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LABORATORY INVESTIGATION OF THE REPELLENCY OF ESSENTIAL OILS OF SOME LOCAL PLANTS AGAINST *ANOPHELES ARABIENSIS* AND *AEDES AEGYPTI* IN ETHIOPIA

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DEDICATION

This work is dedicated to my sister Nigisti Yared

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ABSTRACT

Essential oils extracted by hydro-distillation from six plant species growing in Ethiopia, *Chenopodium ambrosioides* (Chenopodiaceae), *Laggera tomentasa* (Asteraceae), E*ucalyptus camaldulensis* (Myrtaceae), *Cymbopogon citratus* (Poaceae),*Citrus sinensis* (Rutaceae) and *Ruta chalepensis* (Rutaceae), were evaluated for repellency on forearms of human volunteers against *Anopheles arabiensis* and *Aedes aegypti* under laboratory condition (at 10% and 20% concentration). At 10% concentration oils of *L. tomentosa*, *E. camaldulensis* and *Cy. citratus* protected *An. arabiensis* for up two hours with mean protection of 80.87% - 93.45%. *Chenopodium ambrosioides* produced 69.6% protection at the first hour. Two other plants (*R. chalepensis* and *Ci. sinensis*) were only highly effective for 1 hour. With increased concentrations (20%), *L. tomentosa*, *E. camaldulensis* and *Cy. citratus* gave the highest repellency (80.3% - 91%) and the longest duration of protection lasting for three hours against *An. arabiensis*. The repellency of *Ci. sinensis* and *R. chalepensis* only improved slightly from the 10% concentration. *Chenopodium ambrosioides* did not provide significant protection even for one hour at 20% concentration against *An. arabiensis*. At 10 % concentration, only one plant oil (*Cy. citratus*) gave about 91% protection lasting for one hour against *Ae. aegypti*. The remaining oils gave very weak protection starting even after the first hour of application against *Ae. aegypti*. At higher concentration (20%), five more plants except *Ch. ambrosioides* continued to give high protection (81% - 93. 4%) for only one hour of post application against *Ae. aegypti*. It was also observed that *Ae. aegypti* was more tolerant to all the candidate repellent plants than *An. arabiensis* at both concentrations. The experiment also were evaluated the 1:1 combination of essential oils against *An. arabiensis* and *Ae. aegypti*. Almost all blends failed to produce significant protection beginning from the first hour. At 20% concentration there was only a slight improvement at the first hour of the experiments. As for *An. arabiensis*, the combination of oils did not improve their potency as repellents against *Ae. aegypti* even at higher concentration of 20%. DEET, the standard commercial repellent gave much longer repellency (83- 100%) for as long as six hours of the test period against *An. arabiensis*. DEET continued to give > 90 % protection for about six hours against *Ae. aegypti.*

Benshangul region. The number of death was around 100, but the seriousness of the epidemic has not been well known (Mekonnen and Kloos, 2006).

West Nile (WN) virus is a mosquito borne virus of the genus *Flavivirus*, family *Flaviviridae*. WN, Japanese encephalitis, St. Louis encephalitis, Murray Valley encephalitis, and Kunjin viruses (along with other viruses) belong to the Japanese encephalitis serocomplex and are closely related to each other genetically and ecologically. All the viruses are maintained in cycles involving birds as vertebrate hosts and *Culex* species mosquitoes, principally *Culex pipiens* as vectors. WN virus has a wide geographic distribution in Africa, west and central Asia, the Middle East, and Europe. Historically, epidemics have been infrequent and not associated with severe disease (Deubel *et al*., 2001).

Aedes aegypti is the main vector of dengue and urban yellow fever. *Ae. albopictus* is a secondary dengue vector in South-East Asia and the western Pacific. *Ae. polynesiensis* and *Ae. pseudoscutellaris* are arboviral and filarial vectors present in the South Pacific. Other *Aedes* species, including *Ae. africanus* and *Ae. simpsoni sensu lato*, are important vectors of yellow fever outside urban areas (WHO, 2006). *Ae. aegypti* lives in close association with humans, typically breeding in household water-storage containers and other artificial rain-filled containers in the domestic environment, including roof gutters, discarded tyres and food containers. In some towns and cities, underground catchment's basins and storm drains are important larval habitats (Service, 2000). *Ae. albopictus* uses both artificial and natural breeding sites (domestic and per domestic), whereas all the other important *Aedes* species prefer natural breeding sites, such as tree holes, plant axils and coconut husks. The domestic forms of *Ae. aegypti* feed indoors and outdoors, mainly in the early morning and the last 3–4 h of daylight and prefer to rest indoors in isolated places, e.g. under sinks, in curtain folds or in wardrobes. Several species of *Aedes* and *Culex* species are also involved in the transmission of West Nile virus.

Malaria is the most important parasitic disease in the world and remains of highest public health importance. The global incidence of malaria has been estimated at 300-500 million clinical cases annually, causing 1.5 to 2.7 million deaths each year and the disease is a

leading cause of infants, young children and pregnant women mortality in sub-Saharan Africa (WHO, 1995; WHO, 1997; WHO, 2007). More than 90 % of this malaria burden occurs in sub-Sahara Africa (SSA), where severe malaria disease and death mainly occur among young children of rural areas with little access to health services (Snow *et al*., 1999).

Malaria is a disease caused by infection with parasites of the genus *Plasmodium.* Four species of *Plasmodium (P. falciparum, P. malariae, P. ovale and P. vivax)* infect humans and lead to disease (Gilles, 1993). Transmission of the *Plasmodium* parasite is mainly from person to person through the bite of a female *Anopheles* mosquito.

The epidemiological situation of malaria is worsening with the spread of drug resistance in the parasite and insecticide resistance in the vector. More evidence points to significantly increasing malaria morbidity and mortality is Sub-Sahara Africa (SSA) due to the development by *Plasmodium falciparum* of resistance to existing first-line drugs such as chloroquine and sulphadoxine/pyrimethamine (Trape, 2001).

Malaria in Ethiopia generally occurs in low land areas below 2000 meters altitude is considered malarious. Occasionally, malaria transmission also occurs in previously non-malarious areas including areas having above 2500 meters altitude when microclimate and weather conditions favorable for the breeding of mosquitoes prevail. As a result, short-lived severe epidemics occur. Malaria epidemics generally occur immediately after the long rains in September to November and in some places also after the short-lived shower rains in April to May. Three-forth of the land area of the country is considered to be either malarious or potentially malarious, and about 65% of the populations in these areas are at risk of infection (Mitiku *et al.,* 2003). Due to the diversity of topography and variation of climate of the country the nature of the malaria transmission in most parts of Ethiopia is unstable (MOH, 2003). Hence, rampant malaria epidemics that result in huge morbidity and high mortality often occur. Furthermore, the transmission of the disease generally occurs during the peak cultivating and harvesting period of the year and has a tremendous impact on the agriculture productivity. The country had experienced the worst malaria epidemics in 1958 with an estimated three million malaria cases and 150,000 deaths (MOH, 2003).

Lymphatic filariasis (LF) is the second most common vector borne disease, after malaria. LF is caused by three species of nematode parasites, which can be spread by a wide range of mosquito species. The World Health Organization estimated the global burden of infection to be 120 million cases, with 1 billion people being at risk of infection (WHO, 1999). It has a widespread geographic distribution, mainly in the tropical regions of the world. *Wuchereria bancrqfti* is the most common species and accounts for 90% of cases. *Wuchereria bancrofti* parasites are mainly transmitted by *Culex quinquefasciatus* mosquitoes and some species of *Anopheles*. *Brugia malayi* parasites are mainly transmitted by *Mansonia* mosquitoes and confined to East and Southeast Asia, and *Brugia timori* is found only in Timor and nearby islands (WHO, 1999).In Ethiopia lymphatic filariasis is widespread in many regions, particularly, Kaffa, Illubabor, Wollega and Gamo Gofa are considered to be endemic(Hailu *et al*., 2006). Jemaneh and Kebede (1995) described the prevalence of microfilariae in Gambella.

1.2. Major malaria vectors

The *Anopheles* vector is the link between man and the malaria parasite. Because the sexual cycle takes place in the mosquito, it is sometimes called the definitive host. There are over 400 different species of *Anopheles,* but there are only about 70 that are vectors of malaria and of these, about 40 are important (Service, 2000). The most important vectors in the Afro tropical region are the *Anopheles gambiae* complex (which includes *An. gambiae* s.s*, An. arabiensis, An. melas, An. merus, An. bwambae,* and *An. quadriannulatus* A and B) and *An. funestus* (Service, 2000). Among the *An. gambiae* complex, *An. gambiae* s.s is the most important malaria vector and it is probably the world's most efficient vector (Service, 2000). It breeds in sunlit pools, puddles, borrow pits and rice fields. It bites humans both indoors (endophagic) and outdoors (exophagic), and rests mainly indoors (endophilic) but may also rest outdoors. The other important species of the *An. gambiae* complex, *An. arabiensis* has similar breeding and biting habits to *An. gambiae* s.s. except that it tends to occur in drier areas and it exhibits both anthropophagic and zoophagic behaviour depending on the availability of the host..

Forty-two species of Anopheline mosquitoes are recorded in Ethiopia. In Ethiopia the major malaria vectors are *An. arabiensis*. Outside the members of the *An. gambiae* complex; *An. pharoensis, An. funestus* and *An. nili* are considered secondary vectors (Tulu, 1993).

1. 3. Mosquito control

The variable nature of the disease, its vectors, and the vulnerability of particular human population, WHO stresses the need for a range of malaria control approaches in its Global Malaria Control Strategy (WHO, 1993). WHO recommends an integrate approach that relies on early case identification and treatment as well as selective and sustainable prevention measures, including vector control.

Options for vector control include environmental management, chemical control, biological control, genetic control and personal protection methods. 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.

1.3.1. Environmental management

Vector control experts have recognized the value of changing mosquito larval habitats to reduce or eliminate mosquito-borne diseases. Habitat elimination or modification efforts have included general programs to reduce the abundance of all mosquitoes as well as more targeted projects of "species sanitation" directed at the principal malaria vectors (Bruce-Chwatt, 1985). The concept of modifying vector habitat to discourage larval development or human vector contact is generally referred to as environmental management (EM). Source reduction by environmental management, includes drainage, flushing, filling, and rendering river and lake margins unsuitable for Anopheline breeding. These are the classical methods of malaria sanitation, which may be used for all mosquitoes breeding in general or targeted to the specific breeding places of malaria vectors of local importance. They are effective and have long-term effects. However, these methods have relatively high investment costs and

may be cost-effective only in urban areas or some types of development projects (WHO, 1982).

1.3.2. Chemical control

Chemical control of adult female mosquitoes has been the most widely successful vector control method since the 1940s. The most common practice is indoor residual house spraying (IRS), in which the inside walls, the ceiling, and sometimes the outside eaves, porches, and nearby animal sheds is sprayed with a persistent insecticide. The rationale for IRS is based on the behavior of those *Anopheles* species that rest on walls before or after biting humans (Chavasse and Yap, 1997).

DDT and nine other insecticides (two carbamates, two organophosphates and five pyrethroids) commonly used for residual house-spraying in malaria control programmes. The use of DDT for indoor residual spraying has declined substantially over the past 30 years, but this insecticide is still considered valuable for malaria control, mainly because of its low cost relative to alternative insecticides. Despite the development of resistance to DDT in some populations of malaria vector *Anopheles* mosquitoes, DDT remains generally effective when used for house-spraying against most species of *Anopheles* (Walker, 2002).

Recent evidence from Africa indicates that pyrethroid and DDT resistance is more widespread than anticipated. It is believed that the same level of resistance will have a more detrimental impact on the efficacy of IRS (WHO, 2007). Heavy agricultural use of a particular insecticide can reduce its subsequent efficacy for indoor vector control (Georghiou *et al*., 1973). In some cases, resistance created by previous use of DDT and other insecticides for house spraying as well as agricultural uses may also confer cross-resistance to new insecticides or may stimulate the development of multiple-insecticide resistance within the vector population (Beach *et al*., 1989). Research showing some cross-resistance between DDT and pyrethroids raises special concern (Chakravorthy and Kalyanasundaram, 1992; WHO, 1992). The increasing problems associated with resistance argue for resistance

management through the use of multiple vector control methods, including non-chemical tactics (Roberts and Andre, 1994).

1.3.3. Biological control

A wide range of organisms assists to control mosquito populations naturally through predation, parasitism, and competition. Biological control refers to the introduction or manipulation of these organisms to suppress vector populations. At present, the principal biological control agents that have been successfully employed against mosquito are predators, particularly fish, and the bacterial pathogens *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) that attack the larval stages of the mosquito (Das and Amalraj, 1997). Other organisms showing promise include a number of fungal pathogens, the nematode *Romanomermis culcivorax*, and the aquatic plant Azolla (Lacey and Lacey, 1990).

