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Review Article

REVIEW ON LARVICIDAL ACTIVITY OF MEDICINAL PLANTS FOR MALARIA VECTOR CONTROL

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Abstract

Plants have been used since ancient times to repel/kill blood-sucking insects in the human history and even now, in many parts of the world people are practicing plants substances to repel/kill the mosquitoes and other blood-sucking insects. The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositional attractants. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Natural pesticides, especially those derived from plants, are more promising in this aspect. The present study assessed the role of larvicidal activities of various solvent extracts (viz. hexane, chloroform, ethyl acetate, acetone and methanol) of fifty different important medicinal plants. Different plant parts such as leaf, rhizome, bulb, stem and root bark, whole plant and essential oil showed significant larvicidal properties against different mosquito vectors viz., *An. stephensi*, *Ae.* aegypti and *Cx. quinquefasciatus*, *P. duboscqi, An. aambiae*, *A. fluviatilis* etc. This review may open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts.

Key words: Malaria, Larvicidal acitivity, Phytochemicals, Medicinal plants

INTRODUCTION

Malaria is one of the most serious and widespread health problems in many parts of the world, particularly in Africa and Latin America with a high mortality rate. The situation is further complicated by the spread of drug resistant parasites in many parts of the world where *Plasmodium falciparum* is endemic. The multi-drug resistance in *P. falciparum* is a major problem in many countries and the number of drugs available, effective and affordable is very limited[1]. The malaria situation is aggravated by the appearance of strains of *P. falciparum* resistant to antimalarial drugs as well as by the resistance of vector Anopheles mosquitoes to DDT and other insecticides[2]. As malaria vaccines remain problematic, chemotherapy is the most important weapon in the fight against the disease[3,4]. The antimalarial drugs including chloroquine, quinine, mefloquine and artemisinin are currently used to prevent and treat human malaria. Part of the reason for the failure to control malaria, is the spread of resistance to the first line antimalarial drugs, cross-resistance between the limited number of drug families available, and some multidrug resistance[5]. Resistance has emerged to all the classes of antimalarial drugs except artemisinin, an endoperoxide

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antimalarial drug derived as the active component of *Artemisia annua*, a herbal remedy used in Chinese folk medicine for 2000 years "qinghaosu"[6,7,8,9].

Resistance to insecticides

Malaria is one of the most common vector-borne diseases widespread in the tropical and subtropical regions. Despite considerable success of malaria control programs in the past, malaria still continues as a major public health problem in several countries. Vector control is an essential part for reducing malaria transmission and became less effective in recent years, due to many technical and administrative reasons, including poor or no adoption of alternative tools. Of the different strategies available for vector control, the most successful are indoor residual spraying and insecticide-treated nets (ITNs), including long-lasting ITNs and materials. Earlier DDT spray has shown spectacular success in decimating disease vectors but resulted in development of insecticide resistance, and to control the resistant mosquitoes, organophosphates, carbamates, and synthetic pyrethroids were introduced in indoor residual spraying with needed success but subsequently resulted in the development of widespread multiple insecticide resistance in vectors. Vector control in many countries still use insecticides in the absence of viable alternatives. Few developments for vector control, using ovitraps, space spray, biological control agents, etc., were encouraging when used in limited scale[10]. Likewise, recent introduction of safer vector control agents, such as insect growth regulators, biocontrol agents, and natural plant products have yet to gain the needed scale of utility for vector control[11]. Bacterial pesticides are promising and are effective in many countries. Environmental management has shown sufficient promise for vector control and disease management but still needs advocacy for inter-sectoral coordination and sometimes are very work-intensive. The more recent genetic manipulation and sterile insect techniques are under development and consideration for use in routine vector control and for these, standardized procedures and methods are available but need thorough understanding of biology, ethical considerations, and sufficiently trained manpower for implementation being technically intensive methods. Specifically the plant extracts as alternative to chemical applications to fight resistance problem in mosquito. Theoretically, larval control would seem to be an ideal approach to mosquito control as it eliminates mosquitoes before they reach the stage where they can transmit malaria. However, larval habitats may be small, widely dispersed, and transient. Larval control may be implemented through source reduction, chemical larviciding, and through biological control.

Source Reduction

Source reduction is removal or permanent destruction of mosquito breeding sites. The larval habitats may be destroyed by filling depressions that collect water, by draining swamps or by ditching marshy areas to remove standing water. Container-breeding mosquitoes are particularly susceptible to source reduction as people can remove or cover standing water in cans, cups, and rain barrels around houses. Mosquitoes that breed in irrigation water can be controlled through careful water management[12].

Chemical Application

For some mosquito species, habitat elimination is not possible. For these species, chemical insecticides can be applied directly to the larval habitats. This method can be organized quickly, are effective, and can produce results at relatively low cost if used efficiently. They have a special role in control programs for mosquito borne diseases,

particularly at the early stages of intervention to allow other control measures to develop and play effective roles in an integrated strategy[13].

