor. Thetender stems and leaves can be eaten raw, cooked, or pickled (Simopoulos and Salem, 1986; Simopoulos et al., 1992). The edible stems and leaves have a slightly acidic, salty taste, similar to spinach. It is available commercially in both ornamental and culinary cultivars and is widelyused as a potherb in Mediterranean, central European and Asian countries. The aerial parts of the plant are used to alleviate pain and swelling and as an antiseptic (Chan et al., 2000). A recent report indicated that an extract of P. oleracea accelerates wound healing and is used to treat indomethacin and phenylbutazone-induced ulcers (Rashed et al., 2003). Interest in cultivating P. oleracea as a food crop has been stimulated because it contains many bioactive compounds including ascorbic acid, proteins, fatty acids, flavonoids, vitamin E and beta carotene (Ezekwe et al., 1999). The quantity of these compounds in P. oleracea varies with the growing conditions (e.g., planting date, soil quality, fertilization) and the age of the plant (Lim and Quah, 2007). Wild edible P. oleracea plants are found in most parts of Anatolia in Turkey particularly nearcultivated and arid seaside areas. In Anatolia the people consume only wild P. oleraceaas food and use it for medicinal purposes due to economical, traditional and geographical rea-sons. In recent years it has also been widely cultivated in western Turkey for use in salads and as food additives (Ozbucak et al., 2005). P. oleracea has been used to treat the following ailments in humans: alterative, antiseptic, ardor, diuretic, emollient, scurvy and sedative (Baytop, 2000). Chrozophora rottleri (L.) A.Juss. (Family: Euphorbiaceae) Chrozophora rottleri belongs to Euphorbiaceae family commonly known as Suryavarti. The plant occurs naturally through out India, Myanmar, Thailand, Andaman Islands, and Central Java: Malesia. C. rottleri, an erect hairy annual common waste lands, blossoms profusely from January to April. It is an erect herb with silvery hairs; lower part of stem is naked, upper part hairy and has slender tap-root. The three-lobe leaves are alternative, thick and rugose. The plantsare monoecious, the flowers borne in sessile axillary racemes with staminate flowers in upper and pistillate flowers in the lower part of raceme. C. rottleri is traditionally used by the tribes and native medical practitioners for the treatment of various diseases. In Sudan, powdered stems or whole plants are applied to wounds to improve healing. In Ethiopia, an infusion of the seeds and leaves is taken as a laxative The plant is also used medicinally in Saudi Arabia, Pakistan and India (e.g. against jaundice and purifying blood). In Senegal, the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhoea, where as in Kenya, camels graze it. The fruits yield a purplish-blue dye, which is used in East Africa to dye mats (Prota, 2010). In Nepal, juice of the fruit is given in cases of cough and colds (Manandhar and Manandhar, 2002), purifying agent (leaf) and laxative (seed), having bioactive components (Singh, 2010). The leaves are very much beneficial

in treating skin diseases and also used as a depurative agent (Khare, 2007). The seeds are used as cathartic like Ghodtapde and credited with purgative properties. Priyanka et al. (2010), reported that the aqueous extract.