1.3.4. Genetic control method

Recent advances in insect genetic engineering have opened up new possibilities in the genetic control of insect vectors of human diseases. Several genetic methods of mosquito control are being studied under laboratory conditions, a few, including the genetic transformation of *Aedes*, *Anopheles* and *Culex* mosquitoes, and the sequencing of the *Anopheles gambiae* genome, population suppression of mosquitoes using Sterile Insect Techniques (SIT) (Colaman and Alphey, 2004). In addition to these, currently exist in the development and field-testing of the more efficient methods of vector control are sterile male techniques, cytoplasmic incompatibility, interspecific incompatibility, chromosomal translocation, lethal factors and hormonal induced sterility. Genetic control method is a species specific and environmentally non-polluting means of controlling insect populations (Colaman and Alphey, 2004).

1.3.5. Personal protection methods

Because of the nocturnal feeding habits of *Anopheles* mosquitoes, malaria transmission occurs primarily between dusk and dawn. People can usually advise take protective measures to reduce contact with mosquitoes, especially during these hours. Such measures include using mosquito nets, protective clothing and use of repellents.

1.3.5.1 Insecticide-Treated Bed Nets

Insecticide-treated bed nets (ITNs) are a form of personal protection that has repeatedly been shown to reduce severe disease and mortality due to malaria and other mosquito borne diseases in endemic regions. The malaria vector species tend to bite at night, mosquito nets would be expected to protect against them effectively. Mosquitoes can, however, enter through holes in torn nets or can bite human skin in contact with the netting. In order to avoid this problem, nets are treated with insecticides that are safe for humans in close contact. Nets treated with pyrethroids are fairly effective in preventing biting, even if they are torn. Furthermore, treated nets act like mosquito traps baited by the odour of the occupant; thus, when most people in a community are using treated nets, large numbers of mosquitoes are killed (WHO, 2006). Insecticide-treated mosquito nets are expected to be most effective against mosquitoes that bite indoors when people are sleeping. These conditions apply to the world's most important malaria vectors *An. gambiae* and *An. funestus* in Africa. ITNs have also been used successfully outdoors, in areas such as northern Ghana where people to sleep outside in hot weather. In areas highly endemic for malaria, where immunity to the disease is important, very young children and pregnant women are the most vulnerable because their immunity has not yet developed or is temporarily reduced (WHO, 2006).

ITNs have consistently been shown to be very effective and sustainable in reducing malaria morbidity and all-cause mortality in children of different malaria endemic areas (Lengeler, 2004). They have also been shown to be highly cost-effective and are actually one of the most affordable control tools (Curtis *et al*., 2003). Moreover, the successful development of long-lasting ITNs avoids the regular re-treatment of ITNs, every 6 to12 months, which was accompanied by a notoriously low compliance (Guillet *et al*., 2001).

In Ethiopia, the use of ITNs introduced in selected areas as one of the important malaria control measures in 1997/98 on cost recovery basis (MOH, 2000). Since 1998, ITNs have

been introduced throughout most endemic areas a major control strategy (Adhanom *et al.,* 2006). But there are disadvantages: in hot climates, nets may be uncomfortable to use due to poor ventilation, it is used at bedtime but most people are bitten outdoors before bedtime.

1.3.5.2 Repellents

Repellents are normally applied directly to skin or clothing and other fabrics e.g. arm and ankle bands, or mosquito screens.

There are mainly two kinds of repellents, which are described below.

1.3.5.2.1. Synthetic repellents

Synthetic repellents are a common means of personal protection against biting arthropods. The most effective synthetic repellent against a wide range of vectors is DEET (N,N-diethyl-meta-toluamide), an ingredient in many commercially available insect repellents. The actual concentration of DEET varies widely among repellents. DEET formulation as-high-as 50% is recommended for both adults and children above two months of age. Repellents are recommended for people staying outdoors at night for work or leisure and those working in plantations. Repellents are available in various forms (cream, lotion, soap, jelly and oil) and modes of application. Repellents prevent human-biting insect contact by acting as an irritant to the insects. A reduction of the activating or attractive host odors by a repellent could be based on one or more of the following mechanisms: Electrophysiological tests on the relevant receptors of *Ae. aegypti* have shown that the combination of DEET and lactic acid (a component of the host odour) reduces or inhibits the response of the receptor cells involved (Davis, 1985). A change in the olfactory input signals caused by the repellent, with the result that the characteristic host odour pattern generated by the receptor cells is changed by the central nervous system to another pattern which triggers an avoidance reaction (Boeckh *et al*., 1996).

But, synthetic repellents are rarely used to protect communities from malaria and other vector-borne diseases. Cost and sometimes, safety constraints are the main reasons for this situation (Costantini *et al.*, 2004). There have also been concerns over the toxicity of DEET. It irritates the eyes and mucous membrane when applied on the face (Osimitz and Grothaus,

1995). Other disadvantages are associated with the use of DEET that it acts as a solvent of paints, varnishes, and some plastic and synthetic fabrics, which led to the consumer rejection of DEET-based products (Trigg, 1996).

1.3.5.2.2. Plant-derived repellents

Essential oils are naturally occurring substances responsible for the characteristic odor of an aromatic plant. Besides playing a role in the biological processes of the plant, the essential oils in its leaves can serve two additional functions: their odor attracts pollinating insects; and they repel pests, bacteria and viruses which could harm the plant. Some essential oils are found in the peel of fruits such as lemon and orange, or in roots, twigs and other parts of a plant. Plant essential oils, commonly used as fragrances and flavoring agents for foods and beverages, were recommended as an alternative source constituting numerous bioactive phytochemicals that can be potentially used for insect control (Isman, 1999). Monoterpenes, both cyclic and acyclic, are major components of many essential oils, and are the most important group to consider in terms of repelling insects (Choi *et al*., 2002). In addition to this eucalyptol, d-pinene, geraniol, citronella, camphor and linalool are constituent of essential oil of different plant species and have natural repellent properties (Curtis *et al*., 1990).

Generally, essential oil-derived compounds, with a few exceptions, can be applied to humans in a similar way to other conventional insecticides and they tend to be selective and have little or no harmful effects (Mumcuoglu *et al.,* 2002). The promising essential oils with repellent activity are derived from a large number of plants including *Corymbia citriodora* (Collins and Brady, 1993), *Cymbopogon* spp. (Ansari and Razdan, 1995), *Pelargonium citrosum* L (Geraniaceae) (Matsuda *et al.,* 1996), *Mentha piperita* L (Ansari *et al.,* 2000), *Ocimum* spp. (Tawatsin *et al.,* 2001), *Zanthoxylum limonella* (Dennst.) (Rutaceae)(Das *et al.,* 2003), *Conyza newii* Oliv. and Hiern.(Asteraceae)., *Plectranthus marrubioides* Benth. (Labiateae), *Tetradenia riparia* Herba (Lamiaceae), *Tarchonanthus camphoratus* Herba(Asteraceae), *Lippia javanica* (Burm.f.) Spreng.(Verbenaceae), and *Lippia ukambensis* Vatke, (Verbenaceae) (Omolo *et al.,* 2004). Furthermore, the five most effective oils were those of Litsea(*Litsea cubeba* Pers.), Cajeput(*Melaleuca leucadendron*),Niaouli(*Melaleuca*

quinquenervia (Cav.) S.T.), Violet (*Viola odorata* L.), and Catnip(*Nepeta cataria* L.) (Lamiaceae), which induced a protection time of 8hr at the maximum and a 100% repellency against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*(Amir and Mehlhorn, 2006). Several alternative mosquito repellents contain some plant oils such as pennyroyal, citronella, eucalyptus, soybean, or peppermint as putative active ingredients (Barnard and Xue, 2004). In addition to these, essential oils with potential repellent properties have been obtained from plants in several families contains several well-known repellent plants including the Lamiaceae family includes basil (*Ocimum basilicum* L.), mint (*Mentha* spp.), hyptis (*Hyptis suaveolens* L.), lavender (*Lavandula* spp.), sage (*Salvia* spp.), and thyme (*Thymus* spp.). The Myrtaceae family includes eucalyptus and tea tree (*Melaleuca* spp.) and the Poaceae includes citronella, lemongrass and palmarosa (*Cymbopogon* spp.) are used as repellent of mosquitoes (Trongtokit *et al*., 2005). Some promising essential oils citronella, lemon-eucalyptus, neem oil, peppermint oils are currently available in several commercially formulated repellents (Curtis *et al*., 1990; Pitasawat *et al*., 2003).

However, the repellency of these products is commonly lower in both efficacy and duration than that of synthetic repellents, principally DEET. Laboratory studies by Trigg and Hill (1996) on the commercial product of lemon-eucalyptus repellent was almost as effective as DEET against *Anopheles gambiae*, which is the primary malaria vector in Sub-Saharan Africa.

Nowadays, the search for alternative or additional, simple, environmentally safe and cost effective tools is underway in different parts of the world. Plant based repellents that reduce man-vector contact have the advantage that can be cheap, practicable and produced locally. The present investigation was carried out attempt to contribute to the general global effect on the search for better plant-based repellents from essential oil derived from some selected plant found in Ethiopia.

2. OBJECTIVES

2.1. General objectives

The main objective of this study to investigate the potential of the essential oils of some local plants (*Ch. ambrosioides, E. camaldulensis, L. tomentosa, Cy. citratus Ci. sinensis* and *R. chalepensis*) as repellents against some mosquito species in Ethiopia with a view to contribute to vector control efforts.

2.2. Specific objectives

- To evaluate essential oils of the plants mentioned above for their repellency and duration of protection against the laboratory colonies of *Anopheles arabiensis* and *Aedes aegypti.*
- To compare the relative effectiveness of repellency of the essential oils of the plants against laboratory colonies of *An. arabiensis* and *Ae. aegypti.*
- To determine the blend effects of the essential oils against laboratory colonies of *An. arabiensis* and *Ae. aegypti*.

3. MATERIALS AND METHODS

3.1. The test plants

Chenopodium ambrosioides **L. (Chenopodiaceae), Mexican Tea, Wormseed, Amedmada(Amh).**

Chenopodium ambrosioides (*Ch. ambrosioides*) is native to South and Central America. But now this aromatic species can be found scattered throughout continents. It can be easily identified because of its scent, large size, and toothed leaves. It is a herb that grows to a height of 40cm and rich in waste ground, dumps, fields, railroads, roadsides. The flowers are small and green, and the seeds are very small and green when fresh and black when dry. The species has a distinct aromatic smell due to the presence of large quantities of essential oil (Quarles, 2006).

Chenopodium ambrosioides contains essential oils, which were traditionally used to combat intestinal parasites. The oil has been reported to have medicinal and insecticidal properties (Malik and Mujtaba Naqvii, 1984; Su, 1991). Chiasson *et al.* (2004) reported that the essential oil extraction of *Ch. ambrosioides* was effective against the adult and egg stages of *Tetranychus urticae* (spider mites), *Panonychus ulmi* (European red mite) and *Mytus persicae* (peach aphid).

The chemical composition of essential oil obtained from aerial parts of *Ch. ambrosioides* contains alpha-terpinen, p-cymene, cis- β -farnesen, ascaridole and carvacrol are as major constituents (Tapondjiou *et al*., 2002). In Ethiopia, the volatile oils of *Ch. ambrosioides*(leaves) showed the highest larvicidal activities against third and fourth instar *An. arabiensis* larvae after 24 hours exposure (Massebo, 2006).However its repellent properties against mosquitoes and other biting insect has not been evaluated in Ethiopia.

***Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern(Asteraceae), Keskese(Amh)**

Laggera tomentosa is a perennial fragrant bushy herb endemic to Ethiopia, found in Shewa region of Ethiopia. *Laggera tomentosa* and some other species of the genus have a reputation of being used in ethnomedical practices in Asia and Africa (Asfaw *et al.*, 1999). The oil isolated by hydro-distillation was yellowish in color, with the characteristic sweet and persistent odor of the plant. The oil characterized by a high percentage of oxygenated monoterpenes (78%), with chrysanthenone (57.5%), isochrysantenone (fi.8%) filifolone (5.2%) and (Z)-isogeranic acid (4.5%) as the major constituents (Asfaw *et al.*, 2003).

The oil of *L. tomentosa* differed from the oil of the other species in the genus in that it possessed a high percentage of oxygenated monoterpenes and a relatively low percentage of oxygenated sesquiterpenes. *Laggera tomentosa* oil has a strong pleasant smell of chrysantenone and a sweet floral character reminiscent of the plant. Upon biological screening of a series of African medicinal plants, substantial phytotoxic activity was found in the leaves of *Laggera decurrens* (Vahl.) Hepper and Wood (Van Puyvelde *et al*., 1999). However, the essential oil of *L. tomentosa* has not been evaluated against biting arthropods in Ethiopia or elsewhere. There is also no information available on repellency properties other species of *Laggera*.

