

INTRODUCTION

Mosquitoes are one of the most medically significant vectors and they transmit parasites and pathogens which continue to have devastating impact on human beings. Vector borne diseases are major health problem in many countries (Maheswaran *et al.*, 2008). Every year at least 500 million people in the world suffer from one or the other tropical diseases that include malaria, schistosomiasis, trypanosomiasis, lymphatic filariasis, leishmaniasis and dengue (Madhumathy *et al.*, 2007). *Culex quinquefasciatus* is an important vector of filariasis in tropical and sub-tropical regions. According to WHO (1984), about 90 million people worldwide are infected with *Wuchereria bancrofti*, the lymphatic dwelling parasite. In India alone twenty five million people harbour microfilaria and nineteen million people suffer from filarial disease manifestations (NICD, 1990 and Maheswaran *et al.*, 2008).

Most mosquito control programs target the larval and embryonic stages, at their breeding sites with larvicides and ovicides, respectively (Knio *et al.*, 2008). A more efficient approach to reduce the population of mosquitoes would be to target the embryo and larvae. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated in breeding site than they are in the adult stage (Elimam *et al.*, 2009). No part of the world is immune to this risk (Fradin and Day, 2002) which indirectly impediment for the economic development not only for India but also for the entire world. Control of mosquito borne diseases is becoming difficult because of increasing resistance of mosquitoes to pesticides and public health concern over environmental pollution necessitates a continued search for alternative control measures (Ranson *et al.*, 2001).

The most reliable strategy of minimizing the incidence of mosquito-borne diseases is to eradicate and control the mosquito vectors, which is performed principally by systematic treatment of the breeding places through a combination of environmental management and application of larvicides that do not harm other

organisms in the environment (Corbel *et al.*, 2004). However, the most commonly used larvicides are now in dire questions of their sustained dissemination due to the potential environmental pollution caused by them and also the hazards to human health and other non-target organisms, commonly when profusely applied where there are epidemics (Rivero *et al.*, 2010).

An obvious method for the control of mosquito-borne diseases is the use of insecticides and many synthetic agents that have been developed and employed in the field successively. One major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to non target organisms in the environment. The growing toxicity problem, together with the incidence of insect resistance, has called attention for the search of insecticides (Macedo *et al.*, 1997) and also for more detailed studies of naturally occurring insecticides without any side effects (Govindarajan, 2010).

Over the past few decades, the vector has however developed the ability to evade intervention measures, which target adult mosquitoes (Sharp, 1983) thus exacerbating the problem for vector control measures. Current vector control technique involves the use of residual insecticides which are sprayed on to roofs and walls of houses, which is known as indoor residual house spraying that allows for a lethal dose of insecticide to the mosquito once it has rested on a sprayed surface (Walker, 2002). Although many insecticides are known to be very much effective, but they continue to pose a potential health and environmental problem rendering them undesirable and arguably inappropriate for use in public health (Collins and Blackwell, 2000).

Most insecticides are non-selective and can be harmful to other beneficial organisms and to the environment in the form of biomagnifications (Ciccia *et al.*, 2000). Repeated use of chemical insecticides is harmful to human health and environment. Even DEET the worlds most popular and efficient repellent is now reported to be, it won't protect against some dangerous mosquito species and it requires frequent applications (Schmidt, 2005). This low irritancy may represent a serious risk against personal protection and in some cases airway irritation have been reported with the use of these products in indoor application (Fischer and

Bialek, 2002). The number of new insecticides such as carbamates or organophosphates has been used to control mosquitoes but their low repellency allows mosquitoes to remain for sufficient time in the environment (Pennetier *et al.*, 2005). Multiple preparations from naturally occurring sources are repellent to certain insects. Numerous plant products have been reported as insect antifeedants and repellents (Isman, 2006). The use of scientifically proven non-chemical methods and limited use of drug is being considered as safety to environment and human health (Isman, 2006 and Worku *et al.*, 2009).

Commonly advocated approach for preventing mosquito attack is the measure of personal protection. Personal protection method allows an individual to select avoidance techniques, to exclude mosquitoes with physical and chemical barriers, treatment of fabric with toxicants, and the use of topical repellents (Barnard, 2000). Application of repellents to the skin is a common practice of personal protection (Khan *et al.*, 1975). Repellency is known to play an important role in preventing the vector borne diseases by reducing contact of man and vector. Synthetic chemicals and insecticides used for control of vector are causing irreversible damage to the eco-system, as some of them are no-degradable in nature (Das *et al.*, 2003).