Examples of chemical application methods include the following:

- ✓ Targeted residual spraying
- ✓ Larviciding
- ✓ Space spraying

Biological Control

Biological methods consist of the utilization of natural enemies of targeted mosquitoes and of biological toxins to achieve effective vector management. They are typically most feasible with easily identifiable breeding places[14]. Alternatives under this category include the following:

- ✓ Larvivorous fish (e.g., *Gambusia affinis*)
- ✓ Invertebrate predators
- ✓ Nematodes (e.g., *Romanomermis culicivorax*),
- ✓ Protozoa and fungi (e.g., *Laegenidium giganteum*)

Environmental Management

Environmental management is typically applied to reduce the burden of malaria over the long term. These interventions focus on avoiding creation of vector breeding areas, changing natural habitats, or improving human habitation to reduce the abundance of a target vector while creating minimal adverse environmental and social impacts[15,16]. Examples include the following:

- ✓ Marsh alteration
- ✓ Filling, grading, and drainage
- ✓ Vegetative plantings
- ✓ House screening

Personal Protection Measures

Personal protection measures include the use of window screens, ITNs, and repellents (such as DEET) and wearing light-colored clothes, long pants, and long-sleeved shirts. Well-constructed houses with window screens are effective for preventing biting by mosquitoes that bite indoors and may have contributed to the elimination of malaria. Recent evidence suggests that repellents may be effective in reducing malaria transmission and may be appropriate for areas where mosquitoes bite outdoors or early in the evening when people are not using ITNs. However, while repellents are recommended for travelers to malaria-endemic areas, further work to develop repellent formulations that are easily deployed in endemic countries is needed[17].

Other Vector Control Interventions

Fogging or area spraying is primarily reserved for emergency situations such as epidemics. Fogging has not been shown to be effective in any malaria-endemic areas. Fogging and area sprays must be properly timed to coincide with the time of peak adult mosquito activity, because resting mosquitoes are often found in areas that are difficult for the insecticide to reach (e.g., under leaves, in small crevices). In addition, fogging and area spraying will have to

be repeatedly applied to have an impact, and it can easily become too costly to maintain or result in the overuse of insecticides[18]. Oils may be applied to the water surface, suffocating the larvae and pupae. Most oils in use today are rapidly biodegraded. Toxins from the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) can be applied in the same way as chemical insecticides.They are very specific, affecting only mosquitoes, black flies, and midges. Insect growth regulators such as methroprene are specific to mosquito and can be applied in the same as chemical insecticides[19].

Options for vector control include environmental management, chemical control, biological control, and personal protection. An integrated vector control program would incorporate local information about vector distribution and behavior to identify one or more control techniques that would be effective, affordable, and acceptable to local communities. This review examines recent literature on the field effectiveness and, where available, impacts on malaria transmission of the first three types of vector control. Although personal protection, such as use of impregnated bednets or repellants, is clearly important in community-based malaria control programs, these approaches have already been extensively reviewed elsewhere.

Need for herbal insecticide

Mosquitoes are major public health pests throughout the world. Of the 3000 species of mosquitoes recorded worldwide more than hundred species are capable of transmitting various diseases to human[20]. Mosquitoes transmit many medically important pathogens and parasites such as viruses, bacteria, protozoan and nematodes[21]. Mosquito borne diseases are one of the major public health problems today[22]. These diseases are responsible for morbidity, mortality, economic loss and social disruption[23]. The control of mosquito at the immature stage is necessary and efficient in integrated mosquito management because during the immature stages, mosquitoes are immobile[24]. Various measures have been taken to control mosquito menace and one such approach is by killing the mosquitoes at it is larval stages. This is mortality on the basis of using of synthetic and chemical insecticides. Even though these insecticides are effective in controlling mosquitoes they created many environment problems like insecticide resistance, pollution, toxic side effect on human begins[25,26]. Insecticides residual problem together with the insect resistance pose us in the environment to seek attention towards alternative methods[27]. This has necessitated the need of research and development on environmental safe, bio-degradable and indigenous method for vector control. Many herbal products have been evaluated. This review focused on the study of larvicidal effect of mosquito through various medicinal extracts.

Nature of active ingredients responsible for larval toxicity

The plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programme. Kishore *et al*.[28] reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans. They also documented the isolation of several bioactive toxic principles from various plants and reported their toxicity against different mosquito species.

Mode of action of phytochemicals in target insect body

Generally the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signalling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components[29] This in turn, affects insect physiologyin many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway). Rattan[29] reviewed the mechanism of action of plant secondary metabolites on insect body and documented several physiological disruptions, such as inhibition of acetylecholinestrase (by essential oils), GABA-gated chloride channel (by thymol), sodium and potassium ion exchange disruption (by pyrethrin) and inhibition of cellular respiration (by rotenone). Such disruption also includes the blockage of calcium channels (by ryanodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (thymol), hormonal balance disruption, mitotic poisioning (by azadirachtin), disruption of the molecular events of morphogenesis and alteration in the behaviour and memory of cholinergic system (by essential oil), *etc*. Of these, the most important activity is the inhibition of acetylcholinerase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has been observed to be organophosphorus and carbamate resistant, and it is well-known that the alteration in AChE is one of the main resistance mechanisms in insect pests[30].