***Eucalyptus camaldulensis* Dehn. (Myrtaceae) Key beharzaf (Amh)**

Eucalyptus camaldulensis is originated in Australia. It now grows in almost all tropical and subtropical areas and is cultivated in many other climates. Much research has been conducted on the medicinal properties of *Eucalyptus* spp. Of the different species, *E. globulus* has been the most widely studied. *Eucalyptus* is used to treat many human ailments and some livestock ailments. *Eucalyptus* extracts, oils, or fresh leaves are used in steam inhalation treatments, consumed in teas, or used in bathing. The essential oil of lemon eucalyptus (*E. citriodora*) is a well-known commercially available mosquito repellent (Curtis *et al*., 1990). The essential oils of *E. radiata*, *E. globulus* and *E. dives* have reported to repellent activities against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Amer and Mehlhron, 2006).

There are many compounds in *Eucalyptus* spp. While the same compounds exist in many of the species, some compounds can be found in only one or a few species. Within species the quantity of essential oil and the specific compounds in the essential oil and extracts of dry and fresh leaves, buds, mature fruit, and bark vary with the origin of the tree and the age of the leaves. Dagne *et al.* (2000) reported that the oil of the steam distillate of *E. camaldulensis* contains beta-phellandrene, p-cymene and cryptone as major constituents. *Eucalyptus camaldulensis* essential oil (from the leaves) ranges from less than 1 to over 2%. The quantity of 1, 8-cineole in the oil ranges from 15-78%. Other compounds in the leaves include: betulinic acid eucalyptic and eucalyptolic acid oleanolic acid ursolic acid. *Eucalyptus camaldulensis* ethanol extracts showed activity against bacteria and fungi. In Japan essential oil of *E. camaldulensis* has been reported to exhibit repellent activities against *Ae. aegypti* (Watanabe *et al*., 1993). However, *E. camaldulensis* is widely grown in Ethiopia but its repellent activity against mosquitoes has not been evaluated in Ethiopia.

***Cymbopogon citratus* Stapf (Poaceae), Lemmon grass, Tej sar(Amh)**

Cymbopogon citratus (*Cy. citratus*) is one of about 55 species of grass in the genus *Cymbopogon*. It is a fragrant tropical grass. It is said to be indigenous to India where it has been cultivated for its oil since 1888. However, it grows widely in many tropical countries of Asia, America, and Africa. Traditional Indian medicine uses lemongrass for fever and infectious illness. Also used as insecticide, bactericide, antiseptic and food flavoring (Sidibe *et al*., 2001). Ofuya and Okuku (1994) reported the insecticidal activity of *Cy. citratus* oil.

This plant is one of the chief sources of Citral, which is an important raw material of perfumery, confectionery, and beverages. Field tests in Bolivia showed that 25% *Cy. citratus* in ethanol provided 77.93% and 90.67% protection for 3 hours against *An. darlingi* and *Mansonia* spp. Respectively (Moore, 2005). However, laboratory evaluation has shown far lower repellency at only 30 min complete protection (Trongtokit *et al*., 2005).

Cymbopogon winterianus essential oil has been evaluated as a mixture with 5% vanillin against *Ae. aegypti, Cx. quinquefasciatus,* and *An. dirus*. It compared favorably with 25% DEET giving greater than 6 hrs protections against all three-mosquito species in cage

experiments (Tawatsin *et al*., 2001). *Cymbopogon excavatus* evaluated in the laboratory against *An. arabiensis* gave good protection for 2 hrs, but declined to 59.3% protection after 4 hrs, which compares favorably with *Cy. nardus* (Govere *et al*., 2000b). The main component of lemongrass oil is citral, 65-85%, myrcene 12-25%, dipentene, linalool, geraniol and others. Flexuosus – includes citral up to 85%, geraniol, methyl eugenol, borneol (Sidibe *et al*., 2001). The essential oil of *Cy. citratus* has not been evaluated against mosquitoes in Ethiopia.

***Citrus sinensis* (L.) Osbeck (Rutaceae), Orange peel, Birtukan(Amh)**

Citrus sinensis (*Ci. sinensis*) is widely cultivated fruit free in sub-tropics including Ethiopia. The essential of *Ci. sinensis* have repellent activity against mosquito bites(Abdullah *et al*., 2005). Yang and Ma (2005) reported that application of rutaceae oil (*Ci. sinensis*) on mice, under laboratory condition, provided more than 90% protection for 8 hours against *Ae. albopictus*. In some parts of the country an orange peel which is containing volatile oils are burned at night to drive off mosquitoes (Don-Pedro, 1985).

Kidane (2005) found that *Zabrotes subfascitus* was most susceptible to the essential oil of *Ci. sinensis* compared to *Sitophilus* and have lower significant in repellency against *Z. subfascitus*. He also evaluated fumigation toxicity of essential oil of *Ci. sinensis*, which is provided high mortality against *Z. subfasciatus*. The essential oils of *Ci. sinensis* (sweet orange) and *Citrus aurantifolia* (lime) reported have insecticidal activity against mosquito, cockroach and housefly (Ezeonu *et al*., 2001).Limonene found to be the major component of *Ci. sinensis* (Sharma and Tripathi, 2006). While mixed oils of *Citrus aurantium* and mustard oil provided significant protection against *Ae. albopictus* (Das *et al*., 2003). However, its repellency activity has not been evaluated in Ethiopia.

***Ruta chalepensis* L. (Rutaceae), Rue, Tena-Adam (Amh)**

Ruta chalepensis is native to Europe, likely to the Mediterranean region; rue now grows in many parts of the world, including Ethiopia. It is often cultivated as a garden ornamental and as a medicinal plant. Rue is a strongly aromatic evergreen perennial that grows to about three feet. The small, erect bush produces shoots that are a pale green and appear to be covered in

oil glands. It produces small yellow flowers; and its fruit contains volatile oil that gives it a bitter taste.

It occurs widely in Ethiopia and the leaves and fruits are important medically to flavor milk, cottage cheese, coffee and tea. 2-undecanone, 2-nonanone and 2-nonyl acetate were reported to occur as the major constituents of oil from *R. chalepensis* grown in India (Bagchi *et al*., 2003).

In Ethiopia, Atsebeha (2005) evaluated repellency of *R. chalepensis* together with four other plants by traditional means against *An. arabiensis, An. pharoensis* and some culicine mosquitoes. He reported that the thermal expulsion and direct burning of seeds of *R. chalepensi*s were good repellents of mosquitoes. Hadis *et al*. (2003) reported that at 50% concentration of essential oil of *R. chalepensis* 91.6% repellency protection was recorded against *Mansonia* spp. in Gambella area. However, the essential oil of *R. chalepensis* has not been evaluated against *An. arabiensis* and *Ae. aegypti* in Ethiopia.

3.2. Collection of plant material

The parts used for extraction of oil of the six plants and their localities of collection are summarized in Table 1.

Table 1 Description of six essential oils that were obtained from hydro distillation in the laboratory.

Plant species	English name	Plant parts used	Locality of collection
Ch. ambrosioides	Worm seed	Leaves and Seed	Addis Ababa
L. tomentosa	-	leaves	Addis Ababa
E. camaldulensis	Eucalyptus	Leaves	Wondo Genet
Cy. citratus	Lemmon grass	Leaves	Wondo Genet
Ci. sinensis	Sweet Orange	peel	Wondo Genet
R.. chalepensis	Rue	Leaves and Seed	Addis Ababa

3.3. Extraction of essential oils

From these plants volatile oils were extracted by hydro-distillation at the Essential Oil Research Center, Addis Ababa. In this process, the parts were cut into small pieces and placed into a distillation flask with much water. The distillation chamber were heated at about 120^0c and allowed to boil until the distillation was completed. The distillate was collected in a separating funnel in which the aqueous portion separated from the volatile oil. The water layer was slowly drawn off until only the oil layer will remain. The oil was collected in a cylinder at about 4^0c until it was tested for mosquito repellency.

3.4. Test mosquitoes

Healthy adult human volunteers (males) (aged 16–38 years) were used for the experiment. Evaluation of the extracted oils for their repellency was conducted under laboratory conditions against laboratory colonies of female *Anopheles arabiensis* and *Aedes aegypti* (76^{th} and 54^{th} generations, respectively) which are maintained at the Insectary of Aklilu Lemma Institute of Pathobiology, Addis Ababa University. The mosquitoes were kept at

27 ± 2 ^{0}C and 60 to 70% relative humidity. Adults maintained in screened cages were provided with continuous access to 10% sugar solution. Rabbits are used as a source of blood meal for the female mosquitoes. Prior to testing, female mosquitoes (3–5 days old) were starved for 8–12 hr (overnight). Tests were conducted during the day in a dark room using cages (30cm× 30cm× 30cm) (Curtis *et al*., 1987).

3.5. Dose response tests

Thirty nulliparous females (3-5 days old, starved for about 12 hours) were placed into laboratory test cage during all tests. Clean cages and fresh mosquitoes were used for each test. First, acetone treated fore-arm (control arm) was exposed in the cage containing test mosquitoes for 30 seconds, and the number of landing mosquitoes were recorded (to ensure mosquitoes were interested to bite). Mosquitoes were shaken off the arm before they had a chance to imbibe any blood. Immediately there after, the acetone treated fore-arm was removed and the treated other arm was exposed to the same cage after repellent oil formulation were applied evenly between the wrist and elbow and dried for 2 minutes. The forearm was treated with acetone solutions of the test essential oils. The amount of each test oil applied on the arm was determined from the surface of the arm according to the following formula (Frances *et al*., 1998). This resulted between 0.5-0.6ml of the test oils applied on the arm of the volunteers.

$A = (1/3(a+b+c))\times h,$

Where,

A = application area,

a = circumference of the arm at the joint between humerus and radius/ulna,

b = circumference of the arm halfway between joint and wrist,

c = circumference of the arm at wrist,

h = length of the forearm between joint (elbow) and wrist.

In this study four adult (males) volunteers were involved. The arms were first cleaned with water and 95% ethanol, and air-dried before application of the test oils and the control (acetone). A surgical glove was worn on the hand (fingers and palms) during each test to prevent biting on the untreated hand. Each test consisted of two parts where each volunteer used the right arm for treatment and the left arm for control. The dose response tests were conducted on a series of concentrations of the oils (1% to 10%). The concentrations were tested one after the other (from the lowest to the highest) to the caged mosquitoes for particular test oil until no landing/probing was recorded by the concentration of the test oil. The maximum concentration of oil that prevented complete landing/probing during the 30 seconds exposure was then tested for its duration of protection and percentage repellency described below.

3.6. Evaluation of protection time

Protection duration of the essential oils of all candidate plants was determined at 10 %(chief score complete protection at 30 seconds in the dose response seen) and 20 %(v/v) in acetone concentrations for comparison. The control acetone and test oils applied on the forearms were tested in similar way described in dose response test above. Commercial 15% concentration DEET was also tested as a standard reference with the candidate essential oils. Separate cages in which 30 female nulliparous and unfed mosquitoes were used for control and treated arms to avoid contamination of the control cage with oil. The exposure time was for 1 minute and any mosquito landing and biting were counted. The time interval between each exposure was 1 hour. Between the time interval in between the exposure period, volunteers were instructed not to rub, touch, or wet the treated arms and were restricted to stay in a room to minimize the loss of the oils from the treated forearms. The testing period lasted up to 8 hours, depending on the efficacy. Four replicates were conducted for each test of concentrations with an identical test on different days for each of the human volunteers and no one was tested more than one test oil per day.

Duration of protection time for blend effects of 15 combinations of the six essential oils at 10% and 20% concentrations were also tested against mosquitoes. The combinations were

made by mixing equal volume of the oils in 1:1 (v/v) ratio, after which the required test concentration (10% and 20%) v/v in acetone was made for the test.

3. 7. Data analysis

The percentage of repellency was evaluated in both the dose response studies at each time interval in the evaluation of protection time based on the following formula (Yap *et al*., 1998).

% Repellency = $\frac{C-T}{C}x100$, where C = number of mosquitoes landing/biting on control arm; and T = number of mosquitoes landing/biting on the treated forearm.

The median effective dosage (ED_{50}) and ED_{95} of the oils were determined using computer software for probit analysis by SPSS 13.0 for windows. Effectiveness of the test oils was determined by comparing the 95% confidence intervals of the ED_{50} and ED_{95}values.

The percentage repellency among the oils was compared using one-way ANOVA, and the means were separated using Tukey's honestly significant difference (HSD) test at P=0.05.

4. RESULTS

4.1. Dose response studies of the essential oils

This study evaluated the potential of repellency of volatile oils of six plants against laboratory-reared mosquitoes (*An. arabiensis* and *Ae. aegypti*) at different concentrations (1%, 2.5%, 5%, 7.5% and 10%) in dose response studies.