Plants may be a source of alternative agent for control of mosquitoes, because they are very much rich in bioactive chemicals and are active against a limited number of species including specific target insects and are easily biodegradable. Plants are potentially suitable for use in integrated pest management programs (Alkofahi *et al.*, 1989). It is estimated that over 40,000 bioactive compounds are so far been isolated but only about 10,000 of them have been characterized chemically. Many plant extracts of terrestrial origin have been reported to suppress mosquito larval populations (Chavan and Nikam, 1982 and Saxena and Yadav, 1983) and suggested to be advantageous for field use in mosquito control programme (Kalyanasundram and Das, 1985).

India is endowed with a rich wealth of medicinal plants and it is one of the 12 mega biodiversity centres having 4, 50,000 plant species. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic

composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Mitchell and Cotran, 2000). Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects (Manjamalai *et al.*, 2011). In India, around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases. Currently 80% of the world population depends on plant-derived medicine for human alleviation because of its fewer side effects. Medicinal plant based drugs have the added advantage of being simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action (Chin *et al.*, 2006). In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources (Perumalsamy and Gopalakrishnakone, 2007).

India is the birth place of renewed system of indigenous medicines such as Siddha, Ayurvedha and Unani. Traditional system of medicines is prepared from a single plant or combinations of more than one plant. These efficacies depends on the current taxonomic identity of plant species, use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug. Traditional system of medicine continues to be widely practiced. Global estimate indicates that 80% of about 5 billion population cannot afford the products of the western pharmaceutical industry but they offer the uses of traditional medicines which are mainly derived from plant materials. In this modern world, now-a-days plant based drugs are widely used and many countries contribute 40-50% of their total, health budget in the population of novel drugs (Karthishwaran *et al.*, 2010; Sati *et al.*, 2010).

More than 1005 plant species are found to possess insecticidal properties, 384 varieties contain antifeedants, 297 varieties contain repellents and 27 varieties contain attractants and possess growth inhibiting properties. All these features indicate that the plant kingdom is a vast storehouse of potential chemicals for pest control. It is very much believed that insect resistance is less likely to occur because many botanicals contain multiactive compounds. The pest control

principles of botanicals include properties such as antifeedants, insecticides, repellents, chemosterilants, attractants, juveniles, anti-juvenile hormones, moulting, anti-moulting hormones, nematicides, rodenticides, fungicides and bactericides (Rajkumar and Jebanesan, 2004a; Govindarajan, 2010; Mathivanan *et al.*, 2010).

Repellents of synthetic origin may cause skin irritation and affect the dermis (Das *et al.*, 1999). N-N-dimethyl-m-toluamide (DEET), a synthetic mosquito repellent is recognized as one of the few products effective against mosquitoes and biting flies. The efficacy of DEET is providing long-lasting protection against a wide variety of mosquito species (Fradin and Day, 2002; Roberts and Reigart, 2004). Although DEET is an effective repellent against mosquitoes, there are many concerns associated with its use. The human toxicity problems have been reported with DEET, with the symptoms varying from mild to severe (Briassoulis *et al.*, 2001). It is irritating to mucous membranes and concentrated formulations dissolve plastic. DEET may be unsafe for children possibly causing encephalopathy (Abdel-Rahman *et al.*, 2001). Research regarding insect repellent derived from plant extracts is needed to find alternatives that are safer but still effective (Phasomkusolsil and Soonwera, 2010).

Plant products have been used by traditionally human communities in many parts of the world against the vectors and various species of insects. Phytochemicals derived from plant sources can act as larvicidal agents, insect growth regulators, ovipositional attractants, repellents and have deterrent activities observed by many researchers (Babu and Murugan, 1998). Botanical repellents have an important place in protecting man from the bites in insect pests. An effective repellent should be non-irritating and toxic long lasting. The amides, imides, esters and other polyfunctional compounds are known to have good repulsive action (Kalyanasundram and Das, 1985). Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects (Isman, 1995). Because of this much interest has been focused on plant extracts or plant essential oils as potential mosquito repellent agents (Murugan *et al.*, 2003; Yang *et al.*, 2004).

Phytochemicals are advantageous due to their eco-safety, target-specificity and no development of resistance, reduced number of applications, huge acceptability and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programmes. The phytochemicals of plants can be used as insecticides for killing the larvae or adult mosquitoes or as repellents for protection against mosquito bites. The phytochemicals are obtained from the whole plant or specific part of the plant by the extraction with the help of different types of solvents according to the polarity of the phytochemicals viz., aqueous, methanol, chloroform, benzene and acetone, etc. Some phytochemicals act as toxicant both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor of with reproduction or produce an olfactory stimuli thus acting as repellent or attractant (Markouk *et al.*, 2000; Pushpanathan *et al.*, 2008).