Medicinal Plant Extracts as Source of Larvicidal Agent

1. ***Annona squamosa* Linn.** is a small, well-branched tree or shrub from the family Annonaceae is a multipurpose tree with edible fruits and is a source one of the medicine. The leaves of the plants have been used as insecticide, anthelmintic, styptic, externally used as suppurant[31]. The methanol leaf extract of *Annona squamosa* was found to have the most promising larvicidal activity against *Cx. quinquefasciatus* larvae with LC_{50} value 20.26 and LC_{90} value 86.59 ppm[32].
2. ***Allium cepa* Linn.** is a member of the Liliaceae family. Because of their bulbs, tubers and rhizomes, these plants are able to survive under harsh conditions, e.g. winter or dryness. The plant *Allium cepa* Linn. (Liliaceae) are proved to show the antidiabetic, antioxidant, antihypertensive, antithrombotic and hypoglycemic and antihyperlipidemic. The bulb extracts *Allium cepa* showed moderate larvicidal activity (2.92 mg/ml.) with LC_{50} values at 48 hours against *A. fluviatilis*[33].
3. ***Ageratum conyzoides*** L., Asteraceae, is an annual herbaceous plant is widely utilized in traditional medicine as a bacteriocide, antidysenteric, and antilithic. It is used to treat pneumonia, but the most common use is to cure wounds and burns[34]. The larvicidal activity of essential oil of *Ageratum conyzoides* against *Ae. aegypti* achieved 100% mortality at 300ppm concentration[35].
4. ***Acalypha fruticosa* Forssk.** an erect woody shrub, belongs to the family, Euphorbiaceae is one such folklore plant used in traditional system of medicine. This plant species has been used as a folk remedy for the treatment of dyspepsia, skin complaints, jaundice, cholera, sexually transmitted diseases, stomach problems, antipyretic and even as an antidote[36]. *Acalypha fruticosa* mehanolic extracts against adult *P. duboscqi* showed significant mortality rate at ($p < 0.05$) in bioassays with both male and female species with LD_{50} 3.26mg/ml ($\chi^2=7.3$) and 8.95mg/ml ($\chi^2=39.4$) respectively[37].

5. ***Ageratina adenophora* (Spreng.) King & H.Rob.** is one such exotic species belonging to family Asteraceae used as an astringent agent, ther-mogenic and stimulant in folklore medicine. The LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ageratina adenophora* on *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae in 24 h were 144.86, 132.82, 113.08; and 250.70, 231.12 and 198.81 mg/l, respectively[38].
6. ***Andrographis paniculata* (Burm.f) Wall. Ex Nees** is an important medicinal plant of family Acanthaceae that has been effectively used in traditional Asian medicines for centuries. Traditionally, the plant has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects and it is used for the treatment of pharyngolaryngitis, diarrhea, dysentery, and cough with thick sputum, carbuncle, sores, and snake bites[39,40]. The benzene, hexane, ethylacetate, methanol and chloroform leaf extract of *Andrographis paniculata* was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti*[38].
7. ***Ageratum conyzoides*** L. (Asteraceae) is an annual herbaceous plant with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. The weed has been known since ancient times for its curative properties and has been utilized for treatment of various ailments, such as burns and wounds, for antimicrobial properties, for many infectious conditions and bacterial infections, arthrosis, headaches and dyspnea, pneumonia, analgesic, anti-inflammatory, antiasthmatic, antispasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases[41]. The Whole plant extracts of *Ageratum conyzoides* with LC_{50}>4.2mg/ml at 48 hours showed better larvicidal activity against *Aedes aegypti*[42].
8. ***Acalypha indica* L**. (Euphorbiaceae) is a weed widely distributed throughout the plains of India. It has been reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments Chopra[43]. The crude leaf extract of *Acalypha indica* with different solvents, viz. benzene, chloroform, ethyl acetate and methanol showed remarkable larvicidal activity against *An. stephensi*. The LC_{50} values were 19.25, 27.76, 23.26 and 15.03 ppm, respectively[44].
9. ***Breynia vitis-idaea* (Burm.f.) Fischer.** (Euphorbiaceae) is an evergreen, glabrous tree or large shrub. Stem and bark are *used* to cure hydrocoel, inflammation and swellings, stomach ulcers[45]. The leaf crude extracts of *B. vitis-idaea* showed promising result against *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi* which showed 100% mortality in all species at 250 ppm concentration with the LC_{50} values of 98.2 ppm (LCL=68.9–UCL=139.9), 107.79 ppm (LCL=77.4–UCL=149.9) and 115.8 ppm (LCL=86.9–UCL=154.5) respectively[46].
10. ***Blighia sapida* Koenig.** is commonly known as Ackee (English) is a soap berry plant of the family Sapindaceae. It is a perennial herbaceous plant that is commonly employed particularly in developing countries to treat a wide range of disease conditions. The bark pulp serves as liniment for oedema intercostals pains and the root as abortifacient. The juice extracts from the leaves and pulp serve as eye drop in ophthalmic and conjunctivitis[47,48].The stem bark extracts of *Blighia sapida* demonstrated the highest activities (LC_{50} < 2.0mg/ml) against *A. aegyptii* at 24 and 48 hr[49].
11. ***Cassia fistula Linn***. belongs to family Caesalpiniaceae commonly known as Amulthus and in english popularly called "Indian Laburnum" has been extensively used in Ayurvedic system of medicine for various ailments. It posses anti periodic and laxative properties, the leaves are used in jaundice, piles, rheumatism ulcers and also externally skin eruptions, ring worms, eczema. The roots are used in chest pain, joint pain, migraine and blood

dysentery[50]. The LC_{50} of leaf extract of *C. fistula* with different solvents, viz. methanol, benzene and acetone showed remarkable larvicidal activity against *Ae. aegypti* were 10.69, 18.27 and 23.95 mg/l respectively[51].