Table 2 shows the ED_{50} and ED_{95} values of the volatile oils of the six candidate repellent plants species against *An. arabiensis* in dose response studies based on Probit analysis. *Cymbopogon citratus* showed the lowest ED_{50} (3.346%; 95%CI=2.285- 4.236) and ED_{95} (8.69%; 95%CI=7.271 -11.312) values, thus showing highest repellent activity among of the candidate plants. *Citrus sinensis* was the weakest repellent plant with ED_{50} (6.63%; 95%CI= 5.678- 7.775) and ED_{95} (12.06%; 95%CI=10.295-15.389). Based on the 95% confidence interval, there was no significant difference between all the plant oils at ED_{50} value; however, at $ED_{50,}$ *Ci. sinensis and Chenopodium ambrosioides* were significantly different from the remaining plants, although both were not significantly different from each other.

Similarly, Table 3 reveals the results of the dose response analysis of the six volatile oils against *Ae. aegypti.* The ED_{50} values ranged from 4.210 % (95%CI=3.097-5.242) for *Cy. citratus* to 6.726% (95%CI=5.610-8.226) for *Ruta chalepensis*. The ED_{95} values ranged from 10.337% (95%CI=8.571-13.813) for *Cy. citratus* to 13.33(95%CI=11.009-18.147) for *R. chalepensis*. Thus *Cy. citratus* showed the highest level of repellent activity at both values as for *An. arabiensis*. Thus at the level of $ED_{50,}$ three plants (*Cy. citratus, Laggera tomentosa,* and *Eucalyptus camaldulensis)* were significantly different from the remaining three other plants. However, at ED_{95} value, all the six plants exhibited no significant difference between them based on the 95% confidence interval.

Table 2.The ED_{50} and ED_{95} values of essential oils of the six test repellent plants against adults of *An. arabiensis* in dose response studies.

Plant species	ED_{50}(95%CI)	ED_{95}(95%CI)	X^2(df=3)
Cy .citratus	3.346(2.285- 4.236)	8.688(7.271 -11.312)	1.377
E. camaldulensis	3.597 (2.231-4.697)	10.457(8.522-14.499)	5.217
L. tomentosa	4.161(2.952-5.246)	10.770(8.852-14.687)	4.968
R.. chalepensis	6.483(3.972-11.051)	12.041(8.832-31.262)	5.919
Ci. sinensis	6.630(5.678- 7.775)	12.057(10.295-15.389)	4.319
Ch. ambrosioides	6.329(5.243-7.682)	13.007(10.789-17.506)	3.235

Table 3 The ED_{50} and ED_{95} values of essential oils of the six test repellent plants against adults of *Ae. aegypti* in dose response studies.

Plant species	ED_{50}(95%CI)	ED_{95}(95%CI)	X^2(df=3)
Cy. citratus	4.210(3.097-5.242)	10.337(8.571-13.813)	2.943
L. tomentosa	4.433(3.418- 5.399)	10.332(8.695-13.375)	3.215
E. camaldulensis	4.501(3.158-5.767)	11.980(9.667-17.058)	1.904
Ci. sinensis	6.326(5.426- 7.361)	11.527(9.926-14.404)	.509
Ch. ambrosioides	6.650(5.704-7.825)	12.195(10.391-15.555)	.553
R. chalepensis	6.726(5.610-8.226)	13.331(11.009- 18.147)	3.121

4.2. Duration of repellency of the essential oils

This study investigated that the duration of protection of the volatile essential oils of the six repellent plant species against *An. arabiensis* and *Ae. aegypti* under laboratory condition at 10 and 20% concentrations in relation to the standard synthetic repellent (DEET).

The duration of protection at 10 % concentration of each oil against *An. arabiensis* is summarized in figure 1. All the oils from the six plants gave very high protection (> 90%) soon after application. However, three plants (*L. tomentosa, E. camaldulensis* and *Cy. citratus*) protected biting of *An. arabiensis* for up two hours with mean protection of 80.87% - 93.45%. After two hours however, their protection declined from 74% - 75% at three hours post application down to 23% - 31% after six hours of application. *Chenopodium ambrosioides* produced the weakest protection, giving 69.6% protection at the first hour down to zero at six hour post application. However, two other plants *(R. chalepensis* and *Ci. sinensis)* were only highly effective for 1 hour with mean protection of 84% but declined thereafter, from 74% protection at two hour down to 12.5% – 17% protection at six hours post application. DEET, the standard commercial repellent gave much longer repellency (83-100%) for as long as six hours of the test period against *An. arabiensis*. The table in Appendix 1 depicts the trend in repellency effect of the six essential oils at 10% concentration compared with DEET. Statistical comparisons of mean percentage repellency between *Cy. citratus*, *L. tomentosa* and DEET were not significantly different after 1 hour of application ($P>0.05$)(Appendix 1). However, after 2-6 hours of application there was significant difference between all the test oils and DEET ($P<0.05$).

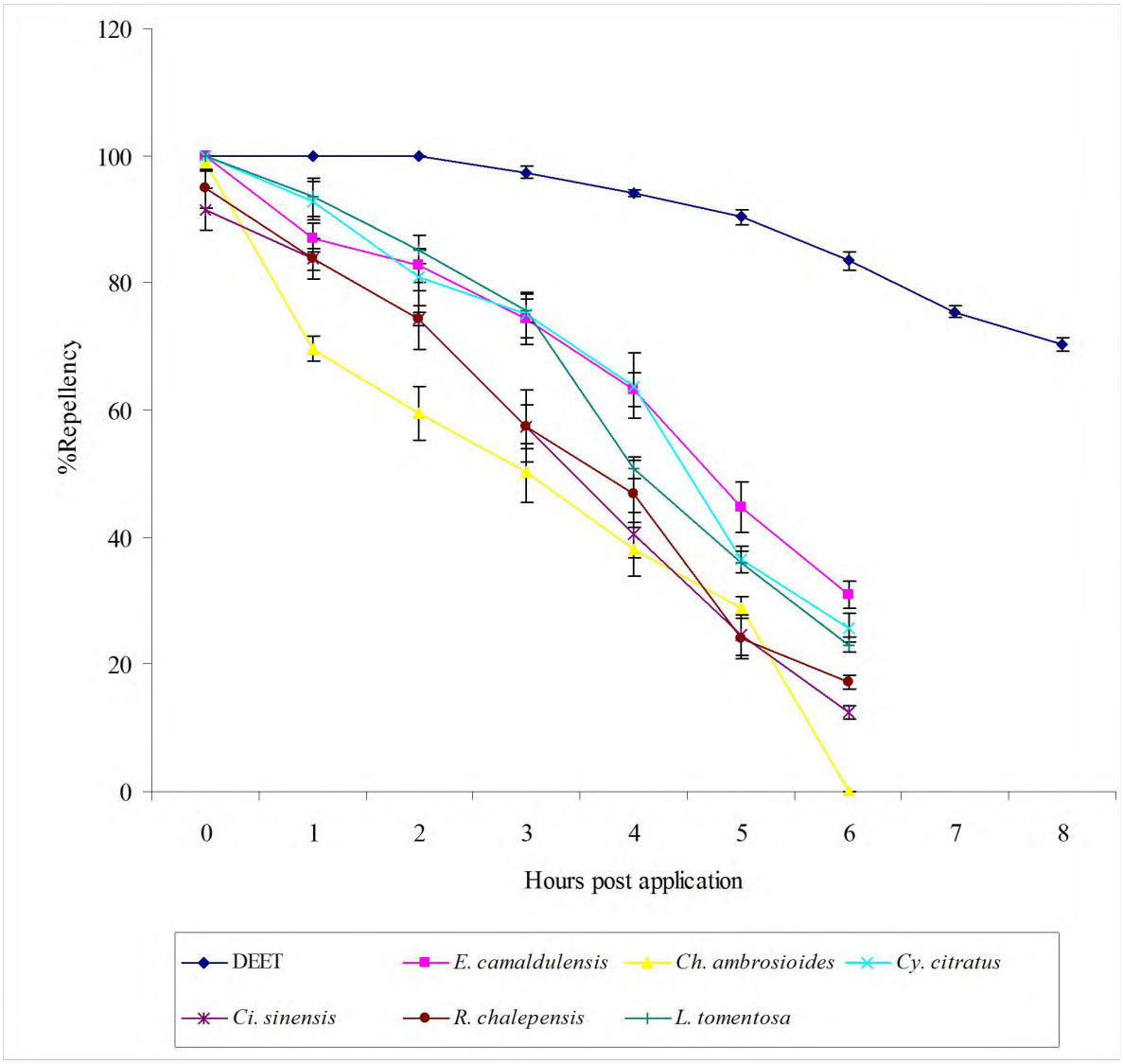

Figure 1 Mean percentage repellency provided by the essential oils of candidate plants and the standard DEET against *An. arabiensis* at 10% test concentration.

The response of *An. arabiensis* to the increased concentration of the oils at 20% is shown in Figure 2. All plants still gave very high repellency immediately after the application of the oils. However, with increased time, the same three plants (*L. tomentosa, E. camaldulensis* and *Cy. citratus*) gave the highest protection (80.3% - 91%) and the longest duration of protection lasting only for three hours. Their efficacy declined afterwards down to 15%- 41% at six post application. However, the repellency of *Ci. sinensis* and *R. chalepensis* only improved slightly from the 10% concentration. Both gave similar efficacy (88 – 89%) at the first hour and close to 80% at the end of the second hour. However, *Ch. ambrosioides* did not provide significant protection even for one hour at 20% concentration. The standard commercial repellent (DEET) still gave higher repellency (83- 100%) for as long as six hours of the test period against *An arabiensis*. The table in Appendix 2 depicts the trend in repellency effect of the six essential oils at 20% concentration compared with DEET. There was no significant difference in efficacy between *E. camaldulensis, Cy. citratus, R. chalepensis, L. tomentosa* and DEET after one hour application($P>0.05$)(Appendix 2). The essential oils of *E. camaldulensis, Cy. citratus, L. tomentosa* and DEET were not significantly different for 2 hours post application. There was no significant difference between DEET and *Cy. citratus* after 3 hours of application ($P>0.05$) (Appendix 2). However, after 4-6 hours of application there was significant difference between six candidate oils and DEET. Also some of them showed significant different between each other.

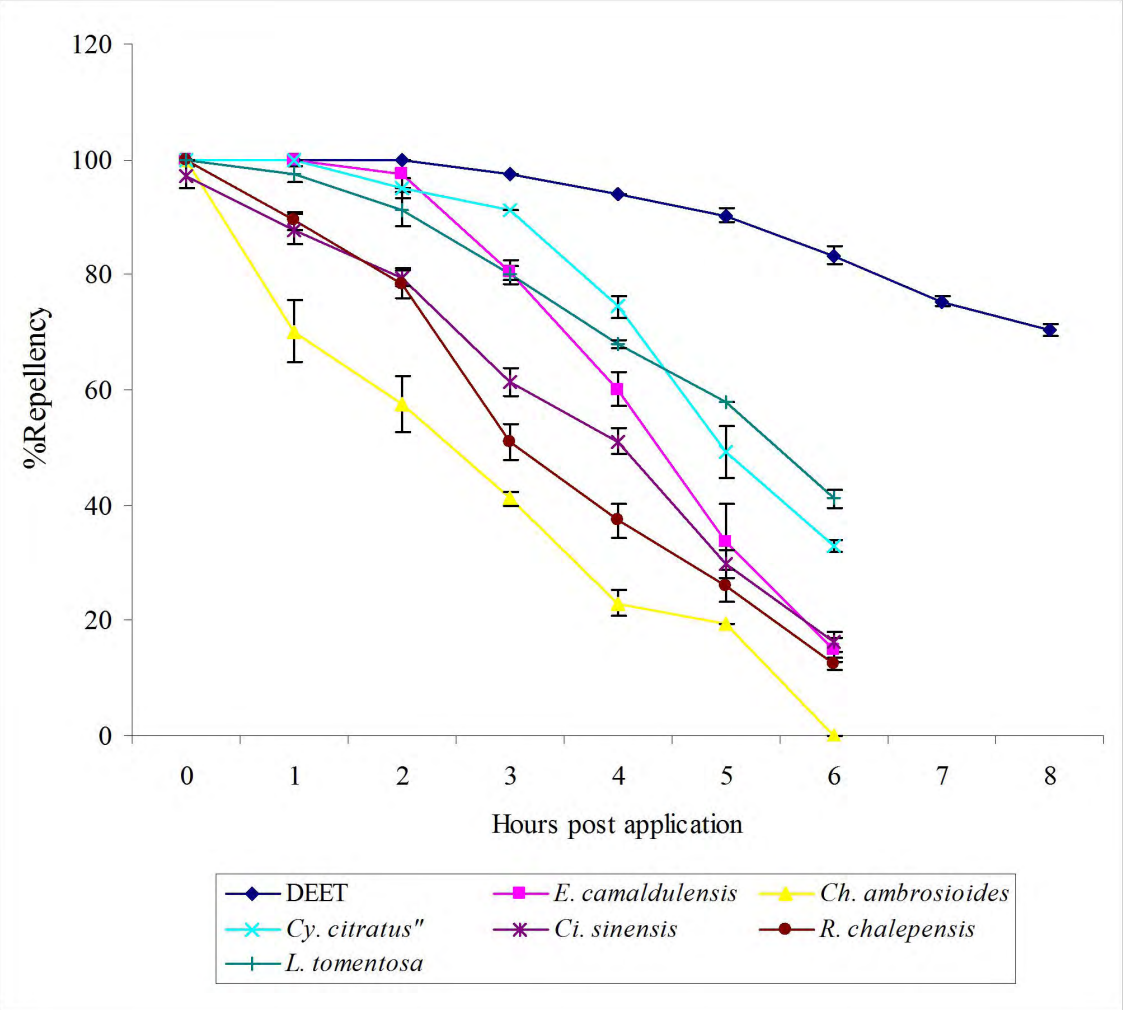

Figure 2 Mean percentage repellency provided by the essential oils of candidate plants and the standard DEET against *An. arabiensis* at 20% test concentration.