FIG 1 Plants used in the study

Portulaca oleracea

Chrozophora rottleri

Flemingia macrophylla

Girardinia diversifolia

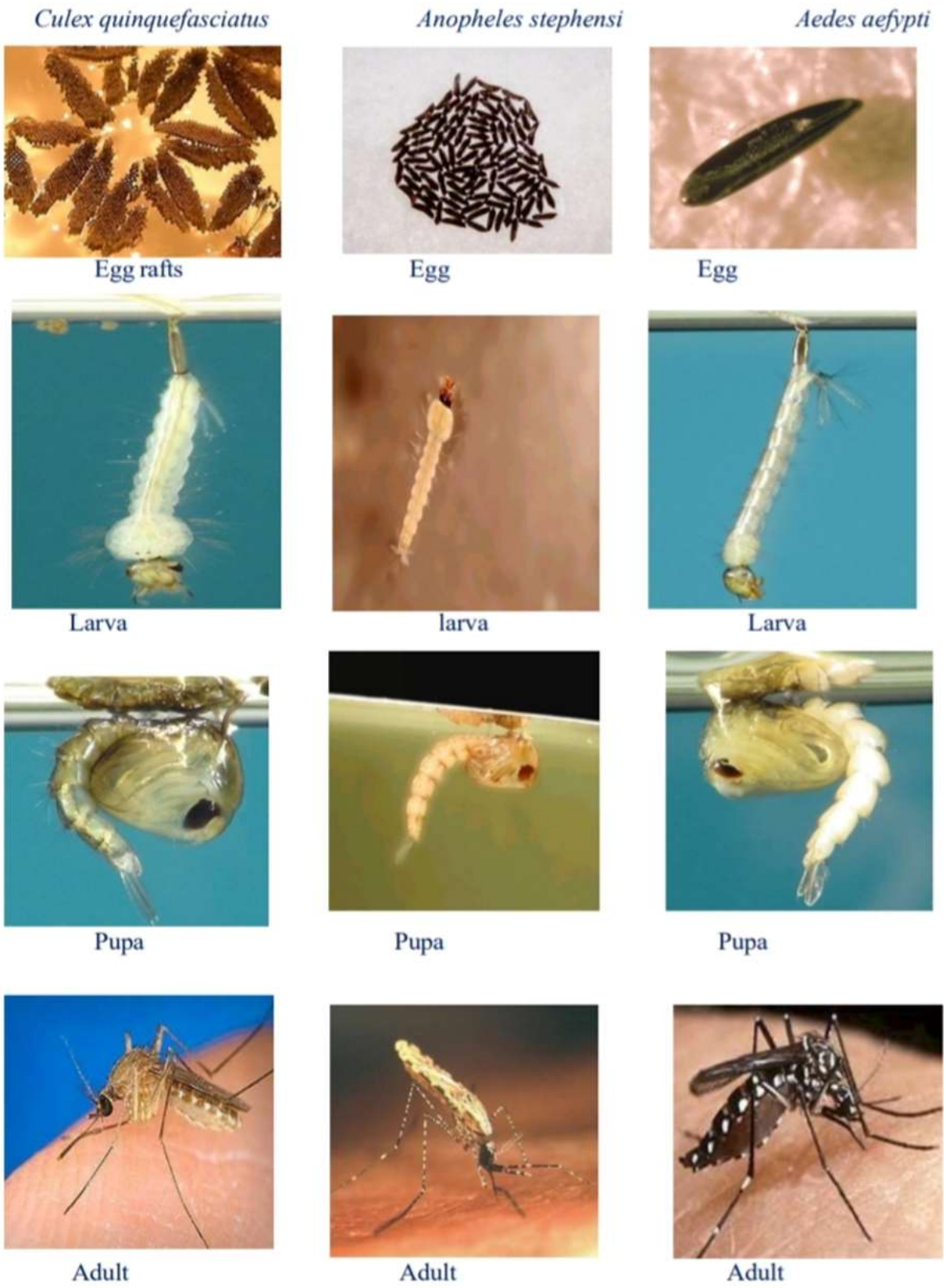

FIG 2Life cycle of vector mosquitoes

Anisops bouvieri

Diplonychus indicus

Gambusia affinis

FIG 3 Non-target organism

Table 1. Plants used in the present study.

S. no	Plant species	Common name	Vernacular name	Plant parts used	Collection area
1	*Portulaca oleracea*	Purslane	Paruppu keerai	Leaf	Nilgiris
2	*Chrozophora rottleri*	Suryavarti	Purapirakkai	Leaf	Nilgiris

Table 2. Yield of the plants with five different solvents.

Sr.no	Plant species	Solvent used	Yield (g)
1	*Portulaca oleracea*	Benzene	103.60
		Hexane	98.52
		Ethyl acetate	117.87
		Chloroform	115.93
		Methanol	132.42
2	*Chrozophora rottleri*	Benzene	84.60
		Hexane	107.38
		Ethyl acetate	99.37
		Chloroform	114.96
		Methanol	136.28

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The eggs of An. stephensi, Ae. aegypti and Cx. quinquefasciatus were collected from vector control laboratory, Annamalai University. The different leaf extracts were diluted in the ethanol to achieve various concentrations. Before treatment the egg rafts of Cx. quinquefasciatus and

the eggs of An. stephensi and Ae. aegypti were counted under microscope individually. Eggs of these mosquito species (100 numbers of 12-18h old eggs) were exposed to each concentration of crude extracts and (100 numbers of 0-6, 6-12 and 12-18h old eggs) the compounds until they hatched or died. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