12. ***Cochlospermum planchonii* Kunth** belonging to the Family Cochlopermacae, which is used in ethno-medicine for the treatment of infertility, diabetes mellitus, premenstrual pain, and management of jaundice, elimination of worms, bilhazias and hepatitis in Senegal[52]. The root extract of *Cochlospermum planchonii* as most larvicidal active extracts causing 100% mortality of larvae at 24 h post-incubation with the LC_{50} were 80–180 ppm against Anopheles and 370 ppm against *Culex quinquefasciatus*[53].
13. ***Cissus populnea* Guill. & Perr** is a strong woody liane or climbing shrub of family Vitaceae, which is Ethno-medicinally used for the treatment of sore breast, indigestion, venereal diseases, intestinal parasites, oedema and eye problems resulting from attack of black cobra. The plant is also used as cathartic, aphrodisiac and antidote to arrow wounds[54] *Cissus populnea* root extract showed 100% mortality of resistant larvae of Anopheles after 6 h contact. The mortality rates range between 6.67 and 100% for Anopheles and 25–100% for *Culex quinquefasciatus*[53].
14. ***Centella asiatica* (Linn.)** is a very important medicinal herb of family Apiaceae used in the orient. It has been used for the treatment of various skin conditions such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhoea, fever, amenorrhea, and diseases of the female genitourinary tract[55]. The leaf extracts *Centella asiatica* showed larvicidal activity with LC_{50} ranged between 6.84 ppm at 19°C and 1.12 ppm at 31°C. LC_{90} varied from 9.12 to 3.63 ppm at the two temperatures, respectively[56].
15. ***Costus speciosus* (J. Konig.) Seem** (Zingiberaceae) an important medicinal and ornamental plant cultivated in India. The rhizomes of *Costus speciosus* are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelminthic, depurative, febrifuge, expectorant, tonic, improve digestion, stimulant herb that clears toxins, rhizomes have anti-fertility, anabolic properties. The rhizome is applied to head for cooling and relief from head-ache, bruised leaves are applied in fever, decoction of stem is used in fever, dysentery, patients with high fever mostly utilize leaf infusion or decoction as a sudorific or in a bath, sap from leaves, young stems are used against diarrhea, cough, cuts, wounds, scabies, antidote for snake bite, jaundice, arthritis, burning sensation, constipation, leprosy, skin diseases, asthma, bronchitis, inflammations, anemia, intestinal warms, worm infection, rash, nose pain, to stop vomiting and spermatorrhoea[57,58]. The rhizome extracts of *Costus speciosus* demonstrated the highest activities (LC_{50}<2.0mg/ml) against *A. aeyptii* at 24 and 48 hr[59].
16. ***Cassia tora* Linn.** (Caesalpinaceae) is a well known oriental herb or under shrub, found as a rainy season weed throughout India. The leaves ad seeds of Cassia tora are useful in leprosy, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders. It has variety of biological/pharmacological activities such as hepatoprotective, anti-inflammatory, antigenotoxic, hypolipidemic, spasmogenic and antinociceptive, antiproliferative, immunostimulatory, hypotensive, purgative, antidiabetic, estrogenic and antiestrogenic, antiulcer, antioxidant, antifungal, antishigellosis, anthelmintic, antimutagenic, antibacterial and antiplasmodial activities[60]. The methanol extracts of *Cassia tora* exhibited >90% larval mortality at 200 ppm on *Ae. aegypti* and *Culex pipiens*[61].
17. ***Callistemon rigidus* (Stiff Bottlebrush)** (family Myrtaceae) is a small trees, used to cure respiratory tract infections, coughing and bronchitis, as well as uses as a general antiseptic[62]. Chloroform fractions of leaf extract of *Callistemon rigidus* R. Br achieved 81.33% of mortality in the highest concentration (1000 ppm) and

5.67% at the lowest concentration (125 ppm) with LC_{50} value of 504.10 ppm. against IV instar larvae of *Aedes aegypti* 24 h post-treatment[63].