The responses of *Ae. aegypti* to the different concentrations of the six oils are shown in Figures 3 and 4. At 10 % concentrations, all plants except *Ch. ambrosioides* gave higher protection (>89%) soon after the fresh application of the oils (Figure 3). However, only one plant oil (*Cy. citratus)* gave about 91% protection lasting for one hour but declined afterwards from 75% at the end of the second hour to only 35% at the end of the fifth hour of testing. The remaining oils gave very weak protection starting even after the first hour of application (52.5% - 78.3% protection), whereas DEET continued to give greater than 90 % protection for about six hours. The table in Appendix 3 depicts the trend in repellency effect of the six essential oils against *Ae. aegypti* at 10% concentration compared with DEET. At 10% concentration there was no significant difference between *Cy. citratus* and DEET against *Ae. aegypti* for 1 hour of post application(Appendix 3). However, after 2-5 hours of application were showed significant difference between six candidate oils and DEET. And also some of them showed significant different between each other.

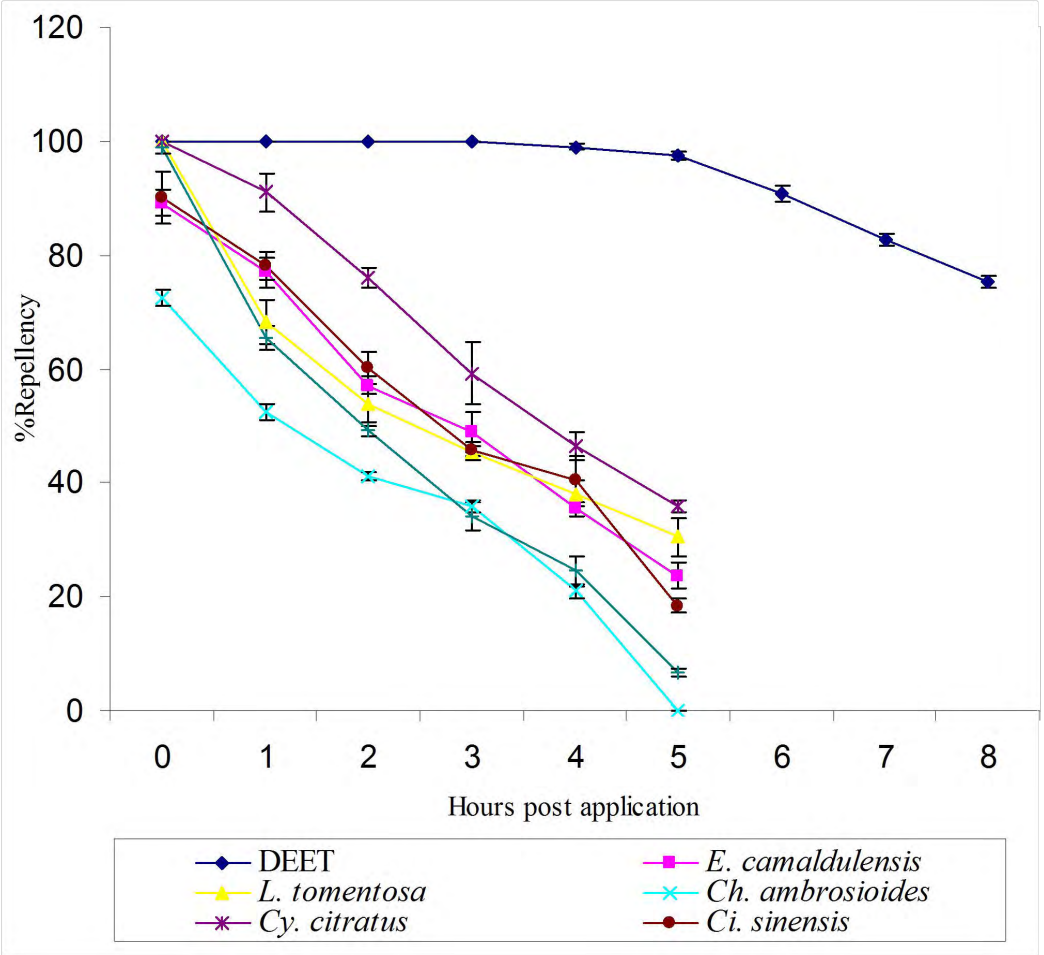

Figure 3 Mean percentage repellency provided by the essential oils of candidate plants and the standard DEET against *Ae. aegypti* at 10% test concentration.

At higher concentration (20%), all plants gave complete protection (>98%) immediately after the application of the oils (Figure 4). However, five more plants except *Ch. ambrosioides* continued to give high protection (81% - 93. 4%) for only one hour of post application but declined from 48% - 78% protection after two hours to as low as 11.3% - 34.2 after 5 hours of application. It was thus observed that increasing the concentration by two fold did not significantly improve the efficacy of the candidate repellent plants against *Ae. aegypti.* Furthermore, it was also observed that *Ae aegypti* was more tolerant to all the candidate repellent plants than *An. arabiensis* at both concentrations (10 % and 20%). DEET was similar in its effect as previously observed. The table in Appendix 4 depicts the trend in repellency effect of the six essential oils against *Ae. aegypti* at 20% concentration compared with DEET. At 20% concentration, there was no significant different between *Cy. citratus* and DEET against *Ae. aegypti* after 1 hour of application($P>0.05$) (Appendix 4). However, between six test oils and DEET were significant differences after 2-5 hours application ($P<0.05$) (Appendix 4).

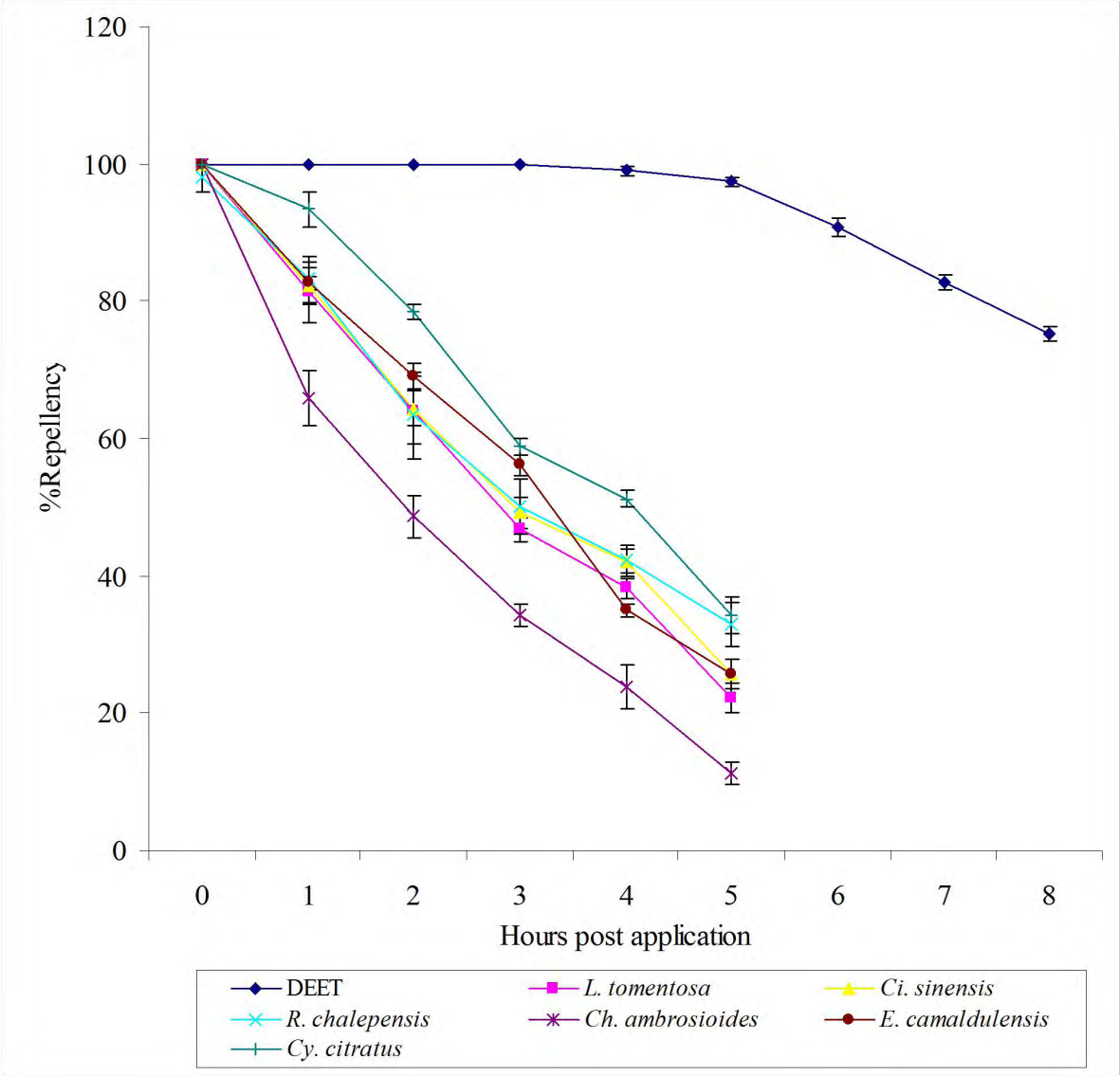

Figure 4 Mean percentage repellency provided by the essential oils of candidate plants and the standard DEET against *Ae. aegypti* at 20% test concentration.

4.3. Blend effect of the essential oils

This aspect of the study evaluated the mean percentage of protection of the 1:1 mixtures of essential oils (blend oils) to see any improvement in repellency due to the blend effects against *An. arabiensis* and *Ae. aegypti* at 10% and 20% concentrations of the mixtures of the oils.

Table 4 summarizes the results of the mean percentage repellency at different hours and 10% concentration of 15 combinations of essential oils against *An. arabiensis* compared to DEET. All the combination of oils produced very high protection (91-100%) soon after the application of the oils. However, only combination of *E. camaldulensis* and *Cy. citratus* gave the longest duration of protection lasting three hours with greater than 80% protection. It was followed by two blends of oils (*Ch. ambrosioides*+ *E. camaldulensis* and *Ch. ambrosioides* + Cy. *citratus)* which lasted only two hours with more than 80% protection against *An. arabiensis*. The remaining blend of oils, although most gave higher protection lasting for only one hour, all declined in their efficacy afterwards giving much less than 80% protection unexpectedly.

At 10% concentration, after 2 hours of application *Ch. ambrosioides* + *Cy. citratus*, *E. camaldulensis* + *Cy. citratus* and DEET were not significantly different in efficacy. There was no significant difference between *E. camaldulensis* + *Cy. citratus* and DEET against *An. arabiensis* for 3 hours of post application ($P>0.05$)(Table 4).

Table 4 Mean percentage protection ±S.E provided by the 1:1 combination of essential oils of test plants and the standard DEET against *An. arabiensis* at 10% concentration.