In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medical herbs, owing to their natural origin, cost effectiveness and lesser side effects (Naik *et al.*, 2003). Medicinal plants are expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds (Sumathi and Parvathi, 2010). Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate (Zaidan *et al.*, 2005).

The only efficient way to control these mosquito-borne diseases is to prevent mosquito bites by controlling mosquito vector populations. Insect repellents are known to play an important role in preventing the mosquito vector by preventing an insect from flying to landing on or biting human and animal skin. The widely used compounds as insect repellents are synthetic chemical repellents that are not safe for humans, especially to children, domestic animals as they may cause skin

irritation, hot sensation, rashes or allergy (Das *et al.*, 2003). Many people prefer to use a repellent that are originated naturally and the demand for natural repellent is gradually increasing everywhere. The use of herbal repellents, especially repellents from herbal essential oils are safe to human and environment and herbal essential oils are reported to have repellency against mosquito adults (Sritabutra *et al.*, 2011).

As plants have been an important source of medicine since thousands of years, its rich resource is decreasing at an alarming rate as a result of over-exploitation. The medicinal value of drug plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body. These chemicals include alkaloids, flavonoids, glucosides, tannins, gums, resins, essential oil, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals etc. Very few of these chemicals are toxic also. Hence, preparation and administration of drugs should be done by experts only. Drugs may be obtained from various parts of the plant (Haraguchi *et al.*, 1999; Sashikumar *et al.*, 2003).

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on human body. The most important of these chemically active constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes (Aiyelaagbe and Osamudiamen, 2009; Edeoga *et al.*, 2005). Natural products, which come out from medicinal plants are important for pharmaceutical research and for drug development as a sources of therapeutic agents. At present the demand of herbal or medicinal plant products has been increasing significantly (Nisha *et al.*, 2011).

Natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites (Castello *et al.*, 2002). Recent studies are involved in the identification and isolation of

new therapeutic compounds of medicinal importance from higher plants (Erturk *et al.*, 2006; Kumar *et al.*, 2007). The most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds. In India, large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexpected. Medicinal plants are at interest to the field of biotechnology, as most of the drug industries depend on parts of plants for the production of pharmaceutical compounds (Velmurugan *et al.*, 2010).

A drug design process begins with knowing the structure of the target protein and then forming a database that contains a collection of compounds that are expected to interact with the target protein. To determine which compounds that have the best interaction with protein target to become candidates for drug synthesis, a series of analyzing techniques is performed by using computer assisted tool. Two of the most well known computational techniques in drug design process are docking and molecular dynamics simulations. Molecular dynamics simulation is a computational approach in which atoms and molecules are allowed to interact with each other during a certain time period so that its behaviour can be observed (Nurbaiti *et al.*, 2010). Fast and inexpensive docking protocols can be combined with accurate but more costly molecular dynamics techniques to predict more reliable protein ligand complexes (Alonso *et al.*, 2006).

Mosquitoes seeking their hosts or mates are exposed to a wide variety of visual, olfactory, gustatory and physical stimuli. Combinations of these preferentially act as cues for host or partner identification and location (Manoharan, 2011). The role of olfaction, however, is currently found to be the major source of this identification among the mosquitoes. The molecular basis of this chemical signal recognition is systematically encoded by a series of proteins. The three major constituents involved in the peri-receptor events include the odorant binding proteins (OBPs), the odorant degrading enzymes (ODE) and the olfactory receptors (ORs) of the sensory neurons (Vogt and Riddiford, 1981). Odorant binding proteins are thought to be the primary proteins involved in the transport of odorants and pheromones to the olfactory receptors (Pelosi *et al.*, 1995; Vogt *et al.*, 1999).

Members of these protein families have been identified in a number of insect species including *C. quinquefasciatus* (Pelletier and Leal, 2009). Odorant-binding proteins (OBPs) are highly expressed in insect olfactory tissues and involved in the first step of odorant reception. An improved understanding of the function of mosquito OBPs with the ligands of plants may contribute in the identification of new ovicidal and repellents and may assist in the development of more efficient and environment friendly mosquito controlling strategies.

Natural products remain an important source of new drugs owing to their natural origin, cost effectiveness and lesser side effects (Cragg *et al.*, 1997). Therefore, characterization of extracts of medicinal plants is necessary, due to its numerous benefits to science and society (Kalaivani *et al.*, 2012).

In view of the above, the present investigation was undertaken with the following objectives:

- To conduct the bioassay studies and to evaluate the efficacy of selected plant extracts for the ovicidal activity against *C. quinquefasciatus*
- To evaluate the repellent activity of selected plant extracts against *C. quinquefasciatus*
- To identify the nature of the phytochemicals by spectral studies
- To find the efficacy of the identified phytochemicals on an *in silico* platform