18. ***Caesalpinia pulcherrima* Linn.** belongs to the family Fabaceaeis an ornamental plant widely used for treatment of various ailments across India. The leaves is said to cure fever, the juice from the flower is said to cure sores, the seeds are said to cure bad cough, breathing difficulty, and chest pain and root is also said to induce abortion in the first trimester of pregnancy[64]. The LC_{50} and LC_{90} values of *Caesalpinia pulcherrima* against early III instar larvae of *Anopheles subpictus* were 159.59 and 207.73 ppm; and *Cx. tritaeniorhynchus* were 245.37 and 299.45ppm, respectively[65].
19. ***Clausena anisata* Willd. Hook. f. ex Benth** (Rutaceae) plant is a tropical shrub traditionally useful as effective remedies against parasitic infections, especially flatworm infestations, such as taeniasis and schistosomiasis, eye complaints; influenza and other respiratory ailments; heart disorders and hypertension; abdominal cramps, constipation and gastroenteritis; hepatic diseases causing bad breath; malaria; diabetes; fevers and pyrexia; boils, rheumatism, arthritis and other inflammatory conditions; headaches, body pains, toothaches and swollen gums; convulsions and some mental disorders; impotence and sterility; blood tonic; and dysentery in cattle[66]. The essential oil from the leaves of *Clausena anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively. The LC_{50} values appeared to be most effective against *Anopheles stephensi* (LC_{50}-23.17, 19.67, 16.95, 11.01, 35.17 ppm) followed by *Aedes aegypti* (LC_{50}-27.69, 21.20, 18.76, 12.70, 38.64 ppm) and *Culex quinquefasciatus* (LC_{50}-32.23, 25.01, 21.28, 14.01, 42.28)[67].
20. ***Cymbopogon citrates* (DC. ex Nees) Stapf (**Poaceae**)** known as Lemon grass is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It is used for the treatment of coughs, constipation, elephantiasis flu, gingivitis, headache leprosy, malaria, ophthalmia, pneumonia, vascular disorders, diarrhoea and stomach ache[68]. The larvicidal activity of essential oil of *Cymbopogon citrus* against *Ae. aegypti* achieved 100% mortality at 200ppm concentration[69].
21. ***Cecropia obtusifolia* Bertol.** (Cecropiaceae) Commonly called "Guarumbo'' is widely used by the Mexican traditional healers for the treatment of diabetes and as an anti-inflammatory agent[70]. *Cecropia obtusifolia* Bertol. is a species of Urticaceae family commonly known as trumpet tree. The methanol leaf extracts of *Cecropia obtusifolia* exhibited >90% larval mortality at 200 ppm on *Ae. aegypti* and *Culex pipiens*[71].
22. ***Emilia sonchifolia* (L.) DC.** (Asteraceae) also known as lilac tassel flower or cupid's shaving brush is an annual herb traditionally used in Brazilian folk medicine to treat asthma, fever, cuts, wounds and rheumatism[72]. Aerial parts of *Emilia sonchifolia* (0.1mg/ml) gave a weak 44.4% mortality against *A. fluviatilis*[27].
23. ***Ervatamia coronaria* Stapf** belonging to the family Apocynaceae, is a glabrous, evergreen tree. In Indian traditional system of medicine, this plant material is widely used as a purgative, tonic to the brain, the spleen and the liver; in the treatment of cancer, wounds, analgesic, antipyretic and anti-inflammatory[73]. The LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67 and 62.08; and 136.55, 127.24 and 120.86 mg/l, respectively[74].
24. ***Eucalyptus globulus* Labill**. is the tallest known tree from family Myrtaceae. The leaves of *Eucalyptus globulus* are the principal source of eucalyptus oil. The oil is antiseptic and is used medicinally as a decongestant for treating catarrh, bronchitis and influenza. It is also used in liniments for bruises, sprains and muscular pains,

and to make herbal tea infusions[75]. Seed and leaf extract of *Eucalyptus globulus* larvicidal activity at a dose of 1000 ppm which caused 100 and 80% mortality to the tested larvae i.e. *Cx. pipiens*[76].

25. ***Ficus benghalensis* L**. (Moraceae) commonly known as the Indian banyan tree is the world's largest tree in terms of its spread with some old trees covering over an acre of ground. Different parts of the tree have been found to possess medicinal properties; leaves are good for ulcers, aerial roots are useful in gonorrhea, seeds and fruits are cooling and tonic, roots of *Ficus bengalensis* are given for obstinate vomiting and infusion and its bark is consider as a tonic, astringent and is also used in diarrhea, dysentery and diabetes[77]. The LC_{50} and LC_{90} values of methanol extract of *Ficus benghalensis* against early III instar of *Cx. tritaeniorhynchus* and *An. subpictus* were 100.88 and 159.76 ppm; and and 56.66, and 85.84 ppm, respectively[78].