Blend oils	Hours post application						
	0	1	2	3	4	5	6
Ca+Ec	**98.91±1.09bc**	**87.99±1.23bcde**	**80.86±1.25cde**	60.14±4.52bcde	47.78±2.22bcd	32.01±1.55bc	20.04±2.36cd
Ca+Cc	**100.00±.00c**	**93.98±3.73cde**	**85.30±5.26ef**	68.63±2.41ef	48.89±3.24cde	30.78±2.85bc	20.80±1.90cd
Ca+Rc	**100.00±.00c**	**93.98±3.73cde**	67.51±4.50abcde	53.69±4.09abcde	43.33±3.54abc	31.85±3.29bc	21.31±1.50cd
Ec+Lt	**96.18±2.62bc**	**82.38±1.77abcde**	72.74±2.81bcde	65.27±3.90def	58.97±2.95de	49.99±4.09d	33.96±1.57e
Ec+Cs	**100.00±.00c**	79.43±5.95abcd	61.97±3.40ab	48.69±3.31abcd	37.17±1.76abc	27.30±1.82ab	20.35±2.02cd
Ec+Rc	**100.00±.00c**	68.40±6.56a	62.32±5.37ab	46.76±5.54abc	39.91±4.12abc	31.07±4.52bc	14.90±1.39ab
Ec+Cc	**100.00±.00c**	**95.44±1.63de**	**82.84±2.77def**	**80.03±2.65fg**	62.86±3.12e	43.61±2.37cd	28.83±1.82de
Cc+Lt	**100.00±.00c**	**84.42±3.89acde**	67.30±2.65abcd	51.39±1.39abcde	39.76±2.65abc	31.74±2.29bc	16.81±1.66bc
Cc+Ra	**100.00±.00c**	75.33±2.89abc	62.14±3.77ab	52.63±1.86abcde	40.04±1.61abc	34.20±1.64bc	16.13±1.59bc
Cs+Lt	**91.47±2.61a**	76.95±3.13abcd	52.13±3.06a	39.46±2.34a	32.35±1.77a	16.25±1.61a	6.00±.54a
Cs+Rc	**99.04±.96bc**	78.34±4.63abcd	64.16±5.27abc	55.07±5.06abcde	41.42±2.66abc	31.31±2.67bc	12.94±1.61abc
Ca+Lt	**100.00±.00c**	**91.28±1.88cde**	77.36±2.50bcde	63.13±3.56cdf	42.47±1.44abc	31.46±2.64bc	26.39±3.15de
Ca+Cs	**100.00±.00c**	79.43±5.95abcd	61.97±3.40ab	48.69±3.31abcd	37.17±1.76abc	27.30±1.82ab	16.36±1.65bc
Rc+Lt	**93.67±2.40ab**	71.60±2.12ab	61.97±3.40ab	44.20±3.65ab	33.84±4.71ab	21.07±2.94ab	8.94±1.23ab
Cc+Cs	**100.00±.00c**	**90.93±2.13cde**	71.51±1.39bcde	56.97±3.32abcde	44.22±3.16abc	29.27±4.66abc	12.50±1.19abc
DEET	**100.00±.00c**	**100.00±.00e**	**100.00±.00f**	**97.31±.95g**	**93.99±.52f**	**90.29±1.29e**	**83.41±1.48f**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at $\alpha = 0.05$).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*

The blend effects of the oils at higher concentration of 20% against *An. arabiensis* are shown in Table 5. It was observed that the same combination of *Cy. citratus* and *E. camaldulensis* oils provided greater than 80.5 % protection for only three hours (similar to the 10% concentration) even though two fold increased the concentration. However, four more mixtures of oils (*Ch. ambrosioides* + *E. camaldulensis, Ch. ambrosioides* + *Cy. citratus, Ch. ambrosioides* + *L. tomentosa* and *Cy. citratus* + *L. tomentosa*) produced more than 83% protection lasting for only two hours, after which they declined in their activity. The remaining mixtures of oils gave also high protection (>80%) but only lasted for one hour declining thereafter in their efficacy. Overall, the combination of oils did not significantly increase their potency against *An. arabiensis* even at higher concentration of 20%.

Table 5 shows there was no significant difference between DEET and *E. camaldulensis* + *Cy. citratus* against *Ae. aegypti* after 3 hours of application. *Chenopodium ambrosioides* + *Cy. citratus, Ch. ambrosioides* +*L. tomentosa, L. tomentosa*+ *Cy. citratus* and DEET were not significantly different in repellency activities for 2 hours of post application. After 1 hour of application *Ch. ambrosioides* + *Cy. citratus, Ch. ambrosioides* +*L. tomentosa, E. camaldulensis* + *Cy. citratus*, *E. camaldulensis* +*Ci. sinensis, Cy. citratus*+ *R. chalepensis, Ci. sinensis*+ *R. chalepensis* and DEET were not significantly different($P>0.05$)(Table 5).

Table 5 Mean percentage protection ±S.E provided by the 1:1 combination of essential oils of test plants and the standard DEET against *An. arabiensis* at 20% concentration.

Blend oils	Hours post application						
	0	1	2	3	4	5	6
Ca+Ec	**98.83±1.18a**	**86.83±3.03abcde**	**83.14±1.10cde**	72.06±2.07c	61.47±1.59de	36.01±1.97ab	21.68±1.87bcde
Ca+Cc	**100.00±.00a**	**96.31±2.136def**	**87.70±4.19def**	68.80±5.62bc	54.96±6.38abcde	42.79±3.58ab	22.69±1.97cde
Ca+Cs	**100.00±.00a**	**86.70±1.46abcde**	71.71±1.69abcd	50.94±1.91a	43.61±1.76abc	30.38±3.43ab	14.19±1.81abc
Ca+Lt	**100.00±.00a**	**95.97±2.64cdef**	**89.94±4.54ef**	59.21±4.51abc	46.41±2.18abcd	36.27±1.70ab	21.93±1.76cde
Ca+Rc	**100.00±.00a**	**85.10±1.89abc**	69.58±1.62abc	53.29±3.87ab	38.78±1.71ab	28.19±2.30ab	10.24±1.42a
Cc+Ec	**100.00±.00a**	**94.12±2.31bcdef**	**83.86±1.96cde**	**80.50±.29d**	44.55±2.06abcd	32.53±2.54ab	23.88±1.72de
Cc+Cs	**100.00±.00a**	**97.30±1.56ef**	78.70±1.87bcde	71.42±1.28c	66.16±2.13e	45.00±2.89b	25.14±2.92de
Cc+Rc	**100.00±.00a**	**93.09±2.75bcdef**	73.45±2.54abcd	59.31±1.68abc	46.64±1.99abcd	32.20±4.73ab	21.06±1.70bcde
Cc+Lt	**100.00±.00a**	**97.57±1.43ef**	**88.76±3.97f**	63.98±4.66abc	55.73±7.86bcde	39.14±3.90ab	26.77±.72de
Ec+Cs	**100.00±.00a**	**86.20±3.29abcd**	77.98±2.84abcde	64.93±2.46abc	53.15±3.97abcde	39.30±3.55ab	28.91±1.78e
Ec+Rc	**100.00±.00a**	**87.34±2.34abcde**	70.72±2.78abc	57.56±5.29abc	46.41±3.88abcd	26.10±5.22a	9.19±1.68a
Ec+Lt	**98.69±1.32a**	**84.44±1.87ab**	76.42±2.44abcde	70.98±3.14c	57.30±3.21cde	41.80±5.10ab	22.81±1.10cde
Cs+Rc	**100.00±.00a**	**90.05±1.91abcdef**	66.23±4.44ab	49.26±2.38a	37.38±3.01a	30.70±3.32ab	13.06±1.25ab
Cs+Lt	**97.56±1.41a**	**83.12±1.72ab**	65.96±4.67ab	50.00±2.27a	47.06±3.29abcd	36.15±3.54ab	19.75±1.40bcd
Rc+Lt	**98.81±1.19a**	**80.70±2.17a**	63.41±1.69a	52.30±1.49ab	41.36±2.30abc	35.57±1.99ab	24.25±1.65de
DEET	**100.00±.00a**	**100.00±.00f**	**100.00±.00f**	**97.31±.95c**	**93.99±.52f**	**90.29±1.29c**	**83.41±1.48f**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at α= 0.05).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*

Table 6 depicts the results of the mean percentage repellency at different hours and 10% concentration of 15 combinations of essential oils against *Ae. aegypti* compared to DEET

As expected, all the combination of oils produced high protection (91-100%) soon after the application of the oils against the bites of *Ae. aegypti*. However, almost all blends failed to produce significant protection beginning from the first hour, although two blends of oils (*Ch. ambrosioides* + *Cy. citratus* and *Cy. citratus* + *R. chalepensis*) resulted in close to 80% protection (Table 6). The remaining blends resulted in 58% to 75% protection at this hour. Subsequently, the percent protection of all blends declined to less than 68 down to 6% at the

end of the experiment at which time DEET was still very effective. There were significant differences in repellency obtained between DEET and all the fifteen 1:1 combined oils for 1-5 hours of after application ($P<0.05$)(Table 6).

Table 6 Mean percentage protection ±S.E provided by the 1:1 combination of essential oils of test plants and the standard DEET against *Ae. aegypti* at 10% concentration.

Blend oils	Hours post application					
	0	1	2	3	4	5
Ca+Ec	**82.7±1.14ab**	61.93±4.31ab	43.05±3.0a	32.14±4.77abc	15.83±3.06a	0.00±.00a
Ca+Cc	**98.86±1.14d**	79.80±2.92c	63.75±2.39cd	47.22±3.58cde	40.99±3.24ef	25.73±1.27cde
Ca+Cs	**97.99±1.16d**	68.46±1.65ab	56.39±4.57abcd	42.22±3.31abcde	30.96±3.50bcde	16.82±3.7bcd
Ca+Rc	**97.56±1.41d**	62.90±3.28abc	45.25±1.68ab	29.87±4.81ab	20.64±3.28ac	6.00±1.57ab
Ca+Lt	**97.64±1.38d**	63.50±2.61abc	56.67±4.00abcd	48.20±3.72cde	39.98±3.92def	28.44±3.8de
Cc+Lt	**98.96±1.04d**	75.46±2.43bc	68.77±.794d	51.97±3.00de	36.27±1.7def	21.72±2.58cd
Cc+Cs	**100.00±.00d**	62.29±.53ab	56.94±1.79abcd	46.11±2.56bcde	31.42±1.31bcde	23.57±.57cd
Cc+Ec	**94.35±2.13cd**	75.34±4.64bc	64.68±4.21cd	56.95±4.17e	49.35±4.01f	36.34±2.99e
Cc+Rc	**98.75±1.25d**	78.89±5.51bc	65.20±2.51cd	28.11±2.01a	17.83±2.25ab	5.21±1.66ab
Ec+Lt	**98.75±1.25d**	57.99±4.77a	46.94±4.54ab	35.62±4.66abcd	25.93±3.32abcd	15.61±1.53bc
Ec+Rc	**81.68±1.45a**	70.09±3.85abc	59.29±.715bcd	43.46±.58abcde	32.77±1.19cde	19.57±4.46dc
Ec+Cs	**89.02±.91abc**	65.13±.62abc	54.22±2.82abcd	43.42±2.52abcde	33.33±2.27cde	23.53±2.40cd
Cs+Lt	**89.53±2.64bc**	61.02±2.12ab	42.03±2.7a	35.13±2.75abc	21.08±4.09abc	14.52±3.01bc
Cs+Rc	**95.29±1.86cd**	62.66±3.96abc	51.04±4.90abc	42.73±3.08abcde	32.72±3.52cde	21.51±1.62cd
Rc+Lt	**90.11±1.84bc**	63.83±1.84abc	47.79±3.17ab	40.60±2.47abcde	31.49±2.13bcde	20.33±1.64cd
DEET	**100.00±.00d**	**100.00±.00d**	**100.00±.00e**	**100.00±.00f**	**99.00±.61g**	**97.44±.66f**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at $\alpha = 0.05$).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*

The blend effects of the oils at higher concentration of 20% against *Ae. aegypti* are shown in Table 7 There was only a slight improvement at the first hour of the experiment where, *Ch. ambrosioides* + *Cy. citratus*, *E. camaldulensis* + *L. tomenosa*, *E. camaldulensis* + *Cy. citratus*, *R.. chalepensis* + *Cy. citratus*, *E. camaldulensis* + *R.. chalepensis* and *Cy. citratus*,+ *Ci. sinensis* gave between 80% and 93% protection. Subsequently, these blends and the remaining blends (which already failed at the first hour) failed to produce significant protection. As for *An. arabiensis,* the combination of oils did not improve their potency as repellents against *Ae. aegypti* even at higher concentration of 20%. In fact, *Ae. aegypti* still appeared to be more resistant than *An. arabiensis* even to the blends of oils at both concentration (10% and 20%) as was the case with single oil applications.

After 1 hour of application *Ch. ambrosioides* + *Cy. citratus*, *E. camaldulensis* + *L. tomentosa*, *E. camaldulensis* + *Cy. citratus*, *R. chalepensis* + *Cy. citratus*, *E. camaldulensis* + *R. chalepensis*, *Cy. citratus* + *C. sinesis* and DEET were not significant different in efficacy($P>0.05$). However, all the mixed oil and DEET showed significant difference after 2-5 hours application ($P<0.05$) (Table 7).