Adulticidal activity

Sugar-fed adult female mosquitoes (5 to 6 days old) were used. The four different plants leaf extracts were diluted with ethanol to make different concentrations. The diluted plants extracts were impregnated on filter papers (140×120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125×44 mm following the method in WHO (1981). One tube served to expose the

Purification and characterization

Glass column of 2.0 × 40 cm was packed with 150 mg silica gel (70-325 mesh) using hexane as solvent. 5 g of active fraction-I was admixed using silica gel (70-325 mesh) 10 g for uniform mixing. The admixture was packed above the column and eluted with solvents of increasing order of polarity gradually from 0-50% ethyl acetate in hexane which was represented in Table 3.4

S. No	% of solvents eluted	Fraction	Weight (g)
1	20% EAc / Hex	I	5.8
2	40% EAc / Hex	II	2.1
3	60% EAc / Hex	III	1.8
4	80% EAc / Hex	IV	2.4
5	100% EAc	V	1.3

Isolation of bioactive fractions.

Table 4. Isolation of pure compounds

S. No.	Number of Fractions	% of Solvent	Volume of Solvent (ml)	TLC Mobile Phase (ml)	TLC
1	1-5	100% Hexane	500	EtoAc :Hex (2:8)	No compound eluted
2	6-12	5% EtoAc : 95 % Hexane	300	EtoAc :Hex (2:8)	Light yellow in colour
3	13-20	10% EtoAc : 90 % Hexane	400	EtoAc :Hex (2:8)	Yellow in colour
4	21-32	15% EtoAc : 85 % Hexane	500	EtoAc :Hex (3:7)	Green in colour
5	33-50	20% EtoAc : 80 % Hexane	400	EtoAc :Hex (3:7)	Green in colour
6	51-69	25% EtoAc : 75 % Hexane	500	EtoAc :Hex (3:7)	Green in colour
7	70-84	30% EtoAc : 70 % Hexane	300	EtoAc :Hex (3:7)	Green in colour
8	85-97	35% EtoAc : 65 % Hexane	300	EtoAc :Hex (4:6)	Yellow in colour
9	98-106	40% EtoAc : 60 % Hexane	300	EtoAc :Hex (4:6)	Yellow in colour
10	107-122	45% EtoAc : 55 % Hexane	300	EtoAc :Hex (5:5)	Light yellow in colour
11	123-132	50% EtoAc : 50 % Hexane	300	EtoAc :Hex (6:4)	Light yellow in colour

RESULTS

Table. Larvicidal activity of different solvent extracts of Portulaca oleracea against Anopheles stephensi.

Solvents	Concentration (ppm)	% of mortality±SD	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Hexane	50 100 150 200 250	25.6±1.2 47.2±0.8 66.5±0.6 89.7±1.2 97.4±0.4	106.01 (94.31-116.42)	207.08 (192.19-226.58)	1.593 n.s.
Ethyl acetate	40 80 120 160 200	24.8±0.8 47.6±0.6 65.4±1.2 86.7±0.4 97.3±0.8	86.53 (76.96-95.04)	170.26 (157.87-186.60)	1.495 n.s.
Benzene	40 80 120 160 200	29.6±1.2 48.2±0.8 67.8±0.6 88.6±0.4 98.1±1.2	81.12 (71.06-89.88)	165.71 (153.32-182.10)	1.146 n.s.
Chloroform	30 60 90 120 150	27.4±0.6 45.9±0.4 68.2±1.2 87.4±0.8 99.3±1.2	62.94 (55.95-69.15)	122.99 (114.12-134.63)	3.526 n.s.

a Values aremean±SD of five replicates No

mortality was observed in the control SD = standard deviation

LC50= lethal concentration that kills 50% of the exposed organisms

LC90= lethal concentration that kills 90% of the exposed organisms

UCL= 95% upper confidence limit

LCL= 95% lower confidence limit $\chi 2$= chi square n.s. = not significant (α=0.05).

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