26. ***Heliotropium indicum* Linn.** a small fragrant evergreen annual herb of Boraginaceae family. The plant is medicinnaly important which is used to cure various diseases like fever, analgesic, inflammation, wound, tumour and flatulence in Indian traditional system of medicine. It has anti-inflammatory, wound healing, antiseptic/ antimicrobial, febrifuge, secretagogue stimulation (of gall bladder functions) and menstruation activator properties. The whole plant is used in high fever, throat infection, ulcerfever, gonorrhea, localized inflammation, reheumatism, ring worm, ulcers, wounds, aphrodiasic, astringent, bitter, expectorant[79,80]. *Heliotropium indicum* leaf extract exhibited larvicidal activity with LC_{50} and LC_{95} values ranging between 180–370 and 342–703 ppm respectively against *Anopheles gambiae*[53].
27. ***Kalanchoe crenata* (Adrews) Haworth** is an ornamental plant belonging to the family Crassulaceae commonly known as “never die” or “Dog’s liver”, this plant is widely used in traditional medicine in the treatment of inflammation, earache, headache, asthma, palpitation, abdominal pain, convulsion and general debility[81]. The leaf extract of ***Kalanchoe crenata*** showed larvicidal activity (LC_{50} 2.24 and 2.28 mg/ml) at 48 hours against *A. fluviatilis* ($p > 0.05$)[82].
28. ***Lantana camara* Linn.** is a small perennial shrub of Verbenaceae family which has been used in traditional herbal medicines for treating a variety of ailments, including cancer, skin itches, leprosey, rabies, chicken pox, measles, asthma and ulcers[83]. The leaves are antimicrobial, fungicidal and insecticidal properties. Acetone extract of *Lantana camara* to be most effective against *Cx. quinquefasciatus* larvae at the dose of 1 ml/100 ml[84].
29. ***Lantana viburnoides* ssp. viburnoides var. kisi (A. Rich) Verdc** (Verbenaceae) is used in traditional medicine as a mosquito repellent and sometimes chewed for treatment of gastrointestinal problems. Gastrointestinal problems are varied and may include microbial infections, parasitic infestation; inflammatory bowel disease, malignancies, peptic ulcers, or simply colic pains. This work seeks to establish proof of the concept on safety and efficacy for treatment of bacterial infections. The crude extract of *Lantana viburnoides* root bark (LC_{50}=7.70ppm in 72h) fractions exhibited different level of mosquito larvicidal activity with subtraction of some fractions resulting in activity enhancement. The active fractions contained furanonaphthaquinones regio-isomers (LC_{50}=5.48-5.70ppm in 72h) and the lantadene triterpenoid camaric acid (LC_{50}=6.19ppm in 72h) as active principles while the lupane triterpenoid betulinic acid (LC_{50}<10ppm in 72h) was obtained from the least active fraction[85].
30. ***Luffa cylindrical* Linn.** commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd is a large monoecious, annual climber is a member of cucurbitaceouse family found wild and also cultivated

throughout the greater parts of India. It is used traditionally in folklore medicines for ailments including jaundice, diabetes, liver diseases, skin diseases and wounds[86]. The toxicity to the III instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Luffa acutangula* showed the LC_{50} values of 839.81 ppm[87].

31. ***Momordica charantia* Linn.** belongs to family Cucurbitaceae, is commonly known as bitter gourd or bitter melon is one such plant that has been frequently used as medicine. Traditionally it has also been used as medicine for several ailments such as antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies[88]. The toxicity to the III instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Memordica charantia* showed the LC_{50} values of 465.85ppm against *Culex quinquefasciatus*[87].
32. ***Mimosa pudica* L.** (Mimosaceae) is a creeping annual or perennial herb. is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, headache, migraine, insomnia, fever and various urogenital infections[89]. The whole plant extracts of *Mimosa pudica* displayed moderate larvicidal activity ($2.0<LC_{50}<4.2$mg/ml) at 24 and 48 hours against *A. fluviatilis*[90].
33. ***Nauclea latifolia* Seem** (Rubiaceae), common name "Bishop`s head", is an ever green multi-stemmed shrub. Traditionally, in West and South Africa infusions and decoctions of the stem bark and leaves of the plant are used for the treatment of malaria, stomach ache, fever, diarrhea and nematodes infections in human and animals[91]. The root extracts of *Nauclea latifolia* displayed moderate) larvicidal activity ($2.0<LC_{50}<4.2$ mg/ml) at 24 and 48 hours against *A. fluviatilis*[90].
34. ***Ocimum sanctum* Linn** (known as Tulsi in Hindi), a small herb of family Lamiaceae is seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The *Ocimum sanctum* L. has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions[92]. The LC_{50} values of *O. sanctum* leaf extract against the larvae of *Ae. aegypti* was 425.94 and *Cx. quinquefasciatus* was 592.60 ppm[93].
35. ***Ocimum gratissimum* L.** (Lamiaceae) is an herbaceous perennial plant commonly known as scent leaf. It is used in the treatment of various diseases like cancer, antinociceptive, anti-inflammatory, antidiahorreal, antibacterial, antifungal, wound-healing and as nephroprotective[92]. The larvicidal activity of essential oil of *Ocimum gratissium* against *Ae. aegypti* achieved 100% mortality at 120ppm concentration with LC_{50} at 60ppm[69].
36. ***Ocimum americans*** is a aromatic annual and perennial herb in the family Lamiaceae commonly known as American basil, is used for stomach spasms, loss of appetite, intestinal gas, kidney conditions, fluid retention, head colds, warts, and worm infections[94]. The larvicidal activity of essential oil of *Ocimum americans* against *Ae. aegypti* achieved 100% mortality at 120ppm concentration with LC_{50} at 67ppm[69].
37. ***Piper longum* Linn.** called as Indian long pepper, is a flowering vine of family Piperaceae is good for aromatic, stimulant, carminative ,constipation, gonorrhea, paralysis of the tongue, advised in diarrhea, cholera, scarlatina

,chronic malaria, viral hepatitis[95]. *Piper longum* seed extracts at 80 mg/l causing complete mortality in *Cx. quinquefasciatus* and 60 mg/l for *Cx. sitiens*[96].