Table 7 Mean percentage protection ±S.E provided by the 1:1 combination of essential oils of test plants and the standard DEET against *Ae. aegypti* at 20% concentration.

Blend oils	Hours post application					
	0	1	2	3	4	5
Ca+Lt	**100.00±.00b**	78.85±3.27cdef	65.86±3.27abcde	55.14±3.54bcd	44.50±3.90cde	33.75±3.61cde
Ca+Cc	**100.00±.00b**	**91.14±3.86fgh**	77.17±4.93de	49.34±2.36abcd	35.21±1.31abc	17.92±2.42ab
Ca+Cs	**100.00±.00 b**	78.19±2.76bcdef	51.19±3.32a	38.60±3.72a	28.10±3.03ab	19.67±3.64abcd
Ca+Rc	**100.00±.00 b**	79.47±2.98cdef	60.54±1.10abcd	49.41±2.77abcd	43.71±2.85cde	34.34±1.34de
Ec+Ca	**100.00±.00 b**	63.71±1.43a	54.98±3.69ab	40.68±2.55ab	34.67±3.11abc	15.46±1.69a
Ec+Lt	**100.00±.00 b**	**83.93±2.43defg**	67.36±2.86abcde	50.17±4.33abcd	26.56±1.56a	18.75±2.55abc
Ec+Cc	**97.73±2.27 b**	**91.08±3.24fgh**	71.43±3.37bcde	60.56±.32cd	49.99±2.27de	32.90±2.07bcde
Ec+Cs	**95.96±1.70 b**	70.83±2.13bcd	59.69±3.74abc	45.46±3.29ab	34.72±2.66abc	23.71±3.99abcd
Cc+Lt	**100.00±.00 b**	79.32±1.72cdef	72.04±3.33cde	63.08±2.71d	52.93±4.13e	40.50±4.17e
Cs+Lt	**94.21±3.45 b**	64.54±3.57ab	54.07±4.04a	42.47±3.47ab	32.35±2.47abc	22.34±2.68abcd
Rc+Lt	**98.81±1.19 b**	66.28±3.18abc	57.25±2.51abc	48.69±1.82abcd	41.97±2.26bcde	25.96±3.28abcde
Rc+Cc	**100.00±.00 b**	**93.28±1.37gh**	79.33±2.73e	53.23±2.83abcd	37.50±2.66abcd	24.17±2.20abcd
Rc+Cs	**100.00±.00 b**	75.87±2.04abcde	56.73±2.67abc	46.57±2.63abc	38.48±3.23abcde	28.77±2.74abcde
Ec+Rc	**86.30±1.73a**	**80.34±1.35defg**	67.50±4.33abcde	53.44±3.56bcd	42.40±4.18bcde	29.32±3.20abcde
Cc+Cs	**100.00±.00 b**	**89.43±3.97efgh**	67.96±3.61abcde	52.50±2.72abcd	41.34±3.13bcde	28.28±4.32abcde
DEET	**100.00±.00 b**	**100.00±.00h**	**100.00±.00f**	**100.00±.00e**	**99.00±.61f**	**97.44±.66f**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at $\alpha = 0.05$).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*

5. DISCUSSION

The present `study was designed to evaluate repellency effect of essential oil of six local plants against laboratory reared *An. arabiensis* and *Ae. aegypti*. In the laboratory study the essential oils were evaluated at 10% and 20% concentration to compare the efficacy of the oils with the standard DEET. In both concentrations all the oils from the six plants gave very high protection (> 90%) soon after application against *An. arabiensis*. While at 10% and 20% concentrations except *Ch. ambrosioides*, all the five oils gave high repellency (>89) immediately after treatment against *Ae. aegypti*.

For *An. arabiensis, Cy. citratus* oils showed highly significant repellent activities at 10% and 20%. At 10% and 20% concentrations, *Cy. citratus* provided 80% protection for 2 hours and 91-100% repellency for 3 hours, respectively, declining much lowers after that. This shows an improved repellency could be obtained by increasing the concentration, however, the same two concentrations of the same plant gave significant protection (above 90 %) for only one hour against *Ae. aegypti* which in this case no improvement was noted even with increased concentration. This also shows that *Ae. aegypti* were not as sensitive as *An. arabiensis* to *Cy. citratus* oil. Sidibe *et al*. (2001) reported the active ingredient of *Cy. citratus* are citral, geraniol, citronellol and linalool. In agreement to this, Oyedele *et al*. (2002) reported the citral with 15%v/v in liquid paraffin exhibited repellent effect against *Ae. aegypti* and geraniol and citronellol used as repellent to mosquitoes(Tyagi *et al*., 1998) and linalool showed repellent to mosquito(Jaenson *et al*., 2006). Field tests in Bolivia showed that 25% *Cy. citratus* in ethanol provided 77.93% and 90.67% protection for 3 hrs against *Anopheles darlingi* and *Mansonia* spp. respectively (Moore, 2005), showing similar level of repellency with the present results obtained.

Similarly, Trongtokit *et al*. (2005) reported that 10% *Cy. citratus* provided 30 minutes complete protection against *Ae. aegypti*. Amer and Mehlhron (2006) also observed that *Cy. citratus* gave 70.3% repellency for 3 hours against *Ae. aegypti*, 100% protection for 8 hours against *An. stephensi* and *Cx. quinquefasciatus* at 20% concentrations. In another study, Oyedele *et al*. (2002) evaluated application of essential oil of *Cy. citratus* on bird's bare skin against *Ae. aegypti*. They reported that 20% and 25% liquid paraffin solution of Lemmon

grass oil exhibited complete protection for an hour. The 1% and 5% concentration oil provided more than 50% repellency for 3 hours while the 10-25% concentrations gave higher protection (>90% repellency) over 3 hours. Ansari and Razdan (1995) also reported the repellent activity of *Cy. citratus* in different mosquito species, protected for 11hours against *An. culicifacies* and for 6-7 hours against *Culex quinquefasciatus*. Related plants (*Cy. martini* and *Cy. nardus)* in India also gave more than 6 hours of protection against *An. culicifacies* and Cx. *quinquefasiatus* (Ansari and Razdan, 1995).

The evaluation of essential oil of *E. camaldulensis* for its repellency against mosquitoes is the first in Ethiopia. At 10% and 20% concentration the oils showed 82% repellency for 2 hours and 80-100% repellency for 3 hour *against An. arabiensis,* respectively. Against *Ae. aegypti*, protections of 76% and 82% repellency were recorded at 10% and 20% concentrations for only one hour, respectively. In Ethiopia, Dagne *et al.* (2000) reported essential oil of *E. camaldulensis* contains beta- phellandrene, p- cymene and 1,8-cineole. These are known to be repellent to mosquitoes (Jaenson *et al*., 2006). On the other hand in Japan Watanabe *et al*. (1993) evaluated the mosquito-repelling activities of 4-isopropylbenzyl alcohol and eucamalol obtained from *E. camaldulensis* oil against *Ae. aegypti* in comparison with DEET. Besides, 7.7% eucamalol provided 75% repellency even after 3 hours treatment and showed superior repellency against *Ae. aegypti* as compared with DEET. However, the present result *E. camaldulensis* showed higher repellency against *An. arabiensis* compared to *Ae. aegypti* at both concentration. In another study conducted in Japan the essential oil from *E. camaldulensis* exhibited significant repellent activities against *Ae. albopictus* (Nishimura *et al*., 1987 cited in Watanabe *et al*., 1993).

The repellency of essential oil of a related plant *E. maculata citriodon* (= *Corymbia citriodoria now*) (lemon eucalyptus) was shown to be as effective as DEET against *An. gambiae* and *An. funestus* Giles. This was due to the main active ingredient, P-menthane-3, 8-diol (quwenling) found in *E. maculata citriodon.* This repellent plant has been found to be effective against mosquitoes, midges, ticks and the stable fly (Trigg, 1996; Trigg and Hill, 1996; Govere *et al*., 2000a). Yang and Ma (2005) reported 15% of *E. globulus* oil provided repellency for at least 5 hours against *Ae. albopictus* when mixed with vanillin, but do not

indicate the percentage of repellency. A report showed by Amer and Mehlhorn (2006) indicated that the protection time of 20% *E. globulus* oil against *Ae. aegypti* are 57.6% for 2 hours and 52.4% for more than 5 hours against *An. stephensi.* Wano (2006) reported the 10% and 20% concentrations of essential oils of *E. globulus* and *E. citriodora* showed lower repellent activities against *An. arabiensis* and *Ae. aegypti* under laboratory study. Therefore, *E. camaldulenis* in the present study can be regarded as superior than some of the above findings, but needs further follow up.

There are no previous reports on the repellency of the essential oil of *L. tomentosa* or any other *Laggera* species elsewhere. In the present study, the repellency of *L. tomentosa* at 10% and 20% concentration provided 85-100% protection for 2 hours and 80-100% repellency was observed after 3 hours application against *An. arabiensis,* respectively. On the other hand, it was much inferior against *Ae. aegypti* by providing only 81-100% protection at 20 % concentration for only one hour. Again, *Ae. aegypti* was shown to be more tolerant than *An. arabiensis* to this plant as observed in other plants as well. Asfaw *et al*. (2003) isolated monoterpens, chrysanthenone, isochrysantenone, filifolone active ingredients from this plant of which the former (also isolated from *Thymus vulgaris)* are known to be have repellent activities against mosquitoes (Choi *et al*., 2002). Therefore, this plant showed promising results and needs further attention and investigation for possible use as a repellent.

Regarding the efficacy of *Ci. sinensis* oil against *An. arabiensis,* no more than one hour of significant protection (> 80%) was recorded at both 10% and 20 % concentration. The sensitivity of *Ae. aegypti* to this plant of both concentrations was some what similar to *An. arabiensis*, although slightly lower. Yang and Ma (2005) reported that application of 1-15% concentrations rutaceae oil (*Ci. sinensis*) on mice, under laboratory condition, provided more than 90% protection for 8 hours against *Ae. albopictus*. Various factors might responsible for this variation, but obvious factor are differences in test subjects and the test mosquito species. Elsewhere, the essential oil of a related fruit *Ci. aurantium* when mixed with mustard oil gave 2, 4, and 5 hours protections at 10%, 20%and 30%, respectively, against *Ae. albopictus* (Das *et al*., 2003). In some countries, orange peels are burned at night to drive off mosquitoes (Don-Pedro, 1985). However, the essential oil of *Ci. sinensis* has been well

studied for its insecticidal properties against a variety of pests and vectors (Ezenou *et al.,* 2001; Tripathi *et al*., 2003).

Similarly, the essential oil of *R. chalepensis* was investigated in the present study. At both concentrations (10% and 20%), *R. chalepensis* provided greater than 83% protection for only one hour against *An. arabiensis*, respectively. Although higher protection was noted for the highest concentration no improvement in the duration of repellency. Against, *Ae. aegypti* only 20% of this plant gave significant protection (about 83%) lasting only one hour. Previously, Hadis *et al*. (2003) reported repellent effects of essential oils of *R. chalepensis* 91.6 and 78% repellency against *Mansonia uniformis* and *M. nigerrima* conducted in Ethiopia under field conditions at 50% and 40% concentrations, respectively. Atsebeha (2005) also evaluated the repellency of this plant by traditional means of applications (direct burning and thermal expulsion of the plant parts) and reported good protections against anopheline and culicine mosqutoes in the field.

With regards to *Ch*. *ambrosioides* it was found to be the weakest of all the tested plants at both 10 and 20 % concentrations against both species of mosquitoes. The results are somewhat surprising in that its essential oils were found to exhibit very effective larvicidal properties against the larvae of both species in the laboratory in Ethiopia (Massebo, 2006). Elsewhere, however, the petroleum ether extract of *Ch. ambrosioides* gave 3 hours protection against mosquitoes at 9% concentration (Venkatachalam and Jebanesan, 2001). The lower repellency of this plant in Ethiopia is either due to the extraction method or the strain of the plant itself.

The present study also evaluated fifteen 1:1 combinations of essential oils against mosquitoes. None of the fifteen combinations of oils, revealed significant blending repellents effects to be regarded better than the single oil application. There was combination of oils which gave as good repellent effects as the single oil application. For example, some of these include combinations of *E. camaldulensis*. and *Cy. citratus* (at 10% and 20% concentrations) *Ch. ambrosioides* + *E. camaldulensis and Ch. ambrosioides* + *Cy. citratus.*

The single application of some oils, especially at 20% gave more than 3 hours of protection, so no marked repellency was noted with the combined oils. This trend is observed for almost all combined oils, showing no synergistic effect by combining oils. This may be due to the antagonistic effects rather than synergetic effects (Howard *et al*., 2003). On the other hand, the combination of 3 or more oils have been known to give greater repellent effects (Howard *et al.,* 2003). In the present study, evaluations of three or more combined oils could not be done due to shortage of time. However, based on the present study, single oil applications with increased concentration of the oils (20% in the case) gave more than three hours of protection. The efficacy of these oils and other plants tested could be better improved if they were extracted by steam distillation which is said to be superior in extracting the active ingredients better than hydro-distillation (Chiasson *et al*., 2001) which is the method used to extract the oils in the present study.