38. ***Piper nigrum* L.** is a flowering vine of family Piperaceae is commonly used for the treatment of pain relief, rheumatism, chills, flu, colds, increase circulation, exhaustion, muscular aches, physical and emotional coldness, nerve tonic and fevers. It has also been advised in diarrhoea, cholera, scarlatina and in solution for a wash for tinea capititis[97]. The larvicidal effect of crude extracts of dried ripened fruits of *Piper nigrum* against *Cx. quinquefasciatus* larval instars. LC_{50} and LC_{90} values as observed for early IV larval instar of *Cx. quinquefasciatus* were 29.11 and 62.37 mg/l and 63.82 and 108.90 mg/l for aqueous and ethanol extracts respectively. A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *Cx. pipiens*[98].
39. ***Phyllanthus amarus* Schum. & Thonn** (Euphorbiaceae) is a plant of the family Euphorbiaceae is a branching annual glabrous herb which has found its traditional usefulness in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds. Further, these are used in the treatment of kidney problems, urinary bladder disturbances, pain, gonorrhea, diabetes and chronic dysentery. Topically, it is used for several skin problems ranging from skin ulcers, sores, swelling and itchiness, wounds, bruises, scabies, ulcers and sores, edematous swellings, tubercular ulcers, ringworm, scabby and crusty lesions[99,100]. The extract of *Phyllanthus amarus* (whole plant) showed (LC_{50} 2.24 and 2.28 mg/ml) larvicidal activity at 48 hours against *A. fluviatilis* ($p > 0.05$)[101].
40. **Phyllanthus emblica L. (Eiphorbiaceae)** commonly known as amla, is one of the foremost plants utilized from antiquity till to date. Traditionally, the fruit is useful as an astringent, cardiac tonic, diuretic, laxative, liver tonic, diuretic, refrigerant, stomachic, restorative, antipyretic, anti-inflammatory, hair tonic and digestive medicine[102]. Ethyl acetate extract of *Phyllanthus emblica* leaves exhibited larvicidal activity with LC_{50} value of 78.89 ppm (99.6%) on *Cx. quinquefasciatus* and followed by *Ae. aegypti* 80.04 ppm (99.5%) after 24h of exposure[103].
41. ***Pentaclethra macrophylla* Benth** (Mimosaceae) is also known as the African oil bean tree used by local human population for various parasitic infections, inflammations, pain and related illnesses. The bark, fruits, seeds and the leaves are used as anthelmintics, for gonorrhea, convulsion and as analgesic[103,104,105]. The bark extracts of *Pentaclethra macrophylla* displayed moderate) larvicidal activity ($2.0<LC_{50}<4.2$mg/ml) at 24 and 48 hours against *A. fluviatilis*[82].
42. ***Schinus terebinthifolius* Raddi.** is a species of flowering plant in the cashew family, Anacardiaceae. It is reported to be an astringent, antibacterial, diuretic, digestive stimulant, tonic, antiviral, and wound healer. It is used for many conditions in the tropics, including menstrual disorders, bronchitis, gingivitis, gonorrhea, gout, eye infections, rheumatism, sores, swellings, tuberculosis, ulcers, urethritis, urogenital disorders, venereal diseases, warts, and wounds[107]. *Schinus terebinthifolia* essential oil displayed activity after 72 h, the mean mortality percentage ranged from 0.5 to 96.75% for *Cx. quinquefasciatus* and 13.75 to 97.91% for *An. gambiae*[108].
43. ***Spondias mombin* L.** is a fructiferous tree belongs to the family Anacardiacae. It grows in the rain forest and in the coastal area. Its bark serves as an emetic, a remedy for diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhea. The flowers and leaves are taken to relieve stomach ache, biliousness, urethritis, cystitis and eye and throat inflammations[109]. The *Spondias mombin* leaf extracts proved to be a

strong candidate for a natural, safe and stable mosquito larvicide to be used in population control of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*. Hexane fraction of *Spondias mombin* Linn showed highest larvicidal activity with LC_{50} of 92.20 and LC_{50} of 326.53ppm against *An. aambiae* and *Cx. quinquefasciatus* respectively[110].