In general, the relative protection of all the oils tested in this study decreased after an hour of application against both *An. arabiensis* and *Ae. aegypti*. This suggests that there is faster loss of repellent effect due to the high volatility of the active ingredients of the essential oils; there are different factors that play determining the effectiveness of any repellent. These include the species of mosquitoes, the user's age, sex, level of activity, temperature, humidity and the density of mosquitoes in a cage.

Finally, the present study revealed that *E. camaldulensis*, *Cy. citratus* and *L. tomentasa* gave good results both at 10% (at least 80% protection for two hours) and 20% concentration (at least 80 % for the three hours) against laboratory colonies of *An. arabiensis*. The Environmental Protection Agency (EPA) of USA has set a 2 hour minimum repellent activity requirement at 80 % protection in order to gain registration (Novak and Gerberg, 2005). However, field evaluations of these oils are mandatory for registrations. Although most plant essential oils are less efficacious than the synthetic repellents, plant oils are believed to be safer than synthetic repellents and can be reapplied repeatedly. However, DEET and other synthetic repellents cannot be reapplied because of safety reasons even though they are effective.

6. CONCLUSION AND RECOMMENDATIONS

The Environmental Protection Agency (EPA) has set 2 hours minimum activity requirement of a plant derived repellent in order to gain registration. According to this the essential oils of *E. camaldulensis*, *Cy. citratus* and *L. tomentasa* gave promising result against *An. arabiensis* for 2 hours and 3 hours protection at 10% and 20% concentration, respectively.

The result of the present study indicated that essential oils of studied local plants have promising protection against mosquito biting. The responsible active ingredients for repellency of the essential oils should be understood, identified and characterized. Moreover, formulation of these oils with vanillin and other complex solvent is of paramount importance in order to improve or enhance their repellency and stability. Prior to practical use of these repellent oils, further research should be conducted on their safety to human health.

Furthermore, further field evaluations are required on some of above promising plants. Laboratory studies are only useful for screening purposes but should be evaluated in field population of mosquitoes. To determine the exact efficacy of these plants. Plant oils attending a minimum 80% protected for at least have known to qualify for registered at field application. Although most plant oils are far in terms the synthetic repellents (e.g. DEET) plant oils are thought to be safe after the reapplied even if the duration of protection is reduced over the DEET and other synthetic repellents which are known to have undesirable side effects

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APPENDICES

Appendix 1 Mean percentage protection ±S.E provided by the essential oils of test plants and the standard DEET against *An. arabiensis* at 10% test concentration.

Oils	Hours post application						
	0	1	2	3	4	5	6
Ec	**100.00±. 00b**	**87.08±2.18b**	**82.60±2.54b**	74.23±4.05bc	63.13±2.63b	44.58±3.93c	30.96±2.10e
Ca	**98.75±1.25ab**	69.59±1.97 a	59.59±4.22a	50.14±4.62a	38.13±4.25a	28.88±1.71ab	.00±. 00a
Cc	**100.00±. 00b**	**92.90±3.08bc**	**80.87±4.47b**	75.00±3.54c	63.83±5.20b	36.47±2.24bc	25.75±2.17de
Cs	**91.58±3.19a**	**83.75±1.73b**	74.29±1.17ab	57.38±3.53ab	40.39±3.55a	24.58±3.14ab	12.50±1.02b
Rc	**94.80±3.12ab**	**83.83±3.16b**	74.22±4.60ab	57.42±5.62ab	46.83±5.28ab	24.01±3.17a	17.25±1.11bc
Lt	**100.00±. 00b**	**93.45±2.95bc**	**85.20±2.27b**	75.68±1.89c	50.85±1.78ab	36.04±1.65bc	23.06±1.21cd
DEET	**100.00±. 00b**	**100.00±. 00c**	**100.00±. 00c**	**97.31±. 95d**	**93.99±. 52c**	**90.29±1.29d**	**83.41±1.48f**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at $\alpha = 0.05$).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*

Appendix 2 Mean percentage protection ±S.E provided by the essential oils of test plants and the standard DEET against *An. arabiensis* at 20% test concentration.

Oils	Hours post application						
	0	1	2	3	4	5	6
Ec	**100.00±. 00a**	**100.00±00c**	**97.50±2.50d**	**80.49±2.20d**	60.04±2.97cd	33.70±6.36a	15.00±2.04b
Ca	**100.00±. 00a**	70.21±5.47a	57.50±4.79a	41.11±1.11a	23.06±2.37a	19.55±. 46a	.00±. 00a
Cc	**100.00±. 00a**	**100.00±00c**	**94.99±1.68d**	**91.22±. 50e**	74.53±1.88e	49.27±4.56b	32.91±1.05c
Cs	**97.03±1.86a**	**87.85±2.6b**	79.58±1.47bc	61.34±2.34c	51.13±2.27c	29.93±2.42a	16.38±1.65b
Rc	**100.00±. 00a**	**89.38±1.49bc**	78.30±2.34b	51.13±3.11b	37.29±3.02b	26.03±2.62a	12.50±1.02b
Lt	**100.00±. 00a**	**97.50±1.45bc**	**91.30±2.97cd**	**80.28±1.15d**	68.02±. 82de	57.92±. 81b	41.11±1.51d
DEET	**100.00±. 00a**	**100.00±00c**	**100.00±. 00d**	**97.31±. 95e**	**93.99±. 52f**	**90.29±1.29c**	**83.41±1.48e**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at $\alpha = 0.05$).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*

Appendix 3 Mean percentage protection ±S.E provided by the essential oils of test plants and the standard DEET against *Ae. aegypti* at 10% concentration.

Oils	Hours post application						
(10%)	0	1	2	3	4	5	6
Ec	**89.08±2.24c**	76.98±2.57c	57.18±1.60bc	49.03±3.53bc	35.37±1.25c	23.75±2.39bc	NT
Lt	**100.00±.00d**	68.19±3.79bc	53.70±3.80bc	45.50±1.66ab	38.15±2.29bcd	30.53±3.40cd	NT
Ca	**72.50±1.44a**	52.50±1.44a	41.13±.66a	35.83±1.02a	20.94±1.39a	.00±.00a	NT
Cc	**100.00±.00d**	**90.97±3.19d**	75.92±1.80d	59.29±5.54c	46.40±2.57d	35.83±1.02d	NT
Cs	**90.06±4.59c**	78.29±2.46c	60.05±2.81c	45.65±.913ab	40.64±4.10cd	18.44±1.18b	NT
Rc	**98.96±1.04cd**	65.42±2.08b	49.41±1.23ab	34.21±2.43a	24.59±2.63ab	6.75±.66a	NT
DEET	**100.00±.00d**	**100.00±.00d**	**100.00±.00e**	**100.00±.00d**	**99.00±.61e**	**97.44±.66e**	**94.90±1.31**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at $\alpha = 0.05$).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis*, NT= Not Tested.

Appendix 4 Mean percentage protection ±S.E provided by the essential oils of test plants and the standard DEET against *Ae. aegypti* at 20% concentration.

Oils	Hours post application						
	0	1	2	3	4	5	6
Lt	**100.00±.00a**	**81.35±4.46b**	64.15±4.99ab	46.80±1.67b	38.21±1.52b	22.18±2.10d	NT
Cs	**100.00±.00a**	**82.24±2.63b**	64.39±2.47ab	49.20±2.24b	42.09±2.29b	25.75±2.17bc	NT
Rc	**97.92±2.08a**	**83.28±3.34b**	63.38±6.21ab	50.15±4.01bc	42.20±1.62b	33.01±3.25c	NT
Ca	**100.00±.00a**	65.97±3.99a	48.64±3.07a	34.30±1.68a	23.90±3.19a	11.25±1.61a	NT
Ec	**100.00±.00a**	**82.67±.90b**	69.11±1.90b	56.16±1.44bc	34.97±1.01b	25.63±2.13bc	NT
Cc	**100.00±.00a**	**93.40±2.53bc**	78.48±1.19b	58.87±1.20c	51.25±1.25c	34.21±2.71c	NT
DEET	**100.00±.00a**	**100.00±.00c**	**100.00±.00c**	**100.00±.00d**	**99.00±.61d**	**97.44±.66d**	**94.90±1.31**

Means in each column (hour) followed by the same letter are not significantly different Tukey HSD homogenous test (at α= 0.05).

Key: Ca= *Ch. ambrosioides,* Ec= *E. camaldulensis,* Cc= *Cy. citratus,* Lt= *L. tomentosa,* Cs= *Ci. sinensis,* Rc= *R.. chalepensis,* NT= Not Tested.

Ruta chalepensis

(Source: carol03205.tripod.com)

Cymbopogon citratus

(Source: www.ceted.org)

Eucalyptus camaldulensis

(Source:www.azarboretum.org)

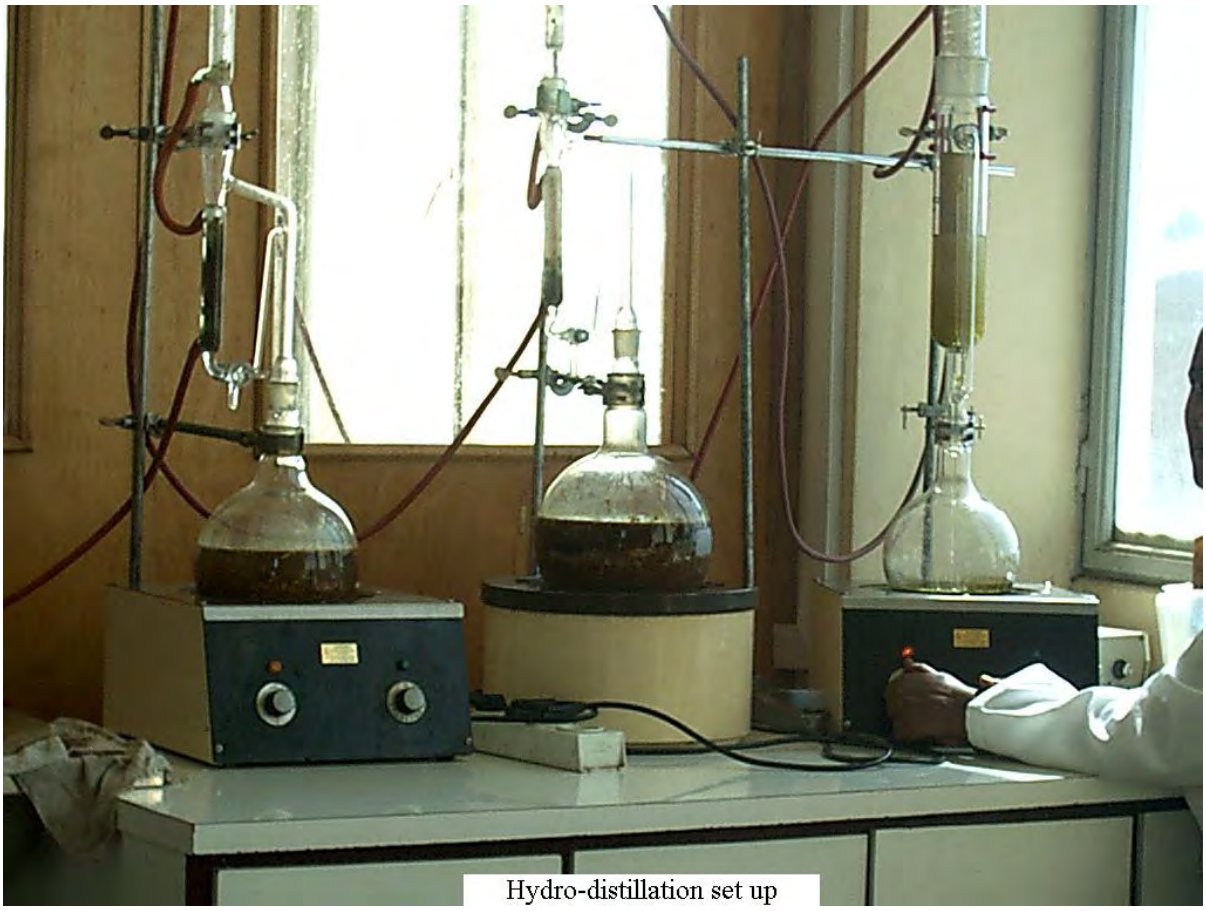
Citrus sinensis

(Source: biology.vassar.edu)

Chenopodium ambrosioides

(Source: www.floradecanarias.com)

Appendix 5.The Test Plants



Appendix 6. Essential oils extraction apparatus

Appendix 7 Human volunteers exposing forearms in the cage