44. ***Solanum trilobatum* Linn**. (Solanaceae) is one of the important medicinal plants, more commonly available in Southern India. It is reported to cure numerous diseases viz., tuberculosis, respiratory problems and bronchial asthma. *S. trilobatum* was reported to harbour hepatoprotective activity, antimicrobial activity, antioxidant activity, cytotoxic activity, haemolytic activity, protective effect, immunomodulatory activity and anti-inflammatory properties[111]. The methnol leaf extract of *Solanum trilobatum* exhibited 100% larval mortality in *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* at 250 ppm concentration[112].
45. ***Tagetes minuta* L.** is a tall upright marigold plant in the sunflower (Asteraceae) family which has several medical benefits such as remedy for colds, respiratory inflammations, stomach problem, anti-spasmodic, anti-parasitic, anti-septic, insecticide and sedative. It is used for chest infections, coughs and catarrh, dilating the bronchi, facilitating the flow of mucus and dislodging congestion and can be used in cases of skin infections[113]. *Tagetes minuta* Linnaeus extracts showed a significant higher mortality to adult sandflies than other extracts with mean mortality of 18.63 (62.1%) and 19.25 (64.2%) in the methanol and ethyl acetate extract bioassays respectively. This contact method gave 90–100% mortality by the 96th hour in 5 mg/ml and above[37].
46. ***Tarchonanthus camphoratus* L.** is a multi–stemmed shrub species of family Asteraceae known as camphor bush for its scent. It is used as a traditional remedy for bronchitis, asthma, headache, inflammation, chilblains or abdominal pains[114]. *Tarchonanthus camphoratus* methanolic extracts against adult *P. duboscqi* showed significant mortality rate at ($p < 0.05$) in bioassays with both male and female species with LD_{50} 24.5 mg/ml ($\chi^2 = 7.19$) and 49.9 mg/ml ($\chi^2 = 3.09$) respectively[37].
47. ***Tricosanthes cucumerina* L.** is a monoecious annual herb climbing branched tendrils of family Cucurbitaceae which is used as an abortifacient, vermifuge, stomachic, refrigerant, purgative, malaria, laxative, hydragogue, hemagglutinant, emetic, cathartic, bronchitis and anthelmintic. The plant is used in the treatment of head ache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrohea, haematuria and skin allergy[115]. The toxicity to the III instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Trichosanthus anguina* showed the LC_{50} values of 839.81 ppm[87].
48. ***Vitex negundo* Linn.** (Verbenaceae) is a woody, aromatic shrub growing to a small tree. Traditionally the leaves of *Vitex negundo* Linn. are documented to possess antibacterial, antitumor, astringent,febrifuge, sedative, tonic and vermifuge. It has been reported to posses potent pharmacological properties likem anti-inflammatory, anti-rheumatic, antibiotic, Hepatoprotective, antioxidant, anticonvulsant, oxidative stress, anti-androgen, snake venom neutralization and anti-allergic activities[116]. The leaf extracts of *Vitex negundo* with LC_{50} value 212.57 ppm showed better larvicidal activity against *Cx. quinquefasciatus*[117].
49. ***Vitex grandifolia* Gürke (**Verbenaceae**)** is an evergreen shrub or tree with a spreading crown. In traditional medicine the bark is used as a stomachic and to treat diarrhoea, bronchial complaints, rickets, sores and fever[118]. *Vitex grandifolia* stem bark extract exhibited larvicidal activity with LC_{50} and LC_{95} values ranging between 180–370 and 342–703 ppm respectively against *Anopheles gambiae*[53].
50. ***Xylopia aethiopica* (Dunal) A. Rich.** is an evergreen, aromatic tree, of the Annonaceae family that is used as a spice and an herbal medicine. The plant bark or fruit has been useful in the treatment of bronchitis and

dysenteric conditions, or as a mouthwash to treat toothaches. It has also been used as a medicine for biliousness and febrile pains. The bark, when steeped in palm wine, is used to treat asthma, stomach-aches and rheumatism[119]. The seed extracts of *Xylopia aethiopica* emonstrated the highest activities (LC_{50}<2.0mg/ml) against *A. aegyptii* at 24 and 48 hr against *A. fluviatilis*[120].

DISCUSSION

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friendly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. This review showed that the crude benzene, chloroform, ethyl acetate and methanol solvent extracts of different parts of medicinal plants such as leaf, rhizome, bulb, stem and root bark, whole plant and essential oil have significant larvicidal properties against different mosquito vectors viz., *An. stephensi*, *Ae.* aegypti and *Cx. quinquefasciatus*, *P. duboscqi, An. aambiae*, *A. fluviatilis* etc. This review focused on fifty different important medicinal plants which show strong larvicidal effect against different mosquito vectors. According to Bowers *et al.*[121] the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. About 80% of the world's population use plant as their primary source of medicine. Natural products are generally preferred to control insect since they are harmless, have no effect on non-target organisms and their bio-degradability[121,122]. To control larvae of mosquito various plant extracts are studied extensively and these studies shows the activity of such plant extracts based on the various significantly plant part, age of plant part, solvent used extraction and mosquito species[123]. Studies on focus on herbs and other medicinal plants due to the historical experimental knowledge and some scientific studies have shown them to be particularly active against certain organisms[124]. Different parts of plants contain a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites which act as larvicidal agent[44,125]. Plant based insecticides provide an alternative to synthetic insecticides because they are generally considered safe, are biodegradable, and can often be obtained from local sources[87].

There are some reports about the resistance to these chemicals in mosquitoes. Therefore we need to identify alternative insecticide substances from natural products. Many scientists reported insecticidal activities of plants belong to different families in different parts of the world. There are several native reports about crude solvent extracts of different parts of plants, essential oils or their chromatographic fractions. They showed various levels of bioactivity against different developmental stages of malaria vectors[126]. Some plants have phytochemicals constituents for the control of mosquitoes. One of the earliest reports of the use of plant extracts against mosquito larvae is extraction of plants' alkaloids like nicotine, anabasine, methyl anabasine and lupinine from the Russian weed in 1933[124]. Some plant families such as Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae and Rutaceae have the maximum potential for development of novel mosquito control agents[127].

The ethno-pharmacological approaches used in the search of new bioactive toxins from plants appear to be predictive compared to the random screening approach. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with new

pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergestic approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology.

CONCLUSION

Application of these extract and fractions to mosquito breeding habitats may lead to promising results in malaria and mosquito management programmes. However, further studies on the bio-guided fractionation of hexane fraction to bring out the most active molecule(s), their insecticidal mode of action, their effects on non-target organisms and the environment and formulations improving the insecticidal potency and stability are needed for their practical use as a naturally occurring mosquito larval control agent.

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